(E)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]-methyl]thio]methyl]benzoic Acid and Related Compounds: High Affinity Leukotriene B_4 Receptor Antagonists

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(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid (**11**, SB 201993) is a novel, potent LTB_4 receptor antagonist. Compound **11** arose from a structure–activity study of a series of trisubstituted pyridines that demonstrated LTB_4 receptor antagonist activity. The placement of an additional methylene unit in the sulfur containing chain linking the pyridine and benzoic acid moieties of lead compound **8** (K_i = 80 nM) resulted in a greater than 10-fold increase in receptor affinity. Additionally, in this new series of compounds, the oxidation state of the sulfur was found to be critical to the activity, i.e., the sulfoxide and sulfone showed substantially lower affinity for the LTB_4 receptor. Compound **11** competitively inhibits the binding of [^{3}H]LTB_4 to LTB_4 receptors on human polymorphonuclear leukocytes with a K_i of 7.1 nM and blocks both the LTB_4-induced calcium mobilization and the LTB_4-induced degranulation responses in these cells with IC_{50} values of 131 and 271 nM, respectively. Compound **11** demonstrated oral LTB_4 antagonist activity as well as topical antiinflammatory activity in the mouse.

Leukotriene B_4 (LTB_4, **1**, Figure 1), a product of 5-lipoxygenase-catalyzed oxygenation of arachidonic acid, has been postulated to be a mediator of a variety of inflammatory diseases. Known pathophysiological responses of LTB_4 include potent neutrophil chemotactic activity, the promotion of adherence of polymorphonuclear leukocytes (PMNs) to the vascular endothelium, stimulation of the release of lysosomal enzymes and superoxide radicals by PMNs, and an increase in vascular permeability.[1,2] Furthermore, the pro-inflammatory actions of LTB_4 have been demonstrated *in vivo*. Topical application of LTB_4 on human skin promotes the infiltration of PMNs and other inflammatory cells,[3] intradermal injection of LTB_4 results in the accumulation of neutrophils at the injection site,[4] and intravenous injection of LTB_4 causes rapid but transient neutropenia.[5] These phenomena coupled with the presence of elevated concentrations of LTB_4 in psoriatic lesional skin,[6] in colonic mucosa associated with inflammatory bowel disease,[7] in synovial fluid from patients with active rheumatoid arthritis,[8] and in gouty effusions[9] support the involvement of LTB_4 in human inflammatory diseases. LTB_4 is produced by mast cells, PMNs, monocytes, alveolar macrophages, peritoneal macrophages, and keratinocytes. The synthesis of LTB_4 can be induced in these cells by the calcium ionophore A23187, opsonized zymosan particles, antigen, the chemotactic peptide formylmethionylleucylphenylalanine (FMLP), aggregated immunoglobins (IgG, IgE, and IgA), and bacterial exotoxins.

The pharmacological effects of LTB_4 are mediated through its interaction with specific cell surface receptors that have been characterized on PMNs, monocytes, U-937 cells, lymphocytes, mast cells, smooth muscle cells, and endothelial cells, as well as on various tissues such as spleen, lung, heart, brain, small intestine, uterus, and kidney.[10] The major pro-inflammatory activity of LTB_4 is thought to involve a receptor-mediated induction of aggregation and the adhesion of inflammatory cells, especially PMNs, to venular endothelial cells. Furthermore, it has been postulated that LTB_4 synergizes with other chemotactic factors, such as C5a, to amplify the inflammatory response.[1] Therefore, the availability of potent and selective LTB_4 receptor antagonists should prove useful in elucidating the role of LTB_4 in human inflammatory diseases.

In recent years, the efforts of several laboratories have been directed toward the identification of high affinity LTB_4 receptor antagonists (Figure 1). Lilly reported that LY223982 (**2**) has an IC_{50} of 12 nM in binding studies using human PMNs. This compound has recently evolved into the xanthone LY292728 (**3**) having a K_i of 0.47 nM in human PMNs.[11] Ono reported that ONO-4057 (**4**) has a K_i of 4 nM for LTB_4 receptors on human PMNs[12] while Roche has reported a related compound, Ro-25-3562 (**5**), as having a K_i of 1 nM.[13] The chiral LTB_4 receptor antagonist SC-53228 (**6**, K_i = 1.3 nM) has been reported by Searle,[14] and Rhône-Poulenc Rorer has reported that RG 14893 (**7**) has a K_i of 2 nM on human PMNs.[15]

The LTB_4 receptor antagonist effort was initiated with the objective to discover novel antiinflammatory agents. As previously reported, using LTB_4 (**1**) as a structural template the program quickly produced compounds with high receptor affinity. However, these initial compounds exhibited partial agonist activity.[16] An effort to reduce the structural similarity between these compounds and LTB_4 led to a class of trisubstituted pyridines possessing moderate LTB_4 receptor antagonist activity. However, more significantly, the compounds were found to be free of LTB_4 agonist activity (e.g.,

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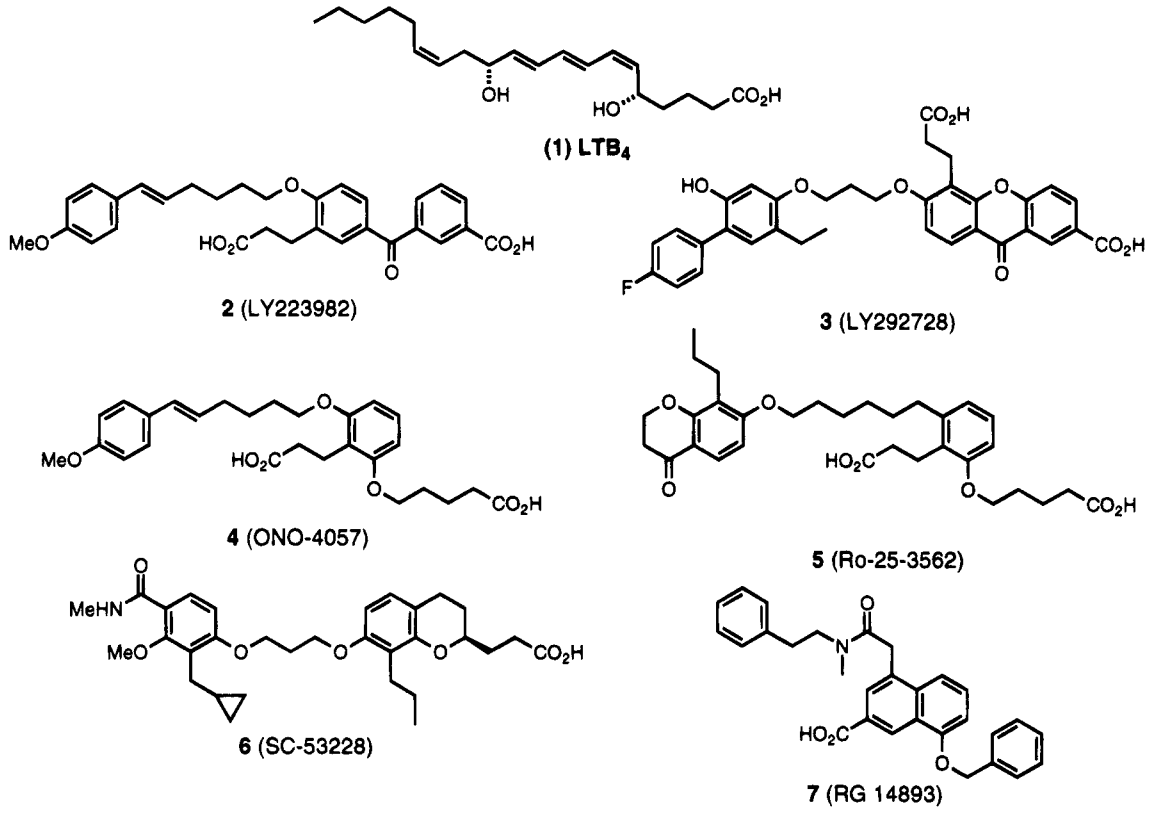


Figure 1. Structures of recently reported LTB_4 receptor antagonists.

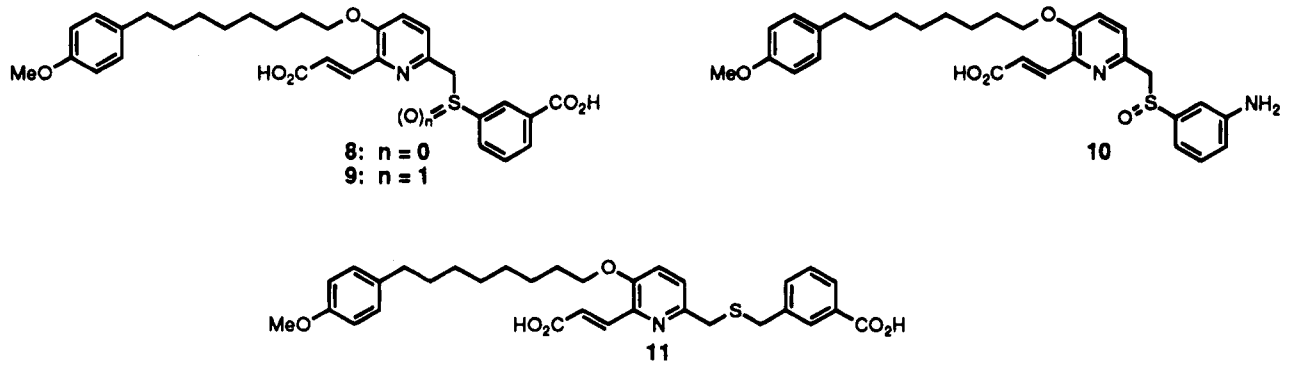
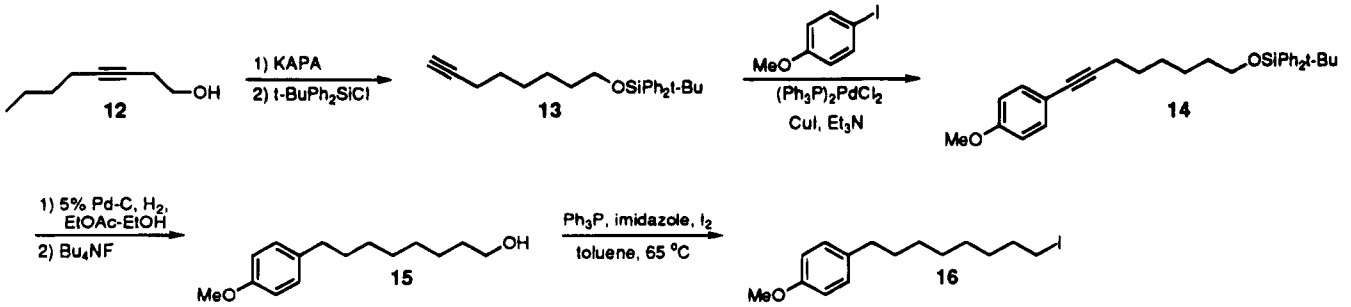


Figure 2.

Scheme 1

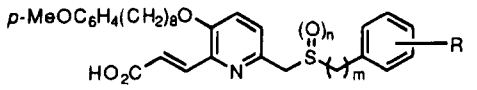


compounds **8** and **9**, Figure 2). Further structure–activity studies led to the discovery of the recently reported high affinity LTB_4 receptor antagonist **10** (SB 201146, $K_i = 4.7$ nM).[17] We report herein the synthesis, biological activity, and SAR of a new series of trisubstituted pyridine LTB_4 receptor antagonists. This study resulted in the identification of (*E*)-3-[[[[6-(2-carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid (**11**, SB 201993) as a potential agent for the treatment of inflammatory diseases in which LTB_4 is involved.[18]

Chemistry

The LTB_4 receptor antagonists of this study were prepared according to Schemes 1–5. The (*p*-methoxyphenyl)octyl iodide (**16**) was prepared as described in Scheme 1 starting from commercially available 3-octyn-1-ol (**12**). This was rearranged to the terminal acetylene

Table 1. Inhibition of [^{3}H]LTB$_4$ Binding and LTB$_4$-Induced Ca^{2+} Mobilization to Human Neutrophils



					IC$_{50}$, nM[a]	
compd	*m*	*n*	R	whole cell binding K_i, nM[a]	Ca^{2+} mobilization	degranulation
8	0	0	3-CO_2H	80 ± 18	550 ± 120	1000
9	0	1	3-CO_2H	167 ± 24	3900 ± 700	3200
11[b]	1	0	3-CO_2H	7.1 ± 1.1	131 ± 23	271 ± 14
29	1	1	3-CO_2H	240	3300 ± 1900	2600
31	1	2	3-CO_2H	53 ± 9	920	1000
34	1	0	4-CO_2H	5.7 ± 0.6	175 ± 69	426
35	1	1	4-CO_2H	161 ± 19	7000	–
36	1	0	2-CO_2H	112 ± 22	710	–

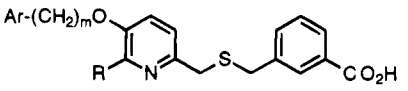
[a] The K_i and IC$_{50}$ values are stated as the mean of at least three determinations ± standard error. All other values are the mean of two concentration response curves. All compounds were tested as dilithium salts. [b] Tested as the free diacid.

via potassium aminopropylamide (KAPA)[19] and the primary alcohol protected as the *tert*-butyldiphenylsilyl ether **13**. The *p*-methoxyphenyl group was then incorporated utilizing a $(Ph_3P)_2PdCl_2$-catalyzed coupling reaction to give **14**.[20] Hydrogenation (H_2, 5% Pd–C) of the alkyne and removal of the silyl protecting group (*n*-Bu_4NF) afforded alcohol **15**. Conversion of the primary alcohol to the corresponding iodide (Ph_3P, I_2, imidazole) provided **16**. The phenyloctyl bromide used in the preparation of compound **37** (Table 2) was prepared according to published procedures.[21]

The thiomethyl benzoates that were not commercially available were prepared as illustrated in Scheme 2. Reaction of methyl 3-(bromomethyl)benzoate (**17**) with thiourea provided the isothiuronium salt **18**. Hydrolysis of **18** followed by re-esterification afforded the benzyl mercaptan **19**.

The synthetic route used in the preparation of the study compounds, exemplified by compound **11**, is outlined in Scheme 3. 2,6-Lutidine-α^2,3-diol (**20**) was oxidized to the corresponding aryl aldehyde **21** using MnO_2. The hydroxy aldehyde **21** was alkylated with the desired lipid tail (e.g., **16**) to provide **22**, which upon treatment with methyl (triphenylphosphoranylidene)-acetate afforded acrylate **23**. This oxidation–alkylation sequence may be reversed; however, increased reaction times are required in both steps. Conversion of **23** to the pyridine *N*-oxide **24** using MCPBA followed by TFAA rearrangement afforded the (hydroxymethyl)-pyridine **25**.[22] Treatment of **25** with $SOCl_2$ provided the

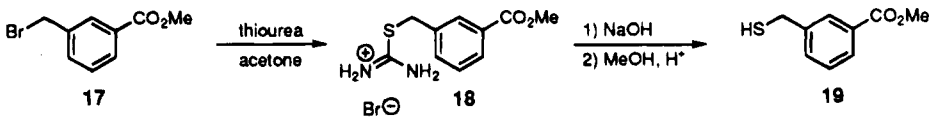
Table 2. Inhibition of [^{3}H]LTB$_4$ Binding and LTB$_4$-Induced Ca^{2+} Mobilization to Human Neutrophils



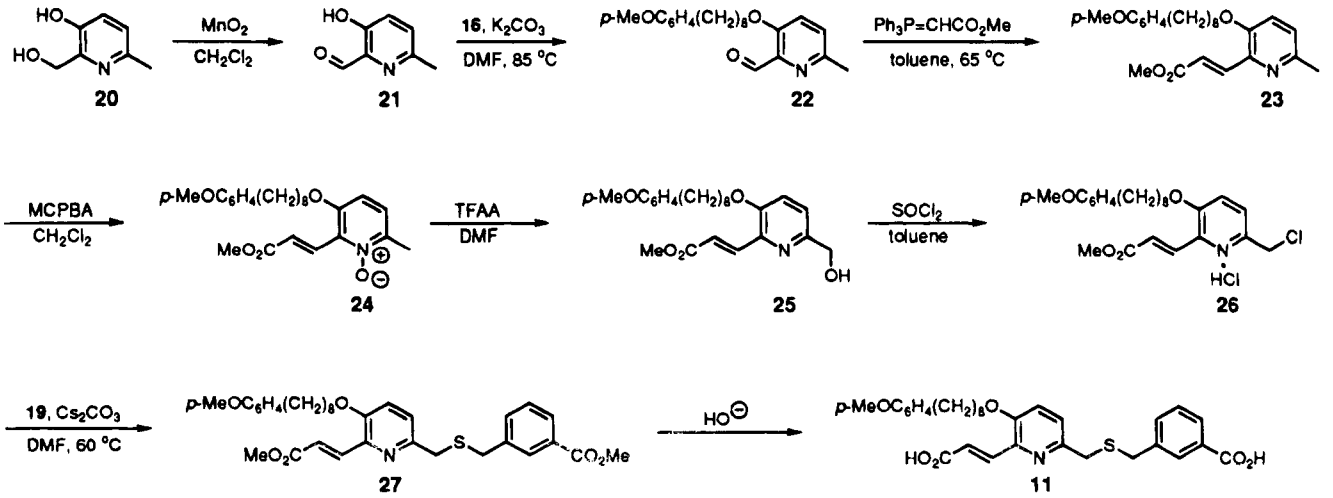
					IC$_{50}$, nM[b]	
compd	*m*	R[a]	Ar	whole cell binding K_i, nM[b]	Ca^{2+} mobilization	degranulation
11	8	CH=CHCO$_2$H	*p*-MeOC$_6$H$_4$	7.1 ± 1.1	131 ± 23	271 ± 14
33	8	CH$_2$CH$_2$CO$_2$H	*p*-MeOC$_6$H$_4$	8	240 ± 60	190
37	8	CH=CHCO$_2$H	C$_6$H$_5$	15.4 ± 4.3	320	500
38	4	CH=CHCO$_2$H	*p*-MeOC$_6$H$_4$	9.2	770 ± 50	770
39	4	CH=CHCO$_2$H	C$_6$H$_5$	4.8	500	1700
40	4	CH$_2$CH$_2$CO$_2$H	*p*-MeOC$_6$H$_4$	28	1900	3800

[a] Acrylates have *E* geometry. [b] See footnote *a*, Table 1.

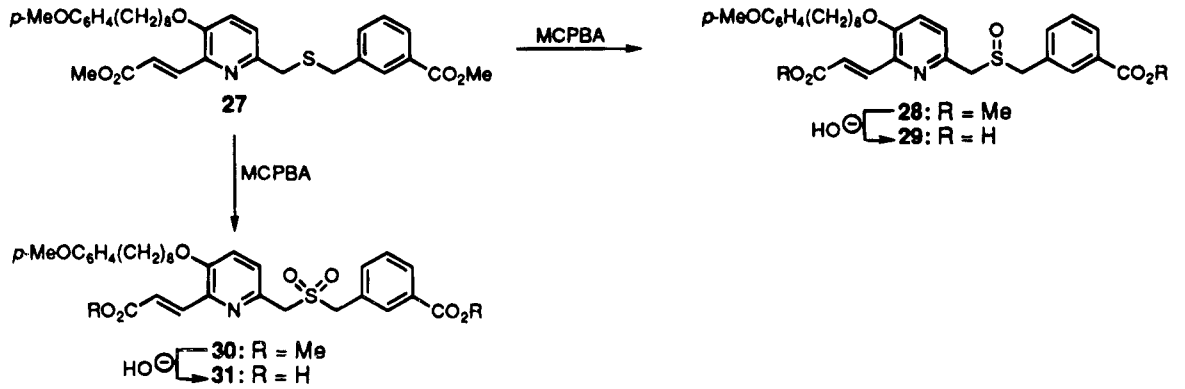
Scheme 2



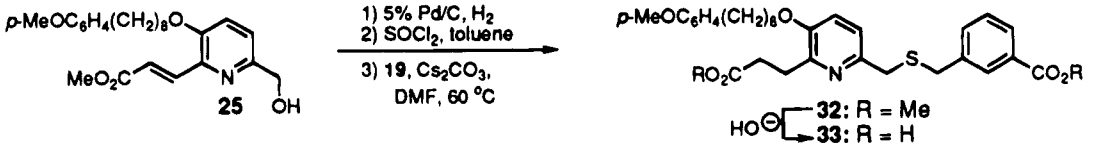
Scheme 3



Scheme 4



Scheme 5



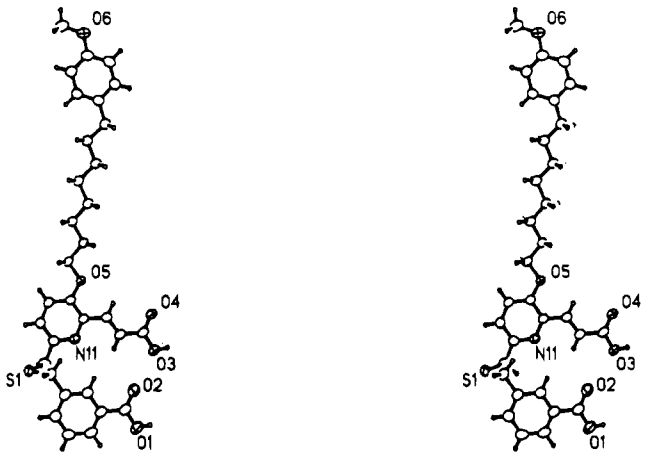


Figure 3. A stereodrawing of molecule **11** from the crystal structure. Non-hydrogen atoms are represented as principal ellipses at the 50% probability level; hydrogen atoms as spheres of arbitrary radius.

(chloromethyl)pyridine **26** as a hydrochloride salt.[23] It was necessary to prepare **26** as a salt since the free base was unstable. Reaction of **26** with mercaptan **19** under basic conditions produced sulfide **27**. Ester hydrolysis then provided target compound **11**. Alternatively (Scheme 4), prior to hydrolysis, ester **27** can be oxidized (MCPBA) to either the sulfoxide **28** or the sulfone **30**. Ester hydrolysis then provided sulfoxide **29** and sulfone **31**. The side chain of acrylate **25** (Scheme 5) was reduced (H_2, 5% Pd/C) and the resulting compound (**32**) converted to the propanoic acid **33** by a sequence analogous to the one used to prepare **11**.

The structure of **11** was verified by single crystal X-ray diffraction and is illustrated in Figure 3. The molecular structure may be described as three planar rings and an extended, essentially planar octyloxy chain. The ring planes lie close to parallel with one another. This conformation places the carboxylic acid groups in a parallel alignment on one side of the molecule. Intermolecular, heterodimeric hydrogen bonding between carboxylic acid groups links molecules of **11** into pairs.

Results and Discussion

The LTB_4 receptor affinities of the test compounds were determined by evaluating the ability of the compounds to compete with the binding of [^{3}H]LTB_4 to receptors on intact human polymorphonuclear leukocytes (PMNs).[24] In addition, compounds were evaluated further in human PMN functional assays where their effect on LTB_4-induced calcium mobilization and LTB_4-induced degranulation was determined.[25] For the present study, the whole cell human PMN binding assay served as our primary test system to determine structure–activity relationships. The functional assays, which are not equilibrium assays, were used to assess antagonist potency and to observe any agonist activity of the test compounds. The data for human PMN binding, human PMN calcium mobilization, and human PMN degranulation are presented in Tables 1 and 2. All compounds in this series were free of LTB_4 receptor agonist activity at concentrations of up to 10 μM (maximum test concentration) as determined in the calcium mobilization functional assay.

As can be seen in Table 1, the inclusion of a methylene unit between the phenyl ring and the sulfur atom of the head group of **8** had a significant effect on the receptor affinity of the compound. Thus, compound **11** was found to have receptor binding affinity approximately 1 order of magnitude greater than that of compound **8**. This increased affinity was demonstrated functionally by a 4-fold increase in potency in the Ca^{2+} mobilization assay. The incorporation of this additional methylene increases the distance between the two carboxylic acid groups. Additionally, there is likely to be a significant change in the conformation of the aryl head group in relation to the pyridine ring. These changes may allow for a more preferred positioning of the carboxylate groups at the LTB_4 receptor. Conversion to the corresponding sulfoxide **29** resulted in a marked decrease in binding affinity as well as in functional potency. The change in activity with oxidation state (**11** → **29**) is opposite to what was found in the series leading to aniline **10** (Figure 2) in which the sulfoxide containing compounds were generally preferred.[17] The activity profile of sulfone **31** also demonstrated the importance

of the sulfide oxidation state in this series of compounds, i.e., oxidation of the sulfur results in decreased receptor affinity. Preparation of the 4-substituted analog **34** afforded a compound possessing similar activity to that of **11** in both binding and functional potency. Although the 3- and 4-substituted analogs had similar binding affinities, the 2-substituted acid **36** showed greatly reduced activity. This further demonstrates the importance of the relative position of the carboxylic acid group.

The compounds presented in Table 2 were prepared in order to further explore the relationship between LTB_4 receptor antagonist activity and structure while remaining within the 3-substituted benzoic acid sulfide containing series (e.g., **11**). Saturation of the acrylate side chain appears to have little effect on the LTB_4 receptor binding affinity of these analogs, compound **33** having similar binding affinity and functional activity to that of **11**. Replacing the tail with phenyloctyl (**37**), likewise, had little effect on activity, decreasing the affinity only slightly. In addition, shortening the tail by a butylene unit (**38**) resulted in a compound with LTB_4 receptor affinity equivalent to parent compound **11**. Again, the (*p*-methoxyphenyl)butyl moiety can be replaced with phenylbutyl (**39**) without loss in activity. This SAR study indicated that the methoxy substituent on the phenyl ring of the tail does not play a major role in the *in vitro* binding affinity of this series of compounds. Additionally, there appears to be considerable latitude in the requirements for the length of this lipid tail, although in the calcium and degranuation functional assays the shorter lipid chain containing compounds (e.g., **38**, **39**) appear less potent. These findings are in contrast to the SAR previously reported for a series of dicarboxylic acid containing LTB_4 receptor antagonists where the length of the lipid tail and the specific terminating group played a critical role in the binding affinity.[11]

An interesting observation was made within the (*p*-methoxyphenyl)butyl series of compounds. In the (*p*-methoxyphenyl)*octyl* series the acrylate side chain can be replaced with that of a propanoic acid without loss in activity (e.g., compare **11** with **33**). However, in the (*p*-methoxyphenyl)*butyl* series, replacement with a propanoic acid chain results in a significant loss of binding affinity (e.g., compare **38** with **40**). The reason for this difference is not directly apparent.

Compound **11** was selected for additional evaluation using both *in vitro* and *in vivo* studies to determine its potential as a therapeutic agent. LTB_4 induces degranulation of human PMNs by activation of low-affinity receptors (i.e., receptors requiring higher concentration of LTB_4 for a response). Neutrophil degranulation was measured by quantitating the release of myeloperoxidase (MPO) induced by 100 nM LTB_4.[26] Compound **11** inhibited LTB_4-induced degranulation in a concentration-dependent manner with an IC_{50} of 271 ± 14 nM (mean ± SEM for four experiments). This potency in degranulation was similar to that of compound **11** in the LTB_4-induced calcium mobilization functional assay (IC_{50} = 131 ± 23 nM).

Compound **11** has demonstrated potent LTB_4 receptor antagonist activity *in vitro*. In order to demonstrate oral LTB_4 receptor antagonist activity, **11** was evaluated in the LTB_4-induced peritonitis assay in the mouse.[27] In this assay, **11** produced dose-related inhibition of LTB_4-induced (250 ng/mL) PMN infiltration with an ED_{50} of 7.1 mg/kg po. The ability of **11** to act as a topical LTB_4 receptor antagonist was also evaluated in the mouse. The topical application of LTB_4 to a mouse ear results in an infiltration of PMNs as indicated by an increase in MPO levels. Topical application of compound **11** (1 mg/ear) to the mouse ear, prior to treatment with LTB_4, resulted in a 92% reduction of PMN infiltration. A similar model using arachidonic acid (AA) applied topically to the mouse ear was also utilized. Activity in this model would be a demonstration of topical antiinflammatory activity. AA-induced inflammation involves both the fluid and the cellular phases of the inflammatory response.[28] Topical application of **11** immediately after administration of 1 mg of AA produced dose-dependent inhibition of both responses with ED_{50} values of 0.39 mg/ear for neutrophil infiltration and 0.58 mg/ear for the edematous response. In addition to demonstrating dose-related antiinflammatory activity in this model, a single dose (1 mg/ear) of compound **11**, applied topically, displayed significant inhibitory activity 24 h following application of arachidonic acid.

LTB_4 is proposed as an important mediator in many inflammatory disease states. However, its specific role in human inflammatory diseases has yet to be established since only a few potent LTB_4 receptor antagonists have been reported in the literature and we are unaware of any complete clinical studies with these compounds. In the present study, (*E*)-3-[[[[6-(2-carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]-methyl]benzoic acid (**11**) was shown to be a potent LTB_4 receptor antagonist both *in vitro* and *in vivo* and has demonstrated topical antiinflammatory actvity in the mouse. Compound **11** should prove to be an important tool in elucidating the role of LTB_4 in human diseases.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were recorded on a Brucker AM-250 instrument with the solvents indicated. All ^1H chemical shifts are reported in δ relative to tetramethylsilane (TMS, δ 0.00) as the internal standard. Elemental analyses were performed by the Analytical and Physical Chemistry Department of SmithKline Beecham. Where analyses are indicated by symbols of the elements, results obtained were within ±0.40%. Mass spectra were determined by the Physical and Structural Chemistry Department of SmithKline Beecham. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F-254 glass backed plates or Whatman KC 18 F reversed phase RP-18 glass backed plates with the solvent systems indicated. High performance liquid chromatography (HPLC) was conducted using an Altex Model 110 gradient liquid chromatograph with a UV wavelength detector set at 254 nM. Flash column chromatography was carried out using silica gel with the solvents as indicated. Carboxylic acid salts were purified by reversed phase medium-pressure liquid chromatography (MPLC) using Altec columns packed with Merck Licroprep RP-18 (25–40 μm) at a maximum pressure of 60 psi. Test compounds were homogeneous as determined chromatographically (TLC) and spectroscopically (^1H NMR, UV). All sulfoxide-containing compounds were prepared in racemic form.

7-Octyn-1-yl *tert*-Butyldiphenylsilyl Ether (13). Potassium hydride (35%) in mineral oil (27 g, 240 mmol) under an argon atmosphere was washed with hexane and treated dropwise with 1,3-diaminopropane. The mixture was stirred

at room temperature until it became homogeneous. The flask was cooled to 0 °C, and 3-octyn-1-ol (**12**; 10 g, 79 mmol) was slowly added. The reaction mixture was then stirred at room temperature for 18 h. The reaction was quenched with H_2O (50 mL), and the product was extracted into Et_2O. The organic layer was washed with 10% HCl and brine and dried ($MgSO_4$). Evaporation gave 9.73 g (97%) as a colorless oil which was used without further purification: ^1H NMR (90 MHz, $CDCl_3$) δ 3.65 (t, J = 5 Hz, 2H, OCH_2), 2.23 (m, 2H, CH_2), 2.0 (m, 1H, acetylenic), 1.7–1.2 (m, 8H, $(CH_2)_4$); IR (neat) ν_{max} 3350, 2930, 2125 cm^{-1}.

To a cooled (0 °C) solution of alcohol obtained above (9.3 g, 73.7 mmol) in DMF (70 mL) under an argon atmosphere was added imidazole (7.5 g, 110 mmol) followed by the dropwise addition of *tert*-butylchlorodiphenylsilane (21 mL, 81 mmol). The reaction was then stirred at room temperature for 2 h. The reaction solution was diluted with Et_2O, washed with H_2O and brine, and dried ($MgSO_4$). Purification by flash column chromatography (3% EtOAc in hexane) provided 24.9 g (93%) of **13** as a colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 7.7 (d, 4H, aryl), 7.4 (m, 6H, aryl), 3.63 (t, 2H, OCH_2), 2.23 (m, 2H, CH_2), 1.97 (t, 1H, acetylenic), 1.6–1.3 (m, 8H, $(CH_2)_4$), 1.05 (s, 9H, *tert*-butyl); IR (film) ν_{max} 3321, 2940, 2125 cm^{-1}.

8-(4-Methoxyphenyl)-7-octyn-1-yl *tert*-Butyldiphenylsilyl Ether (14). To a flame-dried flask containing Et_3N (140 mL) under an argon atmosphere was added 4-iodoanisole (13.3 g, 56.9 mmol), **13** (24.9 g, 68.3 mmol), $(Ph_3P)_2PdCl_2$ catalyst (793 mg, 1.13 mmol), and CuI (431 mg, 2.27 mmol). The resulting mixture was heated at 50 °C for 4 h. Upon cooling to room temperature, the reaction mixture was filtered, the solids were washed with Et_2O, and the solvent was evaporated. The residue was diluted with Et_2O, washed with 5% HCl, H_2O, $NaHCO_3$, and brine, and dried ($MgSO_4$). Purification by flash column chromatography (2% EtOAc in hexane) gave 30 g (93%) of **14** as an orange oil: ^1H NMR (250 MHz, $CDCl_3$) δ 7.7 (d, 4H, aryl), 7.4 (m, 6H, aryl), 7.35 (d, 2H, aryl), 6.8 (d, 2H, aryl), 3.8 (s, 3H, OCH_3), 3.7 (t, 2H, O-CH_2), 2.4 (t, 2H, CH_2), 1.7–1.3 (m, 8H, $(CH_2)_4$), 1.05 (s, 9H, *tert*-butyl).

8-(4-Methoxyphenyl)octan-1-ol (15). Alkyne **14** (30 g, 63.7 mmol) was dissolved in EtOH (125 mL) and EtOAc (125 mL) and treated with 5% Pd–C catalyst (3 g). The reaction mixture was vigorously stirred under an H_2 atmosphere (balloon pressure) for 4 h. The reaction mixture was filtered through a pad of Celite, and the solvent was evaporated. The resulting pale yellow oil was pure by NMR analysis and was used directly for the next step: ^1H NMR (250 MHz, $CDCl_3$) δ 7.7 (d, 4H, aryl), 7.4 (m, 6H, aryl), 7.05 (d, 2H, aryl), 6.8 (d, 2H, aryl), 3.8 (s, 3H, OCH_3), 3.6 (t, 2H, OCH_2), 2.5 (t, 2H, benzylic), 1.75–1.3 (m, 12H, $(CH_2)_6$), 1.0 (s, 9H, *tert*-butyl).

To a cooled (0 °C) solution of silyl ether obtained above (63 mmol) was added tetrabutylammonium fluoride (70 mL, 70 mmol; 1 M solution in THF). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 4.5 h. The solvent was evaporated, and the residue was dissolved in Et_2O. This was washed with H_2O, 5% HCl, $NaHCO_3$, and brine and dried ($MgSO_4$). Purification by flash column chromatography (30% EtOAc in hexane) gave 12.6 g (85%; two steps) of **15** as a white crystalline solid: mp 47–49 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 7.15 (d, 2H, aryl), 6.86 (d, 2H, aryl), 3.85 (s, 3H, OCH_3), 3.68 (t, 2H, O-CH_2), 2.62 (t, 2H, benzylic), 1.75–1.3 (m, 12H, $(CH_2)_6$); MS (CI) *m/e* 254.2 $[M + NH_4]^+$.

1-Iodo-8-(4-methoxyphenyl)octane (16). To a stirred solution of **15** (12.3 g, 52 mmol) in dry toluene (200 mL) under an argon atmosphere was added triphenylphosphine (17.8 g, 67.6 mmol) and imidazole (10.6 g, 156 mmol). After 5 min I_2 (17.1 g, 67.6 mmol) was added. The reaction mixture was then heated at 65 °C for 30 min. Upon cooling to room temperature, the reaction was concentrated to 1/4 volume. The remaining solution was diluted with Et_2O, washed with H_2O and brine, and dried ($MgSO_4$). The solvent was removed, and the resulting residue was dissolved in CH_2Cl_2 and applied to a flash chromatography column; elution with 2% EtOAc in hexane provided 16.3 g (90%) of **16** as a colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 7.08 (d, J = 8.6 Hz, 2H, aryl), 6.82 (d, J = 8.6 Hz, 2H, aryl), 3.78 (s, 3H, OCH_3), 3.17 (t, J = 7.4 Hz, 2H, ICH_2), 2.54 (t, J = 7.6 Hz, 2H, benzylic), 1.85 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 1.31 (m, 8H, aliphatic); MS (CI) *m/e* 364.2 $[M + NH_4]^+$.

3-Hydroxy-6-methylpyridine-2-carbaldehyde (21). 2,6-Lutidine-α^2,3-diol (**20**, 15 g, 107.8 mmol) was suspended in dry CH_2Cl_2 (200 mL) and treated with MnO_2 (47 g, 539 mmol). The reaction was stirred at room temperature for 6 h. The reaction mixture mixture was filtered through a pad of Celite, and the solvent was evaporated. Crude **21** was obtained as a tan solid and was used directly for the next step: ^1H NMR (250 MHz, $CDCl_3$) δ 10.65 (s, 1H, OH), 10.30 (s, 1H, aldehyde), 7.30 (m, 2H, 4,5-pyridyl), 2.55 (s, 3H, methyl).

3-[[8-(4-Methoxyphenyl)octyl]oxy]-6-methylpyridine-2-carbaldehyde (22). To a solution of iodide **16** (16.3 g, 47.1 mmol) in dry DMF (45 mL) under an argon atmosphere was added **21** (7.7 g, 56.2 mmol) and anhydrous K_2CO_3 (32 g, 235 mmol). The reaction mixture was vigorously stirred at 90 °C for 1.5 h. Upon cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O, aqueous NH_4Cl, and brine, and dried ($MgSO_4$). Evaporation provided crude **22** as a dark oil that was used without further purification.

(*E*)-3-[3-[[8-(4-Methoxyphenyl)octyl]oxy]-6-methylpyridin-2-yl]propenoic Acid Methyl Ester (23). Aldehyde **22** obtained above was dissolved in dry toluene (100 mL) under an argon atmosphere and treated with methyl (triphenylphosphoranylidene)acetate (16 g, 48 mmol). The reaction mixture was heated for 1 h at 50 °C. Upon cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, and dried ($MgSO_4$). Purification by flash column chromatography (20% EtOAc in hexane) gave 17.2 g (88%; from **16**) of **23** as a pale yellow oil: ^1H NMR (250 MHz, $CDCl_3$) δ 8.07 (d, J = 15.7 Hz, 1H, vinyl), 7.10 (m, 4H, phenyl, 4,5-pyridyl), 7.07 (d, J = 15.7 Hz, 1H, vinyl), 6.81 (d, J = 8.6 Hz, 2H, phenyl), 3.97 (t, J = 6.5 Hz, 2H, O-CH_2), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, methyl ester), 2.54 (t, J = 7.6 Hz, 2H, benzylic), 2.48 (s, 3H, methyl), 1.85 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 1.37 (m, 8H, aliphatic); MS (CI) *m/e* 412.3 $[M + H]^+$.

(*E*)-3-[3-[[8-(4-Methoxyphenyl)octyl]oxy]-6-methylpyridin-2-yl]propenoic Acid Methyl Ester *N*-Oxide (24). Pyridine **23** (17.1 g, 41.5 mmol) was dissolved in dry CH_2Cl_2 (105 mL) and cooled to 0 °C; 50% mCPBA (15.8 g, 45.8 mmol) was added in three portions over 10 min. The cooling bath was removed, and the reaction mixture was stirred for 15 h at room temperature. The reaction mixture was poured into aqueous $NaHCO_3$ and the product extracted into CH_2Cl_2. The organic extract was washed with H_2O and brine and dried ($MgSO_4$). Crude **24** was obtained as a yellow waxy solid and was used without further purification.

(*E*)-3-[6-(Hydroxymethyl)-3-[[8-(4-methoxyphenyl)octyl]oxy]pyridin-2-yl]propenoic Acid Methyl Ester (25). Pyridine *N*-oxide **24** was suspended in dry DMF (130 mL) and cooled to 0 °C under an argon atmosphere. To this was slowly added trifluoroacetic anhydride (56 mL, 400 mmol). The reaction mixture was maintained at 0 °C for 20 min followed by 18 h at room temperature. The reaction solution was slowly added to a solution of saturated aqueous Na_2CO_3 and stirred for 1 h. The product was then extracted into EtOAc; the combined organic extracts were washed with H_2O and brine and dried ($MgSO_4$). Purification by flash column chromatography (EtOAc:hexane:CH_2Cl_2, 30:20:50) gave 11 g (62%; two steps) of **25** as a waxy solid: ^1H NMR (250 MHz, $CDCl_3$) δ 8.08 (d, J = 15.7 Hz, 1H, vinyl), 7.23 (d, J = 8.6 Hz, 1H, 5-pyridyl), 7.16 (d, J = 8.6 Hz, 1H, 4-pyridyl), 7.09 (d, J = 8.6 Hz, 2H, phenyl), 7.03 (d, J = 15.7 Hz, 1H, vinyl), 6.82 (d, J = 8.6 Hz, 2H, phenyl), 4.69 (d, J = 4.1 Hz, 2H, CH_2OH), 4.01 (t, J = 6.5 Hz, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.78 (s, 3H, methyl ester), 3.62 (t, J = 4.1 Hz, 1H, OH), 2.55 (t, J = 7.6 Hz, 2H, benzylic), 1.85 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.44 (m, 8H, aliphatic); MS (CI) *m/e* 428.2 $[M + H]^+$.

Methyl 3-(Mercaptomethyl)benzoate (19). To a solution of methyl 3-(bromomethyl)benzoate (**17**, 6.9 g, 30 mmol) in dry acetone (10 mL) was added via dropwise addition a solution of thiourea (2.28 g, 30 mmol) in dry acetone (40 mL) at room temperature. After 15 min the precipitated isothiuronium salt **18** was collected by filtration; the solids were washed with

acetone and dried. **18** was then dissolved in H_2O (65 mL), and the pH was adjusted to 10.5 by the addition of 10% NaOH. The mixture was refluxed for 2 h. After cooling to room temperature the solution was extracted with EtOAc, and the organic layer was discarded. The aqueous solution was acidified to pH 1.5 and extracted three times with EtOAc. The organic extracts were dried ($MgSO_4$) and filtered, and the solvent was evaporated. The crude acid was then dissolved in anhydrous MeOH (125 mL) and cooled to 0 °C, and dry HCl gas was bubbled through the solution for 30 min. The reaction was then stirred for 12 h at room temperature. The mixture was concentrated *in vacuo*, and the product was purified by flash column chromatography (5% EtOAc in hexane), providing 1.9 g (35% overall) of **19** as a colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.00 (s, 1H, 2-phenyl), 7.91 (d, J = 7.6 Hz, 1H, 6-phenyl), 7.52 (d, J = 7.6 Hz, 1H, 4-phenyl), 7.39 (dd, J = 7.6 Hz, 1H, 5-phenyl), 3.92 (s, 3H, methyl ester), 3.78 (d, J = 7.7 Hz, 2H, SCH_2), 1.79 (t, J = 7.7 Hz, 1H, SH).

(*E*)-3-[[[[6-[2-(Methoxycarbonyl)ethenyl]-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic Acid Methyl Ester (27). To a cooled (0 °C) solution of $SOCl_2$ (2.5 mL, 35 mmol) in dry toluene (175 mL) under an argon atmosphere was added a solution of **25** (1.5 g, 3.5 mmol) in toluene (10 mL). After 5 min the cooling bath was removed, and the reaction mixture was stirred for 4 h at room temperature. The toluene and excess $SOCl_2$ were evaporated. To this was added dry DMF (5 mL), **19** (600 mg, 3.3 mmol), and anhydrous Cs_2CO_3 (6.6 g, 20 mmol). The reaction mixture was heated at 60 °C under an atmosphere of argon for 1.5 h. Upon cooling to room temperature the reaction mixture was diluted with EtOAc, washed with H_2O, 10% NaOH, H_2O, and brine, and dried ($MgSO_4$). Purification by flash column chromatography (15% EtOAc in hexane) yielded 1.58 g (79%) of **27** as a colorless solid: mp 55–56 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.07 (d, J = 15.8 Hz, 1H, vinyl), 7.99 (s, 1H, 2-phenyl), 7.90 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.54 (d, J = 7.7 Hz, 1H, 4-phenyl), 7.37 (dd, J = 7.7 Hz, 1H, 5-phenyl), 7.28 (d, J = 8.6 Hz, 1H, pyridyl), 7.14 (d, J = 8.6 Hz, 1H, pyridyl), 7.11 (d, J = 8.6 Hz, 2H, phenyl), 7.08 (d, J = 15.8 Hz, 1H, vinyl), 6.82 (d, J = 8.6 Hz, 2H, phenyl), 3.99 (t, J = 6.5 Hz, 2H, OCH_2), 3.91 (s, 3H, methyl ester), 3.81 (s, 3H, OCH_3), 3.78 (s, 3H, methyl ester), 3.71 (s, 2H, SCH_2), 3.68 (s, 2H, SCH_2), 2.55 (t, J = 7.6 Hz, 2H, benzylic), 1.78 (m, 2H, CH_2), 1.5 (m, 10H, aliphatic); MS (CI) *m/e* 592.2 $[M + H]^+$.

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic Acid (11). Dimethyl ester **27** (1.5 g, 2.5 mmol) was dissolved in THF (20 mL) and MeOH (20 mL) and treated with 1.0 M LiOH (11.5 mL, 11.5 mmol). The reaction mixture was stirred under an argon atmosphere for 16 h. The solvent was evaporated, and the residue was dissolved in H_2O. The aqueous solution was extracted with EtOAc and acidified to pH 3.2 with 98% formic acid. The free acid was then extracted into EtOAc. The combined EtOAc extracts were washed with brine and dried (Na_2SO_4). Recrystallization from EtOH provided 1.3 g (90%) of **11** as a light tan solid: mp 113–114 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.23 (d, J = 15.7 Hz, 1H, vinyl), 8.11 (s, 1H, aryl), 7.99 (d, J = 7.6 Hz, 1H, aryl), 7.68 (d, J = 7.6 Hz, 1H, aryl), 7.42 (m, 2H, aryl), 7.25 (d, J = 15.7 Hz, 1H, vinyl), 7.20 (d, J = 9.0 Hz, 1H, aryl), 7.08 (d, J = 8.5 Hz, 2H, aryl), 6.78 (d, J = 8.5 Hz, 2H, aryl), 4.01 (t, J = 6.5 Hz, 2H, OCH_2), 3.76 (s, 3H, OCH_3), 3.55 and 3.51 (singlets, 4H total, CH_2SCH_2), 2.55 (t, J = 7.6 Hz, 2H, benzylic), 1.85 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.38 (m, 6H, aliphatic); HRFAB-MS: m/z 564.2410 $[M + H]^+$. Anal. ($C_{32}H_{37}NO_6S$): C, H, N, S.

(*E*)-3-[[[[6-[2-(Methoxycarbonyl)ethenyl]-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]sulfinyl]methyl]benzoic Acid Methyl Ester (28) and (*E*)-3-[[[[6-[2-(Methoxycarbonyl)ethenyl]-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]sulfonyl]methyl]benzoic Acid Methyl Ester (30). Sulfide **27** (150 mg, 0.25 mmol) was dissolved in dry CH_2Cl_2 (5 mL) under an argon atmosphere and cooled to −20 °C. To this was added 85% *m*-chloroperoxybenzoic acid (52 mg, 0.26 mmol) in two portions 15 min apart. The reaction mixture was stirred for 25 min at −20 °C following the second addition and then quenched with 5% $NaHCO_3$. The product was extracted into CH_2Cl_2, and the organic extracts were dried ($MgSO_4$). Purification by flash column chromatography (20% and 50% EtOAc in hexane) gave 100 mg (67%) of **28** as a white solid and 20 mg (15%) of **30** as a white solid. **28**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.07 (d, J = 15.8 Hz, 1H, vinyl), 8.01 (s, 1H, 2-phenyl), 7.97 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.55 (d, J = 7.7 Hz, 1H, 4-phenyl), 7.46 (dd, J = 7.7 Hz, 1H, 5-phenyl), 7.28 (d, J = 8.6 Hz, 1H, pyridyl), 7.20 (d, J = 8.6 Hz, 1H, pyridyl), 7.07(d, J = 8.6 Hz, 2H, phenyl), 7.05 (d, J = 15.8 Hz, 1H, vinyl), 6.78 (d, J = 8.6 Hz, 2H, phenyl), 4.12 (d, J = 12.8 Hz, 1H, SCH), 4.05 (d, J = 12.8 Hz, 1H, SCH), 4.04 (t, J = 6.5 Hz, 2H, OCH_2), 3.94 (s, 3H, methyl ester), 3.92 (m, 2H, SCH_2), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, methyl ester), 2.56 (t, J = 7.6 Hz, 2H, benzylic), 1.87 (m, 2H, CH_2), 1.40 (m, 10H, aliphatic); MS (CI) *m/e* 608.2 $[M + H]^+$. Anal. ($C_{34}H_{41}NO_7S\cdot{}^1/_4H_2O$): C, H, N. **30**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.23 (s, 1H, 2-phenyl), 8.13 (d, J = 15.8 Hz, 1H, vinyl), 8.08 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.74 (d, J = 7.7 Hz, 1H, 4-phenyl), 7.51 (dd, J = 7.7 Hz, 1H, 5-phenyl), 7.46 (d, J = 8.6 Hz, 1H, pyridyl), 7.24 (d, J = 8.6 Hz, 1H, pyridyl), 7.12 (d, J = 8.6 Hz, 2H, phenyl), 7.11 (d, J = 15.8 Hz, 1H, vinyl), 6.84 (d, J = 8.6 Hz, 2H, phenyl), 4.30 (s, 4H, SCH_2), 4.06 (t, J = 6.5 Hz, 2H, OCH_2), 3.93 (s, 3H, methyl ester), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, methyl ester), 2.56 (t, J = 7.6 Hz, 2H, benzylic), 1.9 (m, 2H, CH_2), 1.5 (m, 10H, aliphatic); MS (CI) *m/e* 624.2 $[M + H]^+$.

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]sulfinyl]methyl]benzoic Acid, Dilithium Salt (29). Sulfoxide **28** (100 mg, 0.165 mmol) was dissolved in THF (1.5 mL) and MeOH (1.5 mL) and treated with 1.0 M LiOH (0.8 mL, 0.8 mmol). The reaction mixture was stirred under an argon atmosphere for 20 h. The solvent was evaporated and the product purified by reversed phase MPLC (H_2O–MeOH gradient). Lyophilization yielded 95 mg (97%) of **29** as a colorless amorphous solid: ^{1}H NMR (250 MHz, MeOH-d_4) δ 7.95 (m, 2H, 2,6-phenyl), 7.82 (d, J = 15.8 Hz, 1H, vinyl), 7.40 (m, 2H, 4,5-phenyl), 7.37 (d, J = 8.6 Hz, 1H, pyridyl), 7.29 (d, J = 8.6 Hz, 1H, pyridyl), 7.10 (d, J = 15.8 Hz, 1H, vinyl), 7.06 (d, J = 8.6 Hz, 2H, phenyl), 6.79 (d, J = 8.6 Hz, 2H, phenyl), 4.36 (d, J = 12.8 Hz, 1H, SCH), 4.25 (d, J = 12.8 Hz, 1H, SCH), 4.08 (m, 4H, OCH_2, SCH_2), 3.73 (s, 3H, OCH_3), 2.54 (t, J = 7.6 Hz, 2H, benzylic), 1.87 (m, 2H, CH_2), 1.55 (m, 4H, aliphatic), 1.37 (m, 6H, aliphatic); MS (FAB) *m/e* 592.2 $[M + H]^+$. Anal. ($C_{32}H_{35}NO_7SLi_2\cdot1^3/_4H_2O$): C, H, N.

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]sulfonyl]methyl]benzoic Acid, Dilithium Salt (31). Sulfone **30** (20 mg, 0.0321 mmol) was dissolved in THF (0.5 mL) and MeOH (0.5 mL) and treated with 1.0 M LiOH (0.2 mL, 0.2 mmol). The reaction mixture was stirred under an argon atmosphere for 20 h. The solvent was evaporated and the product purified by reversed phase MPLC (H_2O–MeOH gradient). Lyophilization yielded 19 mg (97%) of **31** as a colorless amorphous solid: ^{1}H NMR (250 MHz, MeOH-d_4) δ 8.08 (s, 1H, 2-phenyl), 7.96 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.85 (d, J = 15.8 Hz, 1H, vinyl), 7.58 (d, J = 7.7 Hz, 1H, 4-phenyl), 7.39 (m, 3H, 5-phenyl, 4,5-pyridyl), 7.13 (d, J = 15.8 Hz, 1H, vinyl), 7.08 (d, J = 8.6 Hz, 2H, phenyl), 6.82 (d, J = 8.6 Hz, 2H, phenyl), 4.86 (s, 4H, SCH_2), 4.10 (t, J = 6.5 Hz, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 2.52 (t, J = 7.6 Hz, 2H, benzylic), 1.87 (m, 2H, CH_2), 1.55 (m, 4H, aliphatic), 1.40 (m, 6H, aliphatic); MS (FAB) *m/e* 608.2 $[M + H]^+$. Anal. ($C_{32}H_{35}NO_8SLi_2\cdot2^1/_4H_2O$): C, H, N.

3-[[[[6-[2-(Methoxycarbonyl)ethyl]-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic Acid Methyl Ester (32). Acrylate **25** (300 mg, 0.702 mmol) was dissolved in MeOH (3 mL) and treated with 5% Pd–C catalyst (30 mg). The reaction mixture was stirred under an atmosphere of H_2 (balloon pressure) for 5 h. The reaction mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated. Purification by flash column chromatography (EtOAc:CH_2Cl_2:hexane, 25:50:25) gave 265 mg (88%) as a pale yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.09 (m, 4H, phenyl, pyridyl), 6.80 (d, J = 8.6 Hz, 2H, phenyl), 4.62 (s, 2H, CH_2), 3.93 (t, J = 6.5 Hz, 2H, OCH_2), 3.77 (s, 3H, OCH_3), 3.68 (s,

3H, methyl ester), 3.16 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.77 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.54 (t, J = 7.6 Hz, 2H, benzylic), 1.79 (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 1.44 (m, 2H, CH_2), 1.34 (m, 6H, aliphatic); MS (CI) m/e 430.2 $[M + H]^+$.

To a cooled (0 °C) solution of $SOCl_2$ (0.17 mL, 2.33 mmol) in dry toluene (1.5 mL) under an argon atmosphere was added the saturated compound from above (100 mg, 0.233 mmol). After 5 min the cooling bath was removed and the reaction mixture was stirred for 1.5 h at room temperature. The toluene and excess $SOCl_2$ were evaporated. To this was added dry DMF (0.5 mL), **19** (47 mg, 0.258 mmol), and anhydrous Cs_2CO_3 (380 mg, 1.16 mmol). The reaction mixture was heated at 60 °C under an atmosphere of argon for 1 h. Upon cooling to room temperature the reaction mixture was diluted with EtOAc, washed with H_2O, 10% NaOH, H_2O, and brine, and dried ($MgSO_4$). Purification by flash column chromatography (EtOAc:CH_2Cl_2:hexane, 15:25:65) yielded 129 mg (93%) of **32** as a pale yellow oil: ^1H NMR (250 MHz, $CDCl_3$) δ 7.99 (s, 1H, 2-phenyl), 7.92 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.54 (d, J = 7.7 Hz, 1H, 4-phenyl), 7.37 (dd, J = 7.7 Hz, 1H, 5-phenyl), 7.09 (m, 4H, pyridyl, phenyl), 6.88 (d, J = 8.6 Hz, 2H, phenyl), 3.93 (t, J = 6.5 Hz, 2H, OCH_2), 3.91 (s, 3H, methyl ester), 3.78 (s, 3H, OCH_3), 3.71 (s, 2H, SCH_2), 3.65 (s, 3H, methyl ester), 3.64 (s, 2H, SCH_2), 3.14 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.79 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.55 (t, J = 7.6 Hz, 2H, benzylic), 1.80 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.34 (m, 6H, aliphatic); MS (CI) m/e 594.6 $[M + H]^+$. Anal. ($C_{34}H_{43}NO_6S$): C, H, N.

3-[[[[6-(2-Carboxyethyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic Acid, Dilithium Salt (33). Diester **32** (116 mg, 0.195 mmol) was dissolved in THF (2.25 mL) and MeOH (0.75 mL) and treated with 1.0 M LiOH (0.75 mL, 0.75 mmol). The reaction mixture was stirred under an argon atmosphere for 20 h. The solvent was evaporated and the product purified by reversed-phase MPLC (H_2O–MeOH gradient). Lyophilization yielded 111 mg (98%) of **33** as a colorless amorphous solid: ^1H NMR (250 MHz, MeOH-d_4) δ 7.90 (s, 1H, 2-phenyl), 7.83 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.34 (m, 2H, 4,5-phenyl), 7.25 (d, J = 8.6 Hz, 1H, pyridyl), 7.14 (d, J = 8.6 Hz, 1H, pyridyl), 7.07 (d, J = 8.6 Hz, 2H, phenyl), 6.83 (d, J = 8.6 Hz, 2H, phenyl), 4.01 (t, J = 6.5 Hz, 2H, OCH_2), 3.77 (s, 3H, OCH_3), 3.73 (s, 2H, SCH_2), 3.71 (s, 2H, SCH_2), 3.07 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.47 (m, 4H, CH_2, benzylic), 1.81 (m, 2H, CH_2), 1.50 (m, 4H, aliphatic), 1.30 (m, 6H, aliphatic); MS (ES) m/e 566 $[M + H]^+$, free acid; (ES−) m/e 564 $[M - H]^-$, free acid. Anal. ($C_{32}H_{37}NO_6SLi_2 \cdot 2^1/_4H_2O$): C, H, N.

The following compounds were prepared by procedures analogous to those already described.

(*E*)-4-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid, dilithium salt (34): ^1H NMR (250 MHz, MeOH-d_4) δ 7.87 (d, J = 8.6 Hz, 2H, phenyl), 7.79 (d, J = 15.8 Hz, 1H, vinyl), 7.34 (m, 3H, phenyl, pyridyl), 7.23 (d, J = 8.6 Hz, 1H, pyridyl), 7.08 (d, J = 15.8 Hz, 1H, vinyl), 7.06 (d, J = 8.6 Hz, 2H, phenyl), 6.80 (d, J = 8.6 Hz, 2H, phenyl), 4.04 (t, J = 6.5 Hz, 2H, OCH_2), 3.74 (s, 2H, SCH_2), 3.73 (s, 3H, OCH_3), 3.69 (s, 2H, SCH_2), 2.55 (t, J = 7.6 Hz, 2H, benzylic), 1.87 (m, 2H, CH_2), 1.50 (m, 10H, aliphatic); MS (FAB) m/e 576 $[M + H]^+$, 582.3 $[M + Li]^+$. Anal. ($C_{32}H_{35}NO_6SLi_2 \cdot 3H_2O$): C, H, N.

(*E*)-4-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]sulfinyl]methyl]benzoic acid, dilithium salt (35): ^1H NMR (250 MHz, MeOH-d_4) δ 7.98 (d, J = 8.6 Hz, 2H, phenyl), 7.81 (d, J = 15.8 Hz, 1H, vinyl), 7.40 (d, J = 8.6 Hz, 2H, phenyl), 7.39 (d, J = 8.6 Hz, 1H, pyridyl), 7.27 (d, J = 8.6 Hz, 1H, pyridyl), 7.09 (d, J = 15.8 Hz, 1H, vinyl), 7.05 (d, J = 8.6 Hz, 2H, phenyl), 6.77 (d, J = 8.6 Hz, 2H, phenyl), 4.35 (d, J = 12.8 Hz, 1H, SCH), 4.25 (d, J = 12.8 Hz, 1H, SCH), 4.06 (m, 4H, OCH_2, SCH_2), 3.73 (s, 3H, OCH_3), 2.52 (t, J = 7.6 Hz, 2H, benzylic), 1.86 (m, 2H, CH_2), 1.55 (m, 4H, aliphatic), 1.35 (m, 6H, aliphatic); MS (FAB) m/e 592 $[M + H]^+$, 580 $[M + H]^+$, free acid.

(*E*)-2-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid, dilithium salt (36): ^1H NMR (250 MHz, MeOH-d_4) δ 7.89 (d, J = 15.8 Hz, 1H, vinyl), 7.5 (dd, 1H, aryl), 7.3–7.15 (m, 5H, aryl), 7.05 (m, 3H, vinyl, aryl), 6.80 (d, J = 8.6 Hz, 2H, aryl), 4.05 (m, 4H, OCH_2, SCH_2), 3.71 (s, 3H, OMe), 3.65 (s, 2H, SCH_2), 2.50 (t, J = 7.6 Hz, 2H, benzylic), 1.8–1.2 (m, 12H, aliphatic); MS (ES+) m/e 564 $[M + H]^+$, free acid. Anal. ($C_{32}H_{35}NO_6SLi_2 \cdot 2H_2O$): C, H, N.

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[(8-phenyloctyl)oxy]-2-pyridinyl]methyl]thio]methyl]benzoic Acid, Dilithium Salt (37). **37** was prepared by methods similar to those for compound **11** substituting 8-phenyloctyl bromide for 1-iodo-8-(4-methoxyphenyl)octane (**16**): ^1H NMR (250 MHz, MeOH-d_4) δ 7.95 (s, 1H, 2-phenyl), 7.83 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.82 (d, J = 15.8 Hz, 1H, vinyl), 7.39–7.10 (m, 9H, aryl), 7.08 (d, J = 15.8 Hz, 1H, vinyl), 4.06 (t, J = 6.5 Hz, 2H, OCH_2), 3.74 (s, 2H, SCH_2), 3.69 (s, 2H, SCH_2), 2.60 (t, J = 7.6 Hz, 2H, benzylic), 1.88 (m, 2H, CH_2), 1.60 (m, 4H, aliphatic), 1.58 (m, 6H, aliphatic); MS (ES+) m/e 534.2 $[M + H]^+$, free acid; (ES−) m/e 532.2 $[M - H]^-$, free acid. Anal. ($C_{31}H_{33}NO_5SLi_2 \cdot 2^5/_8H_2O$): C, H, N.

The following compounds were prepared by methods similar to those already described substituting 4-(4-methoxyphenyl)-butan-1-ol (Aldrich) or 4-phenylbutan-1-ol (Aldrich) for 8-(4-methoxyphenyl)octan-1-ol (**15**).

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[[4-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid, dilithium salt (38): ^1H NMR (250 MHz, MeOH-d_4) δ 7.94 (s, 1H, aryl), 7.83 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.82 (d, J = 15.8 Hz, 1H, vinyl), 7.41–7.18 (m, 4H, aryl), 7.12 (d, J = 8.6 Hz, 2H, aryl), 7.08 (d, J= 15.8 Hz, 1H, vinyl), 6.82 (d, J = 8.6 Hz, 2H, aryl), 4.0 (t, J = 6.5 Hz, 2H, OCH_2), 3.74 (s, 3H, OMe), 3.71 (s, 2H, SCH_2), 3.67 (s, 2H, SCH_2), 2.62 (t, J = 6.5 Hz, 2H, benzylic), 2.55 (m, 2H, CH_2), 1.7 (m, 4H, aliphatic); MS (ES+) 508.0 $[M + H]^+$, free acid; (ES−) 506.0 $[M - H]^-$, free acid. Anal. ($C_{28}H_{27}NO_6SLi_2 \cdot 2^1/_2H_2O$): C,H,N.

(*E*)-3-[[[[6-(2-Carboxyethenyl)-5-[(4-phenyloctyl)oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid, dilithium salt (39): ^1H NMR (250 MHz, MeOH-d_4) δ 7.94 (s, 1H, aryl), 7.83 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.82 (d, J = 15.8 Hz, 1H, vinyl), 7.41–7.18 (m, 5H, aryl), 7.12 (d, J = 8.6 Hz, 2H, aryl), 7.08 (d, J= 15.8 Hz, 1H, vinyl), 6.82 (d, J = 8.6 Hz, 2H, aryl), 4.0 (t, J = 6.5 Hz, 2H, OCH_2), 3.71 (s, 2H, SCH_2), 3.67 (s, 2H, SCH_2), 2.62 (t, J = 6.5 Hz, 2H, benzylic), 1.78 (m, 4H, aliphatic); MS (ES+) 478.0 $[M + H]^+$, free acid; (ES−) 476.4 $[M - H]^-$, free acid.

3-[[[[6-(2-Carboxyethyl)-5-[[4-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]benzoic acid, dilithium salt (40): ^1H NMR (250 MHz, MeOH-d_4) δ 7.90 (s, 1H, 2-phenyl), 7.83 (d, J = 7.7 Hz, 1H, 6-phenyl), 7.34 (m, 2H, 4,5-phenyl), 7.25 (d, J = 8.6 Hz, 1H, pyridyl), 7.14 (d, J = 8.6 Hz, 1H, pyridyl), 7.07 (d, J = 8.6 Hz, 2H, phenyl), 6.83 (d, J = 8.6 Hz, 2H, phenyl), 4.01 (t, J = 6.5 Hz, 2H, OCH_2), 3.77 (s, 3H, OMe), 3.73 (s, 2H, SCH_2), 3.71 (s, 2H, SCH_2), 3.07 (dd, J = 7.3, 7.2 Hz, 2H, CH_2), 2.47 (m, 4H, CH_2, benzylic), 1.81 (m, 4H, aliphatic); MS (ES+) 510.2 $[M + H]^+$, free acid. Anal. ($C_{28}H_{29}NO_6SLi_2 \cdot 1^1/_2H_2O$): C, H, N.

X-ray Structure Determination. Crystal data for **11**: $C_{32}H_{37}NO_6S$, M_r = 563.69, pale tan plates from ethyl acetate, monoclinic, space group $P2_1/n$, a = 13.513(1) Å, b = 8.863(1) Å, c = 24.851(2) Å, β = 93.29(1)°, V = 2971.4(5) Å^3, Z = 4, d_{calc} = 1.260 g cm^{-3}, μ = 1.328 cm^{-1}, (Cu Kα, λ = 1.5418 Å), T = 223(2) K. Intensity data ($2\theta \leq 125°$, $-9 < h < 15$, $-6 < k < 10$, $-28 < l < 28$) were collected on an Enraf-Nonius CAD-4 diffractometer using variable speed ω–2θ scans. Data were corrected for measured backgrounds, Lorentz, and polarization effects and a maximum 8% variation in the intensities of three standards measured every 3 h of exposure time. Symmetry equivalent data were averaged (R_{int} = 0.033). The structure was solved using SHELXTL-PLUS[29] and refined with SHELXL-92. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included in the final model in calculated positions based on geometrical considerations with isotropic temperature factors assigned as 1.2 (Ueq) of the attached atom (1.4 (Ueq) for methyl hydrogens). The full matrix least-squares refinement (on F^2) converged (max Δ/σ 0.001) to values of the conventional crystallographic residuals R = 0.041 (R_w = 0.110) for all 4738 unique data with a goodness of fit of 1.065. There were 365

variables. A final difference Fourier map was featureless, with maximum residual density between ±0.27 e Å^{-3}.

Biological Evaluation. [^{3}H]LTB$_4$ Binding Assays. [^{3}H]-LTB$_4$ with specific activity of 140–210 Ci/mmol was obtained from New England Nuclear (Boston, MA). Unlabeled LTB$_4$ was synthesized by the Medicinal Chemistry Prep Group at SmithKline Beecham.

Human peripheral blood from healthy aspirin-free donors was phlebotomized into sterile heparinized syringes. PMNs were isolated by the standard Ficoll-Hypaque centrifugation, dextran 70 sedimentation and hypotonic lysis procedure.[30] Cell preparations were >90% neutrophils and >95% viable.

Test compounds were evaluated for the ability to compete with [^{3}H]LTB$_4$ for receptors on intact human PMNs utilizing methods described previously.[23, 31] Equilibrium binding for washed PMNs (10^6 cells) was performed at 0 °C for 20 min in Hanks balanced salt solution with 0.1% ovalbumin and 0.2 nM [^{3}H]LTB$_4$ in a total volume of 500 μL. Total and nonspecific binding of [^{3}H]LTB$_4$ were determined in the absence and presence of 1 μM unlabeled LTB$_4$, respectively. For radioligand competition experiments, increasing concentrations of LTB$_4$ (0.05–10 nM) or test compound (0.1 nM to 10 μM) was included. Unbound radioligand and competing compounds were separated from cell bound ligand by vacuum filtration through Whatman GF/C filters. Cell bound radioactivity was determined by liquid scintillation spectrometry. The percent inhibition of specific [^{3}H]LTB$_4$ binding was determined for each concentration, and the IC$_{50}$ is defined as the concentration of test compound required to inhibit 50% of the specific [^{3}H]LTB$_4$ binding. Concentration response curves (five to eight concentrations) for all compounds were run in duplicate and tested in at least two assays. Values presented are the mean K_i values which were determined from the mean IC$_{50}$ as described by Cheng and Prusoff[32] using the following equation:

$$K_i = \frac{IC_{50}}{[1 + [L]/K_d]}$$

where [L] is the concentration of added ligand and the K_d, as determined from saturation studies, is 0.15 nM. Standard errors are presented for all compounds where three or more concentration response curves were run and indicates the precision of the assay method made.

LTB$_4$-Induced Calcium Mobilization. The functional assay used to determine agonist/antagonist activity of test compounds was LTB$_4$-induced calcium mobilization in intact human PMNs.[33] Cells were washed with 50 mM Tris, pH 7.4 containing 1 mM EDTA. The [Ca^{2+}]$_i$ was estimated with the calcium fluorescent probe fura 2.[34] Isolated PMNs were suspended in Krebs Ringer Hensilet at 2 × 10^6 cells/mL containing 0.1% BSA, 1.1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4 (buffer A). The diacetoxymethoxy ester of fura 2 (fura 2/AM) was added at a concentration of 2 μM and incubated for 45 min at 37 °C. Cells were centrifuged at 225*g* for 5 min and resuspended at 2 × 10^6 cells/mL in buffer A and incubated an additional 20 min to allow complete hydrolysis of the entrapped ester. Cells were centrifuged as above and resuspended at 10^6 cells/mL in buffer A containing 1 mM $CaCl_2$. The cells were maintained at room temperature until used in the fluorescent assay which was performed within 3 h.

The fluorescence of fura 2 containing cells was measured with a fluorometer designed by the Johnson Foundation Biomedical Instrumentation Group. The fluorometer was equipped with a temperature control and a magnetic stirrer under the cuvette holder. Wavelengths were set at 340 nm (10 nm band width) for excitation and 510 nm (20 nm band width) for emission. All experiments were performed at 37 °C with constant stirring. For compound studies, fura 2 loaded cells were centrifuged and resuspended in buffer A containing 1 mM $CaCl_2$ minus BSA at 10^6 cells/mL. For agonist activity, a 2 mL aliquot of PMNs was added to a cuvette and warmed in a water bath to 37 °C. The 1 cm^2 cuvette was transferred to the fluorometer, and fluorescence was recorded for 15 s to ensure a stable baseline before addition of compound. Fluorescence was recorded continuously for up to 2 min after addition of compounds to monitor for the presence of any agonist activity. None of the compounds from the present study demonstrated agonist activity up to 10 μM.

For antagonist studies, varying concentrations of antagonists or vehicle were added to the fura 2 loaded PMNs and monitored for 1 min to ensure that there was no change in baseline fluorescence followed by the addition of 1 nM LTB$_4$. The maximal [Ca^{2+}]/fura 2 fluorescence was then determined for each sample. The [Ca^{2+}]$_i$ was calculated using the following formula as previously described:[23]

$$[Ca^{2+}]_i = 224\ (nM)\ \frac{F - F_{min}}{F_{max} - F}$$

The percent of maximal LTB$_4$ (1 nM) induced [Ca^{2+}]$_i$ was determined for each concentration of compound and the IC$_{50}$ defined as the concentration of test compound that inhibits 50% of the maximal LTB$_4$ response. The concentration response curve for each compound (five to seven concentrations) was run in at least two different assays and the mean IC$_{50}$ presented. An estimate of the precision of the measurements can be assessed by the standard error values presented for the compounds where three or more concentration response curves were run.

LTB$_4$-Induced Degranulation. Several compounds were tested in a second functional assay that requires higher concentrations of the agonist, that is, LTB$_4$-induced degranulation of human PMNs.[35] Degranulation was assessed using 10^6 washed neutrophils, isolated as described above, in 1 mL of Earls balanced salt solution with 20 mM HEPES and 0.1% ovalbumin. Cells were preincubated with 5 μg/mL cytochalasin B for 10 min and antagonist or vehicle for 5 min at 37 °C followed by 100 nM LTB$_4$ for 3 min. Incubation was terminated by placing assay tubes on ice followed by centrifugation (400*g* for 2 min). Myeloperoxidase (MPO) activity in the supernatant fractions was determined kinetically as described by Bradley *et al.*[36] An aliquot (50 μL) of the supernatant was incubated with 0.95 mL of assay reagent containing 0.167 mg/mL *o*-dianisidine dihydrochloride, 0.0005% hydrogen peroxide, and 50 mM potassium phosphate buffer (pH 6.0). Product formation was linear for >2 min and measured spectrophotometrically every 15 s for 2 min in a Beckman DU-70 at 460 nm. A unit of MPO activity was defined as that degrading 1 μmol of peroxide/min at 25 °C. The percent of LTB$_4$-induced (100 nM) degranulation was determined for each concentration of antagonist and the IC$_{50}$ defined as the concentration of test compound that inhibits 50% of the 100 nM LTB$_4$ response. Two concentration response curves were run for each compound using five to seven concentrations of antagonist and the mean value is presented. An estimate of the precision of the method can be assessed with compound **11** where the mean and standard error were determined and presented in Table 1.

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Supplementary Material Available: Fractional atomic coordinates, anisotropic displacement parameters, tables of bond distances, bond and torsion angles, and a stereodrawing of the unit cell for **11** (8 pages); structure factors (11 pages). Ordering information is given on any current masthead page.

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