Notes

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic Acid: A High-Affinity Leukotriene B_4 Receptor Antagonist with Oral Antiinflammatory Activity

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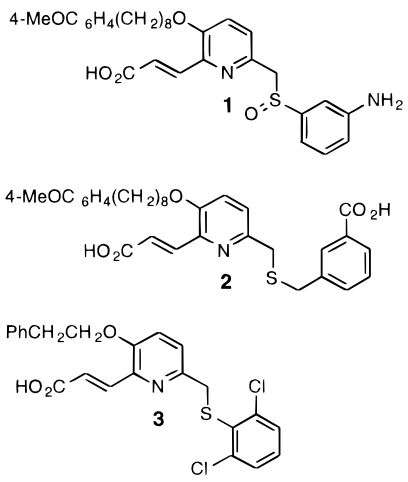
An extensive structure−activity study based around the high-affinity leukotriene B_4 (LTB_4) receptor antagonist SB 201146 (**1**) led to the identification of (*E*)-3-[6-[[(2,6-dichlorophenyl)-thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic acid (**3**). This compound displays high affinity for the human neutrophil LTB_4 receptor (K_i = 0.78 nM), blocks LTB_4-induced Ca^{2+} mobilization with an IC_{50} of 6.6 ± 1.5 nM, and demonstrates potent oral and topical antiinflammatory activity in a murine model of dermal inflammation.

Leukotriene B_4 (LTB_4), a product of 5-lipoxygenase-catalyzed oxygenation of arachidonic acid (AA), has been postulated to be a mediator of a variety of inflammatory diseases.[1] Elevated levels of LTB_4 found in psoriatic lesional skin,[2] colonic mucosa associated with inflammatory bowel disease,[3] synovial fluid from patients with active rheumatoid arthritis,[4] and gouty effusions[5] support the involvement of this mediator in human inflammatory diseases. Known pathophysiological responses of LTB_4 include potent neutrophil chemotactic activity, 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] The pharmacological effects of LTB_4 are mediated through interaction with specific cell surface receptors on inflammatory cells. 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.

Previously, we reported the identification of the novel high-affinity LTB_4 receptor antagonists SB 201146 (**1**, K_i = 4.7 nM)[6] and SB 201993 (**2**, K_i = 7.1 nM).[7] In murine models, these compounds were potent antiinflammatory agents when applied topically and demonstrated oral LTB_4 receptor antagonist activity. However, the compounds did not show significant oral activity in a murine model of inflammation. The *in vivo* test system selected to evaluate antiinflammatory activity was the AA-induced mouse ear inflammation model.[8] In this model, an inflammatory response (neutrophil infiltration) is initiated by the application of 1 mg of arachidonic acid to the mouse ear. Antiinflammatory activity of the test compounds was determined by monitoring the neutrophil marker enzyme myeloperoxidase (MPO). Both SB 201146 (**1**) and SB 201993 (**2**) were potent topical antiinflammatory agents in this model but lacked significant oral activity. The goal of the present study was to identify high-affinity LTB_4 receptor antagonists that demonstrate potent oral antiinflammatory activity. One compound that fully meets these criteria has been identified, i.e., (*E*)-3-[6-[[(2,6-dichlorophenyl)thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic acid, SB 209247 (**3**).



Chemistry

Compounds **8**, **9**, **13**, and **14** were synthesized according to procedures previously described.[7] The phenylethyl-containing compounds of the present study were synthesized according to Scheme 1 (illustrated for compound **3**). Reaction of the pyridyl 2-carboxaldehyde[6] **4** with methyl (triphenylphosphoranylidene)acetate followed by a Mitsunobu-type coupling with phenethyl alcohol provided methylpyridine **5**. Oxidation to the pyridine *N*-oxide, trifluoroacetic anhydride-induced rearrangement, and reaction of the resulting primary alcohol with $SOCl_2$ provided chloride **6**. Conversion of chloride **6** to sulfide **7** was achieved using 2,6-dichlorobenzenethiol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[9] Hydrolysis of the methyl ester and acidification provided **3** as a crystalline solid.[10]

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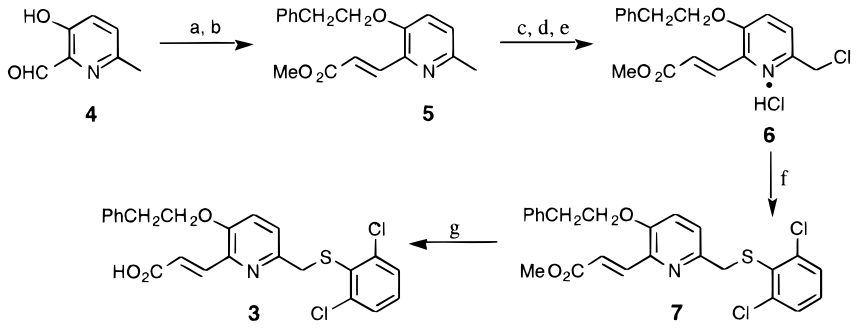
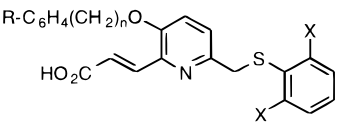
Scheme 1[a]



[a] Reagents: (a) Ph_3PCHCO_2Me, toluene, 50 °C, 85%; (b) $PhCH_2CH_2OH$, DEAD, Ph_3P, 53%; (c) MCPBA, CH_2Cl_2; (d) TFAA, DMF (96%, two steps); (e) $SOCl_2$, toluene, 99%; (f) 2,6-dichlorobenzenethiol, DBU, MeCN, 50 °C, 87%; (g) (i) 1.0 N LiOH, THF, MeOH, (ii) HCO_2H, 60%.

Table 1. Inhibition of [^{3}H]LTB_4 Binding to Human Neutrophils



compd	X	R	*n*	binding K_i, nM[a]
1	–	–	–	4.7 ± 0.4
8[b]	H	4-MeO	4	2.8 ± 0.6
9	Cl	4-MeO	4	1.5 ± 0.4
10[b]	Cl	4-MeO	2	1.2
3	Cl	H	2	0.78 ± 0.16
11[b]	Cl	4-Cl	2	1.3 ± 0.4
12	Cl	4-F	2	1.5 ± 0.3
13[b]	Cl	4-F	1	12
14[b]	Cl	H	3	1.4

[a] The K_i values are stated as the mean of at least three determinations ± standard error. All other values are the mean K_i of two concentration–response curves. [b] Compound tested as a sodium salt.

Results and Discussion

The search for a new class of orally active LTB_4 receptor antagonists started with the aniline sulfoxide **1**. Through an extensive structure–activity study, it was discovered that the requirement for both the aniline head group and the sulfoxide could be eliminated by reducing the length of the lipid chain. Thus, compound **8** (Table 1) containing the (4-methoxyphenyl)butyl tail and the unsubstituted phenyl head group had approximately 2-fold higher affinity for the LTB_4 receptor than **1**. Evaluation of **8** in the AA-induced mouse ear inflammation model revealed that the compound possessed significant, albeit modest, oral activity (Table 2). Additional studies focused on aryl head group substitution revealed that further improvement in affinity could be attained by incorporating a 2,6-dichlorophenyl moiety leading to compound **9** (Table 1). This increase in affinity for the LTB_4 receptor resulted in a corresponding increase in the oral antiinflammatory activity of the compound (Table 2).

Having identified high-affinity compounds possessing K_i values of approximately 1 nM, we focused on further improving the oral antiinflammatory activity. It was postulated that a further reduction in the lipophilic character of these compounds may enhance the oral activity provided the high receptor affinity was retained. To this end, the (4-methoxyphenyl)butyl tail was replaced with (4-methoxyphenyl)ethyl to provide **10** which was determined to be equipotent with **9** in the receptor binding assay. Replacement of the (4-methoxyphenyl)-ethyl group with phenylethyl provided **3**. Compound **3** demonstrated slightly improved receptor affinity over both **9** and **10**; however, a 4-fold increase in oral activity was obtained. In addition to having an ED_{50} of 14.8 mg/kg for inhibiting neutrophil influx in the murine model, **3** inhibited the fluid phase (edema)[8] of the inflammatory response with an ED_{50} of 18.7 mg/kg.

Table 2. Effect of LTB_4 Receptor Antagonists on Arachidonic Acid-Induced Mouse Ear Inflammation[a]

	oral administration		topical administration	
compd	inhibition of MPO, % (dose, mg/kg)	ED_{50}, mg/kg	inhibition of MPO, % (dose, μg/ear)	ED_{50}, μg/ear
1	0 (50)	–	55 (500)	350
2	0 (50)	–	69 (500)	390
8	26 (50)	ND	49 (1000)	ND
9	54 (50)	57.6	82 (1000)	ND
3	70 (25)	14.8	83 (50)	8
12	75 (25)	14.1	ND	ND

[a] ND: not determined. ED_{50} values are the doses required to inhibit 50% of the arachidonic acid-induced inflammation obtained from dose–response curves (4–5 concentrations of antagonist).

Additional modifications within this phenylethyl series resulted in compounds with slightly reduced receptor affinities (i.e., **11** and **12**, Table 1). Compound **12** also demonstrated potent oral antiinflammatory activity exhibiting equivalent potency to that of **3** (Table 2). However, in duration of action and pharmacokinetic studies, compound **3** demonstrated a superior profile to that of **12** (data not shown). Further reduction in the tail length resulted in a compound with 1 order of magnitude weaker affinity for the neutrophil LTB_4 receptor (e.g., compare **12** and **13**, Table 1). In contrast, the phenylpropyl analog **14** was nearly as active in the binding assay as **3**.

In addition to being a high-affinity antagonist, compound **3** demonstrated a high degree of selectivity for the LTB_4 receptor. Compound **3** competes with [^{3}H]-fMLP and [^{3}H]LTD_4 in respective receptor binding assays with IC_{50} values of 3250 and 16 800 nM and with another 14 ligands with IC_{50} values greater than 10 000 nM. Functional activity at the cellular level was demonstrated in human PMNs by blocking the LTB_4-induced Ca^{2+} mobilization and LTB_4-induced degranulation with IC_{50} values of 6.6 ± 1.5 and 53 ± 7 nM, respectively.

Compound **3** was found to be a weak inhibitor of the RBL-1 supernatant 5-lipoxygenase, inhibiting the enzyme with an IC_{50} of 8200 nM. When evaluated as an inhibitor in several other enzyme assays, including CoA

independent transacylase, human phospholipase A_2 (14 and 85 kDa), and protein kinase C (rat brain homogenate, rhPKC-α), **3** was inactive at concentrations up to 10 000 nM.

In summary, (*E*)-3-[6-[[(2,6-dichlorophenyl)thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic acid (**3**) is a selective high-affinity antagonist of the LTB_4 receptor. It selectively blocks the actions of LTB_4 on human PMNs *in vitro*, and *in vivo* it demonstrates potent topical and oral antiinflammatory activity in a murine model of dermal inflammation. Furthermore, compound **3** represents a significant advantage over the earlier aniline sulfoxide **1** in that it is structurally less complex and lacks chiral centers. SB 209247 (**3**) has been selected for further evaluation as a potential orally active antiinflammatory agent.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on either a Brucker AM-250 or AM-400 instrument with the solvents indicated. All ^{1}H NMR 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.

(*E*)-3-[6-Methyl-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic Acid Methyl Ester (5). Aldehyde **4**[7] (20 g, 146 mmol) in anhydrous toluene (250 mL) was treated with methyl (triphenylphosphoranylidene)acetate (48.8 g, 146 mmol) portionwise, and the resulting mixture was heated for 0.5 h at 50 °C. The mixture was cooled to 0 °C and filtered, and the cake was washed with toluene (40 mL) and dried to give 24 g (85%) of (*E*)-3-(3-hydroxy-6-methyl-2-pyridinyl)-2-propenoic acid methyl ester as a gold solid. This material was used directly in the following step.

Hydroxypyridine prepared above (11.3 g, 58.5 mmol), triphenylphosphine (15.3 g, 58.5 mmol), and phenethyl alcohol (7.14 g, 58.5 mmol) were dissolved in anhydrous THF (350 mL) under an argon atmosphere. The resulting solution was cooled to 0 °C, and diethyl azodicarboxylate (10.2 g, 58.5 mmol) was added dropwise over 15 min. The reaction mixture was stirred at 0 °C for 15 min and then allowed to warm to room temperature over 1 h. The reaction mixture was concentrated and the residue triturated with hexane−EtOAc (3:1). The resulting suspension was cooled (0 °C), and the solids were filtered; concentration of the filtrate provided a dark amber oil. Purification by column chromatography (silica gel, 20% EtOAc in hexane) gave 9.2 g (53%) of **5** as a colorless oil that gradually solidified: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.06 (d, J = 15.2 Hz, 1H, vinyl), 7.25−7.34 (m, 5H, aryl), 7.07 (d, J = 1.7 Hz, 2H, pyridyl), 7.00 (d, J = 15.2 Hz, 1H, vinyl), 4.18 (t, J = 7.0 Hz, 2H, CH_2O), 3.82 (s, 3H, OMe), 3.14 (t, J = 7.0 Hz, 2H, benzylic), 2.48 (s, 3H, CH_3); MS (ES+) 297.2 (M + H).

(*E*)-3-[6-(Chloromethyl)-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic Acid Methyl Ester Hydrochloride (6). Pyridyl ester **5** (9.2 g, 31.0 mmol) was dissolved in CH_2Cl_2 (100 mL) and cooled to 0 °C. MCPBA (11.7 g, 33.7 mmol; 50−60%) was added portionwise over 15 min; the cooling bath was removed, and stirring was continued at room temperature for 18 h. The reaction mixture was poured into H_2O (100 mL), and concentrated NH_4OH was added to bring pH to 8.8. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude *N*-oxide (10.4 g) was obtained as a yellow semisolid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.2 (d, J = 16.2 Hz, 1H, vinyl), 7.53 (d, J = 16.2 Hz, 1H, vinyl), 7.26−7.34 (m, 5H, aryl), 7.11 (d, J = 8.8 Hz, 1H, pyridyl), 6.77 (d, J = 8.8 Hz, 1H, pyridyl), 4.26 (t, J = 6.2 Hz, 2H, CH_2O), 3.82 (s, 3H, OMe), 3.17 (t, J = 6.8 Hz, 2H, benzylic), 2.45 (s, 3H, CH_3).

Crude *N*-oxide (9.7 g, 31 mmol) was dissolved in anhydrous DMF (95 mL) under argon, cooled to 0 °C, and treated with TFAA (42 mL, 300 mmol) dropwise over 30 min. The cooling bath was removed, and the reaction mixture was stirred for 18 h at room temperature. The solution was concentrated, and the reaction was quenched slowly by addition to a mixture of Na_2CO_3 (48 g) in H_2O (100 mL) at 20−25 °C with rapid stirring. After 1 h, the solution was poured into a mixture of H_2O (400 mL) and EtOAc (200 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (three times). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The alcohol was purified by column chromatography (silica gel, 33% EtOAc in hexane) to provide 9.3 g (96%, two steps) of alcohol as a waxy solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 15.9 Hz, 1H, vinyl), 7.25−7.33 (m, 5H, aryl), 7.17 (d, J = 1.7 Hz, 2H, pyridyl), 6.99 (d, J = 15.9 Hz, 1H, vinyl), 4.66 (s, 2H, CH_2O), 4.20 (t, J = 6.9 Hz, 2H, CH_2O), 3.82 (s, 3H, OMe), 3.74 (br s, 1H, OH), 3.15 (t, J = 6.9 Hz, 2H, benzylic); MS (ES+) 314.0 (M + H).

The alcohol prepared above (9.0 g, 28.7 mmol) was dissolved in anhydrous toluene (150 mL) under argon and cooled (0 °C). Thionyl chloride (21 mL, 287 mmol) in toluene (20 mL) was added over 15 min. The cooling bath was removed, and stirring was continued for 1 h. The reaction solution was concentrated to remove the excess $SOCl_2$ and toluene; the resulting tan solid was dried under vacuum to give hydrochloride **6** which was used without further purification.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic Acid Methyl Ester (7). Hydrochloride **6** (∼28 mmol) was dissolved in anhydrous MeCN (80 mL) and treated with 2,6-dichlorobenzenethiol (5.7 g, 31 mmol) and DBU (14.9 mL, 100 mmol). The reaction mixture was heated at 50 °C for 1 h under argon and then cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 and brine. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The product was purified by column chromatography (silica gel,20% EtOAc in hexane) to give 11.7 g (87%) of **7** as an off-white solid: mp 53−56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 15.6 Hz, 1H, vinyl), 7.26−7.34 (m, 7H, aryl), 7.11−7.15 (m, 3H, aryl, pyridyl), 7.06 (d, J = 8.6 Hz, 1H, pyridyl), 6.70 (d, J = 15.6 Hz, 1H, vinyl), 4.19 (t, J = 7.0 Hz, 2H, CH_2O), 4.14 (s, 2H, CH_2S), 3.82 (s, 3H, OMe), 3.14 (t, J = 7.0 Hz, 2H, benzylic); MS (ES+) 474 (M + H). Anal. ($C_{24}H_{21}Cl_2NO_3S$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-(2-phenylethoxy)-2-pyridinyl]-2-propenoic Acid (3). Methyl ester **7** (6.9 g, 14.5 mmol) was dissolved in THF (44 mL) and methanol (22 mL) under argon and treated with 1.0 N LiOH (22 mL, 22 mmol). The reaction mixture was stirred for 18 h at room temperature. The solution was concentrated, and the residue was stirred with distilled H_2O (80 mL) for 5 h; the resulting lithium salt was filtered off and washed with H_2O. The salt was suspended in H_2O (100 mL) and treated with HCO_2H to give a pH of 3.5. The free acid was extracted into CH_2Cl_2, and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. Recrystallization from EtOAc provided 4.1 g (60%) of **3** as a white solid: mp 124−125 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 15.7 Hz, 1H, vinyl), 7.28−7.36 (m, 7H, aryl), 7.18 (d, J = 8.6 Hz, 1H, pyridyl), 7.13−7.17 (m, 1H, aryl), 7.08 (d, J = 8.6 Hz, 1H, pyridyl), 6.62 (d, J = 15.7 Hz, 1H, vinyl), 4.19 (t, J = 7.0 Hz, 2H, CH_2O), 4.15 (s, 2H, CH_2S), 3.14 (t, J = 7.0 Hz, 2H, benzylic); MS (ES+) 460.0 (M + H); MS (ES−) 458.0 (M − H). Anal. ($C_{23}H_{19}Cl_2NO_3S$) C, H, N, S.

(*E*)-3-[3-[4-(4-Methoxyphenyl)butoxy]-6-[(phenylthio)methyl]-2-pyridinyl]-2-propenoic acid sodium salt (8): colorless amorphous solid; ^{1}H NMR (250 MHz, MeOH-d_4) δ 7.72 (d, J = 15.7 Hz, 1H vinyl), 7.06 (d, J = 8.6 Hz, 2H, aryl), 7.05−7.31 (m, 7H, aryl, pyridyl), 6.99 (d, J = 15.7 Hz, 1H, vinyl), 6.76 (d, J = 8.6 Hz, 2H, aryl), 4.16 (s, 2H, CH_2S), 3.97 (t, J = 6.7 Hz, 2H, CH_2O), 3.70 (s, 3H, OMe), 2.58 (t, J = 6.7 Hz, 2H, benzylic), 1.78 (m, 4H, aliphatic); MS (ES+) 450.2 (M

+ H, free acid); MS (ES−) 448.0 (M − H, free acid). Anal. ($C_{26}H_{26}NO_4SNa·0.75H_2O$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-[4-(4-methoxyphenyl)butoxy]-2-pyridinyl]-2-propenoic acid (9): colorless solid; mp 129−130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 15.6 Hz, 1H, vinyl), 7.33 (d, J = 8.1 Hz, 1H, aryl), 7.12 (d, J = 8.6 Hz, 2H, aryl), 7.07−7.20 (m, 4H, aryl, pyridyl), 6.84 (d, J = 8.6 Hz, 2H, aryl), 6.62 (d, J = 15.6 Hz, 1H, vinyl), 4.15 (s, 2H, CH_2S), 3.98 (t, J = 6.0 Hz, 2H, CH_2O), 3.78 (s, 3H, OMe), 2.64 (t, J = 7.2 Hz, 2H, benzylic), 1.78−1.85 (m, 4H, aliphatic); MS (ES+) 518.0 (M + H). Anal. ($C_{26}H_{25}Cl_2NO_4S·2.75H_2O$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-[2-(4-methoxyphenyl)ethoxy]-2-pyridinyl]-2-propenoic acid sodium salt (10): colorless amorphous solid; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.70 (d, J = 15.6 Hz, 1H, vinyl), 7.36 (d, J = 8.0 Hz, 2H, aryl), 7.22 (d, J = 7.4 Hz, 2H, pyridyl), 7.15−7.20 (m, 2H, aryl), 7.00 (d, J = 8.5 Hz, 1H, aryl), 6.84 (d, J = 8.0 Hz, 2H, aryl), 6.83 (d, J = 15.6 Hz, 1H, vinyl), 4.14 (t, J = 6.7 Hz, 2H, CH_2O), 4.12 (s, 2H, CH_2S), 3.76 (s, 3H, OMe), 3.05 (t, J = 6.7 Hz, 2H, benzylic); MS (ES+) 490.0 (M + H, free acid); MS (ES−) 487.6 (M − H, free acid). Anal. ($C_{24}H_{20}Cl_2NO_4SNa·2.5H_2O$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-[2-(4-chlorophenyl)ethoxy]-2-pyridinyl]-2-propenoic acid sodium salt (11): colorless amorphous solid; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.67 (d, J = 15.9 Hz, 1H, vinyl), 7.36 (d, J = 7.9 Hz, 2H, aryl), 7.30 (AB quartet, J = 8.4 Hz, 2H, pyridyl), 7.23 (d, J = 8.4 Hz, 1H, aryl), 7.17 (d, J = 8.6 Hz, 2H, aryl), 7.01 (d, J = 8.6 Hz, 2H, aryl), 6.81 (d, J = 15.9 Hz, 1H, vinyl), 4.18 (t, J = 6.7 Hz, 2H, CH_2O), 4.12 (s, 2H, CH_2S), 3.11 (t, J = 6.7 Hz, 2H, benzylic); MS (ES+) 493.8 (M + H, free acid); MS (ES−) 491.8 (M − H, free acid). Anal. ($C_{23}H_{17}Cl_3NO_3SNa·2.95H_2O$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-[2-(4-fluorophenyl)ethoxy]-2-pyridinyl]-2-propenoic acid (12): colorless solid; mp 146−147 °C (EtOAc); ^{1}H NMR (250 MHz, MeOH-d_4) δ 7.67 (d, J = 15.8 Hz, 1H, vinyl), 7.35 (m, 4H, aryl), 7.25 (m, 2H, aryl), 7.05 (m, 3H, aryl), 6.82 (d, J = 15.8 Hz, 1H, vinyl), 4.18 (t, J = 6.7 Hz, 2H, CH_2), 4.12 (s, 2H, pyr-CH_2-S), 3.10 (t, J = 6.7 Hz, 2H, CH_2); MS (ES+) 478 (M + H); MS (ES−) 476 (M − H). Anal. ($C_{23}H_{18}Cl_2FNO_3S$) C, H, N, S.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-[(4-fluorophenyl)methoxy]-2-pyridinyl]-2-propenoic acid sodium salt (13): colorless amorphous solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (m, 4H, aryl), 7.35 (m, 3H, aryl, vinyl), 7.23 (dd, J = 8.9 Hz, 2H, aryl), 7.07 (d, J = 8.4 Hz, 1H, aryl), 6.59 (d, J = 15 Hz, 1H, vinyl), 5.13 (s, 2H, OCH_2), 4.14 (s, 2H, pyr-CH_2-S); MS (ES+) 464 (M + H). Anal. ($C_{22}H_{15}Cl_2FNO_3SNa·2.25H_2O$) C, H, N.

(*E*)-3-[6-[[(2,6-Dichlorophenyl)thio]methyl]-3-(3-phenylpropoxy)-2-pyridinyl]-2-propenoic acid sodium salt (14): colorless amorphous solid; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.74 (d, J = 15.6 Hz, 1H, vinyl), 7.36 (d, J = 7.9 Hz, 2H, aryl), 7.19−7.25 (m, 5H, aryl, pyridyl), 7.11−7.16 (m, 2H, aryl, pyridyl), 6.99 (d, J = 8.4 Hz, 1H, aryl), 6.86 (d, J = 15.6 Hz, 1H, vinyl), 4.13 (s, 2H, CH_2S), 3.97 (t, J = 6.2 Hz, 2H, CH_2O), 2.83 (t, J = 7.5 Hz, 2H, benzylic), 2.12 (quintet, J = 7.2, 6.6 Hz, 2H, aliphatic); MS (ES+) 474.0 (M + H,free acid); MS (ES−) 472.0 (M − H, free acid). Anal. ($C_{24}H_{20}Cl_2NO_3SNa·2.25H_2O$) C, H, N.

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.[11] 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.[12] 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−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 (5−8 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[13] 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 indicate the precision of the assay method made.

LTB$_4$-Induced Calcium Mobilization. The functional assay used to assess antagonist activity of compound **3** was LTB$_4$-induced calcium mobilization in intact human PMNs.[14] 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.[15] 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 225g for 5 min, 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 bandwidth) for excitation and 510 nm (20 nm bandwidth) 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 base line before addition of compound. Fluorescence was recorded continuously for up to 2 min after addition of compound **3** to monitor for the presence of any agonist activity; none was observed.

For antagonist studies, varying concentrations of compound **3** or vehicle were added to the fura 2-loaded PMNs and monitored for 1 min to ensure that there was no change in base-line 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:

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

The percent of maximal LTB$_4$ (1 nM)-induced $[Ca^{2+}]_i$ was determined for each concentration of compound, and the IC$_{50}$ is defined as the concentration of test compound that inhibits

50% of the maximal LTB_4 response. Concentration−response curves (5−7 concentrations) were run in four separate assays, and results are reported as mean $IC_{50} \pm$ SEM.

LTB_4-Induced Degranulation. Compound **3** was tested in a second functional assay that requires higher concentrations of the agonist, that is, LTB_4-induced degranulation of human PMNs.[16] 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 compound **3** 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). MPO activity in the supernatant fractions was determined kinetically as described by Bradley et al.[17] 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 at 460 nm. A unit of MPO activity is 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 compound, and the IC_{50} is defined as the concentration of test compound that inhibits 50% of the 100 nM LTB_4 response. Four concentration−response curves were run for compound **3** using 5−7 concentrations, and the mean $IC_{50} \pm$ SEM is presented.

Arachidonic Acid-Induced Mouse Ear Inflammation Model. For oral studies the test compounds were dissolved in distilled water and administered directly into the stomach. Balb/c mice, weighing 20 g, $N = 6-10$ mice/group for vehicle and typically $N = 5-6$ mice/group for drug treatment, were used for the study. Thirty minutes following vehicle or drug administration, 1.0 mg of arachidonic acid in 20 μL of cold acetone was applied topically to the left ear of the mouse. One hour later, the left treated ear and right untreated ear were measured with a thickness gauge. The difference represents the degree of swelling (edema). The mice were then euthanized with carbon dioxide gas and the left treated ears harvested and placed in dry ice to await MPO analysis.

For topical studies, 1.0 mg/ear AA in 20 μL of cold acetone was applied topically to the left ear for each vehicle and drug group. The number of mice/group used was the same as for the oral study. Immediately following AA application, 1.0 mg/ear of test compound in 25 μL of methanol or acetone was applied topically to the left ear. One hour later, the left and right ears of each group were measured for ear thickness and the mice euthanized. The left ears were harvested for MPO analysis as a measure of neutrophil influx.

MPO was extracted from the ears, and the activity was assayed spectrophotometrically by the method of Bradley[17] (see above). ED_{50} values were derived from multiple dose−response studies and calculated using linear regression.

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