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Discovery of Novel and Potent Leukotriene B_4 Receptor Antagonists. Part 1

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The inhibition of LTB_4 binding to and activation of G-protein-coupled receptors BLT1 and BLT2 is the premise of a treatment for several inflammatory diseases. In a lead optimization effort starting with the leukotriene B_4 (LTB_4) receptor antagonist (**2**), members of a series of 3,5-diarylphenyl ethers were found to be highly potent inhibitors of LTB_4 binding to BLT1 and BLT2 receptors, with varying levels of selectivity depending on the substitution. In addition, compounds **33** and **38** from this series have good in vitro ADME properties, good oral bioavailability, and efficacy after oral delivery in guinea pig LTB_4 and nonhuman primate allergen challenge models. Further profiling in a rat non-GLP toxicity experiment provided the rationale for differentiation and selection of one compound (**33**) for clinical development.

Introduction

Leukotriene B_4 (LTB_4,[a] (6*Z*,8*E*,10*E*,14*Z*)-(5*S*,12*R*)-5,12-dihydroxyeicosa-6,8,10,14-tetraenoic acid, **1**, Figure 1) is derived from the action of several enzymes acting sequentially on arachidonic acid, largely in inflammatory cells. Its effect on the recruitment of inflammatory cells is mediated by agonism of two G-protein-coupled receptors, BLT1 and BLT2.[1] The physiological implications of selective BLT1, BLT2, or dual BLT1 and BLT2 antagonism are not completely understood; however, BLT1 is known to play a key role in the activation and migration of several inflammatory cell types, including neutrophils, macrophages, and lymphocytes.[2] Several recent studies have demonstrated a significant role of BLT2 in inflammatory processes, including mast cell migration, dendritic cell trafficking, and allergic lung inflammation.[1−3] Therefore, blockade of LTB_4 receptors is a potentially useful strategy for the treatment of several pulmonary inflammtory diseases, including asthma, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), and chronic obstructive pulmonary disease (COPD).[4] In addition to extensive pharmacological data characterizing the potential role of LTB_4/BLT pathways in pulmonary diseases, the rationale for treatment of these diseases with LTB_4 antagonists is based in part on the observation of elevated levels of LTB_4 in pulmonary tissues in patients having these diseases. A large body of recent evidence also strongly suggests a role for BLT1 and BLT2 receptors in the initiation and progression of atherosclerosis.[5] Extensive reviews of preclinical and clinical development of small molecules for LTB_4 inhibitory-based therapies have been published.[6,7]

Several compounds that inhibit the action of LTB_4 on BLT1 and/or BLT2 that have entered clinical trials for various inflammatory and oncologic indications are shown in Figure 2. For example, amelubant (BIIL-284) is a carbamate prodrug of an antagonist of both BLT1 and BLT2 receptors; its pharmacology[8] and progress in clinical trials[9] have been thoroughly reported. Phase I studies for treatment of rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) have been disclosed for CP-195543, a dual antagonist of the BLT1 and BLT2 receptors. The preclinical pharmacology has been thoroughly described.[10] The development of LY293111, a selective BLT1 receptor antagonist, for treatment of asthma, IBD, and RA was reported in 2002.[9] Other studies of this

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[a] Abbreviations: ADME, absorption, distribution, metabolism, excretion; ADMET, absorption, distribution, metabolism, excretion, toxicity; AIBN, 2,2′-azobisisobutyronitrile; ALI, acute lung injury; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; BAL, bronchoalveolar lavage; BSA, bovine serum albumin; COPD, chronic obstructive pulmonary disease; cyp, cytochrome p450; $PdCl_2$(dppf), dichloro[1,1′-bis-(diphenylphosphino)ferrocene]palladium(II); DME, dimethoxyethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; GLP, good laboratory practice; GPCR, G-protein-coupled receptor; HBSS, Hank's balanced salt solution; HCl, hydrochloric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; hERG, human ether-a-go-go-related gene; IBD, inflammatory bowel disease; LTB_4, leukotriene B_4; NaOH, sodium hydroxide; PBS, phosphate buffer saline; PK, pharmacokinetic; PMA, phosphomolybdic acid; RA, rheumatoid arthritis; SAR, structure−activity relationship; TBDMS, *tert*-butyldimethylsilyl; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; THF, tetrahydrofuran.

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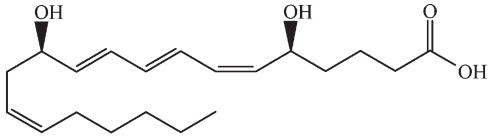
compound for the treatment of some cancers have also been reported.[11,12] The structures of several other compounds that have been studied clinically are also shown in Figure 2.[13]

In 1996, Roche scientists disclosed the structures of a series of potent LTB_4 receptor antagonists. Typical of these molecules is structure **2** (Figure 3).[14] On the basis of its efficacy in an in vivo mechanistic model, this molecule was advanced to canine PK studies.[15] Variability in exposure levels was observed as a function of the feeding state of the dog, rendering it unsuitable for further development. In this paper, we disclose the chemistry efforts to identify a new series of antagonists of the LTB_4 receptors, with the goal of improving the properties of **2** with respect to potency and oral bioavailability and developing new chemistry space around this unique chemical structure.

The strategy for further optimization of this series of LTB_4 inhibitors focused first on the replacement of the 4,6-diarylpyridin-2-ol moiety while leaving the 4-[2-(2-carboxyethyl)-3-hexylphenoxy]butyric acid side chain unchanged. The middle carbon chain length and the position of the two carboxylic acids had previously been optimized during the discovery of **2**. After the synthesis and assay of several small molecule libraries, it became clear that the 3,5-diarylphenoxy system provided the most promising results. In this work, we describe our efforts to optimize this moiety that led to the selection of the asymmetrically substituted derivative **33** for clinical development.

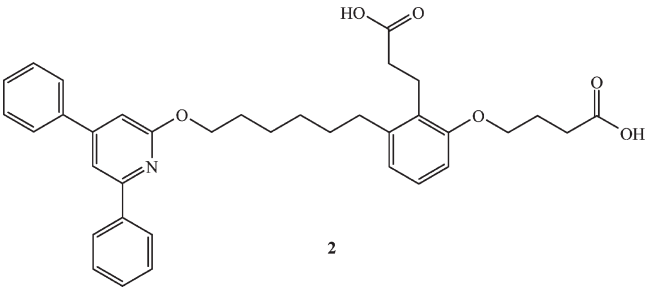


1

Figure 1. Structure of leukotriene B_4 (**1**).

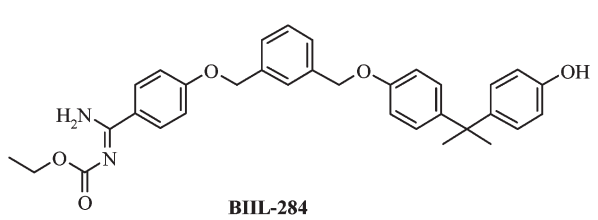
Chemistry

The LTB_4 antagonists were prepared according to the chemistry shown in Schemes 2, 3, and 4.[16] Compound **9** is the common intermediate for the preparation of symmetric and asymmetric analogues, and its synthesis is shown in Scheme 1. The three functionalized alkyl chains of compound **9** were introduced from 2,3-dimethylphenol **3** in nine steps. First, the 2,3-dimethylphenol was alkylated with 4-bromobutyric acid ethyl ester in the presence of lithium hydride in DMSO, resulting in **4**. The more reactive methyl group at the 2-position of **4** could be selectively oxidized with copper(II) sulfate pentahydrate and potassium persulfate in water and acetonitrile to the corresponding aldehyde which was treated with triethyl phosphonoacetate in the presence of sodium ethoxide using a modification of a reported Horner−Emmons condensation condition.[17] At this point, the benzylic bromination was effected with *N*-bromosuccinimide in the presence of AIBN in chlorobenzene to afford compound **5**. The desired TBDMS-protected five-carbon aldehyde intermediate **7** was prepared from monohydroxyl TBDMS-protected 1,5-pentanediol **6**, by a TEMPO-mediated oxidation. The aldehyde was then treated with a Wittig salt generated in situ from compound **5** to provide intermediate **8** in a cis to trans ratio of

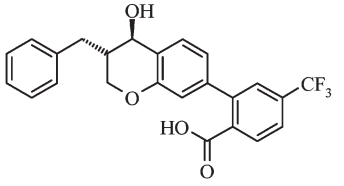


2

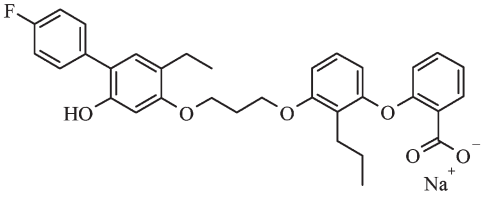
Figure 3. Chemical structure of LTB_4 receptor antagonist (**2**).



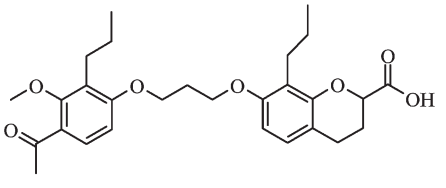
BIIL-284



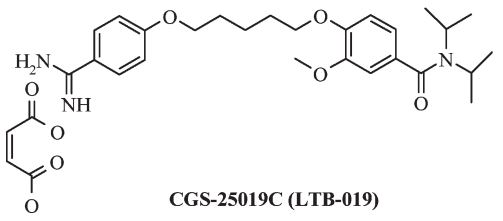
CP-195,543



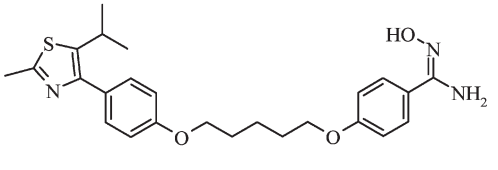
LY-293111



Biomed-101 (SC-41930)



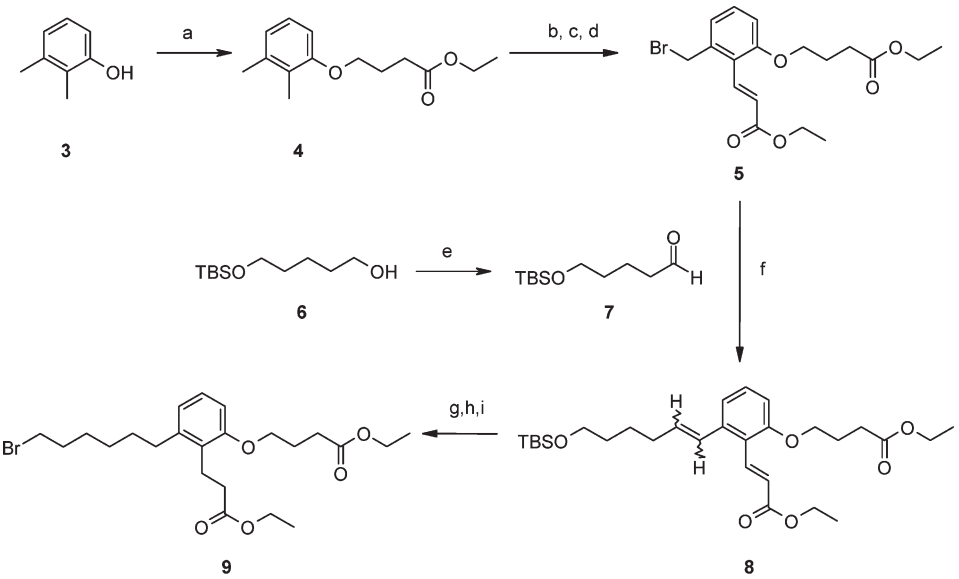
CGS-25019C (LTB-019)



DW-1350

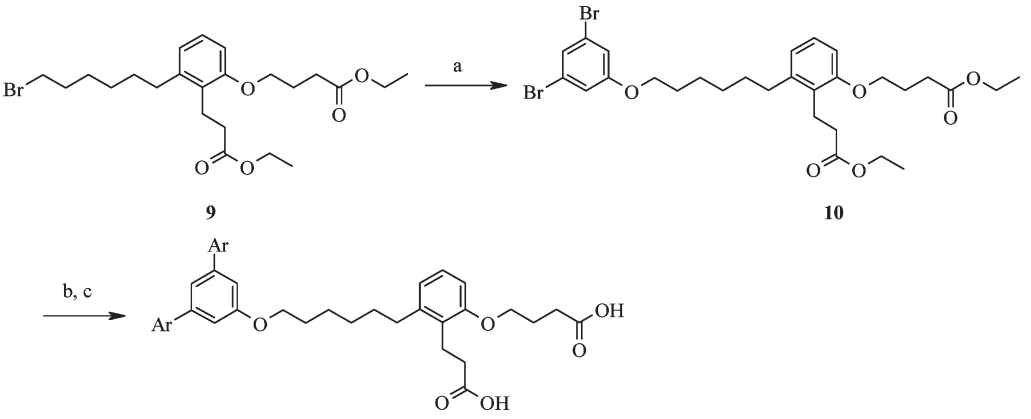
Figure 2. Chemical structures of LTB_4 receptor antagonists that have been reported to have entered clinical development.

Scheme 1. Synthesis of 4-[3-(6-Bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric Acid Ethyl Ester Intermediate[a]



[a] Reagents and conditions: (a) 4-bromobutyric acid ethyl ester, LiH, DMSO, room temp; (b) $CuSO_4 \cdot 5H_2O$, $K_2S_2O_8$, H_2O, CH_3CN, reflux; (c) $(EtO)_2P(O)CH_2CO_2Et$, NaOEt, EtOH, room temp; (d) *N*-bromosuccinimide, AIBN, chlorobenzene, 85 °C, 1 h; (e) *n*-Bu_4NHSO_4, KBr, TEMPO, NaClO, CH_2Cl_2, H_2O, 5−10 °C; (f) PPh_3, CH_3CN, reflux, 1 h, then **5**, epoxybutane, reflux; (g) Pd/C, H_2, EtOAc, room temp; (h) *n*-Bu_4NF, THF, 0 °C to room temp; (i) CBr_4, PPh_3, CH_2Cl_2, 5−10 °C.

Scheme 2. Synthesis of Symmetric 3,5-Bis-phenol Ether LTB_4 Receptor Antagonists[a]



11 Ar = phenyl
12 Ar = 4-fluoro-phenyl
13 Ar = 4-methoxy-phenyl
14 Ar = 3-thiophenyl
15 Ar = 4-pyridinyl
16 Ar = 5-pyrimidinyl
17 Ar = benzo[1,3]dioxol-5-yl
18 Ar = benzo[1,4]dioxin-6-yl

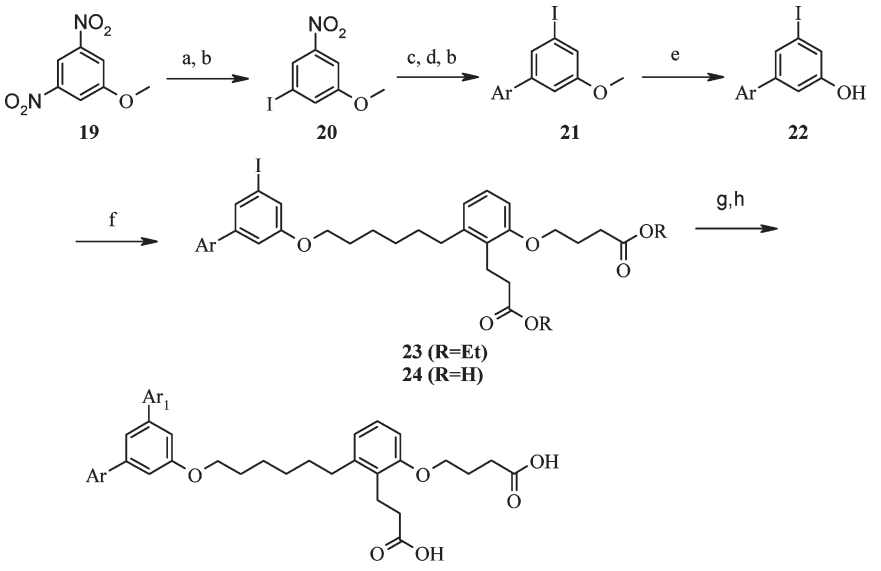
[a] Reagents and conditions: (a) 3,5-dibromophenol, K_2CO_3, DMF, acetone, reflux; (b) Ar-$B(OH)_2$, $Pd(PPh_3)_4/Na_2CO_3$, EtOH or $PdCl_2(dppf)/Cs_2CO_3$, DME, 80 °C; (c) aqueous NaOH, EtOH, room temp.

~1:3. This mixture of cis and trans compounds was converted to the corresponding alkyl bromide intermediate **9** in three steps: hydrogenation of the double bonds, removal of the protecting group, and finally, conversion of the alcohol to the corresponding bromide.

A general synthesis of symmetric 3,5-diarylphenyl ethers is shown in Scheme 2. The common 3,5-dibromo intermediate **10** was prepared from compound **9** and 3,5-dibromophenol in the presence of potassium carbonate in DMF and acetone in 1:2 ratio at reflux temperature. The 3,5-dibromo intermediate **10** was then reacted with various arylboronic acids under Suzuki coupling conditions,[18] and the resulting diesters were saponified to obtain the final symmetric 3,5-bis-phenol ether diacids (**11**−**18**).

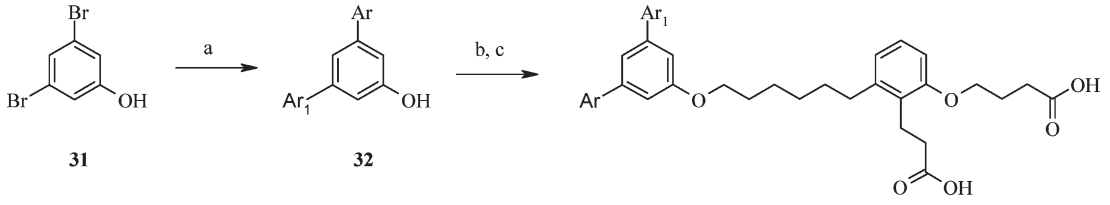
The asymmetrically substituted 3,5-diarylphenyl ether analogues were synthesized using two approaches as shown in Schemes 3 and 4. Several analogues were prepared using a lengthy process starting with 3,5-dinitroanisole (**19**) as shown in Scheme 3. This approach depends on a selective reduction of a single nitro group in 3,5-dinitroanisole (**19**) following a reported procedure using sodium sulfide and sodium bicarbonate in water and methanol to give the 3-amino-5-nitroanisole intermediate.[19] At this point, a classical Sandmeyer reaction of the amine intermediate with sodium nitrite and

Scheme 3. Synthesis of Asymmetric 3,5-Bis-phenol Ether LTB_4 Receptor Antagonists[a]



[a] Reagents and conditions: (a) $NaHCO_3$, Na_2S, H_2O, MeOH, reflux; (b) $NaNO_2$, HCl, KI, H_2O, 0−25 °C; (c) Ar-$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, EtOH, 160 °C, microwave; (d) Zn dust, NH_4Cl, MeOH, H_2O, room temp; (e) TMSCl, NaI, CH_3CN, reflux; (f) 4-[3-(6-bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester, K_2CO_3, DMF, acetone, reflux; (g) Ar_1-$B(OH)_2$, $PdCl_2$(dppf), Cs_2CO_3, DME, 80 °C; (h) aqueous NaOH, EtOH, room temp.

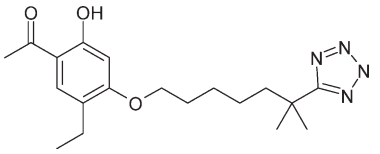
Scheme 4. Synthesis of Asymmetric 3,5-Bis-phenol Ether LTB_4 Receptor Antagonists[a]



[a] Reagents and conditions: (a) Ar-$B(OH)_2$, Ar_1-$B(OH)_2$, $PdCl_2$(dppf)/Cs_2CO_3, DME, 95 °C; (b) 4-[3-(6-bromohexyl)-2-(2-ethoxycarbonylethyl)-phenoxy]butyric acid ethyl ester, K_2CO_3, DMF, acetone, reflux; (c) aqueous NaOH, EtOH, room temp.

hydrochloric acid in the presence of potassium iodide furnished the iodoanisole **20**. The first aryl group was introduced by reaction of **20** with arylboronic acids under Suzuki coupling conditions with microwave irradiation at 160 °C. The second nitro group was reduced to the amine using zinc dust and ammonium chloride under mild conditions. Again, the amine was converted to the corresponding iodide using the same Sandmeyer reaction conditions. The crucial 3-aryl-5-iodophenol intermediate **22** was obtained by treatment of **21** with iodotrimethylsilane generated in situ from chlorotrimethylsilane and sodium iodide in acetonitrile at reflux temperature. The remaining steps were routine: alkylation of **22** with the bromo intermediate **9** to provide **23**, introduction of second aryl moiety using a Suzuki coupling with arylboronic acids, and finally hydrolysis of the diester to provide the desired asymmetrically substituted 3,5-diarylphenyl ethers (**25**−**28**). Alternatively, the iodo intermediate (**23**) was hydrolyzed first to the diacid (**24**) and then the second aryl group was introduced using arylboronic acids under microwave conditions to give compounds **29** and **30**.

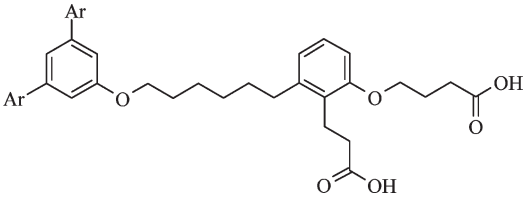
Scheme 4 illustrates a shorter synthetic approach for the preparation of asymmetric 3,5-diarylphenol ethers in one pot from 3,5-dibromophenol (**31**) and two different arylboronic acids using $PdCl_2$(dppf) and cesium carbonate in DME at

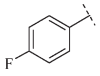
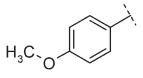
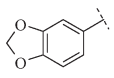
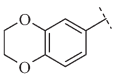


41

Figure 4. Chemical structure of LTB_4 receptor antagonist LY255283 (**41**).

Table 1. Antagonist Activity of Compounds **11**−**18** on BLT-1 Receptors in a HL-60 Cell Line



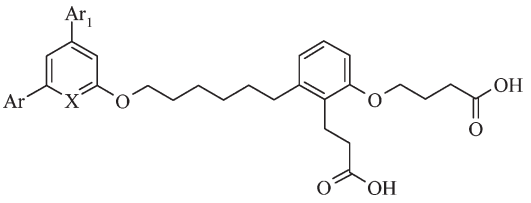
Compound	Ar	IC_{50} HL-60 (nM)
11		0.21
12		8.5
13		31.1
14		0.58
15		0.07
16		5.35
17		5.39
18		139.7

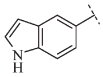
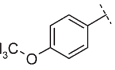
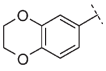
95 °C. The yield of the desired product in this reaction (26−43%) depends on three factors: (1) the relative reactivity of the two arylboronic acids, (2) control of the ratio of the more reactive boronic acid to the less reactive boronic acid (1:1.5, respectively), and (3) the feasibility of the separation of the three possible products. The resulting asymmetric phenols (**32**) were treated with the alkylbromo intermediate **9**, and hydrolysis of the diesters provided the desired diacids (**33**−**40**).

Results and Discussion

The potency of these compounds as BLT1 receptor antagonists was first measured in a functional LTB_4 stimulated calcium flux assay in human HL-60 cells, differentiated toward a neutrophilic phenotype with retinoic acid. HL-60 cells express both BLT1 and BLT2 receptors; however, the reportedly selective BLT2 antagonist LY255283[20] (**41**, Figure 4) had no antagonist activity in this assay in our hands, supporting a role for BLT1 (data not shown). Compound **41** also exhibited no antagonist activity in a neutrophil chemotaxis assay, indicating this response is also mediated by BLT1 (data not shown). The potencies of symmetrical compounds assayed in the HL-60 cell functional calcium flux assay are

Table 2. Antagonist Activity at BLT-1 Receptors Expressed in an HL-60 Cell Line and LTB_4-Evoked Chemotaxis of Human Neutrophils by Compounds **2**, **25**−**30**, and **33**−**40**



Compound	Ar	Ar_1	X	IC_{50} HL-60 (nM)	IC_{50} neutrophil chemotaxis (nM)
2			N	0.48	1.8
25			CH	0.36	0.2
26			CH	0.2	ND
27			CH	0.57	ND
28			CH	0.39	20
29			CH	0.18	0.20
30			CH	13.98	ND
33			CH	0.44	0.10
34			CH	0.58	0.70
35			CH	3.27	ND
36			CH	0.22	0.10
37			CH	0.38	0.20
38			CH	1.19	3.68
39			CH	0.61	7.67
40			CH	60.2	ND

shown in Table 1. It is interesting to note that substitution at the para-position of both aryl rings caused a decrease in inhibitor potency of these molecules as exemplified by the results of compounds **11**, **12**, and **13**. For example, a para substituent as small as fluorine in **12** caused a 40-fold loss in potency relative to the bis-phenyl analogue **11**. The slightly larger methoxy substitution in **13** resulted in an even larger reduction in potency by 150-fold compared to the unsubstituted bis-aryl compound **11**. The bis-4-pyridyl derivative (**15**) was particularly potent, and other heteroaromatic moieties such as the thiophenyl (**14**) and the 3,5-pyrimidinyl (**16**) were well tolerated. Finally, the 26-fold drop in potency of the bis-5-benzo[1,4]dioxolyl (**18**) compared to bis-5-benzo-[1,3]dioxolyl (**17**) indicates that this area is surprisingly sensitive to subtle differences in the size of the two aryl moieties.

In Table 2, the functional calcium flux assay results as well as those for a neutrophil chemotaxis assay are shown for selected unsymmetrically substituted compounds. The potencies of the compounds shown in Table 2 in the two assays correlate within a factor of 2−3 with exception the analogs **28** and **39**; the reasons for this lack of correlation remain unclear. Importantly, the pairing of 5-benzo[1,3]dioxolyl with thiophenyl, phenyl, 4-methoxyphenyl, pyridyl, pyrimidyl, 3-fluorophenyl, or 2-fluorophenyl produced potent compounds **33**−**39**. It is interesting to compare the results of compounds **13** and **35** in which one of the 4-methoxyphenyl moieties was replaced with a benzo[1,3]dioxol-5-y-phenyl; this resulted in an increase in the potency as measured by the calcium flux assay (31 nM vs 3.3 nM). It seems that the nature of the bicyclic ring is also crucial for maintaining good in vitro potency. Comparison of the results of **38** and **30** in which replacement of the 5-benzo[1,3]dioxolyl moiety with an indol-5-yl moiety led to a 12-fold decrease in potency provides further evidence for a unique effect of the five-member ring of the 6−5 system rather than the ring size alone. The results shown in the Tables 1 and 2 are consistent with a binding model in which the one of the aryl moieties preferably has a smaller substitution at either the ortho- or meta-position when the other aryl moiety has a small fused ring at the 3,4-position.

In order to characterize these compounds in greater detail, a small set of analogues was evaluated in a calcium mobilization assay using cloned human BLT1 and BLT2 receptors stably expressed in HEK293 cells to determine their relative selectivity for these receptors (Table 3). The potency of these compounds was consistently less in this assay than their potency in the HL-60 and chemotaxis assays. The high and low affinity of LTB_4 at cloned human BLT1 and BLT2 receptors, respectively, as measured by the EC_{50} in the present studies, is consistent with the well established pharmacology of these receptors. Differences between the types and nature of G-protein-receptor coupling or cell-specific translational modification of the receptor protein may underlie the variability in the effects of LTB_4 antagonists among these cellular assays. The inhibitory effect of LTB_4 antagonists on chemotaxis of primary human neutrophils may represent the most relevant measure of compound efficacy to the in vivo setting. As shown in Table 3, **2** and **11** have ≥15-fold selectivity for the BLT1 receptor while compounds **33**, **38**, and **15** represent potent dual BLT1 and BLT2 antagonists. Subsequent chemistry efforts also identified additional chemical series exhibiting selective BLT2 antagonist activities; these compounds and their results will be described in a future manuscript. The potential clinical significance in human diseases of antagonist activity at either or both of the BLT1 and BLT2 receptors is not well understood at this time. However, the well-established role of the BLT1 receptor in inflammatory processes and the emerging evidence on the importance of BLT2 receptors in diverse inflammatory cell types suggest compounds with selective or dual antagonist activity at these receptors may be therapeutically useful in different disease states with dependence on different cell types. This can only accurately be assessed in the clinical setting in which differential effects of dual or selective antagonists may be observed depending on the role of a particular cell type in a specific human disease. Indeed, currently available animal models do not reproduce the complexity of heterogeneous human diseases and are unlikely to be able to address the importance of dual versus selective BLT1 and BLT2 receptor antagonism. Compounds with differing selectivities represent novel tools with which to further investigate the pharmacology and roles of the BLT1 and BLT2 receptors.

Table 3. Selectivity of Receptor Antagonists at Human BLT-1 and BLT-2[21]

		IC_{50}, nM				
receptor	LTB_4 EC_{50}, nM (**1**)	**2**	**11**	**15**	**33**	**38**
BLT1	1.1	205.0	38.5	71.0	114.0	129.0
BLT2	25.1	3060.0	628.0	143.0	164.0	194.0

In Vitro Safety Characterization. As summarized in Table 4, representative potent molecules in this series (**2**, **11**, **15**, **33**, **38**, and **39**) were further characterized with in vitro ADME and safety assays in order to select compounds for in vivo evaluation. In general, despite high clogP,[22] the log *D* [23] measurements were in an acceptable range. As one would expect for a compounds having two carboxylic acid moieties, these compounds had good solubility in a LYSA assay[24] as well as simulated intestinal fluid[25] at either pH 5 (fed) or 6.5 (fasted) and a negligible level of hERG channel inhibition at 3 μM.[26] Metabolic clearance as estimated by incubation with rat hepatocytes indicated the potential for good to medium metabolic stability.

These compounds were also characterized initially in a cytochrome p450 (cyp) high-throughput panel screen (1A2, 2D6, 2C9, 2C19, and 3A4) using fluorogenic substrates based on published methods.[27] All compounds had

Table 4. Selected ADME and Safety Data for a Selection of BLT-1 and BLT-2 Antagonists

	2	**11**	**15**	**33**	**38**	**39**
clogP	9.12	9.51	6.65	8.75	9.25	9.25
log *D*, pH 7.4	2.51	0.7	1.78	2.34	ND	2.44
FEDSIF, pH 5.0 (μg/mL)	27.7	338	61	32.9	ND	38.1
FASSIF, pH 6.5 (μg/mL)	16.5	27.3	22.7	34.6	ND	27.1
hepatocytes clearance (rat), ((mL/min)/kg)	ND	16.1	14.9	16.2	ND	21.2
hERG binding IC_{50} (μM)	>3	>3	ND	>3	>3	ND
Cyp2C9 (μM) fluorescence method	>12.5	>50	0.34	3.9	>25	7.6
Cyp2C9 (μM) LC/MS method	ND	>12.5	0.90	22.5	ND	ND

Table 5. Rat SDPK ($n = 3$)

	2	15	33	38
dose (po, mg/kg)	10	10	10	10
$T_{1/2}$ (h)	2.9	4.52	2.7	3.1
C_{max} (μM)	2.11	0.31	6.21	2.25
T_{max} (h)	2.3	5	3	5
F (%)	28	10	70	88
dose (iv, mg/kg)	2.5	5	5	5
$T_{1/2}$ (h)	2.1	4.52	2.1	2.4
Vdss (L/kg)	0.3	1.55	0.22	0.98
CL ((mL/min)/kg)	7.53	28.14	5.47	11.6

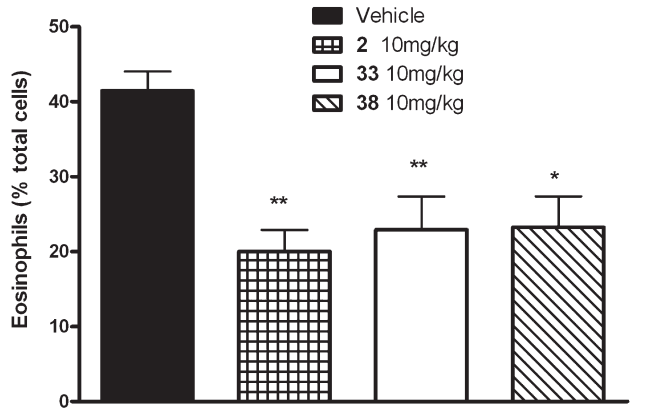


Figure 5. Effect of compounds **2**, **33**, and **38** and on LTB_4 -evoked pulmonary inflammation in guinea pigs when dosed orally (10 mg/kg). Data are the mean ± SEM of 4−20 animals per group: (*) $P < 0.05$ and (**) $P < 0.01$ for compound-treated animals compared to vehicle-treated animals.

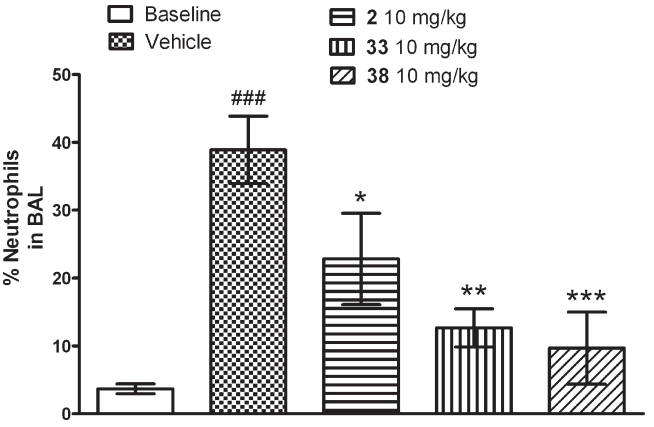


Figure 6. Effect of compounds **2**, **33**, and **38** on percentages of neutrophils in BAL fluid in allergen-challenged nonhuman primates. Baseline BAL data were obtained 24 h prior to allergen challenge. Data are the mean ± SEM of 3−21 animals per group: (###) $P < 0.001$ for vehicle compared to baseline; (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ for analogue-treated animals compared to vehicle-treated animals.

$IC_{50} > 7.5\ \mu M$ against these isoforms with the exception of **15** and **33** at 2C9. These two componds were further characterized in a more rigorous follow-up assay in involving a LC/MS detection metabolite detection protocol, again based on published methods.[28] A strong inhibition of cyp2C9 activity was noted for compound **15** (Table 4) presumably through the interaction of the 4-pyridyl moieties with cyp2C9. In contrast, compound **33** was only a weak cyp2C9 inhibitor using this protocol.

Table 6. Effects of 14-Day Treatment of Compounds **33** and **38** on Clinical Chemistries in Male Rats[a]

day of sampling	vehicle	**33** (40 (mg/kg)/day)	**33** (400 (mg/kg)/day)	**38** (40 (mg/kg)/day)	**38** (400 (mg/kg)/day)
		Clinical Chemistry Parameter: ALT (IU/L)			
5	45 ± 3.6	40 ± 3.4	58 ± 8.9*	40 ± 6.3	69 ± 13.2***
15	45 ± 7.1	38 ± 1.7	125 ± 35.3*	35 ± 1.8	121 ± 58.7*

[a] All data are the mean ± SD of four animals per group: (*) $P < 0.05$ and (***) $P < 0.001$ for compound-treated compared to vehicle-treated animals.

Single Dose Pharmacokinetics in Rats. Several molecules were characterized for oral bioavailability by means of rat single dose pharmacokinetic studies (SDPK) (Table 5). Compared to compound **2**, **33** and **38** showed superior oral bioavailability despite their high molecular weight (>600 Da), high clogP (~9), and the presence of two carboxylic acid groups. The exposure of bis-pyridyl **15** was inferior to that of compound **2** which, coupled with its strong inhibition of cyp2C9, disqualified this molecule from further experimental examination. These results with compound **15** limited any interest in other compounds that also contained the pyridinyl moiety. The volumes of distribution for these molecules are generally low, as are their rates of hepatic clearance.

Mechanistic in Vivo Model of BLT-1/-2 Inhibition. After elimination of compound **15**, the attractive balance of BLT receptor antagonist properieties and good rat PK measured for compounds **33** and **38** prompted us to select them for in vivo efficacy profiling. When guinea pigs are challenged with an aerosol of LTB_4, there is a significant influx of eosinophils into the airway when compared to animals not treated with LTB_4. Oral pretreatment with **2**, **33**, or **38** (10 mg/kg, formulated in 2% Klucel, 1% Tween) significantly attenuated LTB_4-evoked eosinophilic pulmonary inflammation as shown in Figure 5. The effects of these compounds on attenuation of eosinophil levels were not significantly different from each other.

Allergen-Evoked Pulmonary Inflammation in Atopic Nonhuman Primates. Having shown potent activity of compounds **33** and **38** in a mechanistic in vivo model as well as acceptable oral exposure in rat PK models, both compounds were examined for their efficacy in a disease model in nonhuman primates. Allergen challenge with *Ascaris suum* antigen in hypersensitive primates evoked significant increases in percentages of neutrophils in bronchoalveolar lavage (BAL) fluid compared to baseline cell counts (Figure 6). Pretreatment with **2**, **33**, or **38** (10 mg/kg po, 1 h before allergen challenge) inhibited allergen-evoked pulmonary inflammation, resulting in attenuation of allergen-evoked increases in the percentages of neutrophils, with equal efficacy (Figure 6). In similarity to the guinea pig, compounds **33** and **38** could not be differentiated from each other in this nonhuman primate efficacy model.

Differentiation by in Vivo Toxicity Studies. Given the excellent in vivo effects as well as acceptable in vitro properties of both compounds **33** and **38**, a non-GLP toxicity study in rats was conducted as a means of differentiation. To this end, male rats were treated with vehicle, 40 or 400 (mg/kg)/day of compounds **33** and **38** (4 rats per group) for 14 days. All rats survived until the end of the study, and there were no abnormal in-life observations noted for either dose of either compound. There were no effects at either dose on body weight, food consumption, or hematological parameters (data not shown). Treatment-related findings for

Table 7. Metabolite Profiles of Compound **33** in Mouse, Rat, Dog, Monkey, and Human Cryopreserved Hepatocytes[a]

hepatocytes incubation (3 h)	P1 $[M-H]^-$ = 805.3, %	P2 $[M-H]^-$ = 805.3, T	$[M-H]^-$ = 629.3, %	total % metabolism	recovery, %
gender pooled human	15.4	1.9	82.7	17.3	56.7
male CD1 mouse	16.5	0.4	83.1	16.9	40.9
male SD rat	10.7	9.7	79.6	20.4	49.7
male beagle dog	25.0	2.8	72.2	27.8	61.3
male cyno monkey	5.2	2.5	92.3	7.7	50.9
cell-free	ND	ND	100	ND	100.0

[a] Values in the table represent percent of each metabolite and parent remaining after 3 h of incubation based on relative UV absorbance peak area at 232−242 nm, assuming all peaks have same UV extinction coefficients. ND = not detectable.

both compounds consisted of a significant increase in alanine aminotransferase (ALT) and total bilirubin at the higher dose. As shown in Table 6, the increase in ALT was evident after 5 days of treatment and was more pronounced for compound **38** than for compound **33** (53% versus 29%, respectively). After 14 days, the increase in ALT was almost 3-fold for both compounds. Neither compound had significant effects on aspartate aminotransferase (AST) or alkaline phosphatase levels at either dose tested (data not shown). Furthermore, histological evaluation of the livers revealed mild to moderate hepatocyte vacuolation in the group that was treated with 400 (mg/kg)/day of compound **38**, a finding that was not observed in the livers of rats treated with compound **33**. After 14 days of dosing, the 40 (mg/kg)/day dose level of compound **33** corresponded to AUC_{0-24h} and C_{max} values of 90 400 ng·h/mL and 11 300 ng/mL, respectively. Greater than dose-proportional increase in exposure was observed and the 400 (mg/kg)/day dose level of compound **33** corresponded to AUC_{0-24h} and C_{max} values of 2 922 300 ng·h/mL and 262 000 ng/mL, respectively. After 14 days of dosing, the 40 (mg/kg)/day dose level of compound **38** corresponded to AUC_{0-24h} and C_{max} values of 80 571 ng·h/mL and 11 300 ng/mL, respectively. Greater than dose-proportional increase in exposure was observed and the 400 (mg/kg)/day dose level of compound **38** corresponded to AUC_{0-24h} and C_{max} values of 1 871 900 ng·h/mL and 124 000 ng/mL, respectively. The safety margins were calculated to be 45- and 40-fold for compounds **33** and **38**, respectively, in the 40 (mg/kg)/day group and 1461- and 936-fold for compounds **33** and **38**, respectively, in the 400 (mg/kg)/day group based on an efficacious AUC of approximately 2000 ng·h/mL in allergen-challenged nonhuman primates. The high safety margins indicated by these results, in conjunction with the potent in vitro and in vivo data set, prompted the selection of compound **33** for further clinical development. A more detailed discussion of the biological results of **33** has been published.[29]

Further clinical development studies included the identification of the metabolites of **33**. Contrary to expectations based on literature precedent,[30,31] when compound **33** was incubated with human, rat, monkey, mouse, and dog hepatocytes, oxidative cleavage of the 5-benzo[1,3]dioxolyl moiety was not observed to any measurable extent. Rather, the formation of two glucuronide conjugates was observed (P1 and P2) presumably corresponding to different conjugates of the two carboxyl groups (Table 7). These in vitro observations were consistent with in vivo metabolic profiles of compound **33** when dosed at 400 mg/kg in rats. Examination of rat plasma samples indicated that **33** was the major component; the formation of acylglucuronide isomers was observed to levels between 2% and 6% of that for compound **33**. The product of oxidation of the 5-benzo[1,3]dioxolyl ring amounted to less than 1% of the total.

Conclusions

A new series of LTB_4 antagonists was successfully identified and optimized, resulting in compounds with improved potency and bioavailabiliy relative to the starting point (**2**). Compounds having different selectivity profiles between BLT1 and BLT2 receptors were identified. These molecules may be useful as tools to better understand their individual roles in mediating the effects of LTB_4. In vitro ADMET assays as well as rat PK studies guided the selection of the best compounds among many attractive choices for in vivo profiling in a guinea pig mechanistic model. With this assay, it was possible to identify two particularly potent and bioavailable LTB_4 receptor antagonists which were advanced into nonhuman primate efficacy models. To differentiate the better of two good compounds (**33** and **38**), a non-GLP 14-day rat toxicity experiment was conducted. In this way, a rationale was established to select compound **33** for further clinical development as a dual BLT1 and BLT2 receptor antagonist for the potential treatment of inflammatory diseases.

Experimental Section

All reactions were carried out under a nitrogen or argon atmosphere unless otherwise noted. Tetrahydrofuran was distilled over sodium and benzophenone. All other solvents and reagents were purchased from Aldrich and used without further purification unless otherwise noted. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Mercury 300 and Unityplus 400 MHz spectrometers, using residual chloroform as internal standard set to 7.26 ppm; for spectrum obtained for compounds dissolved in DMSO-d_6, residual proton DMSO-d_5 signal was set as 2.54 ppm. Electron impact (EI, 0 eV) and fast atom bombardment (FAB) mass spectra were taken on VG Autospec or VG 70E-HF mass spectrometers, respectively. Biotage silica gel columns or ISCO silica gel columns were used for flash chromatography; columns were run under a 0−5 psi head of nitrogen to assist flow. Thin layer chromatograms were run on glass thin-layer plates coated with silica gel as supplied by E. Merck (E. Merck no. 1.05719) and were visualized by viewing under 254 nm UV light in a view box, by exposure to I_2 vapor, or by spraying with phosphomolybdic acid (PMA) in aqueous ethanol.

LC/MS (liquid chromatography/mass spectroscopy) chromatograms were collected using a Waters ZQ mass detector/LC system. Detectors include the Micromass ZQ spectrometer generally in ES ionization, positive ion mode (mass range, 150 − 1200 amu), a diode array detector, and a PL-ELS 2100 evaporative light scattering detector. HPLC separations are achieved using a reverse phase cartridge column (ES Industries Chromegabond WR C-18 3 μm, 120 Å, 3.2 mm × 30 mm) with a gradient solvent method (mobile phase A, water (0.02% TFA); phase B, acetonitrile (0.02% TFA); gradient 10% B to 90% B). The run times were generally 3 min with a 1 min equilibration time. The 5 μL solution samples were injected with a pump flow rate of 2 mL/min. Other gradient conditions were utilized for samples with extremely short or long retention times. The purity of all

compounds was judged on the percentage of the integrated signal at UV 214 nm. All final compounds submitted for bioassay were at least 95% pure as judged by this method, unless indicated otherwise.

Preparation of 4-[3-(6-Bromohexyl)-2-(2-ethoxycarbonylethyl)-phenoxy]butyric Acid Ethyl Ester (9). a. Preparation of 4-(2,3-Dimethylphenoxy)butyric Acid Ethyl Ester (4).[32] To a solution of 2,3-dimethylphenol (25 g, 204 mmol) in DMSO (205 mL) was added 4-bromobutyric acid ethyl ester (40.96 g, 210 mmol) and lithium hydride (2.0 g, 250 mmol) at room temperature. The resulting light-brown solution was stirred for 2 days. Then the reaction mixture was cooled to 0 °C and water (200 mL) was added slowly. The organic compound was extracted with hexanes (2 × 200 mL). The combined organic extracts were washed with brine solution (150 mL), and the organic solution was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave a light-brown oil. The crude mixture was purified using a Biotage (40L) column, eluting with 5% ethyl acetate in hexanes to isolate 4-(2,3-dimethylphenoxy)butyric acid ethyl ester (45.32 g, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99−7.11 (m, 1H), 6.77 (d, J = 7.55 Hz, 1H), 6.68 (d, J = 8.45 Hz, 1H), 4.15 (q, J = 7.06 Hz, 2H), 3.98 (t, J = 5.89 Hz, 2H), 2.54 (t, J = 7.40 Hz, 1H), 2.27 (s, 3H), 2.14 (s, 3H), 2.08−2.18 (m, 2H), 1.26 (t, J = 7.06 Hz, 3H). ES(+)-HRMS m/e calculated for $C_{14}H_{20}O_3$ (M+)$^+$ 236.1412, found 236.1419.

b. Preparation of 4-(2-Formyl-3-methylphenoxy)butyric Acid Ethyl Ester. A mixture of copper(II) sulfate pentahydrate (21.98 g, 88.06 mmol) and potassium persulfate (71.42 g, 264 mmol) in water (396 mL) was heated to 63−65 °C to give a blue solution. Then a solution of 4-(2,3-dimethylphenoxy)-butyric acid ethyl ester (20.81 g, 88.06 mmol) in acetonitrile (220 mL) was added at the above temperature. The resulting light-green solution was refluxed for 40 min. Then the reaction mixture was cooled to ∼5 °C in order to precipitate most of the inorganic solids. The resulting solids were collected by filtration, and the solid cake was washed with dichloromethane (1.0 L). The two layers of filtrate were separated, and the aqueous layer was extracted with dichloromethane (200 mL). The combined organic extracts were washed with brine solution (150 mL), and the organic solution was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave a brown oil. The crude mixture was purified using a Biotage (40L) column eluting with 5−10% ethyl acetate in hexanes to give 4-(2-formyl-3-methylphenoxy)butyric acid ethyl ester (20.70 g, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.67 (s, 1H), 7.30−7.41 (m, 1H), 6.83 (d, J = 8.45 Hz, 1H), 6.81 (d, J = 7.55 Hz, 1H), 4.15 (q, J = 7.00 Hz, 2H), 4.11 (t, J = 6.30 Hz, 2H), 2.58 (s, 3H), 2.54 (t, J = 7.24 Hz, 2H), 2.08−2.26 (m, 2H), 1.27 (t, J = 7.00 Hz, 3H). EI(+)-HRMS m/e calculated for $C_{14}H_{18}O_4$ (M+)$^+$ 250.1205, found 250.1202.

c. Preparation of 4-[2-((*E*)-2-Ethoxycarbonylvinyl)-3-methyl-phenoxy]butyric Acid Ethyl Ester. Sodium metal spheres (1.6 g, 69.6 mmol) were added to ethanol (100 mL) with stirring at room temperature under a nitrogen atmosphere over 15 min. An exothermic reaction occurred, and the mixture was stirred for another 15 min. After the mixture was cooled to room temperature, triethyl phosphonoacetate (14.7 mL, 73.4 mmol) and 4-(2-formyl-3-methylphenoxy)butyric acid ethyl ester (13.25 g, 52.9 mmol) were added sequentially. During the addition of 4-(2-formyl-3-methylphenoxy)butyric acid ethyl ester, the solution turned brown and the temperature increased to ∼55 °C. The resulting brown solution was stirred for 2 days at room temperature. The reaction mixture was then diluted with water (150 mL), stirred for 1 h, and extracted with hexanes (3 × 100 mL). The combined organic extracts were washed with brine solution (150 mL), and the organic solution was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave a light-yellow oil. The crude oil was taken up in hexanes and ethyl acetate (3:1 ratio), treated with charcoal, and heated gently with a heat gun. After the mixture was cooled to room temperature, the charcoal was filtered off and the filtrate was evaporated under vacuum to give 4-[2-((*E*)-2-ethoxycarbonylvinyl)-3-methylphenoxy]-butyric acid ethyl ester (13.25 g, 78%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 16.00 Hz, 1H), 7.18 (t, J = 8.15 Hz, 1H), 6.83 (d, J = 8.15 Hz, 1H), 6.78 (d, J = 8.15 Hz, 1H), 6.62 (d, J = 16.00 Hz, 1H), 4.27 (q, J = 7.00 Hz, 2H), 4.15 (q, J = 7.00 Hz, 2H), 4.08 (t, J = 6.19 Hz, 2H), 2.54 (t, J = 6.90 Hz, 2H), 2.44 (s, 3H), 2.09−2.24 (m, 2H), 1.35 (t, J = 7.00 Hz, 3H), 1.26 (t, J = 7.00 Hz, 3H). EI(+)-HRMS m/e calculated for $C_{18}H_{24}O_5$ (M+)$^+$ 320.1624, found 320.1626.

d. Preparation of 4-[3-Bromomethyl-2-((*E*)-2-ethoxycarbonyl-vinyl)phenoxy]butyric Acid Ethyl Ester (5). To a solution of 4-[2-((*E*)-2-ethoxycarbonylvinyl)-3-methylphenoxy]butyric acid ethyl ester (8.0 g, 25.0 mmol) in chlorobenzene (190 mL) were added *N*-bromosuccinimide (6.67 g, 37.5 mmol) and 2,2′-azobisisobutyronitrile (AIBN) (591 mg, 3.6 mmol) at room temperature. The resulting solution was heated to 85 °C and stirred for 1 h. Then the reaction mixture was cooled to room temperature and diluted with water (100 mL) and the mixture was extracted with hexanes (3 × 100 mL). The combined organic extracts were washed with brine solution (150 mL) and dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave a crude oil which was purified using a Biotage (40L) column, eluting with 15−25% ethyl acetate in hexanes to give 4-[3-bromomethyl-2-((*E*)-2-ethoxycarbonylvinyl)phenoxy]-butyric acid ethyl ester (7.11 g, 71%) as a low melting solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, J = 16.15 Hz, 1H), 7.27 (t, J = 8.15 Hz, 1H), 7.03 (dd, J = 0.91, 8.15 Hz, 1H), 6.90 (d, J = 8.15 Hz, 1H), 6.73 (d, J = 16.15 Hz, 1H), 4.59 (s, 2H), 4.30 (q, J = 7.05 Hz, 2H), 4.15 (q, J = 7.05 Hz, 2H), 4.09 (t, J = 6.00 Hz, 2H), 2.54 (t, J = 7.20 Hz, 2H), 2.11−2.24 (m, 2H), 1.36 (t, J = 7.05 Hz, 3H), 1.27 (t, J = 7.05 Hz, 3H). ES(+)-HRMS m/e calculated for $C_{18}H_{23}BrO_5$ (M + Na)$^+$ 421.0621, found 421.0621.

e. Preparation of 5-(*tert*-Butyldimethylsilanyloxy)pentanal (7). To a solution of 5-(*tert*-butyldimethylsilanyloxy)pentanol (3.66 g, 15.1 mmol) in dichloromethane (30 mL) were added water (5.6 mL), potassium bromide (202 mg, 1.7 mmol), *n*-tetrabutylammonium hydrogen sulfate (290 mg, 0.84 mmol), and TEMPO (30 mg) at room temperature. The resulting light-brown solution was cooled to ∼5 °C, and a solution of sodium hypochlorite (30 mL, 19.3 mmol, 5%) was added dropwise at this temperature. After the addition of half of the sodium hypochlorite solution, solid potassium carbonate (300 mg) was added to keep the reaction mixture basic. The remaining sodium hypochlorite solution was then added at 5−10 °C. By this point, a precipitate had formed and the reaction mixture was stirred for another 1 h at ∼10−15 °C. Then water (100 mL) was added and the resulting solution was extracted with diethyl ether (2 × 100 mL). The combined organic extracts were washed with brine solution (150 mL), and the organic layer was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave 5-(*tert*-butyldimethyl-silanyloxy)pentanal (3.32 g, 99%) as a light-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.78 (s, 1H), 3.63 (t, J = 6.04 Hz, 2H), 2.47 (td, J = 1.81, 7.24 Hz, 2H), 1.64−1.78 (m, 2H), 1.48−1.62 (m, 2H), 0.90 (s, 9H), 0.05 (s, 6H). ES(+)-HRMS m/e calculated for $C_{11}H_{24}O_2Si$ (M + H)$^+$ 217.1619, found 217.1619.

f. Preparation of 4-[3-[6-(*tert*-Butyldimethylsilanyloxy)hex-1-enyl]-2-((*E*)-2-ethoxycarbonylvinyl)phenoxy]butyric Acid Ethyl Ester (8). A solution of 4-[3-bromomethyl-2-((*E*)-2-ethoxycarbonylvinyl)phenoxy]butyric acid ethyl ester (798 mg, 2.0 mmol) and triphenylphosphine (577 mg, 2.2 mmol) in acetonitrile (12 mL) was heated to reflux for 1 h under a nitrogen atmosphere. Then it was cooled to room temperature and a solution of 5-(*tert*-butyldimethylsilanyloxy)pentanal (606 mg, 2.8 mmol) in 1,2-epoxybutane (22 mL) was added at room temperature and the mixture was again heated to reflux for 15 h. During this period, the mixture first turned to a brick-red color, and at the end of the reaction it had become a pale-yellow solution.

Then the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The residue was dissolved in a solution of ethyl acetate and hexanes (1:3, 150 mL), and the resulting cloudy solution was washed with a mixture of methanol and water (2:1, 225 mL). The aqueous layer was extracted one more time with ethyl acetate and hexanes (1:3, 50 mL). The combined organic extracts were washed with brine solution (150 mL), and the organic solution was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave light-brown oil. The crude mixture was purified using a Biotage (40L) column, eluting with 5% and 15% ethyl acetate in hexanes to give the desired 4-[3-[6-(*tert*-butyldimethylsilanyloxy)hex-1-enyl]-2-((*E*)-2-ethoxycarbonylvinyl)phenoxy]butyric acid ethyl ester (760 mg, 74%) as a trans/cis (~2:1) mixture at six-carbon chain, and it was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84−8.02 (m, 1H), 7.16−7.23 (m, 1H), 6.74−7.09 (m, 2H), 6.63 (d, J = 16.00 Hz, 1H), 6.46 (d, J = 16.00 Hz, 1H), 5.68−6.15 (m, 1H), 4.01−4.34 (m, 8H), 3.49−3.70 (m, 2H), 2.45−2.64 (m, 2H), 2.01−2.34 (m, 4H), 1.19−1.40 (m, 8H), 0.82−0.94 (m, 9H), 0.04−0.12 (m, 6H). Note: Several peaks were broad and were assigned as multiplets; thus, the ratio was assigned approximately on the basis of olefinic proton multiplet integration. ES(+)-HRMS m/e calculated for $C_{29}H_{46}O_6Si$ $(M + Na)^+$ 541.2956, found 541.2953.

g. Preparation of 4-[3-[6-(*tert*-Butyldimethylsilanyloxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy]butyric Acid Ethyl Ester. To a solution of 4-[3-[6-(*tert*-butyldimethylsilanyloxy)hex-1-enyl]-2-((*E*)-2-ethoxycarbonylvinyl)phenoxy]butyric acid ethyl ester (507 mg, 0.977 mmol) in ethyl acetate (10 mL) was added 10% palladium on carbon (350 mg) at room temperature. The resulting black mixture was stirred in the presence of atmospheric hydrogen gas in a balloon for 36 h at room temperature. Then the catalyst was removed by filtration using a filter paper and the residue was washed with hot ethyl acetate (~60 mL). The filtrate was concentrated in vacuo and the resulting residue was dried under high vacuum to give 4-[3-[6-(*tert*-butyldimethylsilanyloxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester (438 mg, 86%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99−7.16 (m, 1H), 6.77 (dd, J = 0.91, 7.85 Hz, 1H), 6.69 (dd, J = 0.91, 8.15 Hz, 1H), 4.08−4.21 (m, 4H), 4.00 (t, J = 6.19 Hz, 2H), 3.60 (t, J = 6.64 Hz, 2H), 2.85−3.02 (m, 2H), 2.43−2.66 (m, 6H), 2.14 (quin, J = 6.72 Hz, 2H), 1.31−1.62 (m, 8H), 1.27 (t, J = 7.24 Hz, 3H), 1.26 (t, J = 7.24 Hz, 3H), 0.90 (s, 9H), 0.05 (s, 6H). ES(+)-HRMS m/e calculated for $C_{29}H_{50}O_6Si$ $(M + Na)^+$ 545.3269, found 545.3267.

h. Preparation of 4-[2-(2-Ethoxycarbonylethyl)-3-(6-hydroxyhexyl)phenoxy]butyric Acid Ethyl Ester. To a solution of 4-[3-[6-(*tert*-butyldimethylsilanyloxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester (438 mg, 0.837 mmol) in THF (12 mL) was added a solution of tetra-*n*-butylammonium fluoride (1.25 mL, 1.25 mmol, 1.0 M in THF) at 0 °C. Then the resulting colorless solution was allowed to warm to room temperature in 2 h and the mixture was stirred for another 2 h at room temperature before being diluted with water (~50 mL). The mixture was extracted with ethyl acetate (2 × 50 mL), and the combined extracts were washed with brine solution (100 mL). The organic solution was dried over anhydrous magnesium sulfate, and the solvent was removed under vacuum after filtration of the drying agent. The crude residue was dried further under high vacuum, and the desired 4-[2-(2-ethoxycarbonylethyl)-3-(6-hydroxyhexyl)phenoxy]butyric acid ethyl ester (342 mg, 99%) was isolated as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (t, J = 8.00 Hz, 1H), 6.75 (d, J = 8.00 Hz, 1H), 6.67 (d, J = 8.00 Hz, 1H), 4.07−4.20 (m, 4H), 3.98 (t, J = 6.04 Hz, 2H), 3.63 (t, J = 6.49 Hz, 2H), 2.88−3.01 (m, 2H), 2.41−2.65 (m, 6H), 2.01−2.17 (m, 2H), 1.35−1.63 (m, 9H), 1.16−1.30 (m, 6H). ES(+)-HRMS m/e calculated for $C_{23}H_{36}O_6$ $(M + Na)^+$ 431.2404, found 431.2404.

i. Preparation of 4-[3-(6-Bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric Acid Ethyl Ester (9). To a solution of 4-[2-(2-ethoxycarbonylethyl)-3-(6-hydroxyhexyl)phenoxy]-butyric acid ethyl ester (349 mg, 0.85 mmol) and carbon tetrabromide (423 mg, 1.26 mmol) in dichloromethane (10 mL) was added triphenylphosphine (281 mg, 1.07 mmol) at ~0 °C. The resulting colorless solution was stirred for 3 h at 5−10 °C. Then the solvent was removed under vacuum and a mixture of ethyl acetate and hexanes (1:3, 50 mL) was added. As a result, a cloudy solution containing some precipitate was formed, and the cloudy solution was transferred into a separatory funnel and was washed with a mixture of methanol and water (2:1, 150 mL). The aqueous layer was extracted one more time with ethyl acetate and hexanes (1:3, 50 mL). The combined organic extracts were washed with brine solution (100 mL), and the organic solution was dried over anhydrous magnesium sulfate. Filtration of the drying agent and removal of the solvent gave a colorless oil which was purified using a Biotage (40M) column, eluting with 10% ethyl acetate in hexanes to give the desired 4-[3-(6-bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester (350 mg, 87.5%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02−7.17 (m, 1H), 6.77 (d, J = 7.55 Hz, 1H), 6.69 (d, J = 8.15 Hz, 1H), 4.16 (q, J = 7.20 Hz, 2H), 4.15 (q, J = 7.20 Hz, 2H), 4.00 (t, J = 6.04 Hz, 2H), 3.42 (t, J = 6.79 Hz, 2H), 2.76−3.10 (m, 2H), 2.43−2.68 (m, 6H), 2.14 (quin, J = 6.70 Hz, 2H), 1.82−1.96 (m, 2H), 1.35−1.64 (m, 6H), 1.27 (t, J = 7.20 Hz, 3H), 1.26 (t, J = 7.20 Hz, 3H). ES(+)-HRMS m/e calculated for $C_{23}H_{35}BrO_5$ $(M + Na)^+$ 493.1560, found 493.1560.

4-{2-(2-Carboxyethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)-hexyl]phenoxy}butyric Acid (11). General Procedure A: Preparation of 4-{3-[6-(3,5-Dibromophenoxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric Acid Ethyl Ester (10). To a mixture of 4-[3-(6-bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester (14.54 g, 30.84 mmol), 3,5-dibromophenol (8.55 g, 33.92 mmol), and potassium carbonate (8.53 g, 61.68 mmol) were added *N*,*N*-dimethylformamide (210 mL) and acetone (420 mL) at room temperature. The resulting suspension was heated to reflux for 2 days. Then the reaction mixture was cooled to room temperature and diluted with water (200 mL). The mixture was extracted with ethyl acetate (2 × 200 mL), and the combined organic extracts were washed with brine solution (200 mL). The organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to give the crude product which was purified using a Biotage column (40L), eluting with 10% ethyl acetate/hexanes to give 4-{3-[6-(3,5-dibromophenoxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric acid ethyl ester (19.61 g, 99%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (t, J = 1.51 Hz, 1H), 7.10 (t, J = 7.85 Hz, 1H), 6.99 (d, J = 1.51 Hz, 2H), 6.77 (d, J = 7.85 Hz, 1H), 6.69 (d, J = 7.85 Hz, 1H), 4.16 (q, J = 7.24 Hz, 2H), 4.15 (q, J = 7.24 Hz, 2H), 4.00 (t, J = 6.04 Hz, 2H), 3.92 (t, J = 6.49 Hz, 2H), 2.91−3.04 (m, 2H), 2.59−2.68 (m, 2H), 2.43−2.58 (m, 4H), 2.14 (qd, J = 6.49, 6.69 Hz, 2H), 1.67−1.83 (m, 2H), 1.36−1.65 (m, 6H), 1.27 (t, J = 7.24 Hz, 6H). ES(+)-HRMS m/e calcd for $C_{29}H_{38}O_6Br_2$ $(M + H)^+$ 641.1108, found 641.1101.

General Procedure B: Preparation of 4-{2-(2-Ethoxycarbonylethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)hexyl]phenoxy}butyric Acid Ethyl Ester. A solution of 4-{3-[6-(3,5-dibromophenoxy)-hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric acid ethyl ester (321 mg, 0.5 mmol) in dimethoxyethane (10 mL) was stirred for 5 min at room temperature under a nitrogen atmosphere. Then tetrakis(triphenylphosphine)palladium(0) (115 mg, 0.1 mmol) was added at room temperature and the resulting light-yellow solution was heated to 80 °C and stirred for 5 min. At this time, a solution of phenylboronic acid (366 mg, 3.0 mmol) in ethanol (10 mL) was added, followed by a solution of sodium carbonate (318 mg, 3.0 mmol) in water (1.0 mL). The resulting light-yellow suspension was stirred for 15 h at reflux. Then the reaction mixture was cooled to room temperature and diluted with water (20 mL) and ethyl acetate (50 mL). The two layers were separated, and the aqueous layer was extracted with ethyl acetate (50 mL). The combined organic extracts were washed with water (100 mL), brine solution

(100 mL) and dried over anhydrous magnesium sulfate. Filtration and concentration of the solvent gave the crude residue which was purified using an ISCO (40 g) column, eluting with 0−50% ethyl acetate/hexanes to afford 4-{2-(2-ethoxycarbonylethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)hexyl]phenoxy}butyric acid ethyl ester (147 mg, 46%) as a light-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.72 (m, 4H), 7.42−7.53 (m, 4H), 7.33−7.41 (m, 3H), 7.12 (d, J = 1.51 Hz, 2H), 7.03−7.12 (m, 1H), 6.78 (d, J = 7.85 Hz, 1H), 6.69 (d, J = 7.85 Hz, 1H), 4.10−4.20 (m, 4H), 4.06−4.12 (m, 2H), 4.00 (t, J = 6.04 Hz, 2H), 2.84−3.05 (m, 2H), 2.59−2.71 (m, 2H), 2.43−2.58 (m, 4H), 2.07−2.20 (m, 2H), 1.77−1.92 (m, 2H), 1.57 (s, 6H), 1.26 (t, J = 7.24 Hz, 3H), 1.26 (t, J = 7.24 Hz, 3H). ES(+)-HRMS m/e calcd for $C_{41}H_{48}O_6$ (M + Na)$^+$ 659.3343, found 659.3343.

General Procedure C: Preparation of 4-{2-(2-Carboxyethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)hexyl]phenoxy}butyric Acid (11). To a solution of 4-{2-(2-ethoxycarbonylethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)hexyl]phenoxy}butyric acid ethyl ester (90 mg, 0.14 mmol) in ethanol (5 mL) was added 1.0 N aqueous NaOH (5 mL) at room temperature. The mixture was heated to 50−55 °C, and the resulting solution was stirred for 3 h. Then the reaction mixture was concentrated and the residue was diluted with water (20 mL) and extracted with diethyl ether (50 mL) to remove any neutral impurities. The aqueous layer was acidified with 1.0 N hydrochloric acid until the solution became acidic. The resulting white solids were collected by filtration and washed with water. After air-drying, 4-{2-(2-carboxyethyl)-3-[6-([1,1′,3,1″]terphenyl-5′-yloxy)hexyl]phenoxy}-butyric acid (71 mg, 86%) was isolated as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (br s, 2H), 7.76 (d, J = 7.55 Hz, 4H), 7.43−7.53 (m, 5H), 7.33−7.43 (m, 2H), 7.17 (d, J = 1.51 Hz, 2H), 7.01−7.09 (m, 1H), 6.70−6.80 (m, 2H), 4.12 (t, J = 6.34 Hz, 2H), 3.94 (t, J = 6.19 Hz, 2H), 2.73−2.90 (m, 2H), 2.59 (t, J = 7.24 Hz, 2H), 2.28−2.46 (m, 4H), 1.87−2.02 (m, 2H), 1.68−1.83 (m, 2H), 1.48 (d, J = 15.70 Hz, 6H). ES(+)-HRMS m/e calcd for $C_{37}H_{40}O_6$ (M + Na)$^+$ 603.2717, found 603.2713.

4-{2-(2-Carboxyethyl)-3-[6-(3,5-dipyrimidin-5-ylphenoxy)hexyl]-phenoxy}butyric Acid (16). a. Preparation of 4-{2-(2-Carboxyethyl)-3-[6-(3,5-dibromophenoxy)hexyl]phenoxy}butyric Acid. To a solution of 4-{3-[6-(3,5-dibromophenoxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric acid ethyl ester (1.5 g, 2.33 mmol) in ethanol (30 mL) was added aqueous 1.0 N sodium hydroxide (25 mL) at room temperature. The resulting suspension was heated to 50−55 °C, and the mixture was stirred for 3 h. Then the reaction mixture was concentrated and the residue was diluted with water (20 mL) and extracted with diethyl ether (50 mL) to remove any neutral impurities. The aqueous layer was acidified with 1.0 N hydrochloric acid, and the organic compound was extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate extracts were washed with brine solution (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to give the crude product which was purified using an ISCO 40 g column, eluting with 0−100% ethyl acetate/hexanes to give 4-{2-(2-carboxyethyl)-3-[6-(3,5-dibromophenoxy)hexyl]phenoxy}butyric acid (1.26 g, 92%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.10 (br s, 2H), 7.35 (t, J = 1.51 Hz, 1H), 7.18 (d, J = 1.51 Hz, 2H), 7.06 (t, J = 7.90 Hz, 1H), 6.76 (d, J = 7.90 Hz, 1H), 6.73 (d, J = 7.90 Hz, 1H), 3.88−4.02 (m, 4H), 2.76−2.86 (m, 2H), 2.53−2.62 (m, 2H), 2.29−2.46 (m, 4H), 1.94 (t, J = 6.34 Hz, 2H), 1.61−1.74 (m, 2H), 1.29−1.56 (m, 6H). ES(+)-HRMS m/e calcd for $C_{25}H_{30}Br_2O_6$ (M + Na)$^+$ 607.0301, found 607.0298.

b. Preparation of 4-{2-(2-Carboxyethyl)-3-[6-(3,5-dipyrimidin-5-ylphenoxy)hexyl]phenoxy}butyric Acid (16). To a solution of 4-{2-(2-carboxyethyl)-3-[6-(3,5-dibromophenoxy)hexyl]phenoxy}butyric acid (150 mg, 0.26 mmol) in ethanol (2 mL) in a microwave tube were added tetrakis(triphenylphosphine)-palladium(0) (29.5 mg, 0.03 mmol), pyrimidin-5-ylboronic acid (371 mg, 3.0 mmol), and potassium carbonate (212 mg, 1.53 mmol) at room temperature. The microwave tube was sealed and heated to 160 °C in a closed microwave for 30 min. Then the reaction mixture was cooled to room temperature and diluted with water (20 mL) and ethyl acetate (20 mL). The two layers were separated, and the ethyl acetate layer was discarded. Then the aqueous layer was acidified with 1.0 N hydrochloric acid and the organic compound was extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with brine solution (20 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated to afford 4-{2-(2-carboxyethyl)-3-[6-(3,5-dipyrimidin-5-ylphenoxy)hexyl]phenoxy}butyric acid (85 mg, 57%) as a light-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (br. s, 2H), 9.30 (s, 4H), 9.22 (s, 2H), 7.82 (s, 1H), 7.49 (d, J = 1.51 Hz, 2H), 6.91−7.18 (m, 1H), 6.67−6.79 (m, 2H), 4.17 (t, J = 6.34 Hz, 2H), 3.94 (t, J = 6.19 Hz, 2H), 2.75−2.87 (m, 2H), 2.54−2.64 (m, 2H), 2.24−2.43 (m, 4H), 1.88−1.97 (m, 2H), 1.68−1.85 (m, 2H), 1.35−1.59 (m, 6H). ES(+)-HRMS m/e calcd for $C_{33}H_{36}N_4O_6$ (M + Na)$^+$ 607.2527, found 607.2527.

4-{2-(2-Carboxyethyl)-3-[6-(5-thiophen-3-ylbiphenyl-3-yloxy)-hexyl]phenoxy}butyric Acid (25). a. Preparation of 3-Methoxy-5-nitrophenylamine. Sodium bicarbonate (5.62 g, 66.87 mmol) was added to a solution of sodium sulfide (5.5 g, 70.58 mmol) in deionized water (60 mL). When the sodium bicarbonate was completely dissolved, methanol (50 mL) was added, and the solution was cooled to 0 °C. A precipitate formed, which was removed by filtration through a Celite pad. Then the filtered solution was added quickly to a solution of 3,5-dinitroanisole (Apin Chemicals Ltd.) (7.36 g, 37.15 mmol) in methanol (50 mL). The resulting suspension was heated to reflux for 30 min, and then the solution was concentrated in vacuo to remove methanol. The aqueous residue was poured into 200 mL of ice−water, and the resulting orange precipitate was collected by filtration. After air-drying, 3-methoxy-5-nitrophenylamine (5.82 g, 93%) was obtained as light-brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.27 (br s, 1H), 6.61 (br s, 1H), 4.09 (br s, 2H), 3.97 (br s, 3H). ES(+)-HRMS m/e calcd for $C_7H_8N_2O_3$ (M + H)$^+$ 169.0608, found 169.0608.

b. Preparation of 1-Iodo-3-methoxy-5-nitrobenzene (20). To a solution of 3-methoxy-5-nitrophenylamine (7.5 g, 44.6 mmol) in water (20 mL) was added concentrated hydrochloric acid (19.95 mL, 267.6 mmol, 36%) at 0 °C. To this was added a chilled solution of sodium nitrite (5.62 g, 81.5 mmol) in water (28.4 mL) dropwise with a vigorous stirring. Then the resulting colored mixture was stirred for 15 min at 0 °C, and a cold solution of potassium iodide (14.81 g, 89.2 mmol) in water (28.4 mL) was added carefully. During this addition, a black-brown solid was formed. After the addition the ice-cold bath was removed, and the reaction mixture was heated to reflux. When the production of purple vapor ceased, the mixture was cooled to room temperature and the organic compound was extracted with dichloromethane (3 × 200 mL). The combined organic extracts were washed with brine solution (300 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Then the crude residue was purified using a LC 120 column, eluting with 0−10% ethyl acetate in hexanes to give 1-iodo-3-methoxy-5-nitrobenzene (10 g, 80%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.70 (s, 1H), 7.56 (s, 1H), 3.88 (s, 3H). EI(+)-HRMS m/e calcd for $C_7H_6INO_3$ (M+)$^+$ 278.9392, found 278.9393.

c. Preparation of 3-(3-Methoxy-5-nitrophenyl)thiophene. To a solution of 1-iodo-3-methoxy-5-nitrobenzene (1.0 g, 3.59 mmol) in ethanol (18 mL) in a microwave tube were added tetrakis-(triphenylphosphine)palladium(0) (837 mg, 0.72 mmol), thiophen-3-ylboronic acid (748 mg, 5.85 mmol), and potassium carbonate (496 mg, 3.58 mmol) at room temperature. Then the mixture was heated to 160 °C in a closed microwave tube for 30 min. After cooling to room temperature, the colored mixture was filtered and the filter cake was washed with water. The filtrate was diluted with 1.0 N HCl, and the mixture was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine solution (100 mL) and dried

over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The crude mixture was purified using an ISCO 80 g column, eluting with 0−10% ethyl acetate in hexanes to give 3-(3-methoxy-5-nitrophenyl)thiophene (776 mg, 92%) as a light-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04−8.07 (m, 1H), 7.65 (t, J = 2.42 Hz, 1H), 7.57 (dt, J = 1.36, 2.42 Hz, 1H), 7.39−7.47 (m, 3H), 3.94 (s, 3H). EI(+)-HRMS m/e calcd for $C_{11}H_9NO_3S$ $(M+)^+$ 235.0303, found 235.0298.

d. Preparation of 3-Methoxy-5-thiophen-3-ylphenylamine. To a mixture of 3-(3-methoxy-5-nitrophenyl)thiophene (3.78 g, 16.07 mmol), zinc dust (10.72 g, 160.7 mmol), and ammonium chloride (12.89 g, 241.1 mmol) were added methanol (50 mL) and water (25 mL) at room temperature. An exothermic reaction began on the addition of water. The suspension was stirred for 1 h, and the reaction mixture was filtered through Celite. The filter cake was washed with water and methanol. The filtrate was concentrated to remove methanol, and the residue was extracted with ethyl acetate (2 × 100 mL). The combined extracts were washed with brine solution (100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was then purified using an ISCO 120 g column, eluting with 0−20% ethyl acetate in hexanes to afford 3-methoxy-5-thiophen-3-ylphenylamine (3.08 g, 93%) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29−7.44 (m, 3H), 6.48−6.60 (m, 2H), 6.21 (br s, 1H), 3.82 (s, 3H), 3.74 (br s, 2H). ES(+)-HRMS m/e calcd for $C_{11}H_{11}NOS$ $(M + H)^+$ 206.0634, found 206.0634.

e. Preparation of 3-(3-Iodo-5-methoxyphenyl)thiophene (21a). To a solution of 3-methoxy-5-thiophen-3-ylphenylamine (2.45 g, 11.93 mmol) in water (7.2 mL) was added a concentrated hydrochloric acid (5.34 mL, 65.2 mmol, 37%) at 0 °C. To this was added a chilled solution of sodium nitrite (1.5 g, 21.7 mmol) in water (9.3 mL) dropwise with a vigorous stirring. Then the resulting colored mixture was stirred for 15 min at 0 °C, and a cold solution of potassium iodide (3.96 g, 23.86 mmol) in water (9.3 mL) was added carefully. During this addition, a black-brown solid was formed. After addition the ice-cold bath was removed, and the reaction mixture was heated to reflux. When the production of purple vapor ceased, the mixture was cooled to room temperature and the organic compound was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with brine solution (200 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Then the crude residue was purified using a LC 80 column, eluting with 0−10% ethyl acetate in hexanes to give 3-(3-iodo-5-methoxyphenyl)thiophene (2.19 g, 58%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.56 (m, 1H), 7.43−7.46 (m, 1H), 7.36−7.41 (m, 1H), 7.30−7.34 (m, 1H), 7.16−7.19 (m, 1H), 7.07 (dd, J = 1.36, 2.26 Hz, 1H), 3.83 (s, 3H). ES(+)-HRMS m/e calcd for $C_{11}H_9IOS$ $(M+)^+$ 315.9419, found 315.9418.

f. Preparation of 3-Iodo-5-thiophen-3-ylphenol (22a). To a suspension of 3-(3-iodo-5-methoxyphenyl)thiophene (2.08 g, 6.7 mmol) and sodium iodide (9.85 g, 65.73 mmol) in acetonitrile (80 mL) was added trimethylsilyl chloride (4.16 mL, 32.86 mmol) at room temperature. Then the resulting light-yellow suspension was heated to reflux for 48 h. Then it was cooled to room temperature and diluted with water (50 mL). The mixture was extracted with ethyl acetate (2 × 75 mL), and the combined ethyl acetate extracts were washed with saturated sodium thiosulfate solution (100 mL) to remove the iodine color and then with brine solution (100 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The crude residue was purified using an ISCO 120 g column, eluting with 0−20% ethyl acetate in hexanes to give 3-iodo-5-thiophen-3-ylphenol (1.92 g, 97%) as a light-brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (dd, J = 1.5, 1.5 Hz, 1H), 7.43 (dd, J = 1.4, 3.0 Hz, 1H), 7.38 (dd, J = 3.0, 5.0 Hz, 1H), 7.31 (dd, J = 1.4, 5.0 Hz, 1H), 7.14 (dd, J = 1.5, 2.4 Hz, 1H), 7.02 (dd, J = 1.5, 2.4 Hz, 1H), 4.83 (br s, 1H). ES(+)-HRMS m/e calcd for $C_{10}H_7IOS$ $(M - H)^+$ 300.9189, found 300.9189.

g. Preparation of 4-{2-(2-Ethoxycarbonylethyl)-3-[6-(3-iodo-5-thiophen-3-ylphenoxy)hexyl]phenoxy}butyric Acid Ethyl Ester (23a). The title compound was prepared in 99% yield from 4-[3-(6-bromohexyl)-2-(2-ethoxycarbonylethyl)phenoxy]butyric acid ethyl ester (2.99 g, 6.35 mmol), 3-iodo-5-thiophen-3-ylphenol (1.92 g, 6.35 mmol), and potassium carbonate (1.75 g, 12.7 mmol) using the general procedure A described for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.44 (br s, 1H), 7.38 (br s, 1H), 7.30−7.36 (m, 1H), 7.17 (s, 1H), 7.04−7.15 (m, 2H), 6.78 (d, J = 7.25 Hz, 1H), 6.69 (d, J = 8.45 Hz, 1H), 4.15 (q, J = 6.87 Hz, 4H), 3.90−4.05 (m, 4H), 2.92−3.02 (m, 2H), 2.64 (t, J = 7.55 Hz, 2H), 2.45−2.59 (m, 4H), 2.07−2.21 (m, 2H), 1.70−1.88 (m, 2H), 1.34−1.67 (m, 6H), 1.26 (t, J = 6.87 Hz, 6H). ES(+)-HRMS m/e calcd for $C_{33}H_{41}IO_6S$ $(M + Na)^+$ 715.1561, found 715.1561.

h. Preparation of 4-{2-(2-Ethoxycarbonylethyl)-3-[6-(5-thiophen-3-ylbiphenyl-3-yloxy)hexyl]phenoxy}butyric Acid Ethyl Ester. The title compound was prepared in 55% yield from 4-{2-(2-ethoxycarbonylethyl)-3-[6-(3-iodo-5-thiophen-3-ylphenoxy)hexyl]-phenoxy}butyric acid ethyl ester (216 mg, 0.31 mmol) and phenylboronic acid (152 mg, 1.25 mmol) using the general procedure B described for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60−7.66 (m, 2H), 7.36−7.52 (m, 7H), 7.04−7.14 (m, 3H), 6.78 (d, J = 7.55 Hz, 1H), 6.69 (d, J = 8.15 Hz, 1H), 4.15 (q, J = 7.09 Hz, 4H), 4.07 (t, J = 6.49 Hz, 2H), 4.00 (t, J = 5.89 Hz, 2H), 2.88−3.04 (m, 2H), 2.59−2.70 (m, 2H), 2.45−2.58 (m, 4H), 2.14 (quin, J = 6.72 Hz, 2H), 1.78−1.91 (m, 2H), 1.41−1.68 (m, 6H), 1.26 (t, J = 7.09 Hz, 3H), 1.26 (t, J = 7.09 Hz, 3H). ES(+)-HRMS m/e calcd for $C_{39}H_{46}O_6S$ $(M + Na)^+$ 665.2907, found 665.2907.

i. Preparation of 4-{2-(2-Carboxyethyl)-3-[6-(5-thiophen-3-yl-biphenyl-3-yloxy)hexyl]phenoxy}butyric Acid (25). The title compound was prepared in 57% yield from the 4-{2-(2-ethoxycarbonylethyl)-3-[6-(5-thiophen-3-ylbiphenyl-3-yloxy)hexyl]-phenoxy}butyric acid ethyl ester (85 mg, 0.13 mmol) and 1.0 N aqueous NaOH (8.0 mL) using the general procedure C described for compound **11**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (br s, 2H), 8.02 (s, 1H), 7.75 (d, J = 7.55 Hz, 2H), 7.62−7.70 (m, 2H), 7.54 (s, 1H), 7.42−7.51 (m, 2H), 7.32−7.43 (m, 1H), 7.25 (s, 1H), 7.10 (s, 1H), 7.05 (t, J = 7.85 Hz, 1H), 6.70−6.78 (m, 2H), 4.10 (t, J = 6.34 Hz, 2H), 3.94 (t, J = 6.04 Hz, 2H), 2.80 (m, 2H), 2.58 (m, 2H), 2.28−2.44 (m, 4H), 1.86−2.02 (m, 2H), 1.75 (m, 2H), 1.30−1.60 (m, 6H). ES(+)-HRMS m/e calcd for $C_{35}H_{39}O_6S$ $(M + H)^+$587.2467, found 587.246. The purity of this compound as measured by HPLC was 80%.

4-{3-[6-(3-5-Benzo[1,3]dioxolyl-5-thiophen-3-ylphenoxy)hexyl]-2-(2-carboxyethyl)phenoxy}butyric Acid (33). General Procedure D: Unsymmetrical 3,5-Diarylphenyl Ethers. a. Preparation of 3-5-Benzo[1,3]dioxolyl-5-thiophen-3-ylphenol (32a). To a mixture of 3,5-dibromophenol (7.55 g, 30 mmol), 3,4-methylenedioxyphenylboronic acid (7.48 g, 45 mmol), 3-thiophenylboronic acid (3.97 g, 31 mmol), dichloro[1,1′-bis(diphenylphosphino)ferrocene]-palladium(II) dichloromethane adduct (3.29 g, 4.0 mmol), and cesium carbonate (48.87 g, 150 mmol) was added 1,2-dimethoxyethane (300 mL) at room temperature under a nitrogen atmosphere. The resulting brown suspension was heated to 95 °C and stirred for 36 h. Then the reaction mixture was cooled to room temperature and diluted with water (200 mL) and ethyl acetate (300 mL). The two layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with water (500 mL) and brine solution (500 mL). The organic layer was dried over anhydrous magnesium sulfate, and filtration of the drying agent and removal of the solvent in vacuo gave the colored residue which was purified using an ISCO (330 g) column, eluting with 20−40% ethyl acetate in hexanes to afford 3-5-benzo[1,3]dioxolyl-5-thiophen-3-ylphenol (3.26 g, 37%) as a low melting white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.90 (dd, J = 1.42, 2.95 Hz, 1H), 7.63 (dd, J = 2.95, 4.99 Hz, 1H), 7.57 (dd, J = 1.42, 4.99 Hz, 1H), 7.34 (t, J = 1.66 Hz, 1H), 7.27 (d, J = 1.81 Hz, 1H), 7.15 (dd, J = 1.81, 8.15 Hz, 1H),

7.01−7.04 (m, 1H), 6.99 (d, J = 8.15 Hz, 1H), 6.85−6.91 (m, 1H), 6.06 (s, 2H). ES(-)-HRMS m/e calcd for $C_{17}H_{12}O_3S$ $(M - H)^-$ 295.0434, found 295.0432.

b. Preparation of 4-{3-[6-(3-5-Benzo[1,3]dioxolyl-5-thiophen-3-ylphenoxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric Acid Ethyl Ester. The title compound was prepared in 98% yield from 3-5-benzo[1,3]dioxolyl-5-thiophen-3-ylphenol (5.05 g, 17.04 mmol), 4-[3-(6-bromo-hexyl)-2-(2-ethoxycarbonylethyl)phenoxy]-butyric acid ethyl ester (8.44 g, 17.89 mmol), and potassium carbonate (4.71 g, 34.08 mmol) using the general procedure A described for compound **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.51 (m, 1H), 7.36−7.44 (m, 2H), 7.31 (d, J = 1.21 Hz, 1H), 7.04−7.13 (m, 4H), 6.97 (s, 1H), 6.89 (d, J = 8.75 Hz, 1H), 6.77 (d, J = 7.55 Hz, 1H), 6.68 (d, J = 7.85 Hz, 1H), 6.01 (s, 2H), 4.09−4.20 (m, 4H), 4.05 (t, J = 6.19 Hz, 2H), 3.99 (t, J = 6.19 Hz, 2H), 2.91−3.04 (m, 2H), 2.58−2.69 (m, 2H), 2.43−2.58 (m, 4H), 2.13 (dt, J = 6.75, 13.36 Hz, 2H), 1.75−1.91 (m, 2H), 1.39−1.68 (m, 6H), 1.20−1.30 (m, 6H). ES(+)-HRMS m/e calcd for $C_{40}H_{46}O_8S$ $(M + Na)^+$ 709.2805, found 709.2808.

c. Preparation of 4-{3-[6-(3-5-Benzo[1,3]dioxolyl-5-thiophen-3-yl-phenoxy)hexyl]-2-(2-carboxyethyl)phenoxy}butyric Acid (33). The title compound was prepared in 95% yield from 4-{3-[6-(3-5-benzo[1,3]dioxolyl-5-thiophen-3-ylphenoxy)hexyl]-2-(2-ethoxycarbonylethyl)phenoxy}butyric acid ethyl ester (21.34 g, 31.06 mmol) and 1.0 N aqueous NaOH (180 mL) using the general procedure C described for compound **11**. Mp =114−116 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (br s, 2H), 8.01 (dd, J = 1.51, 2.95 Hz, 1H), 7.67 (dd, J = 1.51, 5.13 Hz, 1H), 7.63 (dd, J = 2.95, 5.13 Hz, 1H), 7.48 (t, J = 1.36 Hz, 1H), 7.37 (d, J = 1.69 Hz, 1H), 7.25 (dd, J = 1.69, 8.08 Hz, 1H), 7.20 (t, J = 2.00 Hz, 1H), 7.02−7.10 (m, 2H), 7.00 (d, J = 8.08 Hz, 1H), 6.75 (dd, J = 2.87, 8.00 Hz, 2H), 6.07 (s, 2H), 4.09 (t, J = 6.19 Hz, 2H), 3.94 (t, J = 6.34 Hz, 2H), 2.69−2.92 (m, 2H), 2.55−2.65 (m, 2H), 2.28−2.44 (m, 4H), 1.87−2.02 (m, 2H), 1.67−1.82 (m, 2H), 1.36−1.61 (m, 6H). ES(+)-HRMS m/e calcd for $C_{36}H_{38}O_8S$ $(M + Na)^+$ 653.2179, found 653.2183. Elemental analysis ($C_{36}H_{38}O_8S$): C = 68.49 (calcd, 68.55), H = 5.93 (6.07), S = 5.15 (5.08).

In Vitro Cellular Assays. HL-60 Calcium Mobilization. Human leukemia HL-60 cells endogenously expressing BLT1 and BLT2 receptors were cultured in RPMI-1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Prior to the experiment, cells were counted using ViaCount reagent, centrifuged, and resuspended at 2.0×10^5 cells/ml (HL-60). Cells were plated in growth media in 384-well plates containing 1 μM retinoic acid (Sigma Aldrich, St. Louis, MO). On the day of the experiment, media were removed and replaced with loading buffer (Calcium 3 assay kit, Molecular Devices, Sunnyvale, CA) which was prepared by dissolving the contents of one vial (Express kit) into 500 mL of Hank's balanced salt solution (HBSS) containing 20 mM HEPES and 5 mM probenecid and mixed with an equal volume of replacement buffer (HBSS containing 20 mM HEPES, 0.05% BSA, and 5 mM probenecid) and placed in a 37 °C/5% CO_2 incubator for an hour.

Following incubation, an amount 5 μL of the LTB_4 antagonist analogue in question (5×10^{-11} to 3×10^{-6} M final assay concentration) or vehicle was transferred to the cell plates using an automated liquid handling system (fluorometric imaging plate reader, Molecular Devices) and incubated for 30 min (HL-60) at room temperature. During the assay, fluorescence readings were taken every 1.5 s. Five readings were taken to establish a stable baseline, and then 0.5 μM LTB_4 was added. The fluorescence was continuously monitored before, during, and after sample addition for a total elapsed time of 100 s. Responses (increase in peak fluorescence) following agonist addition were determined. The initial fluorescence reading from each well, prior to ligand stimulation, was used as a zero baseline value for the data from that well. The responses are expressed as percent inhibition of the neutral control (wells that received buffer plus DMSO but no test compound).

Human Neutrophil Chemotaxis Assay. Human whole blood was obtained from donors who had given written informed consent, and the human whole blood was layered over an equal volume of Lympholyte-poly (Cedarlane Laboratories Ltd., Ontario, Canada) in a 50 mL centrifuge tube. The tube was centrifuged for 35 min, and the two cell bands at the interface were harvested. The lower band containing polymorphonuclear granulocytes was diluted with assay medium (HBSS with calcium and magnesium, Invitrogen Corporation, CA, and 0.5% bovine serum albumin, Sigma, St. Louis, MO) and centrifuged for 12 min. The pellet was resuspended and a differential cell count performed on a Bayer ADVIA 120 hematology system. Neutrophil purity was 95−97%. Neutrophils at 5×10^6 cells/mL were added to 96-well round-bottom plates and incubated with the LTB_4 antagonist analogue in question (3×10^{-12} to 1×10^{-6} M final assay concentration) for 30 min at 37 °C. Quantification of neutrophil migration toward LTB_4 was determined using modifications to a polycarbonate membrane migration assay kit (Cell Biolabs Inc., CA). LTB_4 (100 ng/mL) was added to the lower feeder tray, and the neutrophils were added to the upper plate assembly. After 90 min at 37 °C, the migratory cell suspensions in the wells of the feeder tray were transferred into a 96-well flat-bottom black plate. CyQuant GR dye solution was added to the black plate to lyse the migrated cells for fluorescent detection. After incubation at room temperature for 30−45 min, fluorescence was read on a Tecan Safire2 microplate reader at 480 nm/520 nm. Inhibition of neutrophil migration was calculated by fitting the percent inhibition relative to the maximum migration minus background to a sigmoidal dose-response equation using Microsoft Excel.

Human BLT1 and BLT2 Calcium Mobilization. Experiments were performed at Multispan Inc. (CA) on cloned FLAG-tagged human BLT1 (Genbank accession number BC004545) and cloned FLAG-tagged human BLT2 (Genbank accession number NM_019839.1) receptors stably expressed in HEK293 cells that were seeded into 96-well plates at 25 000 cells/well the day before the experiment. To determine the EC_{50} of LTB_4 for each receptor, cell culture media were removed from the plate and replaced with 100 μL of Hanks buffer. Then an amount of 100 μL of FLIPR Calcium 4 assay kit (Molecular Devices) was added to the wells and incubated at 37 °C at 5% CO_2 for 60 min. LTB_4 was added (1×10^{-12} to 1×10^{-5} M final assay concentration) to the compound plate and incubated for 1 h. The cell plate was then transferred to FlexStation (Molecular Devices), and within 90 s, LTB_4 was injected automatically into the wells and the calcium response measured. The EC_{50} of LTB_4 was determined using GraphPad Prism software (GraphPad Software, CA) and used as the agonist concentration in the antagonist experiments. In these experiments, the compound plate was incubated for 50 min and then the LTB_4 antagonist analogue **6** added (1×10^{-8} to 1×10^{-5} final assay concentration) and preincubated for 10 min. LTB_4 was then injected into the wells and the calcium response measured. Inhibition of LTB_4-evoked calcium mobilization was calculated by fitting the percentage inhibition relative to the maximum response to a sigmoidal dose-response equation using GraphPad Prism software. The IC_{50} values of LTB_4 antagonist analogues at BLT1 and BLT2 receptors were calculated and statistically compared using Student's unpaired t test using the same software.

Metabolite Characterization of Human, Mouse, Rat, Dog, and Monkey Cryopreserved Hepatocytes. Incubations were carried out with 1.5 million cells/mL hepatocytes in Liebovitz media containing 1% fetal bovine serum (final concentrations). Substrate concentration during incubation was 10 μM, and total incubation volume was 500 μL. Incubations were carried out for 3 h in a 37 °C water bath in glass flat-bottom tubes gently capped while shaking at 40 strokes/min. Incubations were stopped with equal volume methanol, samples were spun at 2000g, and supernatants were collected for analysis. Cell-free incubations

were performed in the same manner except an aliquot of the hepatocytes incubation media was used instead of the cells.

Human hepatocytes (male and female, $N = 10$, product lot no. SX008055/CD1) were obtained from In Vitro. Mouse hepatocytes (male, CD1, product lot no. Mc221) were obtained from CellzDirect. Rat hepatocytes (male, Sprague−Dawley, product lot no. R1000/0510160) were obtained from Xeno Tech LLC. Monkey hepatocytes (male, cynomolgus, product lot no. P2000/XHTcyno112002) were obtained from Xeno Tech LLC. Dog hepatocytes (male, beagle, product lot no. Db139) were obtained from CellzDirect.

In Vivo Assays. LTB_4-Evoked Pulmonary Inflammation in Guinea Pigs. Experiments were performed on male Hartley guinea pigs (250−350 g; Charles River, MA). Animals were dosed with vehicle (2% Klucel LF, 0.1% Tween-80 in water) or 10 mg/kg LTB_4 antagonist analogue orally (po). One hour postdose, animals were placed in a clear plastic chamber and challenged with an aerosol solution of 10 μg/mL LTB_4 for 20 min. Two hours post-LTB_4 challenge, bronchoalveolar lavage (BAL) was performed. Animals were anesthetized (sodium pentobarbital, 40−60 mg/kg, ip), the trachea was cannulated with a 15 gauge tubing adapter, and the lungs were lavaged with 3 × 5 mL of sterile HBSS. The samples were centrifuged at 200*g* for 10 min at 25 °C, and red blood cells were lysed from the resulting pellet with distilled water (1 mL for 30 s) before restoring osmolarity with the addition of 10 mL of HBSS. Samples were centrifuged a second time (200*g*, 10 min, 25 °C), and the resulting pellet was resuspended in 1 mL of HBSS. Total cell number was determined using a Beckman-Coulter Z-1 particle counter. For differential cell counts, an aliquot of the cell suspension was centrifuged in a Cytospin (5 min, 1300 rpm; Shandon Southern Instruments, PA) and the slides were fixed and stained with a modified Wright's stain (Leukostat, Fisher Scientific, PA). Standard morphological criteria were used to classify at least 300 cells under light microscopy. Statistics were performed using two-way ANOVA followed by Bonferroni post-test.

Allergen-Evoked Pulmonary Inflammation in Atopic Nonhuman Primates. Experiments were performed on male cynomolgus monkeys (*Macaca fascicularis*, 4−10 kg; Charles River, MA) exhibiting natural hypersensitivity to *Ascaris suum* antigen. Baseline BAL was performed on all animals 24 h prior to allergen challenge (see below for procedure; alternate sides of lungs were used for pre- and post-BAL procedures). Animals were dosed with vehicle (15% Labrasol in polyethylene glycol) or 10 mg/kg LTB_4 antagonist analogue po. One hour postdose, animals were anesthetized with ketamine/xylazine (10 and 1 mg/kg, respectively, im) and challenged with an aerosol of *Ascaris suum* antigen for 90 s via an endotracheal tube (4−4.5 mm i.d.), at a concentration previously demonstrated to evoke at least a 100% increase in lung resistance. Four hours after allergen exposure, animals were reanesthetized and BAL was performed. A pediatric bronchoscope was inserted transorally into the trachea. The left or right diaphragmatic lobe was lavaged twice with 5 mL of sterile PBS, which was placed on ice immediately after collection and kept cold throughout processing. To determine leukocyte cell differentials, slides were prepared by centrifuging 100 μL of the sample at 950 rpm for 2 min at room temperature (Cytospin-3; Shandon-Lipshaw, PA). The slides were stained using a LeukoStat stain set (Fisher Scientific, PA), and 300 cells were counted. Total leukocyte cell count was performed manually using a standard hemocytometer. Statistics were performed using two-way ANOVA followed by Bonferroni post-test.

Toxicology Studies. Experiments were performed on male Han−Wistar rats (200−230 g; Charles River, MA). Animals were randomized into toxicology groups (vehicle, comprising 2% Klucel LF with 0.1% Tween-80 in water, 40 and 400 (mg/kg)/day **33** or **38**, $n = 4$ each group). Animals were dosed orally once per day for 14 days. In-life observations were recorded for each animal at least twice daily. Body weights were recorded for each animal at least once prior to initiation of treatment and on days 1, 8, and 14. Food consumption was recorded once weekly. Blood samples were collected from toxicity animals for evaluation of hematological and clinical chemistry parameters on day 5 and at scheduled necropsy. At the end of the 14-day study period, animals were euthanized by induction with isoflurane/O_2 anesthesia followed by exsanguination. Organs and tissues were removed and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, mounted on glass slides, and stained with hematoxylin and eosin (H & E). All data were statistically analyzed using Bartlett's nonparametric or Bartlett's parametric test as appropriate. Blood samples were collected from rats under isoflurane/O_2 anesthesia, from the retro-orbital sinus. The plasma was separated after cold centrifugation and stored frozen in polypropylene tubes in a −70 °C freezer until analysis.

Assessment of in Vitro Effect on hERG Channels. Compounds were characterized for their effect on hERG current (IKr) expressed in Chinese hamster ovary (CHO) cells by a non-GLP in vitro assay of current inhibition using a cell-based mammalian expression system. CHO cells were stably transfected with hERG cDNA and selected by G418 resistance. Selection is maintained by including G418 in the culture media. The culture media consist of Ex-cell 301(JRH-14331), 10% FBS (Sigma-Aldrich, catalog no. 8061799), and 0.25 mg/mL G418 (Sigma-Aldrich, catalog no. 8061799). Cells are cultured in 1 L of polypropylene shaker flasks as a suspension with maximum volume of 100 mL at 35−39 °C. Cells are grown with 5% CO_2 applied with a gas flow rate of 10−15 mL/min. Cells are cultured in flasks on a shaker table at 85−100 rpm at a density less than 1.5×10^6 cells/mL. Compounds were were examined at three concentrations (0.3, 3, and 30 μM) for the ability to reduce current amplitude from the hERG potassium channel expressed in CHO cells by automated electrophysiology using the PatchXpress 7000A, with solubility as the limiting factor. If inhibition of current were not represented in these concentrations, further concentrations were selected in order to generate the percent inhibition at low and high concentrations as well as an IC_{50} and IC_{20} value. If hERG current inhibition was less than 50% at the highest concentration tested, the IC_{50} will be reported as an estimate. Compounds were initially dissolved in DMSO to 15 mM and stored at 4 °C before further dilution in DMSO. All testing solutions were made in the recording solution that was composed of NaCl (150 mM), KCl (4 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.2 mM), and HEPES (10 mM). The final DMSO concentration was ≤0.3% DMSO.

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Supporting Information Available: Experimental details and analytical data for compounds **12**−**18**, **26**−**30**, and **34**−**40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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