Design, Synthesis, and Pharmacological Evaluation of Potent Xanthone Dicarboxylic Acid Leukotriene B_4 Receptor Antagonists[1]

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In an effort to develop increasingly potent and specific leukotriene B_4 (LTB_4) receptor antagonists, several xanthone dicarboxylic acids were synthesized and evaluated. Two separate synthetic routes were used to construct a xanthone nucleus containing a regiospecific orientation of each carboxylic acid pharmacophore. These compounds represent the major conformationally-restricted analogues of benzophenone dicarboxylic acids previously shown to antagonize the activation of human neutrophils by LTB_4. The most potent agent was compound **32**, which inhibited the specific binding of [^{3}H]LTB_4 to receptors on intact human neutrophils (IC_{50}, 6.2 ± 0.1 nM), LTB_4-induced luminol-dependent chemiluminescence (IC_{50}, 55 ± 11 nM), aggregation (IC_{50}, 133 ± 42 nM), and chemotaxis (IC_{50}, 899 ± 176 nM). The compound was a poor antagonist of *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine-induced chemiluminescence (IC_{50}, 1599 ± 317 nM) and aggregation (IC_{50}, 2166 ± 432 nM), indicating specificity in the inhibition of LTB_4-stimulated events. Compound **32** (LY210073), which was completely devoid of agonist activity, appears to be one of the strongest inhibitors of LTB_4 receptor binding reported so far.

Introduction

Leukotriene B_4 (LTB_4) has several properties that makes it a very potent proinflammatory mediator.[2] These include the ability to induce neutrophils to migrate in a unidirectional manner, to adhere to endothelial surfaces, to produce reactive oxygen species, and to liberate tissue digesting enzymes from their granules. Enhanced concentrations of this eicosanoid have been observed in tissues of patients with psoriasis,[3] inflammatory bowel disease,[4] rheumatoid arthritis,[5] gout,[6] bronchial asthma,[7] cystic fibrosis,[8] adult respiratory distress syndrome (ARDS),[9] and cerebral hemorrhage.[10] Hence, it seems likely that a potent antagonist of this mediator would be a promising antiinflammatory agent.

Gapinski et al.[11] developed a benzophenone dicarboxylic acid, LY223982 (Chart I), which inhibited the binding of [^{3}H]LTB_4 to receptors on intact human neutrophils nearly as well as nonradioactive LTB_4 (IC_{50} = 13.2 ± 2.2 nM vs IC_{50} = 1.9 ± 0.05 nM) and was 189-fold more selective for the LTB_4 receptor than for the *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) receptor.[12] Structure–activity relationship studies on diacids of this type showed that the most critical attributes for maximum potency consist of a meta-substituted aryl carboxylic acid connected to a 3-phenylpropanoic acid derivative with a lipophilic chain at the ortho position.[11a,b] Benzophenones like LY223982 can exist in a number of conformations due to free rotation about the carbonyl carbon/aromatic carbon bonds, but the application of molecular mechanics has indicated that most of these are limited to four major families.[13] Chaney et al.[14] gained some insight into the conformation most likely to interact with the LTB_4 receptor by comparing the binding affinities of four xanthone analogs (compounds 1–4) of a less potent benzophenone dicarboxylic acid, LY213024 (Chart I). These analogs closely mimicked the four major conformational states of this benzophenone. Compound 4 (IC_{50} = 43 ± 5 nM) was found to be the strongest receptor binder and was even more active than the parent benzophenone LY213024 (Table I).

Table I. Inhibition of Binding of LTB_4 to Receptors on Intact Neutrophils by Xanthone Dicarboxylic Acids

compound	IC_{50}[a] (nM)	relative potency
LY213024	210 ± 24	10
1	1766 ± 113	1.2
2	2097 ± 125	1.0
3	832 ± 35	2.5
4	43 ± 5	49
LY223982	13.2 ± 2.2	159
32 (LY210073)	6.2 ± 0.1	338

[a] The IC_{50}'s were determined by measuring the inhibition of the specific binding of [^{3}H]LTB_4 to intact human neutrophils according to the procedure described in the pharmacological methods. The concentration of [^{3}H]LTB_4 was 0.1 nM. Data for LY223982 is from ref 12 and the results for LY213024 and 1–4 are from ref 14.

This report describes the synthesis of compounds 1–4 and also compound **32** (LY210073, Scheme II), a xanthone analog of LY223982. In an effort to develop more potent LTB_4 antagonists, this latter compound, which is similar to 4 except the hydrocarbon tail has been replaced with the more active 4-methoxystyryl side chain of LY223982, was made and analyzed to see if, like compound 4, it bound more tightly to the receptor than the parent benzophenone. In addition, the effects of these two xanthones on LTB_4- and FMLP-induced aggregation, chemiluminescence, and chemotaxis of human neutrophils were investigated to determine if, like LY223982, they are specific antagonists devoid of any agonist activity. Our results indicate that compound **32** is a more potent and specific LTB_4 antagonist than either LY223982 or compound 4 and one of the most active reported to date.

Chemistry

The syntheses of compounds 1–4 are detailed in Scheme I.[11c] Compounds 1 and 2, containing a carboxylate at the 5-position of the xanthene ring, were both prepared from compound **5**, the Ullmann coupling product of 2-bromobenzonitrile and 3-methoxyphenol. Base hydrolysis of nitrile **5** was followed by demethylation, esterification,

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and allylation under standard conditions to provide the common allyl ether intermediate **9**. Claisen rearrangement of **9** provided a mixture of phenols **10a** and **10b**. The isomers were not separated at this point but submitted to hydroboration/oxidation conditions to afford alcohols **11a** and **11b**, which were separated via HPLC (ratio **11a**:**11b** = 1.4:1). Appendage of the *n*-decyl side chain, alcohol oxidation,[11a] and ester formation provided compound **13a** or **13b**. Xanthone formation was effected using aluminum chloride and oxalyl chloride in methylene chloride at room temperature. Varying degrees of aluminum chloride-mediated cleavage of the *n*-decyl side chain necessitated a further alkylation procedure (performed on the crude cyclization product) to furnish penultimate xanthone ester **14a** or **14b**. Base hydrolysis smoothly provided the target structures **1** and **2**. Beginning with compound **15** (synthesized from 4-bromobenzonitrile and 3-methoxyphenol), preparation of compounds **3** and **4** proceeded along a similar pathway, except that Claisen rearrangement products **20a** and **20b** were separated directly (ratio **20a**:**20b** = 1.6:1), alkylated with the *n*-decyl side chain, and progressed to the target products.

The singular activity displayed by compound **4** (LY264086) prompted development of a more efficient route to the 3,4,7-trisubstituted xanthone nucleus (Scheme II). Modification of a known synthesis of 2-carboxyxanthones[15,16] provided compound **26**, which was demethylated and esterfied under standard conditions to give ester **27**. Of particular note is the use of phosphorus pentoxide/methanesulfonic acid as a substitute for polyphosphoric acid in the cyclization step.[17] Polyphosphoric acid is difficult to work with in large quantities, and the procedure chosen by us in these studies proved to be of high efficiency and was amenable to multigram scale up. The key feature of the route depicted in Scheme II is the regiospecific formation of ortho ester **28**, which utilizes a pivalic acid-catalyzed addition/Claisen rearrangement/ring closure procedure beginning with triethyl orthoacrylate[18] and

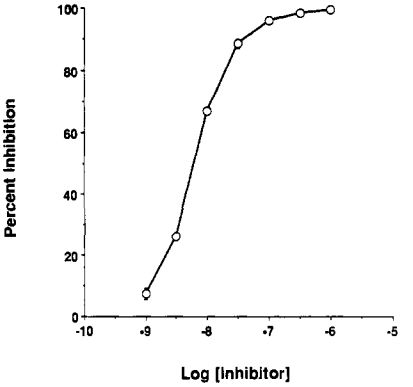


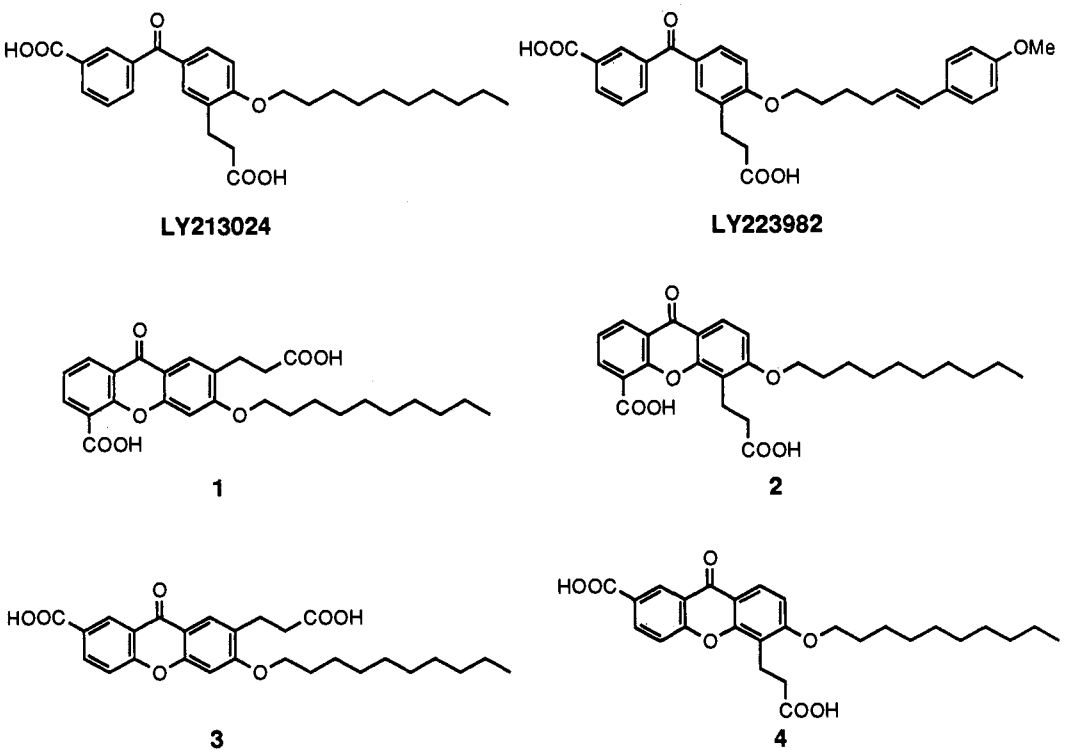
Figure 1. Inhibition of specific binding of [^{3}H]LTB_4 (0.1 nM) to receptors on human neutrophils by compound **32**. Each value is the mean ±SE of results from five individuals.

xanthone **27**. In contrast to the thermal Claisen rearrangement of biaryl ethers **9** and **19**, conversion of **27** to **28** proceeded in 94% yield with exclusive attack at the xanthone C-4 carbon. Ring opening of ortho ester **28** was smoothly effected with dilute aqueous hydrochloric acid to provide phenol **29** in high yield. Differential alkylation with either *n*-decyl iodide or 6-(4-methoxyphenyl)-5(*E*)-hexenyl methanesulfonate,[11b] followed by ester hydrolysis, provided compound **4** or **32**.

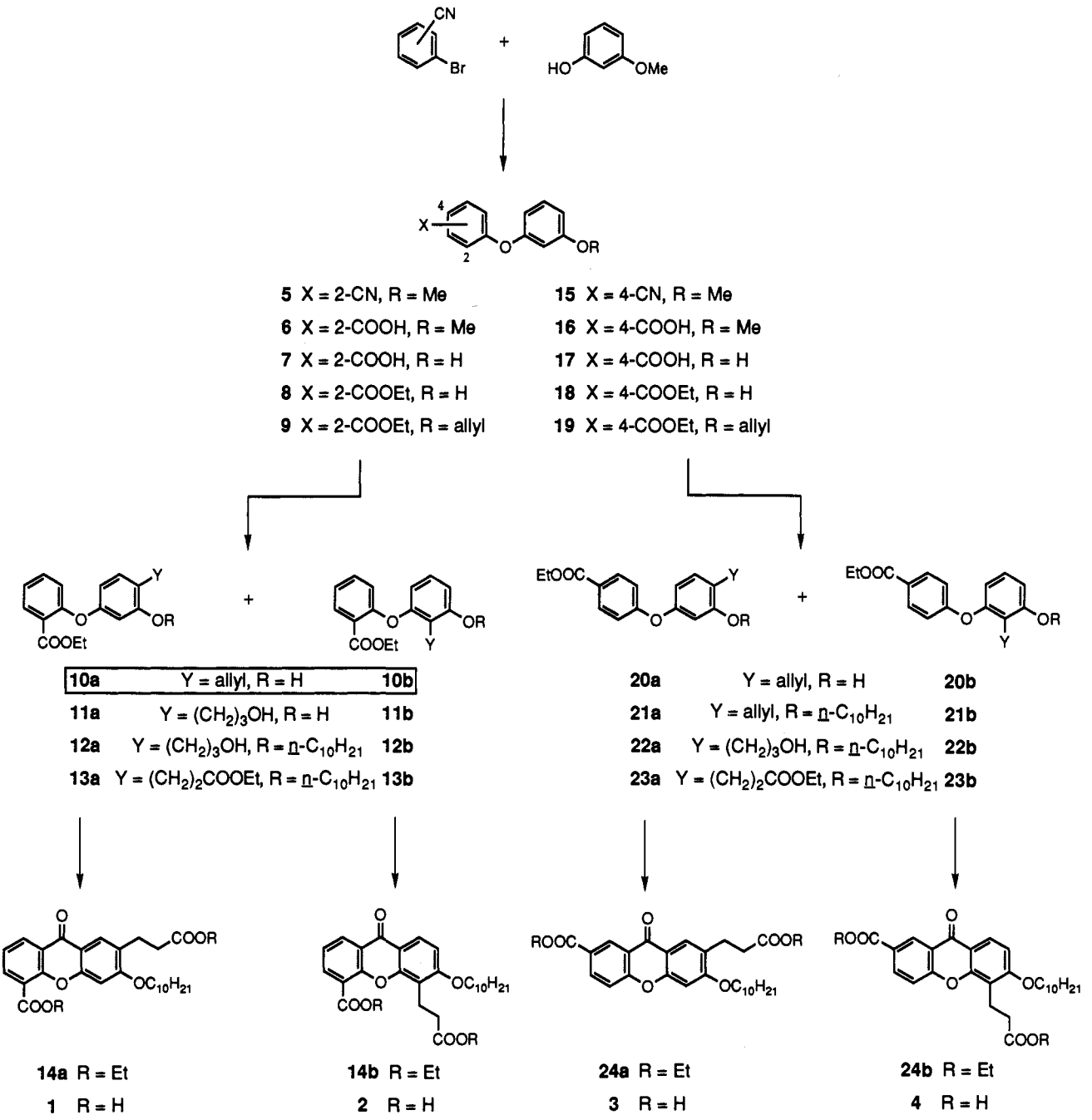
Pharmacological Activity

Measurements of the effect of LY223982 and the xanthone analog **32** on specific binding of [^{3}H]LTB_4 to human neutrophils (Figure 1) revealed that the IC_{50} observed for the xanthone dicarboxylic acid (6.2 ± 0.1 nM) was 2.1-fold lower than the corresponding value reported for the benzophenone dicarboxylic acid (13.2 ± 2.2 nM). Thus, the same pattern was found as observed for the previously studied pair, compound **4** and LY213024. Consequently, compound **32** (LY210073) with a K_i of 5.9 nM, calculated by the method of Cheng and Prusoff,[19] is

Chart I



Scheme I



one of the most potent inhibitors of specific LTB_4 binding found to date.

To determine whether these xanthones were specific antagonists of LTB_4 as previously observed for the benzophenone LY223982, their effects on several cell functions of neutrophils were studied. The first activity investigated was luminol-dependent chemiluminescence induced by stimulating the cells with LTB_4. Compound **32** inhibited the response strongly (IC_{50} = 55 ± 11 nM, Figure 2). However, when cells were stimulated with FMLP, this compound was 29-fold less active (IC_{50} = 1599 ± 317 nM), thus demonstrating selectivity for the LTB_4-induced response. Compound **4** was a less potent antagonist of the LTB_4 response but, more strikingly, was not nearly as specific (Figure 3). The IC_{50} of **4** for the inhibition of LTB_4-stimulated cells was only 2.1-fold lower than the IC_{50} for abating the FMLP-induced chemiluminescence (IC_{50} = 342 ± 59 nM vs. IC_{50} = 734 ± 107 nM). Neither xanthone, **4** or **32**, induced chemiluminescence of the cells at a concentration of 10 μM.

Similar results were obtained when measuring the effects of these two compounds on aggregation of neutrophils stimulated with LTB_4 or FMLP. Again, **32** was found to be a potent specific antagonist of LTB_4 (Figure 4). The IC_{50} for inhibition of LTB_4-induced aggregation was only 133 ± 42 nM, while the corresponding value for suppression of the FMLP-mediated response was 16.3-fold higher (2166 ± 432 nM). Conversely, compound **4** was a less potent and less specific antagonist of LTB_4 (Figure 5). The IC_{50} for inhibition of the LTB_4 response (513 ± 129 nM) was only 2.4-fold lower than the IC_{50} for blockade of the FMLP reaction (1214 ± 413 nM).

A third cell function investigated was unidirectional migration of cells. Both xanthones prevented LTB_4-induced chemotaxis (Figure 6) of human neutrophils, but

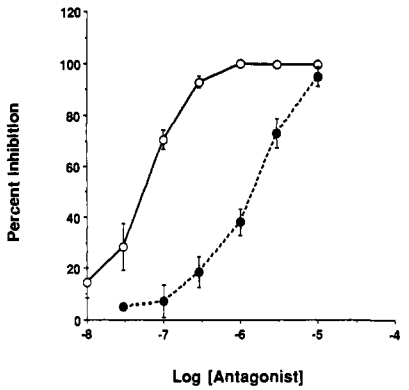


Figure 2. Inhibition of LTB_4- (solid line) and FMLP- (dashed line) induced luminol-dependent human neutrophil chemiluminescence by compound **32**. Each value is the mean ± SE of results from five individuals. The concentration of the agonist was 30 nM.

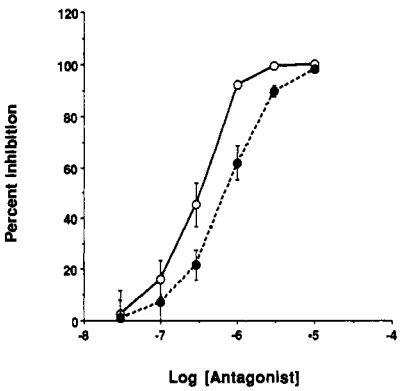


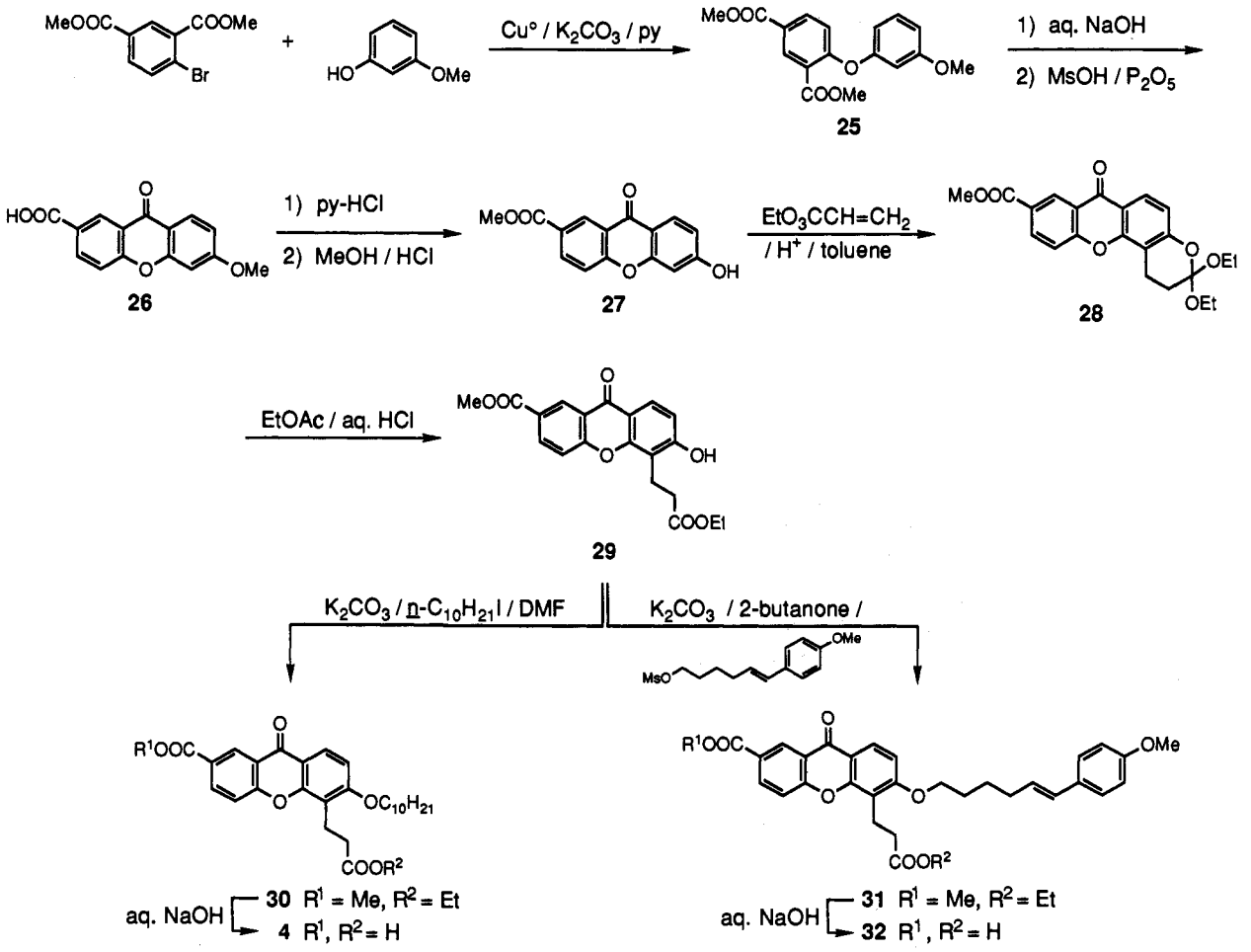
Figure 3. Inhibition of LTB_4- (solid line) and FMLP- (dashed line) induced luminol-dependent human neutrophil chemiluminescence by compound **4**. Each value is the mean ± SE of results from five individuals. The concentration of the agonist was 30 nM.

32 was an 8.8-fold better inhibitor than **4** (IC_{50} = 899 ± 176 nM vs IC_{50} = 7902 ± 2432 nM).

Discussion

These studies with xanthone dicarboxylic acids are an extension of our effort to develop more potent and selective LTB_4 antagonists. Our initial lead compounds were benzophenone dicarboxylic acids. Structure–activity studies indicated that both acid groups were necessary to observe good inhibition of the binding of LTB_4 to its receptor on intact human neutrophils.[11a] The most active compounds had a meta-substituted aryl carboxylic acid attached to a 3-phenylpropanoic acid containing a lipophilic hydrocarbon chain at the ortho position. The most active benzophenone initially synthesized was LY213024, in which the length of the lipophilic side chain was 10 carbons.[11b] Although benzophenones can exist in a number of conformations because of rotation about the phenyl–carbonyl bonds, we hypothesized that the LTB_4 receptor would prefer to bind to just one, and by studying the activity of conformationally rigid analogues we could determine which conformation is preferred. Accordingly, we made four xanthone dicarboxylic acids (compounds **1–4**) that mimicked the major low-energy conformers of

Scheme II



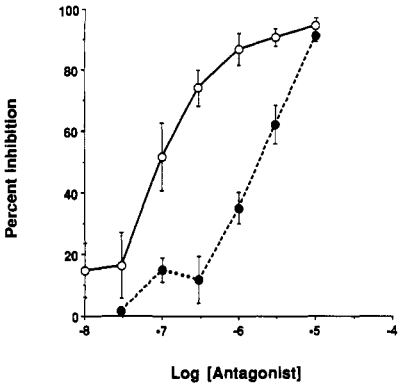


Figure 4. Inhibition of LTB_4- (solid line) and FMLP- (dashed line) induced aggregation of human neutrophils by compound **32**. Each value is the mean ± SE of results from five individuals. The concentration of the agonist was 30 nM.

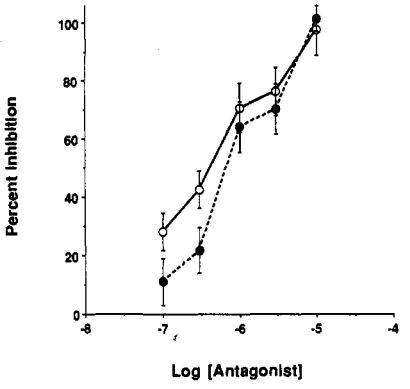


Figure 5. Inhibition of LTB_4- (solid line) and FMLP- (dashed line) induced aggregation of human neutrophils by compound **4**. Each value is the mean ± SE of results from five individuals. The concentration of the agonist was 30 nM.

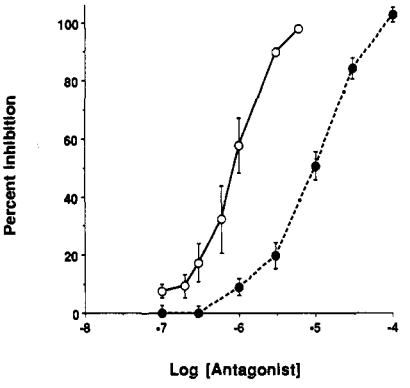


Figure 6. Inhibition of LTB_4-induced chemotaxis of human neutrophils by compound **32** (solid line) and compound **4** (dashed line). Each value is the mean ± SE of results from five individuals. The concentration of the agonist was 10 nM.

LY213024. Studies with these compounds revealed that compound 4 inhibited the binding of [^{3}H]LTB_4 to intact human neutrophils considerably better than compounds 1–3. In fact, compound 4 was found to be a more potent inhibitor than the parent benzophenone LY213024.[14] The reason why the activity of this particular xanthone is superior to the benzophenone is not known, but we have suggested it might be that benzophenones are bound to the receptor in a conformation where the two rings are nearly coplanar, or that the xanthone is in a lower entropic state, or a combination of these.[14]

Testing of additional benzophenone dicarboxylic acids featuring different lipophilic tails led to the synthesis of increasingly potent LTB_4 binding inhibitors. The most active was LY223982, containing a 4-methoxystyryl group.[11b] Based on our experience with the xanthone analogs of LY213024 (compounds 1–4), we explored the possibility that compound **32** (LY210073), the xanthone dicarboxylic acid analog of LY223982 corresponding to compound 4, would also be a better inhibitor of LTB_4 binding than the parent benzophenone. The results given in this study show that compound **32** (IC_{50} = 6.2 nM) is 2.1-fold more potent than LY223982. Moreover, its IC_{50} is only 3.3-fold higher than that of nonradioactive LTB_4.[12]

Although binding studies yield information about the degree to which these compounds are bound to the LTB_4 receptor, they do not distinguish between agonist and antagonist activity. Accordingly, compounds 4 and **32** were tested for their effects on three cell functions of human neutrophils that are activated by binding of LTB_4 to its receptor. Both compounds inhibited LTB_4-induced chemiluminescence, aggregation, and chemotaxis, but as expected from the binding results, compound **32** was 3.9–8.8-fold more potent than compound 4. Neither compound induced chemiluminescence at concentrations as high as 10 μM, indicating no agonist activity.

The specificity of the antagonist activity of the compounds was determined by measuring their effects not only on LTB_4- but also on FMLP-induced activation of human neutrophils. Compound **32** was found to be a very ineffective antagonist of responses stimulated by FMLP. Its IC_{50}'s for inhibiting FMLP-induced chemiluminescence and aggregation were 29- and 16.3-fold higher than the corresponding values for LTB_4-stimulated events. Thus, as found for the parent benzophenone LY223982,[12] the antagonism of **32** appears to be specific for the eicosanoid. In contrast, the antagonist characteristics of **4** were considerably less specific. The IC_{50}'s for blocking chemiluminescence and aggregation induced by FMLP were only 2.1 and 2.4 higher, respectively, than the corresponding values for LTB_4-stimulated cells. The observation that **4** is not a very specific LTB_4 antagonist, while compound **32** and LY223982 are, was unanticipated. Possibly the reason for this is that 4 is not as strongly bound by the LTB_4 receptor as the other two compounds. More likely, the lipophilic hydrocarbon tail on this molecule fits better to a region of the FMLP receptor than the 4-methoxystyryl tail of the other two. We have not studied the specificity of the parent benzophenone of 4, LY213024, but would expect that it too would cross-react extensively with the FMLP receptor.

In summary, these studies have led to the development of a potent, selective LTB_4 receptor antagonist, compound **32** (LY210073), that is as specific for inhibiting LTB_4-induced activation of human neutrophils as LY223982 but more potent. In some tests, **32** was considerably more potent. For example, the IC_{50} for inhibition of LTB_4-induced chemotaxis by **32** was 899 nM compared to a value of 6 μM for inhibition by LY223982.[12] These results also show that increasingly potent LTB_4 antagonists can be synthesized by making rigid analogs that mimic the preferred binding conformation of known antagonists.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were determined on a GE QE-300 spectrometer. All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. The following abbreviations are used to denote signal patterns: s = singlet, d

= doublet, t = triplet, q = quartet, b = broad, m = multiplet. Infrared spectra were determined on a Nicolet DX10 FT-IR spectrometer. Mass spectral data were determined either with a CEC-21-110 spectrometer using electron impact (EI) conditions or with a MAT-731 spectrometer using free desorption (FD) conditions. With the exception of some NMR spectra, all spectroscopic and analytical data were determined by the Physical Chemistry Department (MC525) of the Lilly Research Laboratories. Reported analytical data are within ±0.4% of the theoretical values unless otherwise indicated. High-pressure liquid chromatography (HPLC) purification was performed on a Waters Prep LC-500 using ethyl acetate/hexane gradients. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl immediately prior to use. All reactions were conducted under an argon atmosphere with stirring unless otherwise noted; yields were not optimized.

5-Carboxy-3-(decyloxy)-9-oxo-9*H*-xanthene-2-propanoic Acid (1). (a) Method A: General Procedure for Ullmann Biaryl Ether Coupling. 2-(3-Methoxyphenoxy)benzonitrile (5). A mixture of 2-bromobenzonitrile (100 g, 0.550 mol), 3-methoxyphenol (68.1 g, 0.550 mol), copper bronze (35.0 g, 0.550 mol), and potassium carbonate (75.8 g, 0.550 mol) in pyridine (3 L) was refluxed for 6 days. The reaction mixture was filtered while hot, cooled to room temperature, and concentrated in vacuo. Concentrated hydrochloric acid (1.5 L) was slowly added to the resulting residue. Ethyl acetate and water were added, and the layers were separated. The organic layer was washed several times with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by HPLC using a 10% ethyl acetate/90% hexane gradient to provide 53.4 g (43%) of the desired product as an oil. Anal. ($C_{14}H_{11}NO_2$) C, H, N.

(b) Method B: General Procedure for Nitrile Hydrolysis. 2-(3-Methoxyphenoxy)benzoic Acid (6). A mixture of compound 5 (48.2 g, 0.214 mol) and potassium hydroxide (20.0 g, 0.500 mol) in ethanol and water was refluxed for 18 h. The mixture was cooled to room temperature and concentrated in vacuo. Ethyl acetate and water were added, the layers were separated, and the aqueous layer was acidified with concentrated hydrochloric acid. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. Crystallization of the residue from ethyl acetate/hexane provided 18.8 g (36%) of the desired product: mp 129–131 °C. Anal. ($C_{14}H_{12}O_4$) C, H.

(c) Method C: General Procedure for Demethylation of Phenolic Methyl Ethers. 2-(3-Hydroxyphenoxy)benzoic Acid (7). A mixture of compound 6 (15.0 g, 65.2 mmol) and pyridine hydrochloride (150 g) was melted and maintained at 180–185 °C for 3 h. After the mixture was cooled to room temperature, water was added, and the resulting mixture was stirred at room temperature for 18 h. The mixture was filtered, and the resulting solution was extracted several times with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo. Crystallization of the residue from ethyl acetate/hexane provided 6.88 g (49%) of the desired product: mp 147–149 °C. Anal. ($C_{13}H_{10}O_4$) C, H.

(d) Method D: General Procedure for Esterification. 2-(3-Hydroxyphenoxy)benzoic Acid Ethyl Ester (8). A mixture of compound 7 (17.8 g, 69.0 mmol) and concentrated sulfuric acid (1 mL) in absolute ethanol (250 mL) was refluxed for 2 days. The mixture was cooled to room temperature and concentrated in vacuo. Purification of the resulting residue by HPLC provided 16.8 g (84%) of the desired product as an oil: ^{1}H NMR ($CDCl_3$) 7.92 (dd, J = 8, 2 Hz, 1 H), 7.45 (t, J = 9 Hz, 1 H), 7.21 (t, J = 9 Hz, 1 H), 7.12 (t, J = 8 Hz, 1 H), 7.03 (d, J = 9 Hz, 1 H), 6.52 (dt, J = 9, 2 Hz, 1 H), 6.48 (dt, J = 8, 2 Hz, 1 H), 6.42 (s, 1 H), 5.05 (s, 1 H, OH), 4.27 (q, J = 8 Hz, 2 H), 1.23 (t, J = 8 Hz, 3 H); MS (EI) m/e 258 (p, 53), 213 (72), 121 (100); IR ($CHCl_3$, cm^{-1}) 3400 (b), 1712, 1602, 1579, 1498, 1302, 1140, 966. Anal. ($C_{15}H_{14}O_4$) C, H.

(e) Method E: General Procedure for Phenolic Alkylation. 2-[3-(2-Propenyloxy)phenoxy]benzoic Acid Ethyl Ester (9). A mixture of compound 8 (16.3 g, 63.2 mmol), allyl bromide (7.56 g, 62.5 mmol), potassium carbonate (8.70 g, 63.0 mmol), and potassium iodide (500 mg, 3.01 mmol) in 2-butanone (500 mL) was refluxed for 4 days. The mixture was cooled to room temperature and filtered. The filtrate was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo to provide 17.6 g (93%) of the desired product as an analytically pure oil. Anal. ($C_{18}H_{18}O_4$) C, H.

(f) Method F: General Procedure for Thermal Claisen Rearrangement. Mixture of 2-[3-Hydroxy-4-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (10a) and 2-[3-Hydroxy-2-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (10b). Compound 9 (17.6 g, 59.1 mmol) was heated neat at 200 °C for 3 h. After the mixture was cooled to room temperature, the residue was purified by HPLC to provide 12.5 g (71%) of a mixture of compounds **10a** and **10b** that was used in the subsequent reaction without separation. Anal. ($C_{18}H_{18}O_4$) C, H.

(g) Method G: General Procedure for Olefin Hydroboration/Oxidation. 2-[3-Hydroxy-4-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (11a) and 2-[3-Hydroxy-2-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (11b). A mixture of compounds **10a** and **10b** (6.86 g, 23.0 mmol) from the preceding procedure was dissolved in THF (200 mL) and treated with 0.5 M 9-BBN (70 mL, in THF). After the mixture was stirred for 18 h at room temperature, additional 0.5 M 9-BBN (10 mL, in THF) was added. The mixture was stirred for an additional hour, excess sodium acetate and hydrogen peroxide were added, and the resulting mixture was stirred for 1 h. The layers were separated, and the organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo. Purification of the residue by HPLC provided 2.86 g (39%) of **11a** and 1.93 g (27%) of **11b** as colorless oils.

11a: ^{1}H NMR ($CDCl_3$) 7.92 (d, J = 9 Hz, 1 H), 7.47 (t, J = 8 Hz, 1 H), 7.18 (t, J = 8 Hz, 1 H), 7.03 (m, 2 H), 6.47 (m, 2 H), 4.29 (q, J = 7 Hz, 2 H), 3.68 (t, J = 6 Hz, 2 H), 2.75 (t, J = 6 Hz, 2 H), 1.87 (quintet, J = 6 Hz, 2 H), 1.28 (t, J = 6 Hz, 3 H); MS (EI) m/e 316 (p, 86), 271 (100), 225 (26), 197 (31), 151 (24), 121 (52); IR ($CHCl_3$, cm^{-1}) 3350 (b), 1712, 1603, 1484, 1452, 1302, 976. Anal. ($C_{18}H_{20}O_5$) C, H.

11b: ^{1}H NMR ($CDCl_3$) 7.92 (d, J = 9 Hz, 1 H), 7.43 (t, J = 8 Hz, 1 H), 7.15 (t, J = 7 Hz, 1 H), 7.05 (t, J = 8 Hz, 1 H), 6.87 (d, J = 9 Hz, 1 H), 6.73 (d, J = 9 Hz, 1 H), 6.39 (d, J = 8 Hz, 1 H), 4.34 (q, J = 7 Hz, 2 H), 3.65 (t, J = 6 Hz, 2 H), 2.90 (t, J = 6 Hz, 2 H), 1.93 (t, J = 6 Hz, 2 H), 1.31 (t, J = 6 Hz, 3 H); MS (EI) m/e 316 (p, 56), 252 (57), 225 (86), 197 (100), 150 (96), 133 (74); IR ($CHCl_3$, cm^{-1}) 3350 (b), 1712, 1602, 1485, 1464, 1302, 1084, 987. Anal. ($C_{18}H_{20}O_5$) C, H.

(h) 2-[3-(Decyloxy)-4-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (12a). The desired product was prepared from compound **11a** as an oil in 59% yield using method E and substituting *n*-decyl iodide for allyl bromide/potassium iodide. Anal. ($C_{28}H_{40}O_5$) C, H.

(i) Method H: General Procedure for Alcohol Oxidation and Ester Formation. 2-(Decyloxy)-4-(2-carbethoxyphenoxy)benzenepropanoic Acid Ethyl Ester (13a). A mixture of compound **12a** (490 mg, 1.07 mmol) and Jones reagent (chromic acid solution, 1 mL) was stirred for 1 h. The mixture was diluted with ether, and the resulting solution was washed once with saturated sodium bisulfite solution. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was dissolved in absolute ethanol (5 mL), treated with concentrated sulfuric acid (3 drops), and refluxed for 18 h. The mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate, and the resulting solution was washed once with water. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by HPLC eluting with 5% ethyl acetate/95% hexane provided 110 mg (21%) of the desired product as an oil. Anal. ($C_{30}H_{42}O_6$) C, calcd: 72.26, found: 72.78; H, calcd: 8.49, found: 7.83.

(j) Method I: General Procedure for Xanthone Formation. 3-(Decyloxy)-5-carbethoxy-9-oxo-9*H*-xanthene-2-propanoic Acid Ethyl Ester (14a). A mixture of compound **13a** (700 mg, 1.41 mmol) and aluminum chloride (187 mg, 1.40 mmol) in methylene chloride (10 mL) was treated at room temperature with oxalyl chloride (178 mg, 1.40 mmol) for 1 h. The reaction mixture was poured onto a mixture of ice and dilute hydrochloric acid and stirred for an additional hour. The organic layer was separated, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was alkylated by using method E and substituting *n*-decyl iodide for allyl bromide/potassium

iodide. Purification via HPLC provided 141 mg (19%) of the desired product as a crystalline material: mp 61–63 °C; ^{1}H NMR ($CDCl_3$) 8.54 (d, J = 9 Hz, 1 H), 8.28 (d, J = 9 Hz, 1 H), 8.11 (s, 1 H), 7.44 (t, J = 8 Hz, 1 H), 6.93 (s, 1 H), 4.52 (q, J = 7 Hz, 2 H), 4.14 (m, 4 H), 3.05 (t, J = 6 Hz, 2 H), 2.68 (t, J = 6 Hz, 2 H), 1.89 (quintet, J = 6 Hz, 2 H), 1.48 (m, 5 H), 1.20–1.40 (m, 17 H), 0.88 (t, J = 5 Hz, 3 H); MS (EI) m/e 524 (p, 100), 451 (47), 339 (43), 311 (61); IR ($CHCl_3$, cm^{-1}) 2900, 1726, 1622, 1437, 1264. Anal. ($C_{31}H_{40}O_7$) C, H.

(k) Method J: General Procedure for Ester Hydrolysis. Compound 1. Compound **14a** (130 mg, 0.248 mmol) was dissolved in 2 mL of 1:1 ethanol/water and treated with excess potassium hydroxide at room temperature for 2 h. The mixture was concentrated in vacuo and then diluted with ethyl acetate and water. The aqueous phase was acidified, and the resulting precipitate was collected via vacuum filtration. Recrystallization from ethyl acetate/hexane provided 60 mg (52%) of the desired product as a crystalline material: mp 180–182 °C; ^{1}H NMR (DMSO-d_6) 8.37 (dd, J = 9, 2 Hz, 1 H), 8.22 (dd, J = 9, 2 Hz, 1 H), 7.95 (s, 1 H), 7.52 (t, J = 9 Hz, 1 H), 7.03 (s, 1 H), 4.23 (t, J = 7 Hz, 2 H), 2.90 (t, J = 7 Hz, 2 H), 2.55 (t, J = 7 Hz, 2 H), 1.81 (quintet, J = 6 Hz, 2 H), 1.48 (m, 2 H), 1.18–1.42 (m, 12 H), 0.83 (t, J = 5 Hz, 3 H); MS (EI) m/e 468 (p, 100), 310 (72), 283 (69), 269 (61). Anal. ($C_{27}H_{32}O_7$) C, H.

5-Carboxy-3-(decyloxy)-9-oxo-9*H*-xanthene-4-propanoic Acid (2). (a) 2-[3-(Decyloxy)-2-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (12b). The desired product was prepared from compound **11b** as an oil (pure via HPLC) in 84% yield by using method E and substituting n-decyl iodide for allyl bromide/potassium iodide. Anal. ($C_{28}H_{40}O_5$) C, calcd: 73.65, found: 74.42; H.

(b) 2-(Decyloxy)-6-(2-carbethoxyphenoxy)benzenepropanoic Acid Ethyl Ester (13b). The desired product was prepared from compound **12b** as an oil in 24% yield by using method H. Anal. ($C_{30}H_{42}O_6$) C, H.

(c) 3-(Decyloxy)-5-carbethoxy-9-oxo-9*H*-xanthene-4-propanoic Acid Ethyl Ester (14b). The desired product was prepared from compound **13b** as a crystalline solid in 34% overall yield by using method I: mp 69–70 °C. Anal. ($C_{31}H_{40}O_7$) C, H.

(d) Compound 2. The desired product was prepared from compound **14b** as a crystalline solid in 43% yield by using method J: mp >210 °C; ^{1}H NMR (DMSO-d_6) 8.33 (d, J = 8 Hz, 1 H), 8.24 (d, J = 8 Hz, 1 H), 8.05 (d, J = 9 Hz, 1 H), 7.47 (t, J = 8 Hz, 1 H), 7.22 (d, J = 9 Hz, 1 H), 4.16 (t, J = 6 Hz, 2 H), 3.15 (t, J = 7 Hz, 2 H), 2.50 (m, 2 H), 1.77 (quintet, J = 6 Hz, 2 H), 1.43 (m, 2 H), 1.15–1.38 (m, 12 H), 0.92 (t, J = 5 Hz, 3 H); MS (EI) m/e 467 (p – 1, 33), 421 (26), 310 (90), 282 (100), 269 (33), 127 (32); IR ($CHCl_3$, cm^{-1}) 2918, 1700, 1658, 1620, 1477, 1298, 1096. Anal. ($C_{27}H_{32}O_7$) C, H.

7-Carboxy-3-(decyloxy)-9-oxo-9*H*-xanthene-2-propanoic Acid (3). (a) 4-(3-Methoxyphenoxy)benzonitrile (15). The desired product was prepared from 4-bromobenzonitrile and 3-methoxyphenol as an oil in 36% yield by using method A. Anal. ($C_{14}H_{11}NO_2$) C, H, N.

(b) 4-(3-Methoxyphenoxy)benzoic Acid (16). The desired product was prepared from compound **15** as a crystalline solid by using method B: mp 117–119 °C. Anal. ($C_{14}H_{12}O_4$) C, H.

(c) 4-(3-Hydroxyphenoxy)benzoic Acid (17). The desired product was prepared from compound **16** by using method C. The crude product was used directly in the next step without further purification.

(d) 4-(3-Hydroxyphenoxy)benzoic Acid Ethyl Ester (18). The desired product was prepared from compound **17** as an oil in 72% yield (two steps from compound **16**) by using method D: ^{1}H NMR (DMSO-d_6) 9.83 (s, 1 H, OH), 7.97 (d, J = 9 Hz, 2 H), 7.23 (t, J = 8 Hz, 1 H), 7.05 (d, J = 9 Hz, 2 H), 6.63 (d, J = 8 Hz, 1 H), 6.53 (d, J = 8 Hz, 1 H), 6.46 (s, 1 H), 4.39 (q, J = 7 Hz, 2 H), 1.30 (t, J = 7 Hz, 3 H); MS (EI) m/e 258 (p, 66), 230 (18), 214 (20), 213 (100); IR ($CHCl_3$, cm^{-1}) 3350 (b), 1710, 1599, 1504, 1496, 1483, 1279, 1164, 964. Anal. ($C_{15}H_{14}O_4$) C, calcd: 69.76, found: 68.86; H.

(e) 4-[3-(2-Propenyloxy)phenoxy]benzoic Acid Ethyl Ester (19). Compound **18** (22.3 g, 86.4 mmol) was dissolved in dry dimethylformamide (250 mL) and treated carefully with sodium hydride (60% dispersion in mineral oil, 3.46 g) at room temperature. After the mixture was stirred for 1 h, allyl bromide (10.5 g, 86.7 mmol) was added, and the resulting mixture was maintained at 65 °C for 18 h. The mixture was cooled to room temperature, diluted with ethyl acetate, and washed twice with saturated sodium chloride solution. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo. Purification of the resulting residue via HPLC afforded 21.5 g (83%) of the desired product as an oil. Anal. ($C_{18}H_{18}O_4$) C, H.

(f) 4-[3-Hydroxy-4-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (20a) and 4-[3-Hydroxy-2-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (20b). The desired products were prepared from compound **19** (compound **20a**, 38% yield; compound **20b**, 23% yield) by using method F.

20a: oil; ^{1}H NMR (DMSO-d_6) 9.87 (s, 1 H, OH), 7.96 (d, J = 9 Hz, 2 H), 7.08 (d, J = 8 Hz, 1 H), 7.04 (d, J = 9 Hz, 2 H), 6.50 (m, 2 H), 5.96 (m, 1 H), 5.05 (m, 2 H), 4.28 (q, J = 8 Hz, 2 H), 3.27 (d, J = 7 Hz, 2 H), 1.31 (t, J = 7 Hz, 3 H); MS (EI) m/e 298 (p, 100), 253 (59), 133 (91); IR ($CHCl_3$, cm^{-1}) 3350 (b), 1709, 1600, 1502, 1278, 1163, 1110, 977. Anal. ($C_{18}H_{18}O_4$) C, H.

20b: mp 84–86 °C; ^{1}H NMR (DMSO-d_6) 9.85 (s, 1 H, OH), 7.92 (d, J = 9 Hz, 2 H), 7.09 (t, J = 8 Hz, 1 H), 6.95 (d, J = 9 Hz, 2 H), 6.74 (d, J = 9 Hz, 1 H), 6.47 (d, J = 9 Hz, 1 H), 5.79 (m, 1 H), 4.83 (m, 2 H), 4.29 (q, J = 7 Hz, 2 H), 3.23 (d, J = 7 Hz, 2 H), 1.30 (t, J = 7 Hz, 3 H); MS (EI) m/e 298 (p, 100), 269 (24), 253 (64), 225 (37), 197 (24), 147 (21); IR ($CHCl_3$, cm^{-1}) 3350 (b), 1709, 1605, 1505, 1463, 1280. Anal. ($C_{18}H_{18}O_4$) C, H.

(g) 4-[3-(Decyloxy)-4-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (21a). The desired product was prepared from compound **20a** as an oil in 48% yield by using method E and substituting n-decyl iodide for allyl bromide/potassium iodide. Anal. ($C_{28}H_{38}O_4$) C, H.

(h) 4-[3-(Decyloxy)-4-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (22a). The desired product was prepared from compound **21a** as an oil in 75% yield by using method G. Anal. ($C_{28}H_{40}O_5$) C, calcd: 73.65, found: 72.96; H, calcd: 8.83, found: 9.51.

(i) 2-(Decyloxy)-4-(4-carbethoxyphenoxy)benzenepropanoic Acid Ethyl Ester (23a). The desired product was prepared from compound **22a** as an oil in 23% yield by using method H. Anal. ($C_{30}H_{42}O_6$) C, calcd: 72.26; found: 71.66; H.

(j) 3-(Decyloxy)-7-carbethoxy-9-oxo-9*H*-xanthene-2-propanoic Acid Ethyl Ester (24a). The desired product was prepared from compound **23a** as an oil in 24% overall yield by using method I: ^{1}H NMR ($CDCl_3$) 9.01 (d, J = 2 Hz, 1 H), 8.34 (dd, J = 9, 2 Hz, 1 H), 8.08 (s, 1 H), 7.48 (d, J = 9 Hz, 1 H), 6.82 (s, 1 H), 4.43 (q, J = 7 Hz, 2 H), 4.13 (q, J = 7 Hz, 2 H), 4.07 (t, J = 6 Hz, 2 H), 3.03 (t, J = 6 Hz, 2 H), 2.66 (t, J = 6 Hz, 2 H), 1.88 (quintet, J = 6 Hz, 2 H), 1.15–1.63 (m, 20 H), 0.89 (t, J = 5 Hz, 3 H); MS (FD) m/e 524 (p); IR ($CHCl_3$, cm^{-1}) 3450 (b), 2929, 1718, 1658, 1464, 1224.

(k) Compound 3. The desired product was prepared from compound **24a** as a crystalline solid in 82% yield by using method J: mp >210 °C; ^{1}H NMR (DMSO-d_6) 12.63 (bs, OH), 8.69 (d, J = 2 Hz, 1 H), 8.30 (dd, J = 9, 2 Hz, 1 H), 7.94 (s, 1 H), 7.69 (d, J = 9 Hz, 1 H), 7.17 (s, 1 H), 4.15 (t, J = 7 Hz, 2 H), 2.87 (t, J = 7 Hz, 2 H), 2.74 (t, J = 7 Hz, 2 H), 1.78 (quintet, J = 7 Hz, 2 H), 1.47 (m, 2 H), 1.15–1.25 (m, 14 H), 0.85 (t, J = 7 Hz, 3 H); MS (EI) m/e 468 (p, 94), 310 (82), 283 (100), 269 (60); IR (KBr, cm^{-1}) 2850, 1709, 1693, 1661, 1622, 1463, 1431, 1258, 1133. Anal. ($C_{27}H_{32}O_7$) C, H.

7-Carboxy-3-(decyloxy)-9-oxo-9*H*-xanthene-4-propanoic Acid (4). (a) 4-[3-(Decyloxy)-2-(2-propenyl)phenoxy]benzoic Acid Ethyl Ester (21b). The desired product was prepared from compound **20b** as an oil in 74% yield by using method E and substituting n-decyl iodide for allyl bromide/potassium iodide. Anal. ($C_{28}H_{38}O_4$) C, H.

(b) 4-[3-(Decyloxy)-2-(3-hydroxypropyl)phenoxy]benzoic Acid Ethyl Ester (22b). The desired product was prepared from compound **21b** as an oil in 67% yield by using method G. Anal. ($C_{28}H_{40}O_5$) C, H.

(c) 2-(Decyloxy)-6-(4-carbethoxyphenoxy)benzenepropanoic Acid Ethyl Ester (23b). The desired product was prepared from compound **22b** as an oil in 62% yield by using method H: ^{1}H NMR ($CDCl_3$) 8.02 (d, J = 9 Hz, 2 H), 7.17 (t, J = 8 Hz, 1 H), 6.94 (d, J = 9 Hz, 2 H), 6.73 (d, J = 9 Hz, 1 H), 6.57 (d, J = 9 Hz, 1 H), 4.37 (q, J = 8 Hz, 2 H), 4.08 (q, J = 7 Hz, 2 H), 4.02 (t, J = 7 Hz, 2 H), 2.95 (t, J = 6 Hz, 2 H), 2.50 (t,

J = 6 Hz, 2 H), 1.84 (quintet, J = 6 Hz, 2 H), 1.17–1.58 (m, 20 H), 0.89 (t, J = 5 Hz, 3 H); MS (EI) m/e 498 (p, 66), 313 (39), 312 (100), 197 (24); IR ($CHCl_3$, cm^{-1}) 2920, 1712, 1606, 1585, 1505, 1451, 1280, 1243, 1163, 1076.

(d) 3-(Decyloxy)-7-carbethoxy-9-oxo-9*H*-xanthene-4-propanoic Acid Ethyl Ester (24b). The desired product was prepared from compound **23b** as an oil in 20% overall yield by using method I: ^1H NMR ($CDCl_3$) 9.04 (s, 1 H), 8.38 (d, J = 9 Hz, 1 H), 8.27 (d, J = 9 Hz, 1 H), 7.58 (d, J = 9 Hz, 1 H), 7.00 (d, J = 9 Hz, 1 H), 4.45 (q, J = 8 Hz, 2 H), 4.13 (m, 4 H), 3.31 (t, J = 7 Hz, 2 H), 2.64 (t, J = 7 Hz, 2 H), 1.87 (quintet, J = 6 Hz, 2 H), 1.15–1.55 (m, 20 H), 0.88 (t, J = 5 Hz, 3 H); MS (EI) m/e 524 (p, 57), 338 (100), 149 (32); IR ($CHCl_3$, cm^{-1}) 2929, 1719, 1659, 1609, 1439, 1369, 1277, 1253, 1084.

(e) Compound 4. The desired product was prepared from compound **24b** as a crystalline solid in 83% yield by using method J: mp >210 °C; ^1H NMR (DMSO-d_6) 12.78 (bs, OH), 8.65 (d, J = 2 Hz, 1 H), 8.27 (dd, J = 9, 2 Hz, 1 H), 8.03 (d, J = 9 Hz, 1 H), 7.69 (d, J = 9 Hz, 1 H), 7.17 (d, J = 9 Hz, 1 H), 4.13 (t, J = 6 Hz, 2 H), 3.10 (t, J = 6 Hz, 2 H), 2.42 (t, J = 6 Hz, 2 H), 1.75 (quintet, J = 6 Hz, 2 H), 1.40 (m, 2 H), 1.22 (m, 12 H), 0.82 (t, J = 6 Hz, 3 H); MS (EI) m/e 468 (p, 36), 310 (100), 269 (37); IR (KBr, cm^{-1}) 3300 (b), 2900, 1735, 1688, 1669, 1607, 1439, 1430, 1415, 1290, 1083. Anal. ($C_{27}H_{32}O_7$) C, H.

Alternate Synthesis of 7-Carboxy-3-(decyloxy)-9-oxo-9*H*-xanthene-4-propanoic Acid (4). (a) 3-Carbomethoxy-4-(3-methoxyphenoxy)benzoic Acid Methyl Ester (25). A mixture of 4-bromo-1,3-benzenedicarboxylic acid dimethyl ester (48.0 g, 0.180 mol), 3-methoxyphenol (21.8 g, 0.180 mol), copper bronze (17.2 g, 0.270 mol), and potassium carbonate (29.8 g, 0.216 mmol) in pyridine (750 mL) was thoroughly degassed with nitrogen and refluxed for 24 h. The mixture was cooled to room temperature, filtered, and concentrated in vacuo to reveal a black sludge. This material was dissolved in ethyl acetate, passed down a short column of Florisil (~500 cm^3), and washed twice with saturated copper sulfate solution. The organic phase was concentrated in vacuo to reveal a dark brown oil that was dissolved in methylene chloride and washed twice with 0.5 N aqueous sodium hydroxide solution. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to provide a brown oil. Purification via HPLC provided 17.7 g (32%) of the desired product as a crystalline solid: mp 89 °C. Anal. ($C_{17}H_{16}O_6$) C, H.

(b) 6-Methoxy-9-oxo-9*H*-xanthene-2-carboxylic Acid (26). Compound **25** (17.7 g, 56.0 mmol) was dissolved in 1:1 methanol/THF and treated at room temperature with aqueous 5 N sodium hydroxide solution (20 mL) for 24 h. The mixture was washed once with ether, and the aqueous phase was separated and washed once with methylene chloride. The aqueous phase was acidified with aqueous 5 N hydrochloric acid and extracted with methylene chloride. The second methylene chloride solution was dried over sodium sulfate, filtered, and concentrated in vacuo to reveal a white solid. This material was added to a solution of phosphorus pentoxide (30 g) dissolved in methane sulfonic acid (200 mL) and stirred at room temperature for 24 h.[17] The mixture was poured over crushed ice, resulting in the formation of a cream-colored precipitate that was collected immediately via suction filtration. The solid was dried at room temperature in vacuo to provide 12.7 g (84% for two steps) of the desired product: mp >250 °C. Anal. ($C_{15}H_{10}O_5$) C, H.

(c) 6-Hydroxy-9-oxo-9*H*-xanthene-2-carboxylic Acid Methyl Ester (27). The desired product was prepared from compound **26** as a white solid in 74% yield (two steps) by using method C, followed by method D, and substituting methanol for ethanol: mp >220 °C. Anal. ($C_{15}H_{10}O_5$) C, calcd: 66.67, found: 66.09; H.

(d) 3,3-Diethoxy-2,3-dihydro-7-oxo-1*H*,7*H*-pyrano[2,3-*c*]-xanthene-9-carboxylic Acid Methyl Ester (28). A mixture of compound **27** (3.54 g, 13.1 mmol), triethyl orthoacrylate (4.88 g, 26.2 mmol),[20] and pivalic acid (0.670 g, 6.55 mmol) in toluene (65 mL) was refluxed for 48 h.[18] Upon cooling to room temperature the desired product crystallized from the reaction mixture. The mixture was diluted with hexane and the crystals were collected via vacuum filtration to provide 4.90 g (94%) of the desired product: mp 191–193 °C; ^1H NMR ($CDCl_3$) 9.02 (d, J = 2 Hz, 1 H), 8.36 (dd, J = 9, 2 Hz, 1 H), 8.16 (d, J = 9 Hz, 1 H), 7.55 (d, J = 9 Hz, 1 H), 6.96 (d, J = 9 Hz, 1 H), 3.98 (s, 3 H), 3.79 (m, 4 H), 3.12 (t, J = 7 Hz, 2 H), 2.23 (t, J = 7 Hz, 2 H), 1.23 (t, J = 7 Hz, 6 H); MS (EI) m/e no parent ion, 284 (95), 283 (100), 266 (36), 224 (25); IR ($CHCl_3$, cm^{-1}) 2990, 1722, 1659, 1618, 1593, 1436, 1284, 1254, 1090, 1042. Anal. ($C_{22}H_{22}O_7$) C, H.

(e) 7-Carbomethoxy-3-hydroxy-9-oxo-9*H*-xanthene-4-propanoic Acid Ethyl Ester (29). A mixture of compound **28** (760 mg, 1.91 mmol) and aqueous 1 N hydrochloric acid (0.20 mL) in ethyl acetate (30 mL) was stirred at room temperature for 30 min. The mixture was diluted with ether and washed four times with saturated sodium bicarbonate solution. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to provide 670 mg (95%) of the desired product as a white solid: mp >215 °C; ^1H NMR ($CDCl_3$) 9.07 (s, 1 H, OH), 9.02 (d, J = 2 Hz, 1 H), 8.35 (dd, J = 9, 2 Hz, 1 H), 8.16 (d, J = 9 Hz, 1 H), 7.53 (d, J = 9 Hz, 1 H), 7.04 (d, J = 9 Hz, 1 H), 4.19 (q, J = 7 Hz, 2 H), 3.96 (s, 3 H), 3.21 (t, J = 7 Hz, 2 H), 2.92 (t, J = 7 Hz, 2 H), 1.23 (t, J = 7 Hz, 3 H); MS (EI) m/e 372 (p + 2, 14), 325 (100), 284 (36), 283 (38); IR ($CHCl_3$, cm^{-1}) 3200 (b), 1722, 1658, 1613, 1588, 1436, 1286. Anal. ($C_{20}H_{18}O_7$) C, H.

(f) 3-(Decyloxy)-7-carbomethoxy-9-oxo-9*H*-xanthene-4-propanoic Acid Ethyl Ester (30). A mixture of compound **29** (300 mg, 0.811 mmol), *n*-decyl iodide (239 mg, 0.892 mmol), and potassium carbonate (280 mg, 2.03 mmol) in dimethylformamide (10 mL) was stirred at room temperature for 48 h. The mixture was diluted with ether and washed three times with water. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to give crude product. Purification via silica gel chromatography provided 240 mg (65%) of the desired product as a white microcrystalline solid: mp 80 °C. Anal. ($C_{30}H_{38}O_7$) C, H.

(g) Compound 4. A mixture of compound **30** (220 mg, 0.431 mmol) and aqueous 5 N sodium hydroxide solution (1 mL) in 1:1 methanol/THF (10 mL) was stirred at room temperature for 18 h. The mixture was diluted with water and washed once with ether. The aqueous phase was acidified with aqueous hydrochloric acid, resulting in a white precipitate. Collection of this material via vacuum filtration and recrystallization from methanol provided 130 mg (64%) of the desired product that was identical in all respects to the sample prepared as described above.

7-Carboxy-3-[[6-(methoxyphenyl)-5(*E*)-hexenyl]oxy]-9-oxo-9*H*-xanthene-4-propanoic Acid (32). (a) 7-Carbomethoxy-3-[[6-(methoxyphenyl)-5(*E*)-hexenyl]oxy]-9-oxo-9*H*-xanthene-4-propanoic Acid Ethyl Ester (31). A mixture of compound **29** (290 mg, 0.784 mmol), 6-(4-methoxyphenyl)-5(*E*)-hexenyl methanesulfonate (244 mg, 0.862 mmol),[11b] and potassium carbonate (870 mg, 5.87 mmol) in 2-butanone (25 mL) was stirred at room temperature for 18 h. The mixture was diluted with ether and washed once with water. The organic phase was dried over sodium sulfate, filtered, and concentrated in vacuo to reveal a white solid. Recrystallization from hexane/ethyl acetate provided 270 mg (62%) of the desired product: mp 107–109 °C. Anal. ($C_{33}H_{34}O_8$) C, H.

(b) Compound 32. A mixture of compound **31** (270 mg, 0.484 mmol) and aqueous 5 N sodium hydroxide solution (1 mL) in 1:1 methanol/THF (10 mL) was stirred at room temperature for 24 h. The mixture was diluted with water and washed once with ether. The aqueous phase was acidified with aqueous hydrochloric acid, resulting in a white precipitate that was collected via vacuum filtration to provide 200 mg (80%) of the desired product: mp >215 °C; ^1H NMR (DMSO-d_6) 12.79 (bs, OH), 8.66 (s, 1 H), 8.28 (d, J = 9 Hz, 1 H), 8.04 (d, J = 9 Hz, 1 H), 7.70 (d, J = 9 Hz, 1 H), 7.28 (d, J = 7 Hz, 2 H), 7.19 (d, J = 9 Hz, 1 H), 6.82 (d, J = 8 Hz, 2 H), 6.33 (d, J = 16 Hz, 1 H), 6.10 (m, 1 H), 4.18 (t, J = 5 Hz, 2 H), 3.70 (s, 3 H), 3.12 (t, J = 7 Hz, 2 H), 2.48 (t, J = 7 Hz, 2 H), 2.20 (m, 2 H), 1.79 (m, 2 H), 1.58 (m, 2 H); MS (EI) m/e no parent ion, 311 (17), 190 (31), 148 (72), 122 (100); IR (KBr, cm^{-1}) 2907 (b), 1702, 1609, 1417, 1251, 1083. Anal. ($C_{30}H_{28}O_8$) C, H.

Pharmacological Methods. Inhibition of [^{3}H]LTB$_4$ Binding to Human Neutrophils. The effectiveness of compound **32** to inhibit binding of [^{3}H]LTB$_4$ to human neutrophils was measured by using an adaptation of a radioligand binding assay developed by Goldman and Goetzl.[21] Heparinized venous blood was drawn from normal volunteers, and neutrophils were isolated by standard techniques of Ficoll-Hypaque centrifugation, dextran 70 sedimentation, and hypotonic lysis. Cell preparations were

≥90% neutrophils and ≥90% viable. The binding assay was carried out in silanized 12- × 75-mm glass tubes by adding in the following order: 10 μL DMSO containing different amounts of test compound, 20 μL of radioligand (2.65 nM [^{3}H]LTB$_4$), and 500 μL of cells suspended in Hanks' balanced salt solution without calcium and magnesium containing 0.1% ovalbumin (2×10^7 cells/mL). Thus, the final concentration of [^{3}H]LTB$_4$ was 0.1 nM. A set of three replicate tubes was used for each treatment studied, and the results were averaged. Tubes were incubated at 4 °C for 10 min, and the reaction was terminated by isolating the cells with a Brandel MB-48R harvester. Radioactivity bound to the cells was measured by scintillation spectrometry. Nonspecific binding (3.1 ± 0.2%) was determined by measuring the amount of label bound when cells and [^{3}H]LTB$_4$ were incubated with a 10 000-fold excess of nonradioactive ligand. Appropriate corrections for nonspecific binding were made when analyzing the data. The average specific [^{3}H]LTB$_4$ binding to cells in a tube in the absence of any competitor was 5927 ± 503 dpm (n = 5). Results obtained with compound **32** are expressed as percent inhibition of specific [^{3}H]LTB$_4$ binding at the indicated concentrations. The concentration of DMSO in the incubation mixture (1.9%) had no effect on the binding of radioligands.

Inhibition of LTB$_4$-Induced Human Neutrophil Chemotaxis. Studies were carried out using chemotaxis chambers with a 200-μL blind-end stimulus compartment and fitted with 3 μM Poretics polyvinylpyrrolidone-free polycarbonate membranes. Human polymorphonuclear leukocytes were isolated from citrated venous blood drawn from normal volunteers and resuspended at a density of 12×10^6 cells/mL in Dulbecco's phosphate buffered saline, supplemented with 0.6 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM glucose, and 0.05% human serum albumin. The same buffer was used to make stock solutions of LTB$_4$ (20 nM) and antagonists. Equal amounts of a particular stock solution of antagonist and chemotactic agent were mixed, and 200 μL was added to the lower compartment. Equal parts of the human neutrophil suspension and the stock antagonist solution were mixed and 0.8 mL added to the upper compartment. Thus, the final concentration of LTB$_4$ in the lower compartment was 10 nM, and a total of 4.8×10^6 neutrophils were added to the upper compartment. The chemotaxis chambers were then incubated for 90 min at 37 °C. The number of cells that migrated completely through the filter and dropped into the lower compartment was determined with the use of a Sequoia-Turner Cell-Dyn 900 counter. Cells that passed through the filter by chemotaxis were calculated by subtracting, from the total migrated cells, the number that moved by random motion.

Inhibition of LTB$_4$- and FMLP-Induced Aggregation and Chemiluminescence of Human Neutrophils. A platelet-ionized calcium aggregometer (Chrono-log Corporation) was used to measure aggregation and chemiluminescence of peripheral human neutrophils. Cells (1×10^7 per mL) suspended in Dulbecco's phosphate buffered saline without calcium and magnesium, pH 7.4 (450 μL), were placed in a siliconized cuvette. Cytochalasin B (4.5 μL, 200 μg/mL) was added, and the contents were stirred at 900 rpm and 37 °C. After 2 min, antagonist (4.5 μL) was injected into the cuvette. Calcium and magnesium ions (4.5 μL, 100 mM Ca^{2+}, 50 mM Mg^{2+}) were then added. After another minute, luminol (4.5 μL, 10^{-4} M) and LTB$_4$ or FMLP (4.5 μL, 3 μM) were injected into the cuvette and the subsequent maximum amount of response occurring was measured with the aid of a Compaq 386/20e computer and software supplied by Chrono-log Corporation. Thus, the final concentration of LTB$_4$ or FMLP was 30 nM. Corrections were made for nonspecific aggregation or chemiluminescence occurring in the absence of agonist.

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