Synthesis and Pharmacological Characterization of a Series of Leukotriene Analogues with Antagonist and Agonist Activities[1]

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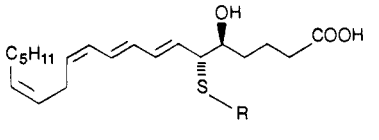
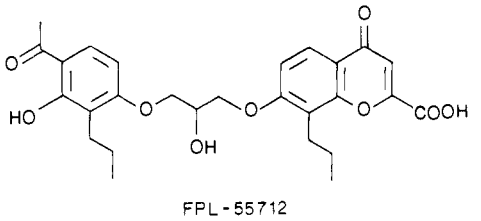
The synthesis and biological characterization of a series of novel leukotriene antagonists and agonists are reported. All of these compounds are derivatives of (5*S*,6*R*,7*Z*)-5-hydroxy-6-mercapto-9-phenyl-7-nonenoic acid. One of the more potent compounds is (5*S*,6*R*,7*Z*)-6-[[(4-carboxy-2-methoxyphenyl)methyl]thio]-5-hydroxy-9-(4-heptylphenyl)-7-nonenoic acid (**3f**). In vitro evaluation of this compound on guinea pig trachea revealed that it is a competitive antagonist of LTD_4 and LTE_4 with pK_B values of 6.4 and 5.8, respectively. On guinea pig ileum, the pK_B values obtained for it with LTD_4 and E_4 were both 7.2. The selectivity of **3f** was shown by its lack of effect on carbachol, histamine, and barium chloride concentration–response curves in guinea pig trachea.

The peptidoleukotrienes **1** have recently been the subject of intense research effort.[2] This interest was prompted by several findings. First, they were identified as the active components of the slow-reacting substances of anaphylaxis (SRS-A), the most potent known constrictors of airway smooth muscle.[3] Second, clinical evaluation with synthetic leukotrienes has provided evidence that they may be important mediators in allergic asthma.[4] As a result, efforts are under way to develop both antagonists of the leukotrienes and inhibitors of their biosynthesis.

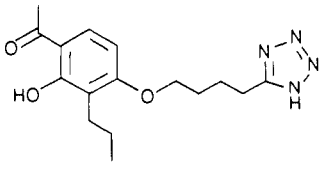
Chemical efforts have focused on two approaches to the synthesis of selective antagonists. The first approach is based on chemical modification of the hydroxyacetophenone FPL-55712, the first known SRS-A antagonist, which was reported in 1973.[5a] This approach led to many antagonists containing the hydroxyacetophenone (HAP) unit.[6] The clinical development of FPL-55712 was hampered by its lack of oral bioavailability and short biological half-life.[5b] The most successful antagonist containing a HAP moiety appears to be LY-171883, which is undergoing clinical evaluation.[7]



1: SR = glutathionyl, cysteinylglycyl, cysteinyl



FPL-55712



LY-171883

The second approach starts with the structures of the leukotrienes. These efforts have led to leukotriene-like agonists as well as leukotriene antagonists.[8] We recently reported a series, **2**, of chemically stable homocinnamyl analogues of the leukotrienes derived from such an approach.[8f] All of our analogues contained the "normal" peptide portions of either LTC_4, D_4, or E_4 and also had the "natural" 5*S*,6*R* stereochemistry. Their activities ranged from moderately potent agonist to weak antagonist. In an attempt to improve the antagonist activity of these homocinnamyl compounds, we decided to explore re-

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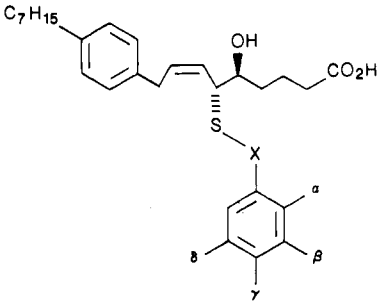
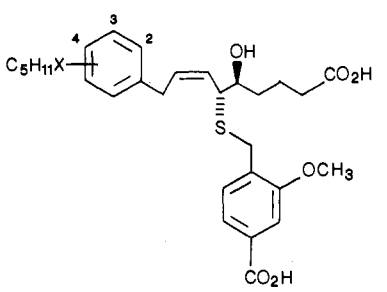
Table I. Structure and Chemical Data of Analogues **3a–q**



	substituent					% yield[a]			
3	X	α	β	γ	δ	ester 9	acid 3	mp, °C acid 3	formula[b]
a	CH_2	H	CO_2H	H	H	81	84	172–174	$C_{30}H_{38}O_5SLi_2 \cdot 1.0H_2O$
b	CH_2	H	H	CO_2H	H	91	93	>230 dec	$C_{30}H_{38}O_5SLi_2 \cdot 1.75H_2O$
c	CH_2	CO_2H	H	H	H	65	82	141–147	$C_{30}H_{38}O_5SLi_2 \cdot 2.75H_2O$
d	–	H	H	$NHCOCH_3$	H	69	74	93–115	$C_{30}H_{40}NO_4SK \cdot 3.5H_2O$
e	–	H	Cl	Cl	H	95	73	>185 dec	$C_{28}H_{35}O_3SCl_2K \cdot 2.0H_2O$
f	CH_2	OCH_3	H	CO_2H	H	89	61	140–152	$C_{31}H_{40}O_6SLi_2 \cdot 3.0H_2O$
g	CH_2	NO_2	H	CO_2H	H	32	43	203–205	$C_{30}H_{37}NO_7SLi_2 \cdot 3.0H_2O$[c]
h	CH_2	F	H	CO_2H	H		95	214–216	$C_{30}H_{37}O_5SFLi_2 \cdot 1.25H_2O$
i	CH_2	NH_2	H	CO_2H	H		50	223–226	$C_{30}H_{39}NO_5SLi_2 \cdot 1.5H_2O$
j	CH_2	H	NH_2	CO_2H	H		71	210–215	$C_{30}H_{39}NO_5SLi_2 \cdot 1.75H_2O$
k	CH_2	H	CO_2H	NH_2	H		72	157–163	$C_{30}H_{39}NO_5SLi_2 \cdot 2.0H_2O$
l	CH_2	NH_2	H	H	CO_2H		71	173–178	$C_{30}H_{39}NO_5SLi_2 \cdot 1.75H_2O$
m	CH_2	H	H	$_E$CH=CHCO_2H	H	32	74	206–210	$C_{32}H_{40}O_5SLi_2 \cdot 1.5H_2O$
n	CH_2	H	$_E$CH=CHCO_2H	H	H	46	60	134–139	$C_{32}H_{40}O_5SLi_2 \cdot 2.25H_2O$
o	–	H	CO_2H	H	H		72	>260 dec	$C_{29}H_{36}O_5SK_2 \cdot 1.0H_2O$
p	–	CO_2H	H	H	H		77	155–160	$C_{29}H_{36}O_5SLi_2 \cdot 2.0H_2O$
q	–	H	NH_2	H	H	90	70	68–71	$C_{28}H_{38}NO_3SK \cdot 0.25H_2O$

[a] Yield of ester 9 is only give for compounds in which is was isolated and purified. [b] Combustion analyses (C, H, N). [c] Calcd, H: 6.94. Found, H: 6.47.

Table II. Structure and Chemical Data of Analogues **3r–x**

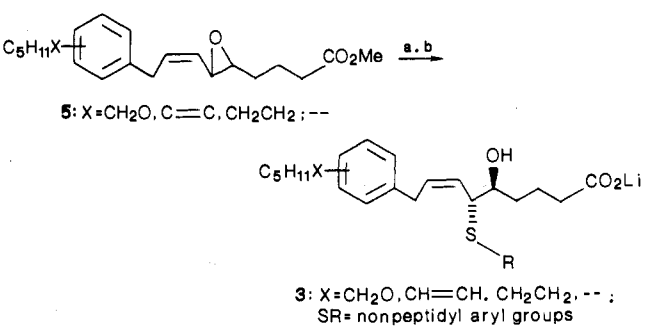


	position	X	% yield acid 3-	mp, °C acid 3-	formula
3r	3	CH_2CH_2	36	165–170	$C_{31}H_{40}O_6SLi_2 \cdot 2.0H_2O$
3s	4	CH_2O	99	163–167	$C_{30}H_{38}O_7SLi_2$
3t	3	$_Z$CH=CH	66	133–138	$C_{31}H_{38}O_6SLi_2 \cdot 3.0H_2O$
3u	3	CH_2O	72	168–173	$C_{30}H_{38}O_7SLi_2 \cdot 1.0H_2O$
3v	4	–	86	163–175	$C_{29}H_{36}O_6SLi_2 \cdot 1.0H_2O$
3w	2	CH_2O	72	160–165	$C_{30}H_{38}O_7SLi_2 \cdot 2.0H_2O$
3x	4	$_Z$CH=CH	88	155–159	$C_{31}H_{38}O_6SLi_2 \cdot 1.25H_2O$

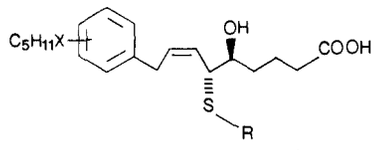
placement of their peptide region with nonpeptidyl groups. The results of those studies are presented in this report.

Our choices for the peptide replacement groups derived from an analysis we had made of the structural relationship between the peptidoleukotrienes and the HAP-type antagonists.[6d] This analysis suggested that the acidic portion of the HAP derivatives might be binding at the peptide recognition sites of the leukotriene receptor. Accordingly, we chose to replace the peptide region in our homocinnamyl analogues[8f] with the acid regions from a series of HAP-type antagonists. The initial aromatic acids (for compounds **3a–c**) came from an in-house series of HAP-derived antagonists,[6d] **4**, which we had worked on simultaneously to our previously reported analogue series, **2**.[8f]

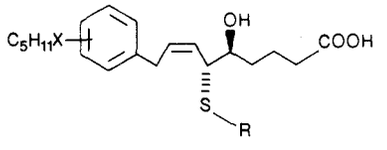
Scheme I[a]



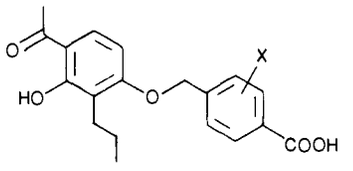
[a] (a) RSH, Et_3N/MeOH; (b) LiOH, MeOH–H_2O.



2: X = CH_2O, CH=CH, CH_2CH_2, --;
SR = glutathionyl, cysteinylglycyl, cysteinyl



3: X = CH_2O, CH=CH, CH_2CH_2, --;
SR = nonpeptidyl aryl groups



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We began by exploring compounds derived from 9-(4-heptylphenyl)-7-nonenoic acid, since this backbone had afforded our most potent agonists. The first compounds were **3a–i** (Table I). Additional structure–activity exploration led to compounds **3j–q** (Table I) and **3r–x** (Table II).

Chemistry

The compounds listed in Tables I and II were prepared as shown in Scheme I. This route is analogous to that used previously for our peptide-containing compounds and conveniently utilizes the previously reported epoxy esters **5**.[8f]

The route also requires access to thiols **6**. These thiols were either commercially available or, in the case of benzylic thiols, prepared from the corresponding toluene precursor via bromination and displacement of the derived benzylic bromide with thiolacetic acid and subsequent solvolysis, or synthesized by published protocols.

For example, thiol **6f** (on route to compound **3f**) was prepared from methyl 3-methoxy-4-methylbenzoate (**7f**) in two steps. Bromination of ester **7f** with bromine in carbon tetrachloride gave the benzyl bromide **8f** in 64% yield. Treatment of bromide **8f** with thiolacetic acid afforded thiol **6f** in 66% yield, via methanolysis of the intermediate thio ester in situ.

Most of the intermediate esters **9** were easily prepared by dissolving epoxy ester **5** and a slight excess of thiol **6** in a mixture of methanol and triethylamine. The resultant esters were, in many cases, used without purification but could be purified by flash chromatography on silica gel.[9]

(9) The structure of the intermediate esters **9**, if unpurified, were usually confirmed by ^1H NMR and mass spectral, analyses, which generally showed minor impurities. If purified, they were also analyzed by combustion analysis. The structures of the thiols **6** and the precursors to them were in general checked by ^1H NMR, IR, and mass spectral analyses. The structures of the test compounds **3a–y** were consistent by ^1H NMR, IR, and combustion analyses. The ionic nature of the isolated salts precluded mass spectral analysis on our instruments.

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Table III. Activity of Analogues **3a–x** on Guinea Pig Tracheal Spirals[e]

	% antagonism of 8nM LTE_4/% basal tension[a] at			
	50 μM	10 μM	5 μM	1 μM
FPL55712			62[g]	
3a		80[d]/32	37/5.8	16 ns
3b		37		
3c	[d]/30	39		
3d		4 ns		
3e		2 ns		
3f		74[d]	31	
3g		24	[d]/0	
3h	[d]/0	63		
3i	[d]/0	66		
3j	[d]/0	51		
3k		68		
3l		39		
3m		38		
3n		34		
3o		[b]/88		
3p		40/10.5		
3q		14 ns		
3r	[d]/0	52	45	
3s		[b]/100		
3t	[d]/0	57		7 ns
3u	[d]/14	86	32[c]	
3v		43		
3w		24		
3x	[d]/0	39		

[a] Basal tension is expressed as percent of an 8 nM LTE_4-induced contraction. [b] Agonist activity was too great to determine if there was any partial antagonism. [c] Determined at 3.3 μM. [d] Denotes that the compound was checked against $BaCl_2$ as agonist at this concentration. [e] Results were statistically significant ($p < 0.05$) except where indicated by ns. All tests were run on a minimum of four tracheal spirals. Reproducibility was, in general, ≤20% of the mean. [g] Determined at 4 μM.

As an example, the intermediate ester **9f**, involved in the preparation of compound **3f**, was obtained analytically pure in 89% yield after chromatography. The intermediate esters **9** were then hydrolyzed with lithium hydroxide in methanol/water. Purification of the resulting salts through reverse-phase chromatography (octyldecylsilyl packing (ODS), methanol/water as eluent) and lyophilization afforded compounds **3a–x** as hygroscopic powders in 30–90% yield. In the case of compound **3f**, the yield at this step was 61%, which corresponds to a 54% yield based on epoxy ester **5f**. However, if the intermediate purification of diester **9f** is omitted and the hydrolysis done directly on crude diester **9f**, compound **3f** is obtained in 79% yield based on the starting epoxy ester **5f**. Because of this improved yield, many of the intermediate esters were not purified and fully characterized.

The test compounds **3a–x**, like the peptide-containing analogues reported earlier,[8f] must be handled as their salts. This is because the free acids are chemically unstable and spontaneously form a γ-lactone between the C-1 carboxyl and the C-5 hydroxyl groups. The alkali metal salts, on the other hand, are stable at room temperature.

These salts were isolated by lyophilization of an aqueous solution to afford a residual powder. The powders were extremely hygroscopic and varied, batch to batch, in the amount of residual water. Although it was awkward to work with such samples, we did not succeed in finding suitable recrystallization conditions.

Biological Results and Discussion

The analogues were evaluated pharmacologically in vitro for their ability to inhibit the contraction induced by 8 nM LTE_4 on isolated guinea pig tracheal strips (see Table III). Furthermore, most of them were evaluated for specificity

of action at the same or higher concentration by using the same protocol with 1.5 mM $BaCl_2$ as the agonist. A significant increase in basal tension during the 10-min preincubation of the test compounds (i.e., before the agonist was added) suggested that these compounds had agonist activity.

In the series of toluic acids (**3a–c**), only the meta acid **3a** showed significant agonist activity at 10 μM whereas both of the benzoic acids **3o** and **3p** showed agonist activity. The ortho toluic acid **3c** also showed significant agonist activity when tested at a higher concentration (50 mM). Furthermore, for both the toluic and benzoic acid series, the ortho analogue had greater agonist activity than the meta analogue. Since we wanted to maximize the antagonist activity, we focused on modifying the para acid **3b**. We found that the addition of an ortho methoxy group **3f** moderately improved activity. We therefore made a series of three ortho-substituted para toluic acids, the nitro (**3g**), fluoro (**3h**), and amino (**3i**) analogues.

If the activity of this series was related to the effect of the substituent on the acidity of the carboxylic acid, we would have expected the rank order of activity to be NO_2 > F > OMe > H > NH_2.[13] This was not the order obtained. In addition, a multiple regression analysis of the data, comparing activity with the σ_m, π, and π_2 values of the aryl substituent, showed little correlation.[13] To probe further the ability of the receptor to recognize these nonpeptidyl pieces, we prepared compounds **3j–q**. These compounds had low affinity for the receptor as antagonists. Although the carboxylate group appeared necessary for activity, its location was relatively unimportant. On the other hand, the apparent correlation between agonist activity and the ortho and meta positions of the carboxylic acid, in both the benzoic acid and toluic acid series, combined with the fact that analogue **3k** did not show agonist activity, implied a greater degree of recognition was necessary to activate the receptor. Finally, the importance of a carboxylic acid for biological activity was confirmed by the lack of either agonist or antagonist activities by compounds **3d**, **3e**, and **3q**.

We next decided to vary the 4-heptyl substituent on the homocinnamyl benzene ring while keeping the methoxytoluic acid portion of compound **3f** constant. In the related peptidyl-substituted analogues, there had been a consistent decrease in activity from para- to meta- to ortho-substituted analogue for a given peptide. For the present compounds, modest decreases in antagonist activity and the absence of intrinsic activity were noted with the following changes: (i) incorporation of a double bond, **3x**; (ii) moving the substituent to the meta position, **3r**, and **3t**; and (iii) shortening the substituent to pentyl **3v**. In a series of hexyl ethers, agonist activity was apparent at a concentration of 10 μM for the para analogue **3s** but not in the meta or ortho analogues, **3u** and **3w**, respectively. The last mentioned was also much weaker as an antagonist. These studies were disappointing in that they neither showed a clear pattern nor provided much direction as to which future modifications might lead to greater antagonist activity.

To better assess the potential of this series compound **3f**, one of the more potent early analogues was evaluated pharmacologically in greater detail. Dissociation constants (pK_B values) were determined for this compound on guinea pig tracheal and/or ileal strips with use of leukotrienes as agonists. In these tissues, treatment with LTD_4, LTE_4, or LTC_4 over the concentration range of 10 pM–0.3 μM afforded a concentration–response curve. Pretreatment of the tissues with 10 μM of **3f** resulted in a parallel shift to the right of the leukotriene response curve, indicative of competitive antagonism. In particular, on guinea pig trachea, the pK_B values obtained with LTD_4 and LTE_4 were 6.43 ± 0.12 (mean ± SEM, n = 8) and 5.80 ± 0.09 (mean ± SEM, n = 8), respectively. It is known that a specific receptor exists for LTC_4 in guinea pig trachea. However, the rapid metabolism of LTC_4 to LTD_4 precludes measurement of in vitro activity at the LTC_4 receptor in the absence of L-serine borate as a metabolic inhibitor.[12] The pK_B value against LTC_4 in the presence of L-serine borate (45 nM) was 5.54 ± 0.18 (mean ± SEM, n = 6). On guinea pig ileum, the pK_B values against LTD_4 and LTE_4 were 7.25 ± 0.16 (mean ± SEM, n = 6) and 7.17 ± 0.13 (mean ± SEM, n = 6), respectively. The selectivity of **3f** for the leukotriene receptor was shown by its inability to affect the carbachol, histamine, and barium chloride concentration–response curves at concentrations that markedly antagonized the leukotriene-induced contractions. In addition, at no time did we see any contraction induced by **3f**. These findings corroborated the antagonism measured in our primary screen (Table III) and showed that analogue **3f** was a specific *reversible antagonist* of the peptidoleukotriene D/E receptor.

In conclusion, we have described a novel series of peptidoleukotriene analogues, the most characterized of which is compound **3f**. This antagonist was more potent in vitro than the first stable antagonist analogue of the leukotrienes ((4*R*,5*S*,6*Z*)-2-nor-LTD_1), which has a pA_2 of 5.2, obtained with LTD_4 on guinea pig trachea.[8a] It is comparable to the prototype LT antagonist FPL-55712, which has a pA_2 of 6.0–6.5 obtained with LTD_4.[10] However, it is significantly weaker than the analogues that have been recently reported by Perchonock.[8c]

Experimental Section

Synthetic Procedures. Melting points were taken on either a Fisher-Johns or a Thomas-Hoover melting point apparatus and are uncorrected. Analytical samples were homogeneous by TLC, IR, and ^1H NMR analyses and afforded results consistent with the assigned structures. In addition, nonionic intermediates were analyzed by mass spectroscopy. Analytical thin-layer chromatography (TLC) was performed either in normal phase on prelayered silica gel GHLF plates (Analtech, Newark, DE) or in reverse-phase mode (RP-TLC) on Whatman MKC 18 reversed-phase TLC plates. Visualization of the plates was accomplished by using UV light and/or phosphomolybdic acid–sulfuric acid charring. Infrared spectra (IR) were taken on either a Perkin-Elmer 727B or 781 spectrometer. Band locations are reported in frequency (cm^{-1}). Proton nuclear magnetic resonance spectra (NMR) were obtained by using either a Bruker WM-250, an IBM NR-80, or a Varian EM-360 spectrometer. Peak positions are reported in parts per million (δ), with use of tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument operating either in the electron impact (EI) or chemical ionization (CI) mode as indicated. Elemental analyses were performed on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of the theoretical values unless otherwise indicated. Flash chromatography was done either straight phase on Kieselgel 60, 230–400 mesh (E. Merck, Darmstadt, West Germany), or reversed phase on J. T. Baker octadecylsilyl (ODS) packing material, 40 μM.

All organic starting materials and reagents were obtained from Aldrich Chemicals and were used without any additional purification unless otherwise indicated. Solvents used were either reagent or HPLC grade and were obtained from either Fisher Scientific or J. T. Baker Chemical Co. Triethylamine (TEA) was distilled from CaH_2 and stored over KOH pellets under nitrogen.

Unless otherwise indicated, all reactions were carried out under an inert atmosphere of nitrogen with vigorous magnetic stirring.

(13) Values for σ_m and π were obtained from *Burger's Medicinal Chemistry*, 4th ed.; Wolf, M., Ed.; Wiley: New York, 1980; Part 1, pp 401–402.

Methyl 4-(Bromomethyl)-3-methoxybenzoate (8f). A stirred solution of 121.2 g (0.67 mol) of methyl 4-methyl-3-methoxybenzoate in 1.4 L of carbon tetrachloride was heated under gentle reflux with a 350-W tungsten lamp and subjected to an air purge by means of a T-tube attached to a water aspirator. A solution of 107.2 g (0.67 mol) of bromine in 500 mL of carbon tetrachloride was added dropwise over 4 h. Evaporation of the solvent gave a light, yellow solid, which was triturated with 500 mL of 1/10 diethyl ether/hexane. The solid was collected by filtration to give 111.7 g (64% yield) of methyl 4-(bromomethyl)-3-methoxybenzoate, **8f**, as a light, yellow solid: mp 87–89 °C; NMR ($CDCl_3$, 60 MHz) δ 3.9 (2 s, 6 H), 4.5 (s, 2 H), 7.4 (m, 3 H).

Methyl 4-(Mercaptomethyl)-3-methoxybenzoate (6f). A 500-mL flask was charged with 26.9 g (0.104 mol) of methyl 4-(bromomethyl)-3-methoxybenzoate, 17.3 g (0.125 mol) of potassium carbonate, and 250 mL of dry degassed methanol. After this mixture was cooled to 5 °C, 11.4 g (0.15 mol) of thiolacetic acid was added over 10 min. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature and stir overnight under nitrogen. By the next morning, a precipitate had formed; the reaction mixture was filtered, the solids were washed with more methanol, and the combined methanol solutions were concentrated in vacuo. The residual oil was partitioned between 300 mL of water and 300 mL of diethyl ether. The layers separated, and the aqueous layer was extracted with diethyl ether (2 × 250 mL). The combined diethyl ether extracts were back washed with 100 mL of water, dried over sodium sulfate, filtered, and concentrated to afford 21.3 g of crude thiol **6f** as a yellow oil. This oil was purified by chromatography on a $1^1/_2$ in. × 12 in. silica gel column with 5/95 diethyl ether/hexanes as eluent. Combination and concentration of the appropriate fractions afforded 16.1 g of thiol **6f**. This was further purified by distillation at 125–130 °C (0.5 mm), giving 14.45 g (66% yield) of analytically pure thiol **6f** as a light yellow oil: R_f 0.29 (1/5 diethyl ether/hexane); IR (CCl_4) 1735, 1290, 1230 cm^{-1}; NMR ($CDCl_3$, 60 MHz) δ 1.9 (t, 1 H, 8 Hz), 3.7 (d, 2 H, 8 Hz), 3.93 (s, 3 H), 7.31 (d, 1 H, 6 Hz), 7.55 (s, 1 H), 7.63 (d, 1 H, 6 Hz); MS-EI, m/e M^+ 212 (60), 179 (100), 149 (45). Anal. ($C_{10}H_{12}O_3S$) C, H.

(5*S*,6*R*,7*Z*)-6-[[(4-Carbomethoxy-2-methoxyphenyl)methyl]thio]-5-hydroxy-9-(4-heptylphenyl)-7-nonenoic Acid Methyl Ester (9f). A 50-mL flask was charged with 91 mg (0.25 mmol) of (5*S*,6*S*,7*Z*)-methyl-5,6-oxido-9-(4-heptylphenyl)-7-noneoate,[8f] **5f**, 1.0 mL of methanol, 0.2 mL of triethylamine, and 108 mg (0.51 mmol) of thiol **6f**. The reaction mixture was stirred under N_2 overnight. The next morning the solvents were removed in vacuo, and the residual solid was purified by chromatography on 9.7 g of silica gel, eluting with a gradient solvent system starting with methylene chloride and changing to 1/10 diethyl ether/methylene chloride as the eluent. Combination of the appropriate fractions afforded 126 mg (89% yield) of the diester **9f**: R_f 0.6 (1/10 diethyl ether/methylene chloride); IR ($CHCl_3$) 1745, 1720 cm^{-1}; MS-CI, m/e 553 (P + 1 - H_2O, 30), 179 (100); NMR ($CDCl_3$, 250 MHz) δ 0.86 (t, J = 7.5 Hz, 3 H), 1.2–1.4 (m, 8 H), 1.5–1.9 (m, 6 H), 2.3 (t, J = 7.5 Hz, 2 H), 2.46 (d, J = 4 Hz, 1 H), 2.54 (t, J = 7.5 Hz, 2 H), 3.23 (d, J = 7.0 Hz, 2 H), 3.65 (s, 3 H), 3.7–3.8 (m, 4 H), 3.85 (s, 3 H), 3.89 (s, 3 H), 5.52 (br t, J = 10 Hz, 1 H), 5.77 (dt, J = 7.0, 10 Hz, 1 H), 7.0 (d, J = 7 Hz, 2 H), 7.07 (d, J = 7 Hz, 2 H), 7.32 (d, J = 8 Hz, 1 H), 7.51 (d, J = 2 Hz, 1 H), 7.59 (dd, J = 2, 8 Hz, 1 H). Anal. ($C_{33}H_{46}O_6S$) C, H.

(5*S*,6*R*,7*Z*)-6-[[(4-Carboxy-2-methoxyphenyl)methyl]thio]-5-hydroxy-9-(4-heptylphenyl)-7-nonenoic Acid (3f). To 91.8 mg (0.16 mol) of diester **9f** dissolved in 1.0 mL of methanol and 0.5 mL of tetrahydrofuran was added a solution of 22.6 mg (0.54 mmol) of lithium hydroxide dissolved in 0.5 mL of water. After 22 h at room temperature, the reaction mixture was acidified to pH 7.5 with dilute acetic acid and partially concentrated to remove the tetrahydrofuran. The resultant solution was purified via chromatography on a 1.5 cm × 12 cm ODS column with 65/35 methanol/water as the eluent. The appropriate fractions were combined and first concentrated to remove the methanol and then lyophilized to afford 60 mg (61% yield) of **3f** dilithium salt trihydrate as a white hygroscopic powder: mp 140–152 °C; NMR ($CDCl_3$, 250 MHz) δ 0.89 (t, J = 7.5 Hz, 3 H), 1.2–1.4 (m, 8 H), 1.5–1.9 (m, 6 H), 2.17 (t, J = 7.5 Hz, 2 H), 2.55 (t, J = 7.5 Hz, 2 H), 3.23 (d, J = 7.0 Hz, 2 H), 3.65–3.85 (m, 4 H), 3.88 (s, 3 H), 5.54 (br t, J = 10 Hz, 1 H), 5.70 (dt, J = 7.0, 10 Hz, 1 H), 7.05 (m, 4 H), 7.29 (d, J = 8 Hz, 1 H), 7.52 (d, J = 8 Hz, 1 H), 7.58 (br s, 1 H). Anal. ($C_{31}H_{40}O_6SLi_2 \cdot 3H_2O$) C, H.

Biological Evaluation Procedures. Guinea pigs were killed by a sharp blow to the head, and the trachea were removed and cut into spiral strips. Each trachea was divided into two sections for paired experiments. Each section was placed in a jacketed 10-mL tissue bath maintained at 37 °C and bathed with modified Kreb's buffer, which was bubbled with 95% O_2 and 5% CO_2. The Kreb's buffer consisted of the following composition (mM): NaCl (119), KCl (4.6), $CaCl_2$ (1.8), $MgCl_2$ (0.5), $NaHCO_3$ (24.9), NaH_2PO_4 (1.0), and glucose (11.1). The bath fluid also contained indomethacin (5 μM). Isometric tension was monitored via a Grass force displacement transducer and displayed on a Beckman dynograph (Model R 612). Resting tension was set at 2 g, and the tissues were allowed to stabilize for 60 min as the bath fluid was changed every 15 min.

The ability of these test compounds to inhibit the LTE_4 (8 nM) contractile response was assessed as follows. After the 60-min equilibration period, the tissues were challenged with 8 nM LTE_4 for 10 min, and the responses were recorded. A 60-min reequilibration period followed during which the tissues were washed and allowed to return to base line. The challenges and reequilibration period were repeated. The test compound was added to the bath at selected concentrations for 10 min. Any significant change in resting tension after the 10-min incubation period was noted. In the presence of test compounds, the tissues were challenged with LTE_4, and the contractile response was recorded. The paired sections of trachea received vehicle to serve as control. Percent inhibition was determined by the following equation

$$\% \text{ inhibition} = (\text{2nd } LTE_4 - \text{3rd } LTE_4)/\text{2nd } LTE_4 \times 100$$

An adjusted percent inhibition was determined by subtracting the percent inhibition obtained with the vehicle treated tissues from that obtained with the drug treated tissues. Significant differences ($p < 0.05$) between the contractile response of the second and third LTE_4 challenges were determined by using the Student's paired t test. To determine specificity of these compounds as leukotriene antagonists, a similar protocol was established where $BaCl_2$ (1.5 mM) was substituted for LTE_4 as the agonist.

Cumulative concentration–response curves were obtained by successive microliter increases in the bath concentration of the agonist. Contractile responses are expressed as a percentage of the response obtainable to a maximally effective concentration of carbachol (30 μM), which was added to the bath after the stabilization period. Following the carbachol challenge, the tissues were washed and allowed 60 min to restabilize before the concentration–response curves were begun. EC_{50} values, the concentration of agonist necessary to produce a contraction equal to 50% of the maximal response, were derived by linear regression. The leukotriene antagonist **3f** was incubated for 30 min prior to starting the concentration–response curves. EC_{50} values were determined in the absence and presence of antagonist and significance ($p < 0.05$) determined using the Student's paired t test. K_b values for the antagonist were determined according to the method of Furchott.[11]

Experiments with isolated guinea pig ileum were prepared as follows: A segment of terminal ileum was removed, the lumen was cleaned, and the tissue was cut into smaller segments of approximately 1–2 cm. Each segment was tied to the bottom of a tissue holder, leaving the lumen open. The ilea were transferred to the tissue baths and attached to transducers by means of thread. Ilea were equilibrated for approximately 60 min under a maintained resting tension of 0.5 g. Ilea were bathed in Tyrodes buffer, which contains atropine sulfate (0.5 μM) and pyrilamine maleate (0.5 μM).

Two sequential concentration–response curves were obtained for LTD_4 and LTE_4. The first curve was control, followed 60 min later by a second curve obtained in either the presence of vehicle or selected concentrations of **3f**. EC_{50} values and K_b values were determined as described above.

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