Leukotriene B_4 (LTB_4) Receptor Antagonists: A Series of (Hydroxyphenyl)pyrazoles

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A series of (hydroxyphenyl)pyrazoles was designed by molecular modeling comparison with the LTB_4 structure and prepared for evaluation as LTB_4 receptor antagonists, culminating in 4-ethyl-5-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]-2-(1*H*-pyrazol-3-yl)phenol (**2**). Using an assay for inhibition of specific [^{3}H]LTB_4 binding to human PMN, it was found that the pyrazole ring could be methylated at N(1) with little loss of activity while methylation at N(2) reduced activity significantly. The structure–activity relationship of the terminal acid group was investigated. Good activity was found with *o*- and *m*-phenylalkanoic acids, chromane carboxylic acid, and tetrazole groups. The best *in vitro* activity was realized with the pyrazole nitrogen unsubstituted and with a six-carbon chain linking the phenyl ether oxygen to the tetrazole group. Compound **2**, having an IC_{50} of 6.4 ± 0.8 nM in the binding assay, was selected for further preclinical evaluation.

Introduction

We recently reported[1] the development of a series of hydroxyacetophenone LTB_4 receptor antagonists represented by **1** (LY255283) (Figure 1). This compound at high doses has shown promising activity in animal models of inflammation.[2] In order to discover a more potent LTB_4 receptor antagonist which might be active at lower doses in our disease models, we designed a new series of compounds to be a better fit to the LTB_4 receptor than compound **1**. We proposed[1] that the acetophenone moiety of compound **1** fits the part of an LTB_4 receptor that binds the triene unit of LTB_4. If this is correct, the acetophenone must be a loose fit to the receptor because the acetophenone unit is only approximately 4.8 Å long (Figure 1) while the triene unit of LTB_4 is approximately 6.2 Å long. We reasoned that replacing the acetyl moiety of **1** with a slightly larger group would give a closer fit to the receptor and that the closer fit might lead to tighter binding because of additional attractive van der Waals interactions between the antagonist and the receptor. The phenylpyrazole group was chosen as our first acetyl replacement because it is close to the length of the LTB_4 triene unit (6.4 Å for **2** vs 6.2 Å for LTB_4) and is readily available synthetically from the corresponding acetophenone.

The ability of compounds to inhibit specific binding of [^{3}H]LTB_4 to human peripheral neutrophils was used as an assay to identify leads and then as a convenient test in subsequent structure–activity relationship (SAR) studies. Exploration of the SAR in the (hydroxyphenyl)-pyrazole series led to the discovery of several new compounds (**2**, **11**, **12**, **15**) that bind to human LTB_4 receptors significantly more tightly than acetophenones such as **1**.

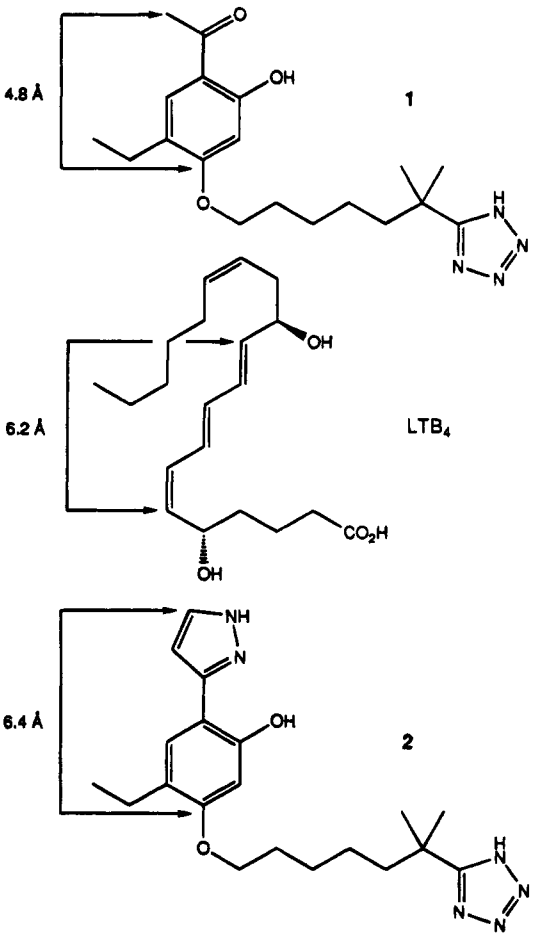

Figure 1. Structures of compounds **1**, **2**, and LTB_4, with estimates of the lengths of the triene equivalents.

Chemistry

The general synthetic pathways for the preparation of the compounds reported are shown in Schemes 1–4. Phenylpyrazoles were prepared by treating the corresponding acetophenones with dimethylformamide (DMF) diethyl acetal at elevated temperature to provide the dimethyl enamino ketones. The enamino ketones were converted to the phenylpyrazoles by treatment with hydrazine or methylhydrazine.[3]

Treatment of the enamino ketone with methylhydrazine gives a mixture of two methylpyrazoles, **3** and **4** (Scheme 1), in approximately equal amounts. The

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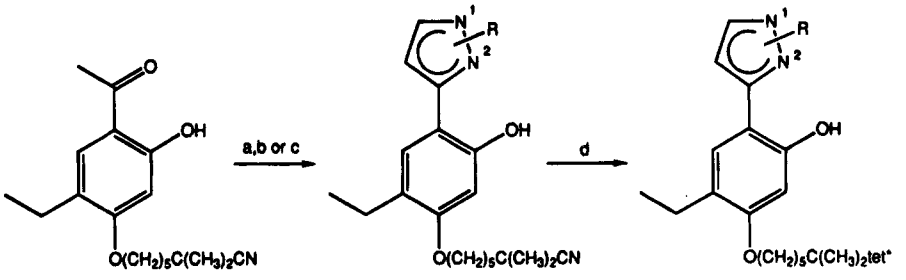
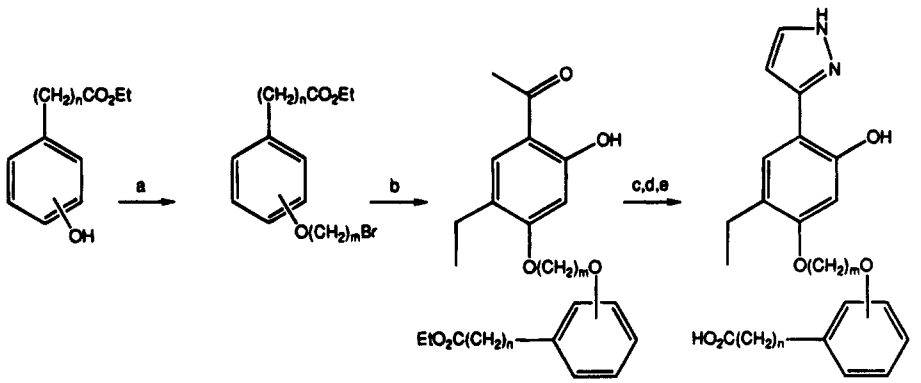
Scheme 1 [a]



18	19 : R = H	2 : R = H
	20 : R = 1-CH_3	3 : R = 1-CH_3
	21 : R = 2-CH_3	4 : R = 2-CH_3

[a] (a) DMF diethyl acetal/Δ; (b) hydrazine hydrate/EtOH/Δ; (c) methylhydrazine/EtOH/Δ; (d) (n-Bu)$_3$SnN$_3$/DEE/Δ. *Abbreviations: tet = 5-(1H-tetrazole).

Scheme 2 [a]



22 : n = 0, *para*	28 : m = 5, n = 0, *para*	36 : m = 5, n = 0, *para*	5 : m = 5, n = 0, *para*
23 : n = 1, *para*	29 : m = 5, n = 1, *para*	37 : m = 5, n = 1, *para*	6 : m = 5, n = 1, *para*
24 : n = 1, *meta*	30 : m = 3, n = 1, *para*	38 : m = 3, n = 1, *para*	7 : m = 3, n = 1, *para*
25 : n = 1, *ortho*	31 : m = 5, n = 2, *para*	39 : m = 5, n = 2, *para*	8 : m = 5, n = 2, *para*
26 : n = 2, *para*	32 : m = 3, n = 2, *para*	40 : m = 3, n = 2, *para*	9 : m = 3, n = 2, *para*
27 : n = 2, *meta*	33 : m = 3, n = 1, *ortho*	41 : m = 3, n = 1, *ortho*	10 : m = 3, n = 1, *ortho*
	34 : m = 3, n = 1, *meta*	42 : m = 3, n = 1, *meta*	11 : m = 3, n = 1, *meta*
	35 : m = 3, n = 2, *meta*	43 : m = 3, n = 2, *meta*	12 : m = 3, n = 2, *meta*

[a] (a) Br(CH$_2$)$_3$Br/K$_2$CO$_3$/KI/Δ; (b) **45**/K$_2$CO$_3$/KI/Δ; (c) DMF diethyl acetal/Δ; (d) hydrazine hydrate/EtOH/Δ; (e) NaOH.

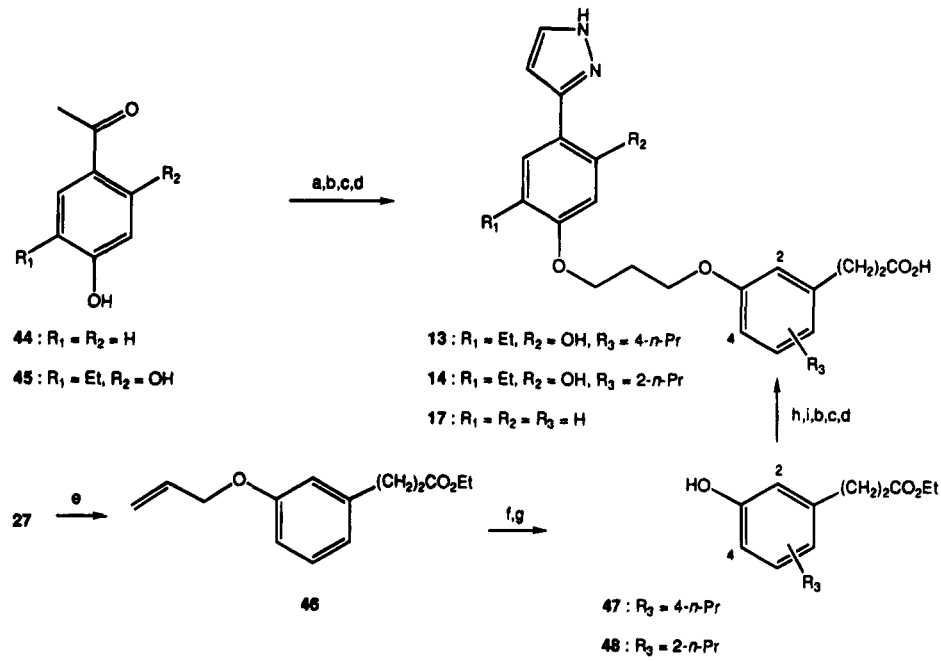
structures were assigned on the basis of the NMR and UV data. The NMR chemical shifts of the two doublets assigned to the pyrazole ring protons of the N(1) methyl compound **3** (∂ 7.78 and 6.70) are very similar to those of the unmethylated pyrazole **2** (∂ 7.88 and 6.70). In contrast, the chemical shifts of the two doublets assigned to the pyrazole ring protons of the N(2) methyl compound **4** (∂ 7.45 and 6.10) are quite different. The upfield shift of one of the pyrazole protons in **4** is consistent with the phenyl and pyrazole rings being noncoplanar in **4**, placing one proton on the pyrazole ring in the shielding region of the phenyl ring. The UV spectra of **3** (λ_{max} = 300, 263, and 211 nm) and of **2** (λ_{max} = 301, 260, and 213 nm) are also very similar, while the spectrum of **4** shows a pronounced shift to shorter wavelengths (λ_{max} = 289, 244, and 206 nm), consistent with the phenyl and pyrazole rings being more nearly coplanar in **2** and **3** than in **4**. AM1 calculations carried out on a model series (3-(hydroxyphenyl)-1H-pyrazole) and the N(1) and N(2) methyl analogs suggest that the phenyl and pyrazole rings of **4** are closer to orthogonal (dihedral angle ≈ 66°) than they are in **2** and **3** (dihedral angles ≈ 46°).

Results and Discussion

The compounds prepared and their activities in the receptor binding test are listed in Table 1.

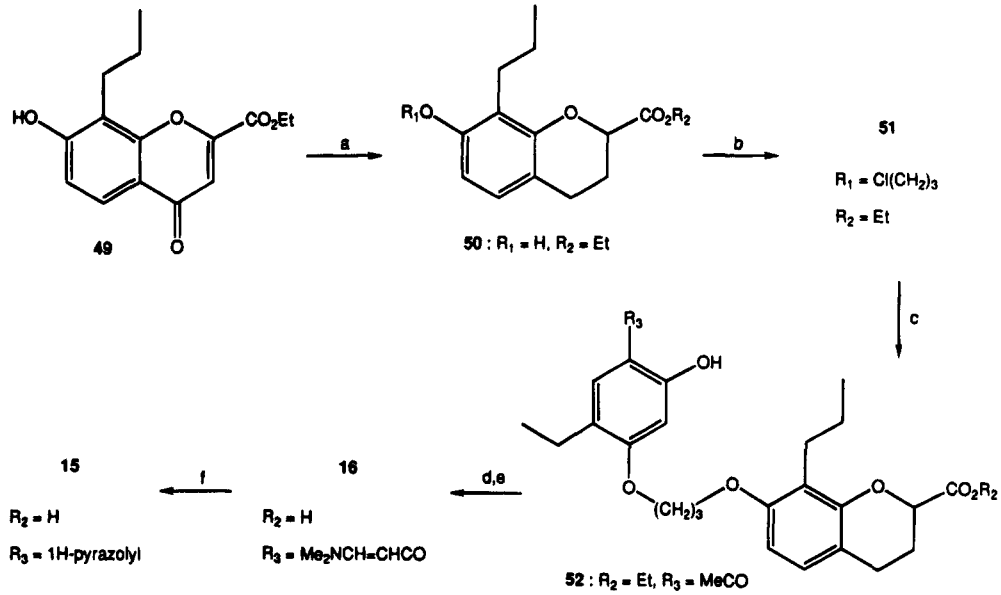
Comparison of the IC_{50}'s of compounds **1** and **2** shows that receptor binding activity is increased approximately 13-fold when the acetyl group of **1** is replaced by the 3-pyrazole ring, supporting the hypothesis that the phenylpyrazole may bind at the triene binding site of the LTB_4 receptor. While this work was in progress, Djuric and Penning[4] reported that similar substitutions of a five-membered ring heterocycle for an acetyl group in the series of 3,4-substituted 2-methoxyacetophenone LTB_4 receptor antagonists represented by **53** (SC-41930, Figure 2) also yielded compounds with increased potency. It was not obvious *a priori* that this substitution would have the same effect in both series, since the

Scheme 3 [a]



[a] (a) **35**/K_2CO_3/KI/MEK; (b) DMF diethyl acetal/Δ; (c) hydrazine hydrate/EtOH/Δ; (d) NaOH; (e) $H_2C{=}CHCH_2Br/K_2CO_3$/KI/MEK; (f) Δ; (g) H_2/Pd/EtOH; (h) $Br(CH_2)_3Br/K_2CO_3$/KI/Δ; (i) **45**/K_2CO_3/KI/Δ.

Scheme 4 [a]



[a] (a) H_2/Pd/HOAc; (b) $Cl(CH_2)_3Br/K_2CO_3$/KI/DMF; (c) **45**/DMSO/MEK/K_2CO_3/KI/Δ; (d) DMF diethyl acetal/Δ; (e) NaOH/EtOH; (f) hydrazine hydrate/EtOH/Δ.

SAR's of the 4,5-substituted 2-hydroxyacetophenones represented by **1** and the 3,4-substituted 2-methoxyacetophenones represented by **53** are known to differ significantly.[5] The fact that substitution of pyrazole for the acetyl group in both series has the same effect on potency suggests that the acetophenones of both series may bind to the LTB_4 receptor as analogs of the triene unit of LTB_4. Possible receptor binding analogies between **1**, **53**, and LTB_4 are suggested in Figure 2. These suggested binding analogies are consistent with the requirement that the phenolic hydroxyl must be alkylated in the 2,3,4-substituted acetophenones while the phenolic hydroxyl must be free in the 2,4,5-substituted series.[5] The knowledge gained in this work, that ring structures could be substituted for the acetyl group in the 2,4,5-substituted acetophenone series, led us directly to the synthesis of the biphenyl class of LTB_4 receptor antagonists, preliminary reports of which have been made recently by other members of our research group.[6]

Methylation of the pyrazole at N(1) (**3**; Scheme 1) had little effect on activity, but methylation at N(2) (**4**) significantly decreased activity. The retention of activity by **3** indicates that a pyrazole NH is not necessary for activity and that the methyl group on N(1) of the pyrazole does not sterically hinder binding of **3** to the LTB_4 receptor. The loss of activity observed in **4** may be due to a greater deviation from coplanarity of the phenyl and pyrazole rings in **4** compared to that in **3**, as suggested by our AM1 calculations. It is also possible that the methyl group on N(2) of the pyrazole sterically hinders binding of **4** to the LTB_4 receptor.

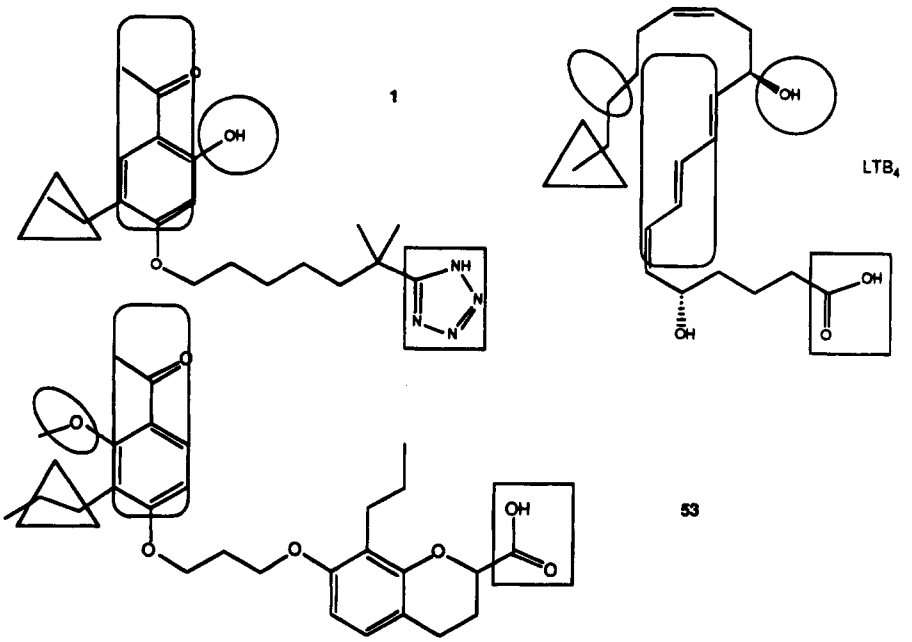


Figure 2. Possible receptor binding analogies between **1**, **53**, and LTB_4.

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

compound	IC_{50} (nM)[a]	compound	IC_{50} (nM)[a]
1	85.1 ± 7.9	**10**	12
2	6.4 ± 0.8	**11**	11
3	9.3	**12**	5.7
4	107	**13**	738
5	82	**14**	834 ± 72
6	55	**15**	4.5
7	54	**16**	319
8	28	**17**	>1000
9	87		

[a] In each experiment, three replicate measurements were made of the extent of inhibition at each compound concentration studied and the IC_{50}'s were calculated assuming linearity from 10% to 90% inhibition. The IC_{50}'s for **1**, **2**, and **14** are the mean ± SEM of values obtained using cell suspensions from three, five, and two individuals, respectively. The IC_{50}'s for **3**–**13** and **15**–**17** were obtained using cells from one individual. The estimated SEM for the latter IC_{50}'s is ±15%.

In pyrazoles containing a phenylacetic acid group (**6** and **7**; Scheme 2), carbon chains of either three or five atoms connecting the two ether oxygen atoms gave the same activity. Examination of molecular models of **6** and **7** suggests that simple staggered conformations of carbon chains of three and five atoms can position one carboxylate oxygen atom of each of these two compounds identically. In pyrazoles containing a phenylpropionic acid group (**8** and **9**; Scheme 2), however, a carbon chain of five atoms connecting the two ether oxygen atoms gave slightly better activity than the carbon chain of three atoms. Examination of molecular models of **8** and **9** suggests that simple staggered conformations do not position carboxylate oxygen atoms of these two compounds identically.

The regiochemistry of the phenylalkanoate acid groups had a clear effect on activity. The *ortho*- and *meta*-substituted isomers (**10** and **11**; Scheme 2) were more active than the *para*-substituted isomer (**7**; Scheme 2). Examination of molecular models of these compounds shows that one carboxylate oxygen atom of **10** and one carboxylate oxygen atom of **11** can be positioned identically while neither carboxylate oxygen of **7** can occupy the position common to the oxygens of **10** and **11**.

A substituted chromanecarboxylic acid group has been found to confer excellent activity in a series of 2,3,4-substituted acetophenone LTB_4 receptor antagonists.[5] Incorporation of this acid moiety into the present series of 2,4,5-substituted acetophenone LTB_4 receptor antagonists also produced a high level of activity in **15** (Scheme 4). Interestingly, a simple phenylpropionic acid analog of this chromanecarboxylic acid group (**12**; Scheme 2) was found to produce a similar high level of activity. However, addition of a propyl group to the phenylpropionate moiety, suggested by the presence of this group in **15**, led to decreased activity in **13** and **14** (Scheme 3). AM1 calculations carried out on model compounds (7-methoxy-8-propyl-2*H*-1-benzopyran-2-carboxylic acid, 3-methoxy-2-(propylphenyl)propionic acid, and 3-(methoxyphenyl)propionic acid) indicate that the bioactive conformations defined by the chromanecarboxylic acid moiety of **15** are also low-energy conformations for the phenylpropionic acid moieties in **12**–**14**. There are several possible explanations for these results. First, while the phenylpropionic acid and the chromanecarboxylic acid may position their carboxyl groups similarly when bound on the LTB_4 receptor, the phenyl rings of the two may be oriented differently, and consequently, the appended propyl groups may be oriented differently as well. It is also possible that the part of the dihydropyran ring of **15** that has no steric counterpart in **12**–**14** may influence the orientation of the chromane ring system in the active site. Alternatively, binding of this additional steric bulk in the chromane ring system may alter the conformation of the receptor, creating a binding site (for the propyl group) which is not accessible when the smaller phenyl ring system is bound. Another possible explanation for the different SAR of the propyl group in the phenylpropionate and chromanecarboxylate series is that there may be two binding sites for carboxylate groups in the LTB_4 receptor and the chromanecarboxylate of **15** may bind to one while the phenylpropionates of **12**–**14** may bind to the other (The existence of two carboxylate binding sites in the LTB_4 receptor has been suggested to explain

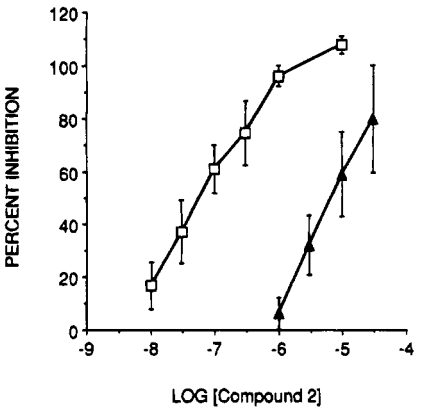


Figure 3. Concentration–inhibition curves of compound **2** on LTB_4 (squares)- and FMLP (triangles)-induced aggregation of isolated human neutrophils (n = 5). Concentration of each agonist was 30 nM.

the enhanced potency of xanthonedicarboxylic acid LTB_4 receptor antagonists.[7]).

The activity of pyrazole **15** (Scheme 4) was compared with the activity of the related acyclic enamino ketone structure **16** (Scheme 4), and the pyrazole was found to be much more active. The reason for this difference in activity is not clear. The dimethylamino group of **16** has no direct counterpart in either LTB_4 or other LTB_4 receptor antagonists prepared to date. Examination of molecular models suggests that in our receptor binding model (Figure 1) the position of the dimethylamino group of **16** is roughly equivalent to the position of a substituent attached to either C(17) of LTB_4 or C(5) of the pyrazole ring of **2**. C(17)-substituted LTB_4 has not been reported, and C(5) of the pyrazole ring is unsubstituted in all of the pyrazoles prepared to date. The dimethylamino group of **16** appears to probe a previously unexplored region of the LTB_4 receptor. One possible explanation for the inactivity of **16** is that the dimethylamino group sterically hinders binding.

A phenylpyrazole lacking the ethyl and hydroxyl groups on the phenyl ring (**17**; Scheme 3) was found to be inactive. Thus the *o*-hydroxyl group and the ethyl group, which were necessary for receptor binding in the hydroxyacetophenone series,[1] are still necessary in the more potent phenylpyrazole series.

Compound **2** was one of the most potent [^{3}H]LTB_4 binding inhibitors among the (hydroxyphenyl)pyrazoles tested. Inhibition of binding to human neutrophils was measured on five different cell preparations. The average IC_{50} was 6.4 ± 0.8 nM, and calculation of the K_D from this result yielded the value 2.3 ± 0.2 nM. We also carried out studies to determine if the compound could antagonize LTB_4-induced cell functions. Figure 3 shows that **2** inhibited LTB_4-induced aggregation of neutrophils (IC_{50} = 65 nM). It also inhibited FMLP (*N*-formyl-L-methionyl-L-leucyl-L-phenylalanine)-induced aggregation of neutrophils (IC_{50} = 7 μM) but only at concentrations 100-fold greater than those required to inhibit LTB_4-induced aggregation. The compound, itself, did not induce neutrophil aggregation over the concentration range studied. These experiments demonstrate that **2** is a selective LTB_4 receptor antagonist. In addition, the ability of **2** to inhibit LTB_4-induced leukopenia in rabbits was measured. The results (Table 2) revealed a dose-dependent inhibition of leukopenia (ED_{50} approximately 0.2 mg/kg), indicating that the compound is also a potent antagonist *in vivo*.

Table 2. Inhibition of LTB_4-Induced Leukopenia by Compound 2

dose of compound **2**[a] (mg/kg)	inhibition[b] (%)
0.02	0.6 ± 0.6
0.2	59 ± 11
2.0	82 ± 0.6

[a] Compound was administered intravenously 2 min before injecting 1 μg of LTB_4. [b] Two rabbits were used for evaluating the effectiveness of the compound at 0.02 and 2.0 mg/kg, and the data were averaged. Three animals were used to obtain the results at 0.2 mg/kg.

In summary, we have developed a series of (hydroxyphenyl)pyrazoles which bind at the human neutrophil LTB_4 receptor more tightly than the related hydroxyacetophenones. The close match between the length of the (hydroxyphenyl)pyrazole unit and the length of the triene unit in LTB_4 suggests that these units may bind to a common site in the LTB_4 receptor. Several members of this series have receptor binding affinities in the nanomolar range and may be useful in exploring the involvement of LTB_4 in inflammatory diseases.

Experimental Section

Chemical Methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were determined with a CEC-21-110 electron-impact mass spectrometer or a MAT-731 spectrometer using field desorption (FD) conditions. Nuclear magnetic resonance (^{1}H NMR) spectra were determined on a GE QE300 spectrometer. Chemical shift values 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, m = multiplet, br = broad. Unless otherwise noted, chromatography was carried out using a medium-pressure FMC pump with Altec columns packed with silica gel (EM Science silica gel 60 230–400 mesh ASTM) at a maximum pressure of 50 psi.

Unless otherwise noted, all organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure using a rotary evaporator. All tri-*n*-butyltin azide reactions were worked up as follows: a mixture was prepared by addition of 10 mL of concentrated HCl to ice followed by addition of water to make 50 mL of solution, to which was added 50 mL of hexane. The reaction mixture was then poured in and the reaction vessel rinsed with EtOAc, which was also added to the aqueous mixture. The mixture was then allowed to stir for about 1 h while warming to room temperature.

Molecular modeling experiments were carried out using Sybyl 5.5 (Tripos Associates, St. Louis, MO) running on a Silicon Graphics workstation. Structures were built and energy minimized using the Sybyl force field, and distances were measured on the resulting structures. Energy minimizations were carried out using AM1 within Sybyl with full geometry optimization and precise calculation.

4-Ethyl-5-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]-2-(1*H*-pyrazol-3-yl)phenol (2). 7-(4-Acetyl-2-ethyl-5-hydroxyphenoxy)-2,2-dimethylheptanenitrile[8] (**18**) (1.0 g, 3 mmol) was combined with 0.92 g of DMF diethyl acetal and heated in an oil bath maintained at 185 °C for 3 h. After cooling, the volatiles were removed under reduced pressure. The crude intermediate was combined with 85% hydrazine hydrate (excess) in ethanol and allowed to stir at room temperature for 4 days. The mixture was concentrated under reduced pressure and subjected to chromatography on silica gel, eluting with EtOAc/hexane (30:70). The pyrazolonitrile **19** was isolated as a crystalline solid (0.44 g, 45%).

Compound **19** (0.44 g, 1.3 mmol) was combined with 1.2 g (excess) of tri-*n*-butyltin azide[8] in diethoxyethane (DEE) (6 mL)

and heated under reflux for 3 days. More azide reagent (0.43 g) was added, and heating was continued overnight. The product was recovered by extraction with EtOAc. The solution was concentrated under reduced pressure and the residual oil triturated with hexane (to remove excess tin reagent). Compound **2** was obtained as a white crystalline solid (55 mg, 11%) by chromatography on C_{18} reverse phase material, eluting with acetonitrile/water/methanol (50:40:10): mp 209–212 °C; ^{1}H NMR (DMSO-d_6) δ 13.01 (s (br), 1H), 10.92 (s (br), 1H), 7.88 (d, 1H, J = 2 Hz), 7.42 (s, 1H), 6.78 (d, 1H, J = 2), 6.46 (s, 1H), 3.89 (t, 2H, J = 6 Hz), 2.48 (t, 2H, J = 6 Hz), 1.62–1.74 (m, 4H), 1.36 (s, 8H), 1.04–1.16 (m, 5H); UV λ_{max} (ethanol) 301 (ϵ = 7670), 260 (ϵ = 14 000), 213 (ϵ = 24 400) nm; MS (M^+) 385. Anal. ($C_{20}H_{28}N_6O_2$) C, H, N.

4-Ethyl-2-(1-methyl-1*H*-pyrazol-3-yl)-5-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenol (3). Compound **18** (1.1 g, 3.5 mmol) was combined with 0.56 g (1.1 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 160 °C for 2 h. The intermediate enamino ketone, which solidified on cooling, was suspended in 20 mL of ethanol, and 0.2 mL (1.1 equiv) of methylhydrazine was added. After stirring at room temperature overnight, the mixture was heated under reflux for 2 h. Another equivalent of methylhydrazine was added, and heating continued for 4 h. The reaction mixture was allowed to stir overnight while cooling to room temperature. The resulting slurry was cooled, filtered, and washed with fresh cold ethanol. After chromatography on silica gel, **20** was isolated in 25% yield (0.31 g) and 0.34 g of **21** was obtained (28%).

Compound **20** (0.31 g, 0.87 mmol) was combined with tri-*n*-butyltin azide (0.9 g, 3.0 equiv) and 4 mL of DEE and heated in an oil bath maintained at 160 °C for about 48 h. Suction filtration and recrystallization from EtOAc/hexane afforded **3** (85 mg, 24%): mp 140–143 °C; ^{1}H NMR (DMSO-d_6) δ 10.56 (s (br), 1H), 7.78 (d, 1H, J = 2 Hz), 7.40 (s, 1H), 6.70 (d, 1H, J = 2 Hz), 6.44 (s, 1H), 3.88 (s, 5H), 2.48 (t, 2H, J = 7 Hz), 1.60–1.74 (m, 4H), 1.36 (m, 8H), 1.10 (t, 5H, J = 7 Hz); UV λ_{max} (ethanol) 300 (ϵ = 8910), 263 (ϵ = 14 500), 211 (ϵ = 24 800) nm; HRMS (FAB, M + H) 399.250500 (399.250850 calcd for $C_{21}H_{31}N_6O_2$).

4-Ethyl-2(1-methyl-1*H*-pyrazol-5-yl)-5-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenol (4). A mixture of 0.34 g (1.0 mmol) of 7-[2-ethyl-5-hydroxy-4-(1-methyl-1*H*-pyrazol-5-yl)phenoxy]-2,2-dimethylheptanenitrile (**21**), which is formed during preparation of compound **3**, tri-*n*-butyltin azide (0.96 g, 3.0 equiv), and 4 mL of DEE was heated under reflux for 48 h. The product was recovered by extraction with EtOAc. The solvent was removed under reduced pressure. The residue was triturated with hexane and ether and subsequently crystallized to give **4** in 35% yield (0.135 g): mp 209–212 °C; ^{1}H NMR (DMSO-d_6) δ 9.66 (s, 1H), 7.46 (d, 1H, J = 2 Hz), 6.96 (s, 1H), 6.52 (s, 1H), 6.10 (d, 1H, J = 2 Hz), 3.88 (t, 2H, J = 6 Hz), 3.64 (s, 3H), 2.43 (t, 2H, J = 6 Hz), 1.64–1.76 (m, 4H), 1.38 (s, 8H), 1.02–1.20 (m, 5); UV λ_{max} (ethanol) 289 (ϵ = 5090), 244 (ϵ = 9000), 206 (ϵ = 26 600) nm; HRMS (FAB, M + H) 399.249600 (399.250850 calcd for $C_{21}H_{31}N_6O_2$).

4-[5-[4-(1*H*-Pyrazol-3-yl)-5-hydroxy-2-ethylphenoxy]-pentoxy]benzoic Acid (5). Ethyl 4-hydroxybenzoate (**22**) (5.20 g, 31.3 mmol), 7.25 g (31.5 mmol) of 1,5-dibromopentane, 13.21 g (95.6 mmol) of K_2CO_3, and 1.04 g (6.27 mmol) of KI, were combined in 50 mL of 2-butanone (methyl ethyl ketone, MEK) and heated under reflux overnight with magnetic stirring. After cooling, water was added and the aqueous layer was extracted with two 50 mL portions of EtOAc. The organic layers were combined and washed with two 50 mL portions of water, 50 mL of 1 N HCl, and 50 mL of brine. The organic layer was dried over K_2CO_3 and $MgSO_4$ and filtered, and the solvent was removed by rotary evaporator, affording bromoalkoxy ester **28** (4.70 g, 47%).

Compound **28** (4.70 g, 14.91 mmol) and 2.73 g (15.15 mmol) of **45** were placed in a 250 mL round-bottom flask, along with 6.22 g (45.0 mmol) of K_2CO_3, 0.45 g (2.71 mmol) of KI, 10 mL of dry DMSO, and 100 mL of MEK. The mixture was heated under reflux overnight with magnetic stirring and cooled, and water was added. The aqueous layer was extracted with EtOAc. The organic layer was washed with 10 mL of 1 N HCl, three 25 mL portions of water, and 25 mL of brine. The organic layer was dried over K_2CO_3 and $MgSO_4$ and filtered, and the solvent was removed by rotary evaporator. The alkoxyacetophenone **36** was isolated as a white crystalline solid (6.05 g, 98%).

Compound **36** (2.76 g 6.66 mmol) and 1.10 g (7.47 mmol) of DMF diethyl acetal were heated neat at 160 °C for 3 h. After cooling, the crystals were taken up in EtOAc and the solution was concentrated under reduced pressure. The crystals obtained were slurried in hexane, and the hexane was decanted by pipette. The excess solvent was removed under reduced pressure. The crude enamino ketone and 0.40 g (7.99 mmol) of 85% hydrazine hydrate were placed in 50 mL of absolute ethanol in a nitrogen atmosphere and heated under reflux overnight with magnetic stirring. The ethanol was removed under reduced pressure, leaving 2.46 g (74%) of the ethyl ester of **5** which was placed in a 100 mL round-bottom flask with 1.02 g (24.31 mmol) of $LiOH{\cdot}H_2O$ and 50 mL of 2:1 isopropyl alcohol/water. The solution was stirred for 4 h at room temperature and then 40 mL of 1 N HCl added. The precipitate was filtered by suction and washed with three 50 mL portions of cold water. The crude product was slurried in hexane and the hexane decanted by pipette, leaving 2.28 g (99%) of **5**; mp 134–136 °C; MS (M^+) 411. Anal. ($C_{23}H_{26}N_2O_5$) C, H, N.

4-[[5-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]-pentyl]oxy]benzeneacetic Acid (6). Ethyl 4-hydroxyphenylacetate (**23**) (3.6 g, 19 mmol) was combined with 1,5-dibromopentane (2.7 mL, 1.0 equiv) and K_2CO_3 (7.8 g, 3.0 equiv) in 60 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction was carried out with EtOAc. The residue after concentration under reduced pressure was subjected to chromatography on silica gel, eluting with EtOAc/hexane (5:95). The bromoalkoxy intermediate **29** was obtained as a colorless liquid (3.0 g, 49%).

Compound **29** (3.0 mg, 9 mmol) was combined with **45** (1.6 g, 1.0 equiv), 3.8 g (3.0 equiv) of K_2CO_3, and 1.0 g (catalytic) of KI in 60 mL of MEK and heated under reflux overnight. After cooling, water was added and the alkoxyacetophenone **37** was isolated by extraction with EtOAc as an off-white crystalline solid (3.2 g, 83%): mp 66–68 °C.

Compound **37** (2.0 g, 5 mmol) was combined with 1.0 mL (1.2 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 160 °C for about 3 h. The residual volatiles were removed under reduced pressure. The crude enamino ketone was combined with 0.6 g of 85% hydrazine hydrate (1.0 equiv) in 10 mL of ethanol and allowed to stir at room temperature overnight. Following concentration under reduced pressure, methylene chloride was added and the mixture filtered, passed through a bed of silica gel, and concentrated under reduced pressure. The crude ethyl ester of **6** (0.2 g 0.4 mmol) was combined with 11 mL of 0.1 N NaOH and a few drops of THF to form a turbid solution. After stirring overnight at room temperature, the mixture was acidified with concentrated HCl. Compound **6** (0.9 g, 48%) was isolated by suction filtration, washed with fresh water, and air-dried: mp 112–114 °C; MS (M^+) 425. Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

4-[[3-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]-propyl]oxy]benzeneacetic Acid (7). Compound **23** (3.6 g, 20 mmol) was combined with 1,3-dibromopropane (2.4 mL, 1.0 equiv) and K_2CO_3 (7.8 g, 3.0 equiv) in 60 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction was carried out with EtOAc. After concentration under reduced pressure, the residue was subjected to chromatography on silica gel, with eluting EtOAc/hexane (5:95). The bromoalkoxy intermediate **30** was obtained as a colorless liquid (3.4 g, 58%).

Compounds **30** (3.4 g, 11 mmol) was combined with **45** (2.1 g, 12 mmol), 4.7 g (3.0 equiv) of K_2CO_3, and 1.0 g (catalytic) of KI in 70 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction carried out with EtOAc. The alkoxyacetophenone **38** was obtained as a crystalline solid (3.6 g, 80%) by chromatography on silica gel, eluting with EtOAc/hexane (10:90): mp 55–57 °C.

Compound **38** (2.0 g, 5 mmol) was combined with 1.0 mL (1.2 equiv) of DMF diethyl acetal and heated in an oil bath

maintained at 160 °C for about 2 h. The residual volatiles were removed under reduced pressure. The crude enamino ketone was suspended in 50 mL of ethanol containing 85% hydrazine hydrate (0.35 g, 1.2 equiv) and allowed to stir at room temperature overnight. The solution was concentrated under reduced pressure and the residue taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. Chromatography on silica gel, eluting with EtOAc/hexane (40:60), afforded the ethyl ester of **7**. The ester was suspended in 20 mL of aqueous 0.1 N NaOH, and a small amount of THF was added to effect a homogeneous solution, which was allowed to stir overnight at room temperature. The solution was acidified with concentrated HCl and extraction carried out with EtOAc. After concentration under reduced pressure, **7** (0.69 g, 87%) was obtained as a crystalline solid: mp 163–165 °C; MS (M^+) 597. Anal. ($C_{22}H_{24}N_2O_5$) C, H, N.

4-[5-[4-(1*H*-pyrazol-3-yl)-2-ethyl-5-hydroxyphenoxy]-pentoxy]benzenepropanoic Acid (8). Ethyl 3-(4-hydroxyphenyl)propionate (**26**) (1.60 g, 8.24 mmol), 2.08 g (9.05 mmol) of 1,5-dibromopentane, 1.16 g (8.37 mmol) of K_2CO_3, and 0.10 g (0.60 mmol) of KI were combined in 50 mL of MEK. The mixture was heated under reflux overnight with magnetic stirring. After cooling, water was added and the aqueous layer was extracted with two 50 mL portions of EtOAc. The organic layers were combined and washed with two 50 mL portions of water, 50 mL of 1 N HCl, and 50 mL of brine. The organic layer was dried over Na_2SO_4 and $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The bromoalkoxy intermediate **31** was obtained by chromatography on silica gel, eluting with EtOAc/hexane (20:80), as a crystalline solid (1.23 g, 44%).

Intermediate **31** (1.23, 3.58 mmol) and 0.68 g (3.77 mmol) of **45** were placed in a 250 mL round-bottom flask, along with 1.30 g (9.41 mmol) of K_2CO_3, 0.35 g (2.11 mmol) of KI, 10 mL of dry DMSO, and 100 mL MEK. The mixture was heated under reflux overnight with magnetic stirring. After cooling, water was added and extraction was carried out with EtOAc. The organic layer was washed with 10 mL of 1 N HCl, three 25 mL portions of water, and 25 mL of brine. The organic layer was dried over Na_2SO_4 and $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The solid alkoxyacetophenone **39** (1.65 g, 3.73 mmol) was used without further purification.

Compound **39** (1.62 g, 3.66 mmol) and 0.62 g (4.21 mmol) of DMF diethyl acetal were heated at 160 °C for 3.5 h. After cooling, the crystals were taken up in EtOAc and the solution was concentrated under reduced pressure. The resultant crystals were then slurried in hexane, and the hexane was decanted by pipette. The excess solvent was removed by rotary evaporator. The crude enamino ketone and 0.84 g (16.78 mmol) of 85% hydrazine hydrate were stirred in 50 mL of absolute ethanol in a nitrogen atmosphere while heating under reflux overnight. The ethanol was removed by rotary evaporator, leaving a white crystalline solid which was recrystallized from ethanol to give 0.94 g (63%) of the ethyl ester of **8** as a white crystalline solid. The ester was placed in a 100 mL round-bottom flask along with 0.53 g (12.6 mmol) of $LiOH·H_2O$ and 50 mL of 2:1 isopropyl alcohol/water. The solution was stirred for 4 h at room temperature and then 40 mL of 1 N HCl added. The precipitate was filtered by suction and washed with three 50 mL portions of cold water. The crude product was slurried in hexane, and the hexane was decanted by pipette, providing 0.41 g (47%) of **8** after drying *in vacuo*: mp 133–135 °C; HRMS (FAB, M + H) 439.221600 (439.223297 calcd for $C_{25}H_{31}N_2O_5$).

4-[3-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]-propoxy]benzenepropanoic Acid (9). Compound **26** (1.12 g, 5.77 mmol), 1.21 g (6.00 mmol) of 1,3-dibromopropane, 0.85 g (6.15 mmol) of K_2CO_3, and 0.08 g (0.48 mmol) of KI were combined in 50 mL of MEK. The mixture was heated under reflux overnight with magnetic stirring. After cooling, water was added and the aqueous layer was extracted with two 50 mL portions of EtOAc. The organic layers were combined and washed with two 25 mL portions of water, 50 mL of 1 N HCl, and 50 mL of brine. The organic layer was dried over Na_2SO_4 and $MgSO_4$ and filtered and the solvent removed by rotary evaporator. The bromoalkoxy intermediate **32** was isolated as a crystalline solid (0.74 g, 40%) by chromatography on silica gel, eluting with EtOAc/hexane (15:85).

Intermediate **32** (0.56 g, 1.78 mmol), **45** (0.32 g, 1.78 mmol), K_2CO_3, (0.53 g, 3.83 mmol), KI (0.07 g, 0.42 mmol), and 10 mL of dry DMSO were combined in a 250 mL round-bottom flask containing 100 mL of MEK. The mixture was magnetically stirred and heated under reflux overnight. After cooling, water was added and the solution extracted with EtOAc. The organic layer was washed with 10 mL of 1 N HCl, three 25 mL portions of water, and 25 mL of brine. The organic layer was dried over Na_2SO_4 and $MgSO_4$ and filtered and the solvent removed by rotary evaporator to give the alkoxyacetophenone **40** as a crystalline solid (0.72 g, 86%).

Compound **40** (0.51 g, 1.32 mmol) and 0.23 g (1.56 mmol) of DMF diethyl acetal were heated at 160 °C for 3.5 h. The mixture was then heated for 45 min without a condenser to allow excess ethanol to boil off. After cooling, the crystals were taken up in EtOAc and the solution was concentrated under reduced pressure. The resultant crystals were slurried in hexane, and the hexane was decanted by pipette. The excess solvent was removed by rotary evaporator. The crude enamino ketone and 0.01 g (0.20 mmol) of 85% hydrazine hydrate were magnetically stirred in 50 mL of absolute ethanol in a nitrogen atmosphere and heated under reflux overnight. The ethanol was removed by rotary evaporator, leaving 0.01 g (0.02 mmol, 27%) of the ethyl ester of **9** as a white crystalline solid. The ester was combined with 24 mL of 2:1 isopropyl alcohol/water containing 0.01 g (0.238 mmol) of $LiOH·H_2O$. The solution was stirred for 3 h, and then, 25 mL of 0.1 N HCl was added. The product **9** (0.01 g, 100%) was isolated as a crystalline solid after filtering by vacuum and washing with three 15 mL portions of cold water: mp 140–144 °C; HRMS (FAB, M + H) 411.192500 (411.191997 calcd for $C_{23}H_{27}N_2O_5$).

2-[3-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]-propoxy]benzeneacetic Acid Disodium Salt (10). Ethyl 2-hydroxyphenylacetic acid (**25**) (7.5 g, 42.0 mmol) was combined with 1,3-dibromopropane (4.3 mL, 1.0 equiv), K_2CO_3 (6.8 g, 1.0 equiv), and 0.5 g of KI (catalytic) in 50 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction was carried out with EtOAc. Chromatography on silica gel, eluting with EtOAc/hexane (5:95), afforded the bromoalkoxy intermediate **33** as a colorless liquid (4.4 g, 35%).

Intermediate **33** (4.4 g, 15.0 mmol) was combined with **45** (2.7 g, 1.0 equiv), 6.0 g (3.0 equiv) of K_2CO_3, and 0.5 g (catalytic) of KI in 60 mL of MEK and heated under reflux overnight. Water was added and extraction carried out with EtOAc. The alkoxyacetophenone **41** was obtained as a white crystalline solid (4.6 g, 79%) after chromatography on silica gel, eluting with EtOAc/hexane (15:85).

Compound **41** (4.4 g, 11.0 mmol) was combined with 2.0 mL (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 150 °C for about 2 h. The residual volatiles were removed under reduced pressure. The crude enamino ketone was combined with 0.7 g (1.0 equiv) of 85% hydrazine hydrate in 30 mL of ethanol and allowed to stir at room temperature for 2 days. After concentration under reduced pressure, the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. The ethyl ester of **10** was isolated as a crystalline solid (1.9 g, 73%) by chromatography on silica gel, eluting with EtOAc/hexane (35:65). To the ester were added 9.0 mL of 2.0 N NaOH (2.0 equiv) and a small amount of THF to facilitate solution. After stirring overnight at room temperature, the solution was homogeneous. The solution was cooled in a refrigerator, filtered, and concentrated under reduced pressure to give **10** as the pale yellow crystalline disodium salt (1.9 g, 96%): mp >180 °C dec; MS (M^+) 441. Anal. ($C_{22}H_{22}N_2O_5Na_2$) C, H, N.

3-[3-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]-propoxy]benzeneacetic Acid (11). Ethyl 3-hydroxyphenylacetate (**24**) (3.6 g, 19.0 mmol) was combined with 1,3-dibromopropane (2.5 mL, 1.0 equiv) and K_2CO_3 (7.8 g, 3.0 equiv) in 60 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction carried out with

EtOAc. The bromoalkoxy intermediate **34** was obtained as a colorless liquid (2.6 g, 46%) by chromatography on silica gel, eluting with EtOAc/hexane (5:95).

Intermediate **34** (2.6 g, 9.0 mmol) was combined with **45** (1.6 g, 1.0 equiv), 3.5 g (3.0 equiv) of K_2CO_3, and 1.0 g (catalytic) of KI in 60 mL of MEK and heated under reflux for 3 days. After cooling, water was added and extraction was carried out with EtOAc. The alkoxyacetophenone **42** was obtained as an off-white crystalline solid (3.5 g, 100%) which was used without further purification.

Compound **42** (3.36 g, 8.4 mmol) was combined with 1.5 mL (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 150 °C for about 5h and then allowed to stir at room temperature over the weekend. Another equivalent of acetal was added, and heating was continued for 2 h. A third equivalent of acetal was added, and heating was continued for 3 h. The residual volatiles were removed under reduced pressure. The enamino ketone was obtained as a viscous yellow oil (1.0 g, 29%) following chromatography on silica gel, eluting with EtOAc/hexane (30:70). The enamino ketone was combined with 0.14 g (1.0 equiv) of 85% hydrazine hydrate in 10 mL of ethanol and allowed to stir at room temperature overnight. Another equivalent of 85% hydrazine hydrate was added, and stirring continued for 24 h. Following concentration under reduced pressure, the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. The ethyl ester of **11** (0.2 g, 44%) was isolated by chromatography on silica gel, eluting with EtOAc/hexane (40:60), as a yellow oil which subsequently crystallized. To the ester were added 16.5 mL of 0.2 N NaOH (3.0 equiv) and a small amount of THF to produce a homogeneous solution which was allowed to stir overnight at room temperature. The mixture was acidified with concentrated HCl and extracted with EtOAc. Compound **11** (0.35 g, 80%) was obtained as a yellow oil, which crystallized upon standing: mp 133–136 °C; MS (M^+) 397. Anal. ($C_{22}H_{24}N_2O_5$) C, H, N.

3-[3-[2-Ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]propoxy]benzenepropanoic Acid (12). Ethyl 3-(3-hydroxyphenyl)propionate (**27**) (19.4 g, 0.1 mol) was combined with 1,3-dibromopropane (10.2 mL, 1.0 equiv) and K_2CO_3 (30.0 g, 2.0 equiv) in 100 mL of MEK and heated under reflux overnight. After cooling, water was added and extraction carried out with EtOAc. The bromoalkoxy intermediate **35** was obtained as a colorless liquid (6.7 g, 21%) after chromatography on silica gel, eluting with EtOAc/hexane (5:95).

Intermediate **35** (6.7 g, 21 mmol) was combined with **45** (3.8 g, 1.0 equiv), 8.7 g (3.0 equiv) of K_2CO_3, and 3.0 g (catalytic) of KI in 100 mL of MEK and heated under reflux for 3 days. Water was added, and extraction was carried out with EtOAc. Following chromatography on silica gel, eluting with a 10–20% gradient of EtOAc in hexane, the alkoxyacetophenone **43** was obtained as an off-white crystalline solid (5.3 g, 60%): mp 66–68 °C.

Compound **43** (5.3 g, 13.0 mmol) was combined with 1.9 mL (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 150 °C for about 2 h. The residual volatiles were removed under reduced pressure. The crude enamino ketone was combined with 0.75 g (1.0 equiv) of 85% hydrazine hydrate in 30 mL of ethanol and allowed to stir at room temperature overnight. After concentration under reduced pressure, the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. The ethyl ester of **12** was isolated by chromatography on silica gel, eluting with EtOAc/hexane (30:70), as a yellow oil (3.8 g, 54%). The ester was combined with 26.1 mL of 1.0 N NaOH (3.0 equiv) and a small amount of THF to facilitate solution. After stirring for about 30 min at room temperature, the solution was homogeneous. After stirring overnight, a copious precipitate had formed. The mixture was acidified with concentrated HCl to about pH 1 and filtered, and the solid was washed with fresh water and air-dried to give **12** as a crystalline solid (3.3 g, 93%): mp 127–128 °C; MS (M^+) 411. Anal. ($C_{25}H_{30}N_6O$) C, H; N: calcd, 6.83; found, 7.64.

(2- and 4-*n*-Propyl-3-hydroxyphenyl)propionic Acids. 3-(3-Hydroxyphenyl)propionic acid (**27**) (19.4 g, 0.1 mol) was combined with 8.7 g (1.0 equiv) of allyl bromide, K_2CO_3 (30.0 g, 2.0 equiv), and 6.0 g of KI in 75 mL of MEK. The mixture was heated under reflux overnight. After cooling, water was added and the crude product was isolated by extraction with EtOAc. Chromatography on silica gel, eluting with a gradient of 5–20% EtOAc in hexane, afforded 3-[3-(allyloxy)phenyl]-propionic acid ethyl ester (**46**) as a colorless liquid (13.3 g, 57%).

Compound **46** (13.3 g, 57 mmol) was heated in a nitrogen atmosphere in an oil bath maintained at 210 °C for 5.5 h. The resultant oil was cooled and subjected to chromatography on silica gel, eluting with EtOAc/hexane (15:85). Ethyl [3-hydroxy-2-(2-propenyl)phenyl]propionate was obtained in 15% yield (2.0 g), and ethyl [3-hydroxy-4-(2-propenyl)phenyl]propionate was obtained in 20% yield (2.7 g).

Ethyl [3-hydroxy-2-(2-propenyl)phenyl]propionate (2.0 g, 8.5 mmol) was combined with 0.25 g of 10% palladium on charcoal catalyst in 25 mL of EtOAc, in a three-necked flask fitted with a septum. Hydrogen was introduced *via* a balloon attached to a needle sufficiently long to reach below the surface of the reaction mixture. The mixture was allowed to stir overnight at room temperature and then purged with nitrogen and filtered through diatomaceous earth. Compound **48** was obtained as a colorless oil (1.85 g, 92%). The NMR spectrum of this intermediate was in accord with the structure assigned.

By the same method, ethyl [3-hydroxy-4-(2-propenyl)phenyl]-propionate (2.7 g, 11.5 mmol) was converted to **47** (2.6 g, 95%), obtained as a colorless oil. The structure of this intermediate was confirmed by NMR spectroscopy.

4-Propyl-3-[3-[2-ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)-phenoxy]propoxy]benzenepropanoic Acid Disodium Salt (13). Compound **45** (2.95 g, 16.4 mmol) was combined with 1-bromo-3-chloropropane (2.8 g, 1.1 equiv), K_2CO_3 (6.7 g, 3.0 equiv), and 0.3 g of KI (catalytic) in 50 mL of MEK and heated under reflux for 3 days. After cooling, water was added and extraction carried out with EtOAc. The crude chloroalkoxy intermediate was isolated by concentration under reduced pressure and used without further purification.

The chloroalkoxy intermediate (2.3 g, 8.9 mmol) was combined with **47** (2.1 g, 1.0 equiv), 2.5 g (2.0 equiv) of K_2CO_3, and 0.3 g (catalytic) of KI in 60 mL of MEK and allowed to stir at room temperature overnight and then heated under reflux for about 5 h. Water was added and extraction carried out with EtOAc. The residue was subjected to chromatography on silica gel, eluting with a 10–15% gradient of EtOAc in hexane, affording the alkoxyacetophenone as a white crystalline solid (0.39 g, 10%). The alkoxyacetophenone was combined with 0.13 g (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 150 °C for about 2 h. The residual volatiles were removed under reduced pressure. The crude enamino ketone was combined with 0.7 g (1.0 equiv) of 85% hydrazine hydrate in 5 mL of ethanol and heated under reflux overnight. After concentration under reduced pressure, the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. The ethyl ester of **13** was isolated by chromatography on silica gel, eluting with EtOAc/hexane (35:65), as a crystalline solid (1.9 g, 73%).

The ester (1.9 g, 4.5 mmol) was combined with 9.0 mL of 2.0 N NaOH (2.0 equiv), and a small amount of THF was added to facilitate solution. After stirring overnight at room temperature, the solution was homogeneous. The mixture was cooled in a refrigerator, filtered, and evaporated to dryness under reduced pressure. Compound **13** (1.9 g, 96%) was obtained as a pale yellow crystalline solid (analyzed as the free acid): mp >200 °C dec; MS (M^+) 453. Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

2-Propyl-3-[3-[2-ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)-phenoxy]propoxy]benzenepropanoic Acid (14). Ethyl (3-hydroxy-2-propylphenyl)propionate (**48**) (1.85 g, 0.78 mmol) was combined with 1,3-dibromopropane (1.98 g, 1.0 equiv) and K_2CO_3 (3.2 g, 3.0 equiv) in 50 mL of MEK and heated under reflux for 3 days. After cooling, water was added and extraction carried out with EtOAc. The solvent was removed under reduced pressure, and the residue was subjected to chromatography on silica gel, eluting with EtOAc/hexane (5:95), affording the bromoalkoxy intermediate as a colorless liquid (0.3 g, 11%).

The bromoalkoxy intermediate (0.3 g, 0.8 mmol) was combined with **45** (0.15 g, 1.0 equiv), 0.35 g (3.0 equiv) of K_2CO_3, and 0.1 g (catalytic) of KI in 20 mL of MEK and heated under reflux overnight. Water was added, and extraction was carried out with EtOAc. The alkoxyacetophenone was isolated as an off-white crystalline solid (0.26 g, 72%) by chromatography on silica gel, eluting with a 10–20% gradient of EtOAc in hexane.

The alkoxyacetophenone (0.26 g, 0.6 mmol) was combined with 0.11 mL (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 160 °C for about 2 h. The residual volatiles were removed under reduced pressue. The crude enamino ketone was combined with 0.05 g (1.0 equiv) of 85% hydrazine hydrate in 20 mL of ethanol and heated under reflux for about 5 h. After concentration under reduced pressure, the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried over $MgSO_4$. The ethyl ester of **14** was isolated by chromatography on silica gel, eluting with a 10–20% gradient of EtOAc in hexane, as a yellow oil (0.15 g, 51%).

The ester (0.15 g, 0.31 mmol) was combined with 6 mL of 0.1 N NaOH (2.0 equiv), and a small amount of THF was added to facilitate solution. After stirring overnight at room temperature, the solution was homogeneous. The mixture was acidified with concentrated HCl to about pH 1 and the product extracted with EtOAc. The carboxylic acid was obtained as a crystalline solid (0.14 g, 100%): mp 125–128 °C; MS (M^+) 453. Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

3,4-Dihydro-8-propyl-7-[[3-[2-ethyl-5-hydroxy-4-(1*H*-pyrazol-3-yl)phenoxy]propyl]oxy]-2*H*-1-benzopyran-2-carboxylic Acid (15). 3,4-Dihydro-8-propyl-7-[[3-[2-ethyl-5-hydroxy-4-[3-(dimethylamino)-1-oxo-2-propenyl]phenoxy]-propyl]oxy]-2*H*-1-benzopyran-2-carboxylic acid (**16**) (1.33 g, 2.6 mmol) was combined with 0.5 g of 85% hydrazine hydrate (3.0 equiv) in ethanol (40 mL). The resultant mixture was allowed to stir at room temperature for about 5 h and then heated under reflux for 45 min. The thick slurry became a homogeneous solution. The solution was allowed to cool and then concentrated under reduced pressure. The residue was taken up in water and the pH adjusted to about 1 with concentrated HCl. Compound **15** (0.46 g, 36%) was separated by extraction with EtOAc and purified by reversed-phase chromatography on C_{18} medium, eluting with methanol/ammonium acetate buffer (80:20): mp 86–89 °C; MS (M^+) 481. Anal. ($C_{27}H_{32}N_2O_6$) C, H, N.

3,4-Dihydro-8-propyl-7-[[3-[2-ethyl-5-hydroxy-4-[3-(dimethylamino)-1-oxo-2-propenyl]phenoxy]propyl]oxy]-2*H*-1-benzopyran-2-carboxylic Acid (16). In a pressure bottle, 7-hydroxy-8-propylchromone-2-carboxylic acid ethyl ester[9] (**49**) (12.1 g, 0.04 mol) was dissolved in 210 mL of acetic acid, and 10% palladium on charcoal catalyst (7.2 g) was added. The bottle was pressurized with 52 psi of H_2 gas and agitated for 23 h. The catalyst was removed by filtration through a Celite pad in a sintered glass funnel and washed with EtOAc. The combined organics were concentrated under reduced pressure, and the resulting oil was azeotroped with toluene, providing 12 g of brown oil. The material was purified by chromatography on a Waters Prep 500 HPLC equipped with silica gel cartridges, eluting with a 5–40% gradient of EtOAc in hexane. 3,4-Dihydro-7-hydroxy-8-propyl-2*H*-1-benzopyran-2-carboxylic acid ethyl ester (**50**) was obtained as a pink oil (10.0 g, 86%).

A solution of **50** (6.6 g, 25 mmol) in 15 mL of dry DMF was stirred in an argon atmosphere at room temperature with solid K_2CO_3 (6.05 g, 1.75 equiv). To this suspension was added 1-bromo-3-chloropropane (9.84 g, 2.5 equiv). The reaction mixture was stirred at room temperature for 20 h and then the reaction quenched with water. The mixture was extracted with EtOAc (three times). 7-[(3-Chloropropyl)oxy]-3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic acid ethyl ester (**51**) (6.12 g, 72%) was obtained by chromatography on silica gel, eluting with EtOAc/hexane (15:85).

To a solution of **45** (2.70 g, 15 mmol) in 30 mL of 5:1 MEK/DMSO at room temperature were added **51** (5.11 g, 1.0 equiv), K_2CO_3 (3.63 g, 1.75 equiv), and KI (5.0 g, 0.20 equiv). The reaction mixture was heated under reflux for 20 h and then cooled to room temperature, the reaction was quenched with water, and the mixture was extracted with EtOAc (three times). Chromatography on silica gel, eluting with EtOAc/hexane (20:80), afforded 3,4-dihydro-8-propyl-7-[[3-(4-acetyl-2-ethyl-5-hydroxyphenoxy)propyl]oxy]-2*H*-1-benzopyran-2-carboxylic acid ethyl ester (**52**) (5.23 g, 73%).

Compound **52** (4.0 g, 8.5 mmol) was combined with DMF diethyl acetal (1.4 g, 1.1 equiv) in a flask equipped with a reflux condenser and heated in an oil bath maintained at 155 °C for about 1.5 h. The volatiles were removed under reduced pressure. The residual red-yellow oil was crystallized from ethanol to give the ethyl ester of **16** (3.4 g, 74%). The ester (1.5 g, 2.8 mmol) was combined with 2.8 mL of 2 N NaOH (2.0 equiv) in 40 mL of ethanol under a nitrogen atmosphere and allowed to stir at room temperature for about 3 h. After chilling, the mixture was filtered and the filtrate washed with fresh, cold ethanol. The filtrate was slurried in water, and the pH was adjusted to about 1 with concentrated HCl. Extraction was carried out with EtOAc. Removal of the solvent under reduced pressure afforded **16** as a yellow solid (1.33 g, 94%): mp 136–138 °C; MS (M^+) 534. Anal. ($C_{29}H_{36}NO_7$) C, H, N.

3-[3-[4-1*H*-Pyrazol-3-yl)phenoxy]propoxy]benzenepropanoic Acid (17). 4-Hydroxyacetophenone (**44**) (6.34 g, 47 mmol), 1,3-dibromopropane (6.2 mL, 2.0 equiv), and K_2CO_3 (7.0 g, 1.1 equiv) were combined in 50 mL of MEK and allowed to stir at room temperature overnight and then heated under reflux for 6 h. The reaction mixture was allowed to cool, water was added, and the product was extracted with EtOAc. The (bromoalkoxy)acetophenone was isolated by chromatography as a colorless oil (5.3 g, 49%).

The (bromoalkoxy)acetophenone was combined with **27** (4.0 g, 1.0 equiv) and K_2CO_3 (5.0 g, 1.75 equiv) in 40 mL of DMF and heated under reflux for 6 h. After cooling, water was added and extraction was carried out with EtOAc. The alkoxyacetophenone (1.95 g, 26%) was obtained as a colorless oil following chromatography on silica gel.

The alkoxyacetophenone was combined with 0.8 g (1.0 equiv) of DMF diethyl acetal and heated in an oil bath maintained at 140 °C for 18 h. The volatile components were removed under reduced pressure. The residue was dissolved in 15 mL of ethanol with 0.5 g (1.6 equiv) of hydrazine hydrate (85%) and heated under reflux overnight. The reaction mixture was cooled, and the solvent was removed under reduced pressure. The residue was taken up in EtOAc, washed with 0.1 N HCl, and subjected to chromatography on silica gel, eluting with EtOAc/hexane (40:60), to give the ethyl ester of **17** (0.53 g, 25%).

The ester was combined with 1.5 mL of 2 N NaOH (2.0 equiv) in 25 mL of methanol. The resultant mixture was allowed to stir at room temperature overnight and then concentrated under reduced pressure. The resultant white powder was dissolved in water, acidified with concentrated HCl, and, then, extracted with EtOAc, affording **17** as a white, crystalline solid (0.145 g, 29%): mp 160–163 °C; MS (M^+) 367. Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

Biological Methods. Inhibition of LTB_4 Binding. The procedure for measuring the ability of compounds to inhibit the binding of LTB_4 to receptors on intact human neutrophils has been described in detail in our previous publication.[1] In brief, concentration–response studies were carried out using an adaptation of a radioligand binding assay developed by Goetzl and Goldman.[10] Ten microliters of DMSO containing different amounts of compound, 20 μL of 2.65 nM [^{3}H]LTB_4, and 500 μL of neutrophils suspended at a concentration of 2 $\times$ 10^7 cells/mL in Hank's balanced salt solution containing 0.1% ovalbumin were added to microcentrifuge tubes which were then incubated for 10 min at 4 °C. Cell-bound radioactivity was subsequently separated from soluble label by adding a 7:2 mixture of dibutyl and dinonyl phthlate (300 μL) and centrifuging for 2 min. After decanting the liquid, the botton tip of the tube containing the pelleted cells was cut off and its radioactivity content measured by scintillation spectromety. Three incubations were carried out at each concentration of compound investigated. The individual measurements of bound label were then averaged (SEM = 1–2%) and the results expressed as a percent inhibition of specific [^{3}H]LTB_4 binding,

after making appropriate corrections for nonspecific binding. The inhibitory activity of most compounds was evaluated on only one cell preparation. However, the variability of measurements from different individuals can be estimated from the extent of inhibition observed with a reference compound that was included in each experiment. At 10^{-5} M, the mean percent inhibition and standard deviation for the reference compound were 93.9% and 3.9%, respectively. At 10^{-6} M, the corresponding values were 56.9% and 6.9%. Assuming a linear correlation between percent inhibition and standard deviation, the following estimates were calculated for the precision at different percentages of inhibition: 90 ± 4.2, 80 ± 5.0, 60 ± 6.6, 40 ± 8.2, 20 ± 9.9, and 10 ± 10.7. In the case of compound **2**, which was tested on five cell preparations, the precision of the measurements was better than these estimates (95.0 ± 0.6 at 10^{-7} M, 73.9 ± 2.6 at 10^{-8} M, and 9.7 ± 2.1 at 10^{-9} M).

The IC_{50} and K_D for compounds **1** and **2** were calculated using Lundon2 (version 3.06; Lundon Software, Chagrin Falls, OH). IC_{50} values for other compounds were calculated assuming that between approximately 10% and 90% inhibition there was a linear relationship between the percent inhibition observed and the concentration of the compound tested. The variability of an IC_{50} value obtained with just one cell preparation was estimated from the standard deviation obtained for six reference compounds whose inhibitory effects were measured on cells from five individuals. The average standard deviation was $15 \pm 4\%$ of the mean IC_{50}.

Inhibition of LTB_4- and FMLP-Induced Aggregation of Human Neutrophils. A platelet-ionized calcium aggregometer (Chrono-log Corporation) was used to measure aggregation of peripheral human neutrophils. Cells (1×10^7/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 1 min, LTB_4 or FMLP (4.5 μL, 3 μM) was injected into the cuvette and the subsequent maximum amount of response occurring 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 occurring in the absence of agonist.

Inhibition of LTB_4-Induced Leukopenia in Rabbits. Studies were carried out using female New Zealand White rabbits weighing 3–3.5 kg. Under anesthesia (methohexital sodium) as needed, catheters were inserted into the left marginal ear vein and the right external jugular vein. Experiments were not started until 3.5 h after the surgery to allow the circulating leukocyte count to return to normal. Leukopenia was induced by injecting 1 μg of LTB_4 through the ear catheter. Blood samples were collected from the jugular catheter and diluted 1:6 with 2% acetic acid/0.01% crystal violet solution and cell counts made with a hemacytometer. Two counts of each blood sample were made and averaged. Compound **2** was dissolved in 0.5 M $NaHCO_3$ and administered intravenously by injection through the ear catheter.

In general, the experimental protocol with each rabbit consisted of sequential measurements of the amount of leukopenia caused by placebo, agonist, and agonist in the presence of compound **2**. This was done as follows. A base-line bleeding was taken and 0.5 mL PBS injected iv into the animal. Blood samples were then collected 0.5, 1, 2, 3, 5, 10, and 15 min later. The rabbit was rested for 15 min and then another base-line bleeding taken before giving LTB_4 iv and again collecting blood samples for 15 min. Finally, after resting the animal for 90 min, another base-line blood sample was taken, and compound **2** was then administered iv to the animal followed 2 min later by injection of agonist and subsequent blood sample collections. A plot was made of the relative cell count (base line = 100) versus time after injection of the agonist, and the area between the base-line and experimental curves from 0–5 min was calculated. The effect of the drug was measured by determining the percent reduction in this area when compound was given.

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