# Biphenylyl-Substituted Xanthones: Highly Potent Leukotriene $B_4$ Receptor Antagonists

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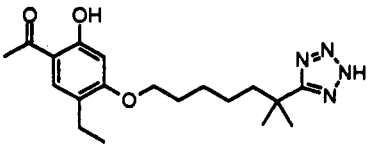
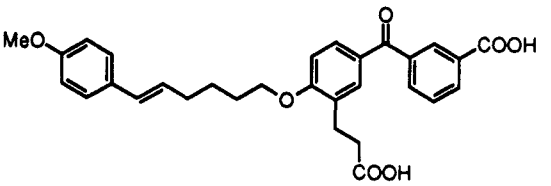
The pharmacologic activity of leukotriene $B_4$ ($LTB_4$) continues to generate intense research interest. $LTB_4$ is known to stimulate degranulation, aggregation, chemotaxis, and chemokinesis of polymorphonuclear leukocytes, as well as promote superoxide generation.[1] The proinflammatory effects of this eicosanoid mediator may play a role in the pathogenesis of several inflammatory diseases such as asthma,[2] inflammatory bowel disease,[3] psoriasis,[4] and gout.[1e,5] We recently disclosed the acetophenone/xanthone $LTB_4$ receptor antagonist LY282210 (compound **4**, Chart I),[6] which evolved from two separate series of compounds represented by LY255283 (**1**, acetophenone class)[7] and LY223982/LY210073 (**2**/**3**, benzophenone/xanthone class).[8] Beyond the general conclusion that two important series of structurally distinct $LTB_4$ antagonists could be hybridized, we also demonstrated that, in the case of xanthone **4**, deletion of the propanoic side chain led to a significant loss of binding affinity. The high in vitro potency observed with **4** in the inhibition of binding of [$^3$H]$LTB_4$ to both human neutrophils ($IC_{50}$ = 4 nM) and guinea pig lung membranes ($K_i$ = 1.2 nM) prompted us to further explore the SAR of the lipophilic portion of the molecule. In keeping with our interest in diacid $LTB_4$ antagonists, we also examined the importance of the aromatic carboxylic acid moiety as it relates to receptor affinity. We now report that substitution of the acetyl group of **4** with phenyl[9] provides a new, highly potent variation of the xanthone class of $LTB_4$ receptor antagonists (compounds **5**–**8**),[10] and that the aromatic carboxylic acid, while unnecessary for high (<10 nM) functional activity, is critical for high (<1 nM) binding affinity.

The synthesis of compound **7** (LY292728, the most potent member of the series), as depicted in Scheme I, is representative of the route employed for xanthones **5**, **6**, and **8**. Appendage of the chloropropyl chain to 4-(benzyloxy)-2-hydroxyacetophenone (**9**) provided ketone **10** in 82% yield. Triethylsilane/trifluoroacetic acid-mediated reduction of the keto functionality in **10** was followed by regiospecific bromination para to the 3-chloropropoxy group, providing bromide **12** in 50% yield for two steps. Application of Suzuki coupling conditions[11] using 4-fluorophenylboronic acid gave biphenyl **13** in 84% yield. While the stability of the primary chloride throughout these last three steps was viewed as a potential issue, such concern proved to be unwarranted. The synthesis of iodide **14** via halogen exchange of chloride **13** (quantitative yield) provided a pivotal intermediate which was easily coupled to phenol **15** (the efficient construction of which has been described earlier[8c]) to give **16** in 69% yield. Debenzylation, hydrolysis, and reversed-phase MPLC afforded biphenylyl-substituted xanthone **7** as its disodium salt in 46% overall yield from **16**.

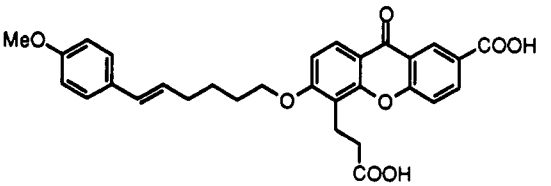
**Chart I**



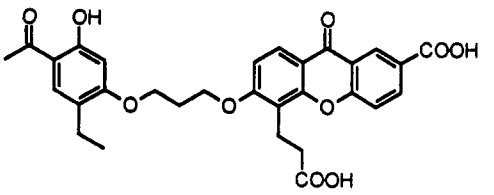
**1**



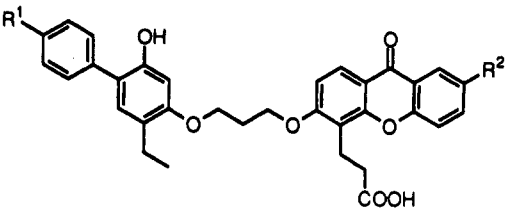
**2**



**3**



**4**



**5** $R^1$ = H, $R^2$ = COOH
**6** $R^1$ = $R^2$ = H
**7** $R^1$ = F, $R^2$ = COOH
**8** $R^1$ = F, $R^2$ = H

Besides inhibition of binding of radiolabeled $LTB_4$ to human neutrophils and guinea pig lung membranes, **5**–**8** were also examined as antagonists of $LTB_4$-induced upregulation of human neutrophil CD11b/CD18 (integrin) receptors (Table I). Compound **5**, where phenyl is directly substituted for the acetyl moiety, exhibited a 7-fold increase in binding affinity for human neutrophils relative to **4**,[6] while an 11-fold increase was observed for guinea pig lung membranes. These results are in line with previous work describing the superior nature of the phenyl group in interaction with a critical pharmacophore of the

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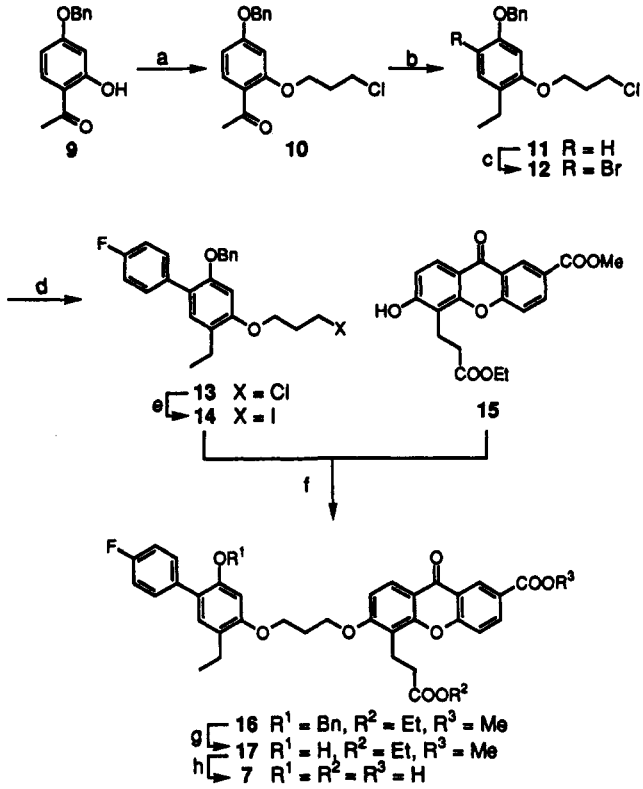
**Scheme I**[a]




[a] (a) 1-bromo-3-chloropropane, $K_2CO_3$, 2-butanone, DMSO; (b) $Et_3SiH$, trifluoroacetic acid, $CCl_4$; (c) NBS, $CCl_4$; (d) 4-fluorophenylboronic acid, EtOH, benzene, aq $Na_2CO_3$, $Pd(PPh_3)_4$ (cat.); (e) NaI, 2-butanone; (f) $K_2CO_3$, DMF; (g) $H_2$, 10% Pd(C), EtOAc; (h) aq NaOH, MeOH, THF.

**Table I.** Inhibition of Specific Binding of [$^3$H]$LTB_4$- and $LTB_4$-Mediated Up-Regulation of Human Neutrophil CD11b/CD18 by Biphenylyl-Substituted Xanthones 5–8

| compd | $K_i$, nM: human neutrophil[14] | $K_i$, nM: guinea pig lung membranes[13a] | human neutrophil CD11b/CD18 up-regulation,[15] $IC_{50}$, nM |
|---|---|---|---|
| 4 | 4.0[6] | 1.2 ± 0.11[6] | 47 |
| 5[a] | 0.57 | 0.11 ± 0.047 | 3.4 ± 0.29 |
| 6[b] | 22 | 12 ± 2.4 | 5.4 ± 0.10 |
| 7[a] | 0.47 | 0.040 ± 0.016 | 1.2 ± 0.10 |
| 8 | 36 | 4.0 ± 1.2 | 1.8 ± 0.040 |
| $LTB_4$ | 1.9 ± 0.050 | 0.12 ± 0.015 | |

[a] Tested as the disodium salt. [b] Tested as the monosodium salt.

$LTB_4$ receptor.[9] This is especially apparent when comparing compound 4 with compounds 5–8 in their ability to inhibit $LTB_4$-induced integrin up-regulation. As previously discussed, the propanoic acid group of the earlier xanthone series is critical for potent receptor binding to both human and guinea pig receptors, as deletion of this side chain in 4 resulted in a weak-binding inhibitor.[6] To ascertain the importance of the aromatic carboxyl group, compound 6 was synthesized. Interestingly, while 40–100-fold less potent in the human neutrophil and guinea pig lung membrane binding assays relative to 5, monoacid 6 still retained potent antagonism against $LTB_4$-induced CD11b/CD18 up-regulation. These observations correlate well with the structure–activity relationships observed for the benzophenone (2)[8a] class of $LTB_4$ receptor antagonists.

Compound 7, the 4-fluoro analogue of 5, displayed somewhat higher activity in vitro, with the most significant gain observed in blocking up-regulation of the CD11b/CD18 receptor. Compound 7 appears overall to be the most potent in vitro $LTB_4$ receptor antagonist yet described. It was especially tenacious in binding to both human neutrophils ($K_i$ = 0.47 nM) and guinea pig lung membranes ($K_i$ = 0.040 nM), a 2–4-fold increase over that of the natural agonist. As predicted, removal of the aromatic carboxylic acid group (compound 8) led to an 80–100-fold loss of human neutrophil and guinea pig lung membrane binding affinity relative to 7. However, as with 5 and 6, functional activity toward the CD11b/CD18 receptor was not significantly affected. We have previously commented on the relationship between the second acid group and the known heterogeneity of the human neutrophil $LTB_4$ receptor.[6] In the present series (compounds 5–8), the secondary aromatic carboxylic acid appears to be necessary only for tight receptor binding to the human neutrophil. However, the lipophilic side chain must also be taken into account, as hydroxyacetophenone diacid 4 is approximately 10 times more potent in inhibiting [$^3$H]-$LTB_4$ binding than $LTB_4$-induced expression of CD11b/CD18, a larger magnitude than is observed with compounds 5 and 7.

The in vivo pulmonary actions of compounds 5 and 7 were evaluated in guinea pig, a species in which inhaled or intravenously adminstered $LTB_4$ produces transient airway constriction.[12] Because of gas trapped distal to obstructed airways, $LTB_4$ challenge results in an increase in excised lung gas volume (ELGV) at the death of the animal.[13] When 5 and 7 were administered at an estimated inhaled dose of 10.0 μg/kg, followed by $LTB_4$ inhalation challenge, ELGV values were reduced by 69 ± 20% and 81 ± 8%, respectively. The 10.0 μg/kg dose is well within the delivery range of current metered dose or dry powder inhalers. Thus, these results suggest the potential for topical application of these agents in pulmonary diseases such as asthma.

Since our earlier observation that the binding domains of $LTB_4$ antagonists represented by 1 and 2/3 may be merged with a gain in overall activity, it has become increasingly apparent that the $LTB_4$ receptor is a very complex entity. In particular, the lipophilic binding site of the receptor appears to tolerate a wide variety of functionality, while the acid-binding domain is sensitive to small changes in antagonist structure. While an antagonist normally requires only one acid group for interaction with the acid-binding domain, compounds with at least two acid groups, such as 5 and 7, tend to display the most potent activity. In summary, we have described a new variation on the xanthone class of $LTB_4$ receptor antagonists which has led to the development of 7 (LY292728), a compound that exhibits potent $LTB_4$ receptor binding activity and inhibition of $LTB_4$-induced events (in vitro and in vivo). Further details on the development of this unique series will appear in forthcoming publications.

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(14) Assay conditions are described in ref 8a. For each compound, an inhibition response study was done in triplicate on cells from a single individual and an $IC_{50}$ value calculated from the results. An estimate of the variation of this value among individuals can be made from results of similar studies done with other compounds in which the inhibitory effect was measured on cells from five individuals. The average standard deviation (standard error for $n = 1$) for six $LTB_4$ antagonists studied in this manner was 15 ● 4% of the mean $IC_{50}$.

(15) Concentration of preincubated antagonist (15 min at room temp) required to provide 50% inhibition of the up-regulated CD11b/CD18 expression of human neutrophils, activated with $1 \times 10^{-9}$ M $LTB_4$ (30 min at 37 °C). CD11b/CD18 expression was determined flow cytometrically by measuring single-cell fluorescence of specific monoclonal-antibody-reacted cells ($IC_{50}$ values are averages of at least four runs). See: Marder, P.; Schultz, R. M.; Spaethe, S. M.; Sofia, M. J.; Herron, D. K. Flow Cytometric Evaluation of the Effects of Leukotriene $B_4$ Receptor Antagonists (LY255283 and SC-41930) on Calcium Mobilization and Integrin Expression of Activated Human Neutrophils. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1991**, *46*, 265–270.