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Communications to the Editor

(+)-1-(3S,4R)-[3-(4-Phenylbenzyl)-4-hydroxychroman-7-yl]cyclopentane Carboxylic Acid, a Highly Potent, Selective Leukotriene B_4 Antagonist with Oral Activity in the Murine Collagen-Induced Arthritis Model

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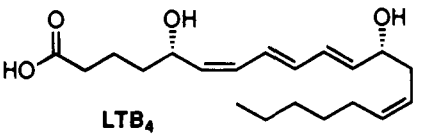
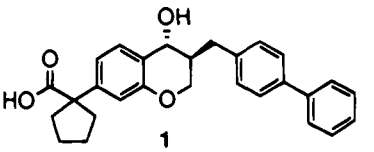
Current treatments for inflammatory diseases such as rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease (IBD) are not satisfactory.[1] The side-effect profiles of steroids and immunosuppressants limit their long-term use in all inflammatory conditions, while in arthritis, NSAIDS offer only palliative relief and do not halt disease progression.[2] Clearly, a novel agent with decreased side effects that suppressed tissue damage would represent a major advance in the treatment of debilitating inflammatory disease. Conceptually, one novel approach would be to inhibit the migration of leukocytes into the inflamed tissue and thereby block delivery by these cells of degradative enzymes, reactive oxygen species, and cytokines.

The migration of leukocytes *in vivo* is regulated by chemotactic factors that control both directional migration of cells (chemotaxis) and upregulation of cell surface adhesion molecules mediating cell–cell contact events required for cellular movement. One such factor is leukotriene B_4 (LTB_4) produced by the 5-lipoxygenase pathway of arachidonic acid metabolism. LTB_4 is a proinflammatory mediator that is synthesized by a number of cell types including mast cells, neutrophils, monocytes, and macrophages. Among its diverse biological effects, LTB_4 stimulates neutrophil aggregation, lysosomal enzyme release, chemotaxis, superoxide production, calcium mobilization, and upregulation of the $\beta 2$ integrin adhesion protein CD11b/CD18.[3] *In vitro*, LTB_4 also selectively stimulates monocytes to produce IL-6[4] and lymphocytes to produce IL-5[4] and induces a hyperadhesive state on endothelial cells[5] and *in vivo* increases vascular permeability.[6] In man, overproduction of LTB_4 has been observed in the rheumatoid synovial tissue,[7] psoriatic skin lesions,[8] inflammatory bowel disease,[9] gout,[10] and the sputum of cystic fibrosis[11] and asthma[12] patients. Given the demonstrated biologic properties of LTB_4, we undertook a program to design potent, orally active LTB_4 antagonists to better define the therapeutic potential for this class of drug in human inflammatory diseases.

Chart 1



LTB_4



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A number of laboratories have directed research toward the identification of LTB_4 antagonists[13] with several structural classes identified to date. In this communication, we wish to describe the structurally novel, selective, and potent LTB_4 receptor antagonist CP-105,696 (**1**) (Chart 1). This compound is the first reported LTB_4 antagonist to display oral activity in a well-defined chronic animal model of rheumatoid arthritis, the murine collagen-induced arthritis model.[14]

The synthesis of compound **1** is as shown in Scheme 1. Chromanone derivative **2**, prepared in three steps from resorcinol,[15] underwent an aldol condensation with

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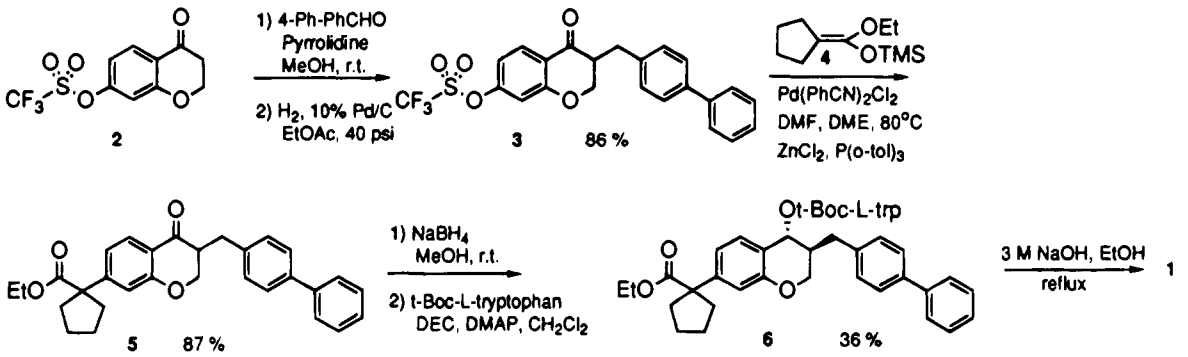
Scheme 1



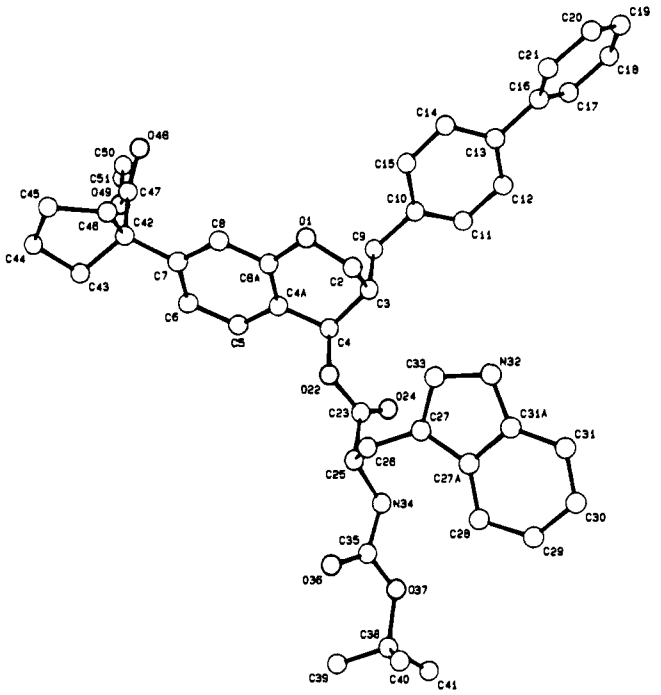


Figure 1.

4-phenylbenzaldehyde in the presence of pyrrolidine in MeOH; subsequent hydrogenaton afforded compound **3**. Introduction of the cyclopentanecarboxylic acid moiety into the 7 position of the chromanone skeleton was accomplished in one step by reaction of **3** with silyl ketene acetal 4[16] in the presence of catalytic amounts of $Pd(PhCN)_2Cl_2$, $ZnCl_2$, and P(*o*-tol)$_3$ (1:1 DMF:DME at 80 °C), affording the crystalline product **5** in 87% yield. This novel reaction allows for the direct formation of quaternary centers adjacent to an aromatic ring in one step via a palladium-catalyzed coupling under neutral conditions.[17] $NaBH_4$ reduction of the ketone afforded a 1.5:1 mixture of *cis*:*trans* alcohol isomers that were separated chromatographically. Resolution was achieved when the desired *trans* isomer was esterified with *t*-Boc-L-tryptophan and the diastereomers were separated via chromatography to afford **6**. Treatment of **6** with 3 M NaOH in refluxing EtOH afforded, after acidification with 1 M H_2SO_4, the final product **1** in 90% yield. The absolute configuration of **1** was deduced from the X-ray crystal structure of **6** (Figure 1).

Compound **1** is a potent inhibitor of the binding of [^{3}H]LTB_4 to whole human neutrophils with a half-maximal inhibition (IC_{50}) of 5.6 nM (n = 8). Using guinea pig spleen membranes or mouse spleen membranes as the source of LTB_4 receptors, 1 is a potent LTB_4 antagonist with IC_{50}s of 9.7 (n = 3) and 30.3 nM (n = 3), respectively. In all cases, the inhibitory activity was dose-dependent.[18] Several bioassays were used to determine the functional potency of our antagonist. Both high- and low-affinity forms of LTB_4 receptor has been characterized that differentially couple to chemotaxis and adhesion molecule expression, respectively.[19] LTB_4-induced chemotaxis of human neutrophils[20] was inhibited by 1 with an IC_{50} of 5.2 nM (n = 3), and LTB_4-induced upregulation of CD11b on human neutrophils[21] was inhibited with an IC_{50} of 430 nM (n = 4). Compound **1** did not show agonist activity in these assays. With human neutrophils, compound 1 (10 μM) did not exhibit any inhibition of the cyclooxygenase enzyme or inhibit chemotaxis induced by C5a, PAF, or IL-8, thus demonstrating a high degree of selectivity for antagonism of LTB_4.

The *in vivo* activity of **1** was assayed in two ways. Injection of LTB_4 (100 ng) intradermally in the mouse causes neutrophil accumulation measured at 4 h.[22] Oral administration of **1** in 0.5% methyl cellulose (dosed 1 h prior to challenge) inhibits this LTB_4-induced response with an ED_{50} value of 4.2 mg/kg (n = 3). At doses of 100 mg/kg, **1** failed to interfere with a similar response to injected interleukin-1, thus demonstrating selectivity for antagonism of LTB_4 *in vivo*. Compound **1** also inhibits neutrophil infiltration in response to LTB_4 (100 ng) in the guinea pig (ED_{50} = 0.26 mg/kg, n = 3) using a similar protocol as above. We next wished to examine 1 in a model of chronic inflammation to determine the role of LTB_4 in a pathologic process that more closely resembles human disease. Compound **1** was tested in the mouse collagen-induced arthritis[23] (CIA) model which exhibits a number of the pathological, immunological, and histological features in common with human rheumatoid arthritis, including a large neutrophil influx prior to flare.[24] In the standard protocol, arthritis is induced by immunizing susceptible strains of mice with heterologous collagen; over the next 30–50 days, a chronic polyarthritic develops. Both incidence and severity of inflammation in each paw were assessed on day 49.[25] Using this protocol (severity score = 3), orally administered doses of compound **1** provided protection from both disease incidence and severity (limb involvement, swelling) versus controls at daily doses of > 1 mg/kg. A more efficient CIA model with a more robust and reproducible disease response[26] can be induced by the administration (0.3 μg) of the cytokine interleukin-1 (IL-1) on days 25 and 26. We tested **1** in this IL-1-exacerbated CIA model, and again, even with a much increased severity score (8–9), 1 was able to abolish

disease at oral doses of ≥10 mg/kg (n = 3). More detailed examination of the mice showed that **1** had prevented both body weight loss associated with disease and histological damage associated with leukocyte influx into the knee joints of the mice. The activity of **1** in the CIA model is most likely attributable to an antiinflammatory effect rather than to inhibition of the immunologic response to collagen since IgG autoantibody titers to type II collagen were not lowered in these experiments.[25]

In conclusion, we report that **1** is a potent, selective LTB_4 receptor antagonist of novel structure that has potent activity in a model of chronic rheumatoid arthritis. Details of structure–activity relationships in this series will be reported in forthcoming publications.

Supplementary Material Available: Spectral and physical data for compound 1, dose–response curves for *in vitro* and *in vivo* assays, and X-ray data on compound **6** (28 pages). Ordering information is given on any current masthead page.

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