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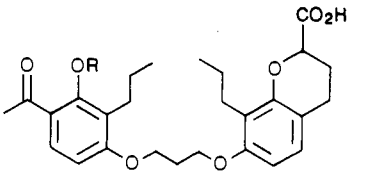
Communications to the Editor

7-[3-(4-Acetyl-3-methoxy-2-propylphenoxy)propoxy]-3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic Acid: An Orally Active Selective Leukotriene B_4 Receptor Antagonist

Sir:

Leukotriene B_4, (5*S*,12*R*)-6,14-*cis*,8,10-*trans*-eicosatetraenoic acid (LTB_4),[1] is believed to be a significant mediator of a number of inflammatory diseases, e.g. gout, psoriasis, and ulcerative colitis.[2] It stimulates aggregation and degranulation of human neutrophils, promotes chemotaxis and chemokinesis of leucocytes, and is a mediator of lysosomal enzyme release and superoxide generation.[3] As part of our program directed toward the synthesis of potential new therapeutic agents for the treatment of inflammatory bowel disease, we have examined the possibility that a receptor level antagonist of LTB_4[4] might be a useful agent for the treatment of ulcerative colitis.[5]

In this communication, we report on the chemical synthesis and biological profile of the first orally active, selective leukotriene B_4 receptor antagonist, SC-41930, 7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxy]-3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic acid (1).



1: R = CH_3
7: R = H

1 was synthesized as shown in Scheme I. Coupling of 2,4-dihydroxy-3-propylacetophenone (2) with 1-bromo-3-chloropropane in dry dimethylformamide in the presence of powdered anhydrous potassium carbonate afforded the crude chloride which was purified by silica gel chromatography and exposed to standard Finkelstein conditions to produce the iodide 3 in 95% yield. Alkylation with the known phenol 4[6] under the same conditions produced 5 in 62% yield as a white solid, mp 113–115 °C. Catalytic hydrogenation of 5 under acidic conditions (5% Pd/C, H_2, 60 psi, 75 °C, AcOH) gave the required reduction product 6 in 70% yield along with a minor amount of byproduct arising from reduction of the acetyl group. Methylation of the phenol (MeI, K_2CO_3, DMF) and subsequent saponification (LiOH, MeOH, H_2O) afforded 1 in 80% yield (for the two steps) as a crystalline solid, mp 69–70 °C.

Assays were developed to evaluate antagonism of the pharmacological activity of leukotriene B_4. Initial testing was done to determine the compound's ability to bind to LTB_4 receptors on human neutrophils. LTB_4 binding to high-affinity receptors is thought to be necessary to elicit a chemotactic response while low-affinity receptors appear to be associated with granular enzyme release (degranulation).[7] Binding to the LTB_4 receptor could produce either agonist or antagonist properties: therefore tests were performed to determine inhibitory effects on in vitro

(1) See, for example: Samuelsson, B. *Science* **1983**, *220*, 568. Lewis, R. A.; Austen, K. F. *J. Clin. Invest.* **1984**, *73*, 889.
(2) Bray, M. A. *Br. Med. Bull.* **1983**, *39*, 249; *Agents Actions* **1986**, *19*, 1. Thorsen, S. *Scand. J. Rheumatol.* **1986**, *15*, 225.
(3) Ford-Hutchinson, A. W.; Bray, M. A.; Doig, M. V.; Shipley, M. E.; Smith, M. J. H. *Nature* **1980**, *286*, 264. O'Flaherty, J. T.; Hammett, M.; Shewmake, T. B.; Wykle, R. L.; Love, S. H.; McCall, C. E.; Thome, M. J. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 552. Goetzl, E. J.; Pickett, W. *J. Immunol.* **1980**, *125*, 1789.
(4) No orally active LTB_4 receptor antagonists have been reported in the literature. However, some in vitro data has been recorded; for example: Goetzl, E. J.; Pickett, W. C. *J. Exp. Med.* **1981**, *153*, 482. Showell, H. J.; Otterness, I. G.; Marfat, A.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 741. Feinmark, S. J.; Lindgren, J. A.; Claesson, H. E.; Malmsten, C.; Samuelsson, B. *FEBS Lett.* **1981**, *136*, 141. Namiki, M.; Igarashi, Y.; Sakamoto, K.; Nakamura, T.; Koga, Y. *Biochem. Biophys. Res. Commun.* **1986**, *138*, 540. Morris, J.; Wishka, D. G. *Tetrahedron Lett.* **1988**, *29*, 143. Herron, D. K.; Bollinger, N. G.; Swanson-Bean, D.; Jackson, W. T.; Froelich, L. L.; Goodson, T. Poster No. 4729, FASEB 72nd Annual Meeting, Las Vegas, 1988.
(5) Sharon, P.; Stenson, W. F. *Gastroenterology* **1984**, *86*, 453.
(6) G.D. Searle and Co. U.S. Patent No. 4,565,882, 1986.
(7) Goldman, D. W.; Gifford, L. A.; Marotti, T.; Koo, C. H.; Goetzl, E. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 200.

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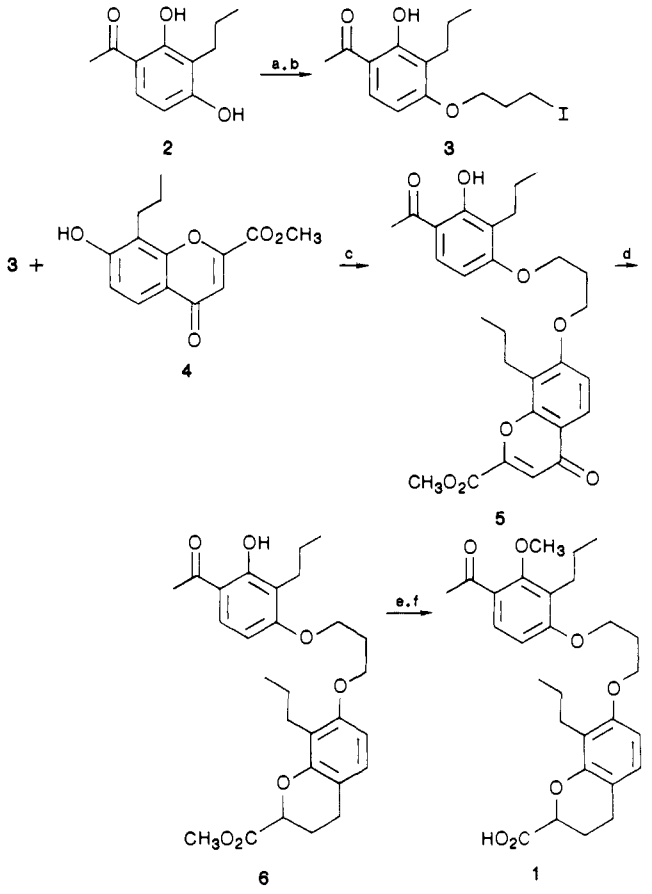
Scheme I[a]



[a] Reagents: (a) 1,3-chlorobromopropane, K_2CO_3, DMF; (b) NaI, acetone, Δ; (c) K_2CO_3, DMF; (d) H_2, Pd/C, AcOH; (e) MeI, K_2CO_3, DMF; (f) LiOH, MeOH, H_2O.

LTB_4-induced human neutrophil chemotaxis and granular enzyme release. The potential antiinflammatory activity in vivo was determined by measuring the inhibitory effect of the compound on LTB_4-induced neutrophil chemotaxis in the dermis of guinea pigs.

1 was shown to exhibit an IC_{50} of $(3 \pm 1) \times 10^{-7}$ M in a human neutrophil LTB_4 receptor binding assay[8] and effectively inhibit LTB_4-induced neutrophil degranulation and LTB_4-induced neutrophil chemotaxis in a modified Boyden Chamber ($IC_{50} = (1 \pm 0.5) \times 10^{-6}$ M and $(2 \pm 2) \times 10^{-6}$ M, respectively).[9] In addition, it inhibited LTB_4-induced intradermal chemotaxis in the guinea pig[10] when administered intragastrically or intravenously 1 h before dermal injection of LTB_4, $ED_{50} = 1.2 \pm 0.2$ μmol/kg.

These results suggest that 1 is able to antagonize the biological action of LTB_4 at both high- and low-affinity sites on its human PMN receptor. It is also able to antagonize the intradermal neutrophil chemotaxis induced by LTB_4 in cavine skin. These observations were followed up in two further in vivo models of inflammation, phorbol ester (PMA) induced ear inflammation[11] (as a model for psoriasis), and acetic acid induced colitis in the guinea pig.[12]

1 had a topical ED_{50} value of 4.1 ± 2.1 μmol/ear when coapplied with PMA as assessed by ear weight as a reflection of edema and myeloperoxidase activity as a reflection of neutrophil infiltration. Compound 1 had an oral ED_{50} value of 41 ± 4.3 μmol/kg when given 30 min before and after induction of colitis by 3% acetic acid. (A 40 mg/kg oral dose of 1 gives blood plasma levels of 7 μg/mL at 1 h and 12 μg/mL at 12 h.)

In addition, 1 was shown to be a selective leukotriene antagonist through its inability to bind to the LTD_4 receptor[13] (4% inhibition at 1×10^{-5} M) and in that at concentrations up to 50 μM it displayed no inhibition of bovine heart phosphodiesterase. The remarkable observation that methylation of the phenolic hydroxyl of a known chroman carboxylic acid leukotriene C_4/D_4 antagonist related to FPL 55712 confers leukotriene B_4 antagonist activity to the structure while abolishing LTC/D_4 antagonist activity is, to our knowledge, unique. The phenol 7 demonstrated no functional antagonist activity in receptor binding, degranulation, and chemotaxis assays at concentrations up to 10^{-5} M.[14] Apparently, phenolic methylation of a leukotriene C_4/D_4 antagonist in this class affords a selective LTB_4 antagonist with no effects on LTC_4/D_4 mediated function.

(8) Lin, A. H.; Ruppel, P. L.; Gorman, R. R. *Prostaglandins* **1984**, *28*, 837.

(9) Human neutrophil degranulation assay: LTB_4-induced neutrophil degranulation was determined by measuring the release of myeloperoxidase (MPO) activity into the incubation medium. Neutrophils (3×10^6) in 1 mL of HBSS solution were preincubated with cytochalasin B (5 μg) at 37 °C for 5 min, followed by preincubation with test compound for 7 min. Neutrophils were then incubated for 2–20 min with LTB_4 (5×10^{-8} M) to induce degranulation. Following incubation, samples were centrifuged, and MPO was extracted from the cell pellets by sonication in phosphate buffer containing 0.4% Triton X-100. Triton X-100 was also added to the supernatants to a concentration of 0.4%. The supernatants and the pellet extracts were then assayed. Modified Boyden chamber chemotaxis: Human neutrophils were isolated from citrated peripheral blood sedimented in 5% dextran, followed by centrifugation on Histopaque sterile solution and hypotonic lysis of erythrocytes. A final cell suspension of 3×10^6 neutrophils/mL of HEPES buffered Hanks balanced salt solution (HBSS) was added to the upper well of a modified Boyden chamber. The lower well (0.2 μL), separated by a polycarbonate membrane, contained HBSS or 3×10^{-8} M LTB_4 in the presence or absence of test compound. Following a 90-min incubation at 37 °C, cells from the lower well were lysed and nuclei counted in a Coulter counter. Percent inhibition was calculated from cell counts corrected for random migration by substracting the mean of the HBSS control. Detailed description of receptor binding, degranulation, and chemotaxis assays will be the subject of a forthcoming publication from Dr. B. S. Tsai's group.

(10) Guinea pig LTB_4-induced dermal chemotaxis: Test compounds were administered intragastrically prior to the injection of leukotriene B_4. LTB_4 was diluted in PBS, and 35 ng in 0.2 mL was injected intradermally into the shaven backs of anesthetized guinea pigs. PBS was injected as control. Four hours later the animals were sacrificed and the skins removed and stored frozen (−70 °C). Injection sites were removed with a skin punch and mechanically homogenized. MPO was extracted with 0.5% hexadecyltrimethylammonium bromide in 50 mM $KHPO_4$ buffer (pH 6.0) by using sonication and freeze–thaw procedures. After centrifugation, enzyme activities in the supernatants were assayed spectrophotometrically. The level of MPO activity was found to increase with the amount of LTB_4 injected.

(11) Bourin, M. C.; Delescluse, C.; Furstenberger, G.; Marks, F.; Schweizer, J.; Klein-Szanto, A. J. P.; Prunieras, M. *Carcinogenesis* **1982**, *3*, 671.

(12) MacPherson, B.; Pfeiffer, C. *Digestion* **1978**, *17*, 135.

(13) LTD_4 receptor binding assay: LTD_4 membrane receptors were prepared from a male guinea pig lung homogenate which was aliquoted and stored at −70 °C. The particular preparation used to test SC-41930 had a K_D of 0.262 nM, yielding 0.447 pmol of receptor/mg of protein. Protein concentrations were adjusted to provide approximately 0.1 nM receptor in the presence of 1 nM of [^{3}H]LTD_4. Incubation was conducted for 30 min at 24 °C, and filtration methods were used to separate bound from free ligand.

Summarily, 1 demonstrates activity in a number of models of inflammation and seems a likely candidate for clinical trials to test the hypothesis that a LTB_4 receptor antagonist may have therapeutic value in the treatment of inflammatory bowel disease and other conditions where LTB_4 is a putative mediator such as psoriasis and ankylosing spondylitis.

(14) O'Donnell, et al. (O'Donnell, M.; Welton, A. F.; Crowley, H.; Brown, D.; Garippa, R.; Cohen, N.; Weber, G.; Banner, B.; Lapresti, R. J. *Adv. Prostaglandin, Thromboxane Leukotriene Res.* **1987**, *17*, 512) describe a related LTC_4/D_4 antagonist that antagonizes LTB_4-induced bronchospasm in guinea pigs. No receptor binding data was reported however.

Stevan W. Djuric,* Paul W. Collins, Peter H. Jones Robert L. Shone, Bie Shung Tsai, Donald J. Fretland Gregory M. Butchko, Doreen Villani-Price Robert H. Keith, Jeanne M. Zemaitis Linda Metcalf, Raymond F. Bauer

Gastrointestinal Diseases Research G.D. Searle and Co. Skokie, Illinois 60077

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Articles

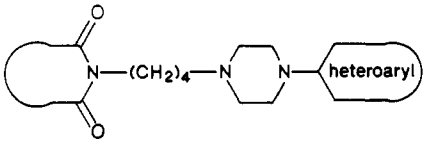
The Thieno[3,2-*c*]pyridine and Furo[3,2-*c*]pyridine Rings: New Pharmacophores with Potential Antipsychotic Activity

James S. New,*,† William L. Christopher,† Joseph P. Yevich,† Rhett Butler,† R. Francis Schlemmer, Jr.,‡ Cam P. VanderMaelen,† and Joseph A. Cipollina†

Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, 5 Research Parkway, P.O. Box 5100, Wallingford, Connecticut 06492-7660, and the Department of Pharmacodynamics, University of Illinois at Chicago, Chicago, Illinois 60612. *Received June 6, 1988*

Two new arylpiperazine derivatives, the 4-(1-piperazinyl)thieno- and -furo[3,2-*c*]pyridine ring systems, have been synthesized and appended via tetramethylene chains to various imide rings. Target compounds from each series were found to have significant activity in the blockade of apomorphine stereotypy and apomorphine-induced climbing, the Sidman avoidance response, and the conditioned avoidance response. In addition, while potent affinity for serotonin 5-HT_1 and 5-HT_2 receptors was observed for both the thieno- and furo[3,2-*c*]pyridine derivatives, the interaction of these molecules with the dopamine D2 receptor was weak. Electrophysiological studies of the lead prototypes from each series, involving compounds **22** and **33**, indicate these two molecules have distinctively different effects on dopamine neurons in areas A9 and A10. Despite the similarity these molecules share in their behavioral indices of antipsychotic activity, it is likely that the thieno- and furo[3,2-*c*]pyridine rings employ different mechanisms to achieve this convergence of biological effects.

The family of chemical structures generically described as the *N*-[(4-heteroaryl-1-piperazinyl)alkyl]-substituted imides (1) has generated clinical candidates with anti-



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psychotic or anxiolytic properties. Noteworthy alumni in this class of psychotropic molecules are the anxiolytic agents buspirone and gepirone and the antipsychotic agent tiospirone.[1-3] The pharmacological profile of these molecules is largely determined by the dominant influence of the heteroarylpiperazine moiety that is common to each of their structures. In the case of buspirone and gepirone, the serotonin agonist properties that mediate the anxiolytic effects of these compounds can be attributed to their 1-(2-pyrimidinyl)piperazine substructure. In tiospirone, the blend of dopamine and serotonin antagonist properties, which arise from its 1-benzisothiazol-3-ylpiperazine moiety, contribute to the antipsychotic activity of the molecule. The function of the imide group in these molecules is less well understood, but its modification in lead optimization studies is commonly pursued toward the fine-tuning of the molecule's biological expression.

Recently, we reported an extension of this structural family in a series of 3-substituted 2-pyridinyl-1-piperazine derivatives.[4] The desired antipsychotic profile of these molecules was shown to be strictly dependent on the electronic and lipophilic properties of the substituent located at the 3-position of the pyridine ring. Inspired by the specificity of this effect and the promising biological activity that resulted from it, we embarked on the synthesis of several hetero-ring-fused pyridine compounds.

The design of the target molecules stemmed from the observation that the lead prototypes in the 3-substituted pyridinylpiperazine study were formulated with X = ni-

† Bristol-Myers Pharmaceutical Research and Development Division.

‡ Department of Pharmacodynamics.

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(2) New, J. S.; Yevich, J. P.; Eison, M. S.; Taylor, D. P.; Eison, A. S.; Riblet, L. A.; VanderMaelen, C. P.; Temple, D. L., Jr. *J. Med. Chem.* **1986**, *29*, 1476.
(3) Yevich, J. P.; New, J. S.; Smith, D. W.; Lobeck, W. G.; Catt, J. D.; Minielli, J. L.; Eison, M. S.; Taylor, D. P.; Riblet, L. A.; Temple, D. L., Jr. *J. Med. Chem.* **1986**, *29*, 359.
(4) New, J. S.; Yevich, J. P.; Temple, D. L., Jr.; New, K. B.; Gross, S. M.; Schlemmer, R. F., Jr.; Eison, M. S.; Taylor, D. P.; Riblet, L. A. *J. Med. Chem.* **1988**, *31*, 618.

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