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ORTHO-ALKOXYPHENOL LEUKOTRIENE B_4 RECEPTOR ANTAGONISTS

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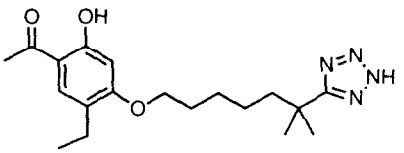
Abstract: A series of ortho-alkoxyphenols containing a tetrazole acid sidechain have been prepared as antagonists of leukotriene B_4 receptors. These compounds were tested as receptor antagonists of human neutrophil and guinea pig lung membrane leukotriene B_4 receptors. Compounds in this series were found to be up to 18-fold more potent than LY255283. These results indicate that the acyl group of the 1,2,4,5 substituted hydroxyacetophenone class of LTB_4 antagonists is not critical to antagonist potency.

Leukotriene B_4 (LTB_4) is a metabolite of the arachidonic acid pathway and binds to specific membrane bound receptors found on both neutrophils and eosinophils.[1] The binding of LTB_4 to neutrophil receptors induces many functional responses which include cell chemotaxis and chemokinesis,[2] aggregation,[3] lysosomal enzyme release,[2] superoxide production,[4] calcium mobilization and upregulation of CD11b/CD18 adhesion proteins.[5] Also, via a receptor mediated mechanism, LTB_4 is known to induce bronchoconstriction in guinea pig airways.[6] Along with LTB_4's effect on cell function, the existence of increased numbers of neutrophils and/or eosinophils as well as increased amounts of LTB_4 in inflammatory tissues has implicated LTB_4 as an important mediator in the inflammatory processes of several diseases such as asthma,[7] inflammatory bowel disease,[8] psoriasis,[9] and arthritis.[10] LTB_4 has also been implicated in adult respiratory distress syndrome (ARDS).[11] In order to delineate the role of LTB_4 in inflammatory diseases, potent and selective antagonists of LTB_4 receptors are needed.

Herron, Goodson and Jackson reported the discovery of LY255283 (**1**) a novel hydroxyacetophenone LTB_4 receptor antagonist.[12] In their analysis of the LY255283 structure and its relationship to the structure of LTB_4 itself, Herron etal. speculated that the strongly hydrogen-bonded hydroxyacetophenone unit of LY255283 provided an extended planar system that behaved as a mimetic of the triene moiety of LTB4.[12] Alternatively, one could speculate that the ketone oxygen of the acyl group provides an important electrostatic interaction at the receptor site, or that the acyl group introduces a positive van der Waals interaction. Within our effort to discover novel LTB_4 receptor antagonists as antiinflammatory agents, we were interested in further defining the role of the ortho-phenol substituent in receptor-antagonist recognition, and subsequently, further delineate the nature of the receptor binding site for this class of antagonists. We report here our work on a series of ortho-alkoxyphenol LTB_4 receptor antagonists.



LY255283

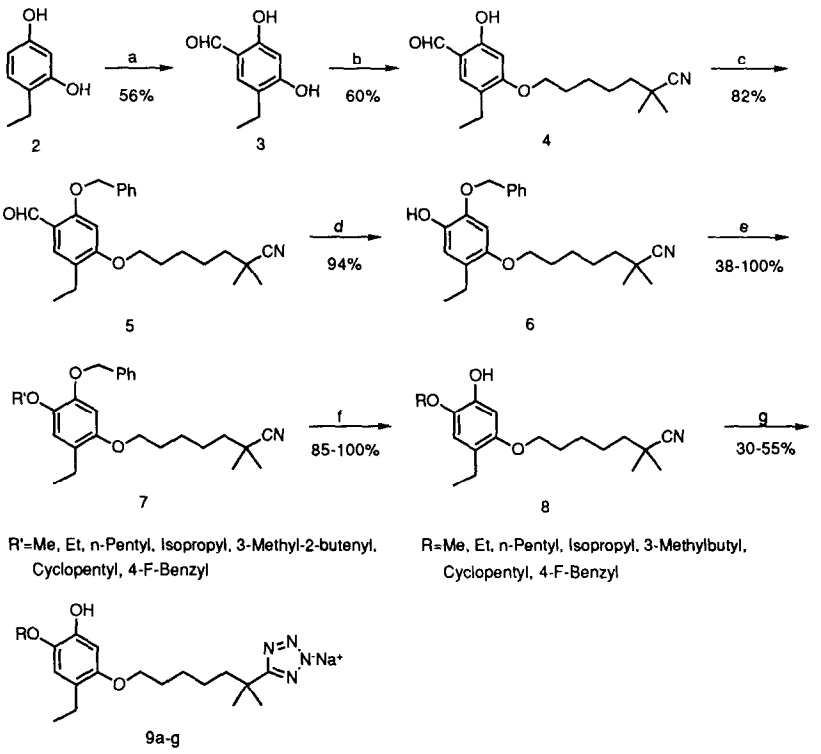
1

The ortho-alkoxyphenols (**9a** to **9g**) were prepared as described in Scheme 1. The commercially available ethylresorcinol was formylated using Vilsmeier conditions to give the aldehyde **3** in 56% yield. The next step required a selective alkylation of the phenol ortho to the ethyl substituent. In the case of the acetophenone series (LY255283), the phenols were easily differentiated by the fact that one of the phenols is strongly hydrogen-bonded to the ketone of the acyl moiety.[12] However, the phenols of aldehyde **3** did not show any chemoselectivity when using conditions which gave chemoselectivity in the acetophenone case. Only a mixture of mono- and dialkylated products were obtained when aldehyde **3** was refluxed with 1-chloro-6-cyano-6-methylheptane or its corresponding iodide in methylethylketone (MEK) or MEK/DMSO in the presence of a base (K_2CO_3).[12] But, when aldehyde **3** was reacted under Mitsunobu conditions with 6-cyano-6-methyl-1-heptanol, the desired monoalkylated product **4** was obtained in 60% yield.[13] The remaining phenol was protected as the benzyl ether. Baeyer-Villiger oxidation of the aldehyde **5** proceeded smoothly to provide the key phenol intermediate **6**. The phenol **6** was then alkylated with the appropriate alkyl halide using NaH in DMF or with an alcohol under Mitsunobu conditions. Susequent removal of the benzyl protecting group (10% Pd/C, EtOAc), and elaboration of the nitrile to the tetrazole ($nBuSnN_3$, ethylene glycol diethylether[14] or NaN_3, $Me_2NCH_2CH_2OH$, HCL, diglyme, 135°C[12]) gave the desired tetrazoles as their free acids. The tetrazole free acids were converted to their sodium salts (2N NaOH/MeOH) and purified by CHP-20 medium pressure chromatography. The sodium salts were obtained as white lyophilates.

The receptor binding affinities of the ortho-alkoxyphenols were evaluated in both human neutrophil[12] and guinea pig lung membrane[15] radioligand binding assays. We observed that the straight chain alkyl ethers (**9a**, **9b**, and **9c**), were especially potent ligands (see Table 1). When compared to the parent hydroxyacetophenone LY255283 (**1**), these straight chain alkyl ethers produced as much as an 18-fold enhancement in binding affinity for human neutrophil receptors and a 5-fold enhancement in affinity for guinea pig lung membrane receptors. Relative to the straight chain ethers, branching either near the ether oxygen or distal to it (compounds **9d**, **9e**, and **9f**) did reduce potency. A benzylether substituent (**9g**) also reduced potency when compared to the straight chain ether derivatives. It is interesting that the ortho-alkoxyphenols bound with higher affinity to receptors on

human neutrophils than to receptors on guinea pig lung membranes. This difference in potency could be related to species or cell specific differences in receptor structure.

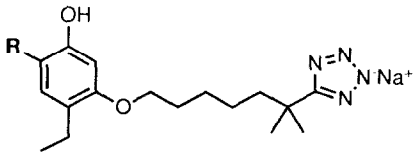
Scheme 1



Reagents: a) $POCl_3$, DMF, CH_2Cl_2; b) DEAD, Ph_3P, THF, 6-cyano-6-methyl-1-heptanol, 25°C; c) NaH, DMF, 18-C-6, benzylbromide, 25°C; d) i) mCPBA, CH_2Cl_2, room temp, ii) NaOH, THF; e) NaH, DMF, 18-C-6, R'Br, 25°C; or R'OH, DEAD, Ph_3P, THF, 25°C; f) 10%Pd/C, H_2(g)(1atm), EtOAc; g) i) tri-n-butyltin azide, ethylene glycol diethylether, or NaN_3, dimethylamino ethanol hydrochloride, diglyme, 135°C, 48-72h, ii) NaOH, CHP-20 chromatography.

We also observed that the ortho-alkoxyphenols antagonized the effect of LTB_4 *in vivo* in a guinea pig model of LTB_4-induced airway obstruction (i.e. lung gas trapping).[16] When given iv, the ED_{50} for inhibition of gas trapping for the ethylether **9b** was 0.3mg/Kg compared to 2.8mg/Kg for the hydroxyacetophenone **1**.

Table 1. Alkoxyphenol Antagonists: Receptor Binding Against [^{3}H]LTB$_4$



Compound No.	R	Human Neutrophil Binding IC_{50} (nM)[17]	Guinea Pig Lung Membrane Binding K_I (nM)[18]
1	CH_3CO	85.1±7.9	77.9±10.4
9a	CH_3O	6.0	25.1±9.2
9b	CH_3CH_2O	4.8	14.2±2.9
9c	$CH_3(CH_2)_4O$	6.6	22.6±5.5
9d	$(CH_3)_2CH_2O$	12.0	52.7±18.2
9e	$(CH_3)_2CH(CH_2)_2O$	35.6	82.5±16.5
9f	Cyclopentyloxy	41.5	109.1±19.9
9g	4-F-Benzyloxy	30.8	110.3±8.5

We have demonstrated for the 1,2,4,5 substituted hydroxyacetophenone class of LTB4 receptor antagonists that the small acyl moiety of LY255283 is not critical for either inherent antagonist-receptor recognition or antagonist potency. In fact, the ortho-ketone substituent can be replaced by an alkoxy group, which in some cases produces significantly more potent antagonists of LTB$_4$ receptors in both human neutrophils and guinea pig lung membranes. Consequently, if one assumes that by replacing the acyl group of LY255283 with an ether substituent, one does not dramatically alter the binding mode of the 1,2,4,5 substituted phenol class of LTB4 receptor antagonists, then it is clear that the region of the receptor which binds the substituent ortho to the phenol can accommodate modest size lipophilic groups larger than an acyl moiety and resembles a hydrophobic cleft.

Therefore, the acyl moiety of LY255283 may be contributing to a van der Waals interaction at the receptor site but in a suboptimal way. Finally, based on the improved affinities of the ether analogues, the ketone oxygen of the acyl group of LY255283 does not appear to contribute to any significant electrostatic interactions with the receptor.

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REFERENCES

1. a) Kreisle, R.A.; Parker, C.W. *J. Exp. Med.* **1983**, *157*, 628-641. b) Goldman, D.W.; Goetzl, E.J. *J. Exp. Med.* **1984**, *159*, 1027-1041. c) Ng, C.F.; Sun, F.F.; Taylor, B.M.; Wolin, M.S.; Wong, P.Y.-K. *J. Immunol.* **1991**, *147*, 3096-3103. d) Sehmi, R.; Cromwell, O.; Taylor, G.W.; Kay, B. *J. Immunol.* **1991**, *147*, 2276-2283. e) Maghni, K.; De Brum-Fernandes, J.; Foldes-Filep, E.; Gaudry, M.; Borgeat, P.; Sirois,P. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 748-789. f) Ng, C.F.; Sun, F.F.; Taylor,B.M.; Wolin, M. S.; Wong, P.Y-K. *FASEB J.* **1990**, *4*, A624.

2. a) Goetzl, E.J.; Pickett, W.C. *J. Immunol.* **1980**, *125*, 1789. b) Palmer, R.M.; Stepney, R.J.; Higgs, G.A.; Eakins, K.E. *Prostaglandins* **1980**, *20*, 411.

3. Ford-Hutchinson, A.W.; Bray, M.A.; Doig, M.E.; Shipley, M.E.; Smith, J.H. *Nature* **1980**, *286*, 264.

4. Palmblad, J.H.; Gyllenhammer, J.A.; Lindgre, J.A.; Malmsten, C.L. *J. Immunol.* **1984**, *132*, 304.

5. Marder, P.; Schultz, R.M.; Spaethe, S.M.; Sofia, M.J.; Herron, D.K. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **1992**, *46*, 265.

6. Hamel, R.; Ford-Hutchinson, A.W. *Prostaglandins* **1983**, *25*, 405-412.

7. a)Aizawa, T.; Tamura, G.; Ohtsu, H.; Takishima, T. *Ann. of Allergy* **1990**, *64*, 287-292. b)Piper, P.J.; Conroy, D.M.; Costello, J.F.; Evans, J.M.; Green, C.P.; Price, J.F.; Sampson, A.P.; Spencer, D.A. *Ann. N.Y. Acad. Sci.* **1991**, *629*, 112-119. c) Sampson, A.P.; Green, C.P.; Spencer, D.A.; Piper, P.J.; Price, J.F. *Ann. N.Y. Acad. Sci.* **1991**, *629*, 437-439. d) Wardlaw, A.J.; Hay, H.; Crimwall, O.; Collins, J.V.; Kay, A.B. *J. Allergy Clin. Immunol.* **1989**, *84*, 19-26.

8. a) Fretland, D.J.; Djuric, S.W.; Gaginella, T.S. *Prostaglandins ,Leukotrienes and Essential Fatty Acids* **1990**, *41*, 215-233. b) Sharon, P.; Stenson W.F. *Gastroenterology* **1984**, *86*, 453-460.

9. a) Ekerdt, R.; Buchmann, B.; Frohlich, W.; Giesen, C.; Heindl, J.; Skuballa, W. *Adv. in Prostagland., Thrombox. and Leukotriene Res.* **1990**, *21*, 565-568. b) Brain, S.D.; Williams, T.J. *Pharmac. Ther.* **1990**, *46*, 57-66. c) Rosenbach, T.; Grabbe, A.; Schwanitz, H. J.; Czarnetzki, B.M. *Br. J. Dermatol.* **1985**, *113*, 157-167.

10. Brain, S.D.; Williams, T.J. *Pharmac. Ther.* **1990**, *46*, 57-66.

11. a) Davis, J.M.; Meyer, J.D.; Barie, P.S.; Yurt, R.W.; Duhaney, R.; Dineen, P.; Shires, G.T. *Surg, Gynecol. and Obstetr.* **1990**, *170*, 405-500. b) Antonelli, M.; Bufi, M.; DeBlasi, R.A.; Crimi, G.; Conti, G.; Mattia, C.; Vivino, G.; Lenti, L.; Lombardi, D.; Dotta, A.; Pontieri, G.; Gasparetto, A. *Intens. Care Med.* **1989**, *15*, 296-301.

12. a) Herron, D.K.; Bollinger, N.G.; Swanson-Bean, D.; Jackson W.T.; Froelich,Ll.; Goodson,T. *FASEB J.* **1988**, *2*, A1110. b) Jackson, W.T.; Boyd, R.J.; Froelich, L.l.; Goodson T.; Bollinger, N.G.; Herron D.K.; Mallett, B.E.; Gapinski, D.M. *FASEB J.* **1988**, *2*, A1110. c) Jackson W.T.; Froelich, L.L.; Goodson T.; Herron, D.K.; Mallett, B.E.; Gapinski,D.M. *Physiologist* **1989**, *31*, A206. d) Herron, D.K.; Goodson, T.; Bollinger, N.G.; Swanson-Bean, D.; Wright, I.; Staten, G.S.; Thompson, A.R.; Froelich, L.L.; Jackson, W.T. *J.Med.Chem.* **1992**, *35*, 1818.

13. Mitsunobu, O. *Synthesis* **1981**, 1-28.

14. Sisido, K.; Nabika, K.; Isida, T.; Kozima, S. *J. Organomet. Chem.* **1971**, *33*, 337-346.

15. Silbaugh, S.; Stengel, P.W.; Cockerham, S.L.; Roman, C.R.; Saussy, Jr., D.L.; Spaethe, S.M.; Goodson, T.; Herron, D.K.; Fleisch, J.H. *Euro. J. Pharmacol.* **1992**, in press.

16. Silbaugh, S.A.; Stengel, P.W.; Dillard, R.D.; Bemis, K.G. *J. Pharmacol. Methods* **1987**, *18*, 295-303. b) Silbaugh, S.A.; Stengel, P.W.; Cockerham, S.L.; Mallett, B.E.; Gapinski, D.M. *Physiologist* **1988**, *31*, A91. c) Stengel, P.W.; Silbaugh, S.A. *Respir. Physiol.* **1986**, *63*, 129-138

17. For each compound, an inhibition response study was done in triplicate on cells from a single individual. For comparison of results from one individual to another, the amount of inhibition of a reference inhibitor, LY177455, was determined at 9.2μM and 0.92μM on each cell suspension. The mean percent inhibition and standard error in IC_{50} values for these compounds if studies had been done with cells from other individuals can be estimated from standard deviations of IC_{50} values obtained for compounds whose effects were measured on cells from five individuals. The average standard deviation for six LTB_4 antagonists studied in this manner was $15\pm4\%$ of the mean IC_{50}.

18. Data are expressed as Mean ± SEM of values obtained from 3 to 10 experiments performed in duplicate as described in reference 15.