*Bioorganic & Medicinal Chemistry Letters*, Vol.3, No.6, pp. 1147-1152, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
© 1993 Pergamon Press Ltd

# ACID UNIT MODIFICATIONS OF 1,2,4,5-SUBSTITUTED HYDROXYACETOPHENONES AND THE EFFECT ON *IN VITRO* AND *IN VIVO* LTB4 RECEPTOR ANTAGONISM

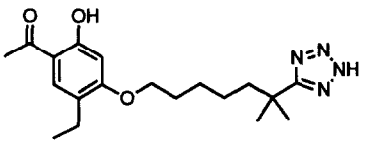
Michael J. Sofia*, Paul Floreancig, William T. Jackson, Philip Marder, David L. Saussy, Jr.#,
Steven A. Silbaugh, Sandra L. Cockerham, Larry L. Froelich,
Carlos R. Roman, Peter W. Stengel, and Jerome H. Fleisch

LILLY RESEARCH LABORATORIES, ELI LILLY AND CO., INDIANAPOLIS, INDIANA 46285.

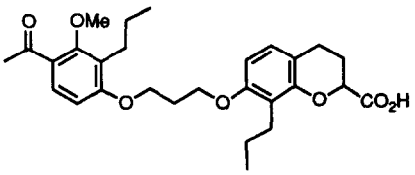
(*Received in USA* 18 *February* 1993)

**Abstract:** 7-[3-(4-Acetyl-2-ethyl-5-hydroxyphenoxy)propoxy]-3,4-dihydro-8-propyl-2H-1-benzopyran-2-carboxylic acid sodium salt **7** (LY247826) and several 7-[3-(4-acetyl-2-ethyl-5-hydroxyphenoxy)propoxy)-2-propylphenoxy] acetic acid analogues were prepared as LTB4 receptor antagonists. When compared with LY255283 and SC-41930, these antagonists showed improved *in vitro* LTB4 receptor antagonism and/or *in vivo* activity.

Leukotriene B4 ( LTB4 ), a product of arachidonic acid metabolism, has received much attention as a potential pro-inflammatory mediator in diseases such as asthma[1], inflammatory bowel disease[2], psoriasis[3] and arthritis[4]. The role that LTB4 plays in these diseases is believed to occur via its ability to act as a chemoattractant to both neutrophils[5] and eosinophils[6] and to activate these cells to release degradative enzymes and toxic oxygen metabolites[5,7]. The development of potent and bioavailable receptor antagonists for LTB4 receptors would provide useful tools to delineate the role of LTB4 in various inflammatory diseases as well as to potentially provide useful antiinflammatory therapies.




**1** (LY255283)




**2** (SC-41930)

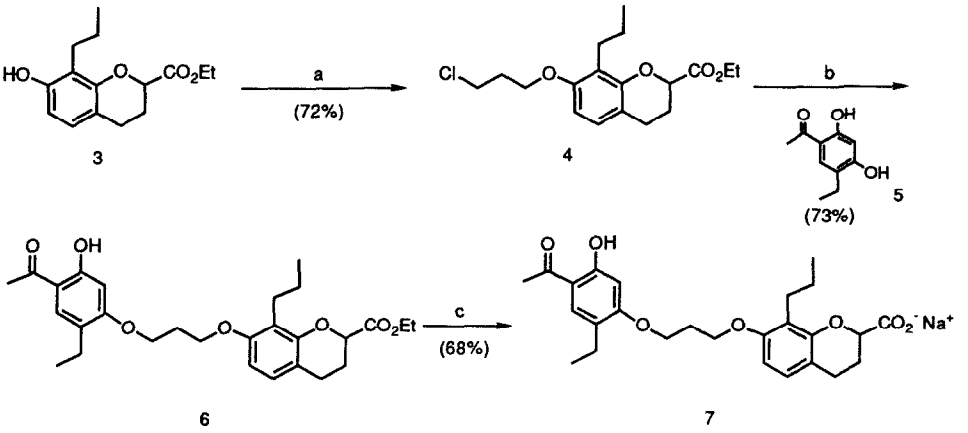
Two of the first reported LTB4 receptor antagonists were **1** (LY255283)[8] and **2** (SC-41930)[9]. In a effort to enhance both the intrinsic and *in vivo* potency of the 1,2,4,5-substituted hydroxyacetophenone series of antagonists represented by LY255283, we were interested in further investigating the structure activity of the acid portion of this class of compounds. The reported SAR of the 1,2,4,5-substituted hydroxyacetophenone series of LTB4 antagonists indicated that the *gem*-dimethyl substitution adjacent to the tetrazole acid moiety was critical for achieving enhanced receptor binding affinity. The class of antagonists represented by SC-41930,

# Current address: Glaxo Research Institute, Research Triangle Park, NC.

although different from LY255283 in the substitution pattern on the acetophenone portion of the molecule, also contain a lipophilic acid moiety, the chroman carboxylic acid. Hence, we decided to study the effect of replacing the *gem*-dimethyl tetrazole acid unit in the LY255283 class of antagonists with the lipophilic chroman carboxylic acid and with several open chain chroman analogues. Reported here are the effects observed on both *in vitro* binding and $LTB_4$-induced functional responses as well as the dramatic effects observed on *in vivo* activity when the chroman acid unit and its simple derivatives are coupled to the hydroxyacetophenone unit of LY255283.

Preparation of the chroman acid hydroxyacetophenone analogue **7** is outlined in Scheme 1. Alkylation of the chroman phenol[9,10] **3** with 1-bromo-3-chloropropane in DMF proceeded cleanly to give the chloride **4**. The bis-phenol **5**[8] was regioselectively alkylated with the chloride **4** via *in situ* Finkelstein exchange in a refluxing methyl ethyl ketone and DMSO solvent mixture containing solid $K_2CO_3$[8]. The hydroxyacetophenone ester **6** was saponified with NaOH, and the resulting sodium salt **7** (LY247826)was purified on CHP-20 resin by eluting with a methanol-water solution.

**Scheme 1**



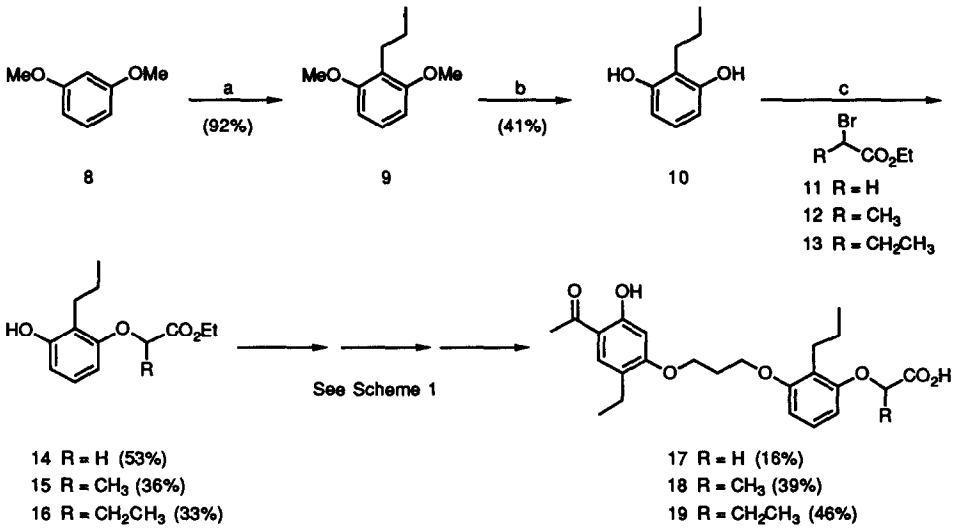

3 4 5 6 7

*Reagents:* a) $K_2CO_3$, DMF, 1-bromo-3-chloropropane, 25°C; b) $K_2CO_3$, KI, MEK, DMSO, reflux, 18h; c) i) NaOH, $H_2O$, Dioxane, 25°C; ii) CHP-20 chromatography.

The open chain analogues of the chroman acid were prepared by first constructing the phenoxy esters **14, 15** and **16** from dimethylresorcinol **8** (see Scheme 2). Regiospecific metallation with n-BuLi followed by addition of propyliodide gave 1,3-dimethoxy-2-propylbenzene **9** in 92% yield. Bis-demethylation of **9** was accomplished by heating in pyridine-HCl[11]. The bis-phenol **10** was obtained as a crystalline material from $CH_2Cl_2$. The desired monoalkylated phenols **14, 15**, and **16** were prepared by alkylation of the sodium

alkoxide of **10** with the corresponding α-bromo ester **11, 12** or **13**. Attachment of the hydroxyacetophenone **5** to the corresponding phenol esters **14, 15** or **16** was accomplished as outlined for the preparation of chroman **7**. The free acids **17** (LY282947), **18** (LY285722) and **19** (LY287126) were obtained after saponification, acidification and crystallization from either diethyl ether/hexane or ethyl acetate/hexane.

**Scheme 2**




*Reagents:* a) i) n-BuLi, THF, -10°C, ii) iodopropane, 25°C, 18h, iii) reflux, 2h; b) pyridine-HCl, 110°C, 17h; c) NaH, THF, 25°C, 17h.

The chroman carboxylic acid **7**, and its acyclic derivatives **17, 18** and **19** were evaluated *in vitro* to determine both intrinsic receptor affinity and antagonism of LTB4-induced functional responses of human neutrophils and guinea pig lung tissues (see Table 1). Attachment of a chroman acid or any of its acyclic derivatives to the 1,2,4,5-substituted hydroxyacetophenone produced as much as a 3-fold enhancement in intrinsic human neutrophil receptor binding affinity[8] and as much as a 15-fold enhancement in binding affinity for guinea pig lung membrane receptors[12] when compared to **1**. Relative to **1** and consistent with the enhancement in intrinsic receptor affinity, the chroman acid **7** showed improvement in the ability to antagonize both LTB4-induced CD11b /CD18 adhesion protein up-regulation in human neutrophils[13] (1.8-fold for **7** vs **1**) and LTB4-stimulated lung parenchyma strip contraction[12] ( 23-fold for **7** vs **1** ). When compound **7** was compared with compound **2** where now the acid portions of the molecules remain the same and the left-hand lipophilic portions of the molecules differ, it was observed that within species, the ratios of the binding affinities of **7** vs **2** correlated well with the ratios of the $IC_{50}$'s for antagonism of CD11b/CD18 integrin up-regulation and $K_B$'s for guinea pig parenchyma strip contraction. However, in every instance whether it be with regard to

receptor affinity or functional antagonism in either human neutrophils or guinea pig tissues, compound **7** was shown to be either equipotent or more potent than SC-41930 (**2**). Within the acyclic series of compounds **17**, **18** and **19**, the ability to antagonize $LTB_4$ induced functional responses in either human neutrophils or guinea pig tissues was not as dramatic as that observed for chroman 7.

**Table 1. Receptor Binding Affinities and Antagonism of $LTB_4$ Induced Functional Responses.**

| Compound No. | Human Neutrophil Binding $IC_{50}$(nM)[15] | Guinea Pig Lung Membrane Binding Ki (nM) | Human Neutrophil CD11b/CD18 Integrin Up-regulation $IC_{50}$ (nM)[16] | Guinea Pig Lung Parenchyma Strip Contraction $K_B$[a] (nM) |
|---|---|---|---|---|
| **7** | 39.6 | 10.3 ± 2.8 | 1600 ± 408 | 8.40 ± 1.67 |
| **17** | 67 | 4.96 ± 0.92 | 4388 ± 275 | 30.0 ± 7.30 |
| **18** | 23 | 18.5 ± 3.4 | 3271 ± 347 | 75.8 ± 26.6 |
| **19** | 36 | 15.5 ± 5.6 | 1941 ± 140 | 85.8 ± 20.7 |
| **1** | 85.1 ± 7.9 | 77.9 ± 10.4 | 2874 ± 470 | 197 ± 43 |
| **2**[14] | 41.3 ±3.1 | 15.4 ± 2.9 | 1860 ± 586 | 22.0 ± 3.3 |

[a]Dissociation constant of receptor-inhibitor complex.

When **7** was evaluated *in vivo* in a guinea pig model of $LTB_4$-induced airway obstruction (*i.e.*, pulmonary gas trapping)[17], a large enhancement in oral potency was observed relative to that found for both **1** and **2**. Compared to **1** ($ED_{50}$'s: iv = 2.8mg/Kg, po = 11.0mg/Kg), chroman acid **7** ($ED_{50}$'s: iv = 0.23mg/Kg, po = 0.50mg/Kg) produced a 10-fold improvement in efficacy when administered iv and a 22-fold improvement in oral efficacy (Figure 1A and B). These superior *in vivo* potencies can be partially attributed to its superior *in vitro* profile relative to **1**. When compared to **2** ($ED_{50}$'s: iv = 0.35mg/Kg, po = 3.5mg/Kg), chroman acid **7** exhibited comparable iv activity. This similarity in iv activity is in agreement with the receptor affinities and functional antagonistic profiles demonstrated for both **7** and **2** (see Table 1). However, in comparison to **2**, chroman acid **7** again demonstrated superior oral efficacy in the pulmonary gas trapping model (Figure 1B). This increased oral activity observed for chroman acid **7** relative to both **1** and **2** can possibly be explained by a more favorable pharmacokinetic or pharmacodynamic profile.

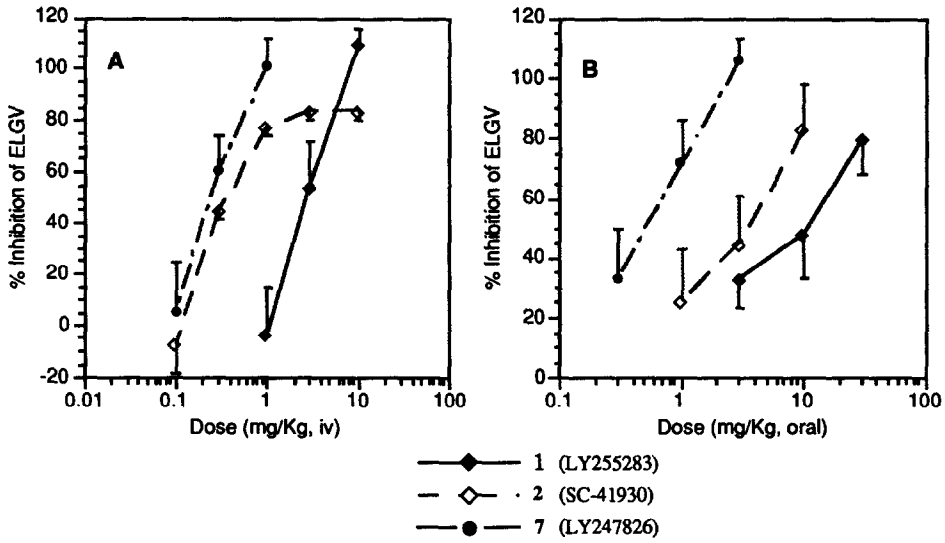



**Figure 1.** **A.** Effect of intravenously administered **1**, **2**, and **7** on inhibition of LTB4-induced increases in excised lung gas volumes (ELGV). Intravenous doses were given 5 min before LTB4 challenge. Values are means ± SEM of 3-14 guinea pigs per group. **B.** Effect of orally administered **1**, **2**, and **7** on inhibition of LTB4-induced increases in excised lung gas volumes (ELGV). Oral doses were administered 2h prior to challenge. Values are means ± SEM of 3-14 guinea pigs per group.

We have demonstrated that one can substitute the *gem*-dimethyl tetrazole acid unit of the 1,2,4,5-substituted hydroxyacetophenone class of LTB4 receptor antagonists, typified by LY255283 (**1**), with a chroman carboxylic acid moiety or one of its acyclic analogues and obtain receptor antagonists with significantly improved receptor affinities and functional antagonistic potencies. Also, the 1,2,4,5-substituted hydroxyacetophenone chroman carboxylic acid analogue **7** was found to be superior to SC-41930 (**2**) *in vivo* in a guinea pig model of LTB4-induced airway obstruction even though the two compounds demonstrated comparable receptor affinities and functional antagonistic responses. As demonstrated by analogues **17**, **18** and **19**, the bicyclic nature of the chroman acid moiety is not critical for retaining receptor binding affinity although there appears to be some minor effects on functional antagonism. Also, it can be inferred from this limited SAR study and our earlier work on *ortho*-alkoxyphenol antagonists[18] that for the receptor-antagonist recognition process, the 1,2,4,5-substituted class of LTB4 receptor antagonist take advantage of a modest size hydrophobic binding region proximal to the acid binding site.

## References and Notes

1. 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.

2. a) Fretland, D.J.; Djuric, S.W.; Gaginella, T.S. *Prostaglandins Leukotrienes and Essent. Fatty Acids* **1990**, *41*, 215-233. b) Sharon, P.; Stenson W.F. *Gastroenterology* **1984**, *86*, 453-460.
3. 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.
4. Brain, S.D.; Williams, T.J. *Pharmac. Ther.* **1990**, *46*, 57-66.
5. 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.
6. a) Shemi, R.; Cromwell, O.;Taylor, G.W.; Kay, A.B. *J. Immunol.* **1991**, *147*, 2276. b) Ng, C.F.; Sun, F.F.; Taylor, B.M.; Wolin, M. S.; Wong, P.Y-K. *FASEB J.* **1990**, *4*, A624.
7. Maghni, K.; De Brum-Fernandes, J.; Foldes-Filep, E.; Gaudry, M.; Borgeat, P.; Sirois,P. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 748-789.
8. a) Herron, D.K.; Bollinger, N.G.; Swanson-Bean, D.; Jackson W.T.; Froelich, L.L.; 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.
9. a) Djuric, S.W.; Collins, P.W.; Jones, P.H.; Shone, R.L.; Tsai, B.S.; Fretland, D.J.; Butchko, G.M.; Villani-Price, D.; Keith, R.H.; Zemaitis, J.M.; Metcalf, L.; Bauer, R.F. *J. Med. Chem.* **1989**, *32*, 1147. b) Tsai, B.S.; Villani-Price, D.; Keith, R.H.; Zemaitis, J.M.; Bauer, R.F.; Leonard, R.; Djuric, S.W.; Shone, R.L. *Prostaglandins* **1989**, *38*(2), 655. c) Shone, R.L.; Djuric, S.W.; Tsai, B.S.; Fretland, D.J.; Gaginella, T.S.; Cook, C.S. *Drugs Future* **1990**, *15*(7), 695. d) Djuric, S.W.; Shone, R.L.; Yu, S.S.T. US Patent No. 4,889,871, **1989**.
10. a) Appeleton, R.A.; Bantick, J.R.; Chamberlain, T.R.; Hardern, D.M.; Lee, T.B.; Pratt, A.D. *J. Med. Chem.* **1977**, *20*(3), 371. b) Miyano, M.; Shone, R.L.; Sohn, D.D. US Patent No. 4,888,356; **1989**.
11. Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1956**, *78*, 1380.
12. Silbaugh, S.; Stengel, P.W.; Cockerham, S.L.; Roman, C.R.; Saussy, D.L., Jr.; Spaethe, S.M.; Goodson, T.; Herron, D.K.; Fleisch, J.H. *Eur. J. of Pharm.* **1992**, *223*, 57.
13. Marder, P.; Schultz, R.M.; Spaethe, S.M.; Sofia, M.J.; Herron, D.K. *Prostaglandins, Leukotrienes and Essent. Fatty Acids* **1992**, *46*, 265.
14. SC-41930 was tested as its sodium salt which was prepared by stirring the free acid in ethanol with excess 2N NaOH followed by purification on CHP-20 reverse phase resin eluting with 50% methanol/water and lyophilization of the product containing eluant.
15. 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±4% of the mean $IC_{50}$.
16. Concentration of pre-incubated antagonist (15 min @ room temp) required to provide 50% inhibition of the up-regulated CD11b/CD18 expression of human neutrophils, activated with $1x10^{-9}$M LTB4 (30min @ 37°C). CD11b/CD18 expression was determined flow cytometrically, by measuring single cells fluorescence of specific monoclonal antibody-reacted cells (13).
17. a) Hamel, R.; Ford-Hutchinson, A.W. *Prostaglandins* **1983**, *25*, 405-412. b) 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.
18. a) Sofia, M.J.; Jackson, W.T.; Saussy, D.L., Jr.;Silbaugh, S.A.; Froelich, L.L.; Cockerham, S.L.;Stengel, P.W. *Biomed. Chem. Lett.* **1992**, *2*, 1669. b) Sofia, M.J.; Saussy, D.L.,Jr.; Jackson, W.T.; Marder, P.; Silbaugh, S.A.; Froelich, L.L.; Cockerham, S.L.; Stengel, P.W. *Biomed. Chem. Lett.* **1992**, *2*, 1675.