*Bioorganic & Medicinal Chemistry Letters*, Vol.2, No.11, pp. 1367-1370, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00


# Synthesis and Pharmacological Activity of Rationally Designed Inhibitors of the Leukotriene $A_4$ Hydrolase Enzyme

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(*Received* 30 *June* 1992)

Abstract. The synthesis of a series of novel Leukotriene $A_4$ analogs containing the oxabicycloheptene nucleus has been achieved. These compounds have been evaluated as inhibitors of the Leukotriene $A_4$ hydrolase enzyme.

Leukotriene $B_4$ ($LTB_4$) has been proposed as a significant pathological mediator of a number of human inflammatory diseases, for example, psoriasis and ulcerative colitis.[1] It is produced in vivo from arachidonic acid by the action of two enzymes, 5-lipoxygenase (5-LO) which produces the labile intermediate leukotriene $A_4$ ($LTA_4$) and leukotriene $A_4$ hydrolase ($LTA_4$-H) which converts $LTA_4$ regio and stereospecifically to $LTB_4$ (Fig. 1)[2]. As the rate limiting step for $LTB_4$ biosynthesis, $LTA_4$-H represents an attractive target for therapeutic agents which interfere with $LTB_4$ biosynthesis.

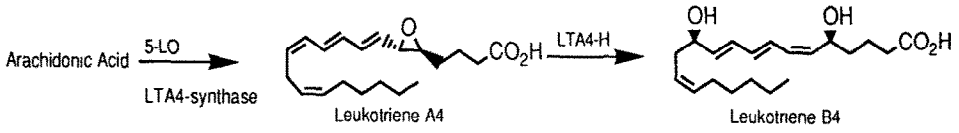
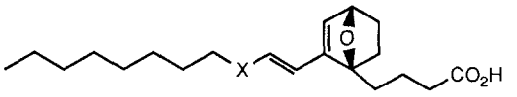



**Figure 1**

Leukotriene $A_4$, itself, has been shown to function as a mechanism based inactivator of $LTA_4$-H and appears to covalently bind to the enzyme during the catalytic cycle[3].

As part of our efforts to design inhibitors of $LTA_4$-H based on the structure of the natural substrate/inhibitor we were attracted to structures **1** and **2** as potential targets.



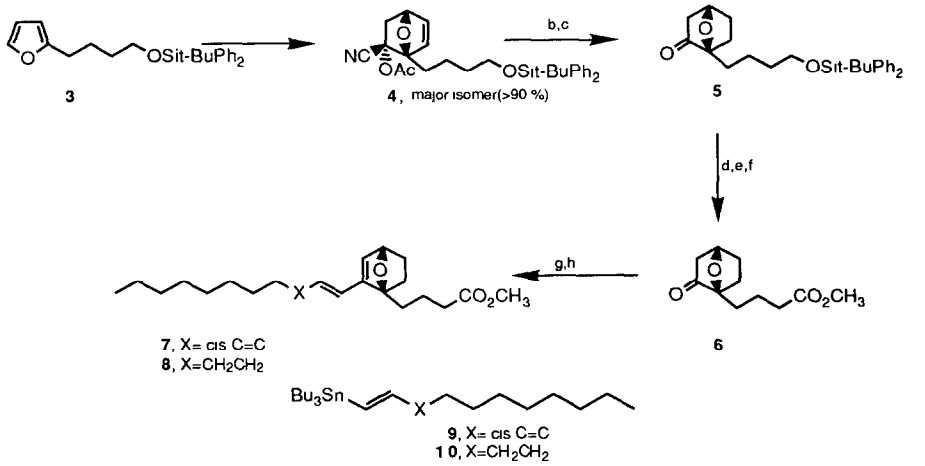

**1**, X= cis C=C
**2**, X=$CH_2CH_2$

Molecular modeling studies suggested that the oxabicycloheptene nucleus might function as an excellent surrogate for the vinyl oxirane present in the natural ligand. In addition, we anticipated that a projected release of approximately 18 kcal of strain energy might provide sufficient impetus for the oxabicycle to undergo nucleophilically triggered ring fragmentation in a manner similar to $LTA_4$ itself during its conversion to $LTB_4$.[4]

The syntheses of **1** and **2** are elaborated in Scheme 1 The somewhat outré ring substitution pattern of the oxabicycloheptene nucleus[5] was constructed through the auspices of a highly regiospecific intermolecular Diels Alder reaction. Substituted furan **3**[6] was treated with 1-cyanovinyl acetate in dichloromethane at ~ 18 kbar for 20 hrs to provide cyano-acetate **4** as the major product and isomer in a yield of 93%. Diimide reduction followed by unmasking of the protected cyanohydrin with methanolic sodium hydroxide afforded the ketone **5** in a yield of 86% for the two steps. Adjustment of oxidation state to access the requisite C-1 (Leukotriene numbering) carboxylic acid ester **6** was accomplished by sequential exposure of **5** to tetrabutylammonium fluoride in tetrahydrofuran, Jones reagent in acetone at 25° and ethereal diazomethane. The overall yield for the three steps was 82%. Compound **6** was subsequently transformed to its corresponding vinyl triflate derivative under standard conditions via conversion to the sodium enolate (NaHMDS, THF, -78°C) and reaction with N-phenyl trifluoromethanesulfonimide (92%). The critical coupling reaction between this vinyl triflate and the appropriate dienyl or vinyl stannane **9** or **10**[7] was accomplished via utilization of the excellent Stille protocol[8], to afford after purification on silica gel the corresponding diene or triene derivatives **7** and **8** in

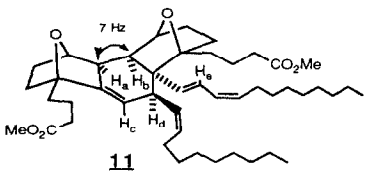
yields of about 50%.[9] Conversion to the sodium salts of **1** and **2** was achieved via careful hydrolysis using 0.25M sodium hydroxide in acetone. The sodium salts were used for bioassay without further purification. Interestingly, the diene and triene esters **7** and **8** displayed a remarkable propensity towards intermolecular Diels Alder dimerisation when stored neat at temperatures as low as -20°C and were, as a result, routinely stored as dilute solutions in ethanol or hexane. The major product isolated from this process (in the case of **7**) was identified to be adduct **11**[10], rather than **12**, based on NOE experiments.[11] Compounds **1** and **2** were evaluated in bioassays established to whether these compounds were inhibitors of leukotriene $B_4$ biosynthesis in isolated enzyme and whole cell screens. Both **1** and **2** were found, disappointingly, to be ineffective at inhibiting the conversion of $LTA_4$ by a purified $LTA_4$-H enzyme.[12] However, both compounds exhibited good inhibitory activity against calcium ionophore induced $LTB_4$ biosynthesis by the promyelocytic leukemia cell line HL-60 at concentrations of $10^{-6}$M. Studies to elucidate the mechanism of action of **1** and **2** in whole cell systems and gain an understanding of why these compounds are not $LTA_4$-H inhibitors are currently in progress.

**Scheme1**




**Reagents**

a) 1-cyanovinyl acetate, 17-20 kbar, $CH_2Cl_2$, 25°C, 20hrs,~ 100%, b) $KO_2CN{=}NCO_2K$, $CH_3OH$, $CH_3CO_2H$, 25°C then c) NaOH, $CH_3OH$, 25°C, 86% for the two steps, d) tetra n- butylammonium fluoride,THF, 25°C, e) Jones oxidation, acetone, 25°C, f) $CH_2N_2$, ether, 0°C, 82% for the three steps, g) NaHMDS, $PhNTf_2$, THF, -78°C, 92%, h) **9** or **10**, cat $Pd(Ph_3P)_4$, LiCl, THF, 50-60°C, 2.5hrs., ~50%.

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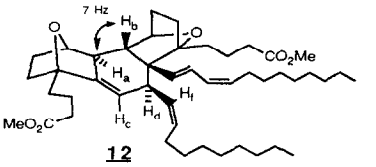


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**Acknowledgements**

The authors would like to thank the Physical Methodology department at Searle for all analytical data (NMR, CMR, IR, MS and microanalysis), Mrs Dolores Weiman and Mrs. Candy Martin for expert secretarial assistance and Dr Paul Collins for valuable suggestions during the course of the work and during the preparation of the manuscript

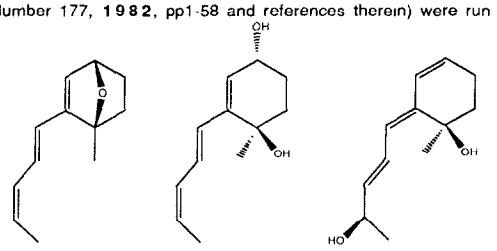
**References**

1 See, for example, A.W. Ford Hutchinson, Crit. Rev. in Immunol., 10 1 (1990).

2. B. Samuelsson, Science, 220 568 (1983).

3 J.F. Evans, D.J. Nathaniel, R.J. Zamboni and A.W Ford Hutchinson, J. Biol Chem., 260 10966 (1985).

4 Molecular mechanics calculations (using Allinger's MM2 Force Field, as described in *Molecular Mechanics*, by U. Burkert and N.L. Allinger, ACS Monograph Series, Number 177, **1982**, pp1-58 and references therein) were run on the model oxabicycloheptene system 13.




13 14 15

The strain energy term was calculated for a minimized structure in which the triene system was in the extended conformation shown in this schematic representation (again using the standard MM2 options described in pages 184-189, of the above reference) and compared to the strain energy terms computed for model systems corresponding to potential hydration products 14 and 15

5 There are few examples of 1, 6 disubstituted 7-oxabicyclo [2 2 1] heptenes in the synthetic literature. Leading references to 7-oxabicyclo [2.2.1] heptanes and their related chemistry and biology include A Warm and P Vogel, Helv. Chim. Acta., 70 690 (1987), D. N. Harris, S.E. Hall, A Hedberg and M L. Ogletree, Drugs of the Future, 13 153 (1988), G Buchbauer and H. Holbick, Chemiker-Zeitung, 115 141 (1991), E.J Corey and I N Houpis, J Am Chem Soc., 112 8997 (1990) and S R Baker and J R Harris, Syn Commun., 21 2015 (1991)

6 3 was prepared in 99% yield by treatment of furan with n-BuLi at -15°C followed by 1-t-butyldiphenylsilyloxy-4-iodobutane and warming to 25° overnight.

7 9 was prepared via a $Pd(PPh_3)_4$ catalyzed coupling of trans-1,2-bis(tri-n-butylstannyl)ethylene[13] and 1-iodo-1Z-decene (prepared from 1-decyne).[14]

8. W J. Scott and J.K. Stille, J. Am. Chem Soc., 108, 3033 (1986).

9 7 IR (neat) 2951, 2925, 2854, 1741, 1459, 1435, 1170 cm $^{-1}$, $^1$H NMR (acetone-$d_6$) δ 6.78 (dd, J=15 7, 11 2 Hz, H10-leukotriene numbering), 6 30 (s, H7), 6 19 (d, J=15 8 Hz, H9), 6 07 (t, J=11 Hz, H11), 5 50 (dt, J=10.6, 7 8 Hz, H12) 4.81 (dd J=4 5, 1 7 Hz, H6), 3.62 (s, 3H), 2 46-1 16 (m, 24H), 0 89 (t, 3H), high resolution mass spectrum, m/e 360.2666 (calcd for $C_{23}H_{36}O_3$, 360.2664).

10 11 $^1$H NMR ($CDCl_3$) δ 6.35 (dd, J=15 6, 10.8 Hz, 1H), 5 94 (t, J=10.8 Hz, 1H), 5 92 (d, J=15.5 Hz, 1H), 5.74 (tt, J=10.6, 1 5 Hz, 1H), 5.48 (dt, J=11.7 Hz, 1H), 5 35 (dt, J=11, 7 5 Hz, 1H), 5 30 (dd, J=4, 2 5 Hz, 1H), 4.60 (d, J=5 2 Hz, 1H), 4.55 (d, J=5 2 Hz, 1H), 2.58 (d, J=7.0 Hz, 1H), 2 44-2 29 (m, 4H), 2 18 (q, J=7 Hz, 2H), 2.11 (dd, J=7 0, 2.5 Hz, 1H), 2 03-1.57 (m, 18H), 1.44-1.17 (m, 29H), 0 88 (2t, 6H), $^{13}$C NMR ($CDCl_3$) δ 174 20, 146 91, 140.37, 133 11, 130 64, 129 24, 128.97, 122.26, 116 50, 91 34, 85 13, 78 17, 77 22, 58 89, 52 56, 51 46, 51.37, 48.44, 46.09, 34 77, 34.42, 33.66, 33.46, 31 92, 31.59, 31.28, 30 73, 30.64, 29 69, 29.57, 29 51, 29 49, 29 43, 29 32, 29.20, 27 76, 27 72, 22 68, 20.83, 20 04, 14 11 Anal calcd for $C_{46}H_{72}O_6 \cdot 1H_2O$: C, 74 75 H, 10 09 Found: C, 75 05 H, 9 79

11 The structure of the major product could not be confirmed based on $^1$H NMR data alone ($J_{ab}$ of ~7Hz would be consistent with either 11 or 12) A NOESY experiment exhibited an NOE crosspeak between protons $H_a$ (δ2 11) and $H_e$ (δ6.35) and a weak crosspeak between the other three vinyl protons of the diene system This would be consistent with structure 11. In addition, no NOE crosspeak was observed between $H_b$ (δ2.58) and $H_f$ (δ5 74) as would be expected for structure 12

12 See, for example, L Orning, G Krivi, and F A Fitzpatrick, J Biol. Chem., 266 1375 (1991)

13 J C Bottaro, R N Hanson, and D E Seitz, J Org Chem., 46, 5221 (1981)

14 See, C Luthy, P Konstantin, and K.G Untch, J Am. Chem Soc., 100, 6211 (1978)