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DESIGN AND SYNTHESIS OF ACHIRAL 5-LIPOXYGENASE INHIBITORS EMPLOYING THE CYCLOBUTYL GROUP

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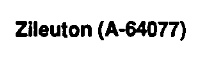
Abstract: *The achiral cyclobutyl group is an effective replacement for the chiral linking group required in hydroxyureas for good in vivo activity against 5-Lipoxygenase. The principle is illustrated in two achiral 5-LO inhibitors: 858C, a more potent inhibitor than Zileuton and, 862C, a very potent 5-LO inhibitor with good oral persistence.*

Leukotrienes are thought to be implicated in a variety of pathophysiological states in man, particularly, those involving inflammation. Reduction of leukotriene biosynthesis *via* inhibition of arachidonate 5-Lipoxygenase (5-LO) offers a possible new approach[1] to combating these diseases and is currently the focus of several groups. To date, Zileuton[2] is the most advanced selective 5-LO inhibitor in clinical development and, although Zileuton is not the most potent or most *in vivo* persistent 5-LO inhibitor known, it has nevertheless shown promising activity in asthma.[3]

We have previously reported[4] our discovery at Wellcome of the N-hydroxyurea 70C which is a very potent 5-LO inhibitor *in vitro* and has a long duration of action *in vivo* following oral administration. 70C was designed to have a favourable metabolic profile, compared with earlier compounds, by the introduction of a F-atom and α-methyl group[5].However, the α-methyl group present in 70C (necessary to block oxidation) introduces a chiral centre into the molecule. Zileuton also contains an α-methyl group in the link between the hydroxyurea and aromatic group. Indeed, an earlier report[6] from the Abbott group noted that an α-methyl group enhanced *in vivo* potency in a series of benzyl acetohydroxamates.

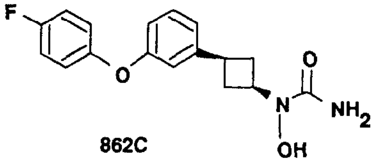
70C

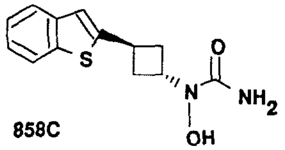


Zileuton (A-64077)

Although the (R)-and (S)-enantiomers of 70C have similar activity against 5-LO, the (S)-enantiomer is cleared more rapidly *in vivo*[4]. This enantioselective metabolism property is likely to be present in other members of the N-hydroxyurea/hydroxamate class of 5-LO inhibitors. In view of the complications involved in development of enantiomers with different properties it would be desirable to remove chirality.

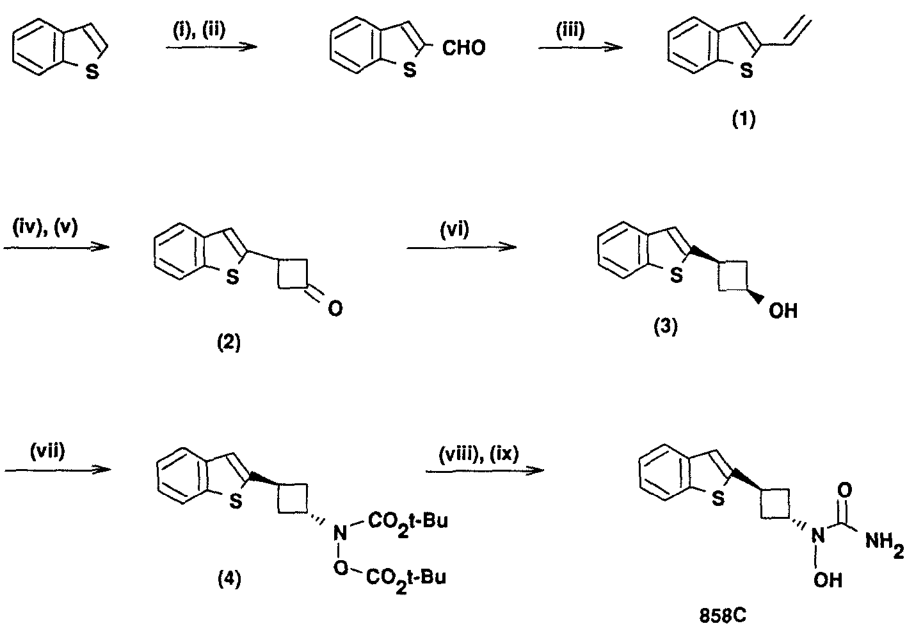
Thus, we report here how we have been able to replace the chiral linker group, in 70C and Zileuton, with the achiral cyclobutyl group, substituted *via* the 1- and 3-positions, to obtain novel achiral 5-LO inhibitors. The principle is illustrated in 858C, where *in vitro* potency against 5-LO has been improved over Zileuton, and in 862C, where high potency and persistent *in vivo* activity against 5-LO *via* oral administration is achieved.


862C


858C

The synthesis of 858C, shown in Scheme 1, began with metallation of benzothiophene and subsequent quenching of the lithio-intermediate with DMF to give the aldehyde in 85% yield. The aldehyde was

Scheme 1 - Synthesis of 858C

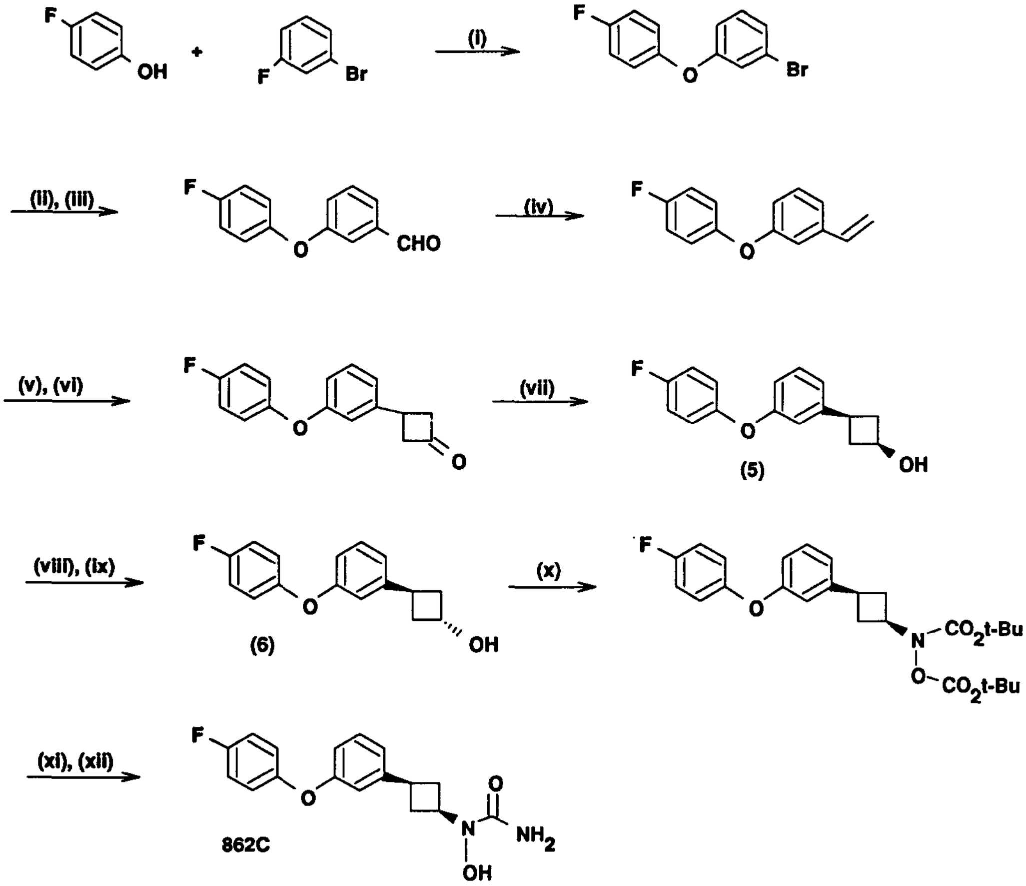


Reagents: (i) BuLi, Et_2O; (ii)DMF; (iii) $MePh_3P^+Br^-$, $KOBu^t$, THF; (iv) Cl_3CCOCl, $POCl_3$, Zn/Cu, Et_2O; (v) Zn, AcOH; (vi) $LiAlH_4$, THF; (vii) $Bu^tO_2CNHOCO_2Bu^t$, DEAD, Ph_3P, THF; (viii) HCl, H_2O, MeOH; (ix) KOCN, HCl, H_2O, THF.

converted to alkene (1) in 65% yield using standard Wittig chemistry, and then subsequent [2 + 2] cycloaddition, with dichloroketene, and reduction of the dichlorocyclobutanone intermediate, gave the cyclobutanone (2) in 26% after flash-chromatography. Reduction of (2) with $LiAlH_4$ gave almost exclusively[7] the *cis*-alcohol (3), which was converted to the N,O-*bis*-BOC-protected hydroxylamine in 65% yield after flash-chromatography. Finally, deprotection under acidic conditions and acylation with cyanic acid (*in situ*) yielded the N-hydroxyurea 858C in 32% yield[13].

Synthesis of the *cis*-fluorophenoxyphenylcyclobutane analogue, 862C, shown in Scheme 2, followed similar chemical methodology to the above. However, to obtain the *cis*-configuration in the target we used a double

Scheme 2 - Synthesis of 862C



Reagents: (i) K_2CO_3, DMF; (ii) BuLi, THF; (iii) DMF; (iv) $MePh_3P^+Br^-$,KOBu^t, THF; (v) Cl_3CCOCl, $POCl_3$ Zn/Cu, Et_2O; (vi) Zn, AcOH; (vii)$LiAlH_4$, THF; (viii) AcOH,DEAD, Ph_3P, THF; (ix) K_2CO_3,H_2O, EtOH; (x) $Bu^tO_2CNHOCO_2Bu^t$,DEAD, Ph_3P, THF; (xi)HCl, H_2O,MeOH; (xii) KOCN,HCl,H_2O,THF.

Mitsanobu-type inversion of the *cis*-cyclobutanol (5) to the *cis*-cyclobutane-N-hydroxyurea, 862C, *via* the *trans*-cyclobutanol (6). It is worth noting that some chemical stages in the synthesis of the fluorophenoxyphenyl derivative were more efficient than in the corresponding benzothienyl case. For example, the [2 + 2] dichloroketene-olefin cycloaddition reaction gave an 84% yield of the fluorophenoxyphenylcyclobutanone, after reduction of the dichlorocyclobutanone. In addition, the final deprotection and acylation steps gave a 41% yield of 862C[14]. The alcohol inversion (5) to (6), however, was less efficient (33%) than expected.

Both 858C and 862C are potent inhibitors of 5-LO in intact human neutrophils (Table below). Interestingly, the presence of cyclobutyl group in 858C has increased *in vitro* potency against 5-LO approximately 10-fold over that of Zileuton. The potency increase with 858C may partly be due to the increase in lipophilicity. However, an alternative explanation is due to the increased separation afforded by the cyclobutane group between the N-hydroxyurea and aromatic groups, a feature we often find in the hydroxamate/hydroxyurea series.

In Vitro Activity Against Human PMN 5-LO[8]

Compound	**IC$_{50}$(μM)^a**	
70C	0.08	(2)
Zileuton	3.3	(3)
858C	0.38	(2)
862C	0.17	(5)

[a]*(Values are means of a number of determintations in parenthesis)*

By way of illustration of the utility of the cyclobutyl group on the *in vivo* properties of the hydroxyurea series, the potent inhibitor 862C was evaluated in the rabbit for persistence of action against 5-LO (figure 1)[9]. Thus, 862C caused substantial inhibition of 5-LO in blood from 1 to 12h, after a single oral dose of 2mg/kg. By 24h, LTB$_4$ levels had returned to normal. 862C appears to be well absorbed and has a good duration of action, being similar in effect to 70C.[4]

Additional evidence for the effectiveness of 862C *in vivo* is shown by the results in Figure 2 where an oral dose of 10mg/kg significantly inhibited the leukotriene-dependent anaphalactic bronchoconstriction in anaesthetised guinea-pig.[10]. Once again 862C appears to have a long duration of action since the inhibition of bronchoconstriction was observed 6h after dosing of the compound. In addition, the duration of 862C is similar to that of 70C reported previously,[11] and more effective than Zileuton, which required 50mg/kg to show significant inhibition.

Figure 1.
TIME COURSE OF THE EFFECT OF 862C ORALLY ON *EX VIVO* PRODUCTION OF LTB4 IN RABBIT BLOOD

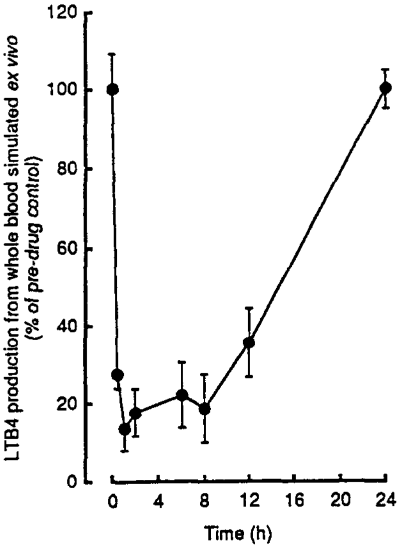
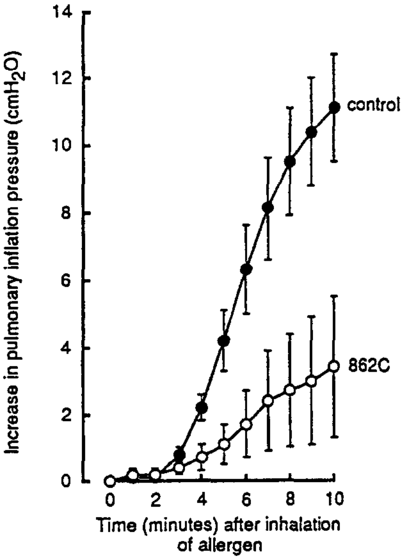


Figure 2.
INHIBITORY EFFECT OF 862C ON LEUKOTRIENE - DEPENDENT BRONCHOCONSTRICTION IN THE GUINEA-PIG



In conclusion, it appears that the ***achiral*** cyclobutyl group offers potency and metabolic advantages similar to chiral linking groups present in some recently discovered potent, orally active 5-LO inhibitors. We have demonstrated this principle with the *achiral* compounds 858C and 862C. In 858C, *in vitro* potency has been increased substantially over Zileuton. In 862C, high potency and good oral persistence have been achieved, to give one of the most orally effective 5-LO inhibitors.

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References:

1. For reviews see, for example, (a) Garland, L. G., Hodgson, S. T. *Advances in Prostaglandin, Thromboxane and Leukotriene Research*; Sameulsson, B.; Dahlèn, S-E.; Fritsch, J.; Hedqvist, P., Eds.; in press, (b) Salmon, J.A.; Garland, L. G. *Progress in Drug Research*; Birkhäuser Verlag: Basel, 1991; Vol 37, pp 9-90, (c) Batt, D. G. *Progress in Medicinal Chemistry*, Ellis, G. P.; Luscombe, D. K., Eds.; Elsevier, 1992; Vol 29, pp 1-63.
2. Carter, G. W.; Young, R.P.; Albert, D.H; Bouska, J.; Dyer, R.; Bell, R. L.; Summers, J. B.; Brooks, D. W. *J. Pharmac. Exp.Ther.*, **1991**, *256*, 929.
3. Israel, E.; Dermarkarian, R.; Rosenberg, M.; Sperling, R.; Taylor, G.; Rubin, P.; Drazen, J.M.. *New Engl. J. Med.*, **1990**, *323*, 1740.
4. Salmon, J.A.; Jackson, W.P.; Garland, L. G. *Advances in Prostaglandin, Thromboxane and Leukotriene Research*; Sameulsson, B.; *et al*, Eds.; Raven, 1990; Vol 21, pp 109-112.
5. Salmon, J. A.; Jackson, W. P.; Garland, L. G. *Therapeutic Approaches to Inflammatory Diseases*; Lewis, A. J.; Doherty, N. S.; Ackerman, N. R.; Eds.; Elsevier, 1989; pp 137-146.

6. Summers, J. B., *et al.*; *J. Med.Chem.*; **1988**, *31*, 1960.
7. 200 MHz 1H-NMR showed 30:1 ratio of *cis*:*trans* alcohols.
8. See Tateson, J. E.; Randall, R. W.; Reynolds, C. H.; Jackson, W.P.; Bhattacherjee, P.; Salmon, J. A.; Garland, L. G.; *Br. J. Pharmacol*, **1988**, *94*, 528. Human peripheral blood leucocytes were isolated from blood taken from normal human volunteers according to methods previously described. The cells were suspended to a cell density of 1×10^6 cells ml^{-1} in indicator-free Hanks' balanced salt solution buffered to pH 7.4 with HEPES (30mM]. Aliquots (0.5ml) of cell suspension were incubated at room temperature for 5 min with either inhibitor or vehicle (DMSO) before the addition of calcium ionophore A23187 (2µM final conc.). The samples were then incubated at 37°C for a further 5 min in a shaking water bath. The reactions were terminated by boiling for 2 min and the samples were then centrifuged to remove cellular debris and the resultant supernatants were then removed for storage at -30°C. The levels of Leukotriene B_4 in the supernatants were estimated using Scintillation Proximity Assay (Amersham International TRF 954).
9. Time course of the inhibitory effect of 862C (2mg/kg orally) on production of LTB4 in rabbit blood stimulated with A23187 *ex vivo*. The marginal ear vein of fasted rabbits (n=3) was cannulated to permit withdrawal of blood samples (2ml per time point). Control bleeds were made prior to administration of 862C as milled suspension in 0.25% celacol, and at the indicated times afterwards up to 24h. Whole blood was stimulated for 30min at 37C by addition of 30ug/ml calcium ionophore (A23187). LTB4 production was measured by specific radioimmunoassay of the plasma fraction (Advanced Magnetics, MA).
10. Guinea-pigs were actively sensitised to ovalbumin (Sigma) and used 14d later. 862C or vehicle was administred as 0.25% celacol suspension at 10mg/kg orally to fasted animals (n=6). Under pentobarbitone anaesthesia, the animals were artificially ventilated. Indomethacin (10mg/kg) and mepyramine (2mg/kg) were given intravenously and, 10min later, the animals were challenged by inhalation of nebulised ovalbumin (10mg/ml, 5s). Leukotriene-dependent bronchoconstriction was monitored as the increase in pulmonary inflation pressure over the subsequent 10min.
11. For full experimental methods see Payne, A. N.; Garland, L. G.; Lees, I.W.; Salmon, J. A., *Br. J. Pharmacol.*, **1988**, *94*, 540.
12. Payne, A. N.; Jackson, W. P.; Salmon, J. A.; Nicholls, A.; Yeadon, M.; Garland, L. G., *New Drugs for Asthma Therapy*, Birkäuser Verlag, Basel, 1991; Vol 34 pp 189-199.
13. Compound 858C was characterised as follows:-
    mp 165-166°C; Found C59.3, H5.3, N 10.4%; $C_{13}H_{14}N_2O_2S$ requires C59.5, H5.4, N 10.7%; ^{1}H-NMR: δ(d_6-DMSO, 200 MHz) 9.35 (1H,s.OH), 7.85 (1H,m,ArH), 7.75(1H,m,ArH), 7.30(3H,m,ArH), 6.32(2H,s,NH_2), 4.92(1H,m,ArC$\underline{H}$), 3.63(2H,m,C$\underline{H}$N). 2.78 and 2.25(4H,m,CH_2); IR vmax(KBr) 3458, 3331, 3136, 1661 cm^{-1}.
14. Compound 862C was characterised as follows - mp 150-152°C; Found: C64.4, H5.4, N8.7%, $C_{17}H_{17}FN_2O_3$ requires C64.5, H5.4, N8.9%; ^{1}HNMR: δ(d_6-DMSO, 200 MHZ) 9.16(1H,s,OH), 7.25, 7.08 and 6.85 (8H,m,ArH), 6.27(2H,s,NH_2), 4.64(1H,m,Ar$\underline{C}$H), 3.07(1H,m,NC$\underline{H}$) and 2.35(4H,m,CH_2); IR vmax(KBr): 3466, 3261, 1645, 1502 and 1200 cm^{-1}.