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INHIBITORS OF LEUKOTRIENE B_4. SYNTHESIS AND SAR OF NEW ß-ENAMINOESTER OPEN-CHAIN ANALOGUES OF ZENECA ZD2138

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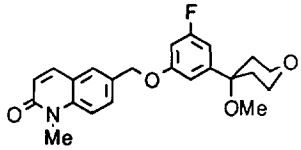
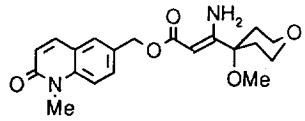
Abstract : A series of methoxy tetrahydropyranyl ß-enaminoesters has been discovered as inhibitors of LTB_4 production. These are unusual open chain analogues of Zeneca ZD2138, the highly potent orally active 5-LO inhibitor under development for the treatment of asthma and rheumatoid arthritis. In vitro, compound **1a** inhibited LTB_4 formation in human whole blood with an IC_{50} of 0.04 µM. In inflamed exudate in the rat **1a** inhibited formation of LTB_4 with an oral ED_{50} 3h after dosing of 3.0 mg/kg.

Leukotrienes (LT's) are a family of potent biologically active molecules that are formed as the result of oxidative metabolism of arachidonic acid initiated by the enzyme 5-lipoxygenase (5-LO). Inhibition of 5-LO blocks the synthesis of LT's and presents potential therapeutic utility for the treatment of a variety of inflammatory conditions including asthma, rheumatoid arthritis, inflammatory bowel disease and psoriasis, in which LT's are believed to play an important role.[1] Many compounds are known which inhibit 5-LO but most of these have poor selectivity for 5-LO or lack oral efficacy. Furthermore, most inhibitors possess redox properties and potentially can interfere with physiologically important redox processes.[2,3]

We have recently described a series of novel 5-LO inhibitors, the methoxytetrahydropyrans, exemplified by Zeneca ZD2138, which are very highly selective and which derive their inhibition from specific and enantioselective interactions with the enzyme.[4,5] Inhibition does not arise from redox or iron chelating properties in this series.[4] ZD2138 was shown to be highly active orally in a number of species and is consequently undergoing clinical evaluation by oral administration in asthma and rheumatoid arthritis.



ZD 2138



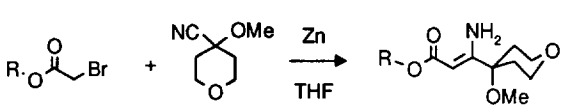
1a

Here we describe the discovery of a new series of β-enaminoesters, which show potent inhibition of LTB_4. They arose from our interest in finding novel inhibitors with greater aqueous solubility than ZD2138 in an attempt to further improve its bioavailability. Structure-activity studies leading to ZD2138 showed that

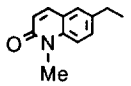
pharmacologically important elements of its structure comprise the carbostyril and methoxytetrahydropyran groups.[6] Thus we investigated structurally varied analogues in which the hydrophobic fluorophenyl ring was replaced by open-chain aliphatic unsaturated ester and ether links. We specifically sought to design structures in which an internal hydrogen bond would mimic the cyclic aromatic ring; following structural and steric considerations this led to the β-enaminoesters discussed here.

Chemistry. ß-Enaminoesters possess particular physical properties owing to an attractive intramolecular hydrogen bond and thus exist predominantly in the **Z** form as can be seen from their NMR spectra[7] (in addition severe steric repulsions disfavour the **E** configuration). These compounds are generally crystalline, fairly soluble and stable in water at pH 7 and therefore appeared intriguing candidates for our studies.

Scheme 1

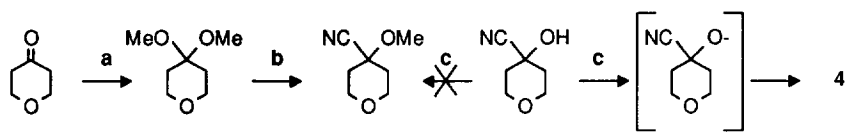


2a-d **3** **1a-d**

R =		
	a	0%
Me	b	82%
Et	c	56%
$NapCH_2$-	d	42%

The synthesis of compounds reported in Table 1 is shown in Scheme 1 using the versatile Blaise reaction[8] which is applicable to the preparation of primary β-enaminoesters by the reaction of organozinc reagents with nitriles. For this purpose we needed the simple but previously unknown 4-cyano-4-methoxytetrahydropyran **3**. All attempts to methylate the cyanohydrin **6**[9] failed, resulting in recovery of the parent ketone **4**. We circumvented this difficulty by the expedient cyanation of dimethyl acetal **5** using *t*-butylisocyanide-$TiCl_4$[10] as shown in Scheme 2. With this method **4** was easily converted into **3**[11] with an overall yield of 50%.

Scheme 2

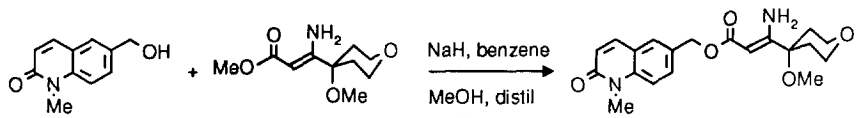


4 **5** **3** **6**

Reagents: (a) $(MeO)_2C(Me)_2$, MeOH, pTSA, 81%; (b) *t*-BuNC, $TiCl_4$, 62%; (c) NaH, MeI, DMF or CH_2N_2.

Using the Blaise reaction, simple unfunctionalised α-bromomethyl esters **2b, c** and **d** (R = CH_3, C_2H_5 and CH_2-naphthyl respectively) were readily reacted with the hindered 4-cyano-4-methoxytetrahydropyran **3** to give the required ß-enaminoesters in yields ranging from 40-80%. However the reaction failed in the case of the bromomethyl carbostyril ester **2a**, required to prepare our target **1a**, presumably due to the presence of the reactive cyclic amide functionality.

We therefore studied the transesterification of the methyl ester **1b** with the poorly-soluble, high-melting carbostyril alcohol **7**,[12] using various conditions. Those including toluene,[13] toluene-DMAP[14] or toluene-pTSA,[15] proved unsatisfactory. Taking into account the deactivation of the ester group in **1b** due to the presence of an intramolecular hydrogen bond, as shown by its IR (CO_2Me at 1670 cm^{-1}) and NMR spectra (bound NH at 8 ppm, free NH at 5,2 ppm), we rendered the alcohol **7** more reactive by preparing its sodium salt *in situ* in benzene solution. This was then reacted with the β-enaminoester **1b** with continuous distillation of the solvent and the required **1a** was indeed formed and isolated in 40% yield. However, it was notable that the reaction has to be initiated with a catalytic quantity of MeOH, which possibly acts by solvation of the sodium alkoxide initially present as a non-reactive aggregate in the benzene solution. 18-Crown-6 also initiates the reaction probably by formation of the "naked" anion. This method is unusual among transesterification methods[16] in that only a single equivalent of the alcohol is required and, as in the present example, may be advantageous in the case of solid, high molecular weight and poorly soluble alcohols used in stoichiometric quantities. The reaction seems to be quite general and has been applied to other functionalised alcohols with yields ranging from 40-70%.



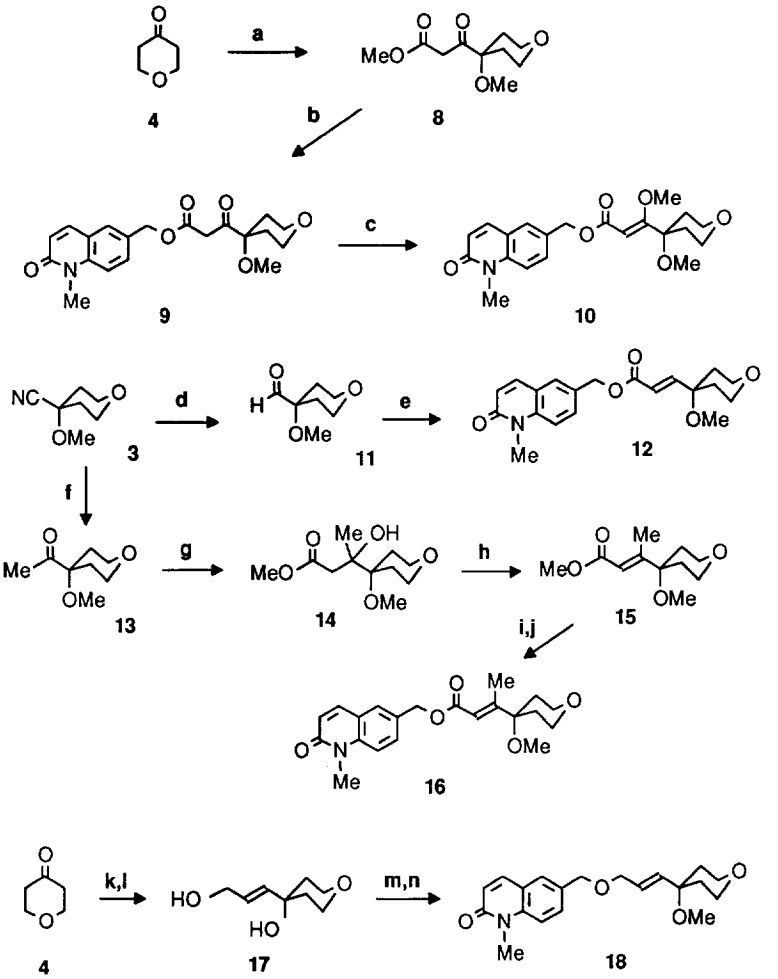
7 **1b** **1a**

For comparison, a series of other open-chain analogues was prepared as follows (Scheme 3). Ester **8** was synthesized from **4**[17] and was smoothly transesterified in refluxing toluene to give **9**. Both ß-ketoesters **8** and **9** exist predominantly in their keto form as drawn. Although **9** can be readily converted into the enol ether **10** we were unable to aminate either **8** or **9** using known conditions.[18] Aldehyde **11** was prepared from nitrile **3** and Wittig reaction gave the parent non-substituted unsaturated ester **12**. Its methyl analogue **16** was derived from the ketone **13**. Finally, the unsaturated ether **18**[19] was prepared via alcohol **17**, again generated from tetrahydropyran-4-one **4**.

Biological results and discussion. Compounds were evaluated as inhibitors of LTB_4 production *in vitro* in human blood and *ex vivo* in rat air pouch inflammatory exudate.[20,21] Table 1 shows a comparison of ß-enaminoesters with ZD2138. Variation of the carbostyril had very little effect on *in vitro* activity although this group appeared to yield the best oral activity. Despite the greater aqueous solubility of this series, expected to lead to more efficient absorption, the overall oral activity was not as high as for ZD2138. Although these β-enaminoesters are uncharacteristically stable under neutral conditions ($t_{1/2}$ ~ 70 days at pH7, 25°C; recrystallisable solids) they do show fairly rapid acid catalysed hydrolysis ($t_{1/2}$ ~ 1h at pH4, 30°C) and may therefore partially hydrolyse in the stomach - thus potentially leading to the drop in oral activity seen.

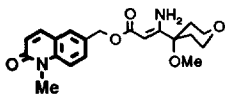
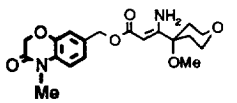
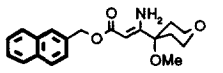
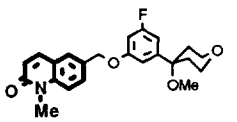
Table 2 shows that in comparison with the other related open chain analogues the ß-enaminoester moiety is superior as an isostere of the fluorophenyl group in the ZD2138 series. Since ß-ketoester **9** exists predominantly in its keto form, steric repulsion between the keto and ester groups would disfavour suitable conformations in this molecule thus leading to the lack of activity observed. Restoration of unsaturation imparts a low degree of activity in **10, 12** and **16** and interestingly the most flexible compound, ether **18**, appears to be more active. However none of these compounds matches enaminoester **1** and none shows any activity in the *ex vivo* test.

Scheme 3



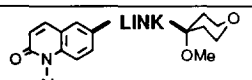
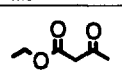
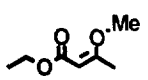
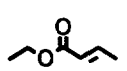
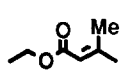
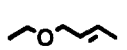
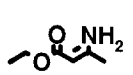
Reagents: (a) see Ref. 17; (b) **7**, DMAP, Δ, 48%; (c) CH_2N_2, 50%; (d) DIBAL, 32%; (e) $CbsCH_2OCOCH{=}PPh_3$, 23%; (f) MeLi, 73%; (g) MeCOOMe/LDA, 87%; (h) $SOCl_2$/py, 57%; (i) $Ba(OH)_2$, Δ, 85%, (j) **7**, DCCI, DMAP, 59%; (k) $HC{\equiv}CCH_2OH$ / BuLi (2 eq.), LiBr, 56%; (l) LAH, 25%; (m) NaH, $CbsCH_2Br$,[12] 0°C, 43%; (n) NaH, MeI, 60%.

Table 1. Comparison of LTB_4 Inhibitory Activity and Aqueous Solubilities of β-enaminoesters with ZD2138

Compound	Structure	IC_{50}[a] Human Blood (μM)	*Ex vivo* ED_{50}[b] Rat Air Pouch (mg/kg po at 3h)	Aqueous[c] Solubility (μM)
1		0.04	3	150
2		0.06	5	25
3		0.07	>5	~50[d]
ZD 2138		0.02	0.3	3.8[d]

[a] Method is described in ref. 20. [b] Ref. 21. [c] Measured over 3 days in buffered water (pH 7.4) at 25°C. [d] Estimated, see ref. 4.

Table 2. Comparison of LTB_4 Inhibitory Activity of **1** with other Open Chain Analogues

Compound		IC_{50} Human Blood (μM)	*Ex vivo* ED_{50} Rat Air Pouch (mg/kg po at 3h)
9		>10	>5
10		2.6	>5
12		1.7	>5
16		2.1	>5
18[19]		0.5	>5
1		0.04	3

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11. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 1.87 (m, 2H), 2.13 (m, 2H), 3.47 (s, 3H), 3.66 (m, 2H), 3.94 (m, 2H). ^{13}C NMR: (100 MHz $CDCl_3$) δ 34.9(2), 52.4(2), 63.8, 77.2, 118.9. IR: 2970, 2240, 1470, 1345, 1160, 1110 cm^{-1}.
12. The alcohol **7** was prepared by hydrolysis (K_2CO_3) of the corresponding bromo derivative previously described by Crawley, G. C.; Edwards, P. N.; E.P. 385662, **1990**. C.A. **1990**, *114*, 228757 k.
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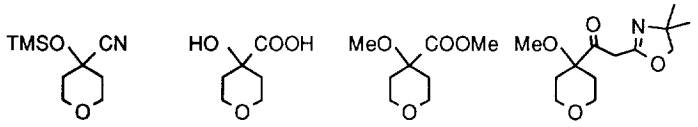
16. Larock, R. C. *Comprehensive Org. Transformations* **1989**, 985.
17. (a) Ketone **4** was treated with trimethylsilyl cyanide and ZnI_2 to give **19** which was quantitatively hydrolysed to the acid with HCl/AcOH. Hydroxy acid **20** was treated with c.H_2SO_4 in methanol to furnish the ester analogue which was etherified with NaH/MeI. The resulting ether **21** was reacted with the lithium salt of Meyers reagent[17b] to give the oxazoline **22** which was converted into the ester **8** by refluxing in methanol with c.H_2SO_4. (b) Meyers, A. I.; Temple, D. L.; Nolen R. L.; Mihelich E. D. *J. Org. Chem.* **1974**, *39*, 2778.



19 **20** **21** **22**

18. (a) The following conditions were tested: ammonia in ethanol,[18b] ammonia in the presence of NH_4NO_3[18c] or HCO_2NH_4[18d] and Ph_3P = $NSiMe_3$ in the presence of TsOH.[18e] (b) Henecka, H.; *Chem. Ber.* **1949**, *82*, 104. (c) Becker, H. G. O. *J. Prakt. Chem.* **1961**, *12*, 294. (d) Southwick, P. L.; Hofmann, G. H.; *J. Org. Chem.* **1963**, *28*, 1332. (e) Kloek, J. A.; Leschinsky, K. L. *J. Org. Chem.* **1978**, *43*, 1460.
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21. Sterile air was injected into the subcutaneous tissue of the backs of anaesthetised rats to form air pouches which were reinflated 3 days later. After a further 3 days the animals were dosed orally with compound and a 1% suspension of zymosan in physiological saline was injected directly into each air pouch. Three hours later lavaged air pouches were analysed for *i*-LTB_4.

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