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SELECTIVE INHIBITION OF LOW AFFINITY IgE RECEPTOR (CD23) PROCESSING: P_1' BICYCLOMETHYL SUBSTITUENTS

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Abstract

Using a variety of α-hydroxy hydroxamic acid derivatives, the size and shape of the S_1' pocket for the CD23 processing metalloprotease has been explored. It has been demonstrated that a P_1' 2-naphthylmethyl group occupies most of the available space and gives excellent selectivity against fibroblast collagenase (matrix metalloproteinase-1, MMP-1) and other MMPs.

Introduction:

CD23, the low affinity IgE receptor, is a type II integral membrane glycoprotein which is known to undergo proteolytic processing with the formation of a number of soluble fragments.[1] Both the intact protein and soluble fragments are implicated in the regulation of IgE production; the former through negative feedback inhibition in B-cells[2] and the latter through their cytokine-like activities.[1] The protease responsible for the cleavage of CD23 to its soluble fragments has been partially characterised as a metalloprotease and our early studies[3,4] demonstrated that it was possible to prepare hydroxamate inhibitors of this enzyme which were selective versus the matrix metalloproteases. In this publication we delineate the SAR for a novel series of inhibitors with enhanced potency and selectivity which have a bicyclomethyl substituent at the P_1' position (Figure 1).

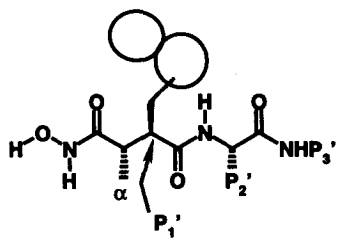

Figure 1

Chemistry

The synthesis of this series of CD23 processing inhibitors, *via* dioxolanones **2**, was analogous to that which has been used previously to prepare matrix metalloproteinase inhibitors, (Scheme 1). Thus reaction of the dianion of (S)-diethyl malate with an appropriate arylmethyl bromide or alkyl triflate gave diester **1**[5] from which dioxolanone **2** could be prepared after hydrolysis and subsequent ketalisation. It is postulated[6] that the stereoselectivity of this dianion reaction arises from chelation between the lithium of the ester enolate and the

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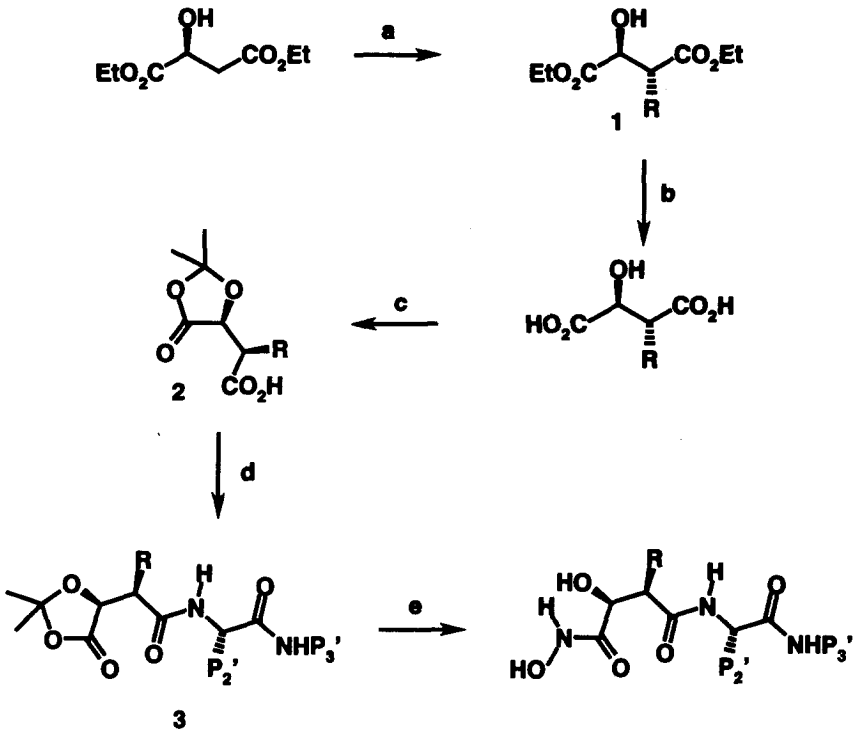
oxygen atom of the adjacent hydroxyl group, giving rise to a cyclic structure where the ester group hinders the approach of the electrophile on one side (Figure 2). This results in selectivities of 3-10:1, R:S. Standard carbodiimide coupling of the dioxolanone **2**, followed by treatment of amide **3** with hydroxylamine in DMF furnished the target compounds. The final compounds could be purified by recrystallisation, removing any residual minor diastereoisomers.

Scheme 1



Reagents: a) i) 2.2eq. LHMDS , THF, -60°C ii) RBr or ROTf; b) 3eq. KOH, dioxane/H_2O; c) 2,2-dimethoxypropane, cat. HCl; d) EDC, HOBT, $H_2NCH(P_2')CONHP_3'$, DMF; e) $HONH_3Cl$, N-methylmorpholine, DMF.

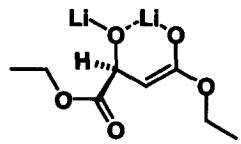


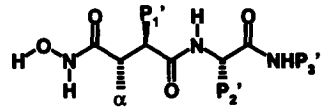
Figure 2

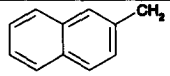
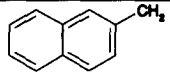
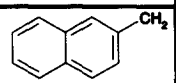
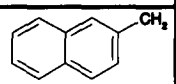
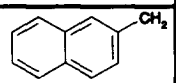
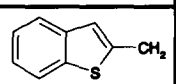
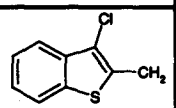
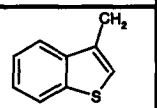
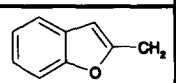
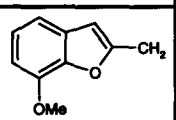
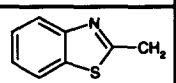
Discussion

Modifications to the critical P_1' group were made, which allowed us to explore the shape and electronic preferences of the S_1' pocket of the CD23 processing metalloprotease. We have fixed the α-substituent as

hydroxy, this having been shown to be beneficial for oral bioavailability in similar matrix metalloproteinase inhibitors, notably Marimastat[7].

Table 1: Inhibitory activities of P_1' bicyclomethyl hydroxamates



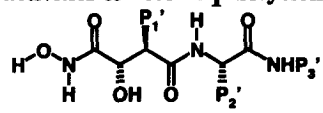
No.	α	P_1'	P_2'	P_3'	IC_{50} Inhibition of CD23 proteolysis (nM)[8]	IC_{50} Inhibition of MMP-1 (nM)
1[3]	H		Bn	Bn	1000	>1000
2	OH		Bn	Me	400	>1000
3	OH		Bn	H	600	>10000
4	OH		^{t}Bu	Me	230	>1000
5	OH		^{t}Bu	H	20	> 10000
6	OH		Bn	H	20	140
7	OH		Bn	H	>10000	NT
8	OH		Bn	Me	>1000	NT
9	OH		Bn	Me	100	<100*
10	OH		Bn	Me	>20000	NT
11	OH		Bn	H	320	500

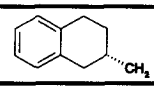
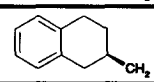
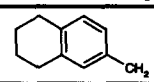
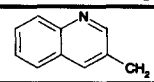
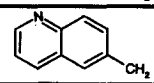
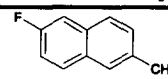
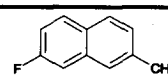
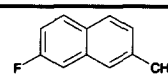
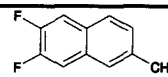
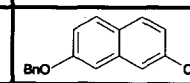
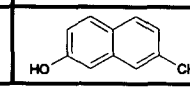
NT = not tested

* Not tested at a lower concentration

From Table 1, comparison of compounds **2** and **4** suggests that a P_2' ^tBu group offers a modest increase in potency when compared to benzyl, but when combined with a P_3' primary amide a ten-fold increase in potency results (compounds **4** vs **5**). The primary amide also generally improves selectivity against collagenase (MMP-1, data not shown). However major changes in potency /selectivity can result from modest alterations in the P_1' group. Thus replacement of the naphthyl ring by a benzothiophene, linked through the 2-position, (compounds **3** vs **6**) resulted in a large increase in potency but a diminution of selectivity. A chloro substituent at the 3-position of the benzothiophene ring, or linkage through the 3-position (compounds **7** & **8**) abolished inhibitory potency, suggesting that both a very precise orientation and ring size are required to maintain potency. In general most of the 5:6 bicyclic systems we prepared gave potent, but non-selective compounds (e.g. **6**, **9** & **11**) and were intolerant of further substitution (e.g. **10**).

Table 2: Inhibitory activities of "6:6" P_1' bicyclomethyl hydroxamates



No.	P_1'	P_2'	P_3'	IC_{50} Inhibition of CD23 proteolysis (nM)[8]	IC_{50} Inhibition of MMP-1 (nM)
12		^tBu	H	70	800
13		^tBu	H	70	1800
14		^tBu	Me	>10000	NT
15		^tBu	H	20	5000
16		^tBu	H	1000	NT
17		Bn	H	220	>10000
18		Bn	H	100	>10000
19		^tBu	H	80	>10000
20		^tBu	H	>1000	NT
21		Bn	H	>20000	NT
22		Bn	H	760	>10000

NT = not tested

As the selectivity of the 5:6 bicyclic systems was disappointing we proceeded to examine analogues based on 6:6 bicyclic compounds. Of the 6:6 bicyclic systems that were synthesised (Table 2) the 1,2,3,4-tetrahydronaphthylmethyl compounds **12** and **13** exhibited only a modest selectivity, with only a minor differentiation in selectivity between the two stereiosomers in the ring. In contrast, the 5,6,7,8-tetrahydronaphthylmethyl compound **14** was inactive. It is possible that the steric requirements deep within the S_1' pocket will not accommodate sp^3 centres particularly well. If the fit of the aromatic ring is indeed tight at this point, then it would explain why any ring substituent is usually deleterious to activity. The 3-quinolinylmethyl derivative **15** was both potent and 250-fold selective for inhibition of CD23 processing, whereas the 6- quinolinylmethyl analogue **16** had considerably less potency. This is consistent with the usual requirement for lipophilic groups within the S_1' pocket of metalloproteases and the idea that the unsubstituted ring is deep within that pocket. The only substitution tolerated on the bicyclic P_1' nucleus was a fluorine atom. Thus in compound **17** there was some modest gain in potency when compared to the unsubstituted analogue (Table 1, compound **3**) and no compromise in selectivity. Moving the fluorine one position around the ring gave compounds **18** & **19** which showed a small increase in potency, compared to compound **17** (although a loss of potency when compared to **5**), but again no change in selectivity. Incorporation of two fluorine atoms **20** gave a marked drop off in potency. It would appear that there is a small amount of room within the pocket to accommodate some extra lipophilicity, but the fit is tight. A large group such as the benzyloxy substituent incorporated in **21,** or a less lipophilic one e.g. the hydroxy group in **22,** is less well tolerated, although in the latter case the compound remained selective and the loss in potency is insignificant when compared to **3**.

Not only do some of these bicyclic P_1' groups impart selectivity against MMP-1, but also against the matrix metalloproteinases MMP-3 (stromelysin) and MMP-9 (gelatinase B, Table 3).

Table 3

No.	IC_{50} Inhibition of CD23 proteolysis (nM)	IC_{50} Inhibition of MMP-3 (nM)	IC_{50} Inhibition of MMP-9 (nM)
5	20	>10000	640
12	70	230	230
13	70	83% @ 10 μM	410
15	20	3000	190
17	220	3000	850

Conclusion

We have shown that the CD23 processing enzyme is tolerant of much larger P_1' groups than MMP-1 and that a 2-naphthylmethyl group occupies most of the available space within the S_1' pocket. Small changes in either orientation or size away from this group dramatically affect either potency and/or selectivity.

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8. For assay methodology see Marolewski, A.E.; Buckle, D.R.; Christie, G.; Earnshaw, D.L.; Flamberg, P.L.; Marshall, L.A.; Smith, D.G.; Mayer, R.J. *Biochem. J.* **1998**, 333, 573.