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SELECTIVE INHIBITION OF LOW AFFINITY IgE RECEPTOR (CD23) PROCESSING

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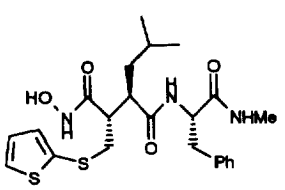
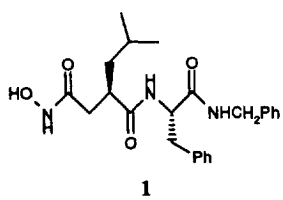
Abstract: A series of hydroxamic acids related to the non-selective matrix metalloproteinase inhibitor Batimastat has been prepared, some members of which are potent inhibitors of the processing of the low affinity IgE receptor (CD 23). Increased activity is obtained by appropriate substitution at the α-position, whilst selectivity is gained by use of a P1' benzyl group in conjunction with a C-terminal primary amide.

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] Whilst the enzyme responsible for intact CD23 processing is not yet known, partial purification and characterisation suggest that it is a metalloprotease of Mr 45-60 kDa.[3,4] Particularly significant has been the observation that processing of CD23 is inhibited by 1,10-phenanthroline and the non-selective metalloprotease inhibitor, Batimastat, whereas generic inhibitors of other protease classes are without effect.[3,4] Moreover, in the preceding publication we explored the broad SAR for the inhibition of CD23 processing in a series of hydroxamate-based analogues and provided evidence to suggest that selectivity from collagenase inhibition was possible.[5] In this communication we have broadened our investigations based on the lead compounds **1** and **2**, for reasons of synthetic expediency, to provide inhibitors having a markedly enhanced potency and/or selectivity for CD23 processing compared to those described previously.



Batimastat



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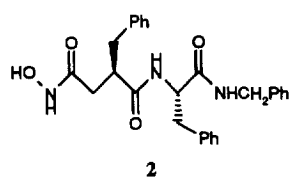

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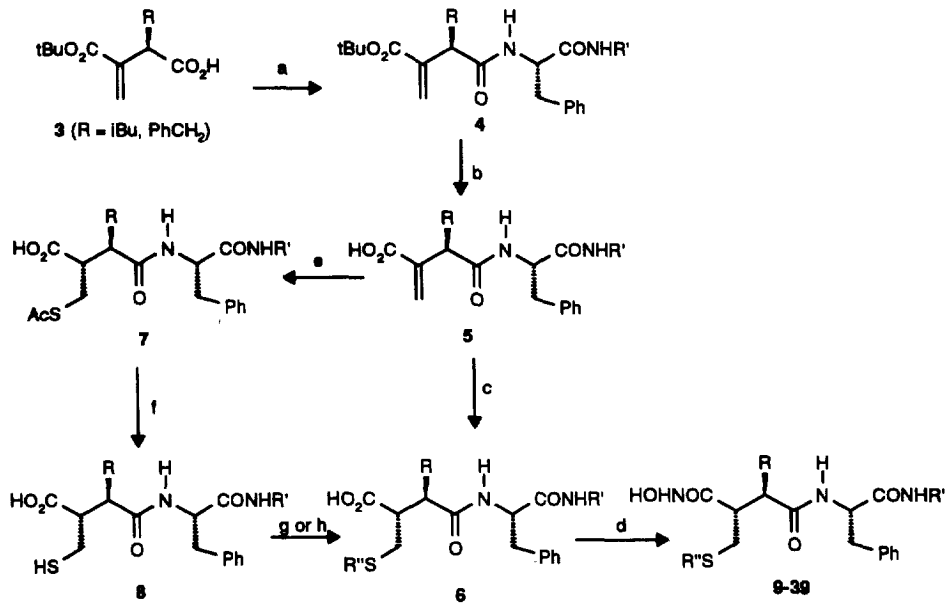
Chemistry:

All of the compounds described were prepared by the route illustrated in Scheme 1 from the common intermediate, **3**.[6] Standard peptide coupling with DEC in the presence of hydroxybenzotriazole readily afforded the α,β-unsaturated esters **4** in high yield and purity and these were deprotected by treatment with trifluoroacetic acid to the corresponding carboxylic acids **5**. At this stage the synthetic procedures diverged, with the method of choice being dependent on the nature of the substituent introduced β to the carboxylate moiety. Thus, in accordance with the findings of others,[6] addition of arylthiolate anions to **5** proceeded with high stereocontrol to provide the arylthioethers **6**, which were isolated as single diastereomers of assumed S-stereochemistry at the α-centre by analogy with Batimastat.[7]

Scheme 1



Scheme 1; Reagents and conditions: a, DEC, HOBt, H-PheNHR', ambient temperature, DMF or THF; b, TFA, MDC, ambient temperature; c, Et_3N, MeOH, R"SH, 60° C; d, DEC, HOAt, N-methylmorpholine, $NH_2OH.HCl$, DMF, ambient temperature or IBuOCOCl, TMS-ONH_2; e, AcSH; f, NaOH, H_2O, EtOH; g, NaOH, MeOH, R"Br or R"Cl; h, NaOH, EtOH, 4-$NO_2C_6H_4F$

Thiolacetic acid has also been shown to add to these α,β-unsaturated acids,[6] and this procedure has been used for the preparation of the adducts **7**. Mild alkaline hydrolysis of **7** gave the free thiol **8**, which was subsequently converted to the products **6** (R" ≠ aryl) upon alkylation with an appropriate alkyl halide. The conversion of the intermediate carboxylic acids **6** into hydroxamic acid derivatives was effected either directly

by treatment with hydroxylamine in the presence of DEC and HOAT,[8] or indirectly *via* the mixed anhydride generated with isobutyl chloroformate followed by treatment with *O*-trimethylsilylhydroxylamine. The benzylthiomethyl derivative **27** prepared by this method was shown to have identical stereochemistry to that produced by the conjugate addition of benzyl mercaptan to the corresponding acid **5**.

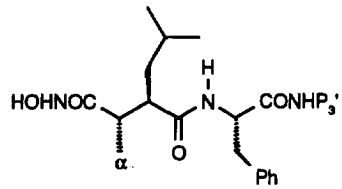
Whilst the majority of compounds were prepared by the above procedures, the carboxy derivatives **14** and **24** were prepared in excellent yield by mild hydrolysis (LiOH, aqueous MeOH) of the corresponding esters **13** and **23**. Control over the isolation of ketone rather than oxime derivatives in the formation of the hydroxamic acids **25, 26, 32** and **36** was effected by the use of one equivalent of hydroxylamine, whereas an excess of reagent was used to generate the oximes **28, 30, 31, 33** and **37**. The oxime ethers **29** and **38** were prepared by treatment of the hydroxamic acids **25** and **36** with methoxylamine in DMF containing a catalytic amount of tosyl chloride. Finally, the intermediate for the nitro compound **21** was prepared by S_NAr reaction of the appropriate thiol **8** with 4-fluoronitrobenzene.

Discussion:

Allergic reactions in animals and man are characterised by high levels of the immunoglobulin IgE, which binds predominantly to high affinity receptors on basophils and mast cells. It is the cross-linking of these IgE-bound receptors, in response to specific antigen exposure, which initiates mediator release and the subsequent allergic response.[9] Strong evidence exists to support a dominant role for CD23 and its soluble fragments in regulating IgE production, making the control of CD23 proteolysis an attractive therapeutic target.[1,2] A significant indicator that this process could indeed be attenuated was afforded by our earlier finding that Batimastat and related hydroxamic acids were effective inhibitors of the proteolytic cleavage, although these lead compounds lacked either potency or appropriate enzymatic selectivity.[5] By selectively modifying key parts of the structure of the lead compounds **1** and **2** it has been possible to address both of these issues successfully, to obtain a range of effective inhibitors of CD23 proteolysis.

Although CD23 is constitutively or inducibly expressed on a variety of different cells, we[3] and others[1,10] have found that the proteolysis is most conveniently studied in RPMI 8866 cells, a readily available Epstein Barr Virus immortalised human B-cell line, which expresses the protein and its associated protease in readily measurable amounts. Moreover, membrane preparations derived from these cells retain the proteolytic activity of intact cells and can be configured to assay potential inhibitors.[3,11] Using such an assay we previously demonstrated that the hydroxamate **1** inhibited the proteolytic cleavage of CD23, albeit with relatively low potency (IC_{50} 1.9 μM) and poor selectivity with respect to the matrix metalloprotease, collagenase (Table 1).[5] An early objective therefore was to enhance both the potency of **1** and its selectivity for the inhibition of CD23 proteolysis.

Table 1: Inhibitory Activity of P_1' isobutyl derivatives

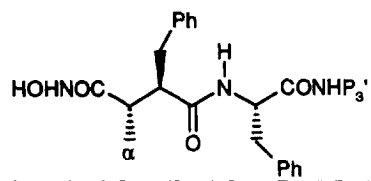


No	α	P_3'	IC_{50} Inhibition of CD23 proteolysis (nM)	IC_{50} Inhibition of Collagenase (nM)
1	H	$PhCH_2$	1900 ± 300	30
9	H	Me	1000-4000	60
10	H	H	3000 ± 500	3000
11	$AcSCH_2$	H	1000-3000	> 10000
12	$HSCH_2$	H	130 ± 30	NT
13	$MeO_2CCH_2SCH_2$	H	50 ± 10	NT
14	$HO_2CCH_2SCH_2$	H	~1000	NT
15	$NCCH_2SCH_2$	H	~100	106
16	$H_2NOCCH_2SCH_2$	H	100-200	NT
17	(2-thienyl)SCH_2	Me	100 ± 50	2.6 ± 1.6
18	(2-thienyl)SCH_2	H	30 ± 4	42 ± 19
19	$PhSCH_2$	Me	40-80	20 ± 12
20	4-HOPhSCH_2	Me	40-80	8 ± 1
21	4-NO_2PhSCH_2	Me	100-300	31 ± 13
22	4-(CO_2Me)PhSCH_2	Me	50-150	NT
23	2-(CO_2Me)PhSCH_2	Me	100 ± 40	21 ± 7
24	2-(CO_2H)PhSCH_2	Me	3700 ± 800	NT
25	$PhCOCH_2SCH_2$	Me	16 ± 5	≤ 100
26	$PhCOCH_2SCH_2$	H	20 ± 7	≤ 100
27	$PhCH_2SCH_2$	Me	30-100	25
28	Ph(C=NOH)CH_2SCH_2	Me	12 ± 3	≤ 100
29	Ph(C=NOMe)CH_2SCH_2	Me	80 ± 30	55% @ 0.01 uM;
30	Ph(C=NOH)CH_2SCH_2	H	100 ± 50	57% @ 0.1 uM;
31	4-ClPh(C=NOH)CH_2SCH_2	H	60 ± 10	34
32	4-PhPhCOCH_2SCH_2	Me	380 ± 100	62% @ 10 uM;
33	4-PhPh(C=NOH)CH_2SCH_2	Me	130 ± 30	58% @ 10 uM;

NT = not tested

Preliminary investigations had shown that selectivity enhancement was possible by appropriate modification of the P_1' substituent,[12] and the benzyl analogue **2** was one of several compounds affording greater than two orders of magnitude improvement (Table 2).[5] Moreover, we have now established that the synthesis of primary amide derivatives also has a marked influence on selectivity (cf **9** and **10**; **17** and **18**), although such modifications have little effect on potency, whereas the C-terminal acid and dimethylamide derivatives of compound **1** were inactive (IC_{50} > 20 μM). As Batimastat (**17**) is approximately tenfold more potent than compound **1** we have explored a range of substituents at the α-position, in an attempt to optimise potency prior to addressing improved selectivity. Many of the substituents investigated (Table 1) significantly increased inhibitory potency, suggesting that this position can accommodate considerable structural change. Substituents containing acidic functions, however, (**14** and **24**) were poorly tolerated, especially when compared to their respective esters **13** and **23**. Of particular interest was the high potency of the phenyl ketone **25** and its oxime derivative **28**, but neither of these two compounds afforded significant selectivity advantage.

Table 2: Inhibitory Activity of P_1' benzyl derivatives



	α	P_3'	IC_{50} Inhibition of CD23 proteolysis (nM)	IC_{50} Inhibition of Collagenase (nM)
2	H	$PhCH_2$	5000 ± 1000	7000
34	H	Me	20000-30000	1250
35	(2-thienyl)SCH_2	H	160 ± 40	930
36	$PhCOCH_2SCH_2$	H	210 ± 40	> 10000
37	$Ph(C{=}NOH)CH_2SCH_2$	H	130 ± 30	≥ 10000
38	$Ph(C{=}NOMe)CH_2SCH_2$	H	100-300	1900

Having established the considerable potency enhancement possible on incorporation of α-substituents into compound **1**, we focused on the intrinsically more selective compound **2** in the anticipation that both potency and selectivity could be elevated simultaneously. That this was indeed possible was illustrated with compounds **35-38** (Table 2) in which selected α-substituents were incorporated into the parent compound, although in this instance the ketone and oxime derivatives **36** and **37** respectively showed no potency enhancement over the thienothiomethyl derivative **35** (cf **25** and **28** with **17**).

Further studies are underway to optimise the inhibitory potency of this series of compounds as inhibitors of CD23 processing, but it is evident from our studies so far that compounds having high potency

can be achieved which can be distinguished from classical inhibitors of matrix metalloproteases such as collagenase.

References & Notes

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6. Compound **3** (R = iBu), was prepared as described in Otsuka patent WO94/21612, and compound **3** (R = Bn) was prepared similarly.
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