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HYDROXAMATE-BASED INHIBITORS OF LOW AFFINITY IgE RECEPTOR (CD23) PROCESSING

Stuart Bailey,[a] Brian Bolognese,[b] Derek R. Buckle,[a] Andrew Faller,*[c] Sally Jackson,[a] Pearl Louis-Flamberg,[b] Mark McCord,[b] Ruth J. Mayer,[b] Lisa A. Marshall,[b] and David G. Smith[c]

SmithKline Beecham Pharmaceuticals; [a] Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, UK, [b] Upper Merion, 709 Swedeland Road, King of Prussia, Philadelphia 19402, USA and *[c]New Frontiers Science Park (North), Third Avenue, Harlow, Essex, CM19 5AW, UK*

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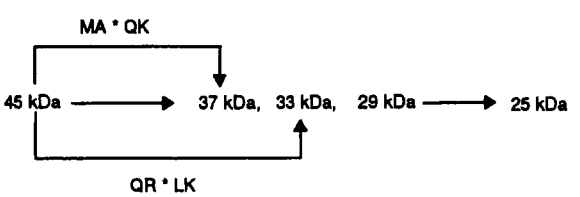
Abstract: A series of hydroxamic acids related to the non-selective matrix metalloprotease inhibitor Batimastat is described, which inhibits the proteolytic cleavage of the low affinity IgE receptor from cell membrane preparations. Limited SAR studies suggest that the structural requirements for effective inhibition are distinct from those required for the inhibition of collagenase.

Introduction:

In addition to its high affinity receptor FcεRI, expressed predominantly on mast cells and basophils, IgE also binds to a low affinity 45 kDa type II integral membrane glycoprotein FcεRII, also known as CD23.[1] In animals and man, CD23 is expressed on various cells, particularly mature B-cells for which it acts as a marker of activation and provides negative feedback inhibition of IgE synthesis.[2] Proteolytic cleavage of intact, cell-bound human CD23 results in the formation of a number of soluble fragments (sCD23) of Mr values 37 kDa, 33 kDa, 29 kDa, 25 kDa and 16 kDa (Scheme 1), the first three of which show potent cytokine-like activities.[3] In particular, these higher molecular weight fragments have been shown to promote IgE synthesis, which contrasts with the attenuation of IgE production displayed by the 16 kDa species.[4] These dual functions of CD23 and its cleavage products emphasise their pivotal role in the control of IgE and identify inhibition of the proteolytic events as a significant target for therapeutic intervention.

Scheme 1. Cleavage pattern of intact CD23



(Major cleavage sites indicated by asterisks)

* Fax +(44) 01279-627628: e-mail: Andrew_Faller-1@sb.com

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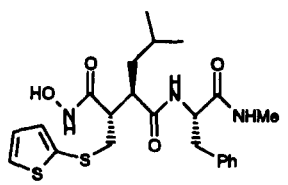
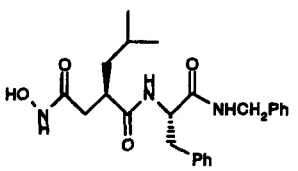
Although the cleavage of CD23 was initially thought to be autocatalytic,[5] evidence now exists to support the presence of an as yet unidentified metalloprotease.[6,7] The ability of the non-specific metalloprotease inhibitors 1,10-phenanthroline, imidazole, Batimastat and related compounds to attenuate proteolysis of CD23 provides particular support for this conclusion.[6] In this *letter* we describe our attempts to delineate the SAR for the inhibition of this protease based on an understanding of the features critical to metalloprotease recognition.[8,9] In an endeavour to simplify Batimastat prior to optimisation it was observed that, whilst removal of the thienothiomethyl moiety resulted in some loss of potency, compounds such as **1** remained effective inhibitors of the proteolysis of CD23 expressed constitutively in membranes derived from RPMI 8866 cells, a human B-cell line,[6] and therefore formed a template for subsequent modification.



Batimastat, BB94



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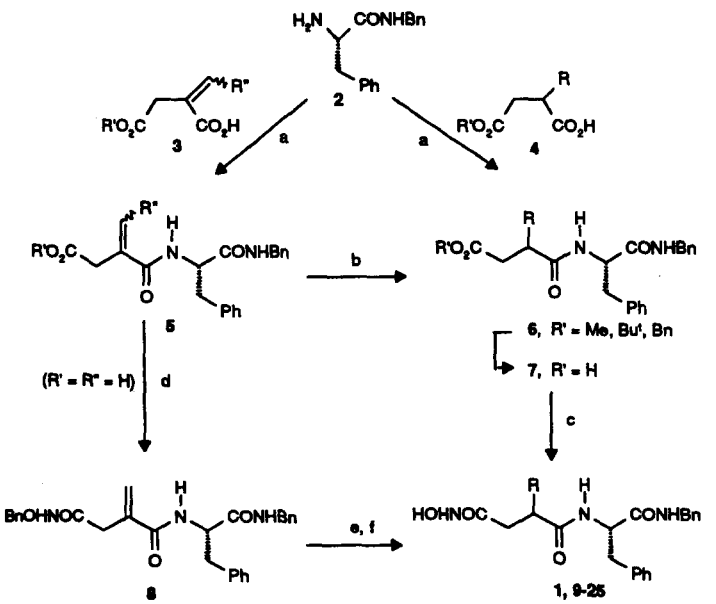
Chemistry:

The starting point for the synthesis of all compounds described was (S)-phenylalanine benzylamide **2**, which was reacted under standard peptide coupling conditions (DEC, HOBt, ambient temperature, DMF or THF) with the monoester of either an α,β-unsaturated succinic acid **3** or a 2-substituted succinic acid **4**, depending on the nature of the eventual functionality at P_1' (*ie.* the centre bearing the R group, Scheme 2, Table 1).[10] Synthesis of the key succinate intermediates **3** followed well documented Stobbe type condensations,[11] whilst those for the saturated analogues **4** involved more diverse procedures. From the unsaturated derivatives **5**, hydrogenation afforded high yields of the corresponding esters **6**, which formed the common intermediate to most of the target compounds. All compounds of formula **6** prepared *via* the alternative routes from either **3** or racemic **4** were necessarily racemic at the P_1' position. A more satisfactory modification to this procedure[12] utilised the Evans chiral auxillary **26** (Scheme 3) to prepare the succinates **4** in high chiral purity, and thus reduced the subsequent need for diastereomer separation. The hydroxy compound **24**, was prepared chirally pure from (R)-malic acid and the regioisomeric acids furnished on condensation with **2** were chromatographically separated to provide **7** (R=OH) prior to hydroxamate formation.

Following conversion of the esters **6** to the corresponding carboxylic acids **7**, introduction of the hydroxamic acid function can be performed in a number of ways. Typically we have preferred the condensation with *O*-trimethylsilyl hydroxylamine (DEC or bromo-tris(pyrrolidino)phosphonium hexafluorophosphate[13]) whereby the unprotected hydroxamic acids **1**, **9-21**, **23-25** were generated in

reasonable yield on aqueous work-up. In contrast to the general synthetic procedures used for these compounds, the P_1' aminated derivative **22** was prepared from the benzhydroxamate **8** and the free hydroxamate released by hydrogenolysis[14] after separation of the individual diastereomers by column chromatography. For other hydroxamates prepared as a mixture of diastereomers (isomeric at P_1'), individual isomers were separated by reverse phase preparative high pressure liquid chromatography at the final stage.

Scheme 2

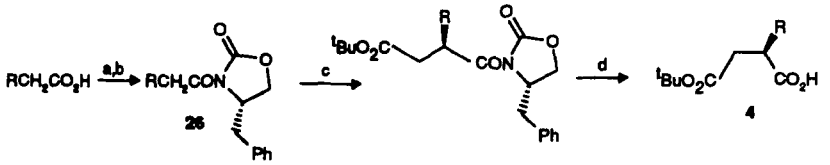


Scheme 2; Reagents: a, DEC, HOBt, ambient temperature, DMF or THF; b, Pd/C, cyclohexene, MeOH/EtOH, reflux, 2 h; c, DEC, HOBt, NH_4OTMS, CH_2Cl_2 or DMF, 25 °C, or bromo-tris(pyrrolidino)phosphonium hexafluorophosphate, NH_4OTMS, CH_2Cl_2, 25 °C or i-BuO_2CCl, $TMSONH_2$, N-methylmorpholine; d, DEC, HOBt, $BnONH_2$, DMF, ambient temperature; e, $NHMe_2$, MeOH, 20 °C, 16 h; f, Pd/$BaSO_4$, cyclohexene, MeOH/EtOH, Δ

Discussion:

The pivotal role of CD23 in the regulation of the immune response is well established,[2,4] but as yet only a single inhibitor of the proteolytic events leading to cleavage of the intact cell surface protein has been described.[6] In our early work, Batimastat, a potent, non-selective matrix metalloprotease inhibitor,[15,16] was shown to inhibit the proteolytic cleavage of constitutive CD23 from human B-cells,[6] with the consequent attenuation of IgE production.[6] In an endeavour to explore further the profile of this inhibitor class we have chosen the related compound **1** as a basis on which to develop subsequent SAR, monitoring the proteolytic events using CD23 expressed on membranes derived from human B-cells.[6,17] Since many compounds of this general type are potent, non-specific inhibitors of matrix metalloproteases, compounds were simultaneously evaluated against human collagenase[18] to establish whether the SAR differed between these two enzymes.

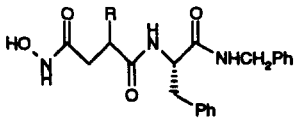
Scheme 3



Scheme 3; Reagents: a, $(COCl)_2$, DMF, CH_2Cl_2; b, ^nBuLi, THF, (S)-4-benzyl-2-oxazolidinone; c, $(TMS)_2NH$, ^nBuLi, $BrCH_2CO_2{}^tBu$, THF; d, LiOH, H_2O_2, MeOH

Initially we chose to investigate the structural preferences at P_1' (*ie.* for the S_1' pocket) since this region is known to be particularly critical for metalloprotease recognition and offers the greatest opportunity for selective inhibitor design.[9] Replacing the isobutyl moiety of **1a** by either hydrogen or methyl, **9** and **10** respectively, resulted in a marked reduction in inhibitory activity against CD23 processing (Table 1), suggesting at least some steric requirement at this position. Increasing steric bulk by phenyl, benzyl, and cyclohexylmethyl, substitution, however, surprisingly caused little change in potency against the CD23 enzyme, whilst resulting in a > 100-fold reduction in potency against collagenase (c.f. **11**, **12a**, **15** with **1a**). Cyclopentylmethyl substitution, by contrast, was tenfold more potent against the CD23 enzyme whilst showing no loss of potency against collagenase (c.f. **17** with **1a**). The similar activities of the benzyl and phenethyl substituents contrasts with the loss of potency in going from cyclohexylmethyl to cyclohexylethyl (**12a**, **13** vs **15**, **16**). Increasing steric bulk still further was less predictable since both the 1-naphthyl **18** and 4-biphenyl **20** analogues were essentially inactive on the CD23 enzyme, whereas the 2-naphthylmethyl compound **19** retained similar potency to that of compound **1**. As expected from established SAR,[19] none of these larger substituents afforded potent collagenase inhibitors. Since the CD23 cleavage site leading to formation of the 37 kDa fragment (see Scheme 1) possesses the glutamine residue at P_1', the corresponding hydroxamate analogue **21** was prepared. Rather surprisingly it showed no inhibitory activity against CD23 proteolysis. Similarly, the four other residues at P_1' containing heteroatoms, **22-25**, were also relatively poor inhibitors. Once again none of these modifications resulted in significant inhibition of collagenase. Consistent with the known preferred (R) stereochemistry for the P_1' substituent of Batimastat and related compounds for the inhibition of matrix metalloproteases,[16] no compound investigated with the opposite configuration inhibited CD23 proteolysis (**1b**, and **12b**).

Table 1: Inhibitory activity of hydroxamic acid derivatives



No.	R	P1' Stereochemistry	Inhibition of CD23 IC_{50} (μM)	Inhibition of collagenase IC_{50} (μM)
	Batimastat	R	0.1± 0.05	0.005
1a	i-Bu	R	1.9± 0.3	0.03
1b	i-Bu	S	>20	> 10
9	H	-	>20	> 10
10	Me	R	>20	> 10
11	Ph	S*	1.6±0.8	17
12a	$PhCH_2$	R	5±1	7
12b	$PhCH_2$	S	>>20	NT
13	$Ph(CH_2)_2$	R	5±1.6	4.1
14	c-hexyl	S*	5-10	1.5
15	c-hexyl CH_2	R	3.7±1.3	4.6
16	c-hexyl $(CH_2)_2$	R	-67% @ 20	> 10
17	c-pentyl CH_2	R	0.2±0.1	0.02
18	1-naphthyl	S*	>20	> 10
19	2-naphthyl CH_2	R	1±0.3	>10
20	4-biphenyl	S*	>20	> 10
21	$(CH_2)_2CONH_2$	R	>20	NT
22	CH_2NMe_2	S*	>20	> 10
23	CH_2SCHMe_2	R	>20	NT
24	OH	R	>20	NT
25	$SCHMe_2$	R	8±4	1.9

* indicates same stereochemistry as Batimastat. NT = not tested.

While these data tend to suggest that some similarity may exist in the active site environment of collagenase and the CD23 processing enzyme, significant differences in their SAR are evident from this work. In particular, two compounds, **11**, and **19**, have been identified, which show low micromolar inhibitory potency against the CD23 processing enzyme while displaying relatively poor inhibitory activity against collagenase. A more detailed investigation of the optimal requirements for inhibition of CD23 processing is reported in the following publication.

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17. The CD23 proteolysis assay was carried out by incubation of various concentrations of the inhibitor with RPMI 8866 cell plasma membrane preparations (Morre, J.; Morre, D.M. *Biotechniques*, **1989**, *7*, 946) (0.75 μg per 50 μl assay) for 1-3 h at 37 °C, followed by quenching by the addition of 50 μl of 10 μM Batimastat. The supernatant was separated by filtration through 0.22 μm Millipore filters and the soluble proteolysis product was assayed by CD23 EIA using MHM6 (DAKO) as the capture antibody and the second antibody as provided by The Binding Site (Birmingham, UK) in their EIA kits for CD23. Inhibition was expressed relative to controls without inhibitor (0 %) and with 5 μM Batimastat added prior to the incubation (100 %). IC_{50} values were determined from the line of best fit of the concentration-response curves.
18. The collagenase assay was carried out by the method of Cawston and Barrett (*Anal. Biochem.*, **1979**, *99*, 340). Thus, various concentrations of the inhibitor were incubated with 13 ng of human recombinant fibroblast collagenase and 0.01 μCi [^{3}H]-acetylated type 1 bovine collagen for 18 h in Tris buffer at pH 7.6 and 37 °C. Unhydrolysed collagen was separated from proteolysis products by centrifugation. The amount of [^{3}H] in the supernatant was determined by scintillation counting and expressed as a percentage of the value obtained for a control sample in the absence of inhibitor. IC_{50} values were determined from the line of best fit of the concentration-response curves.
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