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Inhibition of Matrix Metalloproteinases: An Examination of the S1' Pocket

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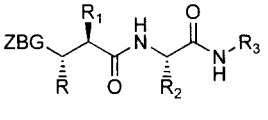
Abstract: Peptidomimetic carboxylate- and hydroxamate-based inhibitors of matrix metalloproteinases containing extended P1' groups have been prepared. Potent inhibition and good selectivity for MMP-2 has been observed for the compounds produced. © 1997, Elsevier Science Ltd. All rights reserved.

The process of matrix remodelling and degradation is performed by a family of calcium and zinc dependent endoproteinases known as the matrix metalloproteinases (MMPs)[1]. To date twelve examples of this family have been identified and these enzymes are divided by protein substrate specificities into the collagenases, gelatinases and stromelysins. Collectively these MMPs can degrade all of the major components of the extracellular matrix. Over production of MMPs is thought to be responsible for a range of biological processes observed in diseases[2] such as the arthritides, tumour metastasis, periodontal diseases, and multiple sclerosis. Many groups are now involved in the design of low molecular weight, orally active matrix metalloproteinase inhibitors (MMPIs)[3]. Among the several types of MMPIs studied the pseudopeptide derivatives **1,** which feature a zinc binding group (ZBG) and mimic the sequence of the natural substrate to the right of the cleavage site (P1' - P3'), have been widely studied. For this class of inhibitor the ZBG is preferably hydroxamic acid for broad spectrum inhibition whilst a carboxylic acid ZBG in combination with certain extended P1' (R_1) groups has been shown by Porter *et al.*[4] to provide selectivity for 72 kDa gelatinase (MMP-2, GelA) and stromelysin (MMP-3, HFS) over fibroblast collagenase (MMP-1, HFC). We have identified two broad spectrum hydroxamate MMPIs, batimastat **2** and marimastat **3**, which are currently in human clinical trials as injectable and orally active agents respectively for the treatment of cancer.



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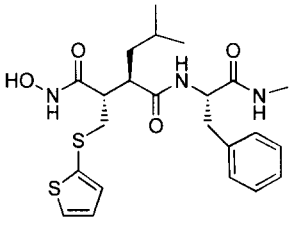
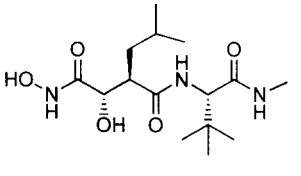


2 (Batimastat)



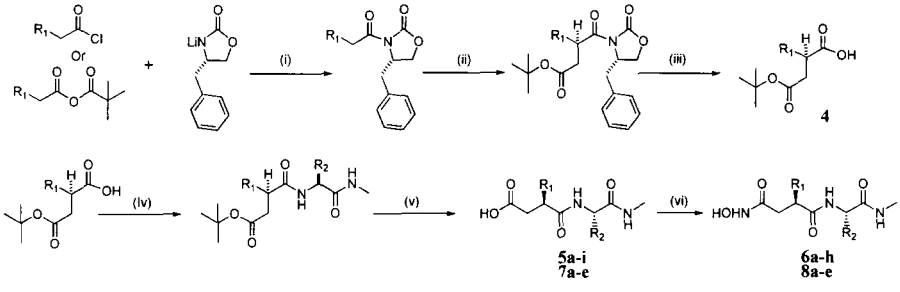
3 (Marimastat)

Recently X-ray crystallographic data have been published for several members of the MMP family[5]. The structures include bound inhibitors which illustrate the interactions necessary for good inhibitor activity. In particular high potency is attributable to: chelation of the active site zinc atom; the presence of a number of backbone amides which hydrogen bond to the enzyme; and the orientation of side chain groups which fill pockets and grooves in the MMPs. Of particular note is the S1' subsite which is the most well defined area of binding and consists of a hydrophobic pocket which varies in depth for the different MMPs. Such structural studies have pointed to the notion that modification of P1' in MMPIs should confer selectivity.

In this paper we report on the synthesis and enzyme selectivity of series of P1' modified succinic acid based carboxylates and hydroxamates.

Chemistry

The general strategy for the synthesis of the P1' modified MMPIs is shown in Scheme 1.



Reagents and conditions: (i) THF/-78°C; (ii) NaHMDS/-78°C; $BrCH_2CO_2{}^tBu$; (iii) H_2O_2/LiOH/THF/0°C; (iv) EDCI/HOBt/DMF/0°C then HPheNHMe or H'BuGlyNHMe; (v) TFA/DCM/4°C; (vi) $BnONH_2$/EDCI/HOBt/DMF then H_2/Pd-C

Scheme 1

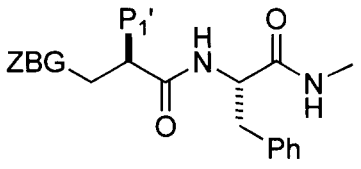
The required chiral succinates **4** were prepared using methods based on the asymmetric alkylation methodology of Evans[6,7]. Coupling of **4** to the N-methyl amides of L-phenylalanine and L-tert-butylglycine followed by TFA hydrolysis of the esters gave the acids **5** and **7** respectively. Conversion of the acids to hydroxamates was achieved by coupling with O-benzyl hydroxylamine followed by careful hydrogenolysis, to avoid over reduction to the amide[8], which furnished **6** and **8**.

The carboxylic acids **5a-i , 7a-e** and the hydroxamic acids **6a-h, 8a-e** were assayed for inhibition of human recombinant HFC, GelA, HFS and matrilysin (MMP-7, Mat)[9].

Results and Discussion

The Celltech group previously discovered differences in the S1' pocket of the MMPs by showing that a 3-phenylpropyl P1' group (e.g. **9**) gives selectivity for HFS and particularly GelA over HFC[4]. To further explore S1' selectivity we chose to produce MMPIs with simple alkyl chains at P1'. Compounds containing both carboxylic acids and hydroxamic acids as the ZBG were initially prepared in a P2' phenylalanine series (Table 1).

Table 1: P1' modified phenylalanine analogues



			IC_{50} nM or %I @ 100,000 nM			
Cpd	ZBG	P1'	HFC	GelA	HFS	Mat
2†	CONHOH	$(CH_3)_2CHCH_2$	5	4	20	6
9	CONHOH	$Ph(CH_2)_3$	1000	15	500	10000
5a	CO_2H	C_6H_{13}	20000	40000	20%	10%
5b	CO_2H	C_7H_{15}	7000	10000	70%	70%
5c	CO_2H	C_8H_{17}	30%	200	5000	20%
5d	CO_2H	C_9H_{19}	7000	600	20000	20%
5e	CO_2H	$C_{10}H_{21}$	70%	1000	70%	50%
5f	CO_2H	$C_{12}H_{25}$	30%	500	1000	10%
5g	CO_2H	$C_{14}H_{29}$	0%	200	50%	20%
5h	CO_2H	$C_{15}H_{31}$	20%	800	0%	30%
5i	CO_2H	$C_{16}H_{33}$	30000	50	50000	NT
6a	CONHOH	C_6H_{13}	20	10	80	2000
6b	CONHOH	C_7H_{15}	400	1	40	2000
6c	CONHOH	C_8H_{17}	150	0.6	450	1000
6d	CONHOH	C_9H_{19}	500	1	30	3000
6e	CONHOH	$C_{10}H_{21}$	20	2	100	2000
6f	CONHOH	$C_{12}H_{25}$	50000	1	40	30%
6g	CONHOH	$C_{14}H_{29}$	50%	30	80	10%
6h	CONHOH	$C_{16}H_{33}$	20%	20	300	20

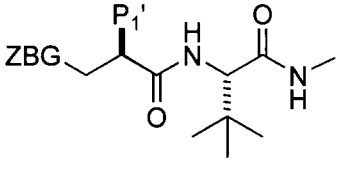
†**2** (Batimastat) also has an alpha 2-thienylthiomethyl modification. NT not tested.

The carboxylic acids all showed weak inhibition against HFC, HFS and Mat and modest activity against GelA. Surprisingly the largest P1' group, C_{16} alkyl (**5i**), gave the greatest degree of inhibition of GelA (IC_{50} 50 nM) combined with excellent selectivity over HFC and HFS. The corresponding hydroxamates all show good inhibition of GelA (IC_{50} 0.6 - 30 nM) with the greatest activity residing in the C_8 alkyl chain compound, **6c**.

Whilst these compounds are generally less active against HFC, HFS and particularly Mat, C_6 (**6a**) and C_{10} (**6e**) show reasonable HFC inhibition (IC_{50} 20 nM) whilst C_7 (**6b**), C_9 (**6d**), and C_{12} (**6f**) have reasonable HFS inhibition.

Having demonstrated that variation of the P1' alkyl chain affects enzyme selectivity we proceeded to prepare a series of related compounds incorporating a P2' t-butyl glycine (Table 2). Again it was found that the C_{16} alkyl P1' group, (**7e**), gave good GelA selectivity in the carboxylic acid series. In the hydroxamic acid series the order of inhibition was generally GelA > HFS > HFC > Mat[10]. The periodicity in HFC and HFS inhibition seen in the phenylalanine series was not observed in the tert-leucine series though a smaller range of alkyl groups was used.

Table 2: P1' Modified t-butyl glycine analogues



			IC_{50} nM or %I @ 100,000 nM			
Cpd	ZBG	P1'	HFC	GelA	HFS	Mat
3†	CONHOH	$(CH_3)_2CHCH_2$	5	6	200	20
7a	CO_2H	C_8H_{17}	20000	900	30000	40000
7b	CO_2H	$C_{13}H_{27}$	5000	100	2000	NT
7c	CO_2H	$C_{14}H_{29}$	40%	50	4000	20%
7d	CO_2H	$C_{15}H_{31}$	8000	90	7000	50%
7e	CO_2H	$C_{16}H_{33}$	30%	30	10000	30%
8a	CONHOH	C_8H_{17}	100	0.7	100	500
8b	CONHOH	$C_{13}H_{27}$	3000	3	60	2000
8c	CONHOH	$C_{14}H_{29}$	3000	0.8	70	50%
8d	CONHOH	$C_{16}H_{33}$	5000	0.6	90	5000
8e	CONHOH	$C_{14}H_{28}OH$	300	0.5	50	500

†3 (Marimastat) also has an alpha hydroxy modification. NT not tested.

Analysis of the recently available X-ray crystal structures reveals that there are two architectural S1' subsite types. One type consists of a deep hydrophobic pocket that forms a channel through the enzyme (e.g. HFS and by homology GelA) and the second type is a shallow S1' pocket by virtue of a larger amino acid residue at 214 (numbering following Browner, 1995[5c]) (e.g. Arg ^{214} in HFC and Tyr ^{214} for Mat). Our experimental selectivities are broadly those that would be expected on the basis of the X-ray analysis, with the exception of the good potency against HFC seen for the C_{10} phenylalanine compound, **6e**. It might be expected that the C_{10} group would be too large to fit into the S1' pocket of HFC, however Sterling Winthrop have reported that long chain phenolic ethers can be accommodated in the HFC pocket. It may be that these groups can produce a conformational change in

the enzyme allowing these chains deeper access to the S1' pocket[11]. The periodicity observed in HFS inhibition for the hydroxamate phenylalanine derivatives may represent a balance between the displacement of water molecules, which are revealed by MMP structural studies to reside in the S1' pocket, and the additional hydrophobic interaction gained. With this in mind we prepared a long alkyl chain terminating in an hydroxyl group, **8e**, within the t-butyl glycine hydroxamate series. This compound showed a similar spectrum of activity to the long chain alkyl compounds.

Compound **7e** is undergoing further pharmacological evaluation due to its high GelA selectivity and promising oral bioavailability in a rat *ex vivo* GelA bioassay[12]. In conclusion, we have demonstrated that within succinyl carboxylate and hydroxamate MMPIs, long alkyl chains at P1' lead to GelA selectivity.

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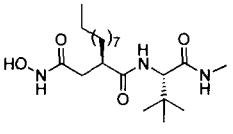

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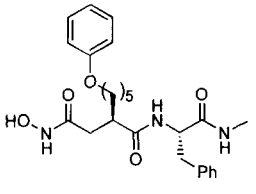

IC_{50} HFC, 26 nM, IC_{50} GelB, 0.31 nM, IC_{50} HNC, <2 nM, IC_{50} HFS, 14 nM

12. Male rats (n=12) were dosed orally with compound **7e** (10 mg/kg), serial blood samples were removed and MMPI activity extracted and assayed against GelA. Concentrations were calculated against a standard curve. The mean blood levels were in excess of 100 ng/ml at 2h post dosing. Bone, E. A.; Askew, M.; Laber, D.; unpublished results.

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