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The identification of β-hydroxy carboxylic acids as selective MMP-12 inhibitors

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ABSTRACT

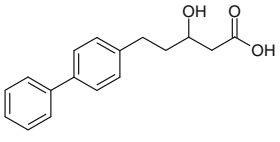
A new class of selective MMP-12 inhibitors have been identified via high throughput screening. Crystallization with MMP-12 confirmed the mode of binding and allowed initial optimization to be carried out using classical structure based design.

The matrix metalloproteinases (MMPs) are a family of zinc containing endoproteinases catalyzing degradation of the extracellular matrix (ECM). As such, they represent potential therapeutic targets for the treatment of a diverse range of pathological conditions including rheumatoid arthritis, atherosclerosis and chronic obstructive pulmonary disorder (COPD).[1] A growing body of evidence has specifically linked MMP-12 (macrophage metalloelastase) with COPD[2] making the development of small molecule MMP-12 ligands of high interest.[3] Historically, MMP inhibitors have been typically non-selective as they have relied on potent metal binding motifs, for example, hydroxamates and reverse hydroxamates, for the majority of their potency. As MMP activity, and indeed activity at other related metallo-enzymes, is required for numerous essential processes within the body, broad spectrum metallo-enzyme inhibition is considered undesirable. As a consequence we sought to identify potent and selective inhibitors of MMP-12.

A high throughput screen of the GSK compound collection identified a number of MMP-12 active compounds. These were refined by removal of molecules with undesirable physiochemical properties (high Mw, clog *P* etc.) and compounds carrying high affinity metal binding motifs (hydroxamates, reverse hydroxamates and thiols). It was anticipated that the use of a weaker zinc binding group, for example, a carboxylic acid, would result in compounds with better selectivity profiles than those in which the binding interaction was dominated by high affinity metal chelation.

An initial measure of selectivity was carried out by determining potency at the closely homologous enzyme MMP-13 (Collagenase-3). This information allowed the identification of a number of classes of MMP-12 selective compounds. Finally, a detailed kinetic profile of exemplars of each of these classes was obtained and analysis of the data using a Lineweaver–Burk plot of $1/v$ against $1/[S]$ allowed classification of the modes of action (competitive, noncompetitive, mixed etc.).

Following these analyses a number of structures were identified as possible synthetic starting points—detailed here is the initial optimization of **1** (Fig. 1).



1

MMP12 IC50=0.52 uM
MMP13 IC50=12.0 uM

Mw=270
HBA=2
HBD=3
clogP=3.19
Solubility=0.96 mg/mL at pH 7.4
Solubility=0.45 mg/ml at pH 6.2
logD=0.55 at pH 7.4
logD=1.43 at pH 6.2
MOA: Competitive/Reversible

Figure 1. Initial profile of the HTS hit 1.

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This encouraging initial data led us to obtain a full selectivity profile for racemic **1** and both of its enantiomers against the GSK panel of metallo-enzymes and other proteinases and to initiate crystallographic studies in order to understand the mode of binding of **1** to MMP-12. Cross screening of **1** and its constitutive enantiomers revealed an encouraging broader selectivity profile. All were inactive against a diverse range of serine, aspartyl and cysteine proteinases. Furthermore, high selectivity was observed against a range of metalloproteinases (Table 1).[4]

Initially it was speculated that **1** was binding with MMP-12 in a mode where both the alcohol and carboxylic acid of the β-hydroxy acid functionality were coordinated to the catalytic zinc, however, this proved not to be the case. Crystallographic investigations revealed that only the carboxylic acid coordinated to zinc and that the hydroxyl group is forming a hydrogen bonded interaction to the Leu181 residue (Fig. 2).[4]

Though Leu181 and its flanking residues are highly conserved in human MMPs[5] it was reasoned that this hydrogen bonding interaction was the key to the selectivity of **1**. This interaction positions the biaryl fragment deep in the S1′ pocket of the MMP-12 where substantial differences in homology between the MMPs exist and previous studies have suggested selectivity can be obtained.[6]

On the basis of these observations two regions of the molecule were identified for preliminary investigation via analogue syntheses, occupancy of the S1′ pocket and the H-bonding interaction to Leu181 (Fig. 3).

Table 1
Inhibition of sample metalloproteinases by **1** and its enantiomers

Metalloproteinase	Inhibition IC_{50} (μM)		
	1	Ent-1	Ent-2
MMP-1	>98	>98	>98
MMP-2	4.52	NT	NT
MMP-3	>98	>98	>98
MMP-7	>98	>98	>98
MMP-9	16.5	10.5	19.1
MMP-12	0.52	0.34	0.79
gp-MMP12	0.91	1.14	4.67
MMP-13	12.0	9.99	36.8
MMP-14	43.5	36.6	>9
TACE	>98	>98	>98
ACE	>250	>250	>250

NT = Not tested; all human enyzymes except gp-MMP12 = guinea pig.

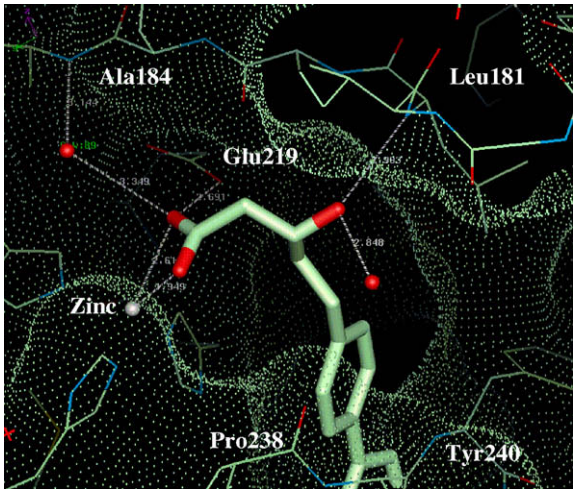


Figure 2. The binding mode of **1** in hMMP-12 determined at 2.0 Å resolution by X-ray crystallographic analysis. H-bond interactions are indicated by dashed lines.

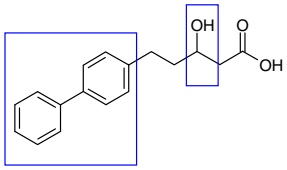


Figure 3. Regions of 1 chosen for modification.

Two types of variation in P1′ were initially investigated, substitution of the terminal aryl ring and complete replacement of the biaryl fragment. Variation in the terminal aryl ring was achieved by the use of Suzuki chemistry on iodide **5**, which was prepared from commercially available benzyl bromide **2** (Scheme 1).[7]

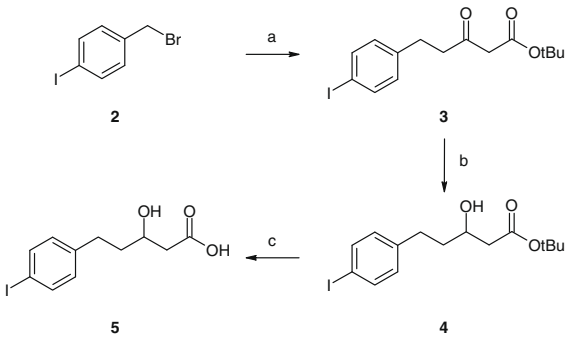
It was found that either thermal or microwave conditions could be successfully employed to couple a diverse range of boronic acids and esters to **5** in the presence of cesium carbonate and a palladium source in DMF. The palladium source of choice was identified as Fibrecat® 1001 which improved the ease of reaction workup and purification (Scheme 2).

Cross screening of a range of the compounds prepared revealed clear SAR for substitution of the terminal aromatic ring (Table 2).

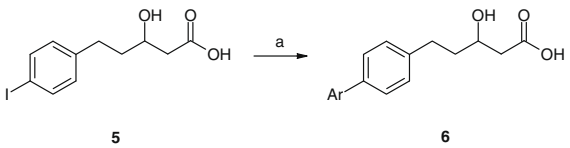
Introduction of a *para* substituent gave clear gains in potency and by suitable choice of Ar it was demonstrated that selectivity could also be varied. Crystallographic analysis[4] of **6c** suggested that the source of the observed potency gain was water mediated hydrogen bonding interactions to residues at the bottom of the S1′ pocket, specifically main chain atoms of Lys233 and Arg249 (Fig. 4).

Variation of the complete biaryl fragment was also investigated using a similar reaction sequence to that described in Scheme 1. Starting from a diverse range of commercially available benzylic halides or alcohols a number of analogues of **1** were prepared (**7a–d**) and subsequently profiled (Table 4).

Crystallographic analysis[4] of **7a** and comparison of its binding mode with that observed for **6c** revealed that introduction of a conformational constraint prevented the biaryl system from adopting

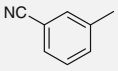
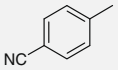
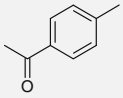
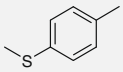
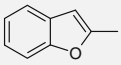


Scheme 1. Preparation of aryl iodide **5**. Reagents and conditions: (a) *t*-butyl acetoacetate, NaH, *n*-BuLi, 0 °C to rt; (b) $NaBH_4$, MeOH, 0 °C; (c) silica gel, toluene, reflux.[8]



Scheme 2. Suzuki coupling of boronic acids/esters to iodide **5**. Reagents and conditions: (a) boronic acid (1.2 equiv), cesium carbonate (2.5 equiv), Fibrecat 1001 (10 mol %), DMF, microwave, 100 °C, 1 h.

Table 2
Sample cross screening data for compounds **1** and **6a–f**

Ar	Compound	Inhibition IC_{50} (μM)		
		MMP-12	MMP-13	MMP-1
	**1**	0.52	12.0	>98
	**6a**	3.39	52.0	>98
	**6b**	0.086	2.45	>98
	**6c**	0.062	0.97	>98
	**6d**	0.058	1.13	>98
	**6e**	0.055	3.76	NT
	**6f**	0.181	50.2	NT

NT = Not tested.

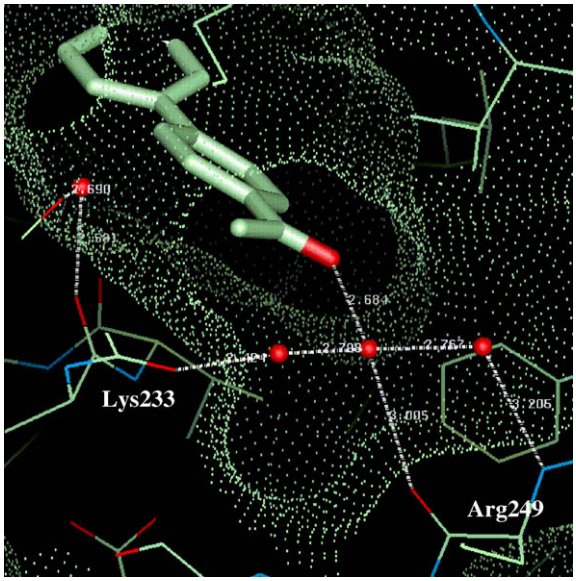


Figure 4. The binding mode of **6c** at the bottom of S1′ pocket of hMMP-12 determined at 1.7 Å resolution by X-ray crystallographic analysis.

a twisted pose along the aryl–aryl axis (Fig. 5). Though the addition of an extra atom is sterically tolerated the restriction in rotation leads to a suboptimal occupation of the S1′ pocket.

Finally, variations of the hydroxyl group, involved in the hydrogen bonding interaction with Leu181, were investigated. Methylation of **4** with methyl iodide followed by Suzuki coupling with

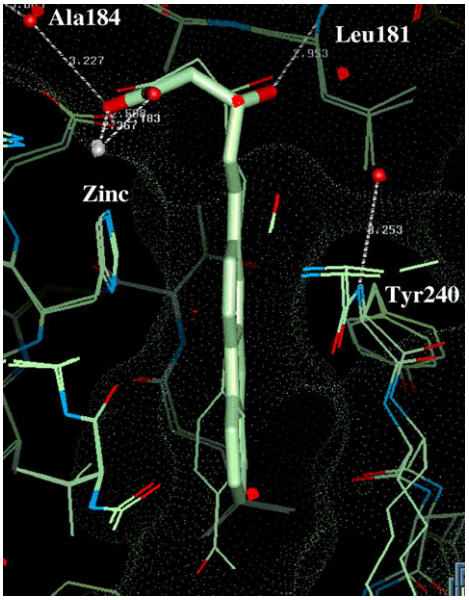
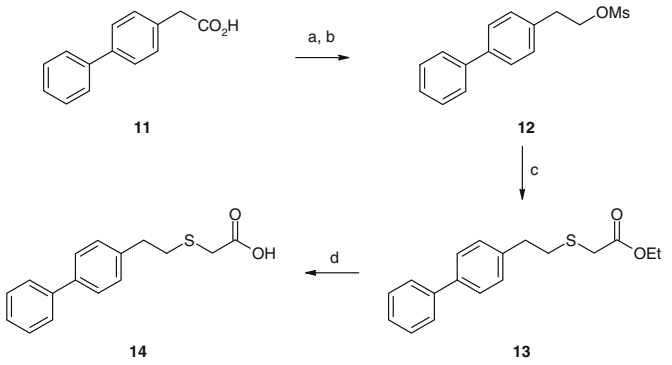


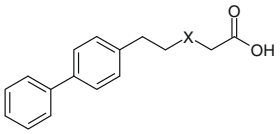
Figure 5. A comparison of the binding modes of **6c** and **7a** (determined at 2.3 Å by resolution X-ray crystallographic analysis).in the S1′ pocket of hMMP-12—**6c** wire, **7a** rods. Note the twist in **6c** compared to **7a**.

phenyl boronic acid and *t*-butyl ester cleavage gave methyl ether **8**. An analogous sequence acylating **4** with acetic anhydride gave ester **9**. The corresponding ketone **10** was prepared from ketone **3** by Suzuki coupling with phenyl boronic acid and subsequent *t*-butyl ester deprotection. A number of corresponding sulfur analogues were also prepared (Scheme 3).



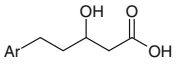
Scheme 3. Preparation of thioether **11**. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C; (b) MsCl, Et_3N, CH_2Cl_2; (c) $HSCH_2CO_2Et$, NaOEt, EtOH; (d) LiOH, THF, H_2O.

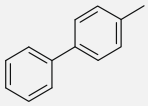
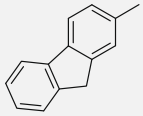
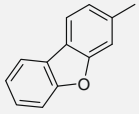
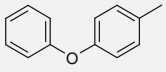
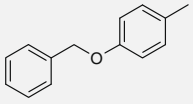
Table 3
Sample cross screening data for compounds **1**, **8–10** and **14–16**



X	Compound	Inhibition IC_{50} (μM)		
		MMP-12	MMP-13	MMP-1
CH_2OH	**1**	0.52	12.0	>98
CH_2OMe	**8**	3.53	>98	>98
CH_2OAc	**9**	54.4	>98	>98
C=O	**10**	7.84	>98	>98
S	**14**	11.8	95.2	>98
S=O	**15**	4.5	94.9	>98
SO_2	**16**	2.49	11.3	>98

Table 4
Sample cross screening data for compounds **1** and **7a–d**



Ar	Compound	Inhibition IC_{50} (μM)		
		MMP-12	MMP-13	MMP-1
	**1**	0.52	12.0	>98
	**7a**	1.15	26.1	>98
	**7b**	20.1	33.5	>98
	**7c**	19.9	41.9	>98
	**7d**	3.75	>98	>98

It was possible to specifically oxidize **14** using 1 equiv of mCPBA to give the corresponding racemic sulfoxide **15** and then by treating **15** with a further equivalent of mCPBA to obtain sulfone **16**. Cross screening of these compounds was then carried out (Table 3).

Whilst the free alcohol was the most potent of the compounds prepared it was demonstrated that a number of viable alternative groups were tolerated.

In summary, we have identified a series of potent, selective MMP-12 inhibitors based around a β-hydroxy acid template. The lower affinity zinc binding group (carboxylic acid) allows excellent selectivity to be obtained over a range of closely related MMPs as the molecules binding interactions are not dominated by metal chelation. On the basis of initial synthetic studies and crystal structures obtained this series appears to be an excellent starting point for further optimization.

Acknowledgements

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References and notes

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4. (a) IC_{50} values are given as the average of at least two replicates ($n \geqslant 2$) with values deviating by less than two fold across the range. Assays were conducted using recombinant metalloproteinases from commercial sources, or generated in-house, with fluorescently tagged peptide substrates. Details available on request. (b) Protein for crystallization was obtained by overexpression of MMP12 residues 106–268 in *E. coli* followed by refolding and standard purification procedures in the presence of a hydroxamate tool compound. Crystals were grown with the tool compound from 100 mM Mes pH 5.2–5.6, 16–20% PEG 5KMME and 200 mM ammonium sulfate. The crystals are P1 with approximate cell dimensions of $a = 46$ Å, $b = 65$ Å, $c = 68$ Å, $a = 66°$, $b = 84°$, $g = 71°$. Complexes of **1**, **6c** and **7a** were obtained by replacement soaking of the crystals. For all three complexes, data was collected at Daresbury synchrotron and density for the ligand was clear and unambiguous. Final Rfactors are 0.184, 0.172, 0.152 and Rfree 0.246, 0.217 and 0.219, respectively, with pdb deposition codes of 2wo8, 2wo9 and 2woa.
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