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STRUCTURE-BASED DESIGN AND SYNTHESIS OF A SERIES OF HYDROXAMIC ACIDS WITH A QUATERNARY-HYDROXY GROUP IN P1 AS INHIBITORS OF MATRIX METALLOPROTEINASES

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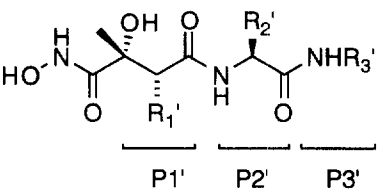
Abstract: Examination of the S1 area of the active site of pro-stromelysin has led us to the design of novel and potent inhibitors of matrix metalloproteinases containing constrained quaternary-hydroxy group at P1. The synthesis and biological activity of these compounds with variations at P1', P2', and P3' will be described.

The MMPs are a family of Zn-dependent endopeptidases involved in the degradation and repair of the major components of the extracellular matrix of connective tissues.[1] To date 14 human enzymes have been identified and are classified according to their substrate specificities as collagenases, stromelysins, and gelatinases.[2] The MMPs have been implicated in a wide variety of diseases, including rheumatoid arthritis, osteoarthritis, periodontal disease, and tumor metastasis.[3] Synthetic inhibitors of these enzymes are considered attractive targets in drug discovery research and the rational design and SAR studies of such inhibitors are currently being pursued by a number of pharmaceutical companies.[4]

In this report, we describe the design, synthesis, and SAR studies of inhibitors of MMP-1, 3, and 9 bearing a quaternary-hydroxy functionality at the P1 position (**I**). Marimastat, a potent broad spectrum MMP inhibitor from the British Biotech Company, bearing a secondary hydroxy substituent in P1, is orally bioavailable and is currently undergoing phase III clinical studies for tumor metastasis.[4c] In our design, we introduced a quaternary-hydroxy group at P1. Modeling in the active site of MMP-3 indicated the presence of a hydrogen bond between the backbone of Ala 165 and the hydroxyl group of the inhibitor, in addition to the regular hydrogen bonding network established for these types of inhibitors (Fig. 1).[5]



The preparation of the inhibitors is outlined in Scheme 1.[6] Introduction of the quaternary center at P1 was achieved by the reaction of an appropriately substituted chiral N-acyl oxazolidinone **A** with methylpyruvate.[7]

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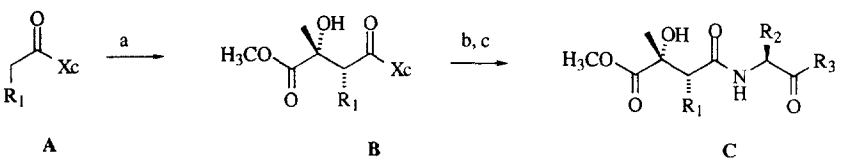
The resultant diastereomeric mixture of aldol adducts **B**, produced in yields of up to 90%, were separated by flash chromatography. Removal of the chiral auxiliary with lithium peroxide at 0 °C over 2 h. proceeded in 50% yield. The resultant acid was reacted with an appropriately functionalized amino acid to afford **C** in 65% yield. Basic hydrolysis proceeded in yields up to 70%, followed by reaction with O-benzylhydroxylamine. Careful hydrogenolysis with Pd/C (10%) in MeOH gave hydroxamic acids **E** in nearly quantitative yields.

Scheme 1



Xc = *S*-(-)-4-benzyl-2-oxazolidinone

Reagents and conditions:

(a) methyl pyruvate, LDA, THF, -78 °C, 2 h, 70 - 91%; (b) LiOH, H_2O_2, THF - H_2O, 2 h, 0 °C, 40 - 50%; (c) TBTU, THF or DMF, 4 - 18 h, 50 -70%; (d) LiOH, THF:H_2O, 0 °C, 1 - 2 h, 60 - 75%; (e) O-benzylhydroxylamine hydrochloride, DCI, THF, 62 - 75%; (f) Pd/C, H_2, MeOH, 1 - 2 h, 90%.

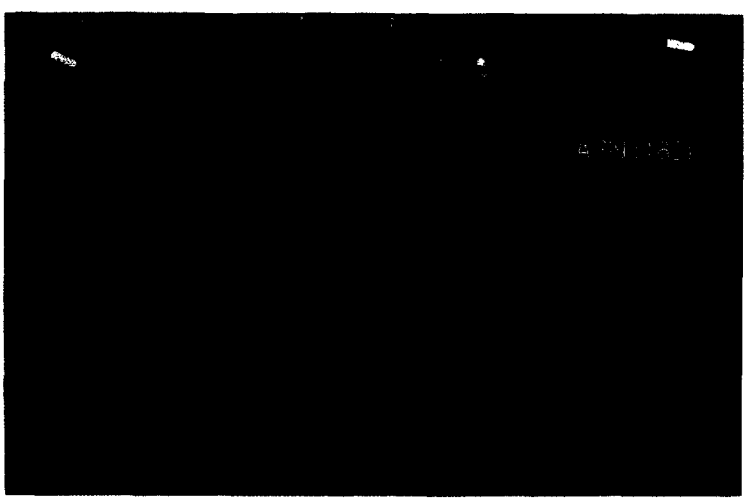
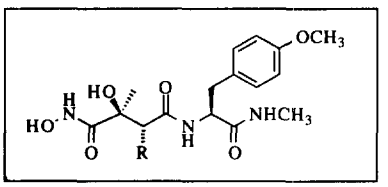


Figure 1. Computer model of **4** in MMP-3 active site. Hydrogen bonded residues of the enzyme are shown for clarity. The model is the average structure during the final three picoseconds of a 30 ps molecular dynamics computer simulation of **4** in the binding domain of MMP-3

Results and Discussion: The initial modelling studies in the active site of MMP-3 suggested that "S" stereochemical orientation of the quaternary-hydroxy center at P1 is essential for activity against MMP-3. As revealed by modelling work (Fig. 1), the hydroxamic acid moiety of inhibitors with the "R" stereochemistry at P1 was moved away from the catalytic Zn and consequently, could not bind effectively. To prove this concept, inhibitors **1** - **3** (Table 1) were prepared. They showed no activity against MMP-3. In contrast, all of the inhibitors with "S" orientation at P1 were active (Tables 2 - 4). "S" stereochemistry at P1 was kept constant throughout the rest of the study.

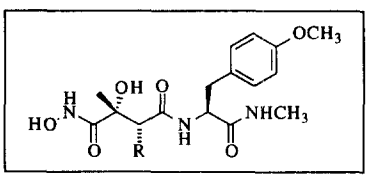
TABLE 1



Compound #	R	MMP-3 (Ki (nM))
1	i-Bu	>10000
2	CH_2Ph	>10000
3	$(CH_2)_2Ph$	>10000

In an effort to determine the optimum spacing in the P1' domain, a series of inhibitors with variations at P1' were prepared. Compound **7**, with phenylpropyl moiety in P1', was the most active inhibitor of MMP-3.[4d] A four fold reduction in MMP-3 activity was observed in compound **4** with isobutyl group in P1' (Table 2). Activity of **4** against MMP-1 assay was slightly improved. The MMP-3 activity was substantially (>100 fold) reduced in **5**, where the P1' substituent was benzyl. This compound was also less active in MMP-1 and MMP-9 assays. The X-ray crystal structures of **4** and **7** (Fig. 3) bound in the catalytic domain of stromelysin-1 were solved.[8] The four fold gain in MMP-3 activity could be explained by the favorable hydrophobic interaction of phenylpropyl group in S1' pocket of the enzyme. Compound **7** has adapted a conformation that allows for the Van der Waals interaction between the phenyl group in P1' and backbone His 201.

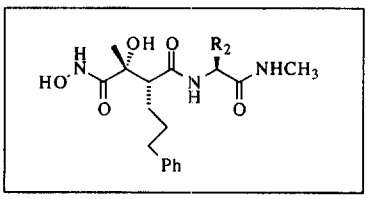
TABLE 2



		Ki (nM)[9]		
Compound #	R	MMP-3	MMP-1	MMP-9
4	i-Bu	13	0.6	<1
5	CH_2Ph	526	85	5
6	$(CH_2)_2Ph$	25	33	2
7	$(CH_2)_3Ph$	3	2	<1

To probe for enhanced activity and selectivity, P2' analogs were prepared (Table 3). The sterically hindered t-Bu group that can presumably suppress the hydrolysis of P2'- P3' amide bond[4] was introduced in **8**. Activity against MMP-3 and MMP-1 was reduced four to six fold. To improve upon water solubility, inhibitors with polar substituents in P2' were prepared. Water soluble **9** (log P = 0) was less active against MMP-3 (Ki = 32 nm). Reduction of activity against MMP-3 was also noted in **10** (Ki = 103 nM). Compounds **11**, **12**, and **13** containing a substituent with hydrogen bonding capabilities in the P2' region did not show improvement in activity against MMP-3 and MMP-1 enzymes. All of the P2' modified compounds were potent inhibitors of MMP-9.

TABLE 3



Compound #	R_2	Ki (nM) MMP-3	MMP-1	MMP-9
7	CH_2-p-OCH_3Ph	3	2	<1
8	tert-Leu	13	13	<1
9	$(CH_2)_4NH_2$	32	15	<1
10	$(CH_2)_3NH_2$	103	18	5
11	$(CH_2)_3NHSO_2N(CH_3)_2$	24	22	2
12	$(CH_2)_3NHC$Omorpholine	70	53	4
13	$(CH_2)_3NHCO_2t$Bu	13	16	2

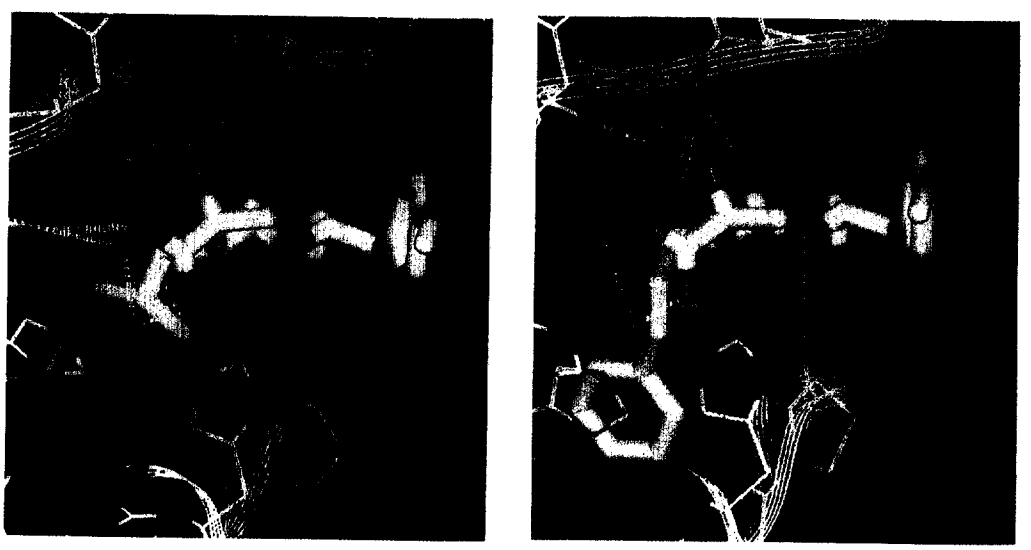
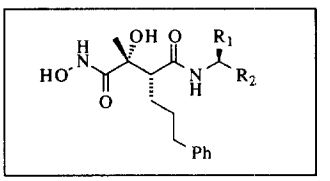


Figure 3. X-Ray crystal structures of **4** (right) and **7** (left), shown in yellow in the catalytic domain of MMP-3. Hydrogen bonds shown as dashed lines, with distances in angstroms.

Placing different substituents in P3' did not lead to enhancement in potency in any of the enzymes tested (Table 4). While the potency of compound **15** has decreased compared to **1**, the selectivity has improved. The N-methyl amide in P3' was found to be the optimal substituent for this series of inhibitors. This finding is consistent with the observation that the S3' subsite of MMP-3 is not well defined and is mostly exposed to the solvent interface of the enzyme.

TABLE 4



Compound #	R1	R2	Ki (nM) MMP-3	MMP-1	MMP-9
7	CH_2-p-OCH_3Ph	$CONHCH_3$	2	2	<1
14	CH_2Ph	CO_2tBu	100	198	34
15	CH_2Ph	COOH	25	167	>213
16	CH_2Ph	$CONHCH_2$(*S*-OH)Ph	7	72	1
17	$(CH_2)_4NH_2$	$CONH(CH_2)_2Ph$	28	25	1
18	$(CH_2)_4NH_2$	CONHCH(*S*-CH_3)Ph	168	282	149

Novel inhibitors of MMP-1, 3, and 9 were synthesized that contain a rigid quaternary hydroxy group at P1. During the course of the SAR study of these inhibitors it was determined: (1) Stereochemical orientation at P1 center is crucial for activity. Compounds bearing "R" stereochemistry at P1 were devoid of activity in MMP-3. In contrast, all of the "S" inhibitors were active. (2) The phenylpropyl group was established as the optimal substituent in P1'region. (3) Hydrophobic substituents at P2' and N-methyl amides at P3' were found to be optimal. (4) Selectivity toward MMP-3 enzyme was observed in compound **15**.

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