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SYNTHESIS AND IDENTIFICATION OF CONFORMATIONALLY CONSTRAINED SELECTIVE MMP INHIBITORS

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Abstract: We have discovered a new series of potent conformationally constrained MMP Inhibitors that are selective for MMP-13 over MMP-1.

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade the major components of the extracelluar matrix. These enzymes can be divided into three subfamilies collagenases, stromelysins, and gelatinases based on which component of the matrix they degrade. The cleavage of collagen, the major constituent of cartilage, is uniquely mediated by the collagenases MMP-1, MMP-8, and MMP-13. MMP inhibitors have the potential to prevent the degradation and loss of cartilage. Preclinical studies using nonselective MMP inhibitors have shown that these agents are able to prevent the destruction of cartilage using two animal models of osteoarthritis; the spontaneous guinea pig and the rabbit partial meniscectomy models.[1,2] Since MMP's play a significant role in human physiology, the use of non-selective inhibitors might be expected to produce a variety of side effects. In fact, during cancer clinical trials with the nonselective inhibitor Marimastat (British Bio-Tech) a dose limiting side effect was observed.[3] This adverse effect was described as fibrosis of the joints. The side effect is believed to be mechanism based and hypothesized to result from inhibition of MMP-1. While this is still speculative many companies have moved away from designing broad spectrum inhibitors to designing ones with specific selectivity profiles for different therapeutic applications. The selective inhibition of MMP-13 over MMP-1 for osteoarthritis treatment is attractive for several reasons, MMP-13 has a more limited distribution in humans than MMP-1, it is located primarily in chondrocytes and has been shown to be up-regulated in patients with osteoarthritis.[4] It has been shown that MMP-13 cleaves type II collagen (the major component of cartilage) at a 10× faster rate then MMP-1.[4] A inhibitor of MMP-13 and MMP-8 with acceptable pharmacokinetics that spares MMP-1 would provide a useful tool to test the above hypothesis.

Chemistry

In a recent paper we outlined the initial discovery and subsequent SAR development of a new series of acyclic thiol based MMP inhibitors[5] that are active and selective for MMP-13 over MMP-1. During the SAR optimization process we found that substitution α and γ to the thiol was tolerated while substitution β was

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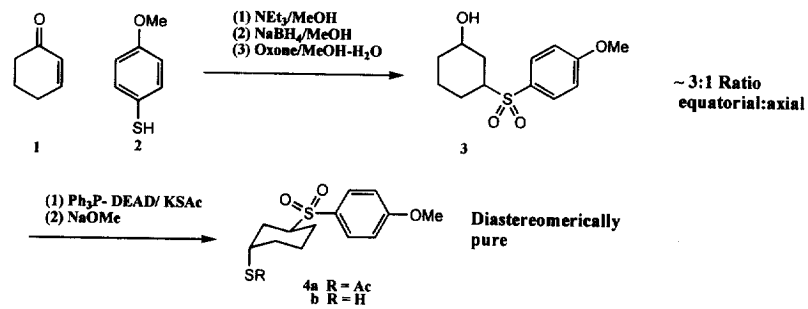
detrimental to potency. We were curious to see if a cyclic thiol analog would maintain high potency against MMP-13 and MMP-8 while eliminating MMP-1 activity.

The synthesis of the target thiol is shown in Scheme 1. Michael reaction of *p*-methoxythiophenol with cylohexenone afforded the β-keto-sulfide in high yield. Reduction with sodium borohydride gave a 76:19 ratio of alcohols with the major, equatorial, isomer arising from the expected axial delivery of hydride. Oxidiation of the sulfide to the sulfone was uneventful and the mixture of sulfone alcohols was subjected to standard Mitsunobu conditions with potassium thioacetate as the sulfur nucleophile. Interestingly we observed that the ratio of products was enriched towards the major (equatorial alcohol–axial thioacetate) isomer. We speculate that the minor isomer underwent partial elimination to olefin. Upon chromatographic purification we obtained diastereomerically pure thioacetate, **4a**. De-protection with sodium methoxide in methanol afforded the free thiol, **4b**.



Scheme 1. Synthesis of Cyclic Thiols.

For biological screening of **4b** and **6** vs the acyclic analog **5** against hMMP-13 and hMMP-1, see Table 1.[6] The cyclohexyl analog, **4b**, is as potent as **5** against hMMP-13 and hMMP-8 while the cyclopentyl analog, **6**, was significantly less active.

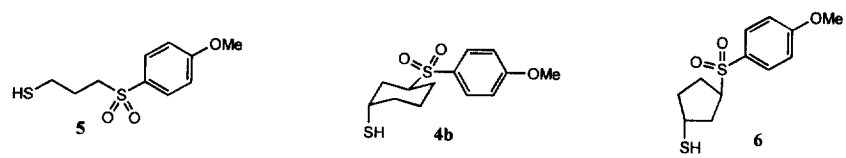


Figure 1. Comparison of Cyclic vs Acyclic Thiol

This potency prompted us to incorporate our more potent right hand pieces. Using the same chemistry that we used in the preparation of the corresponding acyclic thiols[5] we were able to prepare the two cyclic conformationally constrained thiols, **7** and **8**. The enzyme data comparing **7** and **8** to their open chain counterparts **9** and **10** is shown in Table 1. The cyclic thiols, **7** and **8** are essentially equipotent compared to their acyclic

Table 1. IC_{50} (nM) Enzyme data for thiols **4b–10**

Compd	hMMP-13	hMMP-1	hMMP-8	Selectivity 13/1
4b	22	1000	585	45
5	50	>10,000	1100	200
6	500	>10,000	nt	
7	1	1700	8	1700
8	0.4	>10,000	50	25,000
9	0.5	1500	4	3000
10	2	>10,000	36	5000

counterparts. To further understand this phenomenon we undertook crystallographic studies of these thiols bound to hMMP-8. So far only compounds **8** and **9** have produced suitable crystals in complex with MMP-8.

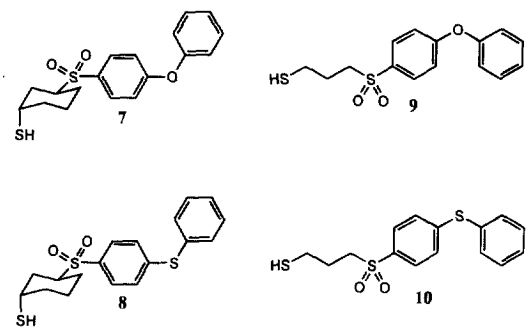


Figure 2. Optimized Cyclic vs Acyclic Thiols

Crystallography

An illustration of the active site of MMP-8 in complex with compounds **8** and **9** is shown in Figure 3. MMP-8 complexes were collected at resolution of 1.57 A and 1.80 A, respectively, and refined to R-factors of 0.19 and 0.17. The thiol in both compounds acts as a fourth ligand to the catalytic zinc, completing a tetrahedral arrangement of ligands which also include the imidazole nitrogens of His 197, 221, and 227. A single sulfonyl oxygen makes a key hydrogen bond to the amide N-H at Leu 180, with a O-N distance of 2.88 A. The remaining sulfonyl oxygen is entirely solvent exposed. Substitution along the backbone of these compounds is consistent with structural information. Elaboration at the α and γ positions is well tolerated, as these are partially solvent exposed. Substitution at the β position is disfavored for steric reasons. In both compounds, the Ph-X-Ph (where X is -O- or -S-) moiety extends into a predominantly hydrophobic cavity that forms P1'.

Figure 3. X-ray crystal structures of 8 and 9 bound to hMMP-8.

Compound **9** (shown in blue) adopts a conformation in the binding site closely approximating that of the more rigid cyclic thiol, **8** (shown in green). This backbone similarity results in a nearly superimposable placement of the sulfonyl group and the P1' substituents in the active site. It was unanticipated to find that the very bulky SO_2PhSPh is occupying an axial position while the free thiol is equatorial.[7] Both **8** and **9** are selective for MMP-13 and MMP-8 over MMP-1, though the -S-Ph compounds demonstrate greater selectivity. Selectivity is due in large part to an Arg in place of Leu 213 in MMP-1, which results in a more shallow S1'. Thus incorporation of groups off the second phenyl ring produce inhibitors with even higher selectivity for MMP-13 and MMP-8 over MMP-1.

In summary, we have developed a novel series of conformationally constrained MMP inhibitors that retain all of the potency of their flexible acyclic counterparts. The use of these thiol based MMP inhibitors in various animal models of osteoarthritis and cancer will be communicated in the future.

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6. Inhibitors were assayed against purified hMMP-13 and hMMP-1 using a enzyme assay based on cleavage of the fluorogenic peptide; MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2. This is similar to conditions described by C. G. Knight, *FEBS Lett.* **1992**, *296*, 263.

7. A referee suggested that the similar activity for the acyclic vs cyclic can be explained by the ring conformation of bound **8**, being thermodynamically unfavorable due to the axial sulfone and that this negates any entropic advantage vs **7**. We thank him for this suggestion.