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Synthesis and evaluation of novel oxazoline MMP inhibitors

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Abstract—MMP inhibitors with novel oxazoline zinc binding groups have been synthesized and evaluated. Selectivity for the inhibition of MMP-9 over MMP-1, MMP-2, and MMP-12 has been achieved in several cases.

Extracellular proteolysis plays a key role in a number of biological processes. Angiogenesis, wound healing, inflammatory reactions, management of the blood brain barrier, and general maintenance of joints, to name a few, all depend on enzymes, which remodel connective tissues in the extracellular matrix. The involvement of matrix metalloproteinases (MMPs) in extracellular degradation has been clearly demonstrated and the number of disease states that these enzymes impact on are many.[1] It is known that MMPs, particularly the gelatinases, mediate extracellular matrix and basement-membrane degradation during the early stages of tumor growth.[2] They also contribute to later stages of tumor growth and promote metastasis. They activate growth factors by inactivating growth-factor-binding proteins and releasing mitogenic compounds from the matrix proteins thus directly inducing tumor-cell proliferation. Inhibition of MMPs, either selective or broad spectrum, has a great potential to be an effective approach to the treatment of a variety of different diseases. As a consequence, there has been a great deal of effort in recent years to design and prepare inhibitors of MMPs, mostly targeted at the right-hand (prime) side of the active site, which contains a hydrophobic S1′ pocket. Key to the activity of nearly all MMPIs is the functional group, which binds the zinc atom in the active site and the P1′ substituent.[3,4]

Some of the best-known MMPIs to date combine a succinate backbone and a zinc-binding group (ZBG) and a fair amount of structure–activity data is now available on these compounds. A hydroxamic acid ZBG has resulted in optimal potencies thus far, and most small molecule MMP inhibitors contain this functionality. But nanomolar inhibition has also been realized with other groups, for example, thiols, carboxylates, mercaptoalcohols,[5] and dithiols.[6] Although many hydroxamate-succinate derived MMPIs show outstanding activity in pre-clinical situations, there has been little efficacy for cancer therapy demonstrated in clinical trials. There are several reasons for the previous failures of the hydroxamates in clinical trials and pharmacokinetics has been a particular problem with hydroxamates.[7] Hence, new ZBGs with better drug-like properties are of current interest. Recent reports demonstrate a continued optimism that MMPI therapy may be beneficial.[8–10] Accordingly, the pursuit of new inhibitor designs is a highly significant endeavor.

Recently, we have developed synthetic methodology for the facile and efficient preparation of chiral 5-vinyloxazolines based on a Pd-catalyzed cyclization and isomerization strategy.[11] We envisioned that our methodology would be amenable for the preparation of MMPIs with novel ZBGs. We hypothesized that the oxazoline moiety, together with another functional group (X), could provide a strong chelating effect for binding to the active site zinc (Fig. 1). P1′ substituents could be placed on the oxazoline 2-position and P2′ groups on the 4-position. We anticipated that the rigidity of the ring might also

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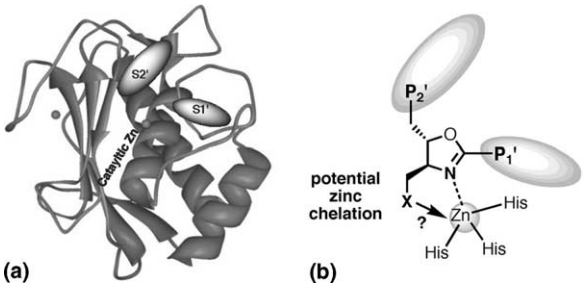
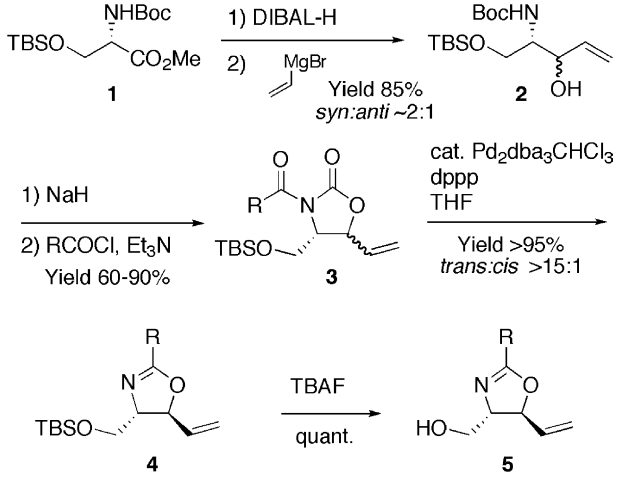


Figure 1. (a) MMP-2 catalytic domain.[12] (b) Hypothetical binding of oxazolines to the active site Zn ion of MMPs.

offer some advantages for good drug-like compounds by restricting bond rotations. Further, the turn angle of the ZBG with respect to the P1′ substituent observed in known X-ray structures matches well with the angle of the oxazoline nitrogen and the 2-position of the ring allowing for a more rigid fit and potential selectivity over other zinc enzymes. To our knowledge, oxazolines have not been utilized as a ZBG in drug-like compounds.

In order to provide a proof of principle for our proposed oxazoline-based MMPIs, we prepared a library of oxazolines that contain minimal functional groups for binding. Structures that possess only a P1′ substituent and a potentially zinc-cordinating oxazoline and alcohol were synthesized as illustrated in Scheme 1. Starting from the *N*- and *O*-protected serine methyl ester **1**, diisobutylaluminum hydride reduction and in situ addition of vinyl magnesium bromide afforded the allylic alcohol **2** in good yield. The diastereoselectivity of this step was generally low, however, it is inconsequential as the alcohol stereocenter was equilibrated by design at a later stage during the Pd-catalyzed oxazoline formation. Cyclization of the alcohol onto the Boc-carbonyl by deprotonation with NaH was followed by acylation of the newly formed oxazolidinone to afford generally good yields of **3**. Key to the synthesis of the target oxazolines was the Pd-catalyzed ring opening of **3**, decarboxylation, and cyclization of the amide to form **4**. This process occurred with rapid equilibration of the intermediate pi-allyl palladium complex and allowed for the formation of the thermodynamically more favored *trans*-oxazolines **4** with high levels of diastereoselectivity (dr > 15). Removal of the *O*-protecting group with tetrabutylammonium fluoride provided the model oxazoline alcohols **5** in quantitative yields. Twenty analogs with variation in the potential P1′ group (R) were prepared and evaluated for biological activity against MMPs (see Table 1).



Scheme 1.

The oxazoline compounds were tested for inhibition of MMP-1, MMP-2, MMP-9, and MMP-12[13] using the fluorogenic MMP substrate 7-(methoxycoumarin-4yl)acetyl-Pro-Leu-Gly-Leu-(*N*-3-[2,4-dinitrophenyl]-L-2,3-diamino-propionyl)-Ala-Arg-NH_2 (Peptide International, Louisville, KY and Biomol International, Plymouth Meeting, PA), in single experiments with full-length enzymes or with catalytic domains in 96-well format.

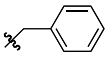
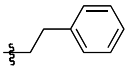
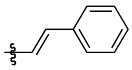
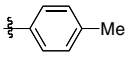
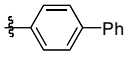
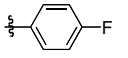
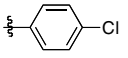
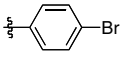
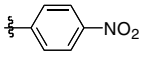
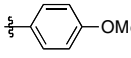
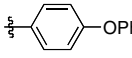
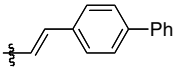
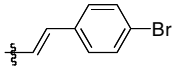
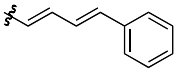
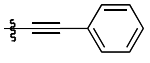
Full-length MMPs in latent form (Q-Biogen-Alexis, San Diego, CA) were activated using freshly prepared 2.5 mM solutions of 4-aminophenyl mercuric acetate (APMA) in 0.1 M NaOH: MMP-9 and MMP-2 required a 2.5 h incubation at 37 °C and MMP-1 required 3.5 h at 37 °C. The substrate (10 μL, final conc. of 5 or 7 μM) and various concentrations of test compounds (10 μL) were incubated in 50 mM HEPES buffer (pH 7.5), 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Brij-35, and 1% v/v DMSO for 2 min, and MMPs (final conc. 0.04–0.25 μg/mL) were added to initiate the reaction. The total volume was 500 μL.

In the 96-well plates (flat-bottom, white plates from Corning, Acton, MA), 20 μL of inhibitor solutions was added to each well and incubated at 37 °C for 45 min. Stock solutions of substrate and of MMP catalytic domains (Biomol International, Plymouth Meeting, PA) were incubated in amber glass vials for 45 min at 37 °C. The assay was initiated by adding 40 μL of diluted MMPs (final concentrations 0.32–1.05 μg/mL) to wells of 96-well plate that contained 20 μL of MMPI and appropriate amount of the fluorogenic substrate stock solutions and HEPES buffer to make the total volume of 200 μL (total DMSO concentration 4%).

In fluorescence measurements, excitation at 328 nm was used, and the time course of emission at 393 nm was monitored for 30–60 min. Fluorescence data were converted to concentrations, which were fitted using equations describing fast or slow inhibition.[14] The inhibition constant K_i in micromolar concentration are presented in Table 1.

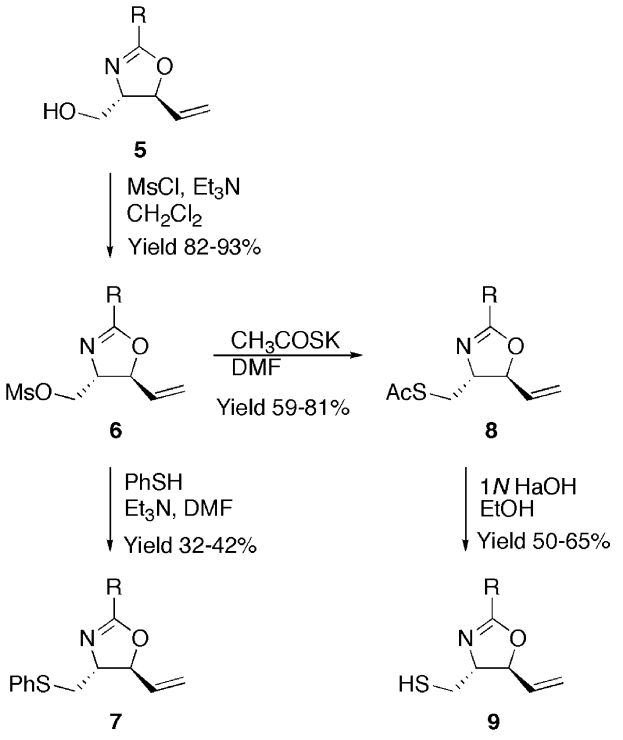
The results of the biological screening of minimum oxazoline structures for MMP antagonist activity are summarized in Table 1. Variation of the 2-oxazoline substituent had a significant effect on the activity. Alkyl groups (entries 1–4) showed no inhibitory activity for MMPs. On the other hand, substituents possessing aromatic groups fared much better. While the simple phenyl substituted compound displayed only slight activity

Table 1. Inhibition of MMP's with oxazolines 5

Entry	R	K_i (μM)			
		MMP 1	MMP 2	MMP 9	MMP 12
1		NA[a]	NA	NA[a]	NA
2				>2000[a]	
3				>2000[a]	
4		NA	NA	NA	NA
5		NA[a]	>2000	>400[a]	>2000
6		>500	NA	6[a,b]	NA
7		NA[a]	NA	NA[a]	
8				40[a,b]	
9		NA[a]	NA	68[a]	NA
10		NA[a]		24[a]	
11		NA[a]	NA	NA[a]	NA
12		>300[a]		33[a]	NA
13		NA[a]	>600	9[a]	>300
14		F[a]	>100	F[a]	>100
15		NA[a]	NA	NA[a]	NA
16				32[a,b]	
17			NA	NA	NA
18		>300	NA	22[a]	NA
19		32	153	8	49
20		46	NA	65	NA

NA = no activity up to solubility limit. F = fluorescence of the compound interfered with the assay. No activity was observed upto the concentration that fluorescence could be compensated for.
[a] Measured with full length enzyme.
[b] Slow inhibition.

(entry 5), the benzyl group (entry 6) showed low micromolar inhibition of MMP-9. This compound was also very selective for MMP-9 over MMP's 1, 2, and 12. As shown in entry 13, the bromophenyl substituent had similar selectivity and activity. The SAR summarized in Table 1 suggests that the 2-oxazoline group

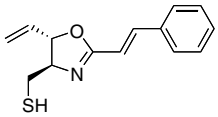
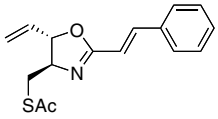
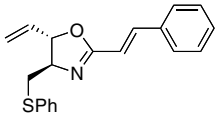
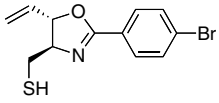
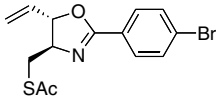
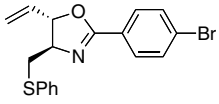
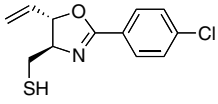
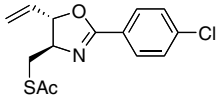
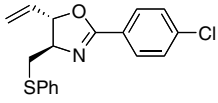
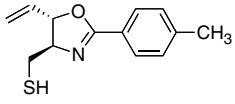
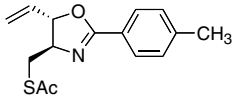
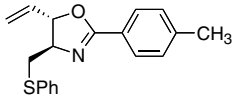
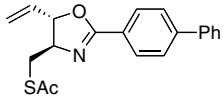
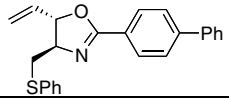


Scheme 2.

may be binding in the hydrophobic S1′ pocket of MMP-9, although a binding mode with the substituent lying in the S1 cleft cannot be ruled out.

Our initial hypothesis for oxazoline-based MMPIs made the assumption that the functional group on the 5-oxazoline substituent would be involved in chelation to the active site zinc. Thus, we envisioned improved binding if the alcohol were replaced with a group that would form stronger bonds to the zinc. As thiols are known to be good zinc binding groups for MMPs, the alcohol was replaced with sulfur containing functional groups as outlined in Scheme 2. Activation with methanesulfonyl chloride afforded **6** in good yields. The phenylsulfides **7** were generated directly from the mesylate by substitution with phenylthiol. Substitution with potassium thioacetate afforded **8** and hydrolysis furnished the oxazoline thiols **9**. The inhibitory activity of sulfur containing oxazolines **7**, **8**, and **9** are summarized in Table 2. Surprisingly, these sulfur derivatives, although still displaying some inhibition, were less active than the original alcohols. For example, as shown in entry 4, the 2-bromophenyl-substituted oxazoline thiol was an order of magnitude less active than the corresponding alcohol (Table 1, entry 13). Further, the substitution of the sulfur made no difference in the level of activity (compare H, Ac, and Ph; entries 4, 5, and 6). This intimates that the 5-thio substituent is not effectively chelating the zinc. If this is so, the binding strength of the ZBG may be less important than having an appropriate P1′ substituent for MMP inhibition. This trend was observed for all of the oxazolines tested. Interestingly, little change in the activity was observed even though large changes in sterics (SH vs SPh) and electronics (SAc) were introduced. This suggests that the 5-oxazoline

Table 2. Inhibition of MMP's with oxazolines **7**, **8**, and **9**

Entry	Oxazoline	K_i (μM)			
		MMP 1	MMP 2	MMP 9	MMP 12
1		92	NA	94	NA
2		NA	>100	>100	>300
3		>100	NA	>100	NA
4		>200		>100	
5		>200	NA	88	NA
6		30[a]		94	
7		>100	NA	>100	NA
8		>100	NA		
9		NA		>200	NA
10		>100	88	>100	>300
11		NA	NA	>100	NA
12		64	NA		NA
13		>400	NA	81	NA
14		NA	NA	>100	

NA = no activity up to solubility limit.
[a] Slow inhibition.

substituent is most likely pointed out of the active site into the solvent.

In conclusion, we have established a new ZBG for inhibition of MMPs. Compounds with only a minimal number binding groups (potential zinc and S1′ binding) demonstrated low micromolar activity against MMP-9. These compounds were also selective for inhibition of gelatinase B over other MMPs. The SAR data is consistent with the supposition that the oxazoline 2-substituent is positioning itself in the S1′ pocket of the enzyme as these substituents would not be expected to bind well into the S1′ pocket of MMP-1 owing to its shallow depth. The oxazoline 5-substituent appears not to chelate with the zinc and the size and electronic nature of this substituent could be varied without significant change in activity.

Acknowledgements

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References and notes

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