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A New Role for Old Ligands: Discerning Chelators for Zinc Metalloproteinases

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This study describes the use of seven nitrogenous ligands that are potent inhibitors of both matrix metalloproteinases (MMPs) and anthrax lethal factor (LF). These ligands are proposed to enhance the affinity of metalloproteinase inhibitors, and improve selectivity for metalloproteins that possess Zn^{2+}, as opposed to other metal ions, in their active sites. For example, MMPs are a well-studied class of Zn^{2+}-dependent hydrolytic enzymes that are involved in the breakdown of the extracellular matrix.[1] MMP activity has been associated with a number of pathologies including, but not limited to, periodontal disease, inflammatory disease, heart disease, and cancer.[1,2] Compounds that can inhibit these enzymes with high potency and selectivity may serve as valuable tools for understanding the role of MMPs in these diseases and as potential chemotherapeutics.

The majority of current matrix metalloproteinase inhibitors (MMPi) utilize a two-component design strategy;[1,3] a peptidomimetic backbone interacts through noncovalent forces with the protein surface, while a zinc-binding group (ZBG) makes coordinate covalent bonds to the active site Zn^{2+} ion. The overwhelming majority of MMPi utilize a hydroxamic acid moiety as the ZBG;[1,4] however, hydroxamates are indiscriminate metal chelators, known to bind a variety of metal ions, including Fe^{3+}.[5] Recently, as part of a program to develop novel MMPi,[6] we described 11 chelators with improved potency for use in full-length inhibitors.[7] These compounds show substantially improved MMP inhibition, but like hydroxamic acids they are avid chelators of a variety of transition metal ions and thereby are not likely to improve the selectivity of MMPi for Zn^{2+}-metalloproteins.[8,9]

In an effort to identify potential ZBGs with both improved affinity and selectivity for the active site Zn^{2+} ion, compounds **1**−**7** were investigated (Figure 1). These compounds consist of a variety of nitrogen and mixed nitrogen/oxygen donor-atom chelators including pyridine- and aza-macrocycle-based ligands. These compounds were chosen for their known binding affinity and preference for late transition metals (e.g., Cu^{2+}, Zn^{2+}) over earlier transition metal and group I/II metal ions.[10−13] For example, compound **4** (dipyridylamine, DPA) binds Zn^{2+} with substantially higher affinity than Fe^{2+} or Mn^{2+} (log β_1 = 7.63, 6.15, and 3.52 for Zn^{2+}, Fe^{2+}, and Mn^{2+}, respectively).[10] Indeed, derivatives of **4** and **6** (1,4,7,10-tetraazacyclododecane, cyclen) have been used as recognition groups for Zn^{2+}-selective fluorescent sensors.[14,15] Furthermore, the hexadentate analogue of **4**, tetra(pyridyl)ethylenediamine (TPEN), is a common reagent for the efficient sequestration of Zn^{2+} from cellular media.[16,17]

To evaluate the hypothesis that these compounds would serve as good ligands for Zn^{2+}-metalloproteinase inhibitors, their efficacy against MMP-3 was evaluated using a fluorescent substrate assay (Table 1).[6,7] Consistent with the rationale described, all of the compounds tested proved to be comparable or better inhibitors of MMP-3 than acetohydroxamic acid (**AHA**); **AHA** represents the most commonly used ligand in MMPi.[1,4] Of particular interest,

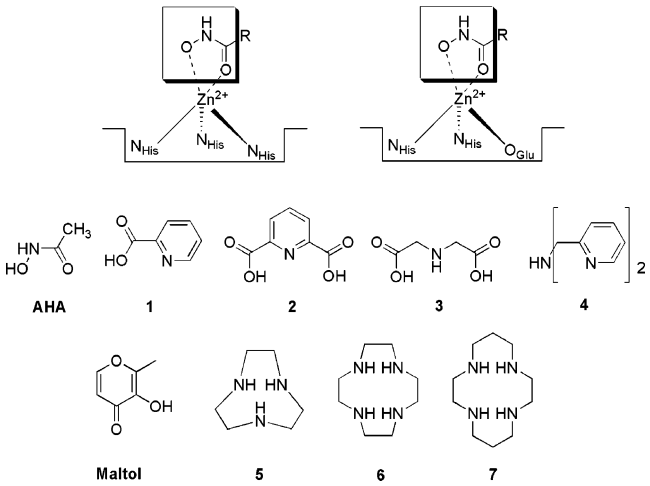

Figure 1. Scheme of MMP (top left) and LF (top right) hydroxamate-inhibited active sites. Nitrogen-donor atom ZBGs (**1**−**7**) and other ligands (**AHA**, maltol) examined in this report (bottom).

Table 1. IC_{50} Values (in μM) for ZBGs against MMP-3 and Anthrax LF Measured Using Fluorescence-Based Assays

ZBG	MMP-3 IC_{50}[a]	LF IC_{50}[a]	potency v. AHA[b]
AHA	25100 (±4000)	11400 (±1000)	n/a
1	181 (±10)	3500 (±300)	139-fold
2	1350 (±160)	1275 (±7)	19-fold
3	6100 (±200)	2690 (±80)	4-fold
4	154 (±13)	5700 (±700)	163-fold
5	136 (±9)	370 (±40)	185-fold
6	180 (±30)	930 (±30)	139-fold
7	1330 (±140)	2920 (±80)	19-fold

[a] Obtained from at least three independent experiments; [b] Based on IC_{50} value from MMP-3 fluorescence assay.

compounds **1**, **4**, **5**, and **6** showed substantially improved potency over **AHA** (139-, 163-, 185-, and 139-fold, respectively). The ligands listed in Figure 1 also showed improved efficacy against MMP-1 (Table S1), indicating that the mechanism of inhibition by compounds **1**−**7** is likely due to binding of the active site Zn^{2+} ion, as opposed to other, unanticipated interactions with the protein active site.

To determine the probable mode of binding of compounds **1**, **4**, and **5** to the active site Zn^{2+} ion in MMPs, modeling studies were performed.[18−20] Compound **1**, picolinic acid, was readily crystallized as the model complex [($Tp^{Ph,Me}$)Zn(**1**)] ($Tp^{Ph,Me}$ = hydrotris(3,5-phenylmethylpyrazolyl)borate). The complex (Figure 2) shows that **1** binds to the active site model in a bidentate fashion through the pyridyl nitrogen atom and the deprotonated carboxylate oxygen atom (Table S2). To elucidate the mode of binding for **4** and **5**, several attempts were made to prepare [($Tp^{Ph,Me}$)Zn(**4**)] and [($Tp^{Ph,Me}$)Zn(**5**)] using a variety of reaction conditions. In all cases the reactions produced $[Zn(\mathbf{L})_2]^{2+}$ (L = **4** or **5**), indicating that these

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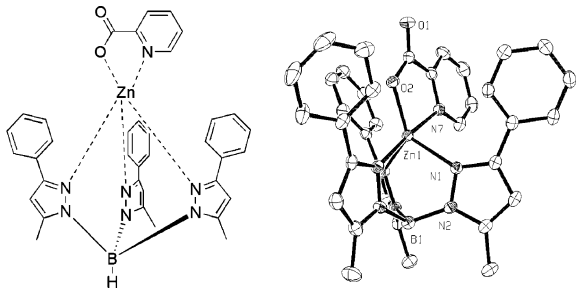

Figure 2. Chemical (left) and structural (right, 50% probability ellipsoids) diagram of [(Tp^{Ph,Me})Zn(**1**)] showing bidentate chelation of the ligand to the zinc(II) ion. Hydrogen atoms and solvent have been omitted for clarity.

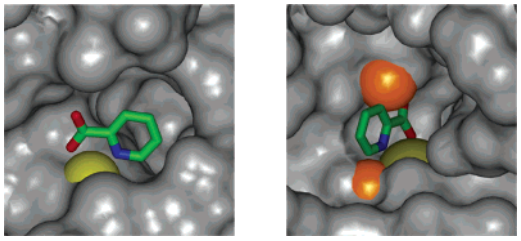

Figure 3. Best fit orientation of **1** superpositioned into the MMP active site (left) and the LF active site (right). The Zn^{2+} ions are shown as gold spheres. Orange coloring (right) shows areas of the protein surface where the ZBG is clashing with the active site residues.

ligands are sufficiently strong chelators for Zn^{2+} that they strip the metal ion from [(Tp^{Ph,Me})Zn(OH)].

To demonstrate that these ligands may be useful for a variety of Zn^{2+} metalloenzymes, compounds **1**−**7** were tested for inhibition against anthrax LF. LF is a Zn^{2+}-dependent endopeptidase that is known to be an important virulence factor for *Bacillus anthracis*.[21] Several compounds currently being investigated as LF inhibitors (LFi) are hydroxamate-based; indeed, some are previously studied MMPi.[22] Again, using a fluorescence-based assay, **1**−**7** demonstrate greater inhibition of anthrax lethal factor than **AHA** (Table 1). Notably, the compounds are generally not as potent against LF as against MMP-3. This difference may be due in part to the rather closed active site of LF, relative to those of the MMPs. This is illustrated by taking the structural parameters (the Zn^{2+} coordination environment and ZBG) from [(Tp^{Ph,Me})Zn(**1**)] and superimposing them[6] into the crystallographically determined MMP-3 (1G4K) and LF (1PWQ) active sites.[22,23] Steric interactions between the ZBG and the protein active site were assessed with the program InsightII (Figure S1). As shown in Figure 3, **1** clearly clashes with the LF protein surface (shown in orange), but has no such steric conflicts in the MMP-3 active site.

Having demonstrated that compounds **1**−**7** could inhibit two distinct classes of Zn^{2+} metalloenzymes, we sought some preliminary determination of whether these ZBGs possessed the desired selectivity. The potential selectivity of these ZBGs was examined by studying the inhibition of the non-heme iron enzyme soybean lipoxygenase (sbLO). sbLO is an Fe^{3+} metalloenzyme that catalyzes the hydroperoxidation of lipids containing a *cis,cis*-1,4-pentadiene motif.[24] sbLO activity, using linoleic acid as a substrate,[25] was monitored in the presence of **AHA**, **1**, **7**, and maltol. **AHA** and maltol (300 μM) were found to significantly inhibit product formation (∼99% and 70%, respectively) relative to control (no inhibitor). In contrast, compounds **1** and **7** had essentially no effect on lipoxygenase activity (2.3% and <1% inhibition, respectively). These results suggest that nitrogen-based ZBGs will show improved specificity for Zn^{2+} metalloproteins over enzymes dependent on other metal ions, such as iron.

In summary, several nitrogen-containing ligands have been identified as useful for the inhibition of Zn^{2+}-metalloproteinases. All of the compounds studied were more potent for MMP-1, MMP-3, and LF than **AHA**. ZBGs **1**−**7** may also show improved selectivity for Zn^{2+}-metalloproteins over other metalloproteins; this has been preliminarily demonstrated by using sbLO and compounds **1** and **7**. The realization of improved zinc-selective inhibition may alleviate some of the side effects that have plagued earlier metalloproteinase inhibitors. The synthesis of full-length inhibitors based on these new ZBGs is currently underway.

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Supporting Information Available: Experimental details for all syntheses, MMP assays, anthrax lethal factor assays, and lipoxygenase assays; X-ray crystallographic files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC reference number 289584 contains the supplementary crystallographic data for this paper. These data can be downloaded free of charge via www.ccdc.cam.ac.uk/conts/retrieving.hmtl (or obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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