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COMMUNICATIONS

Published on Web 06/17/2004

New Beginnings for Matrix Metalloproteinase Inhibitors: Identification of High-Affinity Zinc-Binding Groups

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This report describes 11 new chelators that are potent inhibitors of matrix metalloproteinases (MMPs). MMPs are an important class of zinc-dependent metalloenzymes involved in the hydrolytic breakdown of connective tissue.[1] MMPs have a conserved active site motif, where a tris(histidine)-bound zinc(II) ion acts as the catalytic site for substrate hydrolysis. This group of proteins has important physiological roles in growth, wound healing, and reproduction. MMP activity also has been associated with human pathologies including cancer, arthritis, and heart disease.[1,2] The correlation of MMP activity with these diseases has prompted many efforts to design MMP-selective inhibitors. MMP inhibitors (MPIs) generally follow a two-component design strategy (Figure 1): a peptidomimetic backbone that interacts with "subsites" surrounding the active site is coupled to a metal chelator that binds to the catalytic zinc(II) ion.[1]

Most MPI research has focused on developing the peptidomimetic backbone of these compounds to obtain high potency and selectivity against various MMPs.[1,3] For these inhibitors, the hydroxamic acid functionality has become the zinc-binding group (ZBG) of choice (Figure 1).[1] Although hydroxamic acids have produced potent inhibitors, no hydroxamate-based MPI has successfully completed clinical trials. Indeed, the only medically approved MPI is a non-hydroxamate compound used in the treatment of periodontal disease.[4] The inability of hydroxamates to produce clinically viable compounds has been attributed to low oral availability, poor in vivo stability, and undesirable side effects associated with these compounds.[2] In an attempt to find alternatives to the hydroxamic acid group, 11 compounds were identified as ligands for use in MPIs (Figure 1). The compounds were selected on the basis of some similarities to hydroxamates, such as their ability to form monoanionic five-member chelates. The compounds were also identified due to potential differences, including (a) better hydrolytic stability originating from cyclic structures, (b) potentially improved biological tolerance (e.g., compound **5** is a food additive, Maltol), and (c) proposed increased affinity for the MMP zinc(II) ion due to ligand rigidity[5] and zinc thiophilicity. In light of these criteria, the ligands selected consisted of hydroxypyridinones (**1**−**3**, **6**), hydroxypyridinethiones (**7**−**9**), pyrones (**4**, **5**), and thiopyrones (**10**, **11**).[6−8]

The ability of these compounds to inhibit the hydrolytic activity of MMP-3 (stromelysin) was measured using an established assay that utilizes a fluorescent peptide substrate.[9] The compounds under investigation represent only the ZBG portion of an MPI and, therefore, do not contain the essential peptidomimetic backbone (Figure 1) that confers additional potency and selectivity to complete inhibitors. To establish a benchmark against which to gauge these chelators, acetohydroxamic acid (AHA, Figure 1) was also evaluated as the representative chelator for the majority of current MPIs.[10,11] The IC_{50} values obtained from the kinetic assays are listed in Table 1.

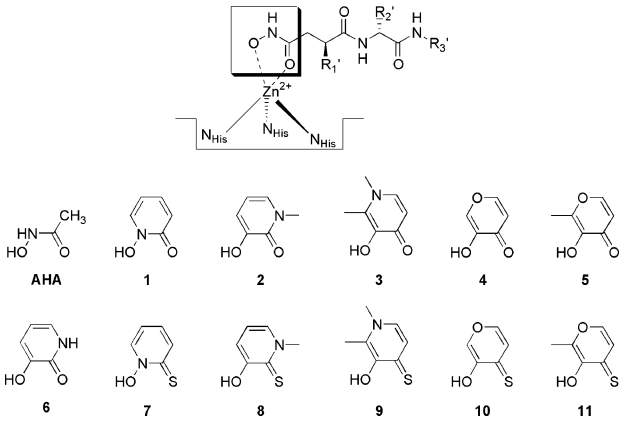

Figure 1. General construct for matrix metalloproteinase inhibitors (MPIs, top with hydroxamate ZBG in box) and new zinc-binding groups (bottom) examined in this study.

Table 1. IC_{50} Values for ZBGs against MMP-3 Measured Using Either a Fluorescence- or Colorimetric-Based Assay

	IC_{50} (μM)		
ZBG	fluorescence[a,b]	colorimetric[a]	potency vs AHA[c]
AHA	25100 (±4000)	−	n/a
1	1600 (±100)	1500 (±10)	16-fold
2	5100 (±200)	−	4.9-fold
4	7200 (±1200)	8300 (±900)	3.5-fold
5	5700 (±100)	16000 (±2000)	4.4-fold
6	5700 (±200)	5000 (±1000)	4.4-fold
7	35 (±3)	20 (±4)	717-fold
8	362 (±3)	−	69-fold
9	137 (±20)	−	183-fold
10	118 (±40)	−	213-fold
11	210 (±20)	−	120-fold

[a] Obtained from at least three independent experiments. [b] Corrected for competitive absorption (see Supporting Information). [c] Based on IC_{50} value from fluorescence assay

The data obtained indicate that the compounds listed in Figure 1 are more effective inhibitors than AHA (**3** could not be evaluated due to low solubility). In addition, O,S mixed donor ligands (**7**−**11**) are all approximately 2 orders of magnitude more potent than AHA, with **7** showing low micromolar activity. To confirm the values obtained from fluorescence-based assays, additional experiments were performed on some ZBGs using a widely used colorimetric-based assay.[12] The IC_{50} values from the colorimetric assays (Table 1) are in good agreement with those obtained by fluorescence measurements, with the exception of compound **5** that shows approximately 3-fold lower potency when determined colorimetrically.

To better characterize the interaction of these ligands with the MMP zinc(II) ion, studies with tris(pyrazolyl)borate model complexes were also performed.[13] Compounds **1**−**3**, **5**−**7**, and AHA had been previously shown to coordinate in a bidentate fashion in

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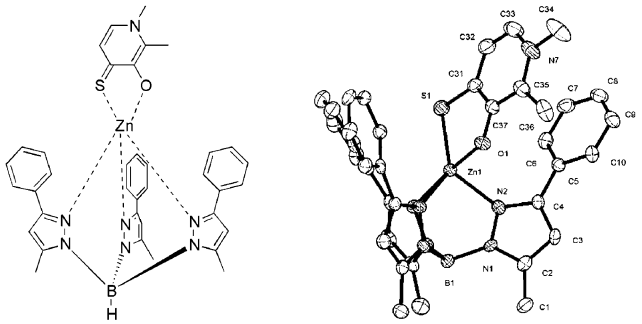

Figure 2. Chemical (left) and structural (right, 50% probability ellipsoids) diagram of [(Tp^{Ph,Me})Zn(**9**)] showing chelation of the O,S ligand to the zinc(II) ion. Hydrogen atoms have been omitted for clarity.

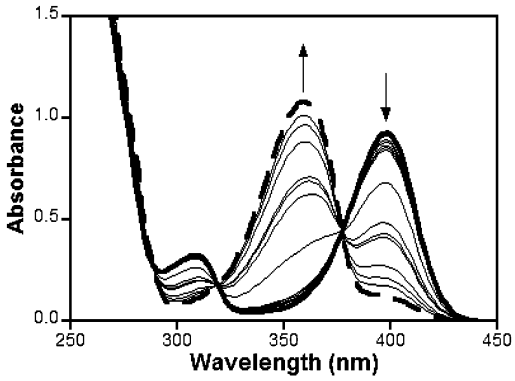

Figure 3. Electronic spectra of [(Tp^{Ph,Me})Zn(**11**)] (60 μM) with increasing amounts of AHA in 1:250 (v/v) DMF:MeOH. The heavy lines are the initial (solid) and final (dashed, free **11**) spectra; arrows indicate changes upon addition of AHA.

the model complex [(Tp^{Ph,Me})Zn(ZBG)] (Tp^{Ph,Me} = hydrotris(3,5-phenylmethylpyrazolyl)borate).[14−16] To confirm that the new O,S ligands presented here also bound in a similar manner, the complexes [(Tp^{Ph,Me})Zn(**8**)], [(Tp^{Ph,Me})Zn(**9**)], and [(Tp^{Ph,Me})Zn(**11**)] were synthesized and structurally characterized; all three showed bidentate chelation to the zinc(II) ion. Figure 2 shows the structure of [(Tp^{Ph,Me})Zn(**9**)] as a representative example of these compounds.

The poor aqueous solubility of compound **3** precluded evaluation of this ligand in MMP assays. This prompted us to use the aforementioned complexes as thermodynamic models of MMP inhibition. [(Tp^{Ph,Me})Zn(ZBG)] complexes in organic solvents were titrated with increasing amounts of AHA in order to obtain equilibrium constants that represent relative binding constants between the bound ZBG and AHA (see Supporting Information). A representative titration between [(Tp^{Ph,Me})Zn(**11**)] and AHA is shown in Figure 3. The results of several such experiments are summarized in Table 2. Millimolar O,O inhibitors showed a ~2.4-fold increase over AHA, while more potent O,S ligands gave binding constants with ~14-fold greater affinity relative to AHA. Although the equilibrium constants obtained do not provide a clear rank order of inhibitor efficacy, they are sufficient to distinguish ligands of high versus moderate potency. On the basis of these results, compound **3** is likely to be a modest inhibitor with an IC_{50} in the low millimolar range. The correlation of IC_{50} values with the relative binding constants supports our contention that the inhibition of MMP-3 is due to direct chelation of these ligands to the active site zinc(II) ion.

Table 2. Relative Binding Affinities of Several Novel ZBGs for a Tris(pyrazolyl)Borate Model Complex

[(Tp^{Ph,Me})Zn(ZBG)]	K_{app}[a]	affinity vs AHA
2	0.32 (±0.01)	3.1-fold
3	0.54 (±0.06)	1.9-fold
5	0.46 (±0.16)	2.2-fold
8	0.072 (±0.010)	14-fold
9	0.078 (±0.006)	13-fold
11	0.067 (±0.009)	15-fold

[a] Obtained from at least two independent experiments.

Several new chelators have been identified for use in MPIs. All of the compounds studied were more potent inhibitors of MMP-3 than AHA, which was used as a benchmark of the commonly used hydroxamate ZBG found in many inhibitors presently under investigation. Mixed O,S chelators were found to be particularly potent, with IC_{50} values down to the low micromolar range. The binding mode of these ligands was found to be bidentate on the basis of the structure of model complexes. In addition, these complexes were found to serve as useful thermodynamic models for broadly ranking the efficacy of different chelators. The synthesis of complete MPIs based on the chelators discussed here is presently underway.

Acknowledgment. We thank Prof. Arnold L. Rheingold and Dr. Lev N. Zakharov (U.C.S.D.) for help with the X-ray structure determinations, and Prof. Francisco Villarreal (U.C.S.D.) for use of his plate readers. This work was supported by the University of California, San Diego, a Chris and Warren Hellman Faculty Scholar award, and an award from the American Heart Association. J.A.L. was supported in part by a GAANN fellowship (GM-602020-03) and an ARCS award.

Supporting Information Available: Experimental details for all syntheses, MMP assays, and titration experiments; X-ray crystallographic files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0485513