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Metalloform-Selective Inhibitors of *Escherichia coli* Methionine Aminopeptidase and X-ray Structure of a Mn(II)-Form Enzyme Complexed with an Inhibitor

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Methionine aminopeptidase (MetAP) enzymes catalyze the nonprocessive removal of the N-terminal methionine from newly synthesized proteins in all types of cells.[1] Prokaryotes have only one MetAP (type I or II), while eukaryotes have two (types I and II) which differ by a small peptide insertion. Inhibitors of MetAPs are of considerable interest as potential antibacterial, antifungal and antiangiogenesis (anticancer) agents.[2−4] All MetAPs require a divalent metal ion such as Mn(II), Fe(II), Co(II), Ni(II), or Zn(II) for activity, but it is not certain which of these ions is most important in vivo.[5−7] Relatively few nonpeptidic MetAP inhibitors are known, and they either show low selectivity among various metalloforms in vitro[8,9] or have not been tested on metalloforms other than the Co(II)-form. New metalloform-selective MetAP inhibitors could be valuable for defining which metals are physiologically important for MetAP activation and could serve as leads for development of new therapeutic agents. We have screened a library of 43 736 small drug-like molecules against *Escherivchia coli* MetAP (*Ec*MetAP1)[10] and report here the discovery and characterization of two groups of potent and highly metalloform-selective inhibitors of the Co-(II)-form, and of the Mn(II)-form, of this enzyme.

High throughput screening on Co(II)-*Ec*MetAP1 generated 786 hits (62% inhibition cutoff) for further characterization by determining IC_{50} values for each of the hits. Similarly, screening on Mn(II)-*Ec*MetAP1 produced 512 hits (54% inhibition cutoff). Finally, the top 64 inhibitors of each enzyme were evaluated against *both* metalloforms for potency and selectivity.

Upon examining the structures of the most potent compounds, two classes of inhibitor structures stood out immediately. Each had a unique structural scaffold and exceptional selectivity for one of the metalloforms (Chart 1 and Table 1). Inhibitors **1**−**3** containing a thiazol-2-yl-oxalamide moiety were Co(II)-form selective, while compounds **4**−**6** comprising 5-phenylfuran-2-carboxylic acid derivatives were Mn(II)-form selective. The Co(II)-selective thiazol-2-yl-oxalamides have structural similarity to some (thiazol-2-yl)picolinamides we reported previously which also show potent inhibitory activity against the Co(II) enzyme.[11] However, no MetAP inhibitors with selectivity for other metalloforms have been reported.[12]

The superb selectivity of compounds **1**−**6** among Co(II)- and Mn(II)-activated *Ec*MetAP1 prompted us to test them against other metalloforms of *Ec*MetAP1. Consistent with our earlier observations,[13] the rank order of inhibitory activity against the Ni(II)-form generally paralleled that against the Co(II)-form. Surprisingly, none of these compounds showed significant activity against the Fe(II) enzyme.

Chart 1. MetAP Inhibitors Identified from High Throughput Screening

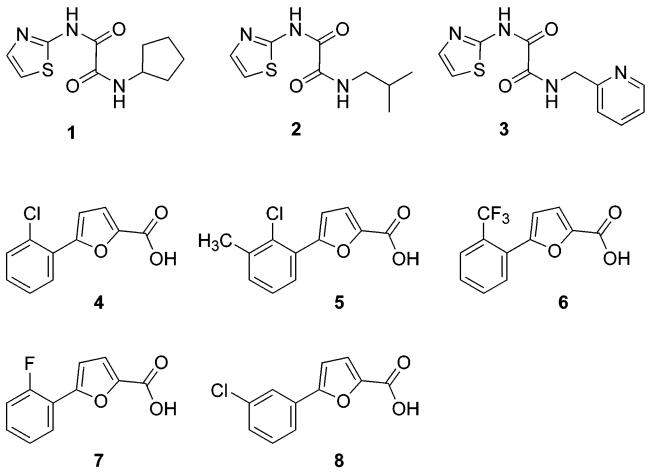

Table 1. Inhibition of Metalloforms of *E. coli* MetAP

	IC_{50} (μM)[a]			
inhibitor	Co(II)	Mn(II)	Ni(II)	Fe(II)
1	0.067	53	1.0	46
2	0.28	108	3.4	118
3	0.073	54	2.0	65
4	154	0.24	>200	182
5	69	0.96	>200	>200
6	73	0.063	>200	195
7	198	14	>200	>200
8	193	19	>200	>200

[a] Assays were carried out by monitoring the hydrolysis of fluorogenic Met-AMC kinetically as described in ref 13. The assay mixture contained apo-*Ec*MetAP1 and one of the metal ions [Co(II), 100 μM; Mn(II), 100 μM; Ni(II), 10 μM; Fe(II), 6 μM]. Relative standard deviations are <25% in all values.

Mn(II)-loaded bacterial MetAPs are catalytically competent,[14] and Mn(II) has been suggested to be the physiologically relevant metal for human type II MetAP.[9] Compounds **4**−**6** are thus the first known inhibitors with potency and selectivity for the Mn(II)-form of *Ec*MetAP1. Among the 5-phenylfuran-2-carboxylic acids evaluated, a distinct requirement for potent inhibitory activity is the presence of an ortho substituent larger than hydrogen or fluorine on the phenyl moiety. The weak activity of compounds **7** and **8** suggests a possible requirement for a noncoplanar conformation of the two aromatic rings for effective binding to the enzyme. A free carboxylate group is also essential since amide or ester derivatives are not active (data not shown).

Understanding how these inhibitors achieve their metalloform selectivity is crucial to improving these inhibitors and to discovering

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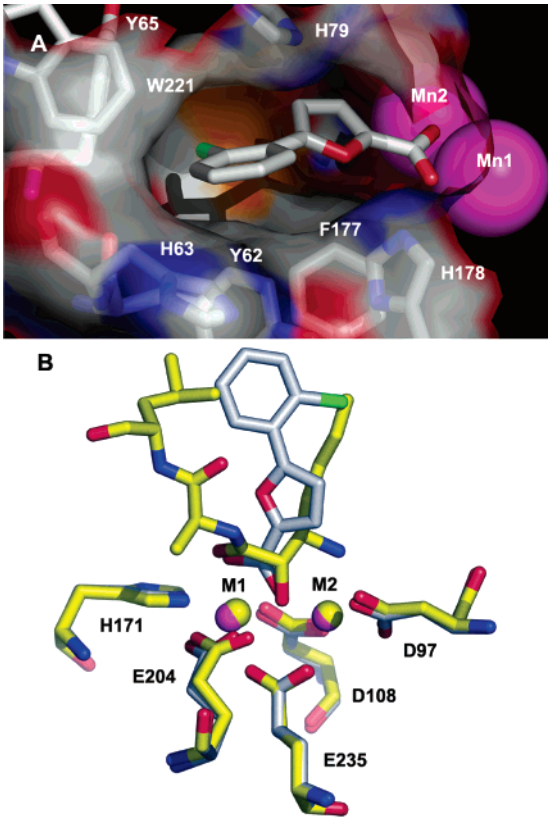

Figure 1. X-ray structure of a di-Mn(II) form of *E. coli* MetAP complexed with the Mn(II)-form selective inhibitor **4**. (A) Close-up view of the inhibitor binding site with Mn(II) ions shown as magenta spheres and the inhibitor and surrounding residues shown as sticks (carbon gray, oxygen red, nitrogen blue, and chlorine green). (B) Metal site overlaid with a previously published di-Co(II)-form (PDB code 3MAT). Metal ligands and the inhibitor in the di-Co(II)-form are colored yellow for carbons. Metal ions are labeled as M1 and M2 and colored magenta for Mn(II) and yellow for Co(II).

inhibitors of other metalloforms. The available X-ray structures of MetAPs are all of the di-Co(II)-form.[5,8] Because the physiologically relevant metal may not be Co(II),[6,7,9] the structures of other metalloforms of MetAPs are critically needed. Thus, we crystallized *Ec*MetAP1 in the presence of a 5-fold excess of Mn(II) and a 10-fold excess of the inhibitor **4** and solved the structure of the resulting complex at 1.5 Å resolution (Figure 1).

Two Mn(II) ions and the inhibitor can be seen clearly at the active site. The inhibitor interacts with both Mn(II) ions through the two oxygen atoms of its free carboxylate group, consistent with the requirement of the free carboxylate for activity. The two oxygen atoms form a bidentate coordination with one of the Mn(II) ions (Mn1), and one of the oxygen atoms also coordinates with the second Mn(II) ion (Mn2). The major portion of the inhibitor resides in the S1 substrate binding pocket and interacts with side chains of Y62, H63, Y65, H79, F177, H178, and W221. As predicted from our initial structure−function analysis of this class of inhibitors mentioned above, the phenyl ring and the furan ring are noncoplanar (dihedral angle = 41.5°) due to the presence of the o-Cl substituent. The phenyl ring is inserted between H79 and Y62, and it fits the cleft tightly in this noncoplanar conformation.[15]

Interestingly, the metal-binding donor atoms and the nearby side chains of this di-Mn(II)-form align very well with those of the previously published di-Co(II)-form,[16] indicating no major changes in geometry upon the replacement of Co(II) by Mn(II). Despite the *apparent* structural similarity of the two metalloforms, compound **4** is over 640-fold more potent toward the Mn(II)-form, while compound **1** is 790-fold more selective for the Co(II)-form of *Ec*MetAP1. Additional studies will be required to understand fully the basis for this selectivity. However, the selectivity pattern in Table 1 is consistent with the known preference of Mn(II) for hard (oxygen) donors, and of Co(II) for softer (nitrogen or sulfur) donors.[17]

In conclusion, we have demonstrated the exceptional power of high throughput screening for discovering potent and selective inhibitors for enzymes such as for MetAPs when no leads are available. Inhibitors discovered this way are providing valuable new tools for mechanistic studies, as well as novel nonpeptidic leads for structure-based drug discovery and development.

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Supporting Information Available: Spectroscopic details (^{1}H and ^{13}C NMR and MS) for the discussed compounds and crystallographic details for the enzyme/inhibitor complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The chemical library of 43 736 small organic molecules was purchased from ChemBridge (San Diego, CA) and selected for structural diversity and drug-like properties. Reactive, unstable, and potentially toxic compounds were eliminated, and all compounds are solids with molecular weights between 150 and 480 and CLogP below 5. A continuous assay with fluorogenic Met-AMC as the substrate was used for the screening, and hydrolysis was monitored by recording fluorescence (ex 360 nm/em 460 nm) every 3 min for 30 min. The 43 736 compounds were screened at 6.67 μg/mL for both Co(II)-*Ec*MetAP1 and Mn(II)-*Ec*MetAP1.
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(15) A coplanar conformation is not favorable because the phenyl ring of the inhibitor will likely collide with Y62 and H63 (3.2 Å)
(16) Alignment of main chain α carbons of this di-Mn(II)-form with a di-Co(II)-form (3MAT) showed a rmsd of 0.422 Å.
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