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Peptidyl hydroxamic acids as methionine aminopeptidase inhibitors

Xubo Hu, Jinge Zhu, Sumant Srivathsan and Dehua Pei*

Department of Chemistry and Ohio State Biochemistry Program, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

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Abstract—A new class of methionine aminopeptidase (MetAP) inhibitors, which contain an internal hydroxamate (*N*-acyl-*N*-alkylhydroxylamine) core as the metal-chelating group, has been designed, synthesized, and tested. The compounds exhibited reversible, competitive inhibition against *Escherichia coli* MetAP as well as human MetAP-1 and MetAP-2. The most potent inhibitor had a K_i value of 2.5 μM and >20-fold selectivity toward *E. coli* MAP.

Protein synthesis universally starts with a methionine or, in the case of bacteria and organelles, an *N*-formylmethionine. Following translational initiation, the N-terminal methionine is frequently removed from nascent polypeptides by methionine aminopeptidase (MetAP).[1] This N-terminal modification has a number of biological functions including the regulation of protein stability through the *N*-end rule pathway and exposure of the penultimate residue for catalytic functions or post-translational modification. MetAP is an essential enzyme in all organisms from bacteria to man.[2–4] Bacteria typically have a single MetAP enzyme, whereas eukaryotes contain two different MetAP enzymes, MetAP-1 and MetAP-2.[2,5] Recently, it has been shown that the antiangiogenic activity of fumagillin and ovalicin is due to its inhibition of the peptidase activity of MetAP-2.[6,7] This finding has led to intense efforts in developing MetAP-2 inhibitors as novel antitumor drugs. There is also a revived interest in developing MetAP inhibitors as new antibacterial agents.

MetAPs are structurally similar metalloenzymes that contain two divalent metal ions per catalytic unit.[8–10] The nature of the metal ion under physiological conditions is still under debate. Historically, both MetAPs were thought to be Co^{2+}-dependent enzymes.[5,11–13] Recent experiments with yeast MetAP-1 have suggested that the in vivo metal may be Zn^{2+},[14] whereas Mn^{2+} has been proposed as the native metal for human MetAP-2.[15] Reconstituted MetAP containing either Co^{2+}, Zn^{2+}, or Fe^{2+} is catalytically active in vitro.[11–16] Since the metal ions are an integral part of the catalytic machinery, the design of metal chelators has been a popular approach to developing MetAP inhibitors. The first class of MetAP inhibitors contain an α-hydroxy-β-aminoacyl (bestatin) group as the metal ligand.[15,17,18] Some of these inhibitors show excellent potency against human MetAP-2, but are generally of only weak to moderate activity (K_i in the μM range) against bacterial MetAP (MetAP-1). A second class contains a triazole group as the metal ligand.[15,19] Interestingly, the potency of a triazole inhibitor has been shown to be greatly dependent on the nature of the metal cofactor.[15] In this work, we report a third class of MetAP inhibitors, which utilize an internal hydroxamic acid to chelate the metal ions.

The crystal structure of *Escherichia coli* MetAP bound with a bestatin inhibitor reveals that the bestatin moiety is engaged in four interactions with the metal center: (i) ligation of the N-terminal nitrogen to Co2, (ii, iii) bridging coordination of the α-hydroxyl group to both metals, and (iv) terminal ligation of Co1 by the keto oxygen of the pseudo-peptide linkage (Scheme 1, structure **I**).[17] We envisioned that an *N*-(α-aminoacyl)hydroxylamine should be capable of engaging in similar interactions with the two-metal center (Scheme 1,

* Corresponding author. Tel.: +1-614-688-4068; fax: +1-614-292-1532; e-mail: pei@chemistry.ohio-state.edu

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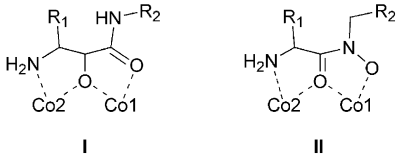
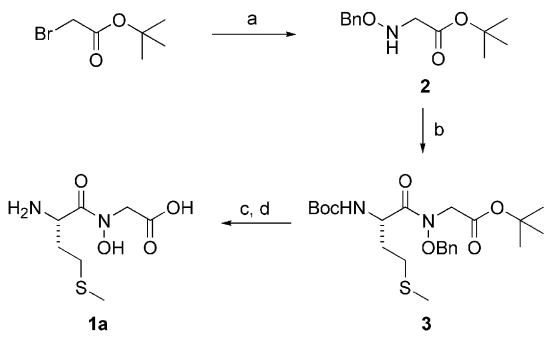


Scheme 1. Mode of binding by bestatin (**I**) and hydroxamic acid inhibitors (**II**) to MetAP.

structure **II**). Moreover, the lower pK_a of the hydroxamic acid relative to that of the bestatin hydroxyl group may improve the binding affinity of the former to the MetAP active site. There are numerous examples of hydroxamate-based metalloenzyme inhibitors. To test the above notion, we initially designed hydroxamate **1a** (Scheme 2), which contains a methionine as the P1 residue, because MetAP is highly specific for methionine at this position.[20] An *N*-hydroxyl glycine is placed at the P1′ site because MetAP requires a small residue at the penultimate position for N-terminal methionyl removal.[21]

Synthesis of compound **1a** is shown in Scheme 2. *tert*-Butyl bromoacetate was incubated with *O*-benzylhydroxylamine to give ester **2**. Condensation of **2** with *N*-Boc-methionine produced amide **3**, which was deprotected by catalytic hydrogenation followed by treatment with trifluoroacetic acid (TFA) to afford the hydroxamate **1a**. Compound **1a** was tested against Co(II)-substituted *E. coli* MetAP (EcMetAP) using a coupled continuous assay.[22] Compound **1a** behaved as a competitive inhibitor of modest potency, with an inhibition constant (K_i) of 170 μM (Table 1).

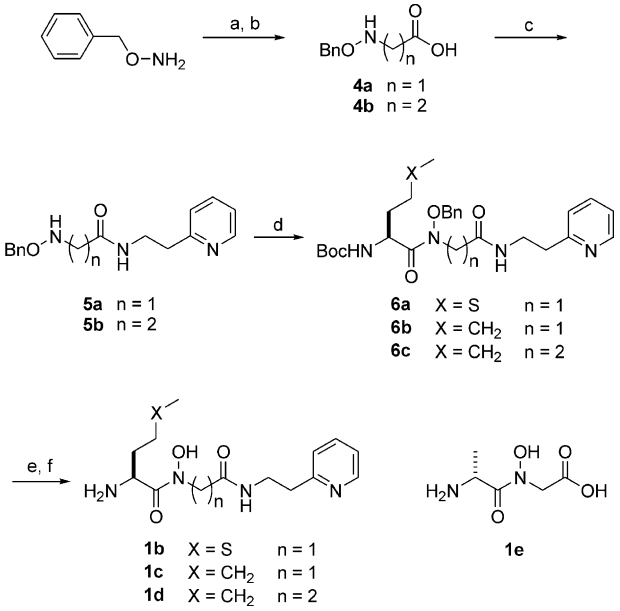
We attributed the poor activity of **1a** to the presence of a negative charge at its C-terminus. In that regard, MetAP requires at least a tripeptide for efficient methionyl cleavage.[11,12] To remove the negative charge in **1a**, we designed compound **1b**, in which a 2-(2-pyridinyl)ethyl group is appended to the C-terminus of **1a**. In addition, we designed compound **1c** to assess the importance of the methionyl side chain to the binding affinity, and **1d** to assess the importance of the linker length (P1′ residue). Compound **1e**, which was originally designed for inhibiting another metalloenzyme, was used as a control. Compounds **1b–e** were synthesized in a similar fashion as **1a** (Scheme 3). *N*-Benzyloxyglycine (**4a**) was obtained by treating compound **2** with TFA. 3-



Scheme 2. Reagents and conditions: (a) $BnONH_2$ (0.8 equiv), Et_3N (2 equiv), CH_3OH, 60%; (b) Boc-Met-OH/HBTU/Et_3N (2 equiv), 71%; (c) H_2, Pd/C; (d) TFA.

(*N*-Benzyloxy)aminopropioic acid (**4b**) was prepared from *t*-butyl acrylic acid, which was first treated with *O*-benzylhydroxylamine followed by cleavage of the *t*-butyl ester by TFA. Condensation of acids **4a** and **4b** with 2-(2-aminoethyl)pyridine gave benzyloxyamines **5a** and **5b**, respectively. Amines **5a** and **5b** were coupled to Boc-methionine or Boc-norleucine to give peptides **6a–c**. Removal of the protecting groups were effected by catalytic hydrogenation followed by TFA treatment to give the hydroxamates **1b–d**. Compound **1e** was prepared in a fashion similar to **1a**.

Addition of the 2-(2-pyridyl)ethyl group to the C-terminus of **1a** rendered compound **1b** a substantially improved inhibitor. It acted as a competitive inhibitor, with a K_i value of 2.5 μM against EcMetAP (Fig. 1 and Table 1). The increased binding affinity is probably due to both removal of the C-terminal negative charge and additional interactions brought about by the pyridylethyl group. On the other hand, replacement of the P1 methionyl side chain with that of norleucine (*n*-butyl) decreased the inhibitor potency by >20-fold



Scheme 3. Reagents and conditions: (a) For **4a**, $BrCH_2CO_2tBu$, Et_3N, CH_3OH, 60%; for **4b**, $CH_2{=}CHCO_2tBu$, Et_3N, EtOH, 70%; (b) TFA–CH_2Cl_2, quantitative; (c) 2-(2-aminoethyl)pyridine, HBTU, Et_3N, 67% (**5a**) or 54% (**5b**); (d) Boc-L-Met-OH or Boc-L-Nle-OH, $ClCO_2tBu$, Et_3N, 62–80%; (e) H_2, Pd/C; (f) TFA–CH_2Cl_2, quantitative yield.

Table 1. Inhibition constants against various Co(II)–MetAPs

Compds	K_i, μM[a]		
	EcMetAP	HsMetAP-1	HsMetAP-2
1a	170±16	390±32	1300±46
1b	2.5±1.0	48±5	91±4
1c	59±6	33±6	164±9
1d	48±10	ND	ND
1e	8.8±2.7	180±6	600±51

[a] Values are means±SD from three independent sets of experiments. ND, not determined.

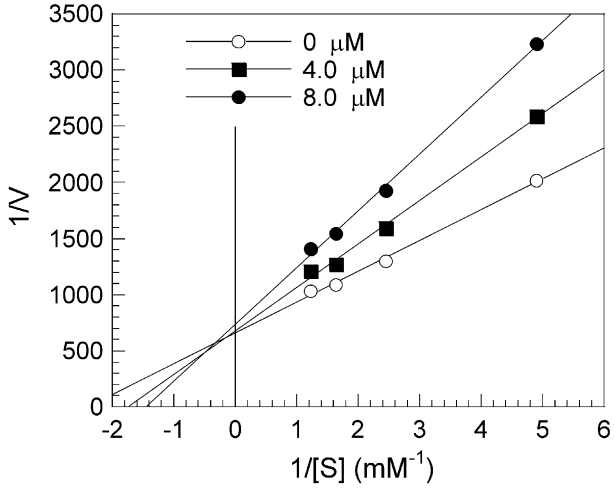


Figure 1. Lineweaver–Burk plot for the inhibition of EcMetAP by compound **1b**. The lines were fitted to the data according to the Michaelis–Menten equation: $1/V = 1/V_{max} + K_M/(V_{max} \cdot [S])$.

($K_i = 59\,\mu M$ for **1c**). It was previously reported that peptides containing N-terminal norleucine are substantially poorer substrates than the corresponding methionyl peptides.[20] Addition of a methylene group to the P1′ residue had little effect on the overall potency (Table 1, compare **1c** vs **1d**). Surprisingly, the control compound **1e**, which contains a D-alanine as the P1 residue, is a significantly better inhibitor than **1a** ($K_i = 8.8\,\mu M$ for **1e**). Note that the only reported EcMetAP inhibitor, (3*R*)-amino-(2*S*)-hydroxyheptanoyl-L-Ala-L-Val-L-Phe-OMe, contains an *n*-butyl side chain in the D-configuration at the P1 position.[17]

The above compounds were next tested against the two human Co(II)-substituted MetAPs, HsMetAP-1 and HsMetAP-2. In general, these compounds are weaker inhibitors against the human enzymes (Table 1). In particular, compounds **1b** and **1e** exhibited >20-fold selectivity toward the bacterial enzyme. These results indicate that it is possible to design inhibitors that are selective against bacterial MetAP as novel antibiotics.

In conclusion, we have shown that *N*-(α-aminoacyl)hydroxylamine derivatives act as metal-chelating inhibitors of both bacterial and humnan MetAPs, with good to moderate potency. A detailed SAR study of the inhibitor structures should lead to a new class of potent, selective MetAP inhibitors as novel antibiotics and antitumor drugs.

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References and notes

1. (a) Meinnel, T.; Mechulam, Y.; Blanquet, S. *Biochimie* **1993**, *75*, 1061. (b) Lowther, W. T.; Matthews, B. W. *Biochem. Biophys. Acta* **2000**, *1477*, 157.
2. Li, X.; Chang, Y.-H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12357.
3. Chang, S.-Y. P.; McGary, E. C.; Chang, S. *J. Bacteriol.* **1989**, *171*, 4071.
4. Miller, C. G.; Kukral, A. M.; Miller, J. L.; Movva, N. R. *J. Bacteriol.* **1989**, *171*, 5215.
5. Arfin, S. T.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. T.; Matthews, B. W.; Bradshaw, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7714.
6. Griffith, E. C.; Su, Z.; Turk, B. E.; Chen, S.; Chang, Y.-H.; Wu, Z.; Biemann, K.; Liu, J. O. *Chem. Biol.* **1997**, *4*, 461.
7. Sin, N.; Meng, L.; Wang, M. Q. W.; Wen, J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6099.
8. Roderick, S. L.; Matthews, B. W. *Biochemistry* **1993**, *32*, 3907.
9. Tahirov, T. H.; Oki, H.; Tsukihara, T.; Ogasahara, K.; Yutani, K.; Ogata, K.; Izu, Y.; Tsunasawa, S.; Kato, I. *J. Mol. Biol.* **1998**, *284*, 101.
10. Liu, S.; Widom, J.; Kemp, C. W.; Crews, C. M.; Clardy, J. *Science* **1998**, *282*, 1324.
11. Ben-Basset, A.; Bauer, K.; Chang, S.-Y.; Myambo, K.; Boosman, A.; Chang, S. *J. Bacteriol.* **1987**, *169*, 751.
12. Chang, Y.-H.; Teichert, U.; Smith, J. A. *J. Biol. Chem.* **1990**, *265*, 19892.
13. Kendall, R. L.; Bradshaw, R. A. *J. Biol. Chem.* **1992**, *267*, 20667.
14. Walker, K. W.; Bradshaw, R. A. *Protein Sci.* **1996**, *7*, 2684.
15. Wang, J.; Sheppard, G. S.; Lou, P.; Kawai, M.; Park, C.; Egan, D. A.; Schneider, A.; Bouska, J.; Lesniewski, R.; Henkin, J. *Biochemistry* **2003**, *42*, 5035.
16. D'souza, V. M.; Holz, R. C. *Biochemistry* **1999**, *38*, 11079.
17. Lowther, W. T.; Orville, A. M.; Madden, D. T.; Lim, S.; Rich, D. H.; Matthews, B. W. *Biochemistry* **1999**, *38*, 7678.
18. Mario, J. P., Jr.; Ryan, M. D.; Thompson, S. K.; Veber, D. F. PCT Int. Appl. 2001, WO0136404; *Chem. Abstr.* **2001**, *134*, 764, 367198e.
19. Mario, J. P., Jr.; Thompson, S. K.; Veber, D. F. PCT Int. Appl. 2001, WO 0124796; *Chem. Abstr.* **2001**, *134*, 761, 295827j.
20. Yang, G.; Kirkpatrick, R. B.; Ho, T.; Zhang, G.-F.; Liang, P.-H.; Johanson, K. O.; Casper, D. J.; Doyle, M. L.; Mario, J. P., Jr.; Thompson, S. K.; Chen, W.; Tew, D. G.; Meek, T. D. *Biochemistry* **2001**, *40*, 10645.
21. Hirel, P.-H.; Schmitter, J.-M.; Dessen, P.; Fayat, G.; Blanquet, S. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8247.
22. Zhou, Y.; Guo, X.-C.; Yi, T.; Yoshimoto, T.; Pei, D. *Anal. Biochem.* **2000**, *280*, 159.