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Synthesis and structure-function analysis of Fe(II)-form-selective antibacterial inhibitors of *Escherichia coli* methionine aminopeptidase

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Abstract

Methionine aminopeptidase (MetAP) is a promising target for the development of novel antibacterial, antifungal and anticancer therapy. Based on our previous results, catechol derivatives coupled with a thiazole or thiophene moiety showed high potency and selectivity toward the Fe(II)-form of *E. coli* MetAP, and some of them clearly showed antibacterial activity, indicating that Fe(II) is likely the physiologically relevant metal for MetAP in *E. coli* and other bacterial cells. To further understand the structure-function relationship of these Fe(II)-form selective MetAP inhibitors, a series of catechol derivatives was designed and synthesized by replacement of the thiazole or thiophene moiety with different five-membered and six-membered heterocycles. Inhibitory activities of these newly synthesized MetAP inhibitors indicate that many five- and six-membered rings can be accommodated by MetAP and potency on the Fe(II)-form can be improved by introducing substitutions on the heterocycles to explore additional interactions with the enzyme. The furan-containing catechols **11–13** showed the highest potency at 1 μ M on the Fe(II)-form MetAP, and they were also among the best inhibitors for growth inhibition against *E. coli* AS19 strain. These findings provide useful information for the design and discovery of more effective MetAP inhibitors for therapeutic applications.

Methionine aminopeptidase (MetAP) plays an important role in removing the N-terminal methionine from nascent proteins in all types of cells and is one of the essential enzymes required for bacterial survival^{1–3}. Inhibitors of MetAPs are of considerable interest as potential antibacterial, antifungal and anticancer agents^{4, 5}. All MetAPs require a divalent metal ion for activation, such as Co (II), Mn (II), Ni (II), Zn(II), or Fe (II), but it is uncertain which of these ions is the most important *in vivo*^{6–8}. Most of current MetAP inhibitors show potent activity *in vitro* but often fail to show potency *in vivo*^{9–11}. Although other factors, such as difficulty in cell-wall penetration, should be considered, it is possible that the lack of cellular efficacy for MetAP inhibitors may be partly due to a disparity between the metalloform of MetAP tested *in vitro* and the one that is physiologically important in cells. For developing MetAP inhibitors as therapeutics, it is critical to clarify the divalent metal ion that activates MetAP in a cellular environment and make sure that the MetAP inhibitors are effective in inhibiting the physiologically relevant metalloform of MetAP.

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Our own work in this field has been focused on discovering unique MetAP inhibitors that can distinguish different metalloforms of MetAP as research tools for the clarification and developing these inhibitors as early leads for antibacterial compounds^{11–14}. By high throughput screening of a large diverse chemical library of small organic compounds, we have identified several MetAP inhibitors with high potency and superb selectivity toward either the Co(II)-form or the Mn(II)-form of *E. coli* MetAP¹². Recently, we discovered additional inhibitors with selectivity for the Fe(II)-form of *E. coli* MetAP^{13, 14}. A unique structural feature for these Fe(II)-form selective inhibitors is the requirement of a catechol moiety for their inhibitory activity. Initial structure–function studies with a series of thiazole and thiophene derivatives leads to the conclusion that Fe(II) is the likely metal used by MetAP in bacterial cellular environment¹⁴. We also obtained an X-ray structure of *E. coli* MetAP in complex with one of the inhibitors and confirmed that these inhibitors directly interact with MetAP at the active site with the catechol moiety chelating with the catalytic metal ions¹⁴. In this paper, we report our extended structure–function studies, in which we kept the essential catechol moiety but included additional five- and six-membered heterocycles in place of the thiazole and thiophene moieties. We observed that some of these derivatives showed improved potency on the Fe(II)-form of purified MetAP and displayed considerable antibacterial activity.

Synthesis of compound **1** is outlined in Scheme 1. The commercially available 2,3-dihydroxybenzoic acid **14** was coupled with Gly-OMe in the presence of HOBt and EDCI to yield compound **15**, followed by dehydration in the presence of POCl₃ to produce compound **1** in 30% yield.

Compounds **2–4** were synthesized by the route illustrated in Scheme 2. The acid **14** was first bis-benzylated with three equivalents of benzyl bromide in acetone to obtain free carboxylic acid **16**¹⁵, followed by treatment of oxlyl chloride with DMF as catalyst to form compound **17**. Compound **18** was obtained by the reaction of methyl α -isocyanoacetate with compound **17** in the presence of triethylamine¹⁶. Hydrogenolysis of compound **18** produced compound **2**. Further basic hydrolysis of **18** gave compound **19**, followed by condensation with appropriate amine in the presence of EDC in DMF, afforded **3a–4a**, which were transferred into compounds **3–4**, respectively, by hydrogenolysis.

Compound **5** was produced by the route shown in Scheme 3. The preparation commenced with the reaction of catechol with benzyl bromide and potassium carbonate in acetone, providing 1,2-dibenzloxybenzene **20** in 80% yield, followed by iodination in the presence of iodine activated by mercuric oxide to yield **21**¹⁷. Subsequent standard Sonogashira coupling reaction employing TMS-Acetylene (TMS = trimethylsilyl) led to the formation of TMS-protected alkyne **22** in 70% yield¹⁸. The desired building block **23** was obtained in 80% yield by deprotection of **22** with K₂CO₃ in methanol at room temperature¹⁹. Then the mixture of benzyl bromide, sodium azide and phenylacetylene **23** in distilled water in the presence of CuI was heated at 65 °C (oil bath) to form compound **24** in 85% yield, followed by hydrogenolysis to yield final compound **5** in 80% yield²⁰.

Synthesis of compounds **6–13** was outlined in Scheme 4. Suzuki coupling of 3,4-dimethoxyphenylboronic acid with appropriate halogenated compounds with a five-membered or six-membered heterocycle yielded compound **6a–13a** (40% – 85% yield)²¹. Demethylation in the presence of BCl₃ yielded compounds **6–13** (30 – 60% yield).

Evaluation of inhibitory activity of these compounds (Table 1) was performed by using purified apoenzyme of *E. coli* MetAP that was activated by Co(II), Mn(II) or Fe(II) during activity assays¹⁴. Previously, we coupled thiazole or thiophene moieties to the catechol moiety¹⁴, and here we present findings on other catechol derivatives with different five- or six-membered heterocyclic rings. It is clear that when the catechol moiety is intact, different heterocyclic rings

can be substituted to maintain or improve potency and selectivity on MetAP. The four five-membered oxazole analogs (**1–4**) maintained the potency and selectivity on the Fe(II)-form of *E. coli* MetAP, so were the three six-membered pyridine and pyrimidine analogs (**6–8**). The outliers were the triazole derivative **5** and the imidazole derivative **9**. The former (**5**) maintained potency on the Fe(II)-form but gained activity on the Co(II)- and Mn(II)-forms, and therefore it lost selectivity. The latter (**9**) did not show activity on all three metalloforms tested. The reason for the inactivity of **9** is not clear, because it is small enough, comparing with **2–4**, to fit into the active site pocket. For **5**, the addition of benzyl group may introduce extra binding interaction to tilt the catechol moiety slightly, affecting its interaction with the metal ions. The four furan-containing compounds **10–13** with an amide group attached to the furan ring are very interesting. By changing substitution on the amide nitrogen, the potency on the Fe(II)-form increased from 14.8 μM for **10** to around 1 μM for **11–13**. This potency is better than thiazoles or thiophenes we reported previously¹⁴. Compounds **11–13** also showed considerable potency on the Co(II)- and Mn(II)-forms. Likely, the side chain groups found additional binding interactions at or near the active site.

MetAP is an essential enzyme in bacteria, and gene knockout experiments showed lethal phenotype when the functional MetAP gene was absent^{2,3}. Conceivably, inhibition of MetAP will lead to growth inhibition of bacterial cells. To minimize complications of cell penetration by these inhibitors, we used *E. coli* strain AS19 to test our MetAP inhibitors, which has unspecified mutations on its cell membrane that make it more permeable to small organic compounds²². Consistent with the data from enzyme inhibition, the better inhibitors of the Fe(II)-form MetAP showed better growth inhibition of the *E. coli* cells, and the best inhibitors for both the enzyme activity and cell growth are **11–13**. Previous experiments by monitoring N-terminal processing of a recombinant GST protein showed that the growth inhibition correlates with MetAP inhibition¹³.

In conclusion, the structure-function analysis of derivatives of initial inhibitors of the Fe(II)-form of *E. coli* MetAP clearly indicates that various five- and six-membered rings can be accommodated by MetAP and potency on the Fe(II)-form, which is the physiologically relevant metalloform¹³, can be enhanced by introducing additional groups to the heterocyclic rings. These findings provide a new starting point for the design and discovery of more potent antibacterial MetAP inhibitors.

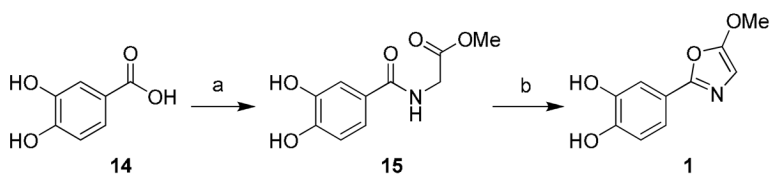
Acknowledgements

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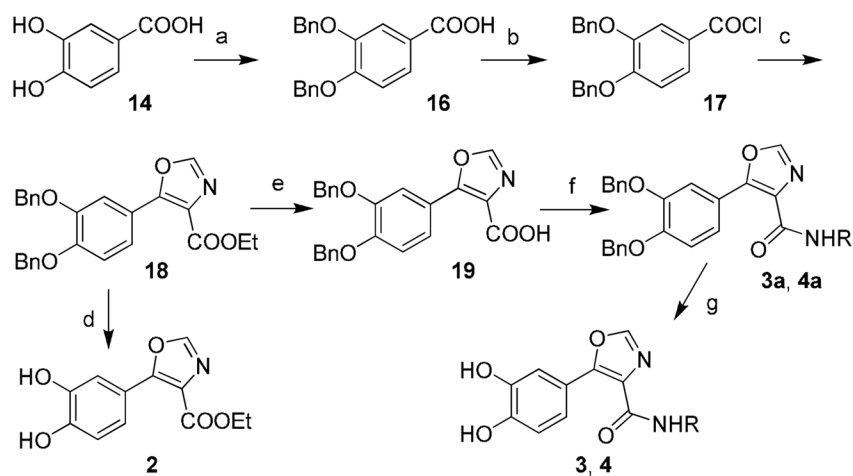
References

1. Bradshaw RA, Brickey WW, Walker KW. Trends Biochem Sci 1998;23:263. [PubMed: 9697417]
2. Miller CG, Kukral AM, Miller JL, Movva NR. J Bacteriol 1989;171:5215. [PubMed: 2670909]
3. Chang SY, McGary EC, Chang S. J Bacteriol 1989;171:4071. [PubMed: 2544569]
4. Vaughan MD, Sampson PB, Honek JF. Curr Med Chem 2002;9:385. [PubMed: 11860363]
5. Griffith EC, Su Z, Niwayama S, Ramsay CA, Chang YH, Liu JO. Proc Natl Acad Sci U S A 1998;95:15183. [PubMed: 9860943]
6. Lowther WT, Matthews BW. Biochim Biophys Acta 2000;1477:157. [PubMed: 10708856]
7. D'Souza VM, Holz RC. Biochemistry 1999;38:11079. [PubMed: 10460163]
8. Walker KW, Bradshaw RA. Protein Sci 1998;7:2684. [PubMed: 9865965]
9. Schiffmann R, Heine A, Klebe G, Klein CD. Angew Chem Int Ed Engl 2005;44:3620. [PubMed: 15880695]
10. Oefner C, Douangamath A, D'Arcy A, Hafeli S, Mareque D, Mac Sweeney A, Padilla J, Pierau S, Schulz H, Thormann M, Wadman S, Dale GE. J Mol Biol 2003;332:13. [PubMed: 12946343]

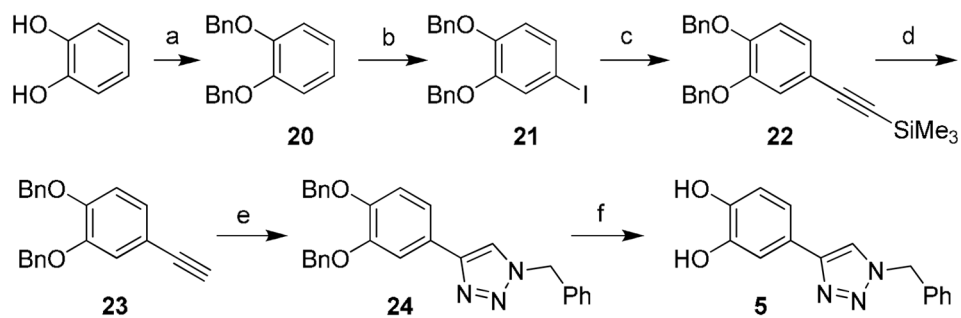
11. Luo QL, Li JY, Liu ZY, Chen LL, Li J, Qian Z, Shen Q, Li Y, Lushington GH, Ye QZ, Nan FJ. *J Med Chem* 2003;46:2631. [PubMed: 12801227]
12. Ye QZ, Xie SX, Huang M, Huang WJ, Lu JP, Ma ZQ. *J Am Chem Soc* 2004;126:13940. [PubMed: 15506752]
13. Chai SC, Wang WL, Ye QZ. *J Biol Chem* 2008;283:26879. [PubMed: 18669631]
14. Wang WL, Chai SC, Huang M, He HZ, Hurley TD, Ye QZ. *J Med Chem* 2008;51:6110. [PubMed: 18785729]
15. Tranchimand S, Tron T, Gaudin C, Lacazio G. *Syn Commun* 2006;36:587.
16. Suzuki M, Iwasaki T, Matsumoto K, Okumura K. *Syn Commun* 1972;2:237.
17. Mee SP, Lee V, Baldwin JE, Cowley A. *Tetrahedron* 2004;60:3695.
18. Muehldorf AV, Guzman-Perez A, Kluge AF. *Tetrahedron Lett* 1994;35:8755.
19. Lee J, Cha J. *Tetrahedron* 2000;56:10175.
20. Wang ZX, Zhao ZG. *J Heterocyclic Chem* 2007;44:89.
21. Pereira R, Iglesias B, de Lera AR. *Tetrahedron* 2001;57:7871.
22. Zorzopulos J, de Long S, Chapman V, Kozloff LM. *FEMS Microbiol Lett* 1989;52:23. [PubMed: 2689280]

**Scheme 1.**

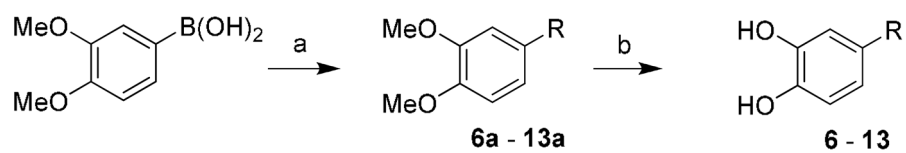
Reagents and conditions: (a) EDCl, HOBT, Gly-OMe, DMF, 70%; (b) POCl₃, 90 °C, 30%.

**Scheme 2.**

Reagents and conditions: (a) BnBr, K_2CO_3 , Acetone, reflux overnight, 90%; then NaOH, H_2O , MeOH, reflux, 2h, 90%; (b) oxalyl chloride, DMF, DCM; (c) Ethyl α -isocyanoacetate, Et_3N , THF, 70%; (d) H_2 , Pd/C, MeOH, 90%; (e) LiOH, MeOH, H_2O , 100%; (f) EDCI, amine, DMAP, DCM, 50–60%; (g) BCl_3 , DCM, $-78\text{ }^\circ\text{C}$ to rt, 40–50%.

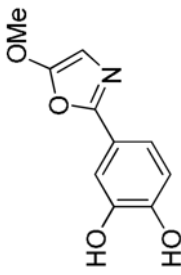
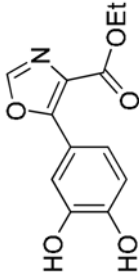
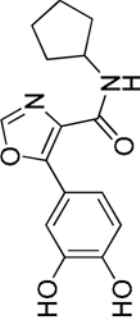
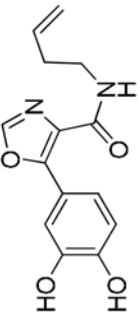
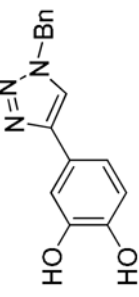
**Scheme 3.**

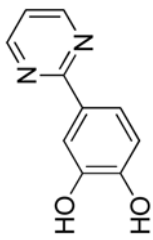
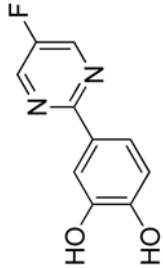
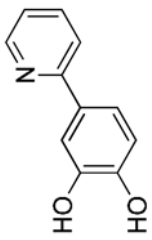
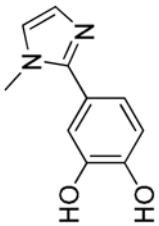
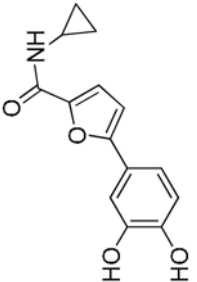
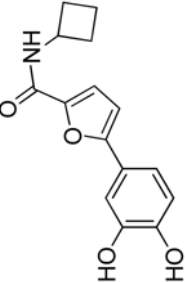
Reagents and conditions: (a) BnBr, K₂CO₃, Acetone, 80%; (b) I₂, HgO, DCM, rt, 15h, 85%; (c) ⁱPr₂NH, 10 mol% (PPh₃)₂PdCl₂, 5 mmol% CuI, TMS-acetylene, reflux, 70%; (d) K₂CO₃, MeOH, 80%; (e) BnBr, NaN₃, 0.1 mol% CuI, H₂O, 65 °C, 85%; (f) H₂, 10% Pd/C, MeOH, 80%.

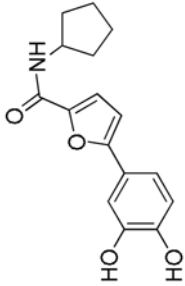
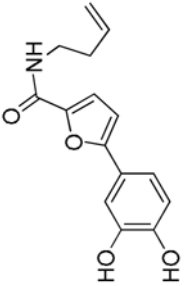
**Scheme 4.**

Reagents and conditions: (a) Pd(PPh₃)₄, 2N Na₂ CO₃, RBr, DMF, 80 °C, 40 – 85%; (b) 1 M BCl₃, DCM, –78 °C to rt, 30 – 60%.

Table 1
Inhibition of different metalloforms of purified *E. coli* MetAP and inhibition of growth of *E. coli* AS19 cells by catechol-containing MetAP Inhibitors^a

Comp	Structure	Inhibition of metal-activated MetAP enzymes, IC ₅₀ , μ M			Inhibition of <i>E. coli</i> cell growth, IC ₅₀ , μ M ^b
		Co(II)	Mn(II)	Fe(II)	
1		>100	>100	11.2	228 (164)
2		>100	>100	14.0	120 (99)
3		>100	>100	11.0	165 (199)
4		>100	>100	13.4	97.2 (99)
5		24.6	19.5	16.1	97.7 (79)

Comp	Structure	Inhibition of metal-activated MetAP enzymes, IC ₅₀ , μM			Inhibition of <i>E. coli</i> cell growth, IC ₅₀ , μM ^b
		Co(II)	Mn(II)	Fe(II)	
6		>100	>100	25.5	>250
7		52.6	>100	10.6	206 (80)
8		>100	>100	19.3	211 (106)
9		>100	>100	>100	>250
10		>100	>100	14.8	55.0 (26)
11		22.5	7.9	1.2	43.9 (29)

Comp	Structure	Inhibition of metal-activated MetAP enzymes, IC ₅₀ , μ M			Inhibition of <i>E. coli</i> cell growth, IC ₅₀ , μ M ^b
		Co(II)	Mn(II)	Fe(II)	
12		30.1	11.8	0.9	23.4 (19)
13		21.2	6.7	1.0	50.8 (39)

^aRelative standard derivations are <20% in all values.

^bNumbers in parenthesis are MIC values in μ g/mL.