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Metal mediated inhibition of methionine aminopeptidase by quinolinyl sulfonamides

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Abstract

Quinolinyl sulfonamides, such as *N*-(quinolin-8-yl)methanesulfonamide (**10**) and *N*-(5-chloroquinolin-8-yl)methanesulfonamide (**11**), were identified as potent methionine aminopeptidase (MetAP) inhibitors by high throughput screening of a diverse chemical library of small organic compounds. They showed different inhibitory potencies on Co(II)-, Ni(II)-, Fe(II)-, Mn(II)-, and Zn(II)-forms of *Escherichia coli* MetAP, and their inhibition is dependent on metal concentration. X-ray structures of *E. coli* MetAP complexed with **10** revealed that the inhibitor forms a metal complex with the residue H79 at the enzyme active site; the complex is further stabilized by an extended H-bond and metal interaction network. Analysis of the inhibition of MetAP by these inhibitors indicates that this is a typical mechanism of inhibition for many non-peptidic MetAP inhibitors and emphasizes the importance of defining in vitro conditions for identifying and evaluating MetAP inhibitors that will be capable of giving information relevant to the in vivo situation.

Keywords: Protein structure; Enzyme inhibition; Metalloenzyme

Methionine aminopeptidase (MetAP) is a ubiquitous metalloenzyme in both eukaryotic and prokaryotic cells. It removes N-terminal methionine residue from newly synthesized polypeptide chains [1]. Two types of MetAP (types I and II) have been identified. Prokaryotes have only one MetAP, while eukaryotes have both type I and type II MetAPs. The essential role played by MetAP in bacteria is demonstrated by the fact that deletion of the single MetAP gene in *Escherichia coli* [2] or *Salmonella typhimurium* [3] is lethal. It is therefore of considerable interest as a potential target for developing novel broad spectrum antibiotics [4]. Interest in MetAP is further enhanced by the discovery that some antiangiogenic compounds, such as fumagillin, are potent inhibitors of human MetAPs [5–8]. MetAP inhibitors have also shown efficacy in preclinical tumor models [9,10].

Although the natural product fumagillin and its structural analogs ovalicin and TNP470 are MetAP inhibitors with nanomolar potency (IC_{50} for TNP470 1.3 nM) [5,11], they covalently modify H231 of human type II MetAP [7,12], which could be responsible for some of the toxicities seen in the clinical trials of TNP470 [13]. Interestingly, although human type I and II MetAPs have the similar substrate specificity [14], fumagillin analogs inhibit only the type II enzyme. The covalent complex of fumagillin with *E. coli* MetAP, which is a type I enzyme, was obtained only at a high inhibitor concentration, which resulted in modification at the equivalent H79 of *E. coli* MetAP [15]. The earliest synthetic MetAP inhibitors, such as AHHpA-Ala-Leu-Val-Phe-OMe derived from bestatin, were peptidic and had only modest activity against *E. coli* MetAP (IC_{50} 5 μM) [16]. More recently, several non-peptidic inhibitors with a triazole [17–19] or thiazole [20–23] moiety have been described, and some of them have achieved nanomolar potency (Table 1). They have been used widely in MetAP research in attempts to define the

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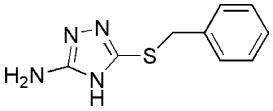
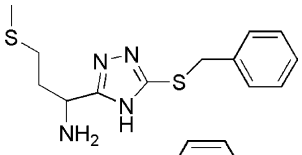
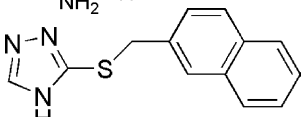
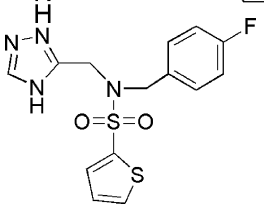
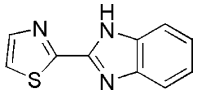
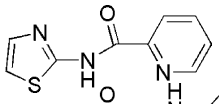
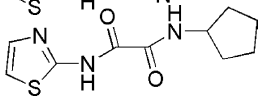
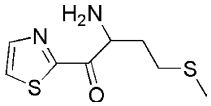
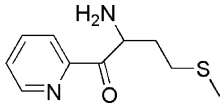
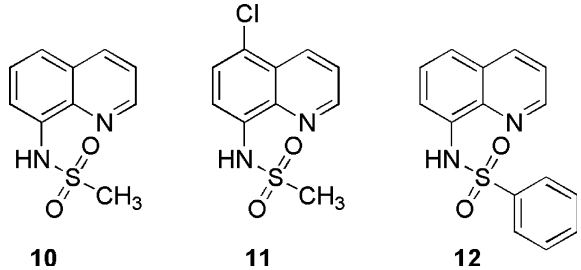
Table 1
Activity of representative non-peptidic MetAP inhibitors

Compound	Structure	IC_{50} (nM)	MetAP tested[a]	Reference
1		599	*Sa*MetAP1	[17]
2		44	*Sa*MetAP1	[17]
3		69	*Hs*MetAP2	[18]
4		8	*Hs*MetAP2	[19]
5		400[b]	*Ec*MetAP1	[20]
6		5000	*Ec*MetAP1	[21]
7		67	*Ec*MetAP1	[22]
8		19,000[c]	*Sa*MetAP1	[23]
9		16,000[c]	*Sa*MetAP1	[23]

[a] All values shown here for the MetAPs tested are in the Co(II)-form. *Hs*MetAP2, human MetAP type II; *Ec*MetAP1, *E. coli* MetAP type I; and *Sa*MetAP1, *Staphylococcus aureus* MetAP type I.
[b] K_i value.
[c] Observed as gem-diols (hydrates) in crystal structures [23].

in vivo metal [18] and test for antibacterial [17,20,21] and antiangiogenic [19] activities. Elucidation of their mechanism of inhibition may help to correlate their in vitro and in vivo activities.

In this paper, we report a novel class of MetAP inhibitors with a quinolinyl sulfonamide moiety (Fig. 1). They



10 **11** **12**

Fig. 1. Quinolinyl sulfonamide inhibitors of *E. coli* MetAP.

are among the most potent non-peptidic MetAP inhibitors yet described. Analysis of the dependence of inhibition on metal concentration, and subsequent analysis of the X-ray structure of the Mn(II)-form *E. coli* MetAP in complex with **10**, revealed that their binding to the enzyme and the resulting inhibition is metal mediated. This seems to be a common mechanism of inhibition for many of the non-peptidic MetAP inhibitors discovered so far. The implications of these findings for the future development of MetAP inhibitors are discussed.

Materials and methods

Enzyme preparation and source of compounds. The recombinant *E. coli* MetAP protein was purified as an apoenzyme [24]. A diverse chemical library of 105,984 small organic compounds used for screening was purchased from ChemBridge (San Diego, CA) and ChemDiv (San Diego,