European Journal of Medicinal Chemistry 47 (2012) 479–484

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

# Structural analysis of inhibition of *Mycobacterium tuberculosis* methionine aminopeptidase by bengamide derivatives

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ARTICLE INFO

*Article history:*
Received 26 September 2011
Received in revised form
3 November 2011
Accepted 9 November 2011
Available online 17 November 2011

*Keywords:*
Metalloenzyme
Drug discovery
Tuberculosis
Antibiotic
X-ray structure

ABSTRACT

Natural product-derived bengamides possess potent antiproliferative activity and target human methionine aminopeptidases for their cellular effects. Using bengamides as a template, several derivatives were designed and synthesized as inhibitors of methionine aminopeptidases of *Mycobacterium tuberculosis*, and initial antitubercular activity were observed. Here, we present three new X-ray structures of the tubercular enzyme *Mt*MetAP1c in complex with the inhibitors in the Mn(II) form and in the Ni(II) form. All amide moieties of the bengamide derivatives bind to the unique shallow cavity and interact with a flat surface created by His-212 of *Mt*MetAP1c in the Mn(II) form. However, the active site metal has significant influence on the binding mode, because the amide takes a different conformation in the Ni(II) form. The interactions of these inhibitors at the active site provide the structural basis for further modification of these bengamide inhibitors for improved potency and selectivity.



## 1. Introduction

Tuberculosis is a deadly disease caused by mycobacterial infection, and *Mycobacterium tuberculosis* is the major tuberculosis pathogen in human. Now, multidrug-resistant and extensively drug-resistant tuberculosis is happening at an alarming rate [1]. To overcome the drug resistance, new antibiotics with a novel mechanism of action are urgently needed. Methionine aminopeptidase (MetAP) is ubiquitous and carries out N-terminal methionine excision from majority of newly synthesized proteins [2]. The importance of this cotranslational modification is underscored by lethality of gene deletion in bacteria, such as *Escherichia coli* [3] and *Salmonella typhimurium* [4]. Therefore, MetAP is a potential target to develop novel antibacterial drugs [5].

*M. tuberculosis* has two MetAP genes (annotated as *mapA* and *mapB* in H37Rv genome). The protein from the *mapB* gene of *M. tuberculosis*, named *Mt*MetAP1c, was purified, and its structures in apoform and in complex with methionine were reported [6]. The structural analysis revealed a SH3 binding motif in its N-terminus, and potential interaction with ribosome through this motif to facilitate cotranslational methionine excision was proposed [6]. We recently further characterized this enzyme for metal activation and inhibition and described three X-ray structures with small molecule inhibitors bound [7]. The other MetAP (from the *mapA* gene) of *M. tuberculosis*, named *Mt*MetAP1a, is shorter at the N-terminus and has no such SH3 binding motif. No structural information for *Mt*MetAP1a has been reported. Both MetAPs of *M. tuberculosis* were active as enzymes when purified [7–9], and their mRNA transcripts were analyzed and showed different levels in log phase and stationary phase [8]. *Mt*MetAP1a gene (*mapA*) expressed more in log phase, while *Mt*MetAP1c gene (*mapB*) showed higher level in stationary phase. It was concluded that the two MetAPs may perform an important function in different growth phases of *M. tuberculosis* [8]. The special characteristics of mycobacterial life cycle may require more than one MetAP enzyme to carry out the important cotranslational modification. Based on comparison of mycobacterial genomes, it was predicted that both *Mt*MetAP1a and *Mt*MetAP1c are essential for *M. tuberculosis* survival *in vivo* and pathogenicity [10].

Eukaryotic cells usually have two MetAPs. Deletion of either of the two MetAP genes in *Saccharomyces cerevisiae* rendered a slow growth phenotype, and lethality was observed only when both genes were deleted [11]. Bengamides are natural products that were isolated from marine sponge [12]. Bengamides A and B (**1** and **2** in Fig. 1) showed nanomolar potency again cancer cell lines [13,14], and bengamides arrest cells at the G1 and G2/M phases of the cell cycle [13,15]. A clinical trial was carried out for anticancer

*Abbreviations:* MetAP, methionine aminopeptidase; *Mt*MetAP1c, *M. tuberculosis* methionine aminopeptidase type 1c; $IC_{50}$, concentration of 50% inhibition.

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doi:10.1016/j.ejmech.2011.11.017

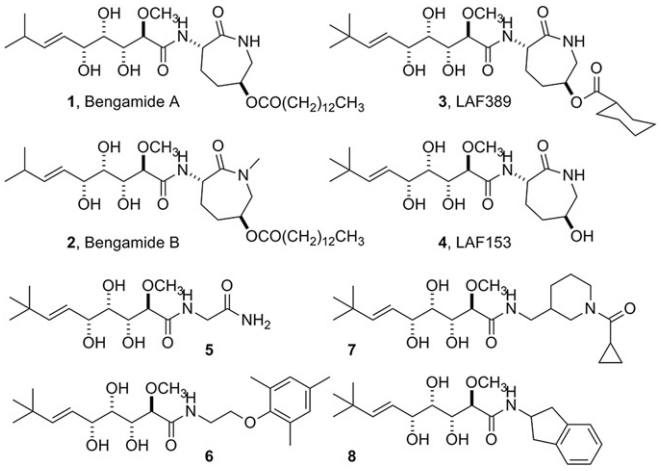


**Fig. 1.** Chemical structures of natural bengamides (**1** and **2**) and their synthetic derivatives (**3** and **4**). Compounds **5**–**9** are some of the newly designed and synthesized bengamide derivatives, used in the X-ray structural studies.

therapy, using the synthetic derivative LAF389 [16] (**3**). Human MetAP1 and MetAP2 were identified as the cellular targets of bengamides by a proteomic approach [17], and bengamides showed no selectivity in inhibition between the two human MetAP enzymes [17].

The unique bound conformation of bengamides at the active site was illustrated by the X-ray structure of a bengamide derivative LAF153 (**4**) in complex with human MetAP2 [17] (pdb code 1QZY) (Fig. 2A). In the dimetalated structure, the triol moiety of LAF153 coordinates with the two Co(II) ions to form two octahedral geometries, which is reminiscent of the binding of a bestatin-derived transition state inhibitor [18]. The spatial arrangement of three hydroxyl groups may uniquely satisfy the coordination requirement and possibly confer the high affinity. On one side of the triol moiety, a *t*-butylalkene substituent occupies the site reserved for the terminal methionine in a peptide substrate, and on the other side, a caprolactam ring beyond the amide bond interacts with residues towards the opening of the active site pocket (Fig. 3A). This unique binding mode of bengamides, coupled with their potent inhibition of MetAP enzymes and cancer cells, makes them an excellent template to develop potent MetAP inhibitors for mycobacterial and other MetAP enzymes as therapeutics.

## 2. Results and discussion

### 2.1. Design of bengamide derivatives for selective inhibition of mycobacterial MetAPs

Considering the importance of proper methionine removal, it is not surprising that MetAP shows stringent specificity for the N-terminal methionine [19,20]. No natural amino acid residues other than methionine are accepted at this position, and the formyl group in formylmethionine must be removed before methionine can be processed [21]. MetAP also shows high selectivity for the penultimate residue and has a strong preference for a small, uncharged amino acid (Gly, Ala, Ser, Thr, Pro, Val, or Cys) as the penultimate residue [22,23]. Therefore, different MetAPs use homologous residues to form the active site as a shallow and mostly hydrophobic pocket to fit the terminal methionine and the penultimate small residue. All MetAPs are homologous in the catalytic domain, and most protein residues inside the pocket are conserved [24],

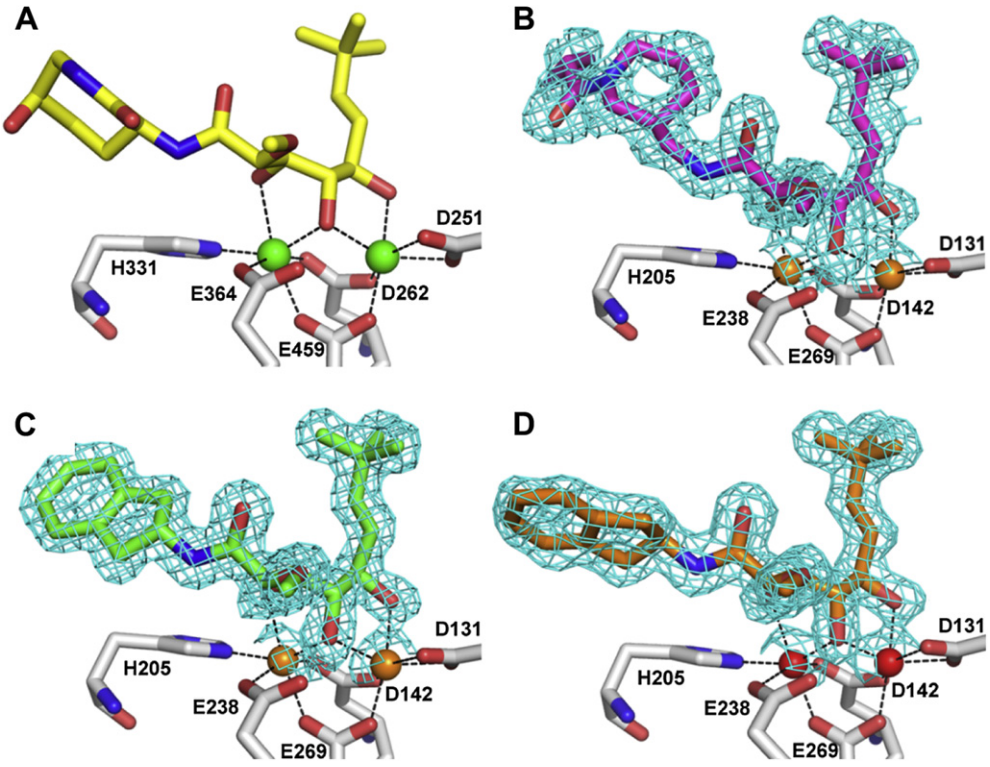


**Fig. 2.** Coordination of bengamide derivatives **7** and **8** with the metal ions at the dinuclear catalytic site, in comparison with LAF153. A. LAF153 with two Co(II) ions in human MetAP2. B. **7** with two Mn(II) ions in *Mt*MetAP1c. C. **8** with two Mn(II) ions in *Mt*MetAP1c. D. **8** with two Ni(II) ions in *Mt*MetAP1c. Inhibitors are shown as sticks, and metal ions are shown as spheres. Coordination between the metal ions and the heteroatoms of the inhibitors or protein residues is shown as dashed lines. Only metal coordinating protein residues are shown. For coloring carbon atoms, LAF153 is yellow, **7** is magenta, **8** is green or orange, and protein residues are grey. For coloring non-carbon atoms, oxygen is red, and nitrogen is blue. For coloring the metal ions, Co(II) is green, Mn(II) is orange, and Ni(II) is red. The electron density ($2F_o - F_c$ map) around the inhibitors is shown as cyan meshes at 1.0 $\sigma$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)