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The identification of inhibitory compounds of *Rickettsia prowazekii* methionine aminopeptidase for antibacterial applications

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ABSTRACT

Methionine aminopeptidase (MetAP) is a dinuclear metalloprotease responsible for the cleavage of methionine initiator residues from nascent proteins. MetAP activity is necessary for bacterial proliferation and is therefore a projected novel antibacterial target. A compound library consisting of 294 members containing metal-binding functional groups was screened against *Rickettsia prowazekii* MetAP to determine potential inhibitory motifs. The compounds were first screened against the target at a concentration of 10 μM and potential hits were determined to be those exhibiting greater than 50% inhibition of enzymatic activity. These hit compounds were then rescreened against the target in 8-point dose–response curves and 11 compounds were found to inhibit enzymatic activity with IC_{50} values of less than 10 μM. Finally, compounds (**1–5**) were docked against *Rp*MetAP with AutoDock to determine potential binding mechanisms and the results were compared with crystal structures deposited within the PDB.

The discovery of chemical therapies for the treatment of bacterial infection continues to be the focus of numerous research programs around the world. Epidemic typhus, a disease often neglected by research programs, is characterized by a characteristic rash that is often accompanied by sustained fever, muscle pain, chills, and delirium. The causative agent of epidemic typhus in humans is *Rickettsia prowazekii*,[1,2] and transmission of *R. prowazekii* generally occurs in crowded populations with compromised sanitation and hygienic practices, as the parasite is transmitted between hosts via the human body louse.[3,4] Because *R. prowazekii* is an obligate intracellular parasite, there are limited clinically effective antibiotic treatments to treat rickettsioses. Additionally, *R. prowazekii* strains resistant to both tetracycline and chloramphenicol antibiotics have been reported,[5] and the identification of novel targets for the development of anti-rickettsial therapeutics is necessary.

To discover effective inhibitory compounds encompassing novel chemical space and complexity while also possessing the necessary and desirable antibiotic activity, research programs should target pathways responsible for necessary functions of bacterial life and proliferation.[6,7] This will lead to two outcomes: first, the optimized antibacterial compounds may exhibit broad spectrum activity against a wide number of distinct bacterial species in the event a universal pathway is successfully regulated, and second, the targeted bacterial species will not have had the opportunity on the evolutionary time scale to develop resistance mechanisms to these compounds.[6] Additionally, if a suitably potent inhibitory scaffold is discovered, derivatization could afford potent compounds tailored to target various infective agents. Recently, methionine aminopeptidase (MetAP), a ubiquitous enzyme responsible for the cleavage of methionine initiatory residues from nascent proteins, has been suggested as a potential broad spectrum antibacterial target.[8] MetAP is a dinuclear metalloprotease, with demonstrated *in vitro* activity when Co, Mn, Fe, Zn, and Ni divalent cofactors are utilized.[9–11] Additionally, current inhibitory motifs demonstrate a significant correlation with cofactor identity and are generally only potent against enzymes binding specific metals.[12,13] Regarding MetAP inhibition resulting in antibacterial outcomes, inactivation of the gene encoding MetAP in *Escherichia coli*[14] and *Salmonella typhimurium*[15] has demonstrated MetAP production and function is necessary for bacterial proliferation. Thus, the inhibition of MetAP is a proposed mechanism of antibacterial activity, and compounds demonstrated to potently bind MetAP have bactericidal activity against species in whole cell *in vitro* assays.[16–19] However, MetAP is present in all

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eukaryotic life forms, and selective inhibition of bacterial MetAPs is formidable. Indeed, human and bacterial isoforms have significant conservation, with *Homo sapiens* and *E. coli* isoforms of MetAP type 1 demonstrating 47% sequence identity.[20] Additionally, many of the residues composing the substrate binding pocket are conserved between human and bacterial MetAPs, resulting in difficulties associated with isoform selective binding of inhibitors (Fig. 1).[20]

We therefore set out to determine inhibitory motifs capable of regulating the activity MetAP from *R. prowazekii*. Because *R. prowazekii* is an obligate intracellular pathogen, the parasite cannot survive for extended periods outside of a host. Consequently, screening campaigns targeting *R. prowazekii* must therefore be performed within host cells,[21] affording the bacteria an additional resistance mechanism; the host cells must first absorb the compounds, which must then be absorbed by the bacteria. For this reason, *R. prowazekii* is resistant to a wide number of commercially available antibiotics and few antibiotics are approved to treat this infection.[19] Thus, *Rp*MetAP was chosen as the enzymatic target and was expressed and purified according to our previously published methods with slight modifications[21–25] (see Supplementary Content). Additionally, the choice of the metal cofactor to be utilized in the enzymatic activity assay is important, as inhibitory values are dependent upon cofactor identity. Here, Mn(II) was used for our enzyme assays as it is the suggested native cofactor in human isoforms[11] and is the cofactor present in available crystal structures of *Rp*MetAP.[21]

With the enzyme in hand, enzymatic activity was monitored via a fluorescence-based activity assay where methionine-aminomethylcoumarin (Met-AMC) was utilized as the substrate.[18,21] Enzymatic activity cleaves the peptide bond of the Met-AMC substrate, resulting in free AMC, which is fluorescent (excitation: 360 nm; emission: 460 nm) (Scheme 1). Because only the product of enzymatic activity is fluorescent, an increase in fluorescence over time is directly related to enzyme activity. The addition of inhibitory compounds and the resulting decrease in fluorescence is a measure of inhibitory activity, where activity is calculated as the slope of a fluorescence versus time plot as compared to an uninhibited control.[26]

The members of the compound library screened against the *Rp*MetAP enzyme were chosen based upon published inhibitory scaffolds and compounds possessing functional groups capable of binding to the metal cofactors.[27] Typically, MetAP inhibitors contain functional groups capable of potently coordinating the metal cofactors found within all species of MetAP.[20] These functional groups generally include carboxylic acid, 2,2′-bipyridyl, 1,2,4-triazolo, thiazolo, or oxamide derivatives. A commercially available library was therefore assembled from Otava Chemicals catalog to include compounds possessing these (or derivatives of these) functional groups. Additionally, reactive functional groups (alkyl halides, hydrides, oxidizing agents, etc.) were intentionally excluded from the screening library to minimize the likelihood of false positive results.

Initially composed of 294 individual chemical species, the inhibition activities were assessed using a single-point screening (10 μM compound). Compounds found to exhibit inhibition greater than 50% were considered initial hits and were rescreened in 8-point dose–response curves to determine IC_{50} values. The final determinant for hit motifs was IC_{50} values <10 μM. A total of 11

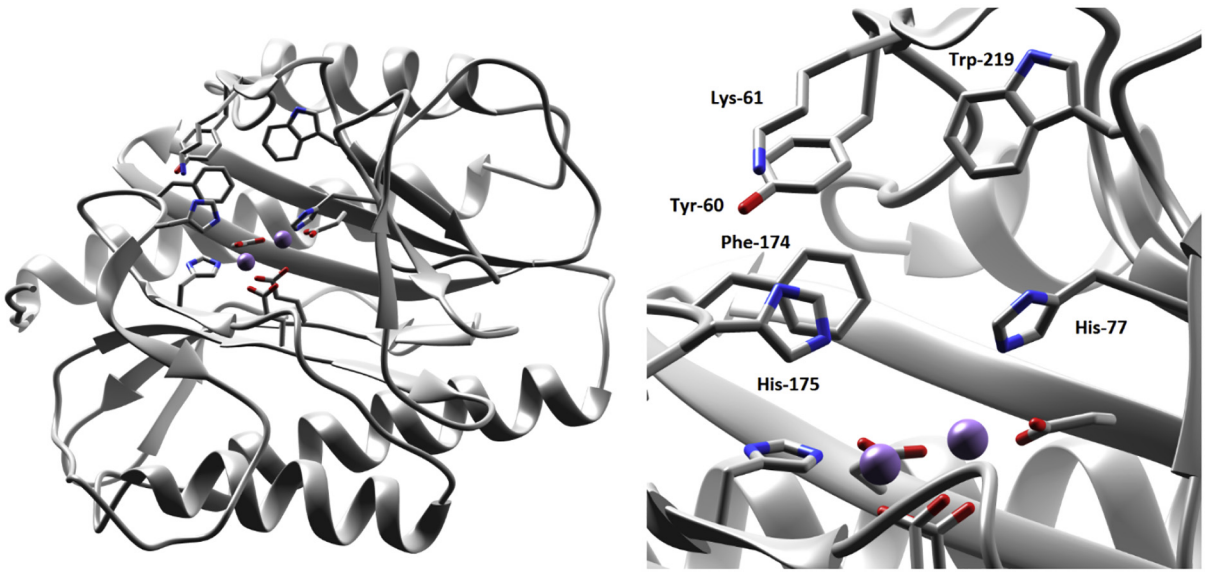
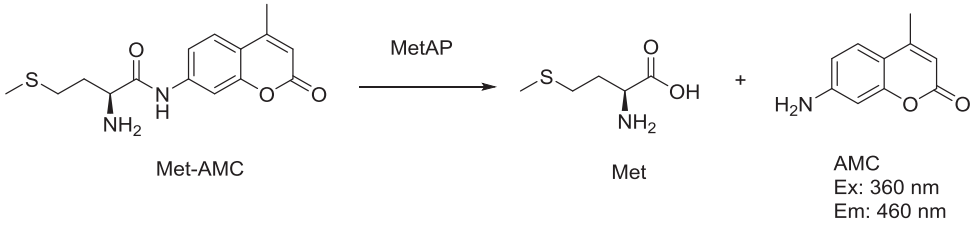


Fig. 1. Left: *Rp*MetAP with active site residues displayed; Mn(II) cofactors are shown as purple spheres. Right: Zoom-in of the *Rp*MetAP active site with bound Mn(II) cofactors (PDB 3MX6[21]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Scheme 1.