Bioorganic & Medicinal Chemistry Letters 25 (2015) 3301–3306

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel broad-spectrum inhibitors of bacterial methionine aminopeptidase

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ARTICLE INFO

Article history:
Received 13 April 2015
Revised 20 May 2015
Accepted 22 May 2015
Available online 30 May 2015

Keywords:
Antibacterial
Methionine aminopeptidase
Azepinone
Structure-based design
Drug discovery

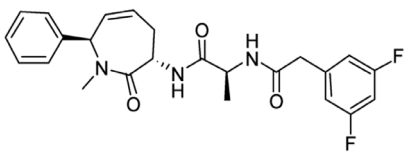
ABSTRACT

With increasing emergence of multi-drug resistant infections, there is a dire need for new classes of compounds that act through unique mechanisms. In this work, we describe the discovery and optimization of a novel series of inhibitors of bacterial methionine aminopeptidase (MAP). Through a high-throughput screening campaign, one azepinone amide hit was found that resembled the native peptide substrate and possessed moderate biochemical potency against three bacterial isozymes. X-ray crystallography was used in combination with substrate-based design to direct the rational optimization of analogs with sub-micromolar potency. The novel compounds presented here represent potent broad-spectrum biochemical inhibitors of bacterial MAP and have the potential to lead to the development of new medicines to combat serious multi-drug resistant infections.

Bacterial protein translation has been an abundant source of antibacterial targets including many different classes of drugs that treat serious clinical infections. With resistance to currently prescribed antibiotics increasing, however, there is a need for new classes of compounds that inhibit novel biochemical targets. In prokaryotic organisms, protein translation is initiated with a formylmethionine residue, which is converted to methionine during protein elongation by the metalloenzyme, peptide deformylase (PDF).[1] Inhibitors of PDF containing metal chelating moieties have shown in vivo efficacy, however concerns of toxicity remain.[2] An important step in the co- or posttranslational protein maturation is hydrolysis of the N-terminal methionine by the metalloenzyme, methionine aminopeptidase (MAP).[3,4] MAP is a ubiquitous enzyme found in all prokaryotes and gene deletion studies have shown it to be essential for *Escherichia coli*[5] and *Salmonella typhimurium*.[6] Our own unpublished gene deletion studies have confirmed this essentiality in *Streptococcus pneumoniae* and *Haemophilus influenza* (data not shown). Therefore, MAP is a validated target for the development of broad-spectrum antibacterial drugs with a novel mechanism of action that could provide therapeutic options to combat multidrug-resistant bacterial pathogens.[7]

There are two major types of MAP enzymes. Type I enzymes are found in eubacteria, while both type I and II enzymes are found in eukaryotes.[8] In humans, type I and, in particular, type II MAP have been considered as oncology targets, and type II MAP is known to be inactivated by the anti-angiogenesis compound fumagillin.[9] This compound and its subsequent analogs have been found to inhibit both endothelial cell growth as well as tumor growth in vivo.[10] Similarity of human type I MAP to bacterial MAP is moderate (30–40%), with even lower similarity observed for the type II MAP (20%). Engineering selectivity for bacterial MAP should be achievable given this sequence divergence and the fact that crystal structures of both human[11] and bacterial[12] MAP enzymes have been published.

MAP's enzymatic hydrolysis of native proteins requires the assistance of divalent metal ions that serve as a cofactor for the catalytic reaction. MAP activation has been accomplished with Co(II), Mn(II), Ni(II), and Fe(II).[13] Although several MAPs were initially



Azepinone Amide

Eco	30 ± 3 μM
Hin	60 ± 9 μM
Spn	46 ± 5 μM
Sau	>200 μM

Figure 1. High throughput screen azepinone amide hit (**1**). Resynthesis of the HTS hit confirmed moderate biochemical activity against three MAP isozymes. Eco = *E. coli*. Hin = *H. influenzae*. Spn = *S. pneumoniae*. Sau = *S. aureus*.

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http://dx.doi.org/10.1016/j.bmcl.2015.05.061
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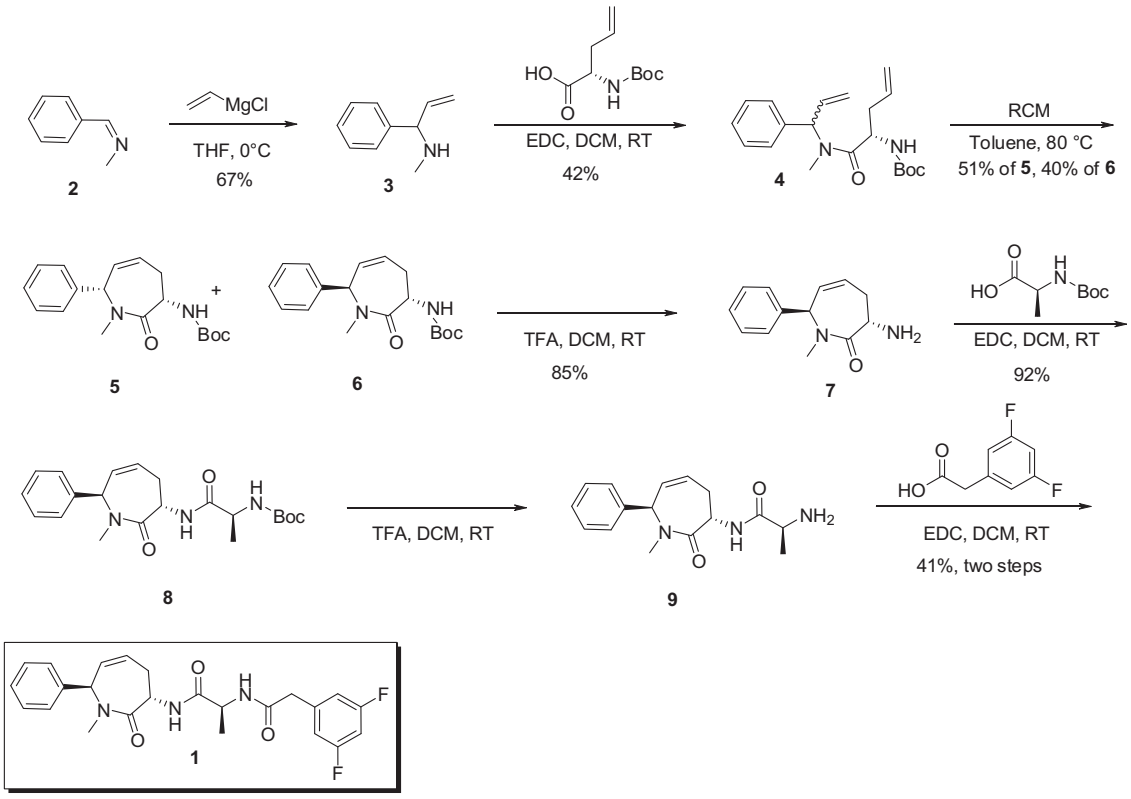
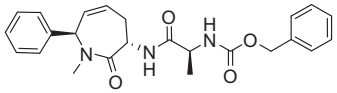
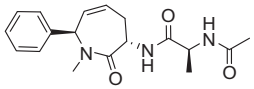
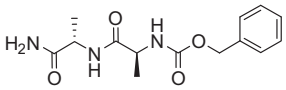
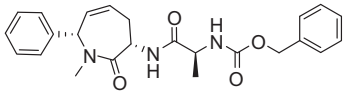
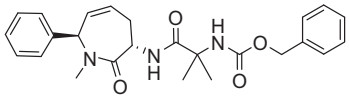
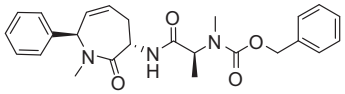
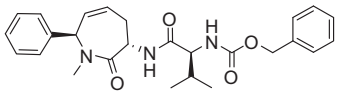
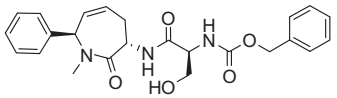


Scheme 1. Synthesis of azepinone amide hit (**1**).

Table 1
Structure–activity relationship of azepinone analogs

Compound number	Structure	Eco IC_{50} (μM ± SE)	Hin IC_{50} (μM ± SE)	Spn IC_{50} (μM ± SE)
10		8.1 ± 1	11.3 ± 1	7.4 ± 1
11		>200	>200	>200
12		>200	>200	>200
13		>200	>200	>200
14		>200	>200	>200
15		>200	>200	>200
16		>200	>200	>200
17		102 ± 18	132 ± 27	71.4 ± 13

IC_{50} values are best-fit values ± standard error (SE).