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Beta-aminoketones as prodrugs for selective irreversible inhibitors of type-1 methionine aminopeptidases

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ABSTRACT

We identified and characterized β-aminoketones as prodrugs for irreversible MetAP inhibitors that are selective for the MetAP-1 subtype. β-Aminoketones with certain structural features form α,β-unsaturated ketones under physiological conditions, which bind covalently and selectively to cysteines in the S1 pocket of MetAP-1. The binding mode was confirmed by X-ray crystallography and assays with the MetAPs from *Escherichia coli*, *Staphylococcus aureus* and both human isoforms. The initially identified tetralone derivatives showed complete selectivity for *E. coli* MetAP versus human MetAP-1 and MetAP-2. Rational design of indanone analogs yielded compounds with selectivity for the human type-1 versus the human type-2 MetAP.

Protein biosynthesis is initiated with methionine (in eukaryotes) or N-formylmethionine (in prokaryotes, mitochondria and chloroplasts). Methionine aminopeptidases (MetAPs) are metalloproteases that remove the initiator methionine from a nascent polypeptide chain. MetAPs play a key role in the N-terminal processing of proteins and are essential in the control of folding, subcellular localization and degradation processes of proteins.[1,2] MetAPs are divided into two subtypes: Bacteria possess only the isoform 1 whereas eukaryotic cells contain both isoforms and archaea only the isoform 2.[3] Since it has been shown that the deletion of the MetAP gene is lethal for bacterial cells,[4,5] the prokaryotic MetAPs are considered as targets for the development of antibacterial drugs. Inactivation of the human MetAP-2 (HsMetAP-2), for example, by fumagillin and its derivatives, causes an inhibition of neoangiogenesis.[6] Fumagillin is an irreversible inhibitor that binds to HsMetAP-2 by alkylation of a histidine residue in the active site.[7] Inhibition of HsMetAP-1 was reported to cause a cell cycle delay in the G2-to-M transition phase and induce apoptosis in leukemia cell lines. Therefore, MetAP-1 may serve as target for the development of new anticancer drugs.[8]

Although the substrate recognition features of MetAPs are practically identical, the binding sites show a certain degree of variability. Most significant for the work described here is the presence of cysteine residues near the active site of isoform-1 MetAPs. These can be targeted by electrophilic functional groups such as α/β-unsaturated ketones. Cys59 (numbering for *Escherichia coli* MetAP), is exclusively present in some bacterial MetAPs and not in the human isoforms, and can be targeted by cysteine-reactive electrophiles. While the present manuscript was prepared, other authors reported the targeting of an active-site cysteine in MetAPs with highly reactive maleimides.[9]

We here report the discovery, optimization and characterization of a novel class of MetAP inhibitors with a covalent binding mode and selectivity for type-1 MetAPs. Initial inhibition assays with the lead compound **1** at *E. coli* MetAP revealed a time-dependent binding mode. This led us to hypothesize that under physiological (or assay) conditions (pH >7), **1** eliminates piperidine and forms the α/β-unsaturated ketone **1b**, which binds to cysteine residues near the active site of *E. coli* MetAP (Scheme 1).

To further elucidate the binding mode of the lead compound **1** and to create a basis for rational optimization, we determined the X-ray structure of **1** in complex with *E. coli* MetAP (PDB accession code: 4PNC). As expected, the unsaturated ketone **1b** is responsible for inhibition of the enzyme. Surprisingly, however, we found that only a single cysteine residue is alkylated by **1b**, whereas the other four solvent-exposed cysteines near the active site remain unaffected (see Fig. 1).

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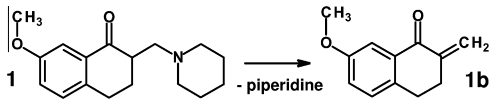

Scheme 1. Elimination of piperidine from the lead compound **1**.

The ketone group of the tetralone system is oriented towards the metal binding site of the protease, but does not directly interact with the metal cofactors. However, a water molecule between the carbonyl oxygen and the two water molecules that coordinate to the two cobalt ions in the active site may mediate electronic or acid-base effects of the metal ions on **1b** and therefore trigger the reaction to the nucleophilic sulfhydryl group. In combination with the sterically ambitious and rigid tetralone ring, this effect is probably responsible for the selective binding of the inhibitor to Cys59.

The following derivatizations of the lead compound **1** had two aims. First, to increase the affinity to bacterial MetAPs, in order to explore the potential of this compound class as antibiotic agents; Second, to create compounds that inhibit the human MetAP-1. The latter enzyme does not contain a cysteine residue in the position of *E. coli* Cys59 and is therefore resistant towards compound **1** (see Table 1). However, a homolog of the *E. coli* Cys70 residue is present in human MetAP-1, and docking simulations indicated that a geometric change from a tetralone to an indanone moiety could result in compounds that selectively target Cys70.

The data compiled in Table 1 allow the following conclusions on the SAR:

- All inhibitors are highly selective for type-1 MetAPs. The human MetAP-2, which does not contain a reactive cysteine residue in this position of the active site, is not inhibited by any of the compounds.
- Inhibition of type-1 MetAPs is directly linked to the formation of α/β-unsaturated ketones, because all stable compounds (**8**–**14**, **20**) do not inhibit MetAPs.
- In accordance with the design considerations outlined above, the indanone congeners are potent inhibitors of the human MetAP-1 and *Staphylococcus aureus* MetAP.
- The antibacterial effect of the compounds is also linked to the formation of α/β-unsaturated ketones, but does not correlate with their inhibitory potential against MetAPs (cf. compounds **18**, **21**, **24**).
- Substituent effects (cf. the indanones **21**–**24**) have a significant effect on the half-lifes of the compounds and therefore on the formation of the reactive α/β-unsaturated ketones and the inhibitory potential of the aminoketones.

For some indanone analogs, the elimination products were also characterized to elucidate whether the differences in inhibitory activity are related to the elimination behavior or a target-specific recognition. As can be seen from Table 2, the inhibitory activity against *E. coli* MetAP is fairly constant for all analogs and therefore the substitution pattern appears to have only minor influence on the target recognition. For the human MetAP-1, however, there is a notable difference in inhibitory potencies that relates to the substituents on the aromatic moiety (cf. **21b** vs **23b**).

To study the unspecific reactivity of the α/β-unsaturated ketones towards thiol nucleophiles, we determined the second-order rate constants for the reaction with glutathione (see Table 2 above and Table S-2 in the Supplementary material). The tetralones **3b** and **4b** are considerably more reactive than all indanones. The unspecific reactivity within the indanone series is highly variable, which is most probably due to electronic influences of the substituent on the aromatic moiety. Especially remarkable is the combination of low unspecific reactivity and high inhibitory potential for compound **21b**. The inhibitory activity of **21b** against

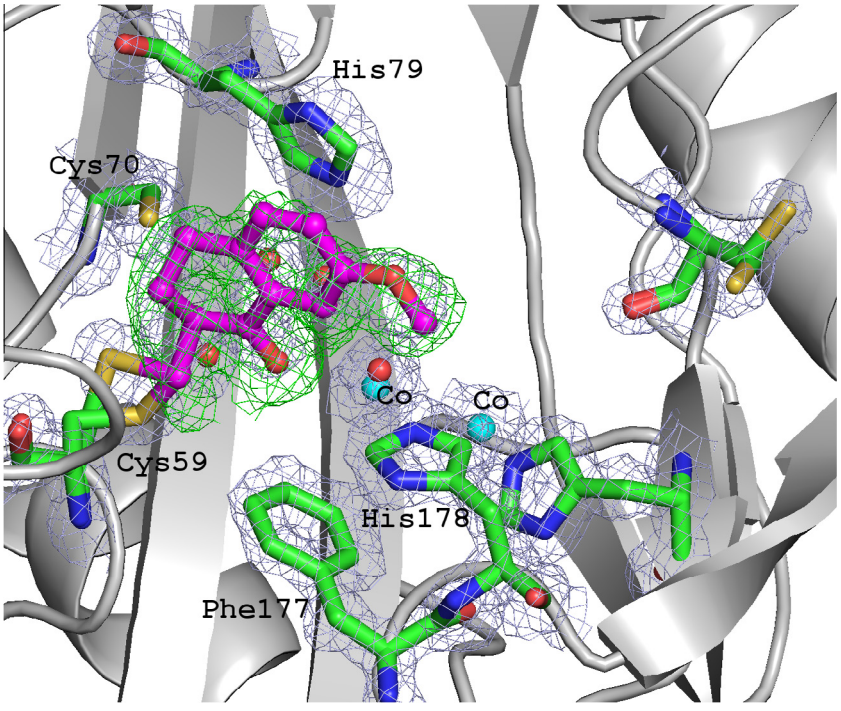

Figure 1. X-ray structure of the unsaturated ketone, derived from compound **1**, bound to *E. coli* MetAP (PDB accession code: 4PNC). The inhibitor is covalently bound to Cys59 and positioned between His79 and His178. Cys70 and the other two cysteines are not modified. The side chain of Cys59, the target cysteine of the inhibitor, is present in two slightly different, alternative conformations. In the background are the two catalytic cobalt ions and three water molecules (lightblue and red spheres, respectively). The 1fo1fc electron density map, calculated with REFMAC5 in absence of the inhibitor, is shown in green.