The Physiological Form Of MetAP2 Can Be Inhibited Through Binding To Either Of The Two Active-Site Metals

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INTRODUCTION

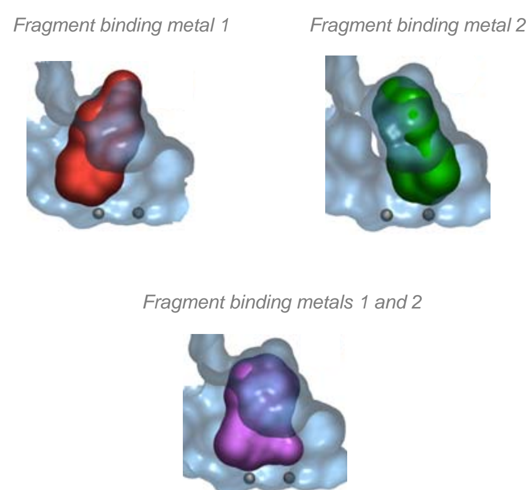
Methionine aminopeptidases (MetAP) are metalloenzymes that remove the N-terminal initiator methionine from newly synthesised polypeptides allowing essential post-translational modifications such as acetylation and myristoylation to take place. MetAP2, one of the two eukaryotic forms of the enzyme, was identified as the target of fumagillin, a natural product with anti-angiogenic properties that inhibits the proliferation of endothelial cells. Clinical activity has been seen for a semi-synthetic analogue of fumagillin, TNP470, suggesting MetAP2 is a good target for inhibiting angiogenesis.

In vitro, MetAP2 appears to have sites for two divalent metal ions within its active site, but there has been much discussion around the identity and number of metal ions actually present in the physiological states of the various MetAPs. An understanding of the physiologically relevant metalloform of the enzyme is essential for designing inhibitors that are active in cells. We have used tool compounds that bind the active site metals in diverse ways to investigate the relevance of the two potential metal binding sites in MetAP2.

METAL BINDING FRAGMENTS

The manganese form of the MetAP2 enzyme was screened using a fragment-based approach, Pyramid™. Multiple low molecular weight fragments were identified that bind the metals at the active-site of MetAP2 in diverse ways.

Figure 1. Fragments Binding To The Active Site Metals Of MetAP2

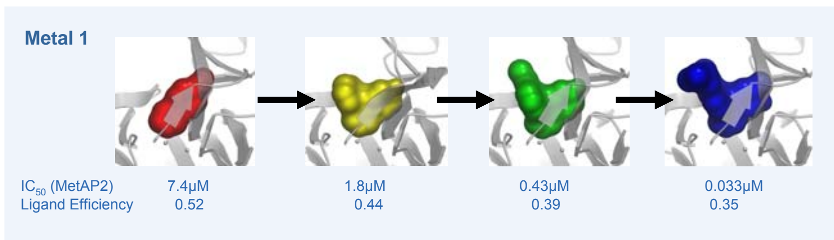


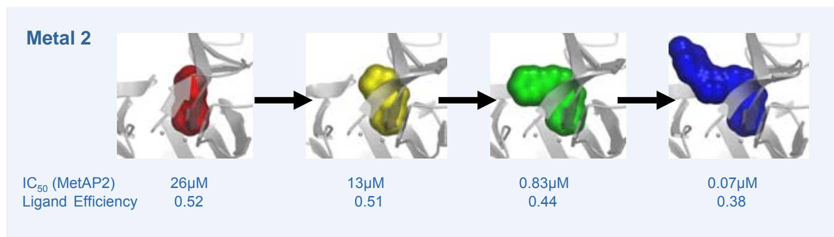
FRAGMENT OPTIMISATION

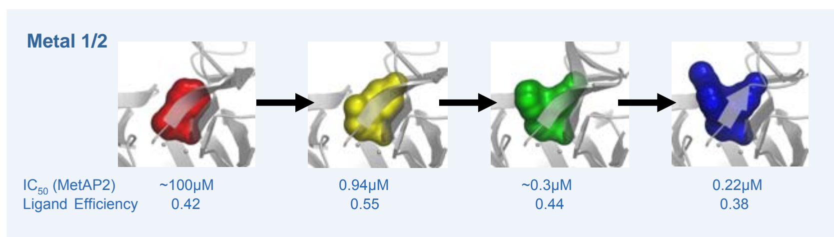
Three hit series were selected for further optimisation by structure-based drug design. These represented three different binding modes to the metals of the MetAP2 active site, binding metal one only (M1), metal two only (M2) and both metals 1 and 2 (M1/2). Each fragment series was optimised to give potent lead compounds, which maintained their respective binding modes to the active-site metals.

Figure 2. Optimisation Of Three Fragment Hits Into Potent Lead Molecules

IC_{50}s, Ligand Efficiencies and MetAP2-ligand crystal structures are shown for key steps in the optimisation process of each lead series.







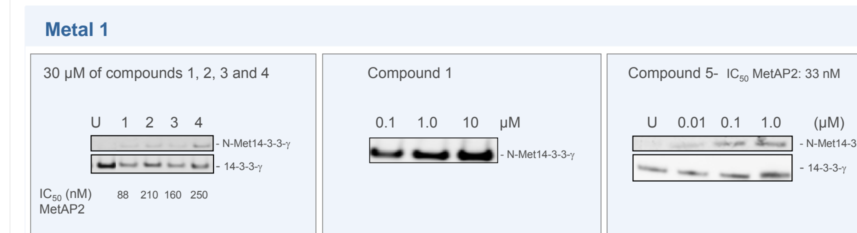
MetAP2 activity of the manganese form of the enzyme was measured to determine IC_{50}s.

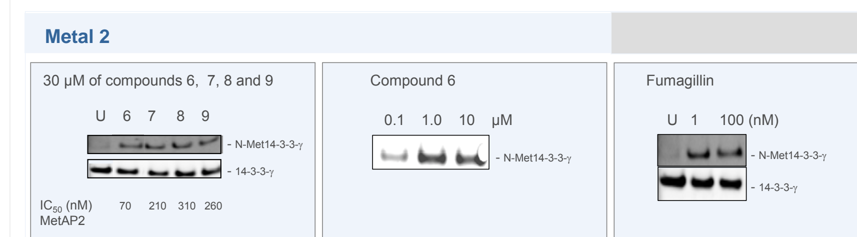
CELLULAR INHIBITION OF METAP2

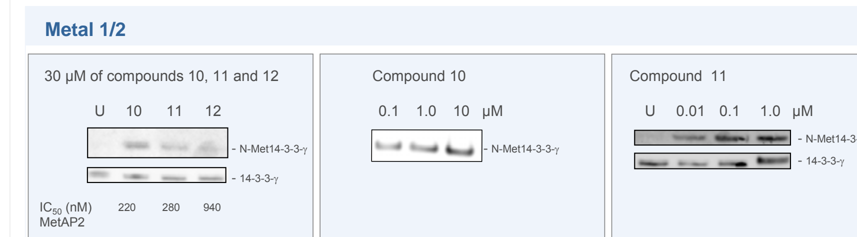
Potent compounds from each series, with anti-proliferative activity against human umbilical vein endothelial cells (HUVECs), were used as tool molecules to investigate the mechanism of action of the different metal binding modes in cells. Levels of the methionylated, unprocessed form of 14-3-3-γ, a MetAP2 substrate, increased in cells treated with M1, M2 or M1/M2 binding compounds indicating a block in processing and inhibition of MetAP2.

Figure 3. MetAP2 Is Inhibited In Cells By Potent Compounds From All Three Series

Levels of methionylated 14-3-3-γ, as determined by western blot, are shown in HUVECs treated with potent compounds from each lead series







HUVECs were treated with the indicated concentrations of compounds for 48 h. Cell extracts were immuno-blotted with a monoclonal antibody to methionylated 14-3-3-γ. Fumagillin, a known potent inhibitor of MetAP2, is shown as a control and U are untreated cells.

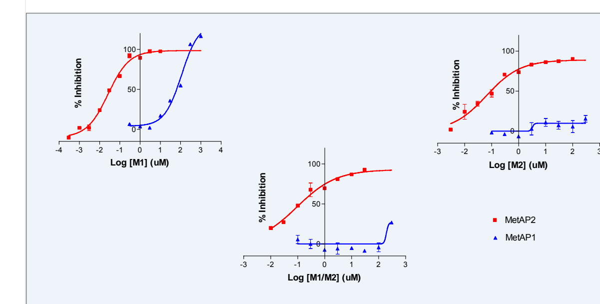
PROFILE OF LEAD COMPOUNDS

Lead compounds were tested for potency against the MetAP2 and MetAP1 enzymes *in vitro* and their inhibitory activity in a HUVEC proliferation assay. All three series inhibited HUVEC proliferation and showed significant selectivity for MetAP2 over MetAP1.

Table 1. Selectivity Of Lead Compounds From Each Series

	MetAP2 IC_{50} (nM)	Fold-Selectivity over MetAP1	HUVEC Proliferation IC_{50} (μM)
M1	33	> 1000	0.3
M2	70	> 4000	5.9
M1/M2	220	> 1000	2.0

Figure 4. Selectivity Of Lead Compounds From Each Series



CONCLUSIONS

- We have identified and optimised chemical series which bind in diverse ways to the active site metals of MetAP2.
- Each of these series selectively inhibited MetAP2 over MetAP1 and inhibited the processing of a MetAP2 substrate, 14-3-3-γ, in cells.
- These data indicate that the physiological form of MetAP2 can be inhibited by compounds which bind solely to either of the two active-site metals and suggests that both these metals are present in the intra-cellular form of the enzyme.
- The three lead series presented here have potential for further optimisation and provide multiple approaches for inhibition of this key angiogenic target through their diverse modes of binding to the active-site metals.

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