ANTI-ANGIOGENIC ACTIVITY OF FRAGMENT-DERIVED INHIBITORS OF METAP2

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INTRODUCTION

Methionine amino peptidase (MetAP) 2 is the target of the anti-angiogenic natural product fumagillin and so is believed to play a role in angiogenesis. MetAPs are metalloenzymes which cleave the N-terminal methionine from newly formed polypeptides. This allows essential post-translational modifications, such as myristoylation and acetylation, to take place thus generating fully functional proteins.

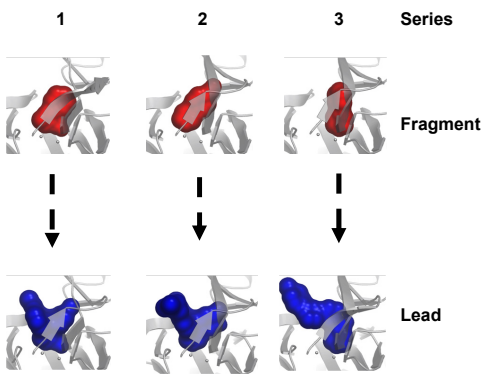
Analogues of fumagillin have shown activity in several different disease models, where angiogenesis may be relevant, including oncology. Semi-synthetic analogues of fumagillin, such as TNP-470, have shown evidence of antitumor activity in the clinic but poor pharmacokinetic properties and neurotoxic effects have limited their development. Nevertheless MetAP2 remains a promising oncology target and inhibitors with improved properties should have potential as anti-angiogenic agents.

We have screened MetAP2 using our fragment-based screening approach (Pyramid™) and identified multiple low molecular weight fragments, which bind at the active site of MetAP2 in diverse ways. Three of these were optimised to novel hit series using structure-based drug design and their anti-angiogenic properties are described here.

OPTIMISATION OF FRAGMENTS

Three novel hit series for MetAP2 were optimised to potent sub-100 nM lead compounds using structure based drug design.

Optimisation of fragment hits to potent lead compounds

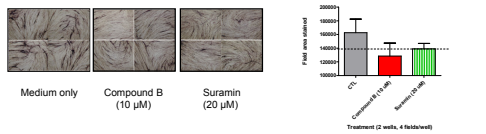


ANTI-ANGIOGENIC PROPERTIES OF METAP2 LEAD COMPOUNDS

The anti-angiogenic properties of Series 1 were investigated further, both *in vitro* and *in vivo*. Treatment of human umbilical vein endothelial cells (HUVEC) with lead compounds appeared to decrease tubule formation in these cells.

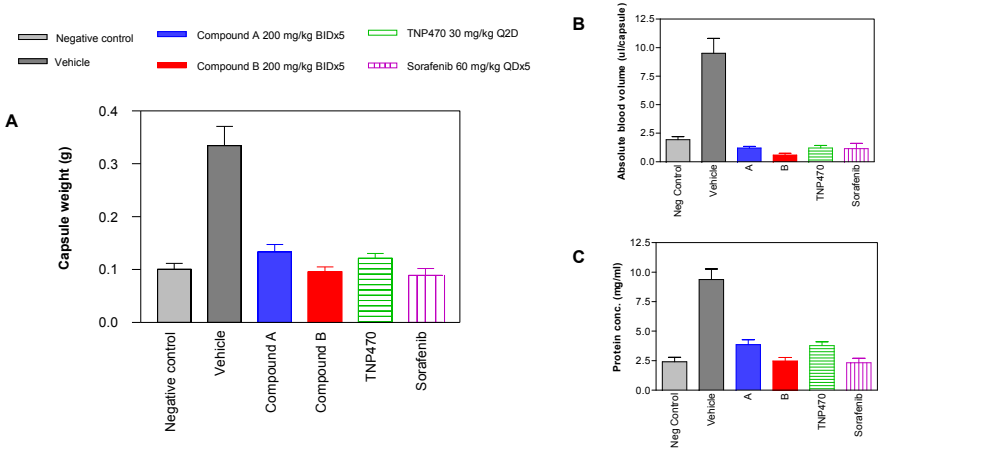
Two compounds (A & B) were selected for testing in an *in vivo* mouse angiogenesis model (AngioChamber™, VivoPharm, Pty Ltd, Kent Town, Australia), where FGF-loaded capsules are implanted subcutaneously in mice. Compounds were dosed orally at 200 mg/kg *bid* for five days and their activity compared to TNP-470, dosed subcutaneously at 30 mg/kg *q2d* and sorafenib dosed orally at 60 mg/kg *qd*. Capsule weight, blood volume and protein concentration were significantly decreased for compound-treated mice compared with vehicle-treated mice indicating significant inhibition of vascularisation. Overall the data suggest that the MetAP2 lead series described here has anti-angiogenic properties in this mouse model.

Inhibition of tubule formation in HUVECs



HUVECs were cultured with compound for 9 days. Tubules were visualised using the Angiokit (TCS Cell works) CD31 antibody and alkaline phosphatase detection. AngioSys image analysis software was used to measure stained tubule area.

Inhibition of angiogenesis in an *in vivo* mouse AngioChamber™ model



Groups of eight mice, randomised by body weight, were implanted with AngioChambers™. Vehicle was administered orally to two groups, one of which was implanted with bFGF-loaded AngioChambers™ (Vehicle) and the other with AngioChambers™ with no bFGF (Negative control). At the end of the study wet weight of capsules was recorded (A) and protein (B) and haemoglobin (C) content was analysed by Bradford and Drabkin assay, respectively.

PROFILE OF LEAD COMPOUNDS

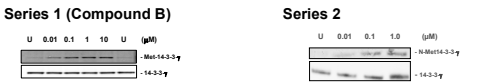
Compounds from each series were selective inhibitors of MetAP2 over MetAP1 and inhibited proliferation of HUVECs.

Levels of the methionylated-14-3-3γ, a MetAP2 substrate, were shown to increase in HUVECs treated with these compounds demonstrating the inhibition of MetAP2 in these cells.

Lead Series Properties

Series	MetAP2 IC_{50} (μM)	MetAP1 IC_{50} (μM)	HUVEC Proliferation IC_{50} (μM)	Cellular MetAP2 inhibition
1	<0.030	7.6% I at 100 μM	0.13	Yes
2	0.033	110	0.3	Yes
3	0.070	16% I at 300 μM	5.9	Yes

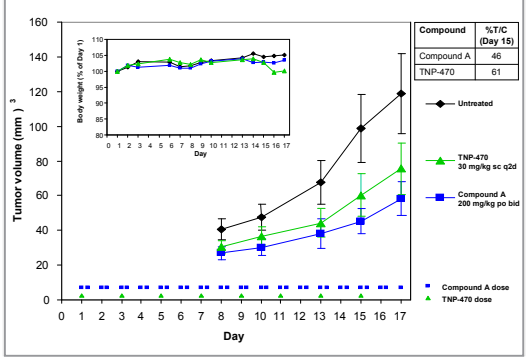
Inhibition of MetAP2 in cells



INHIBITION OF TUMOR GROWTH

Compound A was further evaluated in a mouse HCT116 tumor xenograft model, where cells were inoculated subcutaneously and treatment started on the following day. Tumor growth was inhibited in treated mice compared with control and inhibition was greater in those mice treated with Compound A compared with TNP-470. Compound A was well tolerated.

Inhibition of tumor growth in HCT116 xenograft model



SUMMARY AND CONCLUSIONS

- Three fragment-based lead series have been identified, which potently inhibit MetAP2 *in vitro* and in cells.
- These small molecule, non-covalent MetAP2 inhibitors may have improved properties over the semi-synthetic fumagillin analogues such as TNP470.
- One of these series has been tested in models of angiogenesis and has promising anti-angiogenic properties.
- Preliminary data suggest that these compounds also have anti-tumor activity and warrant further testing *in vivo* to evaluate their anti-tumor properties.
- The compounds presented here have potential for further optimisation and evaluation in both angiogenesis and other indications where MetAP2 is a target.

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