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. MetAP2s 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 to 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.

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 FGFR-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 q3d 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 tube formation in HUVECs

We have screened MetAP2 using our fragment-based screening approach (Pyramid™) and identified multiple low molecular weight fragments, which bind to 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.

Inhibition of angiogenesis in an in vivo mouse AngioChamber™ model

Series MetAP2

<table>
<thead>
<tr>
<th>Series</th>
<th>MetAP2</th>
<th>MetAP1</th>
<th>HUVEC Proliferation</th>
<th>Cellular MetAP2 inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.030</td>
<td>7.6%</td>
<td>100 µM</td>
<td>0.19</td>
</tr>
<tr>
<td>2</td>
<td>0.033</td>
<td>90%</td>
<td>100 µM</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>0.070</td>
<td>16%</td>
<td>100 µM</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Series 1 (Compound B)

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 potentiate inhibit MetAP2 in vitro and in cells.

• These small molecule, non-covalent MetAP 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.

Acknowledgements: The AngioChamber™ experiment was carried out by VivoPharm Pty Ltd, Australia.

© Astex Pharmaceuticals Inc. Poster presented at Targeted Anticancer Therapies: March 8-10 2012 Poster can be downloaded from www.astx.com©