Development of a potent class of small molecule inhibitors of the MDM2-p53 protein-protein interaction

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INTRODUCTION

In response to cellular stress, the p53 tumour suppressor is activated to modulate cell cycle progression, DNA repair, and apoptosis. p53 activity is tightly regulated by MDM2, an E3 ubiquitin ligase that targets p53 for proteasomal degradation. Inhibition of the MDM2-p53 interaction in tumours carrying wild-type p53 prevents its degradation and can reactivate p53 to elicit an anti-cancer effect. Development of small molecule inhibitors of the MDM2-p53 interaction remains a promising strategy for cancer therapy and a number of these compounds are in clinical development.

An isoindolinone series, identified by the Northern Institute for Cancer Research (NICR)1, has been used as a starting point for the development of novel potent MDM2-p53 inhibitors. Structure-based drug design was applied during lead optimisation to gain potency whilst also focusing on stabilizing the main metabolically labile position and reducing lipophilicity. This approach led to the identification of promising lead compounds meritting further optimisation of the series.

Further lead optimisation resulted in potent isoindolinone MDM2 inhibitors

### Optimisation of early lead compounds resulted in isoindolinone MDM2 inhibitors with good oral bioavailability

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (nM) MDM2 inhibition</th>
<th>GI50 (μM) 72h</th>
<th>T½ (min) Cpd 3</th>
<th>Non-deuterated</th>
<th>Deuterated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 1</td>
<td>12</td>
<td>0.49</td>
<td>&gt;2500</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Cpd 4</td>
<td>3.8</td>
<td>0.20</td>
<td>20% at 30μM</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

A. Isoindolinones are inhibitors of MDM2 and show activity in p53WT SJSA-1 cell line. GI50 values were determined in a Cell Titre Glo assay after 72 hours of compound treatment.

B. Cpd 1 displayed metabolic clearance in vitro following incubation in human and mouse liver microsomes (LM) at 37°C. Cpd 1 was also a CYP3A4 substrate. Very high plasma protein binding (PPI) resulted in low, unmetabolised plasma clearance (C1p) and poor oral bioavailability (PO %F) in the mouse. Cpd 1 was administered to mice by the IV and oral route at 1 and 5mg/kg, respectively. Cpd 1 rapidly disappeared in mouse liver microsomes, generating a carboxylic metabolite which was identified as Cpd 2.

C. The major metabolic clearance route of Cpd 1 was phase 1 oxidation of the C3 sidechain to a carbonylic acid metabolite (Cpd 2).

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<tr>
<th>Compounds</th>
<th>IC50 (nM) MDM2 inhibition</th>
<th>GI50 (μM) 72h</th>
<th>T½ (min) Cpd 5</th>
<th>Cpd 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 5</td>
<td>&lt;1 nM</td>
<td>&lt;1 nM</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Cpd 6</td>
<td>&lt;1 nM</td>
<td>&lt;1 nM</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>

A. Deuterating the C3 sidechain of Cpd 3, to give Cpd 4, reduced the rate of oxidation; half-life in human and non-human primate (NHP) liver microsomes was increased and the in vitro CYP3A4 half-life also increased from 6 to 14 minutes.

B. Oral bioavailability in NHP increased from 8%, for the non-deuterated Cpd 5, to 31%, for the deuterated Cpd 4. Compounds were administered to NHP by the oral route at 5mg/kg.

C. Cpd 5 was tested in a panel of wild-type p53 cell lines and potently inhibited growth of a range of haematological and solid tumour cell lines. Growth inhibition assays (72 hours) were quantified using the sulforhodamine B (SRB) assay.

### SUMMARY AND CONCLUSIONS

- Small molecule inhibitors of the MDM2-p53 interaction reactivate p53 in tumours carrying wild-type p53 and have an anti-cancer effect.
- Both isoindolinones and RG7388 are specific for p53WT and induce p53 and its target genes, with IC50 <1 nM against MDM2 in cell-free ELISA assays and EC50 <30 nM for p53 induction in SJSA-1 osteosarcoma cells.
- Good oral bioavailability was achieved by blocking phase 1 metabolism on the C3 sidechain of the isoindolinone series.
- Strong induction of p53, together with an increase in the expression of p53 target genes MDM2 and p21 following oral administration of Cpd 6, resulted in efficacy in an SJSA-1 xenograft model.

REFERENCES