Abstract No. 1652

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 meriting further optimization of the series.

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

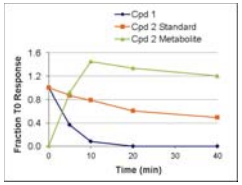
A.

Compounds	IC_{50} (nM) MDM2 inhibition	GI_{50} (μM) SJSA-1 $p53^{WT}$	GI_{50} (μM) SJSA-1 $p53^{mut}$
Cpd 1	12	0.49	18
Cpd 4	3.8	0.20	20% at 30μM

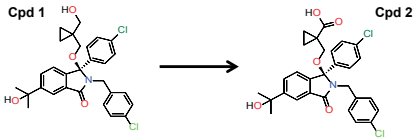
B. Cpd 1 DMPK

% Human PPB		99.7
% Mouse PPB		99.8
Human LM T½ (min)		<5
Mouse LM T½ (min)		10
CYP3A4 T½ (min)		<5
Mouse	Clp (mL/min/kg)	25
	PO %F	7

C. Cpd 1 incubated in Mouse Liver Microsomes



D.



A. Isoindolinones are inhibitors of MDM2 and show activity in $p53^{WT}$ SJSA-1 cell line. GI_{50} values were determined in a Cell Titre Glo assay after 72 hours of compound treatment.

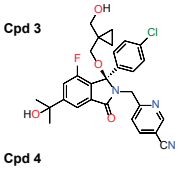
B. Cpd 1 displayed metabolic clearance *in vitro* following incubation in human and mouse liver microsomes (LM) at 1μM. Cpd 1 was also a CYP3A4 substrate. Very high plasma protein binding (PPB) resulted in low, restricted plasma clearance (Clp) and poor oral bioavailability (PO %F) in the mouse. Cpd 1 was administered to mice by the IV and oral route at 1 and 5mg/kg, respectively.

C. Cpd 1 rapidly disappeared in mouse liver microsomes, generating a carboxylic metabolite which was identified as Cpd 2.

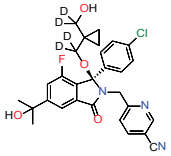
D. The major metabolic clearance route of Cpd 1 was phase 1 oxidation of the C3 sidechain to a carboxylic acid metabolite (Cpd 2).

Deuteration of the C3 sidechain of Cpd 3 resulted in good oral bioavailability in non-human primate

Cpd 3



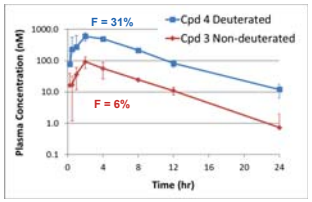
Cpd 4



A.

In vitro T½ (min)	Cpd 3 Non-deuterated	Cpd 4 Deuterated
Human LM	18	52
NHP LM	7	17
CYP3A4	6	14

B. Cpd 3 and 4 NHP Oral Exposure: 5mg/kg PO

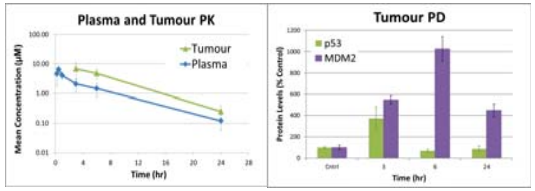


A. Deuterating the C3 sidechain of Cpd 3, to give Cpd 4, reduced the rate of oxidation; half-life in human and non-human primate (NHP) liver microsomes was increased and the *in vitro* CYP3A4 half-life also increased from 6 to 14 minutes.

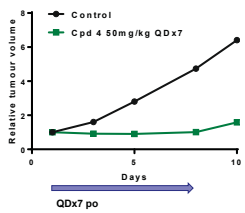
B. Oral bioavailability in NHP increased from 6%, for the non-deuterated Cpd 3, to 31%, for the deuterated Cpd 4. Compounds were administered to NHP by the oral route at 5mg/kg.

Cpd 4 displayed good oral activity in a SJSA-1 human osteosarcoma xenograft model in mice

A Cpd 4 administered at 50mg/kg PO to mice bearing SJSA-1 xenografts



B.



A. Cpd 4 displayed good plasma exposure and tumour distribution following single oral administration at 50mg/kg to mice bearing SJSA-1 human osteosarcoma xenografts. Target engagement was confirmed in SJSA-1 tumours; p53 stabilisation and activation of target genes, including MDM2 induction. Levels of p53 and MDM2 were measured by a Mesoscale Discovery (MSD) assay.

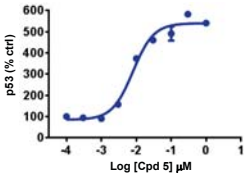
B. Cpd 4 was orally-active in the SJSA-1 human osteosarcoma xenograft model following daily dosing (QD) at 50mg/kg to mice for 7 days.

Further lead optimisation resulted in potent isoindolinone MDM2 inhibitors

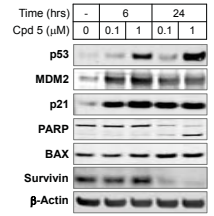
A.

Compounds	IC_{50} (nM) MDM2 inhibition	EC_{50} (nM) p53 induction
Idasanutlin (RG7388)	<1 nM	13
Cpd 5	<1 nM	26
Cpd 6	< 1 nM	3.5

B. Cpd 5 p53 Induction



C. Cpd 5 SJSA-1 Cell Line



A. Cpds 5 and 6 showed comparable potency to RG7388, against MDM2 in cell-free ELISA assays and for p53 induction in SJSA-1 osteosarcoma cells. Cpd 5 and 6 in a cell-free ELISA assay had an IC_{50} <1 nM against MDM2 (n ≥ 3).

B. In an MSD assay Cpd 5 demonstrated an EC_{50} <30 nM for p53 induction in SJSA-1 cells after two hours.

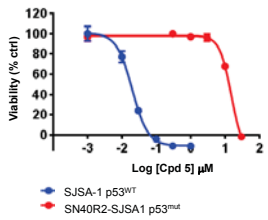
C. Cpd 5 modulates the expression of p53 pathway-related genes in SJSA-1 cells.

Isoindolinones are selective for wild-type p53

A.

Cell lines / Compounds	GI_{50} (μM) SJSA-1 $p53^{WT}$	GI_{50} (μM) SJSA-1 $p53^{mut}$	GI_{50} (μM) A2780 $p53^{WT}$	GI_{50} (μM) A2780 $p53^{mut}$	GI_{50} (μM) HCT-116 $p53^{WT}$	GI_{50} (μM) HCT-116 $p53^{-/-}$
RG7388	0.038	9.5	0.077	8.3	0.200	7.9
Cpd 5	0.011	12	0.017	11	0.019	9.4
Cpd 6	0.025	15	0.047	15	0.099	15

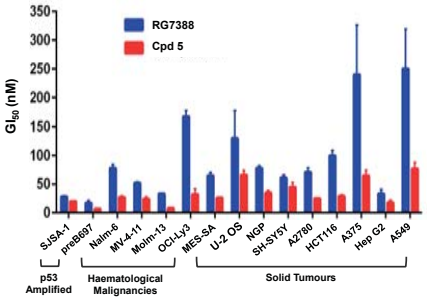
B. Cpd 5 Cell Growth Inhibition



A. Cpd 5, Cpd 6 and RG7388 are specific for $p53^{WT}$ cells. Viabilities of three paired isogenic $p53^{WT}$/$p53^{mut}$ or $p53^{WT}$/$p53^{-/-}$ cell lines were analysed following 72 hours of compound treatment using the sulforhodamine B (SRB) assay (n ≥ 3).

B. Cpd 5 strongly inhibited the viability of $p53^{WT}$ SJSA-1 osteosarcoma cells, but not that of the paired isogenic $p53^{mut}$ SN40R2 cells. Cell viability was measured after 72 hours of compound treatment using the SRB assay.

C. $p53^{WT}$ Cell Line Panel

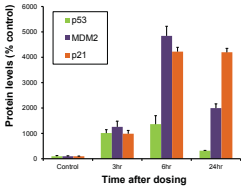


C. Cpd 5 was tested in a panel of wild-type p53 cell lines and potently inhibited growth of a range of haematological and solid tumour cell lines. Growth inhibition assays (72 hours) were quantified using the SRB (monolayer cells) assay or XTT cell proliferation kit II (Roche).

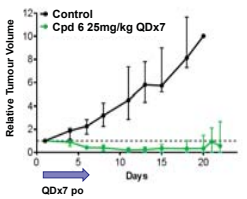
Cpd 6 showed regression in a SJSA-1 human osteosarcoma xenograft model in mice

Cpd 6 administered at 25 mg/kg PO to mice bearing SJSA-1 xenografts

A.



B.



A. Induction of tumour p53, p21, and MDM2 in SJSA-1 xenografts following single oral administration of Cpd 6 at 25 mg/kg to mice. Levels of p53, MDM2 and p21 were measured by a MSD assay.

B. Tumour regression in the SJSA-1 human osteosarcoma xenograft model following daily dosing (QD) of Cpd 6 at 25mg/kg to mice for 7 days.

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 $p53^{WT}$ and induce p53 and its target genes, with IC_{50} <1 nM against MDM2 in cell-free ELISA assays and EC_{50} <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

1. Hardcastle *et al.* (2011), *J Med Chem* 54:1233-43

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