opnMe by Boehringer Ingelheim

MDM2–p53 antagonist

BI-0282

Boehringer Ingelheim

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Summary

BI-0282 is a small molecule antagonist of the MDM2 and p53 protein-protein interaction. BI-0282 shows good permeability and cellular potency and is suitable for oral dosing in *in vivo* studies.

Chemical Structure

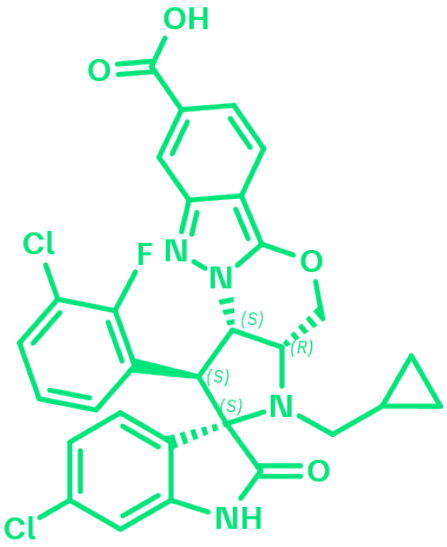


Figure 1: 2D structure of BI-0282, an antagonist of the protein-protein interaction between MDM2 and p53.

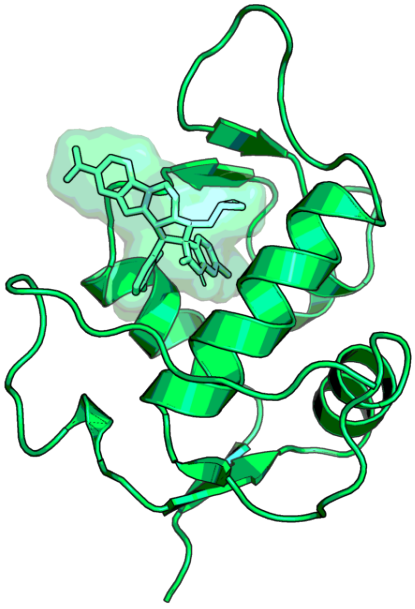


Figure 2: 3D structure of the complex of MDM2 with BI-0282, as resolved by X-ray crystallography.

Highlights

BI-0282 is a small molecule antagonist of the protein-protein interaction between the tumor suppressor p53 and its negative regular MDM2, leading to p53 activation in TP53 wild-type tumors[1,2]. BI-0282 binds to the p53 binding pocket located at the N-terminus of the MDM2 protein, thereby inhibiting the binding of p53 to MDM2. However, BI-0282 does not inhibit the E3 ubiquitin ligase activity of the MDM2 protein. BI-0282 shows good permeability and cellular potency and is suitable for oral dosing in *in vivo* studies.

Target information

p53 is a pivotal tumor suppressor[3]. As a transcription factor p53 regulates multiple downstream target genes that are involved in cell cycle arrest or senescence, promote DNA repair or initiate apoptosis. In healthy cells p53 protein levels are kept at a low basal level, which is achieved by rapid proteasome-mediated degradation. In cells that are exposed to stress or that are damaged, p53 is rapidly activated. In tumor cells the gene encoding p53 (TP53) is frequently mutated[4]. In fact, the TP53 gene is one of the most frequently mutated genes in human cancer with about 50% of all cancers having mutations or deletions in this gene. In the remaining 50% of human cancers the function of p53 is frequently attenuated by other mechanisms, including overexpression/amplification of its key negative regulator MDM2, an E3 ligase that regulates p53 function and protein stability by three main mechanisms. First, MDM2 binds to the transactivation domain of p53 and represses transcriptional activity of p53. Secondly, MDM2 transports the transcription factor p53 from the nucleus to the cytoplasm and third, the E3 ligase function of MDM2 facilitates proteasome-mediated degradation of p53[5]. BI-0282 is an antagonist of the protein-protein interaction between MDM2 and p53, restoring the p53 tumor suppressor activation in tumors with p53 wild-type status.

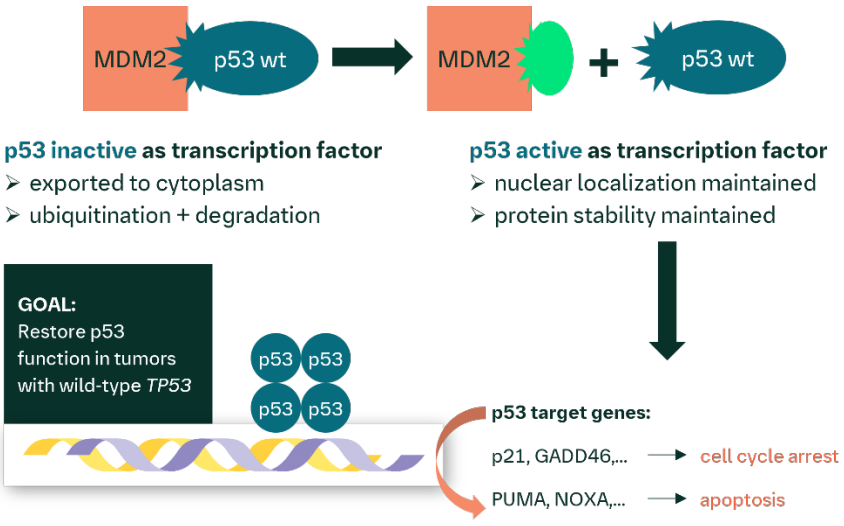


Figure 3: Mechanism of action of BI-0282, a small molecule that blocks the interaction of p53 with its negative regulator MDM2.

In vitro activity

BI-0282 exhibits single digit nanomolar potency (IC_{50} = 5 nM) in an ALPHASCREEN assay, which measures the interaction of human MDM2 with a human p53-derived peptide. Cellular potency was assessed in an MDM2-amplified, p53 wild-type cancer cell line and a good antiproliferative activity was demonstrated (SJSA-1; IC_{50} = 152 nM).

Probe name / Negative control	BI-0282	BI-0283
MW [Da][a]	593.44	593.44
MDM2::p53 Alpha assay (IC_{50}) [nM][b]	5	454
Celluar potency SJSA-1 (IC_{50}) [nM][c]	152	7,408

[a]The molecule is supplied in salt form; for the molecular weight of the salt, please refer to the vial label.
[b]Assay conditions: ALPHASCREEN Assay: This assay was developed to identify compounds which interfere with the p53-MDM2 interaction and thus restore p53 function. For profiling compounds are diluted to a final start concentration of 25 µM followed by 10 subsequent 1:5 dilution steps. Compounds are tested in duplicates. The assay is run on a fully automated robotic system in a darkened room below 100 Lux. To 5 µl of compound dilution (final dilution in the assay 1:400, final DMSO concentration 5%) 5 µl of MDM2/p53 peptide mix are added into columns 1-23, 5 µl of assay buffer into column 24. After an incubation time of 15 minutes at room temperature 5 µl of bead mix are added. Plates are kept at room temperature in a darkened incubator.
After a 30 minute incubation time the signal is measured in a PerkinElmer Envision HTS Multilabel Reader using the AlphaScreen specs from PerkinElmer. Each plate contains 16 wells of a negative control (diluted DMSO instead of test compound; w protein peptide mix; column 23) and 16 wells of a positive control (diluted DMSO instead of test compound; w/o protein peptide mix column 24). As internal control a known inhibitor of MDM2/p53 interaction is used in the same compound dilution scheme. IC_{50} values are calculated and analyzed in the MEGASTTAR IC_{50} application using a 4 parametric logistic model.
[c]Assay conditions: Cytotoxicity Assay CellTiter Glow SJSA-1: 2000 SJSA-1 cells are seeded in 180 µl RPMI + 10% FCS, + Penstrep, into a 96-well plate, flat bottom. The plates are incubated at 37 °C in a CO_2 incubator overnight. The compounds are diluted to the appropriate start concentration between 10 and 100 µM. The cells are incubated with the compounds for 3 days. Then, 30 µl of CellTiter Glow are added to each well, it is agitated for 30 minutes, and the luminescence is measured. IC_{50} values are calculated using the Smiley program (based on GrapPad Prism) or the MEGASTAR IC_{50} Application.

In vitro DMPK and CMC parameters

BI-0282 is a small molecule with a high lipophilicity and low aqueous solubility. It has a good stability in liver microsomes and hepatocytes and is highly bound to plasma proteins. The absorptive permeability of BI-0282 is good, with a low efflux ratio as determined by the Caco2-assay. No relevant inhibition of the hERG channel and CYP3A4 was observed by subjecting BI-0282 to the according assays.

Probe name / Negative control	BI-0282	BI-0283
logD @ pH 7.4. / logP	2.7/ 6	n.d.
Solubility @ pH 6.8 [mg/ml]	0.020	0.015
Caco-2 permeability @ pH 7.4 [$*10^{-6}$ cm/s]	39	n.d.
Caco-2 efflux ratio	2.1	n.d.
Microsomal stability (human/mouse/rat) [% Q_H]	<24/ 40 / <23	<24/ 33 / <23

Hepatocyte stability (human/mouse/rat) [% Q_H]	10/19/6	16/16/17
Plasma Protein Binding (human/mouse/rat) [%]	>99.8/>99.9/>99.9	n.d.
hERG [inh. % @ 1 µM]	4	n.d.
CYP 3A4 Mid/(Tes/Nif) (IC_{50}) [µM]	48/48/>50	n.d.
CYP 2C8 (IC_{50}) [µM]	9	n.d.
CYP 2C9 (IC_{50}) [µM]	18	n.d.
CYP 2C19 (IC_{50}) [µM]	>50	n.d.
CYP 2D6 (IC_{50}) [µM]	>50	n.d.
CYP 2B6 (IC50) [µM]	>50	n.d.
CYP 1A2 (IC50) [µM]	>50	n.d.

In vivo DMPK parameters

BI-0282 shows high permeability and low systemic clearance, resulting in a high bioavailability in mice and rats. It demonstrated a dose-linear PK with low variability in AUC and C_{max} across species.

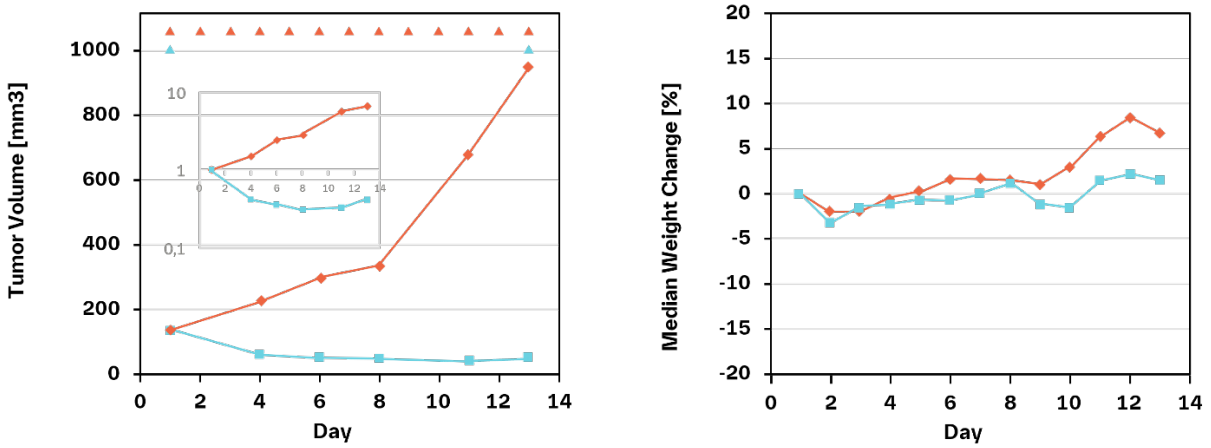
BI-0282	Mouse	Rat
Clearance [% Q_H][a]	11.8	3.1
Mean residence time after *i.v.* dose [h][a]	6.5	3.8
t_{max} [h][b]	2.8	1.7
C_{max} [nM][b]	2,700	12,000
V_{ss} [l/kg][a]	3.8	0.5
F [%]	97	39

[a] *i.v.* dose 5 mg/kg
[b] *p.o.* dose 25 mg/kg

In vivo pharmacology

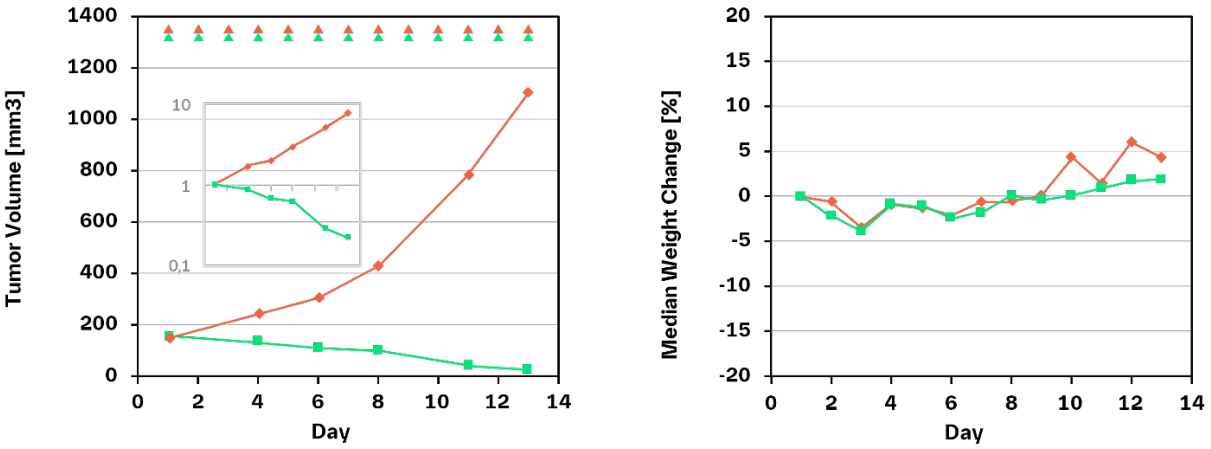
In vivo efficacy has been assessed in the p53 wild-type SJSA-1 cell-line derived xenograft (CDX) model that carries an MDM2 amplification and has been widely used also externally to assess efficacy of MDM2-p53 antagonists.

Efficacy of BI-0282 was assessed in this osteosarcoma model, using two different oral dose-schedules: a daily oral dose-schedule or a single-oral dose schedule. The minimal efficacious dose to achieve tumor regression was a daily oral dose of 15 mg/kg, corresponding to an AUC_{eff} of 37,000 nMh or a single oral dose of 50 mg/kg, corresponding to an AUC_{eff} of 181,000 nMh.



		Dose [mg/kg]	Schedule	AUC_{0-24h} [nMh]	TGI [%]	Regressions [x/8]	Mortality [x/8]
BI-0282		50	Single dose	181,000	111	8	0

Figure 4: Efficacy of BI-0282 after a single oral dose in the SJSA-1 xenograft model



		Dose [mg/kg]	Schedule	Weekly dose [mg/kg]	AUC_{0-24h} [nMh]	TGI [%]	Regressions [x/8]	Mortality [x/8]
BI-0282		15	qd	105	37.000	113	8	0

Figure 5: Efficacy of BI-0282 after daily oral dosing the SJSA-1 xenograft model

Negative control

BI-0283 is the enantiomer of BI-0282. As distomer, BI-0283 is almost inactive and can be used as an ideal negative control.

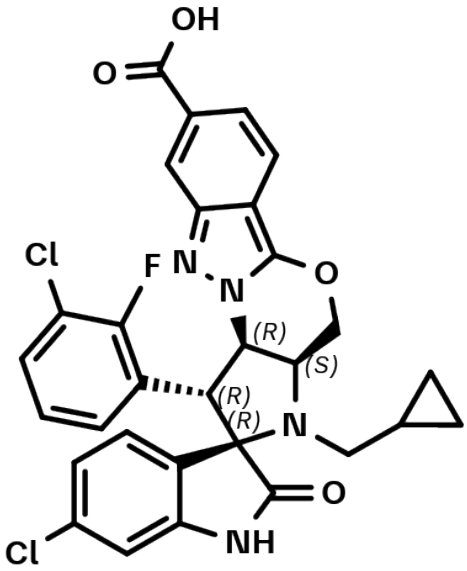


Figure 4: 2D structure of BI-0283, which serves as a negative control.

Selectivity

The selectivity of BI-0282 was assessed in the SafetyScreen44™, where it hit 3 out of 44 targets more than 50% at the high concentration of 10 µM. BI-0282 was further tested in the Invitrogen kinase panel (number of kinases: 31) and 0 kinases were hit more than 50% at 10 µM.

Selectivity data available	BI-0282	BI-0283
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No

Reference molecule(s)

Other available tool compounds: BI-0252.

Supplementary data

2D structure files can be downloaded free of charge from opnMe

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