opnMe by Boehringer Ingelheim

KRAS switch I/II pocket inhibitor

BI-2852

Boehringer Ingelheim

Table of contents

Summary 2
Chemical Structure 2
Highlights 3
Target information 3
In vitro activity 4
In vitro DMPK and CMC parameters 4
In vivo DMPK parameters 5
In vivo pharmacology 5
Negative control 5
Selectivity 6
Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein 6
Supplementary data 6
References 6

Summary

The *in vitro* tool compound BI-2852 is a potent nanomolar inhibitor of the KRAS switch I/II pocket and directly inhibits both the active and inactive forms of KRAS.

Chemical Structure

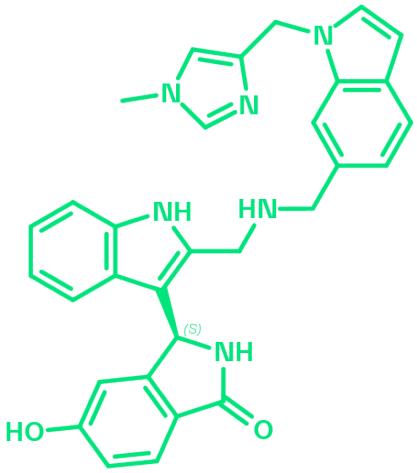

Figure 1: 2D structure of BI-2852

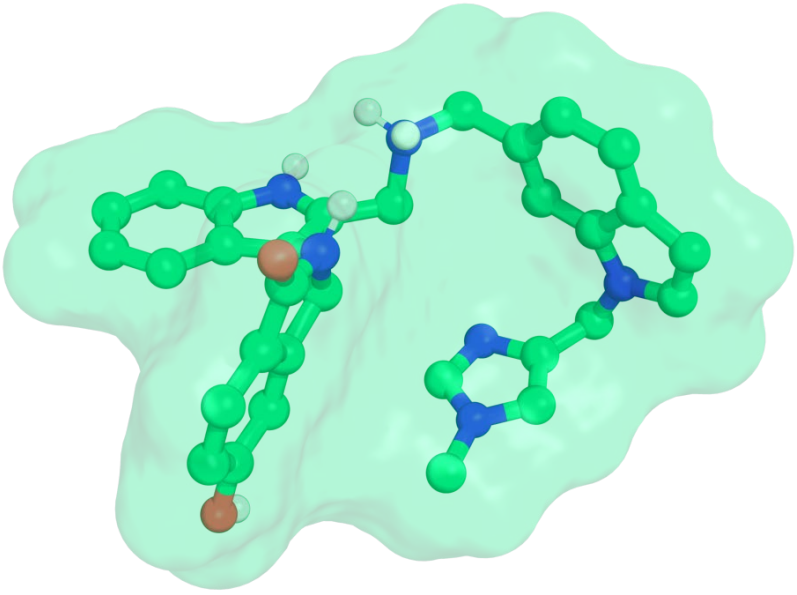

Figure 2: BI-2852, 3D conformation

Highlights

BI-2852 is a potent nanomolar inhibitor of the KRAS switch I/II pocket. It directly targets and inhibits active GTP-bound KRAS. This compound is suitable for *in vitro* experiments and may be an excellent tool for testing KRAS-related biological hypotheses. pERK modulation and antiproliferative effects were observed in $KRAS^{mut}$ cells (NCI-H358) treated with BI-2852[5,6].

Target information

The three human *RAS* genes, *KRAS*, *NRAS* and *HRAS* encode four different RAS proteins (KRAS-4A, KRAS-4B, NRAS and HRAS) which belong to the protein family of small GTPases. The RAS proteins function as molecular switches between active GTP-bound and inactive GDP-bound conformations. RAS is the most frequently mutated oncogene in human cancers (~27%) with activating mutations mainly in codons 12, 13 and 61. The main mutation in codon 12 causes RAS activation by interfering with GAP binding and GAP-stimulated GTP hydrolysis. *KRAS* mutations rates are high in pancreatic (~90%), colorectal (~45%) and lung adenocarcinomas (~35%)[1,2]. KRAS could serve as an excellent drug target for many cancers, but direct inhibition of oncogenic RAS has proven to be challenging[1-6].

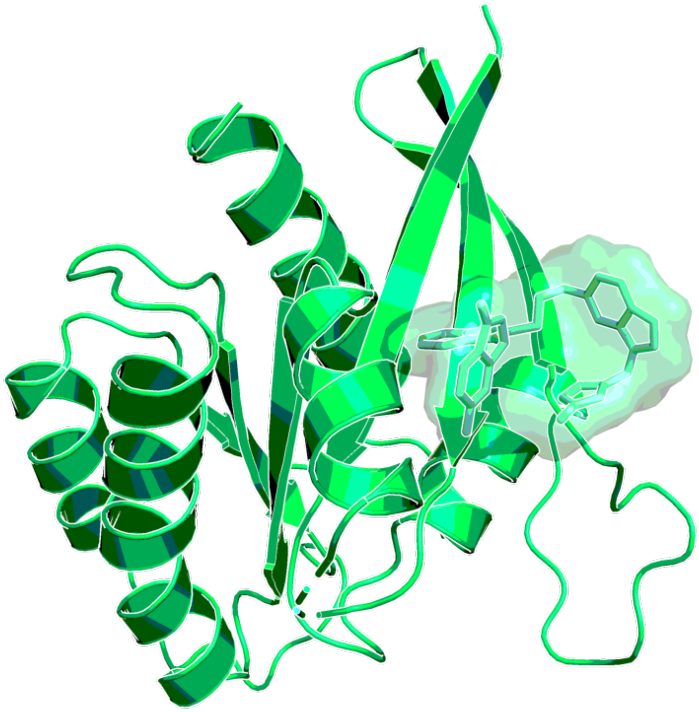

Figure 3: Complex of KRAS with BI-2852

In vitro activity

BI-2852 binds to KRAS^{G12D} with a KD of 740 nM (ITC) and inhibits GTP-KRAS^{G12D} binding to effectors like SOS1, CRAF and PI3K with an IC_{50} of 490, 770 and 500 nM. BI-2852 displays an IC_{50} of 490 nM in a GTP-KRASG12D::SOS1 AlphaScreen (AS) assay leading to low micromolar inhibition (EC_{50} of 5.8 µM) of pERK (in H358 cell line)[5].

PROBE NAME / NEGATIVE CONTROL	BI-2852	BI-2853
MW [Da, free base][a]	516.6	516.6
ITC (K_D) GCP-KRAS^{G12D} [µM][b]	0.74	n.a.
ITC (K_D) GCP-KRAS^{wt} [µM][b]	7.5	n.a.
ITC (K_D) GDP-KRAS^{G12D} [µM][b]	2.0	n.a.
ITC (K_D) GDP-KRAS^{wt} [µM][b]	1.1	n.a.
AS (IC_{50}) GTP-KRAS^{G12D}::SOS1 [nM][b]	490	4400
AS (IC_{50}) GTP-KRAS^{G12D}::CRAF [nM][b]	770	n.a.
AS (IC_{50}) GTP-KRAS^{G12D}::PI3K [nM][b]	500	n.a.
AS (IC_{50}) GDP-KRAS^{G12D}::SOS1 [nM][b]	260	2500
AS (IC_{50}) GTP-KRAS^{wt}::SOS1 [nM][b]	490	n.a.
EC_{50} pERK H358 cells (2 h) [µM][b]	5.8	>50
EC_{50} H358 cells (low serum) [µM][b]	6.7	n.a.

[a] For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs
[b] For assay conditions please refer to reference 5

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-2852	BI-2853
logP @ pH 11	2.6	2.6
Solubility @ pH 6.8 [µg/mL]	18	21
Caco-2 permeability AB @pH7.4 [*10^{-6} cm/s]	5.0	<1.15
Caco-2 efflux ratio	2.1	n.a.

Microsomal stability (human/mouse/rat) [% Q_H]	91 / 93 / 90	92 / 95 / 86
Hepatocyte stability (human/mouse/rat) [% Q_H]	12 / 21 / 25	12 / 69 / 52
Plasma Protein Binding (human/mouse/rat) [%]	98.8 / 99.5 / 98.5	98.7 / 99.1 / 98.6
CYP 3A4 (IC_{50}) [µM]	4.4	n.a.
CYP 2C8 (IC_{50}) [µM]	8.4	n.a.
CYP 2C9 (IC_{50}) [µM]	4.8	n.a.
CYP 2C19 (IC_{50}) [µM]	11.0	n.a.
CYP 2D6 (IC_{50}) [µM]	15.0	n.a.

In vivo DMPK parameters

No data available, BI-2852 is an *in vitro* tool compound.

In vivo pharmacology

No data available, BI-2852 is an *in vitro* tool compound.

Negative control

BI-2853 is the less active enantiomer of BI-2853. It shows no effect on cells and is around 10-fold less potent in the AS assays.

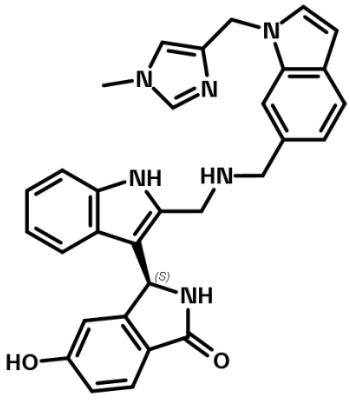

Figure 4: BI-2853 which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	BI-2852	BI-2853
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

The X-ray crystal structure of target in complex with BI-2852 is available (PDB code: 6GJ8)[5,6].

Supplementary data

2D structures can be downloaded free of charge from opnMe.

References

1. Wang Y., Kaiser C. E., Frett B., and Li H. Targeting Mutant KRAS for Anticancer Therapeutics: A Review of Novel Small Molecule Modulators *J. Med. Chem.* 2013, 56, 5219–5230. DOI: 10.1021/jm3017706, PubMed: 23566315.

2. Cox D., Fesik S. W., Kimmelman A. C., Luo J., Der C. J. Drugging the undruggable RAS: Mission possible? *Nat. Rev. Drug. Discov.* 2014, 13, 828-851. DOI: 10.1038/nrd4389, PubMed: 25323927.

3. Stephen A. G., Esposito D., Bagni R. K., McCormick F. Dragging ras back in the ring *Cancer. Cell* Mar 17, 25(3), 272-281. DOI: 10.1016/j.ccr.2014.02.017, PubMed: 24651010.

4. Hobbs G. A., Der C. J., Rossman K. L. RAS isoforms and mutations in cancer at a glance *J. Cell Science* 2016, 129, 1287-1292. DOI: 10.1242/jcs.182873, PubMed: 26985062.

5. Kessler D., Gmachl M., Mantoulidis A., Martin L. J., Zoephel A., Mayer M., Gollner A., Covini D., Fischer S., Gerstberger T., Gmaschitz T., Goodwin C., Greb P., Häring D., Hela W., Hoffmann J., Karolyi-Oezguer J., Knesl P., Kornigg S., Koegl M., Kousek R., Lamarre L., Moser F., Munico-Martinez S., Peinsipp C., Phan J., Rinnenthal J., Sai J., Salamon C., Scherbantin Y., Schipany K., Schnitzer R., Schrenk A., Sharps B., Siszler G., Sun Q., Waterson A., Wolkerstorfer B., Zeeb M., Pearson M., Fesik S. W. and McConnell D. B. Drugging an "undruggable" pocket on KRAS *PNAS* 2019, 116 (32), 15823-15829. DOI: 10.1073/pnas.1904529116, PubMed: 31332011.

6. Kessler D., Bergner A., Böttcher J., Fischer G., Döbel S., Hinkel M., Müllauer B., Weiss-Puxbaum A., McConnell D. B. Drugging all RAS isoforms with one pocket *Future Medicinal Chemistry* 11 Aug 2020. DOI: 10.4155/fmc-2020-0221, PubMed: 32779487.