KRAS switch I/II pocket inhibitor | BI-2852

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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

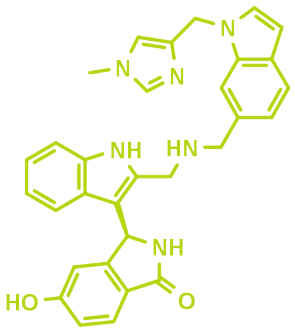

Figure 1: 2-D structure of BI-2852

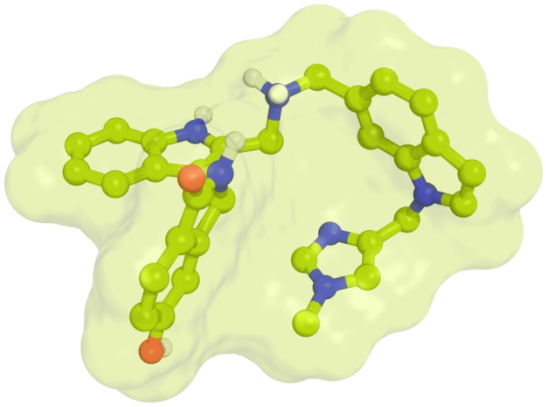

Figure 2: BI-2852, 3D conformation

Highlights

BI-2852 is a potent inhibitor for *in vitro* use that directly targets GTP-bound KRAS, which is the major form present in cancer cells carrying KRAS mutations. BI-2852 binds to $KRAS^{G12D}$ with a KD of 740 nM (ITC), inhibits GTP-$KRAS^{G12D}$ binding to effectors like SOS1, CRAF and PI3Kα with an IC_{50} of

490, 770 and 500 nM. BI-2852 showed pERK(2h) modulation and antiproliferative effects in $KRAS^{mut}$ cells (NCI-H358) at 5.8 µM and 6.7 µM.[5]

Together with the structurally similar negative control BI-2853 which is also available via opnMe.com, the probe BI-2852 can serve as an excellent small molecule tool inhibitor of KRAS for testing biological hypotheses *in vitro*.

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. After more than three decades of intense effort, the first anti-RAS therapies have just reached clinical application in the beginning of 2019.[1-5]

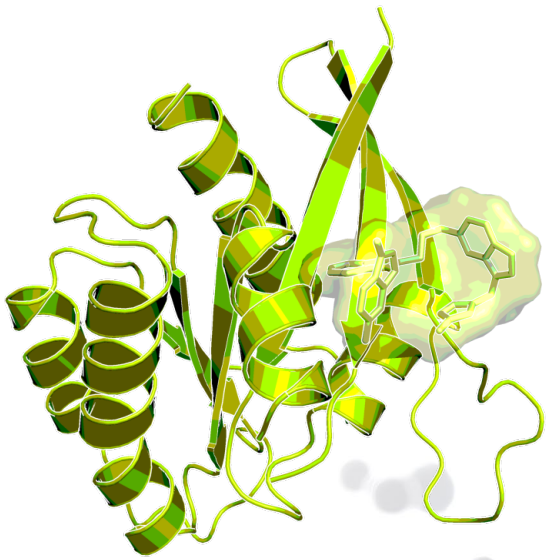

Figure 3: Complex of KRAS with BI-2852

In vitro activity

BI-2852 displays an IC_{50} of 490 nM in a GTP-KRASG12D::SOS1 AlphaScreen (AS) assay leading to low micromolar inhibition of pERK (in H358 cell line)[5].

PROBE NAME / NEGATIVE CONTROL	BI-2852	BI-2853
MW [Da]	516.6	516.6
ITC (K_D) GCP-$KRAS^{G12D}$ [µM][a]	0.74	n.d.
ITC (K_D) GCP-$KRAS^{wt}$ [µM][a]	7.5	n.d.
ITC (K_D) GDP-$KRAS^{G12D}$ [µM][a]	2.0	n.d.
ITC (K_D) GDP-$KRAS^{wt}$ [µM][a]	1.1	n.d.
AS (IC_{50}) GTP-$KRAS^{G12D}$::SOS1 [nM][a]	490	4400
AS (IC_{50}) GTP-$KRAS^{G12D}$::CRAF [nM][a]	770	n.d.
AS (IC_{50}) GTP-$KRAS^{G12D}$::PI3Kα [nM][a]	500	n.d.
AS (IC_{50}) GDP-$KRAS^{G12D}$::SOS1 [nM][a]	260	2500
AS (IC_{50}) GTP-$KRAS^{wt}$::SOS1 [nM][a]	490	n.d.
EC_{50} pERK H358 cells (2 h) [µM][a]	5.8	> 50
EC_{50} H358 cells (low serum) [µM][a]	6.7	n.d.

[a] for assay conditions please refer to reference 5

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-2852	BI-2853
logP	2.6	2.6

Solubility @ pH 6.8 [µg/ml]	18	21
CACO permeability @pH7.4 [*10^{-6} cm/s]	5.0	< 1.15
CACO efflux ratio	2.1	n.d.
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	n.d.	n.d.
MDCK efflux ratio	n.d.	n.d.
Microsomal stability (human/mouse/rat) [% QH]	91 / 93 / 90	92 / 95 / 86
Hepatocyte stability (human/mouse/rat) [% QH]	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.d.
CYP 2C8 (IC_{50}) [µM]	8.4	n.d.
CYP 2C9 (IC_{50}) [µM]	4.8	n.d.
CYP 2C19 (IC_{50}) [µM]	11.0	n.d.
CYP 2D6 (IC_{50}) [µM]	15.0	n.d.

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.

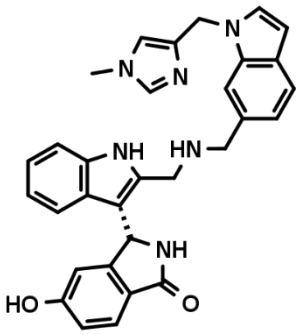

Figure 4: BI-2853 which serves as a negative control

Selectivity

Selectivity data are not available at this point in time.

BI-2852	SELECTIVITY DATA AVAILABLE
Cerep®	No
Panlabs®	No
Invitrogen®	No
DiscoverX®	No
Dundee	No

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

The Xray crystal structure of target in complex with BI-2852 is available (PDB code: 6GJ7)[5].

Summary

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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.

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.

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.

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.

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**, Latest articles, 1-7. DOI: 10.1073/pnas.1904529116