SOS1::KRAS inhibitor | BI-3406

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Summary

BI-3406 is a potent and selective Son of Sevenless 1 (SOS1) :: Kirsten rat sarcoma viral oncogene (KRAS) protein interaction inhibitor for research. The small molecule inhibitor BI-3406 binds to the catalytic site of SOS1, inhibiting the interaction with RAS-GDP. This significantly reduces formation of GTP-loaded KRAS (activated KRAS), thereby inhibiting downstream MAPK signaling. The SOS1::KRAS inhibitor shows pharmacokinetic (PK) properties that are suitable for *in vivo* testing in rodent species but is expected to have low brain penetration[1].

Chemical Structure

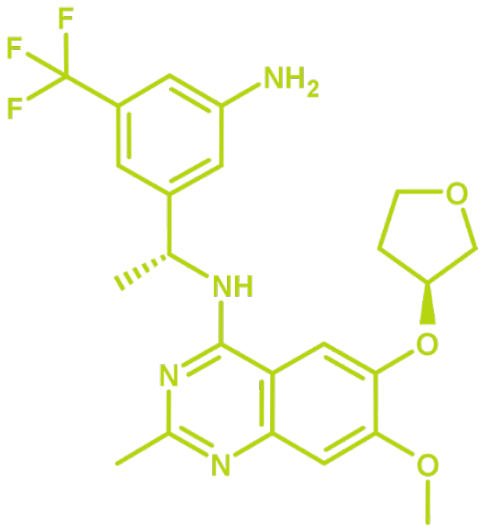

Figure 1: 2-D structure of BI-3406, a SOS1::KRAS inhibitor | BI-3406

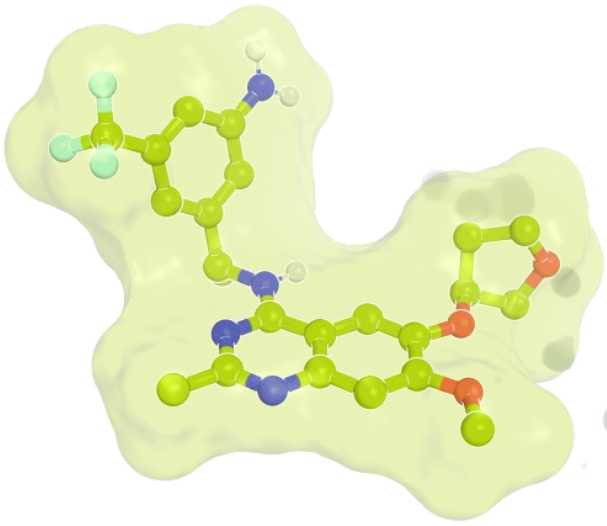

Figure 2: BI-3406, 3D conformation, as observed in complex with SOS1 (PDB code: 6scm)

Highlights

BI-3406 is a highly potent and selective SOS1::KRAS protein interaction inhibitor[1]. This small molecule reduces the formation of GTP-loaded KRAS and inhibits MAPK pathway signaling *in vitro* and *in vivo*. It has good solubility in water and is highly permeable in the Caco2 assay. Its PK properties make it suitable for *in vivo* testing in rodent species.

Target information

Aberrant activation of Kirsten rat sarcoma viral oncogene homolog (KRAS) by deregulated upstream signaling, loss of GTPase-activating protein function, or oncogenic mutations results in increased GTP-bound KRAS and persistent signaling through downstream effector pathways in cancer.[2,3] KRAS is the most frequently mutated oncogene in three of the deadliest cancers, as it occurs in approximately 90% of pancreatic cancer, 40% of colorectal cancer and 25% of non-small cell lung cancer cases (Data from cBioPortal v3.7.4 accessed Sept 14, 2021)[11,12].

RAS family small GTP/GDP binding proteins, such as KRAS, have a weak intrinsic GTPase activity and slow nucleotide exchange rates. Two classes of enzymes have evolved to facilitate cycling between the active GTP-bound state and the inactive GDP-bound form. GTPase Activating Proteins (GAPs) increase the intrinsic GTPase activity of RAS family proteins, leading to the formation of GDP bound RAS (e.g. NF1), whereas guanine nucleotide exchange factors (GEFs), such as Son of Sevenless 1 (SOS1), directly interact with KRAS and release GDP, enabling GTP binding and re-activation. Cancer-associated mutations in KRAS further suppress the intrinsic and GAP-induced GTPase activity leading to an increased population of signaling competent GTP loaded KRAS molecules.[3-6]

Selective inhibition of SOS1, a GEF for the RAS family of small GTPases, significantly reduces formation of GTP-loaded KRAS (activated KRAS) and thereby inhibits downstream MAPK signaling. SOS1 needs to be recruited to the membrane where it binds to Shp2 and Grb2 to act as GEF. Depleting SOS1 decreased the survival of tumor cells carrying a KRAS mutation or amplification whereas, no effect was observed in KRAS non addicted wild-type cells.[8]

Reduction of RASGDP and an anti-proliferative cytostatic effect was demonstrated with this SOS1::KRAS inhibitor in KRAS addicted cell lines *in vitro* using 3D proliferation assays as well *in vivo* in KRAS mutant mouse models.

Preclinical data showed enhanced pathway modulation and synergistic anti-tumor effects following vertical pathway inhibition of BI-3406 with either Mitogen-activated protein kinase inhibitors (MEKi; e.g. trametinib, binimetinib, cobimetinib, selumetinib or BI 3011441) or KRASG12C inhibitors (MRTX849, AMG510 or BI 1823911). Furthermore, SOS1::KRAS treatment sensitizes tumor cells to enhanced DNA damage in combination with irinotecan.[9]

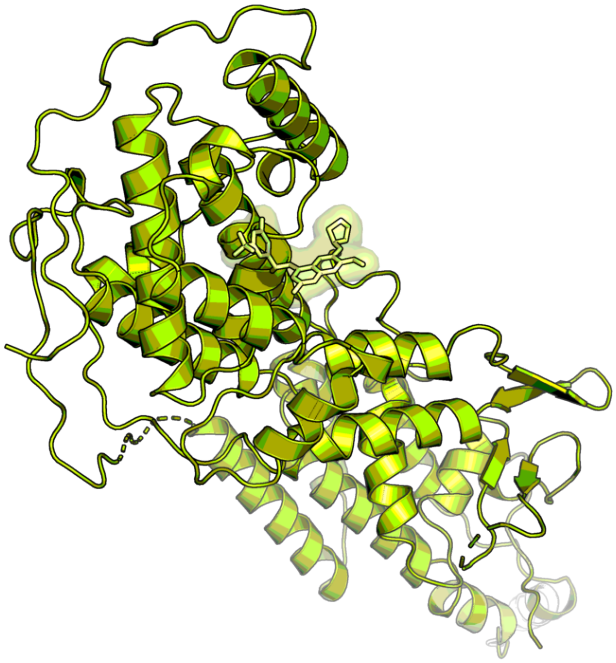

Figure 3: BI-3406 in complex with SOS1 (PDB code:6scm)

In vitro activity

BI-3406 inhibits the interaction of SOS1 with GDP-loaded KRAS with an IC_{50} of 5 nM. In KRAS mutant NCI-H358 cells, the small molecule SOS1::KRAS inhibitor inhibits pERK formation with an IC_{50} of 4 nM and cellular proliferation with an IC_{50} of 24 nM.[1] The small molecule SOS1::KRAS inhibitor has no species selectivity versus mouse KRAS mutant cell lines.[10]

PROBE NAME / NEGATIVE CONTROL	BI-3406	BI-0178
MW [Da]	462.5	335.4
SOS1 (IC_{50}) [nM][a]	4	>100,000
pERK (DLD-1, $KRAS^{G13D}$) (IC_{50}) [nM][a]	24	n.a.
Proliferation (DLD-1, $KRAS^{G13D}$) (IC_{50}) [nM][a]	36	n.a.
Proliferation (H520, $KRAS^{wt}$) (IC_{50}) [nM][a]	>10,000	n.a.

[a] assay conditions see ref. 1,10

In vitro DMPK and CMC parameters

BI-3406 has good solubility in water at acidic or neutral pH. It showed high permeability in Caco2 assay but significant efflux in the MDCK permeability assay.

PROBE NAME / NEGATIVE CONTROL	BI-3406	BI-0178
logP	4.2	n.d.
Solubility @ pH 6.8 [µg/ml]	84	12
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	15.3	n.d.
CACO efflux ratio	1.75	n.d.
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	1.7	40
MDCK efflux ratio	25	1.7
Microsomal stability (human/mouse/rat) [% Q_H]	44/81/48	<23/78/78
Hepatocyte stability (human/mouse/rat) [% Q_H]	12/56/28	n.d.
Plasma protein binding (human/mouse/rat) [%]	98.8/98.4/99.0	n.d.
hERG [inh. % @ 1 µM]	11.2	n.d.
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2C8 (IC_{50}) [µM]	18	24.9
CYP 2C9 (IC_{50}) [µM]	15	>50
CYP 2C19 (IC_{50}) [µM]	13	>50
CYP 2D6 (IC_{50}) [µM]	11	35.9

In vivo DMPK parameters

PK properties in rodent animal species are suitable for once or twice daily oral dosing in acute or sub-chronic *in vivo* experiments, but is expected to have low brain penetration[1]. BI-8668 has a good DMPK profile and showed 33% inhibition of fluid absorption at 3 µg/kg in an airway fluid absorption assay in Wistar rats.

BI-3406	MOUSE	RAT
Clearance [% Q_H][b]	61	16
Mean residence time after *iv* dose [h]	0.5	2.6
t_{max} [h]	0.8	3.3
$C_{max,\ dose\ normalized}$ [nM]	85	237
F [%]	100	100
V_{ss} [l/kg]	1.7	1.7

In vivo pharmacology

BI-3406 was studied in several KRAS mutant CRC, PDX and NSCLC PDX and CDX models.[1,10]

Negative control

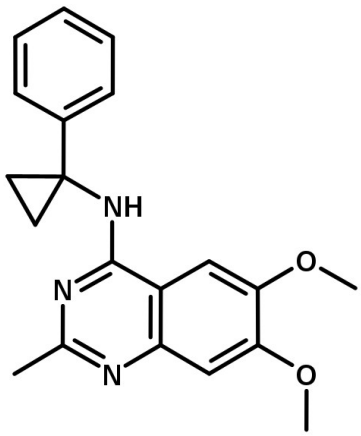

Figure 4: BI-0178 which serves as a negative control

Selectivity

BI-3406 was shown to be selective versus SOS2 (IC_{50} > 10µ M), selective in a panel of 368 kinases (no off-target hits at 5 µM) and moderately selective in a panel of 44 other off-targets (10 hits at 10 µM, IC_{50} (alpha A1 antagonism)= 6 µM).

SELECTIVITY DATA AVAILABLE	BI-3406	BI-0178
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	No	No

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

The Xray crystal structure of target in complex with BI-3406 is available (PDB code: 1NVU)[1].

Reference molecule(s)

BAY-293

Supplementary data

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

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