BI-9321 NSD3-PWWP1 antagonist | BI-9321

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Summary

BI-9321 is the first potent and highly selective antagonist of the PWWP1 domain of NSD3. It allows investigating the biological function of this multivalent epigenetic regulator as part of *in vitro* studies.

Chemical Structure

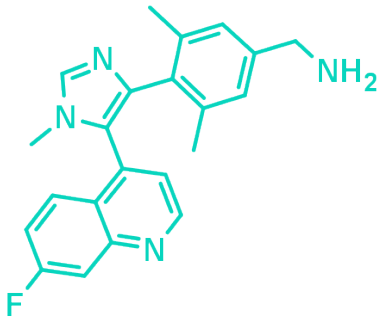

Figure 1: 2-D structure of BI-9321, an epigenetic regulator of NSD3

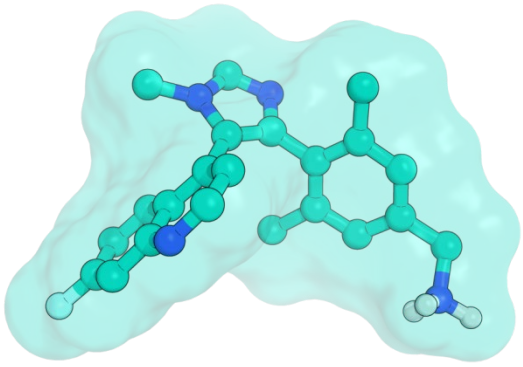

Figure 2: BI-9321, 3D conformation, as observed in complex with the NSD3-PWWP1 domain

Highlights

BI-9321 was discovered in collaboration with the Structural Genomics Consortium (SGC). It represents a potent, selective and cellular active antagonist of the NSD3-PWWP1 domain. Initial binders to the proposed methyl-lysine binding site of the PWWP1 domain of NSD3 were identified applying fragment based screening (FBS) methods. Consecutively, structure-based optimization yielded in BI-9321, a potent antagonist of the PWWP1 (Pro-Trp-Trp-Pro) domain of NSD3 (Nuclear Receptor Binding SET Domain 3). High selectivity of BI-9321 was confirmed using *in vitro* assays and quantitative chemical proteomics. Cellular target engagement was confirmed with FRAP (Fluorescence Recovery After Photobleaching) and BRET (Bioluminescence Resonance Energy Transfer) at 1 µM. Treatment of MOLM-13 cells with BI-9321 results into the downregulation of Myc mRNA in cells and reduced proliferation which cannot be observed with the available negative control BI-9466.

Target information

Human NSD3 is encoded by the *WHSC1L1* gene, located in the 8p11-p12 amplicon, which is frequently amplified in cancer different tumor types. The methyltransferase NSD3 is a multi-domain epigenetic regulator that exists in three isoforms (long, short and the testis-specific Whistle). Both NSD3 long and Whistle isoforms contain the SET domain, with lysine methyltransferase activity, as well as several chromatin binder motifs so called “reader domains”, including PHD and two PWWP domains named PWWP1 and PWWP2. The NSD3-short isoform contains only the first PWWP domain.

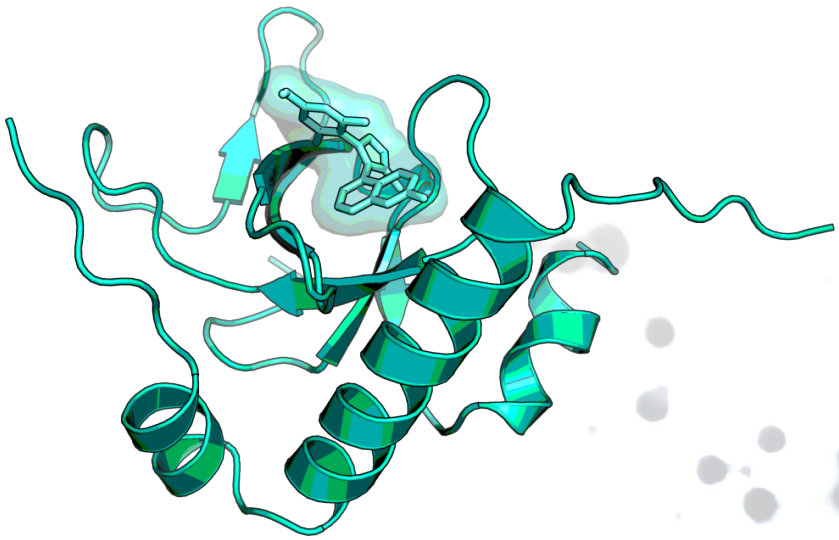

Figure 3: BI-9321 in complex with NSD3

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-9321	BI-9466
MW [Da]	360.4	295.4
MW [Da] HCl salt (BI-9321 will be delivered as the HCl salt)	396.9	331.9
TR-FRET[a] (IC_{50}) [nM]	203	120 000
SPR (K_d)[b] [nM]	166	144 000
ITC (K_d)[c] [nM]	445	n.d

[a] 1x PBS; 0.05% Tween20; 0.1 % BSA; filtered

[b] 50 mM TRIS, pH 8.0; 150 mM NaCl; 1 mM TCEP; 0.005 % Tween 20; 2% DMSO

[c] 20 mM HEPES, 100 mM NaCl, 3% DMSO, pH 8.0

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-9321	BI-9466
logP	n.d.	n.d.
Solubility @ pH 6.8 [µg/ml]	> 100	n.d.
CACO permeability @ pH7.4 [$*10^{-6}$ cm/s]	16	n.d.
CACO efflux ratio	2.8	n.d.

Microsomal stability (human/mouse/rat) [% QH]	<23/<24/<24	n.d.
Hepatocyte stability (human/mouse/rat) [% QH]	<10/n.d./31	n.d.
Plasma protein binding (human/mouse/rat) [%]	41.7/43.5/45.5	n.d.
CYP 3A4 (IC_{50}) [µM]	19	n.d.
CYP 2C8 (IC_{50}) [µM]	5.4	n.d.
CYP 2C9 (IC_{50}) [µM]	1.3	n.d.
CYP 2C19 (IC_{50}) [µM]	< 0.2	n.d.
CYP 2D6 (IC_{50}) [µM]	23	n.d.

In vivo DMPK parameters

No data available, BI-9321 is an *in vitro* tool.

In vivo pharmacology

No data available, BI-9321 is an *in vitro* tool.

Negative control

BI-9466 is a closely related analogue of BI-9321, exhibiting a more than 500 fold weaker affinity as determined by TR-FRET and SPR. No target engagement up 100 µM could be observed with protein and ligand based BRET assays.

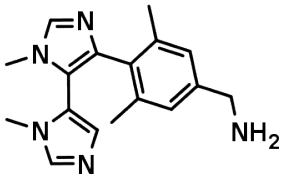

Figure 4: BI-9321 which serves as a negative control

Selectivity

A kinase panel is available on opnme.com. BI-9321 did not hit any of the 31 tested kinases.

BI-9321	SELECTIVITY DATA AVAILABLE
Cerep®	No
Panlabs®	No
Invitrogen®	Yes
DiscoverX®	No
Dundee	No

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

The X-ray crystal structure of NSD3-PWWP1 in complex with BI-9321 is available (PDB code: 6G2O)[1].

Reference molecule(s)

No other molecules available.

Summary

BI-9321 is a potent and highly selective antagonist of the PWWP1 domain of NSD3. It is a first in class chemical probe, targeting the methyl-lysine binding site, developed in collaboration with the Structural Genomics Consortium (SGC). BI-9321 exhibits an *in vitro* potency of 200 nM and cellular target engagement at around 1 µM. In MOLM-13 cells BI-9321 downregulates Myc mRNA and impairs proliferation. A closely-related compound, BI-9466 is 500-fold less active (TR-FRET assay) and is a recommended negative control. Both compounds should be used in parallel in a dose response range between 0.1 and 20µM.

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

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