opnMe by Boehringer Ingelheim

NSD3-PWWP1 antagonist

BI-9321

Boehringer Ingelheim

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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

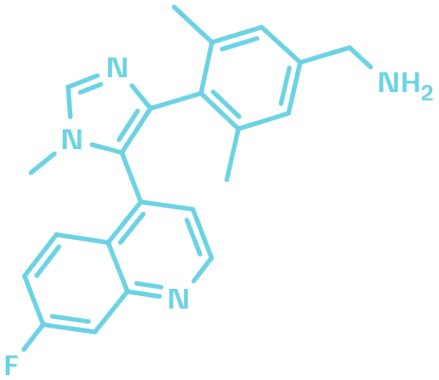

Figure 1: 2D structure of BI-9321, an epigenetic regulator of NSD3

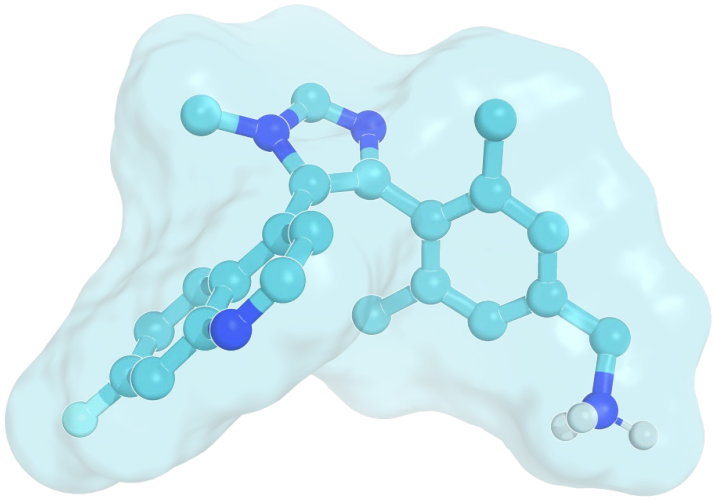

Figure 2: BI-9321, 3D conformation, as observed in complex with the NSD3-PWWP1 domain

Highlights

BI-9321 is a potent, highly selective antagonist of the PWWP1 domain of NSD3. This first-in-class chemical probe has shown an *in vitro* potency of 200 nM and cellular target engagement at around 1 nM. It is suitable for *in vitro* experiments. Myc mRNA downregulation and reduced proliferation was observed in MOLM-13 cells treated with BI-9321.

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. 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.
BI-9321 was designed in collaboration with the Structural Genomics Consortium (SGC).

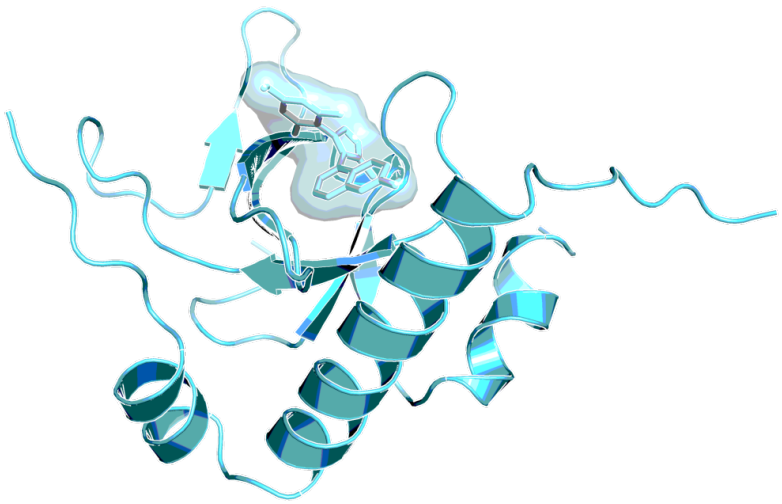

Figure 3: BI-9321 in complex with NSD3

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-9321	BI-9466
MW [Da, free base][a]	360.4	295.4
TR-FRET[b] (IC_{50}) [nM]	203	120 000
SPR (K_d)[c] [nM]	166	144 000
ITC (K_d)[d] [nM]	445	n.d

[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] PBS; 0.05% Tween20; 0.1 % BSA
[c] 50 mM TRIS, pH 8.0; 150 mM NaCl; 1 mM TCEP; 0.005 % Tween 20; 2% DMSO
[d] 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
logD @ pH 11	1.5	- 0.16
Solubility @ pH 6.8 [µg/mL]	> 100	n.d.
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	16	n.d.
Caco-2 efflux ratio	2.8	n.d.
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / <24 / <24	n.d.
Hepatocyte stability (human/mouse/rat) [% Q_H]	<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.

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.

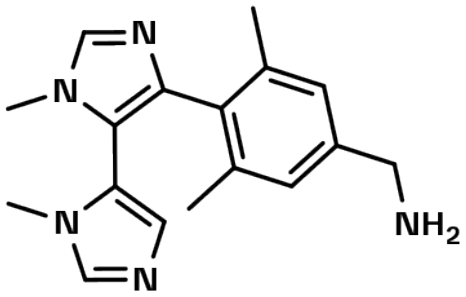

Figure 4: BI-9466 which serves as a negative control

Selectivity

A kinase panel is available on opnMe.com. BI-9321 did not hit any of the 44 targests tested in SafetyScreen44™ and 31 tested kinases.

SELECTIVITY DATA AVAILABLE	BI-9321	BI-9466
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 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.

Supplementary data

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

References

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