opnMe by Boehringer Ingelheim

BRD9 inhibitor

BI-9564

Boehringer Ingelheim

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Summary

BI-9564 binds with high affinity to BRD9 (K_D(ITC) = 14 nM), displays good cellular potency and an excellent selectivity versus most BET family members.

Chemical Structure

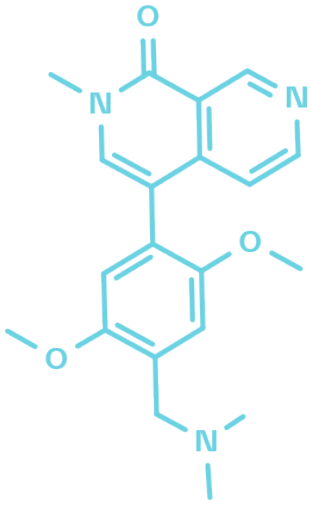

Figure 1: 2D structure of BI-9564, a BRD9 inhibitor

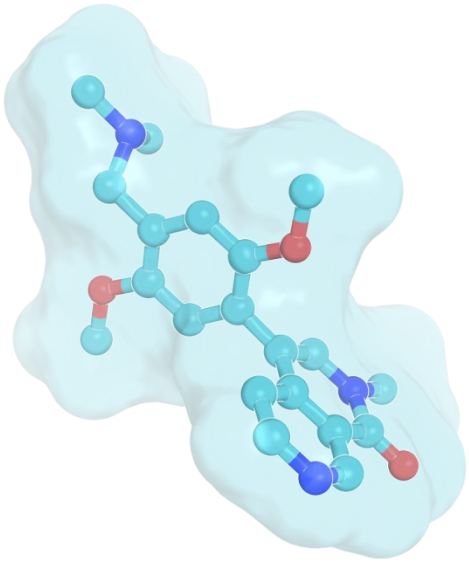

Figure 2: BI-9564, 3D conformation, as observed in complex with BRD9 by X-ray crystallography

Highlights

BI-9564 is a highly potent and selective BRD9 inhibitor: This compound binds with high affinity to BRD9 and with lower affinity to the closely related BRD7. BI-9564 is an attractive tool to study BAF complex biology both *in vitro* and *in vivo*, as it has a good ADME-PK profile and high oral bioavailability[1]. In a disseminated AML mouse model, it showed efficacy at oral doses of 180 mg/kg. BI-9564 was developed in collaboration with the Structural Genomics Consortium (SGC).

Target information

The mammalian switch/Sucrose Non-Fermentable (SWI/SNF) complex is one of four mammalian chromatin remodelling complexes. Recurrent inactivating mutations in certain subunits of this complex have been identified in different cancers. Despite its known roles in tumor suppression, the mammalian SWI/SNF complex has recently received attention as a potential target for therapeutic inhibition[2].

The human bromodomain family encompasses 61 domains, found on 46 proteins and BRD9 and BRD7 proteins containing a single acetyl-lysine reader bromodomain and are components of the chromatin remodelling SWI/SNF BAF complex. A recent study highlighted a role of another SWI/SNF subunit, BRD9, in leukemia growth. The BRD9 bromodomain (BD) was shown to be required for the proliferation of acute myeloid leukemia (AML) cells[3].

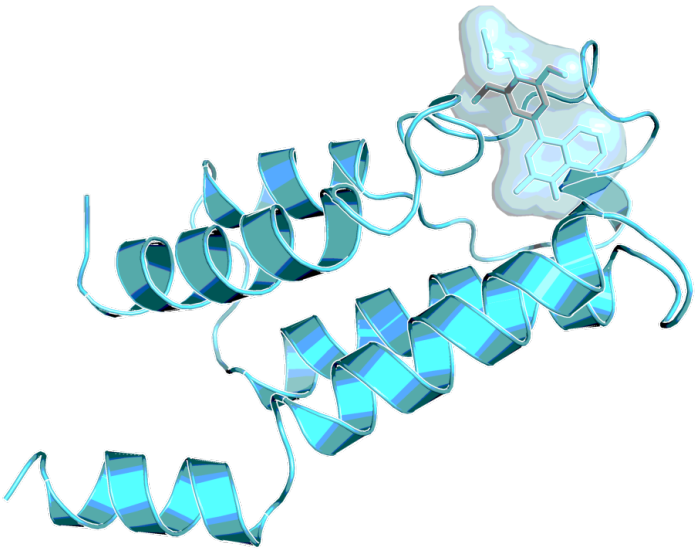

Figure 3: BRD9 with BI-7273, as observed by X-ray[1]

In vitro activity

The compound binds with high affinity to BRD9 K_D(BRD9, ITC) = 14 nM and with lower affinity to closely related BRD7 K_D(BRD7, ITC) = 239 nM. CECR2 was the only other identified off-target (K_D(CECR2, ITC) = 258 nM), but with no effect in cells at 1 µM (FRAP assay). BI-9564 is completely negative on BET family members in the AlphaScreen (>100 µM).

PROBE NAME / NEGATIVE CONTROL	BI-9564	BI-6354
MW [Da, free base][a]	353.4	279.3
ITC(BRD9) (K_D) [nM][b]	14	n.d.
ITC(BRD7) (K_D) [nM][b]	239	n.d.
AlphaScreen(BRD9) (IC_{50}) [nM][b]	75	27,192
AlphaScreen(BRD7) (IC_{50}) [nM][b]	3,410	81,896
AlphaScreen(BRD4-BD1) (IC_{50}) [nM][b]	>100,000	>100,000

[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 detailed assay conditions see Ref. 1

In vitro DMPK and CMC parameters

BI-9564 has an attractive ADME/PK profile for *in vivo* proof-of-concept studies, namely, high solubility at pH 6.8, moderate to high *in vitro* metabolic stability, low plasma protein binding, and no cytochrome P450 inhibition.

PROBE NAME / NEGATIVE CONTROL	BI-9564	BI-6354
logD @ pH 11	2.0	0.6
Solubility @ pH 7 [µg/mL]	>90	>59
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	11.4	63
Caco-2 efflux ratio	4.0	0.7
Microsomal stability (human/mouse/rat) [% Q_H]	<24 / 35 / <23	<24 / n.a. / <23
Hepatocyte stability (human/mouse/rat) [% Q_H]	17 / 56 / 17	n.a. / n.a. / 23

Plasma Protein Binding (human/mouse/rat) [%]	42 / 35 / 23	n.a. / n.a. / 62.8
CYP 3A4 (IC_{50}) [µM]	>50	n.a.
CYP 2C8 (IC_{50}) [µM]	>50	n.a.
CYP 2C9 (IC_{50}) [µM]	>50	n.a.
CYP 2C19 (IC_{50}) [µM]	>50	n.a.
CYP 2D6 (IC_{50}) [µM]	49	n.a.

In vivo DMPK parameters

BI-9564 showed moderate to high absorptive permeability and moderate *in vivo* plasma clearances upon *i.v.* dosing. BI-9564 displayed high oral bioavailability.

BI-9564	MOUSE
Clearance [% Q_H][a]	59
Mean residence time after *i.v.* dose [L/kg][a]	0.6
t_{max} [h][b]	0.7
C_{max} [nM][b]	5,400
F [%][b]	88
V_{ss} [L/kg][a]	2.1

[a] *i.v.* dose: 5 mg/kg
[b] *p.o.* dose: 20 mg/kg

In vivo pharmacology

BI-9564 showed efficacy at oral doses of 180 mg/kg in a disseminated mouse model of AML with a median TGI value of 52% on day 18, which translated into an additional survival benefit compared to that of the control group[1].

Negative control

BI-6354 is available as an *in vitro* negative control. It shows only very weak potency on BRD9 and BRD7 and no potency on BRD4.

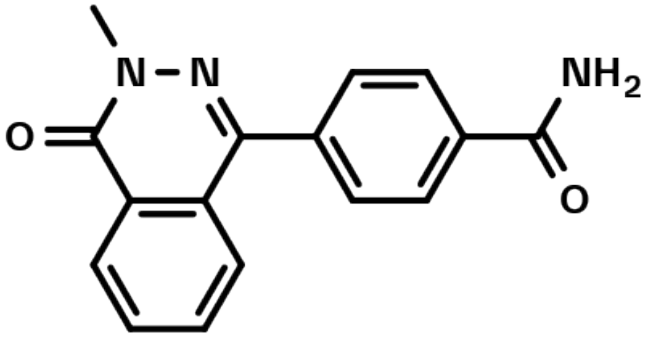

Figure 4: BI-6354 which serves as an *in vitro* negative control

Selectivity

BI-9564 was screened on 48 bromodomains, 55 GPCRs and a large kinase panel (324 kinases). Beside BRD9 and BRD7, CECR2 was the only bromodomain off-target (258 nM, ITC), but with no cellular effect at 1 µM in FRAP assay. All GPCRs except M1(h) (75%) and M3(h) (86%) showed less than 40% crtl inhibition at 10 µM. From the 324 kinases only 3 kinases (ACVR1, TGFBR1, ACVR2B) showing an % ctrl inhibition of > 40%, for which the measured IC_{50} values were > 5 µM.

SELECTIVITY DATA AVAILABLE	BI-9564	BI-6354
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	Yes	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-9564 is available (PDB code: 5F1H)[1].

Reference molecule(s)

LP99[4], I-BRD9[5], BI-7273[1], "compound 28"[6,7].

Supplementary data

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

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