Orally bioavailable BET inhibitor | BI 894999

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Summary

BI 894999 (amredobresib) is a small molecule oral BET inhibitor suitable for *in vitro* and *in vivo* studies. BET family proteins are key regulators of transcription.

Chemical Structure

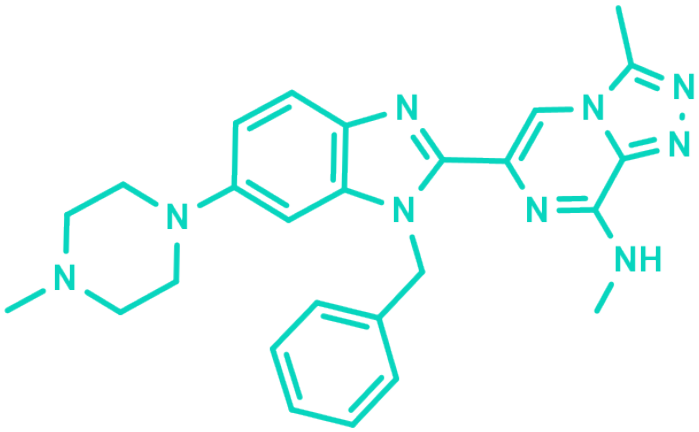

Figure 1: 2D structure of BI 894999, an orally bioavailable BET inhibitor

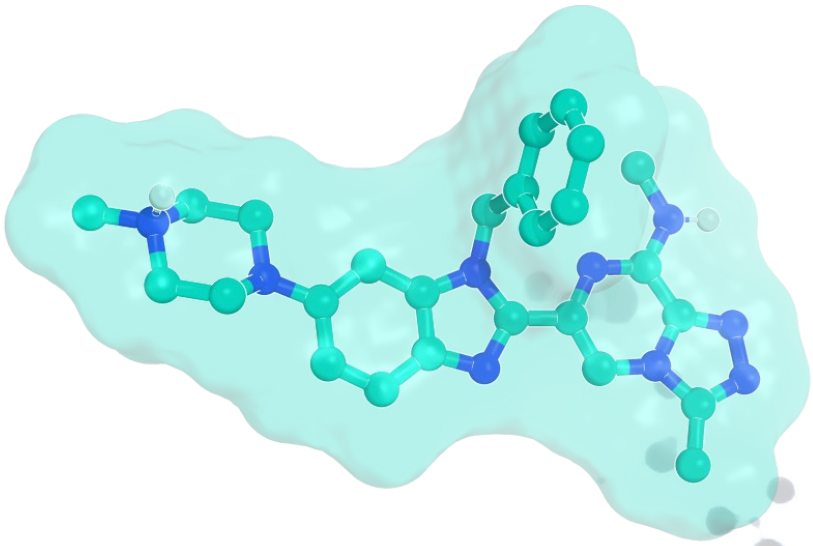

Figure 2: 3D conformation of BI 894999, as seen in BRD4^{BD1}-BI 894999 x-ray structure

Highlights

BI 894999 (amredobresib) is a potent inhibitor of the interaction between a histone H4-derived peptide with acetylated lysines and the bromodomains $BRD4^{BD1}$ (IC_{50} 5 ± 3 nM) and $BRD4^{BD2}$ (IC_{50} 41 ± 30 nM). It only inhibits BET family members and is selective versus 24 other bromodomains. It complements the BET PROTAC MZ1 which is also available on opnMe. **Please note that due to its potency, BI 894999 should be handled with great caution.**

Target information

BI 894999 is a low molecular weight compound which inhibits bromodomain 4 (BRD4) as well as other members of the bromo- and extra-terminal domain (BET) family (BRD2, BRD3 and BRDT) with high selectivity and potency. BRD4 activates transcriptional elongation, thereby contributing to gene expression, at least partially by activating P-TEFb (consisting of CDK9 and CyclinT1). In this process, BRD4 functions as an antagonist of a ribonucleoprotein complex (7SK/HEXIM-1, LARP7, MEPCE), which binds to P-TEFb and functionally inhibits it. BRD4 activates P-TEFb, which then can phosphorylate Ser2 on paused RNA Pol II, leading to transcriptional elongation.

BRD4 is considered to be a general transcriptional regulator. However, pharmacological inhibition of BET proteins has shown therapeutic activity in different models of cancer (mouse xenografts).

BI 894999 has been developed as an oral formulation for investigation in patients with malignancies. Its potency was determined on tumor cells *in vitro* and *in vivo*. The closely related molecule BI-6953 can be used as a negative control.

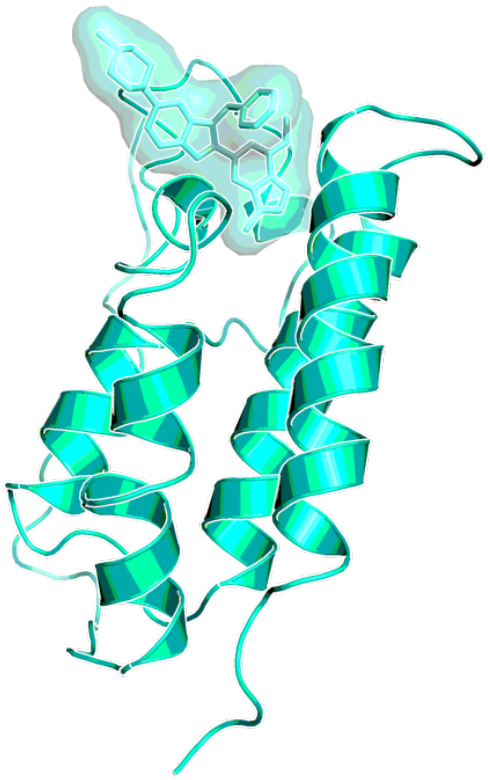

Figure 3: X-ray of BI 894999 in BRD-BD1 (PDB code: 8PIQ).

In vitro activity

Competitive displacement of a histone peptide (acetyl-histone H4 Lys5, 8, 12, 16) from human bromodomain by the test-compounds is measured by using the AlphaLISA® bead-based proximity assay.

BI 894999 inhibited the binding of:

- Bromodomain $BRD4^{BD1}$ to acetylated Histone (Lys5, 8, 12, 16) with an IC_{50} of 5 ± 3 nM
- Bromodomain $BRD4^{BD2}$ to acetylated Histone (Lys5, 8, 12, 16) with an IC_{50} of 41 ± 30 nM
- Bromodomain $BRD2^{BD1}$ to acetylated Histone (Lys5, 8, 12, 16) with an IC_{50} of 33 ± 12 nM

The effect of the BET inhibitor BI 894999 was also tested on numerous malignant cell lines of various origin and ranged from single digit nM to µM[1-3].

The cellular potency of BI 894999 was determined in cell proliferation assays. A panel consisting of >50 acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), multiple myeloma (MM), diffuse large B-cell lymphoma (DLBCL, ABC and GCB-type), and T-cell lymphoma (TCL) cell lines was

tested. All cell lines showed an GI_{50} <100 nM, with most of the cell lines displaying single digit nM GI_{50} values.

NUT carcinoma cell lines, carrying BRD-NUT fusions, were exquisitely sensitive to BI 894999 with GI_{50} values in the single digit nM range (geomean from 0.9 to 2.6 nM).

No data were generated for the negative control BI-6953.

PROBE NAME / NEGATIVE CONTROL	BI 894999
MW [Da]	467.6
$BRD4^{BD1}$ ALPHA LO (IC_{50}) [nM][a]	6
$BRD4^{BD2}$ ALPHA LO (IC_{50}) [nM][a]	39
$BRD2^{BD1}$ ALPHA LO (IC_{50}) [nM][a]	33
BRD9 AS LO ECHO (IC_{50}) [nM][a]	3,059
BRD7 AS LO ECHO (IC_{50}) [nM][a]	81,199
MV-4-11 MYC (EC_{50}) [nM][a]	3
ALAMAR BRD4 MV-4-11 (EC_{50}) [nM][a]	9
MYC_MOLP-8 (EC_{50}) [nM][a]	5
ALA HCT116 (EC_{50}) [nM][a]	>250

[a] For assay conditions see reference 1

In vitro DMPK and CMC parameters

BI 894999 is a highly potent BET inhibitor with overall good DMPK properties. No data were generated for the negative control BI-6953.

PROBE NAME / NEGATIVE CONTROL	BI 894999
logP pH = 11	3.5
Solubility @ pH 6.8 [µg/ml]	54
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	7.9
MDCK efflux ratio	14
Microsomal stability (human/mouse/rat) [% Q_H]	32/44/<23
Hepatocyte stability (human/mouse/rat) [% Q_H]	12/51/7
Plasma protein binding (human/mouse/rat) [%]	65.6/54.2/74.7
hERG [inh. % @ 1 µM]	8.9
CYP 3A4 (IC_{50}) [µM]	>50
CYP 2C8 (IC_{50}) [µM]	>50
CYP 2C9 (IC_{50}) [µM]	>50
CYP 2C19 (IC_{50}) [µM]	>50
CYP 2D6 (IC_{50}) [µM]	>50

In vivo DMPK parameters

BI 894999 is a highly potent BET inhibitor with oral bioavailability in mice and rats.

BI 894999	MOUSE	RAT
Clearance [% Q_H][b]	71	28

Mean residence time after iv dose [h]	0.5	5.4
t_{max} [h][c]	0.5	3
C_{max} [nM][c]	300	1,500
F [%]	23	40
V_{ss} [l/kg]	1.9	6.1

[b]mouse 0.5 mg/kg IV; rat 1 mg/kg IV

[c]mouse 2 mg/kg PO; rat = 10 mg/kg PO

In vivo pharmacology

BI 894999 was also tested in several tumor cell line derived xenografts (CDX) as well as patient-derived xenografts (PDX). The results of these *in vivo* experiments were supportive to initiate a clinical trial (NCT02516553). BET pathway modulation markers HEXIM1 and HIST2H2BF have been used preclinically and throughout the clinical trial.

Negative control

BI-6953 features a substitution of a NH group by a N-Methyl group, resulting in a molecular weight of 481.6 Da. Due to this substitution, BI-6953 is no longer able to bind to the different bromodomains and therefore serves as a good negative control.

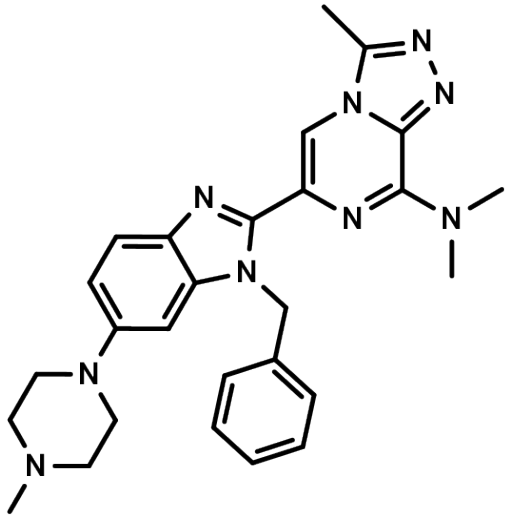

Figure 4: 2D structure of BI-6953, which serves as a negative control

Selectivity

SELECTIVITY DATA AVAILABLE	BI-894999	BI-6953
SafetyScreen44™ with kind support of eurofins	yes	Data in the process of being generated
Invitrogen®	Yes	n.d.
DiscoverX®	Yes	n.d.

Only BZD/CENTR/R is hit > 50% inhibition (51%) in the SafetyScreen 44. In addition to BRD2-4 (0-1% ctrl), TAF1 (9% ctrl), BRDT (21% ctrl) and CREBBP (25% ctrl) are hit in the DiscoverX panel.

Reference molecule(s)

BI 894999 is potentially similar or inferior to JQ1, OTX015, CPI-0610 or GSK525762[11,12]

Supplementary data

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

References

1. Gerlach D., Tontsch-Grunt U., Baum A., Popow J., Scharn D., Hofmann M. H., Engelhardt H., Kaya O., Beck J., Schweifer N., Gerstberger T., Zuber J., Savarese F., Kraut N. The novel BET bromodomain inhibitor BI 894999 represses super-enhancer-associated transcription and synergizes with CDK9 inhibition in AML. *Oncogene*. **2018**, 37(20):2687-2701. DOI: 10.1038/s41388-018-0150-2, PubMed.
2. Tontsch-Grunt U., Rudolph D., Waizenegger I., Baum A., Gerlach D., Engelhardt H., Wurm M., Savarese F., Schweifer N., Kraut N. Synergistic activity of BET inhibitor BI 894999 with PLK inhibitor volasertib in AML in vitro and in vivo. *Cancer Lett*. **2018**, 421:112-120. DOI: 10.1016/j.canlet.2018.02.018, PubMed.
3. Tontsch-Grunt U., Traexler P. E., Baum A., Musa H., Marzin K., Wang S., Trapani F., Engelhardt H., Solca F. Therapeutic impact of BET inhibitor BI 894999 treatment: backtranslation from the clinic. *Br J Cancer*. **2022**, 127(3):577-586. DOI: 10.1038/s41416-022-01815-5, PubMed.
4. Bartholomeeusen K., Xiang Y., Fujinaga K., Peterlin B. M. Bromodomain and extra-terminal (BET) bromodomain inhibition activate transcription via transient release of positive transcription elongation factor b (P-TEFb) from 7SK small nuclear ribonucleoprotein. *J Biol Chem*. **2012**, 287(43):36609-16. DOI: 10.1074/jbc.M112.410746, PubMed.
5. Filippakopoulos P., Qi J., Picaud S., Shen Y., Smith W. B., Fedorov O., Morse E. M., Keates T., Hickman T. T., Felletar I., Philpott M., Munro S., McKeown M. R., Wang Y., Christie A. L., West N., Cameron M. J., Schwartz B., Heightman T. D., La Thangue N., French C. A., Wiest O., Kung A. L., Knapp S., Bradner J. E. Selective inhibition of BET bromodomains. *Nature* **2010**, 468(7327):1067-73. DOI: 10.1038/nature09504, PubMed.
6. Shi J., Vakoc C. R. The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell*. **2014**, 54(5):728-36. DOI: 10.1016/j.molcel.2014.05.016, PubMed.
7. Jung M, Gelato KA, Fernández-Montalván A, Siegel S, Haendler B. Targeting BET bromodomains for cancer treatment. *Epigenomics*. **2015**, 7(3):487-501. DOI: 10.2217/epi.14.91, PubMed.
8. Rathert P., Roth M., Neumann T., Muerdter F., Roe J. S., Muhar M., Deswal S., Cerny-Reiterer S., Peter B., Jude J., Hoffmann T., Boryń L. M., Axelsson E., Schweifer N., Tontsch-Grunt U., Dow L. E., Gianni D., Pearson M., Valent P., Stark A., Kraut N., Vakoc C. R., Zuber J. Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature*. **2015**, 525(7570):543-547. DOI: 10.1038/nature14898, PubMed.
9. Morel D., Jeffery D., Aspeslagh S., Almouzni G., Postel-Vinay S. Combining epigenetic drugs with other therapies for solid tumours - past lessons and future promise. *Nat Rev Clin Oncol*. **2020**, 17(2):91-107. DOI: 10.1038/s41571-019-0267-4, PubMed.
10. Bechter O., Schöffski P. Make your best BET: The emerging role of BET inhibitor treatment in malignant tumours. *Pharmacol Ther*. **2020**, 208:107479. DOI: 10.1016/j.pharmthera.2020.107479, PubMed.
11. Shorstova T., Foulkes W. D., Witcher M. Achieving clinical success with BET inhibitors as anti-cancer agents. *Br J Cancer* **2021**, 124(9):1478-1490. DOI: 10.1038/s41416-021-01321-0, PubMed.
12. Duan Y., Guan Y., Qin W., Zhai X., Yu B., Liu H. Targeting Brd4 for cancer therapy: inhibitors and degraders. *Medchemcomm*. **2018**, 9(11):1779-1802. DOI: 10.1039/c8md00198g, PubMed.