CDK8 inhibitor | BI-1347

Table of contents

Summary	2
Chemical Structure	2
Highlights	2
Target information	3
In vitro activity	4
In vitro DMPK and CMC parameters	4
In vivo DMPK parameters	5
Selectivity	6
Negative control	5
Reference molecule(s)	6
Summary	6
Supplementary data	7
References	7

Summary

BI-1347 is a selective nanomolar CDK8 inhibitor, suitable for testing biological hypotheses *in vitro* and also *in vivo*.

Chemical Structure

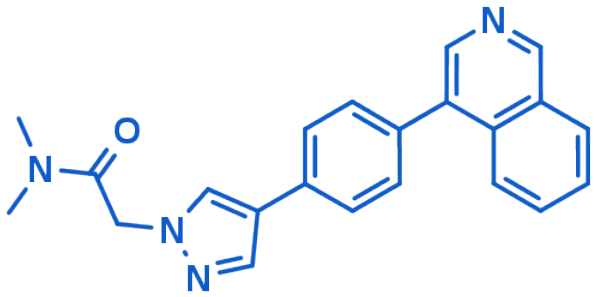

Figure 1: 2-D structure of BI-1347, an inhibitor of CDK8

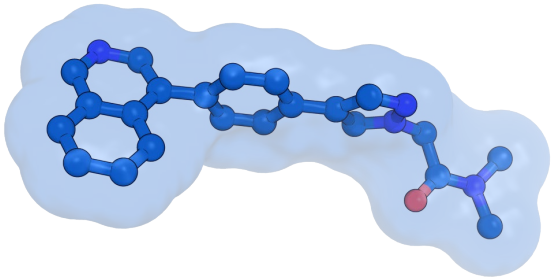

Figure 2: 3-D structure of BI-1347, 3D conformation as observed in the X-ray structure of the complex with CDK8 (see Figure 3)

Highlights

Cyclin-dependent kinase 8 (CDK8) is a Mediator complex-associated transcriptional regulator. Mediator is a multiprotein assembly containing up to 30 subunits that consist of four modules each: head, middle, tail and CDK/Cyclin and serves as a hub for diverse signaling pathways to regulate

gene expression.[2] In addition CDK8 inhibition escalate the native activities of Natural Killer cells (NK cells), promoting their tumor surveillance and cytotoxicity.[3]

BI-1347 is a selective inhibitor of CDK8/cyclinC with an IC_{50} of 1 nM. BI-1347 with its good DMPK profile showed tumor growth inhibition in an *in vivo* xenograft model. Together with the also available structurally similar compound BI-1374, which can be used as negative control due to much weaker potency BI-1347 can serve as an excellent small molecule inhibitor for testing biological hypotheses like the role of the Mediator complex in human pathologies of as a tool targeting NK cells for anticancer immunotherapy *in vitro* and *in vivo*.

Target information

CDK8 and its closely related paralog CDK19 are transcription-regulating cyclin C–dependent kinases. CDK8 and CDK19 are components of the Mediator complex, which provides a link between transcriptional regulators, the RNA polymerase II (Pol II) transcription machinery and gene-specific transcription.[4]

CDK8 and CDK19 form the kinase module of the Mediator complex along with MED12 and MED13. Recently, several subunits have been implicated in a growing number of human diseases including developmental disorders and cancer. Most evidence has been gathered for the kinase module subunit CDK8, which has been linked to colon and pancreatic cancer and was also investigated as a potential target in cancer therapy.[2]

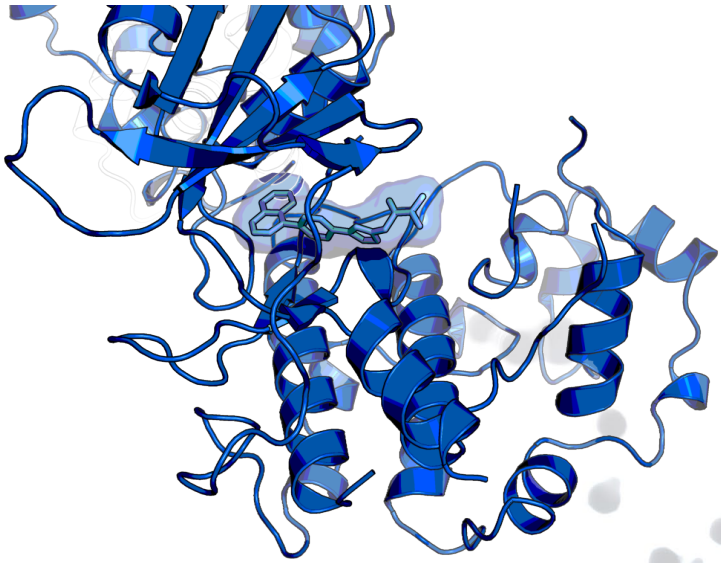

Figure 3: BI-1374 1374 in complex with inhibitor BI-1374 (X-ray structure solved at Boehringer-Ingelheim)

In vitro activity

BI-1347 is a potent and selective CDK8 inhibitor with an IC_{50} of 1 nM.

PROBE NAME / NEGATIVE CONTROL	BI-1347	BI-1374
MW [Da]	356	346
CDK8/cyclinC inhibition (IC_{50}) [nM]	1	671
Inhibition of pSTAT S727 in NK-92 cells (IC_{50}) [nM]	3	>10,000
Secretion of Perforin in NK-92 cells (EC_{50}) [nM]	10	n.d.
Inhibition of proliferation in MV-4-11b cells (IC_{50}) [nM]	7	n.d.
Inhibition of proliferation in NK-92 cells (IC_{50}) [nM]	>10,000	n.d.

In vitro DMPK and CMC parameters

PROBE NAME	BI-1347	BI-1374
Solubility FaSSIF / FeSSIF [µg/ml]	11 / 280	n.d.
Caco permeability @ pH 7.4 [$*10^{-6}$ cm/s]	95	n.d.
Caco efflux ratio	1.1	n.d.
Human hepatocyte clearance [% Q_H]	17	n.d.
Plasma protein binding 10% FCS [%]	64	n.d.
Plasma protein binding human [% Q_H]	98	n.d.

In vivo DMPK parameters

PROBE NAME	BI-1347	
Species	mouse	rat
Dose *i.v.* / *p.o.* [mg/kg]	1 / 10	1 / 3
CL [% Q_H]	15	14
Mean residence time after *iv* dose (l/kg)	0.6	0.7
F [%]	93	69
V_{ss} [l/kg]	0.5	0.4

Negative control

The compound BI-1374 can be used as an *in vitro* negative control (CDK8 IC_{50} = 671 nM)

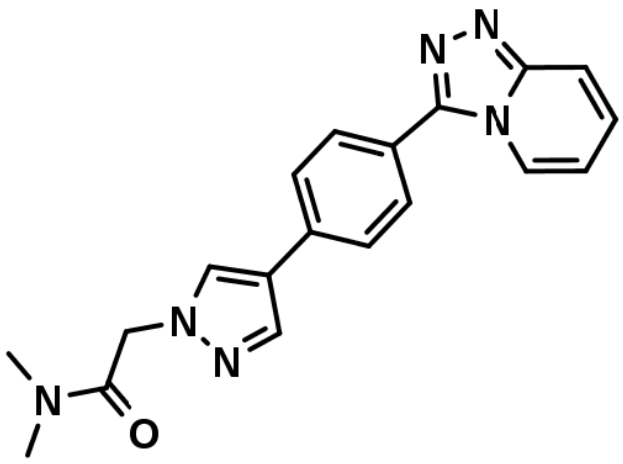

Figure 4: Chemical structure of the negative control BI-1374

Selectivity

Extensive external screens available (also see supplementary data):
Invitrogen® panel: 369 kinases screened @ 10 µM
Selected IC_{50} measured @ Invitrogen®:
CDK8 IC_{50} = 1.5 nM; CDK11 IC_{50} = 1.7 nM; MLCK IC_{50} = 531 nM; AURKB IC_{50} = 809 nM; FLT3 IC_{50} = 1360 nM; ICK IC_{50} = 2390 nM; STK16 IC_{50} = 3550 nM
Eurofins Safety Panel 44™ External screen covering 44 targets: @ 10 µM

SELECTIVITY DATA AVAILABLE	BI-1347	BI-1374
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

CCT251545, SEL120-34A

Summary

Cyclin-dependent kinase 8 (CDK8) is a Mediator complex-associated transcriptional regulator. Mediator is a multiprotein assembly containing up to 30 subunits that consist of four modules each: head, middle, tail and CDK⁄Cyclin and serves as a hub for diverse signaling pathways to regulate gene expression.[2] In addition CDK8 inhibition escalate the native activities of Natural Killer cells (NK cells), promoting their tumor surveillance and cytotoxicity.[3]

BI-1347 is a selective inhibitor of CDK8/cyclinC with an IC_{50} of 1 nM. BI-1347 with its good DMPK profile showed tumor growth inhibition in an *in vivo* xenograft model. Together with the also available structurally similar compound BI-1374, which can be used as negative control due to much weaker potency BI-1347 can serve as an excellent small molecule inhibitor for testing biological hypotheses like the role of the Mediator complex in human pathologies of as a tool targeting NK cells for anticancer immunotherapy *in vitro* and *in vivo*.

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

1. Marco H. Hofmann, Harald Engelhardt, Sebastian Carotta, Heribert Arnhof, Dirk Scharn, MarcKerenyi, Moritz Mayer, Gerhard Gmaschitz, Georg Egger, Christian Engelhardt, MichaelSanderson, Maria A. Impagnatiello, Renate Schnitzer, Mark Pearson, Darryl McConnell, NorbertKraut and Jürgen Moll. Development of selective and potent CDK8 inhibitors that increase NK cellactivity, which translates in tumor surveillance [abstract]. In: Proceedings of the AmericanAssociation for Cancer Research Annual Meeting 2017; 2017 Apr 1-5; Washington, DC.Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl):Abstract nr 4630. DOI: 10.1158/1538-7445.AM2017-4630.
2. Johannes Bragelmann, Niklas Klumper, Anne Offermann, Anne von Massenhausen, DianaBohm, Mario Deng, Angela Queisser,Christine Sanders, Isabella Syring, Axel S. Merseburger,Wenzel Vogel, Elisabeth Sievers, Ignacija Vlasic, Jessica Carlsson, Ove Andren, Peter Brossart,Stefan Duensing, Maria A. Svensson, Zaki Shaikhibrahim, Jutta Kirfel and Sven Perner Pan-Cancer Analysis of the Mediator Complex Transcriptome Identifies CDK19 and CDK8 asTherapeutic Targets in Advanced Prostate Cancer *Clin Cancer Res*, **2016**, *23(7)*, 1829-1840. DOI:10.1158/1078-0432.CCR-16-0094, PubMed.
3. Sebastian Carotta, Targeting NK Cells for Anticancer Immunotherapy: Clinical and PreclinicalApproaches *Front. Immunol.* **2016**, *7*, 1-10 DOI: 10.3389/fimmu.2016.00152, PubMed.
4. Trevor Dale, Paul A Clarke, Christina Esdar, Dennis Waalboer, Olajumoke Adeniji-Popoola,Maria-Jesus Ortiz-Ruiz, Aurélie Mallinger, Rahul S Samant, Paul Czodrowski, Djordje Musil, Daniel Schwarz, Klaus Schneider, Mark Stubbs, Ken Ewan, Elizabeth Fraser, Robert TePoele, Will Court, Gary Box, Melanie Valenti, Alexis de Haven Brandon, Sharon Gowan, Felix Rohdich, Florence Raynaud, Richard Schneider, Oliver Poeschke, Andree Blaukat, Paul Workman, Kai Schiemann, Suzanne A Eccles, Dirk Wienke & Julian Blagg A selective chemical probe for exploring the role of CDK8 and CDK19 in human disease *Nat. Chem. Biol.* **2015**, *9*, 206–209 DOI: 10.1038/nchembio.1952, PubMed.
5. The compound numbers mentioned herein are a reference to the numbering system employed in: Gollner A., Heine C., Hofbauer K. S. Kinase Degraders, Activators, and Inhibitors: Highlights and Synthesis Routes to the Chemical Probes on opnMe.com, Part 1. *ChemMedChem* **2023**, Published online ahead of print. DOI: 10.1002/cmdc.202300031, PubMed.