ALK5 inhibitor I BI-4659

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Summary

BI-4659 inhibits Alk5 with an IC_{50} value of 19 nM and blocks the cellular phosphorylation of Smad2/Smad3 with an EC_{50} of 185 nM. BI-4659 is a suitable tool for testing biological hypotheses *in vitro*.

Chemical Structure

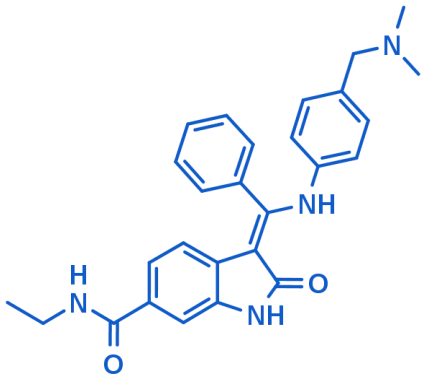

Figure 1: 2-D structure of BI-4659, an Alk5 inhibitor

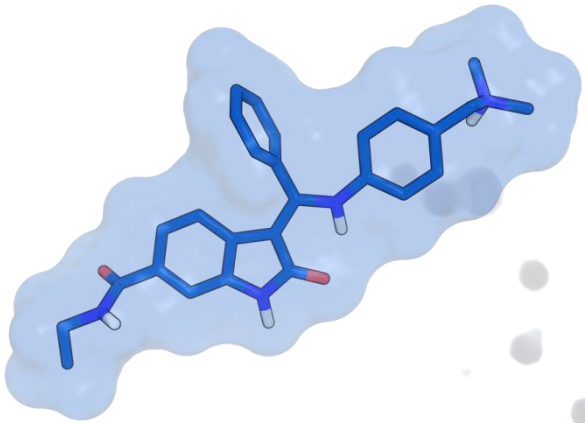

Figure 2: BI-4659, 3D conformation

Highlights

BI-4659 is a potent inhibitor of Alk5 (TGFßR1) with an IC_{50} value of 19 nM and shows selectivity against a broad panel of other kinases (see Selectivity section). BI-4659 blocks the phosphorylation of Smad2 and Smad3 in HaCaT cells with an EC_{50} of 185 nM. Together with the offered negative control BI-4101, BI-4659 is a suitable tool to test biological hypotheses *in vitro*.[1] (BI-4659 = compound **47i**, BI-4104 = compound **48** in reference 1).

Target information

Transforming growth factor β (TGFβ) is a pluripotent cytokine involved in the regulation of various biological processes such as cell proliferation, differentiation, migration, adhesion, apoptosis, and epithelial-to-mesenchymal transition (EMT). Therapeutic approaches to inhibit its signaling by targeting TGFβ receptor I (TGFβRI/Alk5) are discussed for the treatment of diseases such as idiopathic pulmonary fibrosis (IPF) and cancer.[2,3,4]

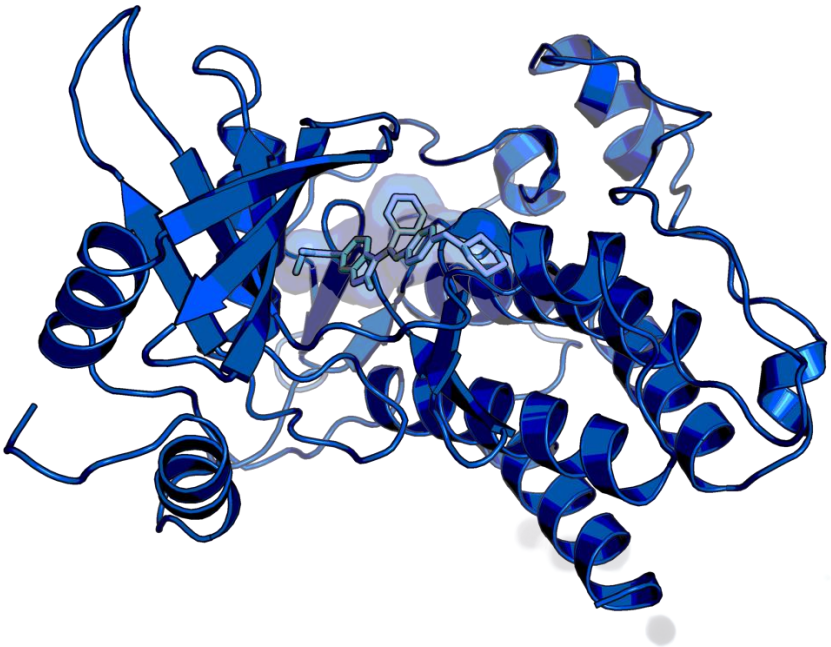

Figure 3: Crystal structure of Alk5 complexed with a close analog of BI-4659 (PDB code 2X7O).[1]

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-4659	BI-4101
MW [Da]	440.5	418.5
Inhibition of Alk5 (Kinase Glow assay, IC_{50}) [nM][a]	19	>50000
Inhibition of phosphorylation of Smad2 and Smad3 in HaCaT cells (EC_{50}) [nM]	185	n.d.

[a] for detailed assay conditions see reference 1

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI-4659			BI-4101
Solubility @ pH 6.8 [µg/ml]	2			n.d.
Solubility @ pH 4 [µg/ml]	84			n.d.
Solubility @ pH 2 [µg/ml]	71			n.d.
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	8			n.d.
CACO efflux ratio	6			n.d.
Microsomal stability (human/mouse/rat) [% Q_H]	26	23	<22	n.d.

Hepatocyte stability (human/mouse/rat) [% Q_H]	n.d.	n.d.
Plasma protein binding (human/mouse/rat) [%]	n.d.	n.d.
hERG [inh. % @ 10 µM]	38.5	n.d.
hERG (IC_{50}) [µM]	>10	n.d.
CYP 3A4 (IC_{50}) [µM]	>50	n.d.
CYP 2C8 (IC_{50}) [µM]	>50	n.d.
CYP 2C9 (IC_{50}) [µM]	>50	n.d.
CYP 2C19 (IC_{50}) [µM]	>50	n.d.
CYP 2D6 (IC_{50}) [µM]	>50	n.d.

In vivo DMPK parameters

BI-4659 showed very high clearance in rat and therefore is not suited for *in vivo* studies

BI-4659	RAT
Clearance [% Q_H][a]	123
Mean residence [l/kg][a]	1.7
t_{max} [h][b]	3.3
C_{max} [nM][b]	27.7
F [%][b]	50

V_{ss} [l/kg][a]	9

[a] *i.v.* 4.5 mg/kg

[b] *p.o.* 45 mg/kg

Negative control

BI-4101 shows no inhibition of Alk5 in the Kinase Glow assay (IC_{50} > 50 µM) and therefore is a suitable negative control for *in vitro* experiments.[1] (BI-4101 = Compound **48** in reference 1.)

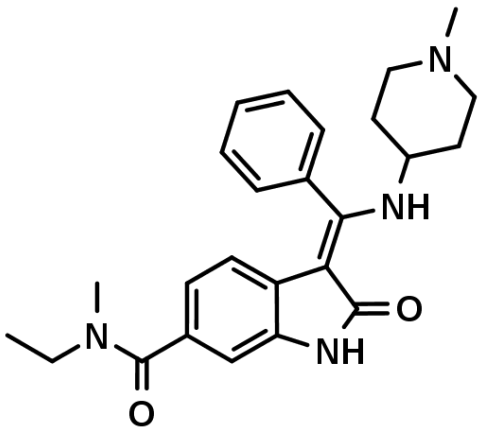

Figure 4: BI-4101 which serves as a negative control

Selectivity

BI-4659 shows good selectivity in kinase panels. The compound shows no inhibition of 218/232 kinases tested at 2 µM. Cross-reactive kinases (%inhibition data at 0.2 µM): ABL1 (67), BLK (85), CSF1R (FMS) (82), FGR (91), FLT3 (66), FYN (74), LCK (100), LYN A (79), LYN (78), MAP4K5 (KHS1) (77), MELK (80), NTRK3 (TRKC) (78), RET (79), SNF1LK2 (96), YES1 (90).

Selectivity data is available on opnMe.com for download free of charge.

BI-4659	SELECTIVITY DATA AVAILABLE
Cerep®	No
Panlabs®	No
Invitrogen®	Yes

DiscoverX®	Yes
Dundee	Yes

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

No in-house structure is available for BI-4659, but for related compound (PDB code 2x7o).

Reference molecule(s)

SB-505124, SB-525334, GK6604, SD-208, LY-2157299, EW-7197, GW788388 and others. For a review on Alk5 kinase inhibitors in oncology see reference 5.

Summary

BI-4659 is a potent and selective inhibitor of Alk5 (IC_{50} = 19 nM) which effectively blocks cellular phosphorylation of Smad2/Smad3 in HaCaT cells (EC_{50} = 185 nM). We also offer BI-4101 as negative control. BI-4659 (5 mg) can be ordered free of charge together with BI-4101.[1,6]

Supplementary data

Selectivity data and 2-D structure files can be downloaded free of charge from opnMe.

References

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2. Anne-Charlotte de Gouville and Stephane Hue Inhibition of ALK5 as a new approach to treat liver fibrotic diseases *Drug News Perspect*, **2006**, *19*, 85-90. DOI: 10.1358/dnp.2006.19.2.977444, PubMed.

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4. Rik Derynck and Ying E. Zhang Smad-dependent and Smad-independent pathways in TGF-beta family signaling *Nature* **2003**, *425*, 577-584. DOI: 10.1038/nature02006, PubMed.

5. Leona E. Ling, Wen-Cherng Lee TGF-Beta Type I Receptor (Alk5) Kinase Inhibitors in Oncology *Curr. Pharm. Biotechnol.*, **2011**, *12*, 2190-2202. DOI : 10.2174/138920111798808257, PubMed.

6. Patrick Baum , Ramona Schmid , Carina Ittrich, Werner Rust, Katrin Fundel-Clemens, Susanne Siewert, Martin Baur, Lisa Mara, Lore Gruenbaum, Armin Heckel, Roland Eils, Roland E. Kontermann, Gerald J. Roth, Florian Gantner, Andreas Schnapp, John E. Park, Andreas Weith, Karsten Quast, Detlev Mennerich Phenocopy – A Strategy to Qualify Chemical Compounds during Hit-to-Lead and/or Lead Optimization *PLoS One*, 2010, 5, e14272. DOI: 10.1371/journal.pone.0014272, PubMed.