opnMe by Boehringer Ingelheim

IRAK4 inhibitor

BI1543673

Boehringer Ingelheim

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Summary

BI1543673 is a potent and selective IRAK4 inhibitor suitable for both *in vitro* as well as *in vivo* use. BI-4326, a structurally close analog, is available as a negative control.

Chemical Structure

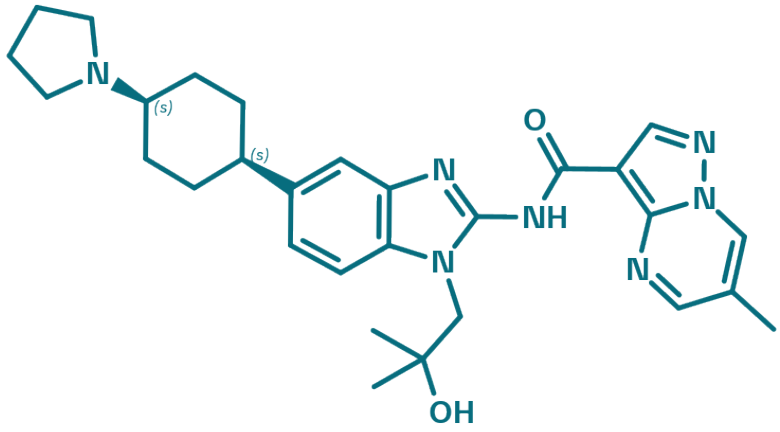

Figure 1: 2D structure of BI1543673, an IRAK4 inhibitor

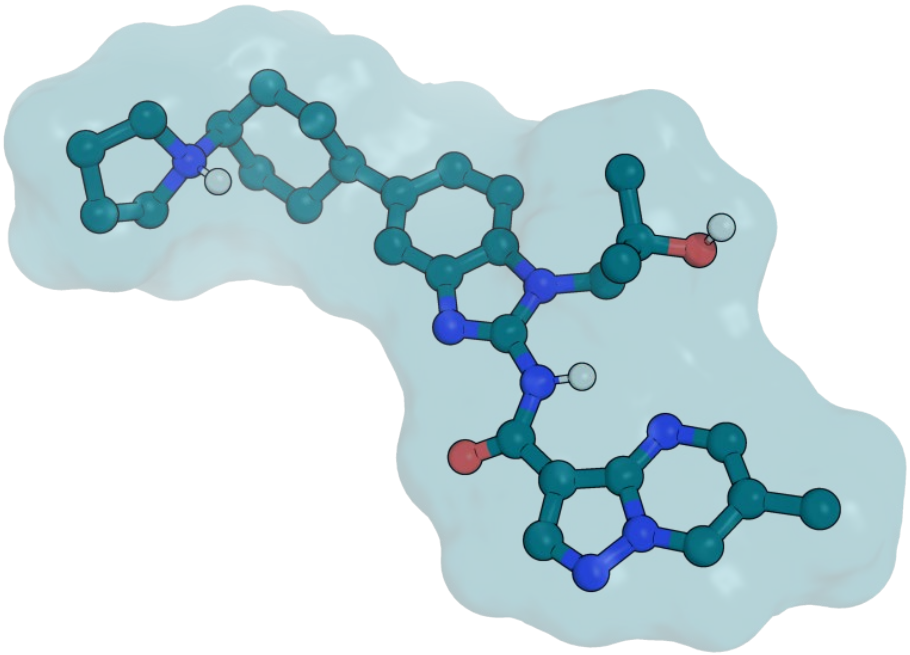

Figure 2: BI1543673, 3D conformation

Highlights

BI1543673 is a potent IRAK4 inhibitor with a unique selectivity profile, including selectivity versus IRAK family members. It is suitable for both *in vitro* as well as *in vivo* use. BI1543673 reduced inflammatory responses to both TLR4 and TLR7/8 stimulation in human lung tissue studied *ex vivo*[1]. BI-4326, a structurally close analog, is available as a negative control.

Target information

The family of interleukin-1 receptor-associated kinases (IRAKs) consists of 4 members (IRAK1, 2, 3, 4) that play an important role in the immune response to pathogens via toll-like receptor (TLR) signaling.

Specifically, IRAK4 is a key node in innate inflammatory signaling directly downstream of the TLR, and interleukin-1 (IL-1) family of receptors. IRAK4 is expressed in T and B lymphocytes and has been reported to play an important role in the cross talk between the innate and adaptive immune systems. IRAK4 has both a kinase dependent signaling role as well as a scaffolding role in a larger signaling complex including proteins such as myeloid differentiation primary response gene 88 (MYD88) and IRAK1.

From a pathophysiology perspective, it has been shown that IRAK4 is involved in cellular processes underlying inflammation, autoimmunity, as well as drug resistance. Targeting IRAK4 for the development of inhibitors and PROTAC degraders may present a novel research strategy against defined disease conditions[2].

The protein kinase activity of IRAK4 plays a key role in the further downstream inflammatory signaling, for example of TLR4, 7, and 8[3,4].

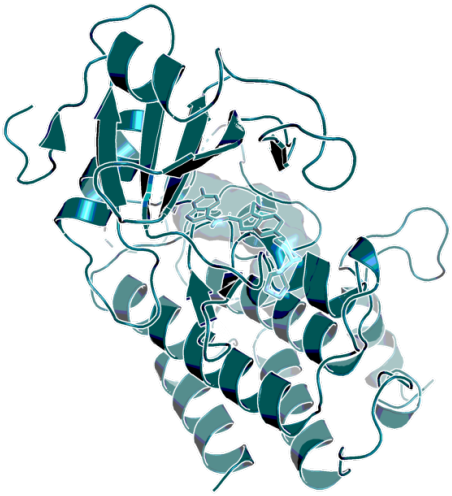

Figure 2: Model of the complex of IRAK4 with BI1543673, based on a proprietary X-ray structure with a closely related inhibitor

In vitro activity

BI1543673 is a potent IRAK4 inhibitor with a unique selectivity profile, including selectivity versus IRAK1.

Probe name / Negative control	BI1543673	BI-4326
MW [Da][a]	515.7	629.69
IRAK4 (IC_{50}) [nM][b]	6.9	>3,000
IRAK1 (IC_{50}) [nM][c]	>100	>3,000

[a] The molecule is supplied in salt form; for the molecular weight of the salt, please refer to the vial label.

[b] Z'-LYTE Assay conditions: The 2X IRAK4 / Ser / Thr 07 mixture was prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl2, 1 mM EGTA, 2 mM DTT, 0.02% NaN3. The final 10 µL Kinase Reaction consisted of 3.45 - 20 ng IRAK4 and 2 µM Ser / Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl2, 5 mM MnCl2, 1 mM EGTA, 1 mM DTT, 0.01% NaN3. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45,000 dilution of Development Reagent A was added. The Development Reagent was diluted in Development Buffer. Z'-LYTE™ Kinase Assay Kits | Thermo Fisher Scientific - DE

[c] Adapta Assay conditions: The 2X IRAK1 / Histone H3 (1-20) peptide mixture was prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, 1 mM EGTA. The final 10 µL Kinase Reaction consisted of 3.5 - 30.5 ng IRAK1 and 100 µM Histone H3 (1-20) peptide in 32.5 mM HEPES pH 7.5, 0.005% BRIJ-35, 5 mM $MgCl_2$, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix was added.
The Detection mix consisted of EDTA (30 mM), Eu-anti-ADP antibody (6 nM) and ADP tracer. The detection mix contained the EC60 concentration of tracer for 5-150 mM ATP. Adapta® Universal Kinase Assay and Substrates | Thermo Fisher Scientific - DE

In vitro DMPK and CMC parameters

BI1543673 is a well soluble compound with good metabolic stability profile across species. It shows medium plasma protein binding, yet based on high efflux ratios in permeability assays, distribution into the brain via crossing the blood brain barrier is limited.

Probe name / Negative control	BI1543673	BI-4326
logD @ pH 7.4	0.9	n.a.
Solubility @ pH 7 [µg/mL]	>167	>157
Caco-2 permeability AB @ pH 7.4 [$*10^{-6}$ cm/s]	0.8	4.6
Caco-2 efflux ratio	19.1	9.8
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	<1.9	n.a.
MDCK efflux ratio	8.3	n.a.
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / <23 / <22	<23 / <23 / <22
Hepatocyte stability (human/mouse/rat) [% Q_H]	<4 / 81 / 26	n.a.

Plasma Protein Binding (human/mouse/rat) [%]	70.3 / 37.6 / 38.1	96.4 / 71.7 / 62.5
hERG (IC_{50}) [µM]	>10	n.a.
CYP 3A4 (IC_{50}) [µM]	>50	>50
CYP 2C8 (IC_{50}) [µM]	>50	>50
CYP 2C9 (IC_{50}) [µM]	>50	>50
CYP 2C19 (IC_{50}) [µM]	>50	>50
CYP 2D6 (IC_{50}) [µM]	>50	>50
CYP 1A2 (IC_{50}) [µM]	>50	>50
CYP 2B6 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

BI1543673 shows acceptable to low oral bioavailability in mouse and rat, respectively. We recommend the use of mouse models for *in vivo* investigations.

BI1543673	Mouse[a]	Rat[b]
Clearance [% Q_H][a]	56	140
Mean residence time after *i.v.* dose [h][a]	0.97	3.8
t_{max} [h][b]	1.7	2.1
C_{max} [nM][b]	519.4	67.8
F [%][b]	52	22
V_{ss} [L/kg][a]	2.9	22.0

[a] *i.v.* dose: 0.58 mg/kg, *p.o.* dose: 4.9 mg/kg
[b] *i.v.* dose: 0.58 mg/kg, *p.o.* dose: 5.8 mg/kg

In vivo pharmacology

In vivo activity of BI1543673 was tested in an LPS-induced lung inflammation model. Briefly, C57BL/6 mice very challenged with the nebulized solution of 1 mg/ml LPS for 30 min. Four hours after the LPS challenge, animals were euthanized, venous blood samples were collected, and a lung lavage was performed (HBSS). Cell counts in the lavage fluid and cell differentiation were determined using a hematocytometer following the suppliers' instructions. TNF-α concentration in the lavage fluid was measured by enzyme-linked

immunosorbent assay (ELISA). BI1543673 was dissolved in 0.5% hydroxyethylcellulose (pH 4). Mice were treated with BI1543673 or vehicle orally 1h before LPS challenge.

Treatment with BI1543673 resulted in a marked inhibition of neutrophil influx into the lung and TNF-α production.

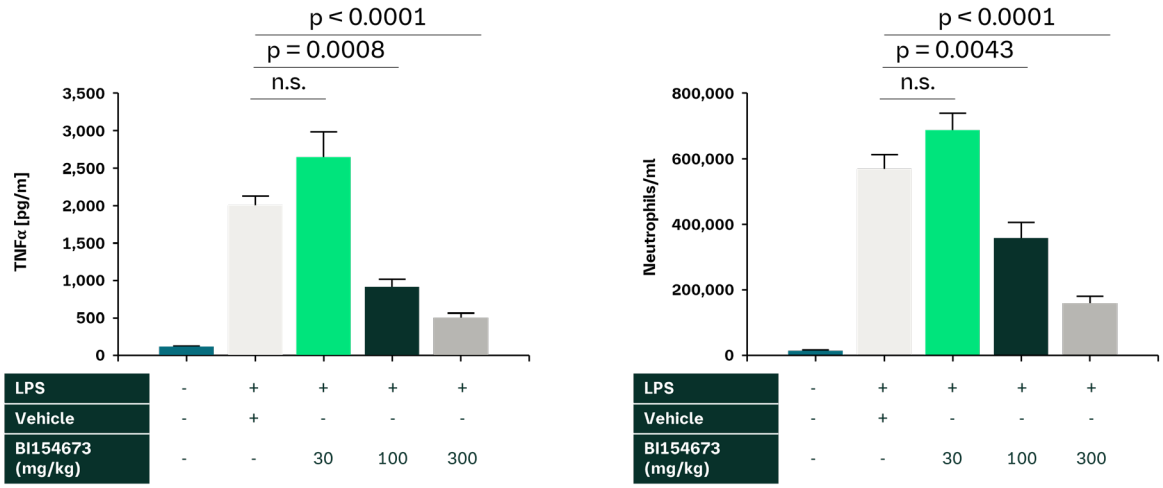

Figure 3: TNF-α concentration and neutrophil count in the absence of stimulation, following treatment with vehicle and different concentrations of BI1543673. Based on these results we recommend a dose of 300mg/kg for *in vivo* studies

Negative control

BI-4326, a structurally close analog, can be used as negative control.

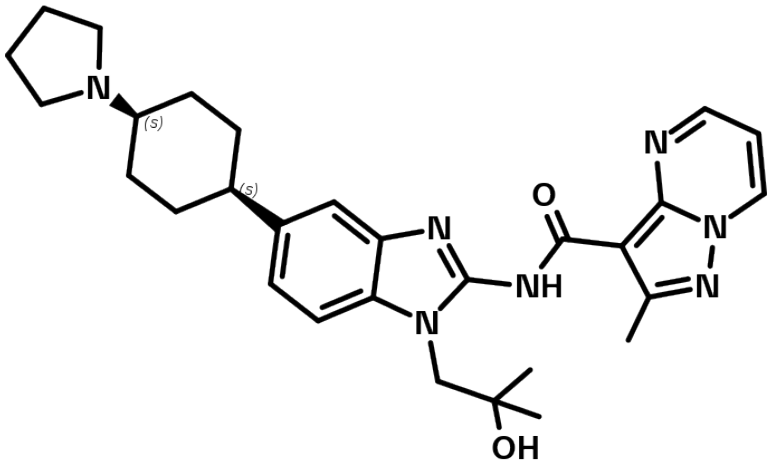

Figure 4: BI-4326 which serves as a negative control

Selectivity

BI1543673 inhibited 4 out of 389 tested kinases by greater than 50% at a 1-µM concentration (IRAK1, IRAK3, NTRK1 and IRAK4 itself) in the SafetyScreen44™. The negative control BI-4326 inhibited 5 out of 44 tested kinases by greater than 50% at a 10µM concentration (ACE (hum), M3/H, M1/H, SLC6A4/H and M2/H).

Selectivity data available	BI1543673	BI-4326
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

The commercially available tool compounds zimlovisertib (PF06650833)[5], BAY1834845 (zabedosertib), and BAY1830839[6] can serve as reference molecules.

Supplementary data

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

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