opnMe by Boehringer Ingelheim

Ketohexokinase KHK

BI-9787

Boehringer Ingelheim

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Summary

BI-9787 is a potent, highly permeable and selective KHK inhibitor, suitable for *in vitro* and *in vivo* use. The structurally similar BI-2817 is provided as a negative control.

Chemical Structure

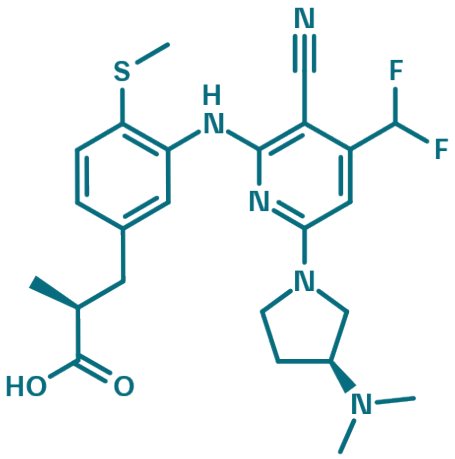

Figure 1: 2D structure of BI-9787, a zwitterionic KHK inhibitor

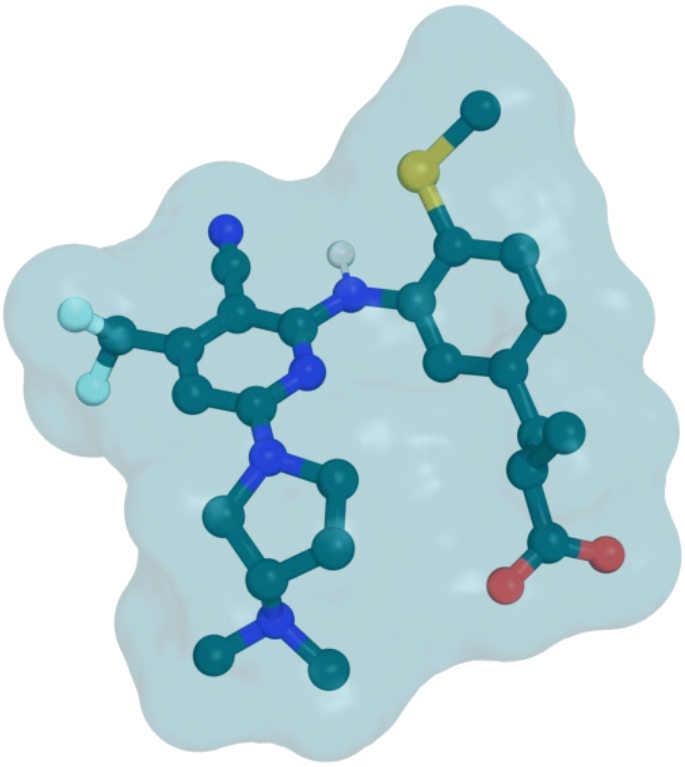

Figure 2: BI-9787, 3D conformation

Highlights

BI-9787 is a potent, structurally distinct, zwitterionic KHK inhibitor, suitable for use *in vitro* as well as *in vivo*. It combines high potency and high permeability with a favourable oral rat PK and high target selectivity. In addition, we also provide BI-2817, which can serve as a negative control for *in vivo* and *in vitro* studies that utilize BI-9787 as a chemical probe.

Target information

Ketohexokinase (KHK) is a kinase that catalyses the phosphorylation of fructose forming fructose-1-phosphate. In humans, KHK exists as two isoforms, KHK-A and KHK-C, whereby the former has a lower affinity for fructose and is expressed in various tissues[1,2]. The higher fructose affinity KHK-C is expressed predominately in the liver, pancreas, kidneys, and small intestine[1]. Since fructose metabolism occurs mainly in the gut wall and, in instances of high fructose consumption, the liver, pancreas, and kidneys, KHK-C is thought to play a key role in metabolic disorders, implicating KHK-C as a driver of metabolic dysfunction[2].

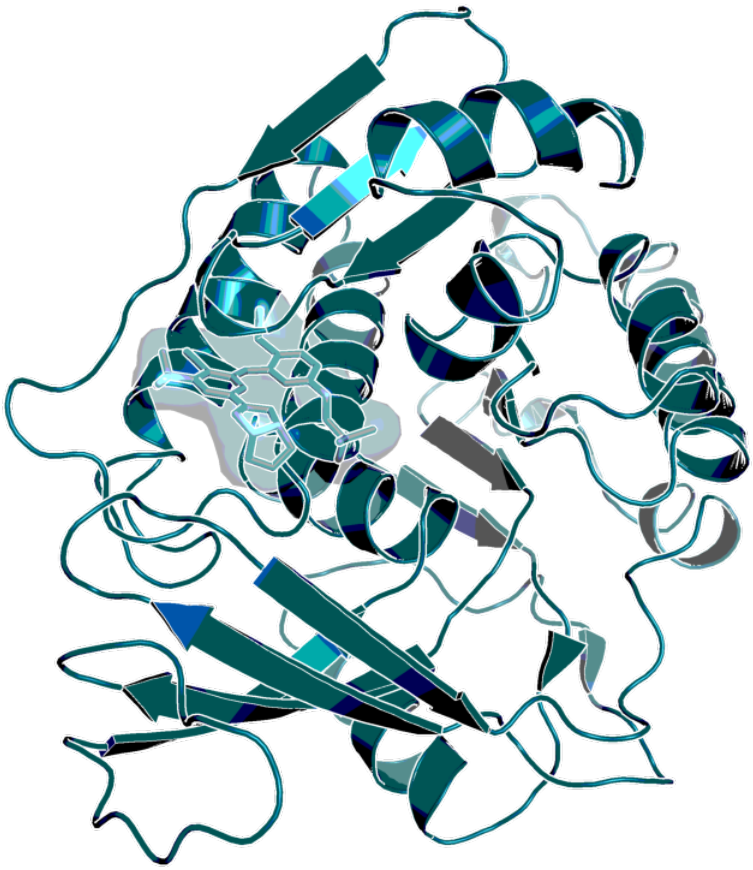

Figure 3: X-ray structure of a close BI-9787 analog (referred to as cpd28 in Heine *et al.*[3]), in association with KHK-C (PDB code: 8OMJ).

In vitro activity

The zwitterionic structure of BI-9787[3] displays a combination of high enzymatic and cellular potency, high microsomal stability and significantly improved selectivity.

PROBE NAME / NEGATIVE CONTROL	BI-9787	BI-2817
MW [Da, free base][a]	489.6	447.5
hKHK-C (IC_{50}) [nM][b]	12.8	5,029
hKHK-A (IC_{50}) [nM][b]	12	n.a.
MouseKHK-C (IC_{50}) [nM][b]	20	n.a.
RatKHK-C (IC_{50}) [nM][b]	3.0	8870
F1P HepG2 (IC_{50}) [nM][c]	123	12,256
F1P mouseHep (IC_{50}) [nM][c]	59	n.a.

[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] **Assay conditions: Kinase activity of recombinant His-tagged KHK isoforms.**
The enzymatic activity of recombinant human KHK-C, human KHK-A, rat and mouse KHK-C was determined using the ADP-GLOTM Kinase Assay kit from Promega as described in the instructions. In brief, 1.25 µg/ml His tagged human KHK-A was incubated for 60 min at room temperature with 15 mM D-fructose. His-tagged human KHK-C (1 µg/ml) and His-tagged mouse KHK-C (0.625 µg/ml) were incubated for 60 min at room temperature with 400 µM D-fructose and 200 µM ATP. His-tagged rat KHK-C (0.5 µg/1.5 ml) was incubated for 60 min at room temperature with 100 µM D-fructose and 200 µM ATP.
For all incubations the following assay buffer was used: 50mM HEPES pH 7.4, 4mM MgCl2, 20mM KCl, 0.01% Tween 20, 1mM DTT. The enzymatic reaction was stopped and developed using the ADP-GLOTM Kinase kit according manufacturers description and the results were analyzed using the luminescence signal determined by a multiplate reader from EnVision. Signals from samples with enzyme and substrates alone were reported as 100 % and signals from samples with enzyme alone were reported as 0 %

[c] **Assay conditions: Quantitative determination of fructose-1-phosphate in HepG2 cells and primary mouse hepatocytes.**
HepG2 cells (B.B Knowles, Wistar Institute) were incubated with test compound or solvent (DMSO) in medium (EMEM, 10 mM NEAA, 8 mM glutamine, 10 % FCS) for 30 min at 37°C under 5 % CO2. D-fructose was added to a final concentration of 15 mM and cells were incubated for further 60 min under the same conditions. Cells were put on ice washed with phosphate buffered saline and lysed in 10 mM ammonium acetate. Cell protein was precipitated with acetonitrile and an aliquot of the supernatant was analyzed for fructose-1-phosphate using the RapidFire-MS/MS (RIAS) technology. Fructose-6-phosphate (0.1 µM) in the samples was used as internal standard for the quantification.
Primary mouse hepatocytes (Fisher Scientific) were incubated in Williams Medium E (0,1 mg/ml Gentamycin, 0.1 µM Dexamethasone, 2 mM L-glutamine, 17 nM insulin-transferrin-selenite supplement-G). All other reagents and procedures were identical with the protocol described for HepG2 cells

In vitro DMPK and CMC parameters

BI-9787 and BI-2817 show good cell permeability, good hepatocyte stability across species and high plasma protein binding.

PROBE NAME / NEGATIVE CONTROL	BI-9787	BI-2817
logD @ pH 11	0.9	0.6
High Throughput Solubility @ pH 7 [µg/mL]	19	87
Caco-2 permeability AB @pH7.4 [*10^{-6} cm/s]	22	19
Caco-2 efflux ratio	1.9	1.7
Microsomal stability (human/mouse/rat) [% QH]	<23 / <23 / <22	25 / <23 / <22
Hepatocyte stability (human/mouse/rat) [% QH]	24 / 25 / 13	23 / 15 / 10
Plasma Protein Binding (human/mouse/rat) [%]	>99.9 / 99.4 / 99.5	99.6 / 95.7 / 95.0
hERG (IC_{50}) [µM]	1.6	>10
CYP 3A4 (IC_{50})[µM]	>50	>50
CYP 2C8 (IC_{50})[µM]	26.8	>50
CYP 2C9 (IC_{50})[µM]	>50	>50
CYP 2C19 (IC_{50})[µM]	>50	>50
CYP 2D6 (IC_{50})[µM]	>50	>50

In vivo DMPK parameters

BI-9787 exhibits good clearance and mean residence time in rat together with good bioavailability.

BI-9787	RAT
Clearance [% Q_H][a]	22
Mean residence time after *i.v.* dose [h][a]	2

t_{max} [h][b]	0.4
C_{max} [nM][b]	2,620
F [%]	68
V_{ss} [L/kg][a]	1.8

[a] *i.v.* dose: 0.5 mg/kg
[b] *p.o.* dose: 5 mg/kg

Negative control

BI-2817 was designed as inactive analogue of BI-9787, resulting in a tool compound that is structurally very close to BI-9787 and displays almost identical *in vitro* PK parameters.

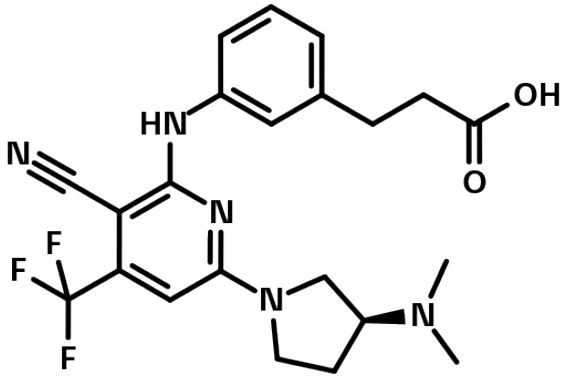

Figure 4: BI-2817 which serves as a negative control.

Selectivity

BI-9787 was tested on 44 targets in a selectivity panel and showed ≥100fold selectivity for 43 targets. Against PDE4D2, it still maintains an estimated selectivity factor of at least 30-fold (81% inhibition at 10 µM).
The comparably selective negative control BI-2817 showed in only 3 out of 44 targets inhibition with more than 50% at 10 µM (N_Neuro_A, H1/Pyril/HS, BZD/Centr/R).

SELECTIVITY DATA AVAILABLE PROBE NAME / NEG. CONTROL	BI-9787	BI-2817
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No

DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

PF-06835919[4–7]

Supplementary data

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

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