opnMe by Boehringer Ingelheim

Orally bioavailable 3-phosphoglycerate dehydrogenase (PHGDH) inhibitor

BI-9593

Boehringer Ingelheim

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Summary

BI-9593 is the first orally bioavailable PHGDH inhibitor suitable for cellular and *in vivo* profiling. BI-9594 is available as a negative control.

Chemical Structure

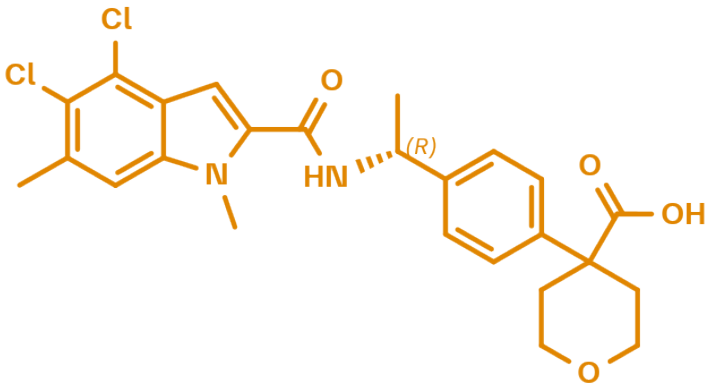

Figure 1: 2D structure of BI-9593, an orally bioavailable PHGDH inhibitor

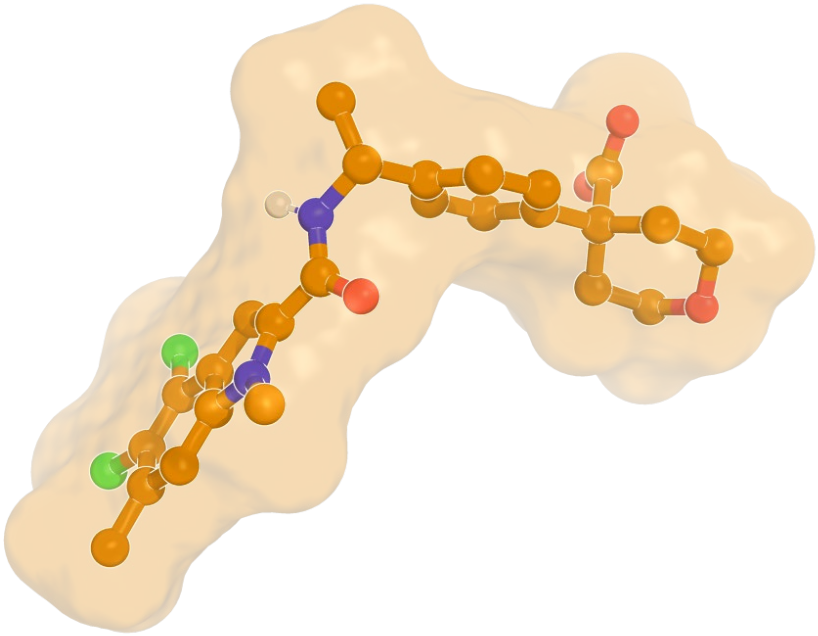

Figure 2: BI-9593, 3D conformation

Highlights

To our knowledge, BI-9593 is the first orally bioavailable PHGDH inhibitor, characterized by high potency (IC_{50} = 7 nM) and selectivity. With its unique profile, it complements the previously shared BI-4924 and its esterified prodrug BI-4916. Its stereoisomer BI-9594 has significantly reduced potency in both *in vivo* and cellular assays, and is suitable as a negative control.

Target information

PHGDH (3-phosphoglycerate dehydrogenase) catalyzes the first step of *de novo* serine biosynthesis downstream of glycolysis and is the rate limiting enzyme for the pathway[1]. PHGDH converts 3-phosphoglycerate (3-PG) to 3-phosphohydroxypyruvate (3-PHP) in a NAD-dependent manner and is expressed in high levels in the brain, heart, kidneys, liver, and skeletal muscle. Patients with loss-of-function mutations in PHGDH suffer from neurodevelopmental symptoms such as microcephaly, a finding which is replicated in murine models with brain-specific PHGDH deletions[2].

PHGDH is also amplified or overexpressed in a subset of tumors, most frequently melanoma and triple-negative breast cancers. Cells with amplified or overexpressed PHGDH show an elevated serine synthesis and are relatively resistant to serine starvation while showing some dependency on PHGDH activity[3-6].

Although a plethora of published data support the contributions of the serine biosynthesis pathway to tumorigenesis, as well as to the development of the central nervous system, the precise molecular mechanisms of PHGDH's role in disease remain unclear. BI-9395 can help elucidating the role of PHGDH *in vivo*, while BI-4924 and its prodrug BI-4916 with better permeability characteristics are suitable for cellular studies.

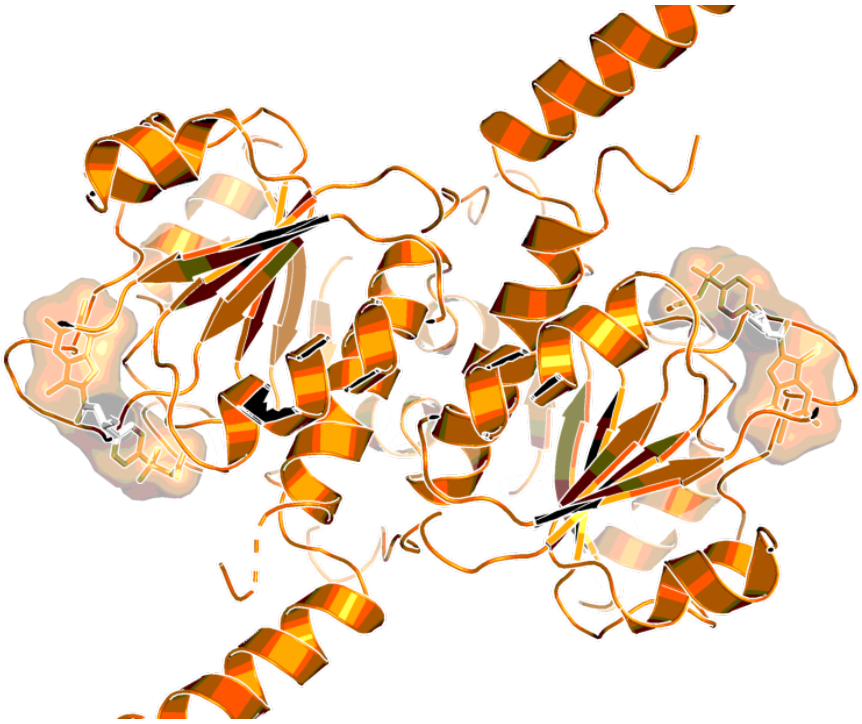

Figure 3: X-ray structure of BI-4924, a close analog of BI-9593, binding in PHGDH (PDB code:6RJ6)

In vitro activity

BI-9593 displays an IC_{50} of 7 nM in a PHGDH FL His 500/500 NAD/3-PG diaphorase/resazurin assay and shows modulation of cellular ^{13}C serine biosynthesis over 72 h with an IC_{50} of 1.4 µM.

PROBE NAME / NEGATIVE CONTROL	BI-9593	BI-9594
MW [Da, free base][a]	489.4	489.4
PHGDH FL His 500/500 NAD/3-PG Diaphorase/Resazurin Assay (IC_{50}) [nM][b]	7	160
PHGDH (3-Phosphoglycerate dehydrogenase length version with His-tag) ^{13}C Serine MS Assay 50 µl (IC_{50}) [nM]	1,360	> 100,000

[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] PHGDH (3-phosphoglycerate dehydrogenase) FL His HighConc 500/500 NAD/3-PG diaphorase/resazurin Assay
[c] All assay conditions are in reference 1

In vitro DMPK and CMC parameters

Both BI-9593 and the negative control BI-9594 are highly permeable compounds without efflux liabilities. The excellent microsomal stability qualifies both compounds for *in vivo* profiling.

PROBE NAME / NEGATIVE CONTROL	BI-9593	BI-9594
logD @ pH 11	2.2	1.2
Solubility @ pH 7 [µg/mL]	38	29
Microsomal stability (human/mouse/rat) [% Q_H]	< 24 / < 24 / 28	< 24 / < 24 / < 23
Hepatocyte stability (mouse) [% Q_H]	15	n.a.
Plasma Protein Binding (human/mouse) [%]	> 99.8 / > 99.9	n.a. / 99.8

In vivo DMPK parameters

BI-9593	MOUSE
Clearance [% Q_H][a]	6.0
Mean residence time after *i.v.* dose [h][a]	6.2
t_{max} [h][b]	1.7
C_{max} [nM][b]	29,200
F [%][b]	100
V_{ss} [L/kg][a]	1.9

[a] *i.v.* dose: 1 mg/kg
[b] *p.o.* dose: 25 mg/kg

In vivo pharmacology

PK properties in mice are suitable for twice daily oral dosing in acute or sub-chronic *in vivo* experiments, resulting in >90% inhibition of *de novo* serine synthesis for up to 20 h in the spleen of mice treated with a single dose of 250 mg/kg. The compound is well tolerated, as assessed by measuring body weight change.

Negative control

BI-9594, is a stereoisomer with >20-fold and >70-fold lower potency in biochemical and cellular assays respectively and can be used as negative control.

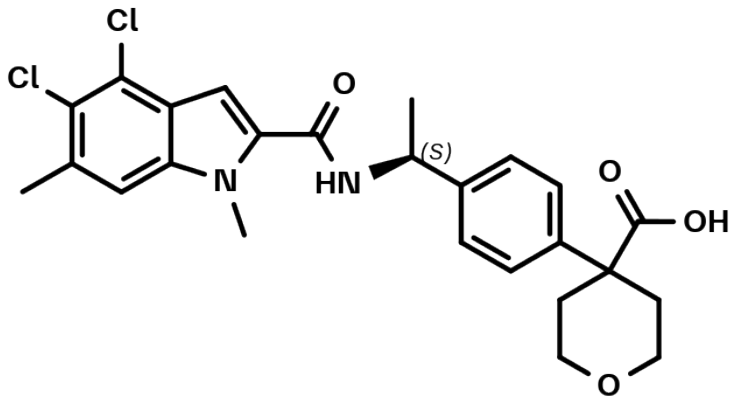

Figure 4: BI-9594 which serves as a negative control

Selectivity

BI-9593 hits 4 out of 44 targets with > 50% inhibition. Two GPCRs namely A2A/H and CCKA/H are hit with 62% and 95% inhibition respectively. In addition, BZD/CENTR/R and PDE3A are hit with 68% and 85% inhibition respectively. The negative control BI-9594 hits 3 out of 44 targets with > 50% inhibition, showing a different selectivity pattern. It hits 5HT2B with 65% inhibition, CCKA/H with 59% inhibition and GCORTICOID/H with 67% inhibition in radio displacement assay.

SELECTIVITY DATA AVAILABLE	BI-9593	BI-9594
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No

DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

The 1st generation PHGDH inhibitor BI-4924 can be used as a reference molecule.

Supplementary data

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

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