opnMe by Boehringer Ingelheim

GSK-3 inhibitor

BI-5521

Boehringer Ingelheim

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Summary

BI-5521 is a potent and selective ATP-competitive small molecule inhibitor of glycogen synthase kinase 3 (GSK-3) with demonstrated *in vivo* activity. The *N*-alkylated derivative BI-4481 is also available as negative control.

Chemical Structure

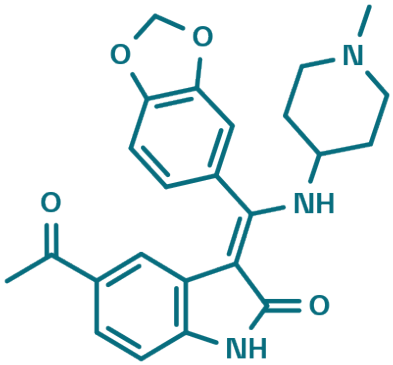

Figure 1: 2D structure of BI-5521, a potent and selective GSK-3 inhibitor

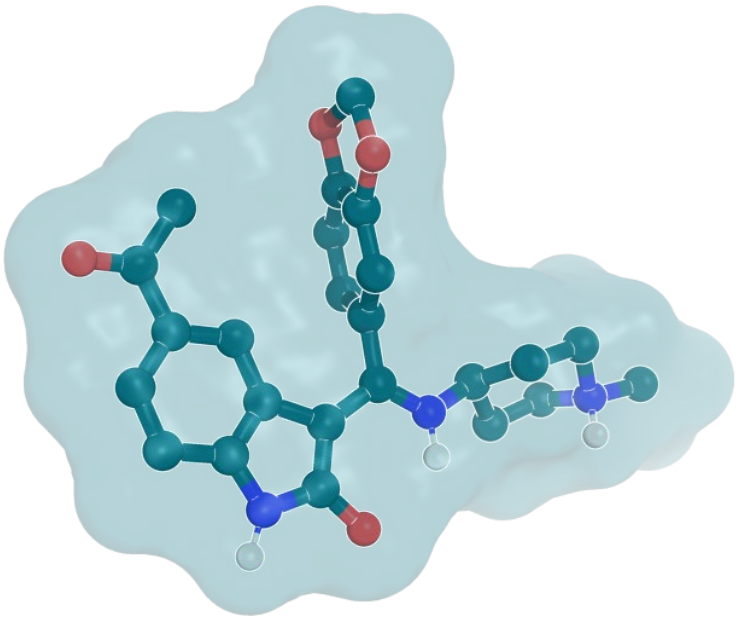

Figure 2: BI-5521, 3D conformation

Highlights

BI-5521 is a highly potent and selective non-covalent ATP-competitive inhibitor of glycogen synthase kinase (GSK-3)[1]. It is similarly active on both GSK-3 isoforms (GSK-3α and β) with single-digit nanomolar potency and has demonstrated glucose-lowering efficacy in both acute and subchronic settings in rodents. This compound has a decent PK profile *in vivo* and may be a useful tool for the validation of GSK-3 as a therapeutic target.

Target information

Glycogen synthase kinase (GSK-3) is a constitutively active serine/threonine kinase that phosphorylates a large number of proteins in a variety of different pathways. In mammalian tissues, GSK-3 exists as two isoforms (GSK-3α and GSK-3β) that share 98% homology of their kinase domains. GSK-3β has been implicated in various diseases such as diabetes, inflammation, cancer, amyotrophic lateral sclerosis (ALS), Alzheimer's and Parkinson's diseases, and bipolar disorders[2-5]. Moreover, GSK-3 inhibitors serve as tools for regenerative medicine through increasing self-renewal and/or differentiation of stem cells[5].

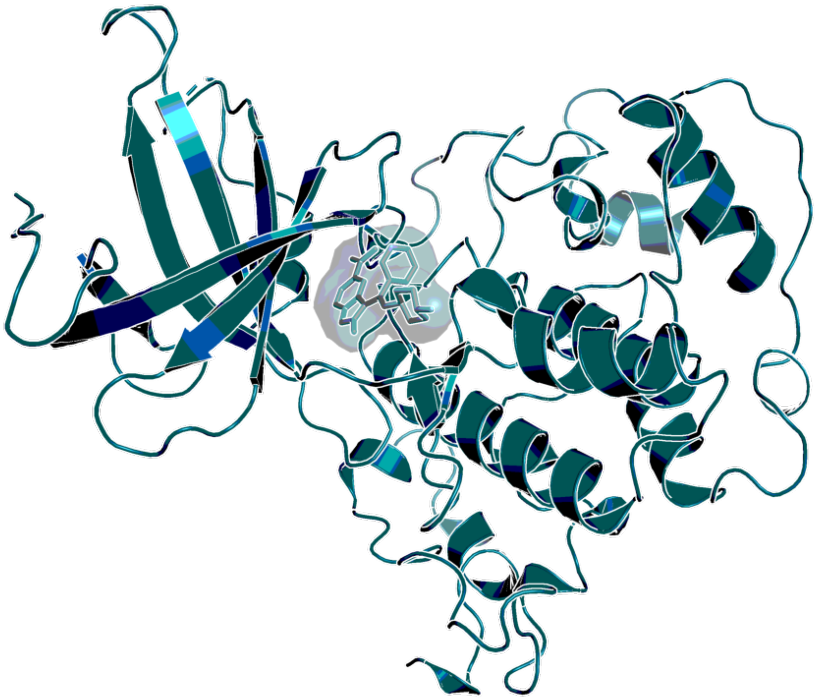

Figure 3: Crystal structure of GSK-3 complexed with BI-91BS, a close analog of BI-5521 (PDB code 6GJO).

In vitro activity

BI-5521 inhibits GSK-3β with an IC_{50} of 1.1 nM. The *N*-Ethyl analog BI-4481, which can be used as a negative control, shows an activity of >10,000 nM in this assay.

PROBE NAME / NEGATIVE CONTROL	BI-5521	BI-4481
MW [Da, free base][a]	419.5	448.5
Inhibiton of GSK-3β (IC_{50}) [nM][b]	1.1	>10,000
Inhibiton of GSK-3α (IC_{50}) [nM][c]	2.0	n.d.
Inhibiton of GSK-3β (DC_{50}) [nM][c]	5.0	n.d.
Stimulation of Glycogen Synthesis rate in C3A cells (EC_{50}) [nM]	3.0	n.d.
Cytotoxicity (IC_{50}) [nM]$_{50}$[c]	390	>50,000

[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] Inhouse assay: recombinant human GSK-3 was incubated with ^{33}P-ATP and a 21 AA-peptide from Glycogen Synthase bearing the phosphorylation sites for GSK-3. The radiolabelled P-GS peptide was absorbed using a filter and was quantified in a counter.

[c] In vitro cytotoxicity assay using the U937 cell line and the colorimetric EZ4U assay.

In vitro DMPK and CMC parameters

BI-5221 is a permeable but poorly soluble tool compound with moderate microsomal stability. Efforts to improve the solubility led to the discovery of BI-4481, the negative control.

PROBE NAME / NEGATIVE CONTROL	BI-5521	BI-4481
logD @ pH 11	2.2	3.4
Solubility @ pH 6.8 [µg/mL]	76	90
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	10.3	30.1
Caco-2 efflux ratio	4.6	0.7
Microsomal stability (human/mouse/rat) [% Q_H]	26 / 48 / 35	52 / >88 / 83
CYP 3A4 (IC_{50}) [µM]	>50	n.a.

CYP 1A2 (IC_{50}) [µM]	>50	n.a.
CYP 2C9 (IC_{50}) [µM]	>50	n.a.
CYP 2C19 (IC_{50}) [µM]	12.9	n.a.
CYP 2D6 (IC_{50}) [µM]	>50	n.a.

In vivo DMPK parameters

Pharmacokinetic parameters of BI-5521 in rats

BI-5521	RAT
Clearance [mL/min/kg][a]	32
Mean residence time after *i.v.* dose [h][a]	2.9
t_{max} [h][b]	4
C_{max} [nM][b]	15.5
V_{ss} [L/kg][a]	5.6
F [%]	17

[a] *i.v.* dose: 4.2 mg/kg
[b] *p.o.* dose: 0.42 mg/kg

In vivo pharmacology

BI-5521 showed acute efficacy in ZDF rats.

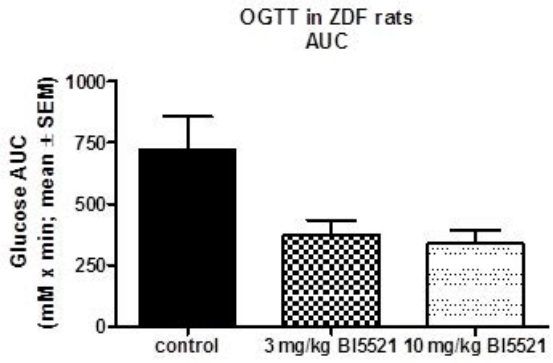

Figure 4: Decreased plasma glucose levels during an oral glucose tolerance test in ZDF rats

BI-5521 showed subchronic efficacy in db/db mice

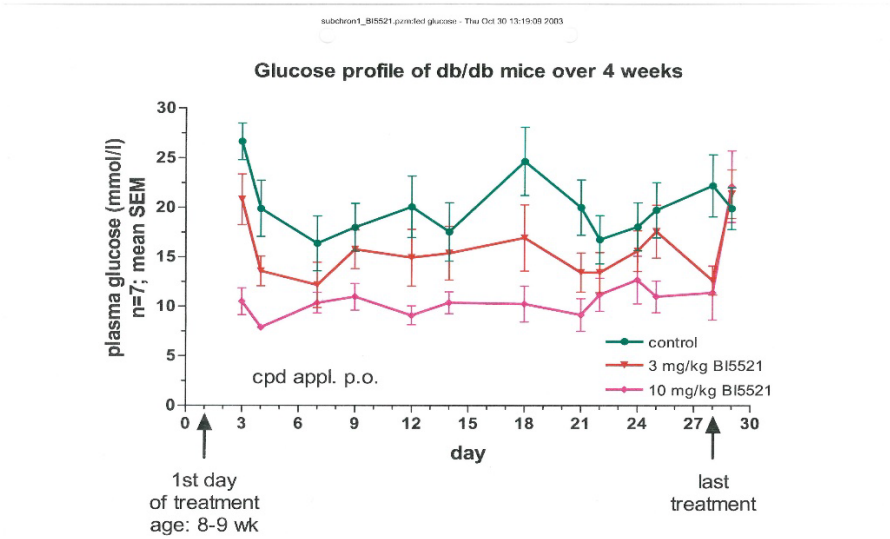

Figure 5: Decreased plasma glucose levels over 4 weeks in db/db mice.

Negative control

The *N*-Ethyl analog BI-4481 can be used as negative control ($IC_{50\ GSK\text{-}3\beta}$ >10,000 nM).

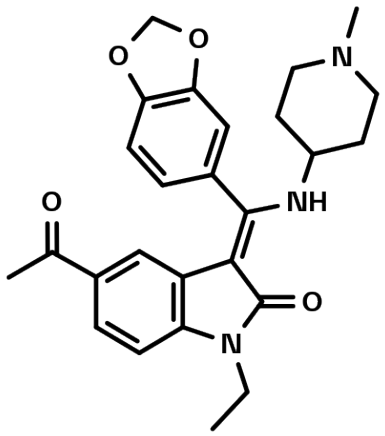

Figure 6: BI-4481 serves as a negative control.

Selectivity

BI-5521 is similarly active on both isoforms GSK-3α and GSK-3β.
Selectivity against other protein kinases:

- No selectivity against DYRK1A (99% inhibition @500 nM)
- ≥100fold selectivity against CDK2/CyclinA, MAP3K7_K7IP1, MAPKAPK1A, ROCK1
- >1000fold selectivity against all other targets tested

Selectivity against non-kinase targets:

- >500 fold selectivity against all targets of a 62 target panel (please see Supplementary Data for detailed information)

Negative control BI-4481 hits 4 from 44 with >50% inhibition @ 10µM (HERG, M1/H, PDE4D2, BETA2/HUM).

SELECTIVITY DATA AVAILABLE	BI-5521	BI-4481
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	Yes	No
DiscoverX®	No	No
Dundee	Yes	No

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

A crystal structure of GSK-3 complexed with BI-91BS, a close analog of BI-5521, is available (PDB: 6GJO).

References molecule(s)

Pharmacological inhibitors of GSK-3 described in the literature can be classified as either ATP competitive, non-ATP-competitive (allosteric) or substrate competitive inhibitors.
Among these, three molecules so far reached clinical trials: the ATP competitive inhibitors AZD1080 and LY2090314 and Tideglusib with a non-ATP-competitive binding mode[2,4].

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

1. Heckel A., Roth G. J., Kley J., Hoerer S., Uphues I Aryl-containing 5-acylindolinones, the preparation thereof and their use as medicaments **2005**, US Patent 2005/0203104, (BI-5521 corresponds to example 1).

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4. Khan I., Tantray M. A., Alam M. S., Hamid H. Natural and synthetic bioactive inhibitors of glycogen synthase kinase, *European Journal of Medicinal Chemistry* **2017**, *125*, 464-477. DOI: 10.1016/j.ejmech.2016.09.058, PubMed: 27689729.

5. Palomo V., Perez D. I., Roca C., Anderson C., Rodríguez-Muela N., Perez C., Morales-Garcia J. A., Reyes J. A., Campillo N. E., Perez-Castillo A. M., Rubin L. L., Timchenko L., Gil C., Martinez A. Subtly Modulating Glycogen Synthase Kinase 3 β: Allosteric Inhibitor Development and Their Potential for the Treatment of Chronic Diseases *J Med Chem*

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