HSD17B13 inhibitor | BI-3231

Table of contents

Summary

BI-3231 is a highly potent and selective inhibitor of human and mouse of HSD17B13. BI-0955 is available as negative control.

Chemical Structure

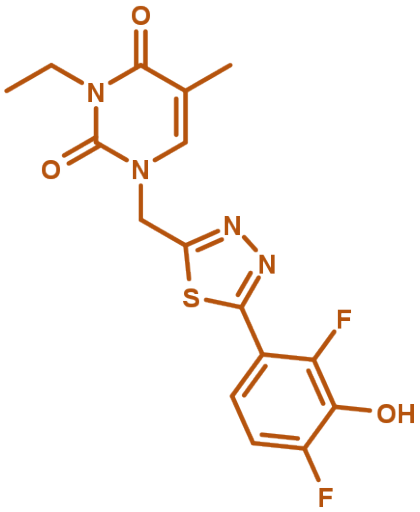

Figure 1: 2D structure of BI-3231.

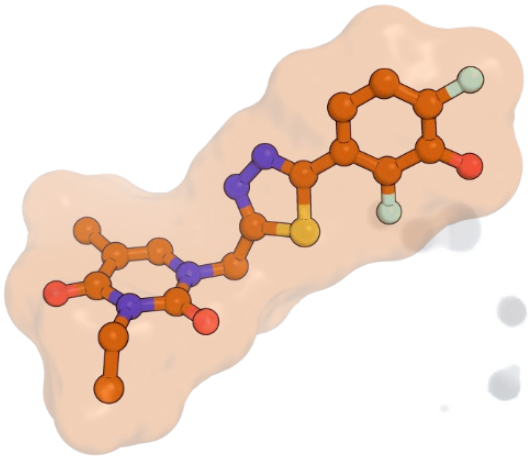

Figure 2: Bound 3D conformation of BI-3231 from predicted complex with HSD17B13 (see Fig. 3).

Highlights

BI-3231 is a highly potent inhibitor of human and mouse HSD17B13 with good selectivity against other HSD17B family members, such as HSD17B11. This compound has been extensively characterized both *in vitro* and *in vivo* and has shown good water solubility and permeability, as well as medium metabolic stability in human and mouse hepatocytes. Thus, it may be an excellent tool to explore the unknown biological function of HSD17B13.

Target information

Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) is a lipid droplet-associated member of the 17-beta hydroxysteroid dehydrogenases (HSD17B) family, primarily expressed in hepatocytes.[2]

Genome wide association studies (GWAS) revealed a strong genetic link between HSD17B13 and liver diseases, such as non-alcoholic fatty liver disease (NAFLD),[3,4] non-alcoholic steatohepatitis (NASH) and cirrhosis.[5-8]

Although its biological function and endogenous substrates are unknown, HSD17B13 acts on a broad range of lipid substrates,[9] including estradiol.[5] NAD^+ (nicotinamide adenine dinucleotide, oxidized form) plays an important role as co-factor.[1,5]

Sequence similarities between HSD17B13 and other HSD17B isoforms indicate HSD17B11 as the closest homolog (85% sequence similarity based on MOE[10] alignment), and thus was used to assess the selectivity of BI-3231. The overall sequence identity of murine and human HSD17B13 is 75% (source www.uniprot.org), displaying a higher conservation of 92% in the putative binding sites of NAD^+ and BI-3231 based on computational modelling approaches.[1]

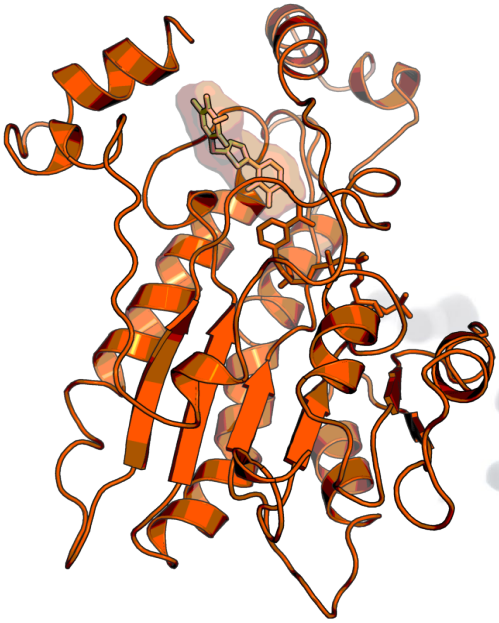

Figure 3: Binding model of BI-3231 in HSD17B13.[1] BI-3231 is shown with molecular surface, the co-factor is shown without surface in stick representation.

In vitro activity and mode of inhibition studies

BI-3231 shows low nanomolar potency in human and mouse HSD17B13 *in vitro* assays and good selectivity against HSD17B11. On-target binding was confirmed by a strong temperature shift in a human HSD17B13 thermal shift assay.[11] Interestingly, binding of BI-3231 to HSD17B13 only takes place in the presence of NAD^+ (see Figure 4). These results indicate that binding of NAD^+ is a prerequisite for the binding of BI-3231. Furthermore, the mode of inhibition was investigated via cross-titrations of BI-3231 and NAD^+ revealing an uncompetitive mode of inhibition of BI-3231 against NAD^+ (see Figure 5).[1,12,13,14]

PROBE NAME / NEGATIVE CONTROL	BI-3231	BI-0955
MW [Da]	380.37	394.40
Enzymatic hHSD17B13 assay (IC_{50} / K_i) [nM][a]	(1)* / 0.7	>10,000
Cellular hHSD17B13 assay (IC_{50}) [nM][a]	11	>10,000
Enzymatic hHSD17B11 assay (IC_{50}) [nM][a]	>10,000	>10,000
Enzymatic mHSD17B13 assay (IC_{50} / K_i) [nM][a]	(14)* / 0.5	>10,000
DSF hHSD17B13 (temperature shift) [K][a]	16.7	n. d.

[a] IC_{50} and K_i values are geometric means of multiple independent measurements (nd = not determined). *Real IC_{50} value unclear due to limits of the assay wall; K_i values (NAD^+) should be used for comparison. For more detailed assay conditions please see literature.[1]

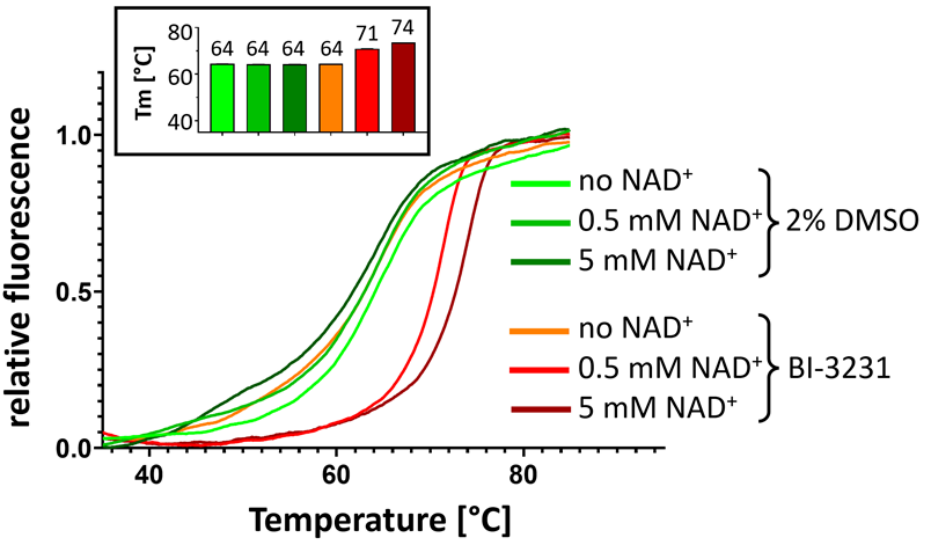

Figure 4. NAD^+ dependency of BI-3231 binding: hHSD17B13 melting curves from Thermal Shift Assay experiment (nanoDSF) in the presence of 2% DMSO or BI-3231 at increasing NAD^+ concentrations (0 mM, 0.5 mM and 5 mM) showed thermal stabilization by BI-3231 only in the presence of NAD^+. Inset: corresponding melting temperatures.

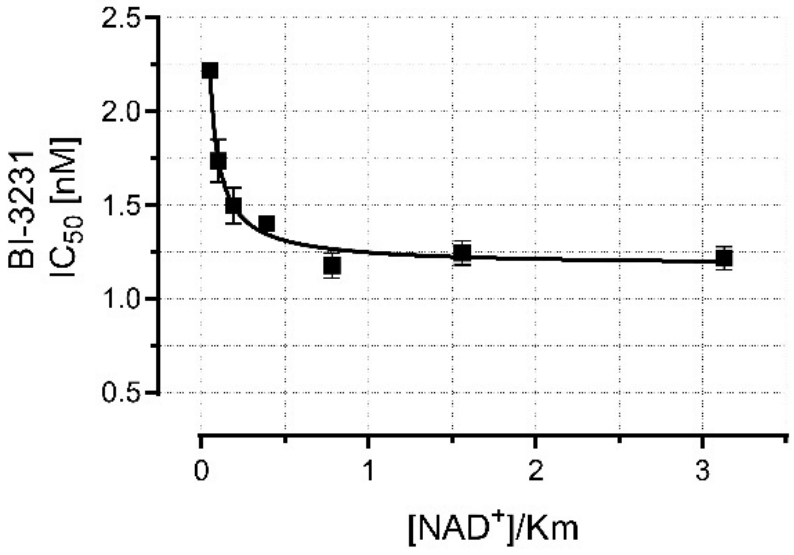

Figure 5. Cross-titrations of BI-3231 at various NAD^+ concentrations indicate an uncompetitive mode of inhibition of BI-3231 against NAD^+.

In vitro DMPK and CMC parameters

BI-3231 shows good water solubility and permeability as well as medium metabolic stability in human and mouse hepatocytes.[1]

PROBE NAME / NEGATIVE CONTROL	BI-3231	BI-0955
logD @ pH 2, 11	1.83, -1.14	3.25, 3.21
Solubility @ pH 6.8 [µg/ml]	>81	<1
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	18	83
CACO efflux ratio	1.2	1
Microsomal stability (human/mouse) [% Q_H]	<23 / 25	59 / >88
Hepatocyte stability (human/mouse) [% Q_H]	58 / 57	68 / 97
Plasma protein binding (human/mouse) [%]	90.9 / 87.1	96.8 / 91.9
hERG [inh. % @ 10 µM]	>10	n. d.
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	45.8
CYP 2D6 (IC_{50}) [µM]	>50	>50

In vivo DMPK parameters

BI-3231 was thoroughly profiled *in vivo* (rodent PK, tissue distribution & bile excretion).[1] Given the high clearance and short half-life of BI-3231, a tailored approach such as multiple daily administrations or the development of an extended-release formulation might be needed to maintain relevant target exposure in subchronic animal models.

PROBE NAME	BI-3231		
Clearance [a] [% Q_H]	130		
V_{ss} [a] [L/kg]	1.4		
Route of administration	i.v. [a]	p.o. [b]	s.c. [c]
Mean residence time, MRT [h]	0.2	2.6	2.1
t_{max} [h]	0.03	0.25	0.5
F [%]	100	10	89

PK Parameters in mouse: [a] Dose i.v.: 5 µmol/kg, [b] Dose p.o.: 50 µmol/kg, [c] Dose s.c.: 80 µmol/kg

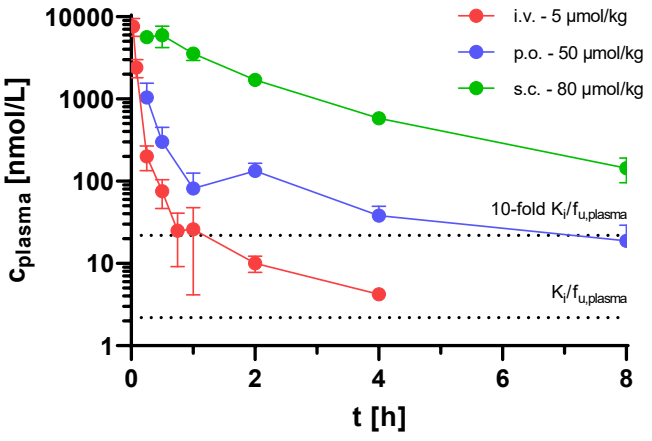

Figure 6. *In vivo* pharmacokinetics and tissue distribution of BI-3231 in mice (n = 3, SD indicated by error bars). The plasma pharmacokinetics after intravenous and oral administration in mice was characterized by a bi-phasic and rapid plasma clearance that exceeded the hepatic blood flow and a low oral bioavailability of 10%. Bioavailability was significantly increased by subcutaneous dosing. Relevant systemic exposure corresponding to >10-fold *in vitro* mouse K_i in unbound plasma concentration could be maintained over 8 hours in mice.

Negative control

BI-0955 is the methylated analog of the active probe BI-3231. It does not have any detectable activity in the HSD17B13 *in vitro* assays.

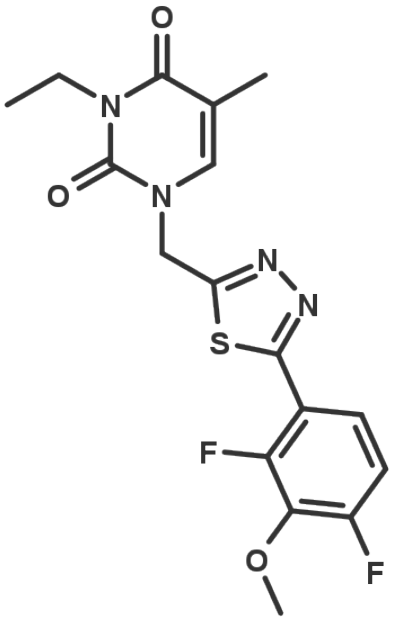

Figure 7: BI-0955 serves as a negative control.

Selectivity

BI-3231 was tested against a panel of 44 receptors and showed an inhibition > 50% @ 10µM on 1 target (COX-2@CE). Negative control BI-0955 hits 2 targets (5HT2B/H and COX-2@CE).

SELECTIVITY DATA AVAILABLE	BI-3231	BI-0955
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Supplementary data

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

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