BI-3231, a Well-Characterized Chemical Probe for HSD17B13, is Available for Free at opnMe.com

Sven Thamm, Marina K. Willwacher, Gary E. Aspnes, Tom Bretschneider, Nicholas F. Brown, Silke Buschbom-Helmke, Thomas Fox, Emanuele M. Gargano, Daniel Grabowski, Christoph Hoenke, Damian Matera, Katja Mueck, Stefan Peters, Sophia Reindl, Doris Riether, Matthias Schmid, Christofer S. Tautermann, Aaron M. Teitelbaum, Cornelius Trünkle, Thomas Veser, Martin Winter, and Lars Wortmann*

Boehringer Ingelheim Pharma GmbH & Co.KG, 88397 Biberach an der Riß, Germany
Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut 06877-0368, United States

Correspondence: Sven.Thamm@boehringer-ingelheim.com, Lars.Wortmann@boehringer-ingelheim.com

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- **HSD17B13 is a lipid-droplet associated member of the family of 17ß-hydroxysteroid dehydrogenases (HSD17B), primarily expressed in hepatocytes[1,2]**
- **HSD17B13 acts on a range of lipid substrates, using NAD^+ as co-substrate, but the physiologically relevant substrate and function of HSD17B13 are unknown[3]**
- **Genome wide association studies in patients revealed HSD17B13 as a potential target to treat metabolic dysfunction-associated steatohepatitis (MASH) and other liver diseases[3]**
- **We discovered and characterized BI-3231, a specific Chemical Probe for HSD17B13[4] and made it available for free at opnMe.com**
- **BI-3231 was accepted as Donated Chemical Probe by the SGC (Structural Genomics Consortium) and received a 4-star rating at ChemicalProbes.org**

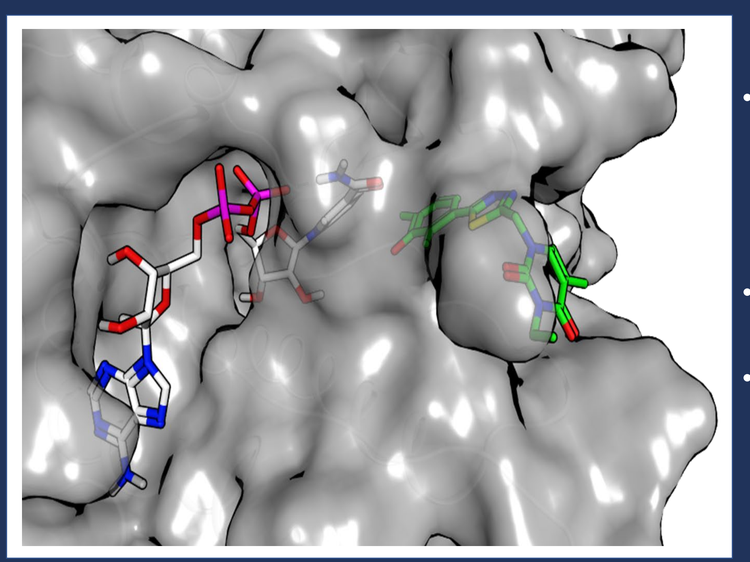
BI-3231, a Chemical Probe to study HSD17B13

- *In vitro* studies demonstrated an uncompetitive mode of inhibition of **BI-3231** with regards to the HSD17B13 co-substrate NAD^+
- Plasma exposure in mice after s.c. administration of **BI-3231** covered 10-fold K_i (unbound) over eight hours
- **BI-3231** is the first potent and selective HSD17B13 inhibitor with equal potency for human and mouse protein
- **BI-3231** showed a good *in vitro* physicochemical, pharmacokinetic and safety profile. However, metabolic stability in hepatocytes remains moderate.

BI-3231 was already successfully used by the research community to study HSD17B13:

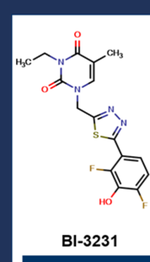
- **BI-3231**, an enzymatic-inhibitor of HSD17B13, reduces lipotoxic effects induced by palmitic acid in murine and human hepatocytes[5]

BI-3231 docked into X-ray Structure



- Crystal structures of full length HSD17B13 in complex with its NAD^+ co-substrate and inhibitors were published recently[6]
- Successful docking of **BI-3231** into crystal structure
- The difluorophenol moiety makes a stacking interaction with the nicotinamide of the co-substrate NAD^+

In vitro Profile of BI-3231



In vitro Pharmacology	IC_{50}	K_i
hHSD17B13 enzyme [nM]	(1 ± 0.5)*	0.7 ± 0.2
hHSD17B11 enzyme [nM]	> 10000	
hHSD17B13 DSF [K]	16.7	
hHSD17B13 cell [nM]	11 ± 4	
mHSD17B13 enzyme [nM]	(14 ± 2)*	0.5 ± 0.1

PhysChem	
MWt	380
clogP	1.3
TPSA	90
S_w pH 6.8	213 µM
LE	0.45
LipE	7.25

DMPK / Tox	
hERG IC_{50} [µM]	> 10
MBI	no
GSH adducts (hLM incub.)	no
PPB h / m [%]	91 / 77.5
Mode of inhibition (NAD^+)	
uncompetitive	

MetStab	CL [mL/min/kg]	Q_H [%]
LM h / m	< 5.3 / 12.1	< 23 / 25
Hep h / m	12.2 / 51.6	58 / 57

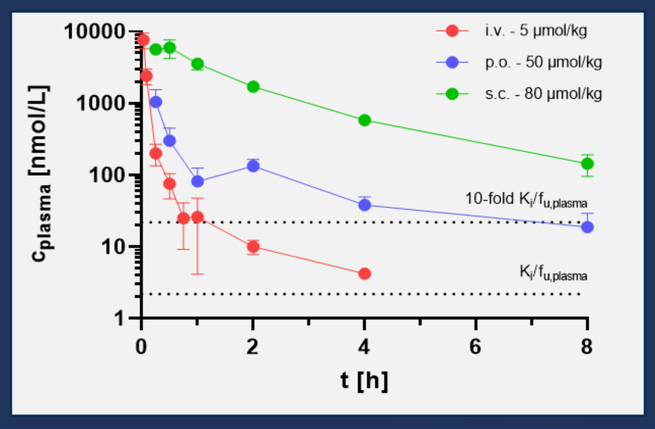
Caco-2, A-B [nm/sec]	Efflux ratio
18	1.2

CYP Inhibition [µM]						
3A4	2D6	2C19	2C9	2C8	2B6	1A2
> 50	> 50	> 50	> 50	> 50	> 50	> 50

- Highly potent human and mouse HSD17B13 inhibitor
- Good selectivity versus HSD17B11 and versus targets in CEREP safety panel
- Good *in vitro* physico-chemical, safety and DMPK properties
- Stability in human and mouse hepatocytes remains moderate

* Real IC_{50} value unclear due to limits of the assay wall. K_i values (NAD^+) should be used for comparison.

In vivo PK of BI-3231

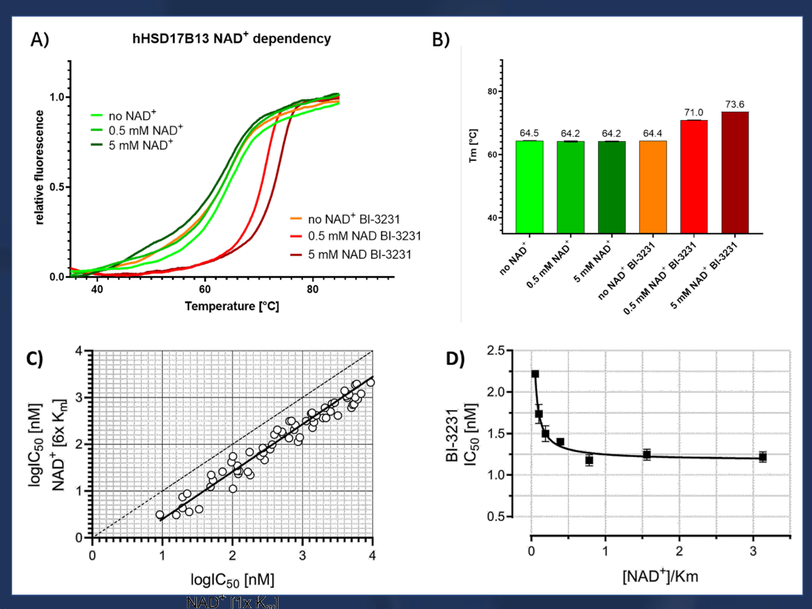


BI-3231 Pharmacokinetic properties in mice

i.v. PK				p.o. PK		s.c. PK	
CL [mL/min*kg]	CL [%Q_H]	V_{ss} [L/kg]	$AUC_{DN,i.v.}$ [nM*h/D]	$AUC_{DN,p.o.}$ [nM*h/D]	F_{oral} [%]	$AUC_{DN,s.c.}$ [nM*h/D]	$F_{s.c.}$ [%]
110	130	1.4	153	16	10	136	89

- PK after i.v. and p.o. administration in mice was characterized by a bi-phasic and rapid plasma clearance exceeding the hepatic blood flow
- Systemic exposure (> 10-fold *in vitro* mouse K_i) in unbound plasma concentration could be maintained over 8 hours in mice after s.c. dosing
- For tissue distribution and bile excretion studies see publication[4]
- For investigation of extended-release formulations see publication[7]

Mode of Inhibition Studies with BI-3231



- On-target binding of **BI-3231** was confirmed by nanoDSF
- **BI-3231** indicated an uncompetitive mode of inhibition against NAD^+
- Formation of the HSD17B13 / NAD^+ complex is a prerequisite for binding of the inhibitor **BI-3231**
- With increasing NAD^+ concentrations, the potencies of HSD17B13 inhibitors improve

BI-3231 and its Publication are available for free

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Accepted Donated Chemical Probe[8]

4-star rating as high-quality *in vitro* and *in vivo* probe[9]

Free publication[4] Download

[1]Marchais-Oberwinkler *et al.*, J. Steroid Biochem. Mol. Biol. **2011**, 125 (1-2), 66-82; [2]Ma *et al.*, Hepatology **2020**, 73, 1701–1716; [3]Abul-Husn *et al.*, N. Engl. J. Med. **2018**, 378, 1096–1106; [4]Thamm *et al.*, J. Med. Chem. **2023,** 66, 4, 2832–2850; [5]Alcober-Boquet *et al.*, Am. J. Physiol. Cell Physiol. **2024**, 326 (3), C880-C892; [6]Liu *et al.*, Nat. Commun. **2023,** 14, 5158; [7]Block *et al.*, Eur. J. Pharm. Sci. **2024,** 196, 106733; [8]www.thesgc.org; [9]www.chemicalprobes.org