FAS inhibitor | BI 99179

Table of contents

Summary

BI 99179 is a potent and selective inhibitor for *in vitro* and *in vivo* validation of FAS as a therapeutic target for lipid metabolism related diseases.

Chemical Structure

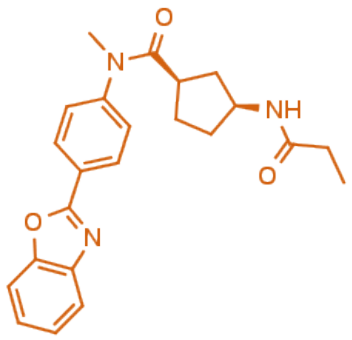

Figure 1: 2-D structure of BI 99179, an inhibitor of FAS

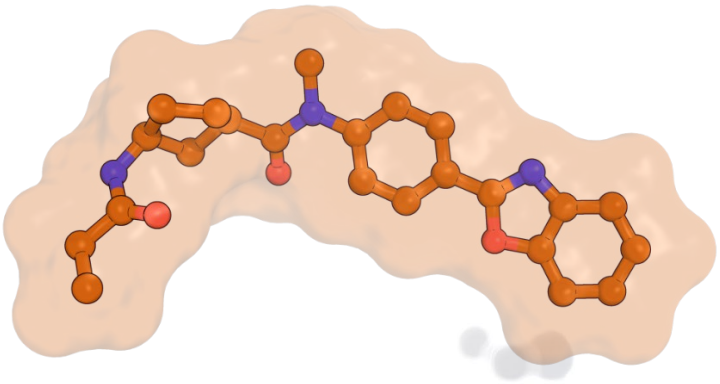

Figure 2: 3-D structure of BI 99179, an inhibitor of FAS

Highlights

BI-99179 is a highly potent and selective non-covalent inhibitor of FAS (IC_{50} = 79 nM). It has significant peripheral and central exposure upon oral administration in rats, where it also showed acute efficacy. This compound may be used for *in vitro* and *in vivo* validation of FAS as a therapeutic target for lipid metabolism-related diseases.

Target information

Mammalian type I fatty acid synthase (FAS) is a key enzyme for lipogenesis and highly expressed in lipogenic tissues. While most tissues, except liver and adipose tissue, have low levels of FAS expression and activity, FAS is over expressed in many cancers.[2, 3]

FAS inhibition could be a potential way to treat obesity.[4]

It has been reported that inhibitors of FAS reduce the production of sebum in sebocytes,[5] suggesting topical FAS inhibition as a potential anti-acne approach.

The reported involvement of FAS in the mechanisms of viral infection and replication suggests that FAS inhibition could be applied as an antiviral principle. [8, 9, 10]

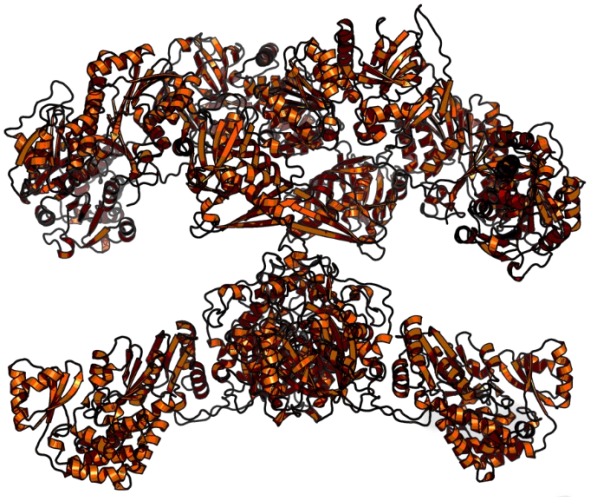

Figure 3: Structure of full length fatty acid synthase, (PDB code: 2vz9.pdb).[6]

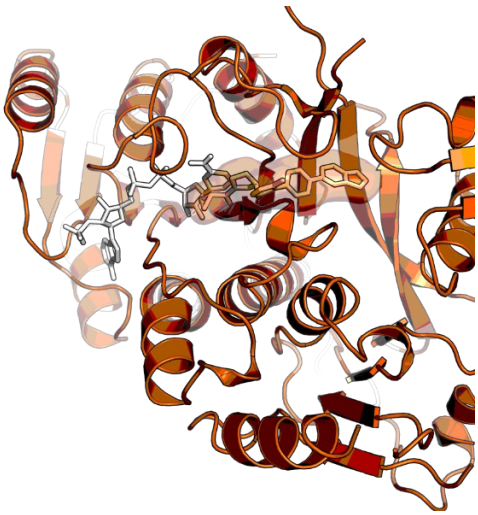

Figure 4: Human Fatty Acid Synthase Psi/KR Tri-Domain with GSK2194069 bound next to NADPH (grey; PDB code: 4piv)

The two identical subunits each comprise an acyl carrier protein (ACP) domain and six different catalytic domains.

BI 99179 most probably binds to the ketoacyl reductase domain (evidence: inhouse enzymatic data and analogy to the published co-crystal structure of the human KR domain with GSK2194069)[7]

In vitro activity

BI 99179 inhibits the FAS enzyme isolated form HeLa cells with an IC_{50} of 79 nM. The optical antipode which can be used as a negative control shows an activity of >3000 nM in this assay.

PROBE NAME / NEGATIVE CONTROL	BI 99179	BI 99990[e]
Inhibition of FASN* (IC_{50}) [nM]	79	>3,000
Inhibition of [^{14}C]acetate incoorporation[§] mouse N-42 cells (IC_{50}) [nM]	570	>10,000
Inhibition of [^{14}C]acetate incorporation[§] hum. H1975 cells (IC_{50})	180	>10,000
Cytotox. (LDH release from U937 cells) (IC_{50}) [nM]	>30,000	n.d.

*Human FAS enzyme isolated from HeLa cells

[§] Cells incubated with compound for 1h, ^{14}C-acetate in Krebs-Ringer-buffer incubation for 4h, Methanol:$CHCl_3$ 1:1 extraction, measurement in ß-counter;

[e] Please refer to the section negative control

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI 99179	BI 99990
Aqueous solubility @ pH 7 [µg/ml]	>39	>39
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	94	n.d.
CACO efflux ratio	0.9	n.d.
Microsomal stability – rat, human [% Q_H]	<27	33
Plasma protein binding rat [% Q_H]	97.6	n.d.

In vivo DMPK parameters

PROBE NAME / NEGATIVE CONTROL	BI 99179	BI 99990
$t_{1/2}$ [h]	3.0	n.d.
t_{max} [h]	0.5	0.5
C_{max} [nM]	2,110	317
$AUC_{0\text{-}inf}$ [nMh]	9,350	2,160

F [%]	46	n.d.
CL [ml/min/kg]	8.2	n.d.
V_{ss} [l/kg]	1.6	n.d.
$C_{brain,2h}$ [nM]	1,300	n.d.
$C_{CSF,2h}$ [nM]	50	n.d.

Pharmacokinetic parameters of BI 99179 in male Han/Wistar rats *fasted upon oral application of 4 mg/kg

In vivo pharmacology

BI 99179 showed acute efficacy in rats

- Increased hypothalamic [Malonyl-CoA] (10 or 100mg/kg; 2h /24h post dose (p.d.))

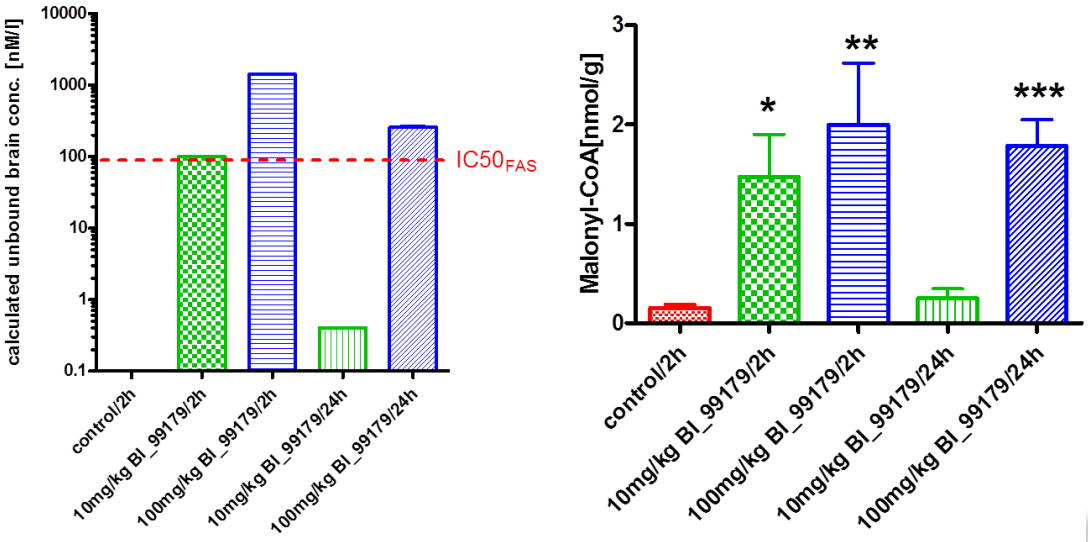

Figure 5: hypothalamic [Malonyl-CoA] 2h/24h p.d.; $_{cbr,unbound}$

- Decreased cumulative food intake 100mg/kg; 24h p.d.

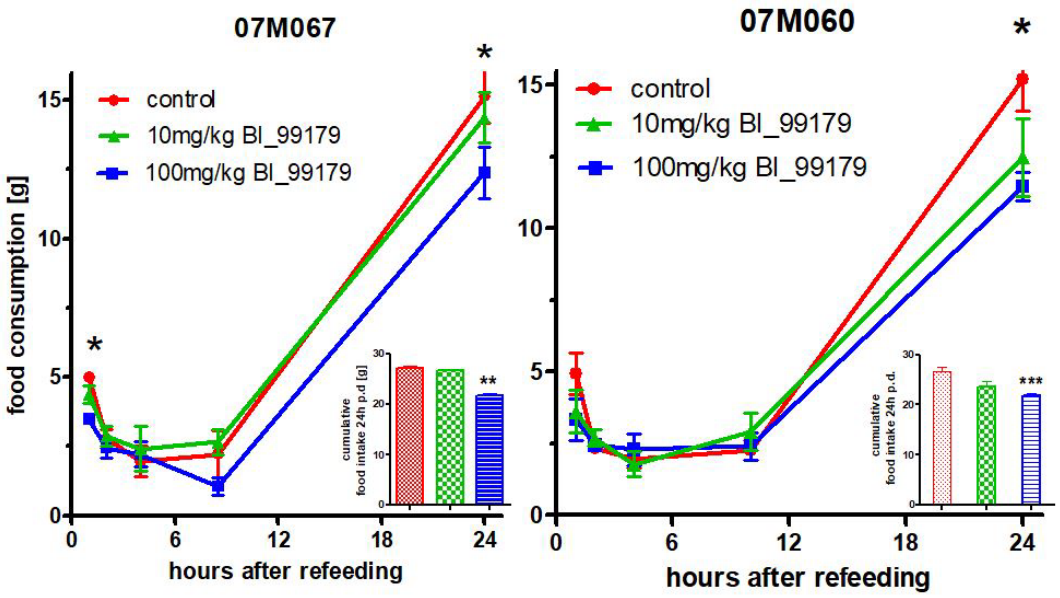

Figure 6: Pharmacologic POC in Han Wistar rats; Food intake with refeeding after 24h fast

BI 99179 showed adverse effects beginning at day 4 (30mg/kg), day 9 (3mg/kg)

- Reddened and swollen mouth (not seen with 50mg/kg BI 99990)
- Reddened and swollen eye lids (not seen with 50 mg/kg BI 99990)
- Salivation (less pronounced with BI 99990)
- Hair loss (less pronounced with BI 99990)

BI 99990 showed additional adverse effects at 50mg/kg, therefore the compound is not suitable for further studies in rats.

Negative control

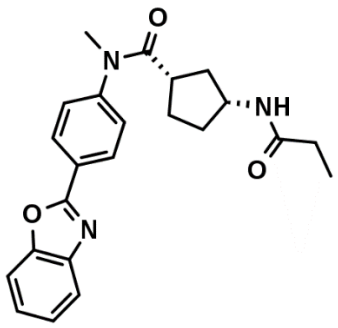

Figure 7: BI 99990 which serves as a negative control

The optical antipode BI 99990 can be used as negative control ($IC_{50\ FAS}$ => 3000 nM). We tested BI 99990 also *in vivo* to assess if the observed adverse effects for BI 99719 are rather compound or target specific with the result that the adverse effects have not been observed or have been less pronounced with BI 99990. (For more details see *in vivo* pharmacology section.) BI 99990 showed additional adverse effects at 50mg/kg, therefore the compound is not suitable for further studies in rats and should be only used *in vitro*.

Selectivity

No closely related mammalian proteins as potential off-targets.

Selectivity on non-related targets: External screen covering 30 targets: <20% inhibition @ 10 µM for all targets. (Please see Supplementary data for detailed information)

High selectivity confirmed in >100 in-house screens @ Boehringer Ingelheim (including >10 enzymes, >10 GPCRs, >10 kinases, and 5 ion channels).

SELECTIVITY DATA AVAILABLE	BI-99179	BI-99990
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

See references 3 and 11

Supplementary data

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

References

1. Kley J. T., Mack J., Hamilton B., Scheuerer S., Redemann N. Discovery of BI 99179, a potent and selective inhibitor of type I fatty acid synthase with central exposure *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5924-5927. doi:10.1016/j.bmcl.2011.07.083, PubMed.

2. Flavin R., Peluso S., Nguyen P. L., Loda M. Fatty acid synthase as a potential therapeutic target in cancer *Future Oncol.* **2010**, *6*, 551-562. doi: 10.2217/fon.10.11, PubMed.
3. Kridel S. J., Lowther W. T., Pemble C. W. IV Fatty acid synthase inhibitors: new directions for oncology *Expert Opin. Invest. Drugs* **2007**, *16*, 1817-1829. DOI:10.1517/13543784.16.11.1817, PubMed.
4. Loftus T. M., Jaworsky D. E., Frehywot G. L., Townsend C. A., Ronnett G. V., Lane M. D., Kuhajda F. P. Reduced Food Intake and Body Weight in Mice Treated with Fatty Acid Synthase Inhibitors *Science* **2000**, *288*, 2379. DOI: 10.1126/science.288.5475.2379, PubMed.
5. D'Arcangelis, A. D., et al. U.S. Patent 53631, 2005.
6. Maier T., Leibundgut M., Ban N. The Crystal Structure of a Mammalian Fatty Acid Synthase *Science* **2008**, *321*, 1315-1322. DOI: 10.1126/science.1161269, PubMed.
7. Hardwicke M. A., Rendina A. R., Williams S. P., Moore M .L., Wang L., Krueger J. A., Plant R. N., Totoritis R. D., Zhang G., Briand J., Burkhart W. A., Brown K. K., Parrish C. A. A human fatty acid synthase inhibitor binds β-ketoacyl reductase in the keto-substrate site *Nature Chemical Biology* **2014**, *10*, 774-779.doi:10.1038/nchembio.1603, PubMed.
8. Tongluan N., Nio Y., Akahori Y., Kim S., Watashi K., Wakita T., Hijikata M. Involvement of fatty acid synthase in dengue virus infection *Virology Journal* **2017**, 14, 28 DOI: 10.1186/s12985-017-0685-9, PubMed.
9. Okamura H., Nio Y., Akahori Y., Kim S., Watashi K., Wakita T., Hijikata M. Fatty acid biosynthesis is involved in the production of hepatitis B virus particles *Biochemical and Biophysical Research Communications* **2016**, 475, 87-92 DOI:10.1016/j.bbrc.2016.05.043, PubMed.
10. Ohol Y. M., Wang Z., Kemble G., Duke G. Direct Inhibition of Cellular Fatty Acid Synthase Impairs Replication of Respiratory Syncytial Virus and Other Respiratory Viruses *PLoS One* **2015**, *10*(12) DOI: 10.1371/journal.pone.0144648, PubMed.
11. Jones S. F., Infante J. R. Infante Molecular Pathways: Fatty Acid Synthase *Clin. Cancer Res.* **2015**, *21*, 5434-5438 DOI: 10.1158/1078-0432.CCR-15-0126, PubMed.