

FAS inhibitor

BI 99179

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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

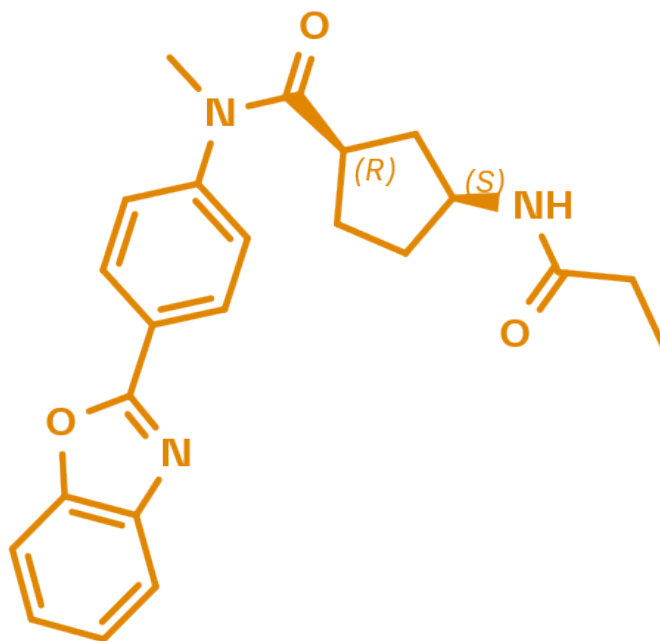


Figure 1: 2D structure of BI 99179, an inhibitor of FAS

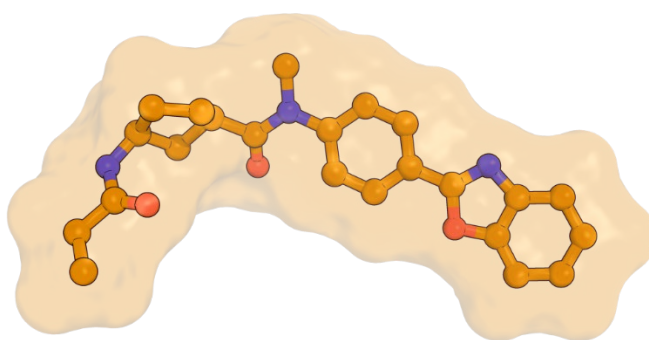


Figure 2: 3D structure of BI 99179, an inhibitor of FAS

Highlights

BI-99179 is a highly potent and selective non-covalent inhibitor of FAS ($IC_{50} = 79 \text{ nM}$). 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 obesity⁴.

It has been reported that inhibitors of FAS reduce the production of sebum in sebocytes⁵, 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}.

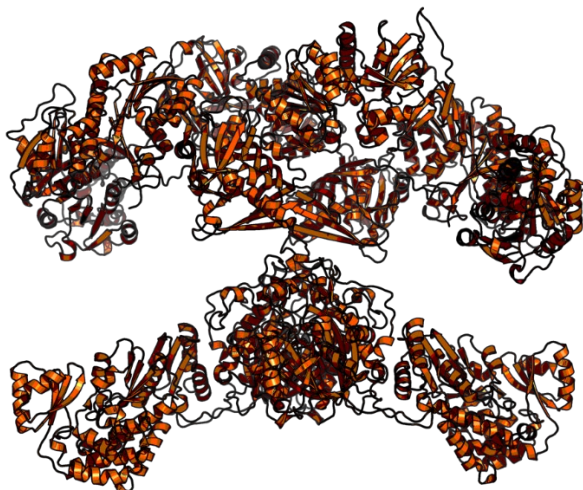


Figure 3: Structure of full length fatty acid synthase, (PDB code: 2vz9.pdb)⁶.

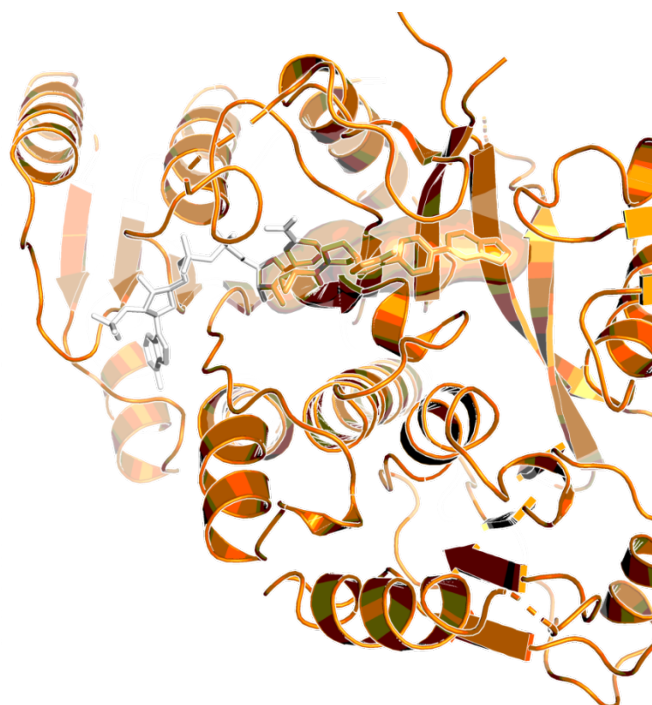


Figure 4: Human Fatty Acid Synthase Psi/KR Tri-Domain with GSK2194069 bound next to NADPH (grey; PDB code: 4piv)

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 from 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
MW [Da, free base] ^a	391.5	391.5
Inhibition of FAS (IC_{50}) [nM] ^b	79	>3,000
Inhibition of [¹⁴ C] acetate Incorporation mouse N-42 cells (IC_{50}) [nM] ^c	570	>10,000

Inhibition of [¹⁴ C] acetate Incorporation hum. H1975 cells (IC ₅₀) ^c	180	>10,000
Cytotox. (LDH release from U937 cells) (IC ₅₀) [nM]	>30,000	n.d.

^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 Human FAS enzyme isolated from HeLa cells

^c Cells incubated with compound for 1h, ¹⁴C-acetate in Krebs-Ringer-buffer incubation for 4h, Methanol:CHCl₃ 1:1 extraction, measurement in β-counter

In vitro DMPK and CMC parameters

PROBE NAME / NEGATIVE CONTROL	BI 99179	BI 99990
logD @ pH 11	3.3	3.3
Solubility @ pH 7 [µg/mL]	>39	>39
Caco-2 permeability AB @ pH 7.4 [$\cdot 10^{-6}$ cm/s]	94	n.a.
Caco-2 efflux ratio	0.9	n.a.
Microsomal stability (human) [% Q _H]	<24	33
CYP 3A4 (IC ₅₀) [µM]	27.2	n.a.
CYP 2C9 (IC ₅₀) [µM]	11.9	n.a.
CYP 2C19 (IC ₅₀) [µM]	21.7	n.a.
CYP 2D6 (IC ₅₀) [µM]	>50	n.a.

In vivo DMPK parameters

RAT	BI 99179	BI 99990
Clearance [mL/min/kg] ^a	8.2	n.a.
V _{ss} [L/kg]	1.6	n.a.
t _{max} [h] ^a	0.5	0.5
t _{1/2} [h]	3.0	n.d.

C _{max} [nM]	2,110	317
C _{brain,2h} [nM]	1,300	n.a.
C _{CSF,2h} [nM]	50	n.a.
F [%]	46	n.a.

^a i.v. dose: 0.4 mg/kg

^b p.o. dose: 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.))

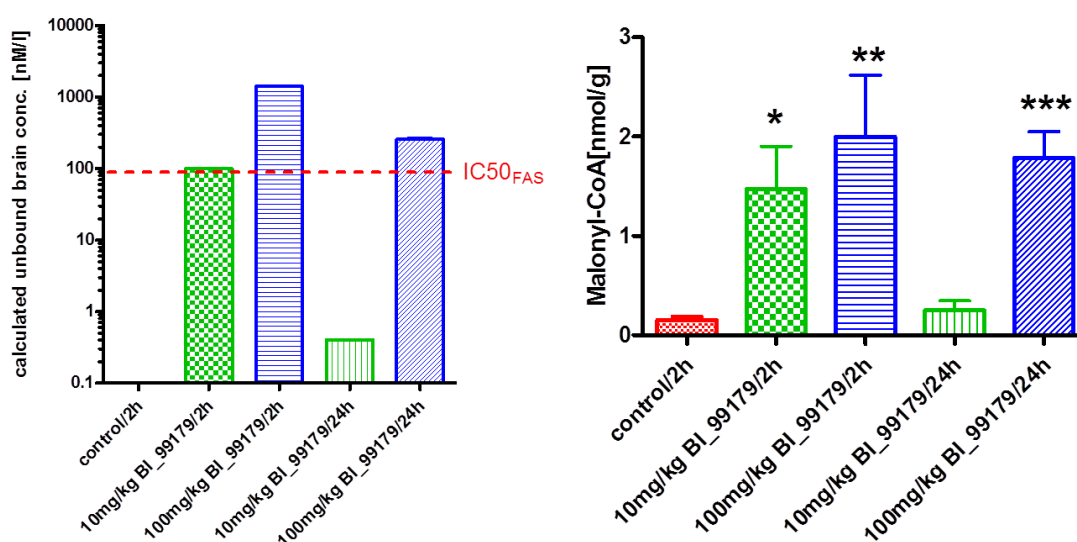


Figure 5: hypothalamic [Malonyl-CoA] 2h/24h p.d.

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

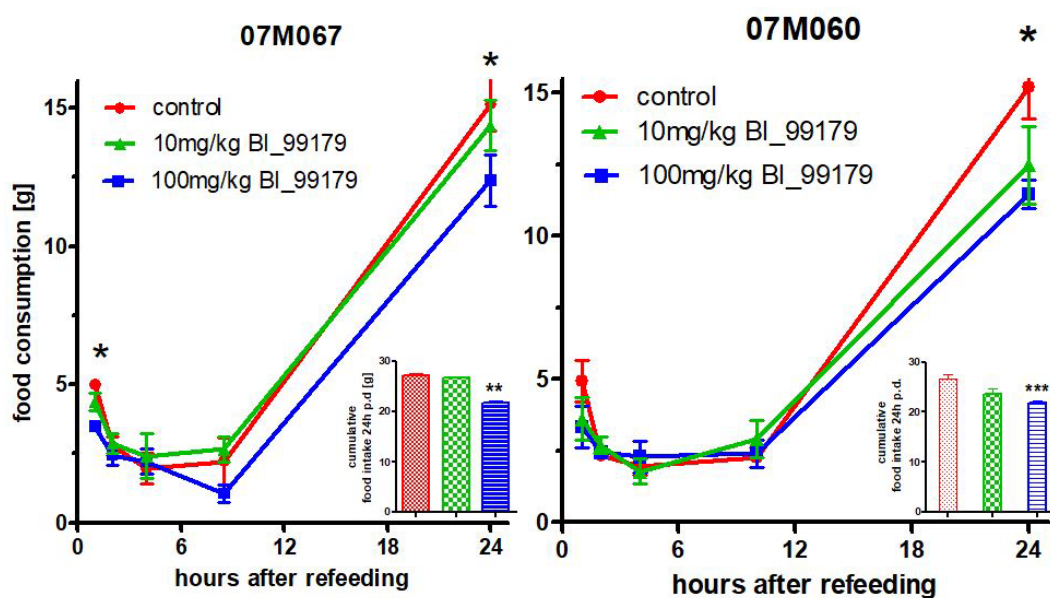


Figure 6: Pharmacologic POC in Han Wistar rats; Food intake with refeeding after 24h fast

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

- Reddened and swollen mouth (not seen with 50 mg/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 50 mg/kg, therefore the compound is not suitable for further studies in rats.

Negative control

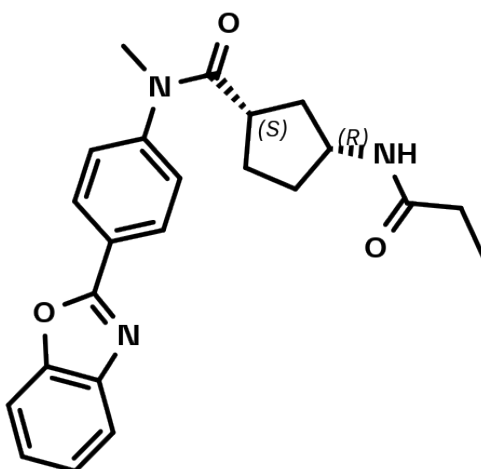


Figure 7: BI 99990 which serves as a negative control

The optical antipode BI 99990 can be used as negative control ($IC_{50\text{ FAS}} \Rightarrow 3000\text{ nM}$). 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 50 mg/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
PDSP ¹²	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

See references 3 and 11

Supplementary data

2D 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(19), 5924–5927. DOI: [10.1016/j.bmcl.2011.07.083](#), PubMed: [21873051](#).
2. Flavin R., Peluso S., Nguyen P. L., Loda M. Fatty acid synthase as a potential therapeutic target in cancer *Future Oncol* **2010**, 6(4), 551–562. DOI: [10.2217/fon.10.11](#), PubMed: [20373869](#).
3. Kridel S. J., Lowther W. T., Pemble C. W. Fatty acid synthase inhibitors: New directions for oncology *Expert Opin Investig Drugs* **2007**, 16(11), 1817–1829. DOI: [10.1517/13543784.16.11.1817](#), PubMed: [17970640](#).
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(5475), 2379–2381. DOI: [10.1126/science.288.5475.2379](#), PubMed: [10875926](#).
5. D’Arcangelis A. D., Bajor J., Campbell K., Knaggs H. U.S. Patent **2005**, [US20050053631](#).
6. Maier T., Leibundgut M., Ban N. The crystal structure of a mammalian fatty acid synthase *Science* **2008**, 321(5894), 1315–1322. DOI: [10.1126/science.1161269](#), PubMed: [18772430](#).

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 *Nat Chem Biol* **2014**, 10(9), 774–779. DOI: [10.1038/nchembio.1603](https://doi.org/10.1038/nchembio.1603), PubMed: [25086508](https://pubmed.ncbi.nlm.nih.gov/25086508/).
8. Tongluan N., Ramphan S., Wintachai P., Jaresitthikunchai J., Khongwichit S., Wikan N., Rajakam S., Yoksan S., Wongsiriroj N., Roytrakul S., Smith D. R. Involvement of fatty acid synthase in dengue virus infection *Virol J* **2017**, 14(1), 28. DOI: [10.1186/s12985-017-0685-9](https://doi.org/10.1186/s12985-017-0685-9), PubMed: [28193229](https://pubmed.ncbi.nlm.nih.gov/28193229/).
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 *Biochem Biophys Res Commun* **2016**, 475(1), 87–92. DOI: [10.1016/j.bbrc.2016.05.043](https://doi.org/10.1016/j.bbrc.2016.05.043), PubMed: [27178211](https://pubmed.ncbi.nlm.nih.gov/27178211/).
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), e0144648. DOI: [10.1371/journal.pone.0144648](https://doi.org/10.1371/journal.pone.0144648), PubMed: [26659560](https://pubmed.ncbi.nlm.nih.gov/26659560/).
11. Jones S. F., Infante J. R. Molecular Pathways: Fatty Acid Synthase *Clin Cancer Res* **2015**, 21(24), 5434–5438. DOI: [10.1158/1078-0432.CCR-15-0126](https://doi.org/10.1158/1078-0432.CCR-15-0126), PubMed: [26519059](https://pubmed.ncbi.nlm.nih.gov/26519059/).
12. Kroeze W. K., Sassano M. F., Huang XP., Lansu K., McCorvy J. D., Giguère P. M., Sciaky N., Roth B. L. PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome *Nat Struct Mol Biol.* **2015**, 22(5):362-9. DOI: [10.1038/nsmb.3014](https://doi.org/10.1038/nsmb.3014), PubMed: [26011111](https://pubmed.ncbi.nlm.nih.gov/26011111/).