GPR40 agonist | BI-2081

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Summary

BI-2081 is a partial GPR40 agonist with a high *in vitro* potency (EC_{50} = 4 nM) and a good *in vitro* and *in vivo* PK profile.

Chemical Structure

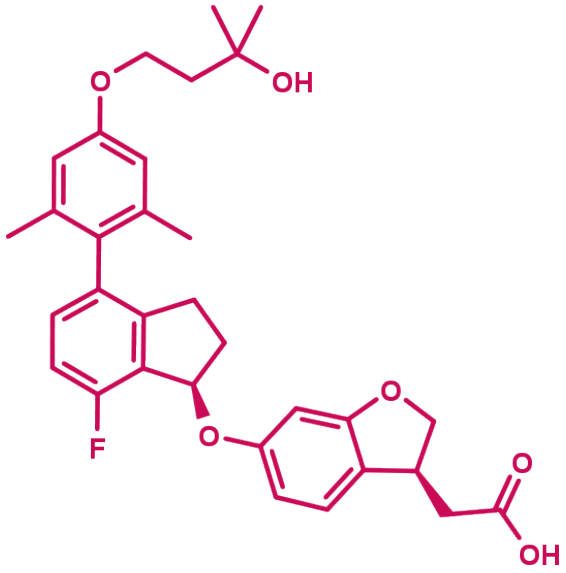

Figure 1: 2-D structure of BI-2081, a potent GPR40 agonist.

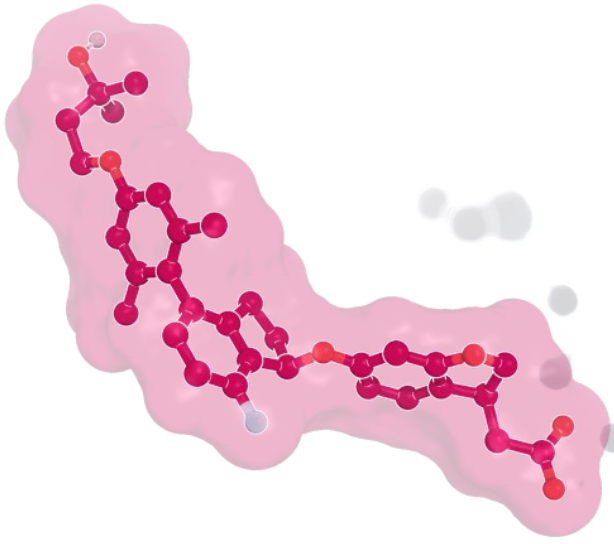

Figure 2: BI-2081, 3-D low energy conformation.

Highlights

BI-2081 is a partial agonist GPR40 with a good *in vitro* potency, reducing the plasma glucose concentration in Zucker diabetic fatty (ZDF) rats. It possesses a good *in vitro* and *in vivo* PK profile, which makes an oral application with high bioavailability possible. The structurally related BI-0340 is a suitable negative control due to its significant lower potency on GPR40.

Target information

The GPR40, also known as free fatty acid receptor 1 (FFA1), is a member of the rhodopsin family of G-protein coupled receptors and it interacts predominantly with the $G\alpha_q$ subunit.[1] The receptor is related to other fatty acid receptors (i.e. GPR43/FFA2 and GPR41FFA3) and shares an overall sequence homology of up to 50% with this family.[2] GPR40 is highly expressed in the β-cells of the pancreas. Additionally, it can be found in the brain and the GI tract.[3] The receptor is activated by medium to long chain saturated and unsaturated fatty acids (C_{12}-C_{20}). Activation of GPR40 leads to an increase of intracellular Ca^{2+} concentrations via the IP_3 pathway and stimulates the insulin release in the presence of glucose. The GPR40 agonist should have a low risk of hypoglycemia due to this glucose-stimulated insulin secretion (GSIS).[4]

Figure 3: Structure of GPR40 with an agonist related to BI-2081, as revealed by X-ray crystallography (PDB code 4HPU).

In vitro activity

BI-2081 is a partial agonist on GPR40 and shows a high cellular potency in the human IPOne assay (EC_{50} = 3-5 nM). The plasma shift with 4.5% HSA on the human GPR40 is fourfold with BI-2081. The cellular potency of BI-0340 (EC_{50} = 1230 nM) on the human GPR40 receptor is more than 200-fold lower compared to the probe BI-2081.

PROBE NAME / NEGATIVE CONTROL	BI-2081	BI-0340
MW [Da]	534.62	568.65
Ki [nM] hGPR40	23	-
IPOne (EC_{50}) human [nM][a/b]	5/3	1230/-
IPOne (EC_{50}) rat [nM][a/b]	302/20	3630/-
IPOne (EC_{50}) mouse [nM][b]	31	-
IPOne (EC_{50}) dog [nM][b]	6	-
IPOne (EC_{50}) cyno [nM][a]	76	-

[a] Stimulation of 1321N1 cells, which express the GPR40 receptor, followed by measurement of the IP1 accumulation by fluorescence.

[b] Stimulation of 1321N1 cells, which express the GPR40 receptor, followed by measurement of the IP1 accumulation by fluorescence. Differs mainly from assay **a** by different cell preparation and LiCl containing stimulation buffer. More detailed information can always be obtained via the "Contact us" formular.

In vitro DMPK and CMC parameters

BI-2081 has a good permeability and a high plasma protein binding. It displays a high stability in microsomes in human and rat cells, but seems to have a lower stability in hepatocytes. However, this *in vitro* result does not correlate to the low clearance *in vivo* which was observed in rats.

PROBE NAME / NEGATIVE CONTROL	BI-2081	BI-0340
$logD_{7.4}$	3.9	2.6
Solubility @ pH 6.8 [µg/ml]	28	100
CACO permeability @ pH 7.4 [$*10^{-6}$ cm/s]	65.2	4.1
CACO efflux ratio	1.2	1.6
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	2.5/17	-
MDCK efflux ratio	6.8	-
Microsomal stability (human/rat) [% Q_H]	<23/<22	61/>88
Hepatocyte stability (human/rat) [% Q_H]	90/67	-
Plasma protein binding (human/rat) [%]	>99.7/>99.8	-
hERG [inh. % @ 10 µM]	22	-
CYP 3A4 (IC_{50}) [µM]	40	-
CYP 2C8 (IC_{50}) [µM]	5.4	-
CYP 2C19 (IC_{50}) [µM]	>50	-
CYP 2D6 (IC_{50}) [µM]	>50	-
MBI 3A4 (25 µM) [%Ctrl]	92	-

In vivo DMPK parameters

BI-2081 possesses high bioavailability and an overall good PK profiles in rats. The observed *in vivo* clearance is low despite the *in vitro* measured low stability in hepatocytes.

BI-2081	Rat[a]
Clearance [% Q_H][a]	6.9
Mean residence time after i.v. dose [h]	4.1
Mean residence time after p.o. dose [h]	4.2
t_{max} [h]	1.17
C_{max}_dn [nM]	930
F [%]	79
V_{ss} [l/kg]	0.9

[a] Rat doses: 1 µmol/kg i.v.; 10 µmol/kg p.o.

In vivo pharmacology

An acute oral glucose test (oGTT) in male Zucker diabetic fatty (ZDF) rats was performed with BI-2081. We observed a strong glucose lowering effect as well as an increase of the plasma insulin level compared to the untreated ZDF rats. The compound reduced the glucose level in this disease-related model by 71% ($AUC_{0\text{-}180\ min}$) with ED_{50} = 0.7 mg/kg and ED_{100} around 10 mg/kg. No significant change of the plasma glucose level was observed in GPR40 KO mice compared to the WT, which shows the on-target-related specificity. Another study on normal fasting rats showed that there was no significant difference in glucose levels between BI-2081 treated rats and the control group, supporting the low risk of hypoglycemia due to the glucose-dependent mode of action on GPR40.

We observed a significant lowering of HbA1c (ΔHbA_{1c} = −1.8%) after treating male ZDF rats with BI-2081 in a subchronic 30 day study (10 mg/kg b.i.d.). We could additionally observe in the same study that BI-2081 lowers plasma lipids such as total cholesterol (39%), triglycerides (25%) and free fatty acids (34%). The body weight of the treated rats was reduced by 14% after 30 days without any effect on food consumption.

IN VIVO STUDY	OBSERVED EFFECT
oGTT in 8-10 old male ZDF rats	- ED_{50} = 0.7 mg/kg - Estimated ED_{100} = ~10 mg/kg - E_{max} = 71% Inhibition ($AUC_{0\text{-}180\ min}$)
subchronic study male ZDF rats: 10 mg/kg bid, 30 day	- ΔHbA_{1c} = −1.8% - ΔPlasma Cholesterol = −39% - ΔPlasma Triglycerides = −25% - ΔFree Fatty Acids = −34% - ΔBody Weight = -14%

Negative control

The negative control BI-0340 has a similar structure to BI-2081, but it is more than 200-fold less potent on human GPR40 compared to BI-2081 in the IPOne assay.

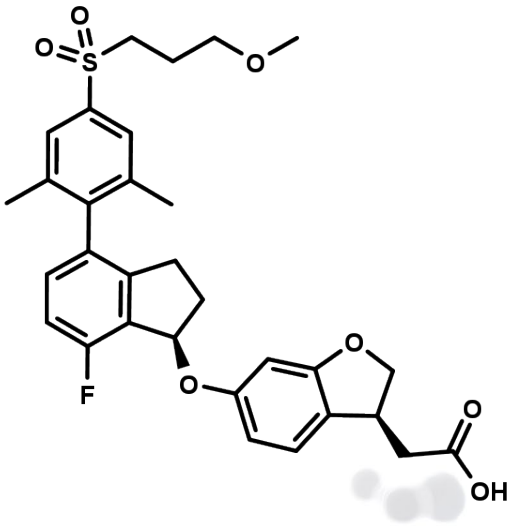

Figure 4: BI-0340 which serves as a negative control

Selectivity

The selectivity profile for BI-2081 was assessed with the Eurofins Safety Panel 44™ assay. BI-2081 had an affinity towards adrenergic α_{2A} (K_i = 1.3 µM), histamine H_1 (K_i = 3.1 µM), CysLT1 (69% inh. @ 10 µM) and thyroid hormone (rat, K_i = 3.5 µM).

SELECTIVITY DATA AVAILABLE	BI-2081	BI-0340
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Reference molecule(s)

Fasiglifam hemihydrate (TAK875)

Supplementary data

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

References

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2. Costanzi S., Neumann S., Gershengorn M. C. Seven Transmembranespanning Receptors for Free Fatty Acids as Therapeutic Targets for Diabetes Mellitus: Pharmacological, Phylogenetic, and Drug Discovery Aspects *J. Biol. Chem.* **2008**, 283(24), 16269-16273. DOI: 10.1074/jbc.R800014200, PubMed.
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4. Poitout V., Lin D. C.-H. Modulating GPR40: therapeutic promise and potential in diabetes *Drug Discov. Today* **2013**, 18(23-24), 1301-1308. DOI: 10.1016/j.drudis.2013.09.003, PubMed.