opnMe by Boehringer Ingelheim

GPR40 agonist

BI-2081

Boehringer Ingelheim

Table of contents

Summary

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

Chemical Structure

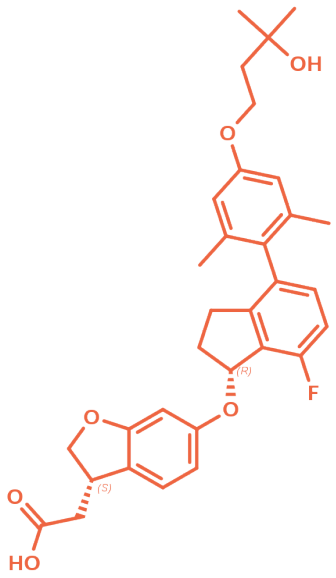

Figure 1: 2D structure of BI-2081, a potent GPR40 partial agonist.

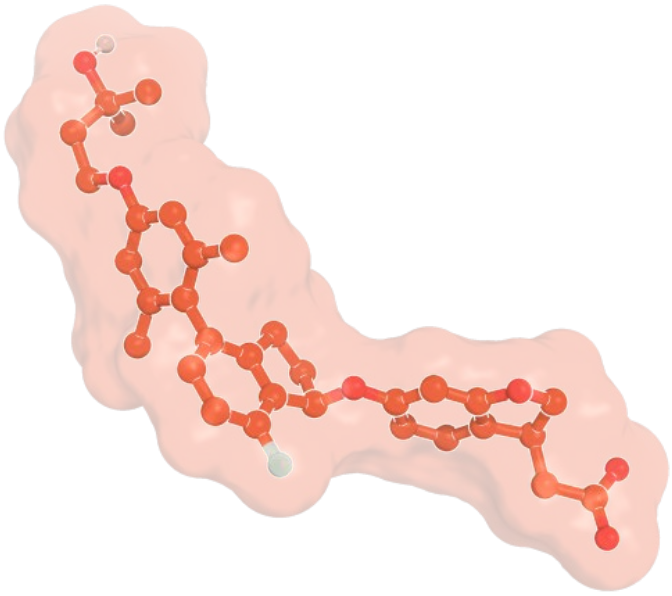

Figure 2: BI-2081, 3D low energy conformation.

Highlights

BI-2081 is a partial agonist on GPR40 with a good *in vitro* potency (EC_{50} = 4 nM). The activation of GPR40 allows a glucose depending insulin secretion and BI-2081 significantly reduces the plasma glucose concentration in Zucker Diabetic Fatty (ZDF) rats. This mode of action allows a high anti-hyperglycemic efficacy with low risk for hypoglycemia. BI-2081 possesses a good *in vitro* and *in vivo* PK profile, which makes an oral application with high bioavailability possible. The structural 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 receptor 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 GPR41/FFA3) 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. GPR40 agonist should have a low risk of hypoglycemia due to this glucose-stimulated insulin secretion (GSIS)[4].

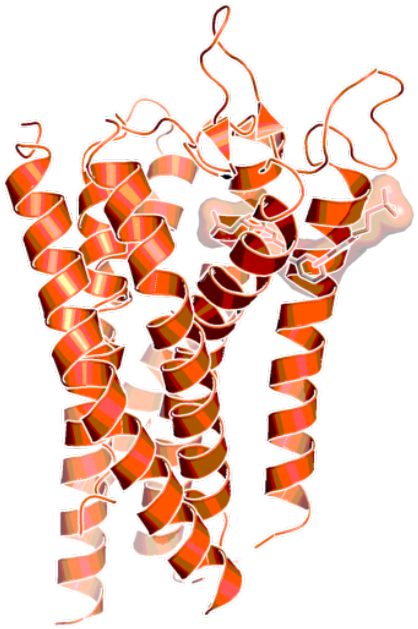

Figure 3: Structure of GPR40 with an agonist related to BI-2081, as revealed by X-ray crystallography (PDB code: 4HPU).

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, free base][a]	534.6	568.7
Ki hGPR40[nM]	23	-
IPOne (EC_{50}) human [nM][b/c]	5/3	1230/-
IPOne (EC_{50}) rat [nM] [b/c]	302/20	3630/-
IPOne (EC_{50}) mouse [nM][c]	31	-
IPOne (EC_{50}) dog [nM][c]	6	-
IPOne (EC_{50}) cyno [nM][b]	76	-

[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] Stimulation of 1321N1 cells, which express the GPR40 receptor, followed by measurement of the IP1 accumulation by fluorescence
[c] 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 over all tested species, 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 several species.

PROBE NAME / NEGATIVE CONTROL	BI-2081	BI-0340
logD @ pH 7.4	3.9	2.6

Solubility @ pH 6.8 [µg/mL]	28	100
Caco-2 permeability AB @pH7.4 [*10^{-6} cm/s]	65.2	4.1
Caco-2 efflux ratio	1.2	1.6
Microsomal stability (human/rat/dog) [% QH]	<23 / <22 / <20	61 / >88 / n.a.
Hepatocyte stability (human/rat/dog) [% QH]	90 / 67 / 94	n.a.
Plasma Protein Binding (human/rat/dog) [%]	>99.7 / >99.8 / >99.7	n.a.
hERG [inh. % @ 10 µM]	22	n.a.
CYP 3A4 (IC_{50})[µM]	40	n.a.
CYP 2C8 (IC_{50})[µM]	5.4	n.a.
CYP 2C19 (IC_{50})[µM]	>50	n.a.
CYP 2D6 (IC_{50})[µM]	>50	n.a.
MBI 3A4 (25 µM) [%Ctrl]	92	n.a.

In vivo DMPK parameters

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

BI-2081	RAT
Clearance [%Q_H]	3.1
Mean residence time after *i.v.* dose [h]	3.9
C_{max}[nM][a]	930
t(h)	1.2
F [%]	79
V_{ss} [L/kg]	0.7

[a] *i.v.* dose: 0.5mg/kg
[b] *p.o.* dose: 5 mg/kg

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 00-fold less potent on human GPR40 compared to BI-2081 in the IPOne assay.

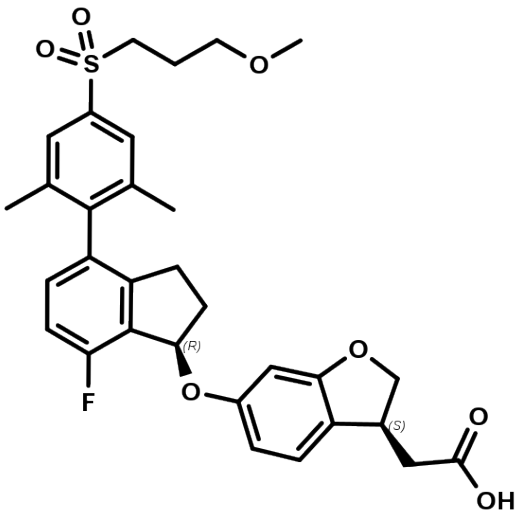

Figure 4: BI-0340 which serves as a negative control

Selectivity

The selectivity profile for BI-2081 was tested in SafetyScreen44™. 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).
Negative control BI-0340 hits 1 from 44 in SafetyScreen44™ (GCORTICOID/H > 50% at 10µM).

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

Selectivity data can be downloaded free of charge from opnMe.

References

1. Surgand J.-S., Rodrigo J., Kellenberger E., Rognan D. A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors *Proteins* **2006**, 62(2), 509–538. DOI: 10.1002/prot.20768, PubMed: 16294340.

2. Costanzi S., Neumann S., Gershengorn M. C. Seven transmembrane-spanning 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: 18385136.

3. Khan M. Z., He L. The role of polyunsaturated fatty acids and GPR40 receptor in brain *Neuropharmacology* **2017**, 113(Pt B), 639–651. DOI: 10.1016/j.neuropharm.2015.05.013, PubMed: 26005184.

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: 24051395.