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GPR142 Agonist

BI-1046

Boehringer Ingelheim

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Summary

BI-1046 is a selective and highly potent GPR142 agonist that qualifies for *in vivo* pharmacology experiments due to its high cellular potency and permeability. A negative control is available (BI-4420).

Chemical Structure

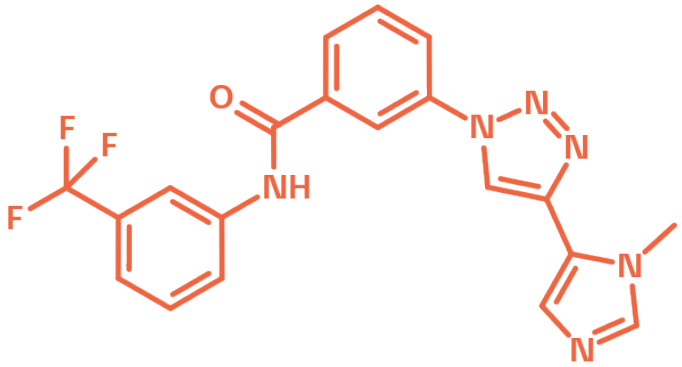


Figure 1: 2D structure of BI-1046, a highly potent GPR142 agonist.

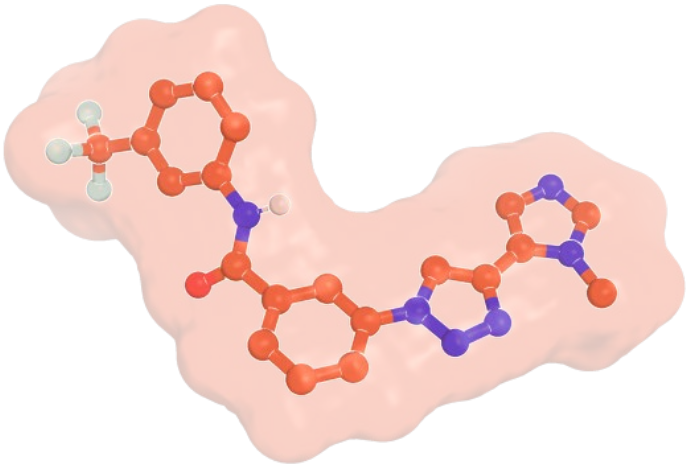


Figure 2: BI-1046, 3D conformation

Highlights

BI-1046 has been identified as a potent and selective GPR142 agonist which increases Gq-coupled signalling in cells of recombinant and endogenous (mouse insulinoma cell line Min-6) receptor expression. The GPR142 agonistic activity of BI-1046 has been demonstrated to potentiate glucose-stimulated insulin secretion from mouse islets, a physiological response that translates into improved glucose tolerance in mice when investigated in an acute oral glucose tolerance test.

Target information

GPR142 is a tryptophan-activated Gq-coupled receptor with highly enriched expression in pancreatic islets[1]. Both natural[2] and synthetic ligands[3-8] for this GPCR increase insulin secretion in a glucose-dependent fashion and improve *in vivo* glucose homeostasis in animals. Additionally, synthetic GPR142 agonists induce proliferative and anti-apoptotic responses in ß-cells from mouse and human dispersed pancreatic islets[9].

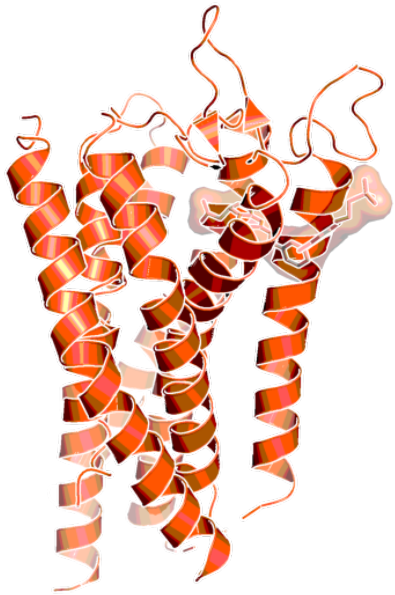


Figure 3: 3D structure of a class A GPCR (GPR40, 4phu.pdb). The structure of GPR142 or closely related GPCRs is unknown.

In vitro activity

BI-1046 displays high potency with an EC_{50} of 0.05 nM in an IP1-based cellular assay to test the effect of the compounds on GPR142. It is also highly active (EC_{50} <0.266 nM) on the respective mouse protein.

PROBE NAME / NEGATIVE CONTROL	BI-1046	BI-4420
MW [Da, free base][a]	412.4	398.3
Human GPR142 IP1 (EC_{50}) [nM][b]	0.05	>30,000
Mouse GPR142 IP1 (EC_{50}) [nM][b]	<0.266	3,166
Min-6 IP1 (EC_{50}) [nM][c]	3	n.a.
GSIS mouse islets (EC_{50}) [nM][d]	3,500	n.a.

[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] HEK293 IP1 assay

The activity of the compounds was tested using the following assay which determines the generation of inositol monophosphate, which is a stable metabolite of IP3 induced by activation of a phospholipase C (PLC). This assay allows monitoring activity of compounds acting on Gq-coupled receptors. The principle of the assay is based on the competition between IP1 generated in the cells with d2-labeled IP1 for binding to a monoclonal anti-l P1 Tb2+ cryptate that allows for measuring quantitative changes using Homogeneous Time Resolved Fluorescence (HTRF). LiCI is added to the cell as stimulation buffer, causing IP1 to accumulate upon receptor activation.

The open reading frame encoding the human GPR142 receptor (primary accession number: NM_181790; Gene ID: 350383) or the mouse GPR142 (primary accession number: NM_181749.1) were cloned into the pcDNA5/FRT/TO vector for stable, and inducible expression using the Flp-ln™ T-REx™ technology in HEK293 FLPinTREX cells. For stable expression, HEK293 FLPinTREX cells were co-transfected with the vector encoding the human or mouse GPR142 and the pOG44 plasmid that constitutively expresses the Flp recombinase for stable integration of the GPR142 into the genome of the Flp-ln™ T-REx™ host cell line. Stably expressing clones were obtained by hygromycin (100µg/ml) selection. To test for GPR142 agonist activity the human/mouse GPR142 receptor cell clone was thawed at 37°C and immediately diluted with cell culture medium (Hams F12 medium; 10% FBS, 15 µg/ml Blasticidin, 100µg/ml Hygromycin B). After centrifugation, the cell pellet was resuspended in medium, and distributed into the assay well plates (10,000 cells/well; 20 pl/well). Receptor expression was induced by adding 0.1 µg/ml doxycyclin to the culture medium. The plates were incubated for one hour at room temperature, followed by a 24 hours incubation at 37°C/5% CO_2. After washing the cells in the plate twice with assay buffer (10 mM HEPES, 1 mM $CaCl_2$, 0.5mM $MgCl_2$, 4.2mM KCl, 146mM NaCl, 5.5mM Glucose, 50mM LiCl and 0.1% BSA, pH 7.4) 20 µl buffer remained in the wells after washing), 10 µl per well of test compound diluted in assay buffer was added to the wells. The assay plate was incubated for 60 minutes at 37°C. Then 5 µl per well of Anti-IP1-Cryptate Tb solution (prepared by 1:33 dilution of stock with Lysis buffer from IP-One Kit) and 5 µl per well of IP1-d2 (Prepared by 1:33 dilution of stock with Lysis buffer from IP-One Kit) dilution were added, followed by another 60 minutes incubation (light protected, room temperature). The emissions at 615 nm and 665 nm (excitation wavelength: 320 nm) were measured on the EnVision™ reader (PerkinElmer). The ratio between the emission at 665 nm and 615 was calculated by the reader.

Each assay microtiter plate contained 8 wells in with vehicle controls instead of compound (100% CTL; low values, negative control) and 8 wells with a reference GPR142 agonist (200% CTL; high values; positive control). An IP1 standard curve was prepared according to the manufacturer.

The ratio between the emission at 665 nm and the emission at 615 nm (Em665/Em615 ratio) was calculated and the signals for the test items were normalized using the positive and negative controls by the following formula: 100 - (100 x[(ratio(sample) - ratio(low))/(ratio(high) - ratio(low))].

[c] Min-6 IP1 assay

The activity of the compounds was tested applying the mouse insulinoma cell line Min-6, which endogenously expressed GPR142.

[d] Glucose-stimulated insulin secretion

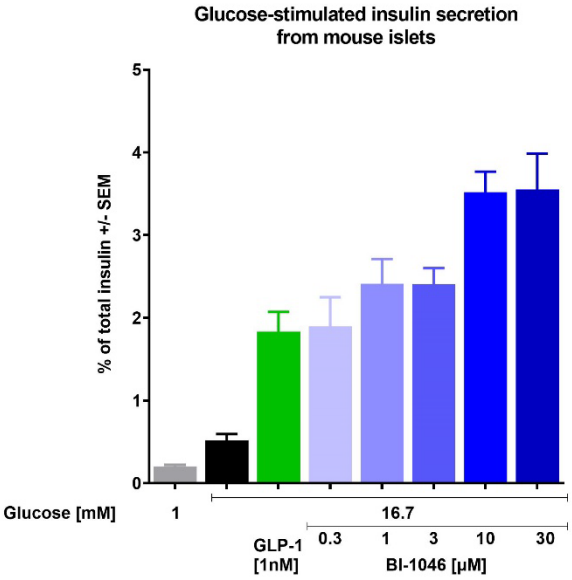
Islet isolation

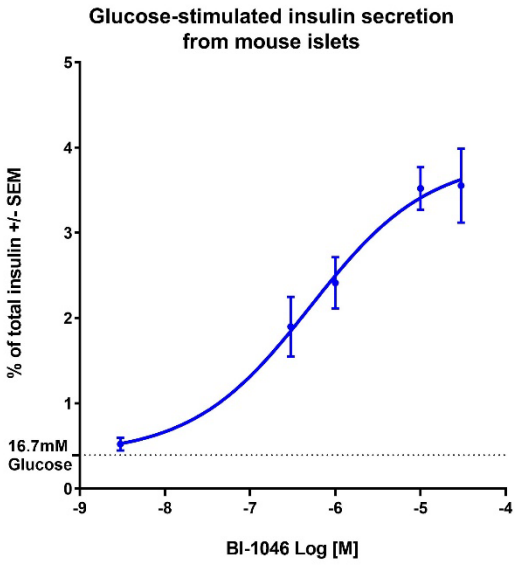
Mouse islets were isolated as described as in reference 10 by injection of collagenase (solution containing 0.1mg/mL DNAse) into the pancreas of anesthetized animals via the common bile duct. After injection of ice-cold collagenase, the pancreas was

removed and digested at 37°C for 12min. Collagenase activity was stopped by the addition of ice-cold KRBH with 2% BSA and the tissue was washed 3 times with KRBH followed by a centrifugation at 400g. Islets were obtained by gradient centrifugation using Histopaque 1077 and resuspended in RPMI. After multiple washes including a cellstrainer step to reduce remaining acinar tissue, islets were cultured for 24h in RPMI1640 containing 10% FCS, GlutaMax, and Penicilin/Streptomycin before use.

Static insulin secretion

Static insulin secretion assays with mouse islets were performed after 24 hours culture of the islets upon isolation in RPM1640 with 10% FCS, Glutamax with 11.1 mM Glucose. Islets were transferred to KRBH containing 0,1% BSA and 2.8mM glucose for 30 min. Islets (5 per well) were then transferred to into a 96-well plates with V-shaped bottom containing the respective glucose concentration and test compound. After 30 min incubation 50µL were sampled to determine the secreted insulin. Insulin content of the islets was measured after HCl/Ethanol lysis of the islets overnight. Insulin was determined using the HTRF. The amount of insulin secreted was normalized (in % of total) to the insulin content determined form the lysed islets.





In vitro DMPK and CMC parameters

BI-1046 is a GPR142 agonist with high permeability on Caco-2 and MDCK cells. It further shows high stability in rat microsomes and moderate stability in rat hepatocytes.

PROBE NAME / NEGATIVE CONTROL	BI-1046	BI-4420
logP @ pH 11	4.12	n.a.
logD @ pH 2 / 11	2.2 / 3.2	4.6 / 4.5
Solubility @ pH 6.8 [µg/mL]	<1.0	<1.0
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	48.3	19.0
Caco-2 efflux ratio	0.69	0.36
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	7.6	28
MDCK efflux ratio	4.87	0.157

Microsomal stability (human/mouse/rat) [% Q_H]	32 / 63 / <22	76 / 81 / 66
Hepatocyte stability (human/mouse/rat) [% Q_H]	11 / - / 44	n.a.
Plasma Protein Binding (human/mouse/rat) [%]	99.7 / 99.3 / 99.2	n.a.
hERG [inh. % @ 1 µM]	42.9	n.a.
CYP 3A4 (IC_{50}) [µM]	1.7	>50
CYP 2C8 (IC_{50}) [µM]	<0.2	5.0
CYP 2C9 (IC_{50}) [µM]	0.56	>50
CYP 2C19 (IC_{50}) [µM]	1.3	>50
CYP 2D6 (IC_{50}) [µM]	0.94	>50

In vivo DMPK parameters

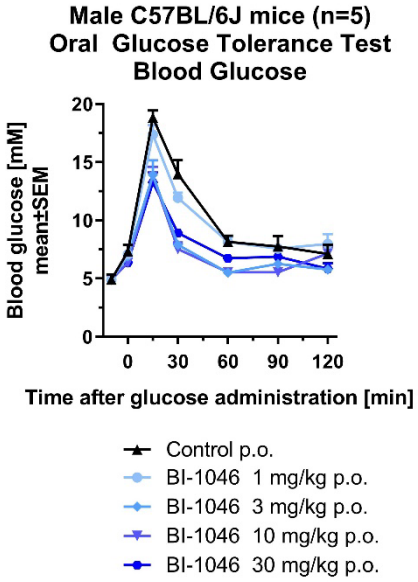
BI-1046 is a medium clearance compound that qualified as an *in vivo* molecule.

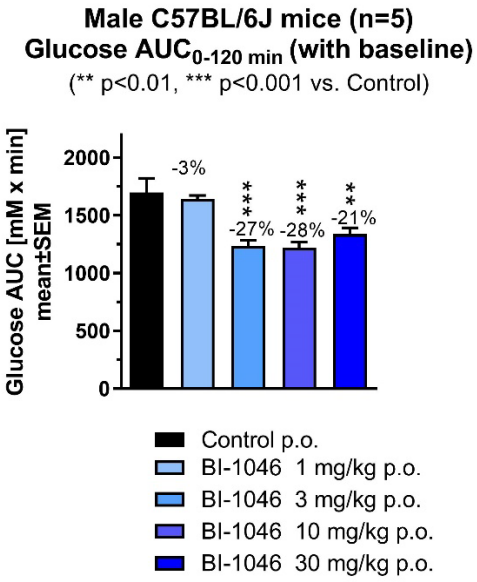
BI-1046	MOUSE	RAT
Clearance [% Q_H][a]	31.2	45.1
Mean residence time after *i.v.* dose [h][a]	0.34	0.96
t_{max} [h][b]	0.8	n.a.
C_{max} [nM][b]	2624	n.a.
F [%]	38	n.a.
V_{ss} [L/kg][a]	0.56	1.80

[a] *i.v.* dose: 0.4 mg/kg
[b] *p.o.* dose: 10mg/kg

In vivo pharmacology

In an oral glucose tolerance test, BI-1046 improved glucose tolerance in mice (male C57BL/6J) with an ED_{50} of about 1.3 mg/kg. The maximal efficacy (Emax) for the glucose excursion (area under the curve) was determined with about 30% (Emax) compared to vehicle.





Negative control

BI-4420 is a structurally related negative control of BI-1046. The 2-furanyl substituent in BI-4420 compared to the N-Methyl imidazolyl ring renders it inactive on both human and mouse GPR142.

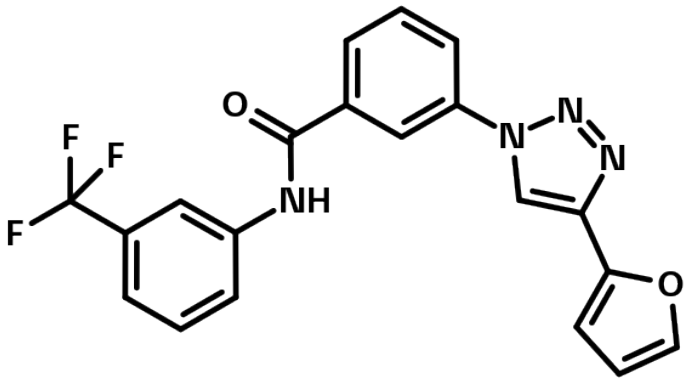


Figure 4: BI-4420, which serves as a negative control

Selectivity

BI-1046 was shown to be selective on GPR142 (EC_{50} (human) = 0.05 nM; EC_{50} (mouse) < 0.266 nM). Most importantly, it was shown to be selective against a related GPCR GPR40 (EC_{50} > 10µM for human and mouse GPR40). In selectivity panels, it showed 3 hits with >80% inhibition in the SafetyScreen44™ panel and 2 hits with >80% inhibition on the Invitrogen® kinase panel.
Negative control BI-4420 hits 6 from 44 with >50% in the SafetyScreen44™ panel at @10µM (5HT2B, COX-2, HERG, CCKA/H, NA+/SITE2/R, Alpha1AH).

SELECTIVITY DATA AVAILABLE	BI-0146	BI-4420
SafetyScreen44™ with kind of support of eurofins	Yes	Yes
Invitrogen®	Yes	Yes
DiscoverX®	No	No
Dundee	No	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein.

Not available.

Reference molecule(s)

Several synthetic GPR142 agonists have been reported in the literature[3-6].

LY3325656, a GPR142 agonist developed by Eli Lilly & Company has been investigated in a phase1 clinical trial for type 2 diabetes[7,8].

Supplementary data

2D structures can be downloaded free of charge from opnMe.

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