

GPR55 inverse agonist

BI-8310

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Summary

BI-8310 is a potent and efficacious GPR55 inverse agonist with properties supporting its use as a suitable tool compound for *in vitro* studies. BI-6227, a structurally related inactive analog, serves as a negative control.

Chemical Structure

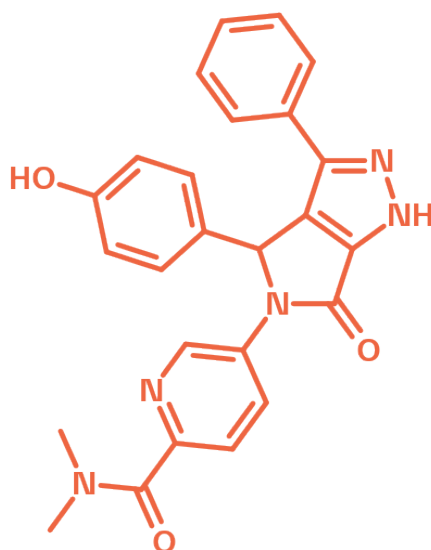


Figure 1: 2D structure BI-8310

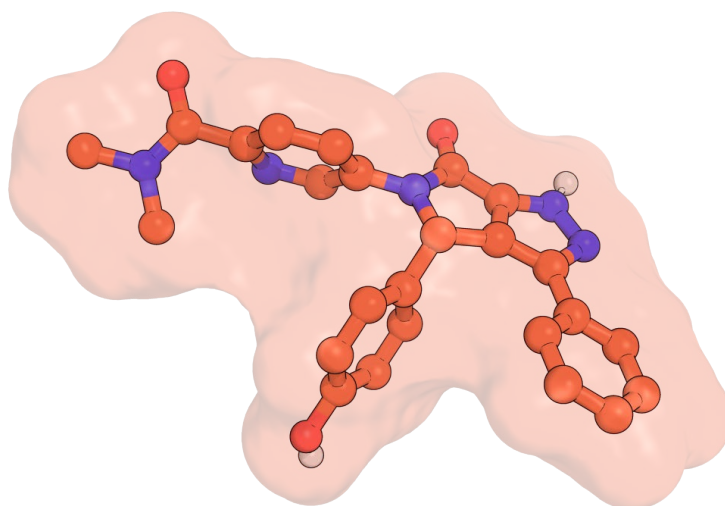


Figure 2: GPR55 in complex with the (S)-enantiomer of BI-8310, as determined by cryo-EM

Highlights

BI-8310 is a potent and efficacious GPR55 inverse agonist with favorable physicochemical properties and an *in vitro* DMPK profile supporting its use as a robust tool compound. It exhibits inverse agonist activity against the constitutive activity of GPR55 and is also active in assays using the endogenous ligand LPI. A cryo-EM structure of GPR55 in complex with the active (S)-enantiomer of BI-8310 is available, enabling structure-based investigations. The structurally related compound BI-6227 serves as a negative control.

Target information

GPR55 is a lipid-activated G protein-coupled receptor (GPCR) whose pharmacological characterization has been hindered by the limited availability of suitable tool compounds¹⁻³. Lysophosphatidylinositol (LPI) is considered its most likely endogenous ligand, although definitive *in vivo* evidence is still lacking. As a result, GPR55 has not yet been fully deorphanized⁴. Unlike many classical GPCRs activated by neurotransmitters or hormones, GPR55 is modulated by bioactive lipids and certain cannabinoid-related compounds⁵. However, its classification as a cannabinoid receptor remains controversial due to inconsistent pharmacological profiles⁶.

GPR55 signalling predominantly engages $G\alpha_{13}$ and downstream pathways such as RhoA (Ras homolog family member A) and Rho-associated kinases (ROCK), influencing cytoskeletal dynamics, calcium mobilization and cellular motility^{7,8}. Notably, GPR55 exhibits high constitutive activity via $G\alpha_{13}$ signalling⁹ and is widely expressed in the central nervous system, gastrointestinal tract, liver, adrenal glands, and immune cells. This distribution suggests roles in diverse physiological processes including energy metabolism, inflammation, and immune regulation⁷.

Recent cryo-electron microscopy (cryo-EM) studies⁹ have resolved GPR55 in complex with different ligands, revealing key structural features such as N-linked glycosylation near the ligand binding pocket as well as a cholesterol binding pocket directly contacting ligands. These insights provide a foundation for rational, structure-based ligand design.

Despite growing interest in GPR55 as a target for mechanistic and drug discovery research, significant challenges persist. These include limited availability of selective tool compounds, incomplete understanding of its *in vivo* functions, and the complexity of its lipid-mediated signalling^{9,10,11}. Addressing these gaps may support further investigation of GPR55 biology and pharmacology.

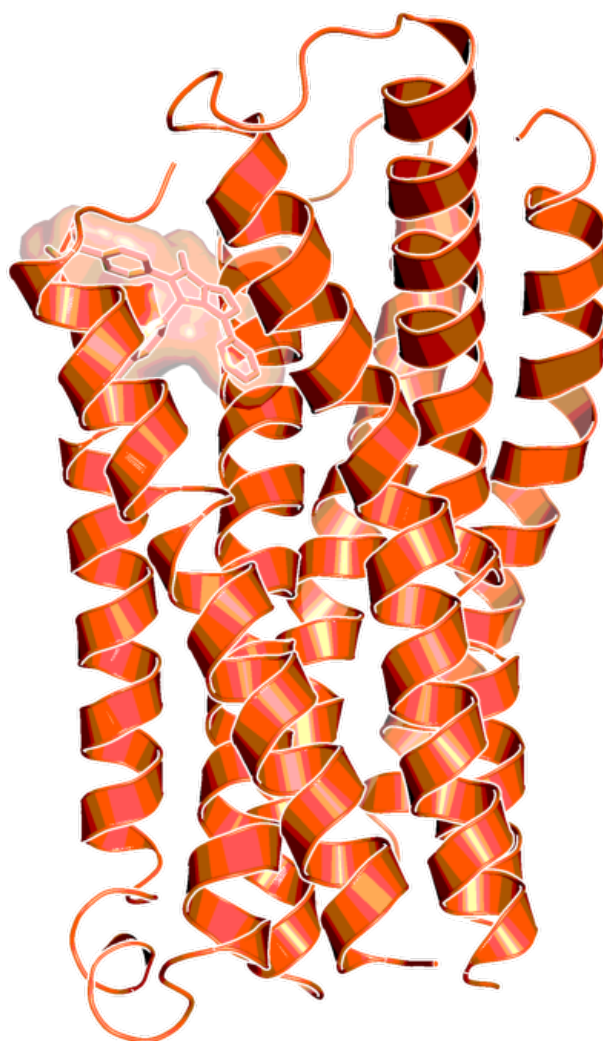


Figure 3: Bound conformation of the active BI-8310 enantiomer, as observed in complex with GPR55 (Boehringer in house cryo-EM structure)

***In vitro* activity**

BI-8310 exhibits potent inverse agonist activity with an IC_{50} value of 263 nM and an efficacy (E_{max}) of -53% in a bioluminescence energy transfer (BRET)-based G_{13} protein dissociation assay targeting constitutive receptor activation. BI-8310 shows a ~3-fold reduction in potency when tested against the 80% effective concentration (EC_{80}) of LPI. In contrast, the structurally related compound BI-6227, is inactive at concentrations up to 100 μ M. In a β -arrestin2 recruitment assay (PathHunter), BI-8310 showed a potency of 610 nM against the EC_{80} of LPI. The *S*-enantiomer of BI-8310 represents the eutomer. However, BI-8310 is provided as the racemate because its pure enantiomers rapidly racemize in aqueous solution.

Probe name / Negative control	BI-8310	BI-6227
MW [Da] ^a	439.5	425.4
Evaluated versus constitutive activity, BRET-based G ₁₃ protein dissociation assay (TRUPATH) ³ (IC ₅₀) [nM]	263	>100,000
Evaluated versus constitutive activity, BRET-based G13 protein dissociation assay (TRUPATH) ³ (E _{max})	-53%	-
Evaluated versus EC ₈₀ of LPI, BRET-based G13 protein dissociation assay (TRUPATH) ³ (IC ₅₀) [nM]	871	>100,000
Evaluated versus EC ₈₀ of LPI, β -arrestin2 recruitment assay (PathHunter) ³ (IC ₅₀) [nM]	610	>10,000

^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.

In vitro DMPK and CMC parameters

BI-8310 exhibits a favorable *in vitro* DMPK profile with adequate solubility, good metabolic stability, and no relevant CYP inhibition, supporting its use as a robust *in vitro* tool compound despite its low membrane permeability.

Probe name / Negative control	BI-8310	BI-6227
clogD @ pH 7.4	2.0	1.8
Solubility @ pH 7 [μ g/mL]	17	>92
Caco-2 permeability AB @ pH 7.4 [$\times 10^{-6}$ cm/s]	<0.4	<0.5
PAMPA @pH 7.4 [$\times 10^{-6}$ cm/s]	0.007	0.006
Microsomal stability (human/mouse) [% Q _H]	37 / <23	65 / <23
CYP 3A4 (IC ₅₀) [μ M]	>50	>50
CYP 2C8 (IC ₅₀) [μ M]	>50	42
CYP 2C9 (IC ₅₀) [μ M]	>50	3.4
CYP 2C19 (IC ₅₀) [μ M]	>50	>50
CYP 2D6 (IC ₅₀) [μ M]	>50	>50
CYP 1A2 (IC ₅₀) [μ M]	>50	>50
CYP 2B6 (IC ₅₀) [μ M]	>50	>50

Negative control

BI-6227, a structurally close analog, can be used as negative control.

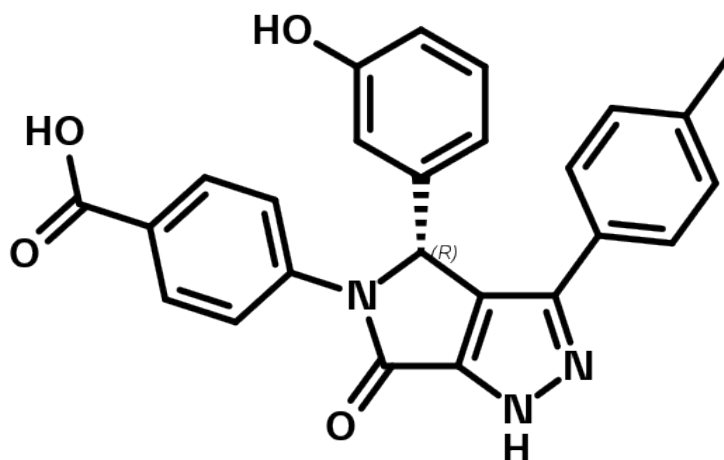


Figure 4: BI-6227 which serves as a negative control

Selectivity

In the SafetyScreen44™ panel, BI-8310 showed >50% inhibition at 10 µM for 2 of 44 tested targets: human 5-HT2B and adenosine A2A receptors.

BI-6227 inhibited 1 out of 44 tested targets with > 50% at 10 µM concentration (DELTA2/H) tested in SafetyScreen44™.

Selectivity data available	BI-8310	BI-6227
SafetyScreen44™ with kind support of  eurofins	Yes	Yes

Reference molecule(s)

Other available tool compounds are CID16020046^{1,2} and ML193^{12,13}.

Supplementary data

2D structure files can be downloaded free of charge from [openMe](https://openme.boehringer-ingelheim.com).

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