opnMe by Boehringer Ingelheim

GCH1 Inhibitor

AXSP0056

Boehringer Ingelheim

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Summary

AXSP0056 is an allosteric inhibitor of human GTP Cyclohydrolase I. It can be used as a chemical probe for *in vitro* and *in vivo* studies alongside the negative control D X 0373.

Chemical Structure

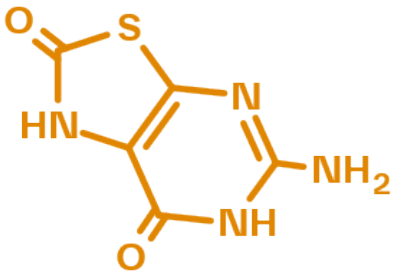

Figure 1: 2D structure of AXSP0056

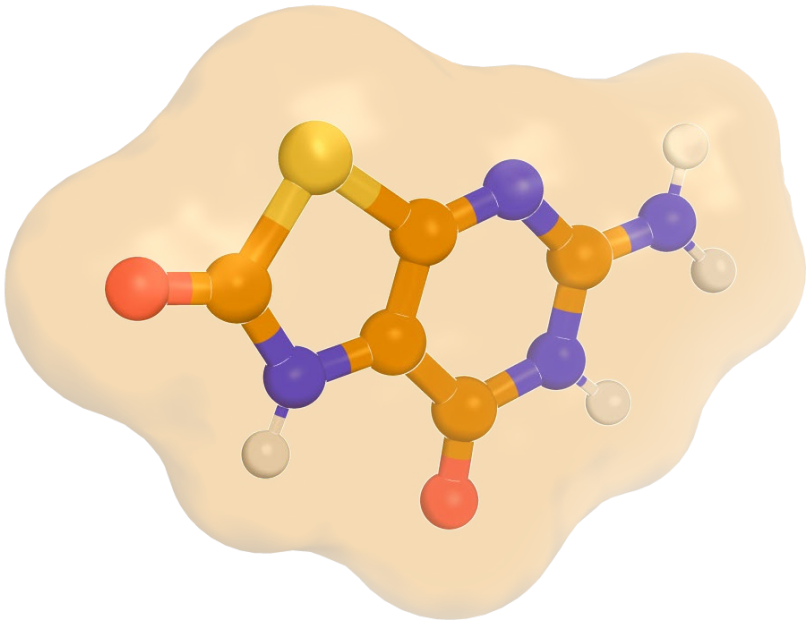

Figure 2: AXSP0056, 3D structure, as observed in complex with GCH1, revealed by cryo-EM1

Highlights

AXSP0056 acts as a mimic of tetrahydrobiopterin (BH4) by occupying the allosteric site of GCH1 which binds BH4 under physiological conditions in its feedback inhibition mode. It utilizes its aminopyrimidinone moiety to bind to key residues in the allosteric pocket. It is a single digit micromolar inhibitor. Its moderate inhibitory activity has been demonstrated in functional assays and biophysical assays (thermal shift, STD NMR).

Target information

GTP cyclohydrolase I (GCH1) catalyzes the conversion of GTP to dihydroneopterin triphosphate (H2NTP). This reaction is the first and rate-limiting step involved in the *de novo* synthesis of tetrahydrobiopterin (BH4)[2]. BH4 plays key roles in phenylalanine catabolism and the biosynthesis of serotonine and catecholamine-type neurotransmitters like dopamine or norepinephrine by functioning as an essential cofactor for hydroxylases of the aromatic amino acids phenylalanine, tyrosine, and tryptophan[3,4].

Furthermore, BH4 is a co-factor for the family of nitric oxide synthases (NOS)[5], which produce the cellular signaling molecule nitric oxide that helps to modulate vascular tone and insulin secretion. Nitric oxide has also been implicated in inflammation as well as in the regulation of immune responses[6].

The human GCH1 sequence comprises 250 amino acids and forms a 270-kDa, D5-symmetric homodecameric functional enzyme complex in solution[7-10]. The enzymatic activity of GCH1 is tightly regulated and has been shown to involve GCH1 and a regulatory protein, now known as GTP-cyclohydrolase-I-feedback-regulatory protein (GFRP), which simultaneously functions as a positive and negative regulator of GCH1[8]. The effects of GFRP on GCH1 occur via formation of heteromeric protein complexes between GCH1 and GFRP, which are dependent on the intracellular concentrations of the effector molecules phenylalanine or BH4. Elevated phenylalanine levels lead to stimulation of GCH1 activity, whereas BH4, the end product of the biosynthesis pathway, inhibits GCH1 in a feedback inhibition type mode[9]. Mammalian GCH1 shows cooperative enzymatic activity. Complex formation with GFRP-Phe leads to increased activity at lower substrate concentrations and eliminates substrate cooperativity. Conversely, GCH1 alone is allosterically inhibited by BH4. In the presence of GFRP, the inhibitory effect of BH4 is boosted and occurs at lower, physiologically relevant BH4 concentrations. The GCH1-GFRP system can therefore be regarded as a metabolic sensor that establishes BH4 and aromatic amino acid homeostasis.

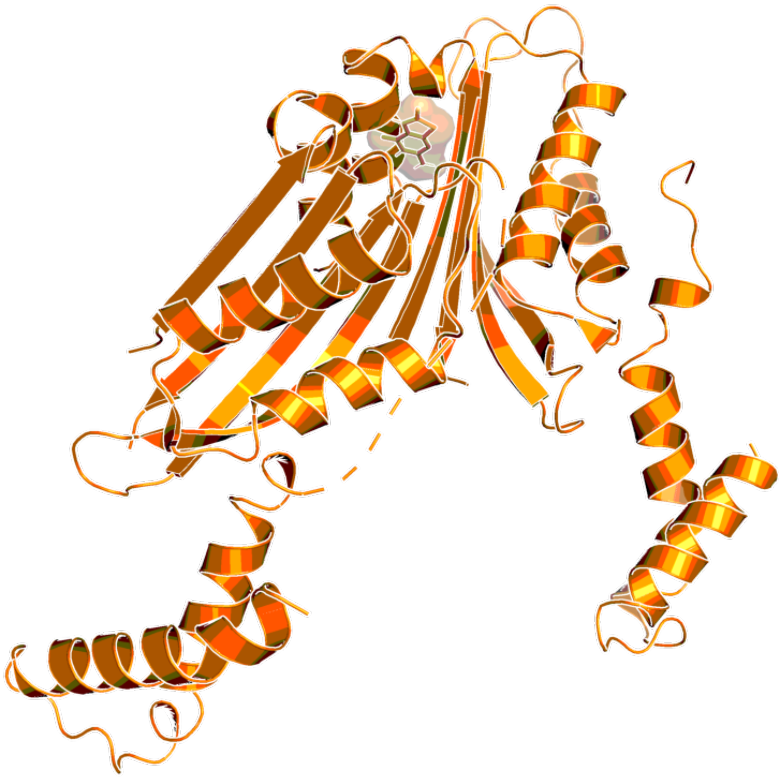

Figure 3: GCH1 is a homodecameric enzyme with D5 symmetry forming a donut-shaped structure. AXSP0056 binds to the allosteric site at the interface of two GCH1 subunits, as revealed by protein crystallography[1]

In vitro activity

AXSP0056 displays an IC_{50} of 2.1 µM in functional assays measuring formation of products of the reaction catalyzed by GCH1.

Probe name / Negative control	AXSP0056	D X 0373
MW [Da]	184.2	236.3
H2NTP Product formation (IC_{50}) [nM][a]	2,100	n.d.
Formate product formation [%CTR] at 150µM compound[b]	25	n.d.

[a] GTPCH-I specific enzyme activity was determined as described in reference 1
[b] NMR sample preparation for the functional assay was performed as described in reference 1

In vitro DMPK and CMC parameters

AXSP0056 shows moderate solubility and good microsomal stability in human, mouse and rat.

Probe name / negative Control	AXSP0056	D X 373
Log D @ ph2	-1.6	-
Solubility @ pH 6.8 [µg/ml]	>46	>1,000
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	No valid analytical method	25
Caco-2 efflux ratio	No valid analytical method	1
Microsomal stability (human/mouse/rat) [% Q_H]	<23 / <23 / <22	<23 / <23 / <22
Hepatocyte stability (mouse/rat) [% Q_H]	12 / <2	72 / 55
Plasma Protein Binding (human/mouse/rat) [%]	<70.9 / <24.3 / <43.7	- / <16.2 / <16.7
CYP 3A4 (IC_{50}) [µM]	>50	<50
CYP 2C9 (IC_{50}) [µM]	14.1	<50
CYP 2D6 (IC_{50}) [µM]	>50	<50

In vivo DMPK parameters

AXSP0056 reaches plasma levels of 1-4-fold IC_{50} for 2 hours after oral application. At this concentration it shows side effect-free efficacy in a CFA model. The application of 100mg/kg AXSP0056 intraperitoneally leads to plasma levels ~10-fold above minimal effective plasma concentration, however, this dose is associated with adverse effects.

AXSP0056	Rat
Clearance [% Q_H][a]	n.d.
Mean residence time after iv dose [h]	n.d.
t_{max} [h]	0.25

C_{max} [nM]	2,318

[a] rat *p.o.* dose: 1.8 mg/kg

Negative control

While structurally similar to AXSP0056, the negative control D X 373 features a di-methylation on the exocyclic nitrogen atom which prohibits binding to the GCH1 allosteric site.

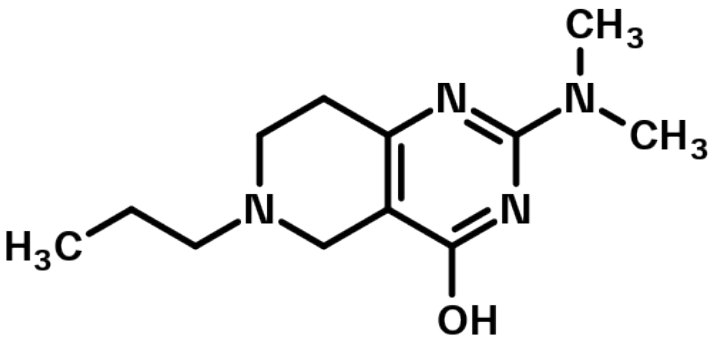

Figure 4 : D X 373 serves as a negative control

Selectivity

In the SafetyScreen44™ at a high concentration of 10µM AXSP0056 hits 2 of 44 targets. All targets measured for D X 373 in the SafetyScreen44™ are below 50% inhibition.

Selectivity data available	AXSP0056	D X 373
SafetyScreen44™ with kind support of eurofins	Yes	Yes
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

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

The X-ray crystal structure of GCH1 in complex with AXSP0056 has been resolved and is available under PDB code: 6Z88[1].

Supplementary data

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

References

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