TPP Riboswitch activator | BI-5232

Table of contents

Summary

BI-5232 is a drug-like high affinity activator of the thiamine pyrophosphate (TPP) riboswitch. It has demonstrated activity both against the native TPP riboswitch and an orthogonal variant that can no longer be activated by its natural ligand.

Chemical Structure

Figure 1: 2D structure of BI-5232, a high affinity activator of the TPP riboswitch. Single enantiomer with stereochemistry as shown.

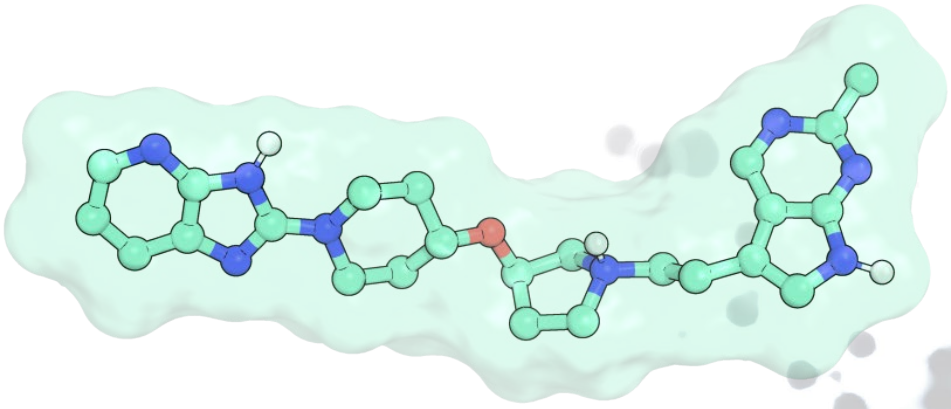

Figure 2: BI-5232, 3D conformation, as observed in the modelled complex with the TPP riboswitch.

Highlights

BI-5232 is a synthetic TPP riboswitch ligand that has demonstrated activity against an orthogonal TPP riboswitch that can no longer be activated by its natural ligand. Designed through structure-based approaches, it is one of the first drug-like, high-affinity riboswitch modulators. As a proof-of-concept molecule, it represents a first step towards precise temporal control of gene expression, making it an attractive research tool that should inspire further research in the field.

Target information

Riboswitches are small, structured RNA molecules, consisting of a ligand-sensing aptamer domain joined to a ribozyme domain, which regulates gene expression in response to aptamer binding. Riboswitches are promising tools to regulate gene expression for scientific and, potentially, therapeutic purposes[1]. However, there remains a demand for orthogonal, i.e., engineered aptamers that bind drug-like ligands without being activated by the native activators. Such synthetic riboswitches hold the potential to display inducible activity.

The TPP riboswitch class was one of the earliest riboswitches identified[2] and is the most widespread riboswitch in nature, being distributed among archaebacteria, eubacteria as well as eukaryotes[3,4,5]. Especially the *Escherichia coli* thiM aptamer has been studied intensively and has been the subject of several structural and mechanistic studies providing insights into the mechanism of ligand binding[6]. It is composed of two helical sections connected via a three-way junction to a closing stem that is stabilized upon binding of the natural ligand, thiamine pyrophosphate[6,7]. TPP acts as a bridge that connects the two helical regions and locks them in a 'Y-shape'[6,7]. TPP is an active form of vitamin B1, an essential participant in many protein-catalysed reactions. The TPP-sensing riboswitch, controls genes responsible for importing or synthesizing thiamine and its phosphorylated derivatives. Structure-guided randomization of a specific binding region of the thiM aptamer, led to an aptamer that binds BI-5232 with very high selectivity, while its natural ligand TPP could no longer bind[8].

BI-5232 is a drug-like non-natural ligand of the thiM aptamer of the TPP riboswitch with potencies near equal to TPP itself. It has been shown to cellularly induce transgene expression in constructs using both the native aptamer as well as the site-directed mutant which does not bind TPP. It therefore represents a tool compound for both the native as well as the orthogonal riboswitch. Further research is warranted to come up with a fully "synthetic toolbox for conditionally controlling gene expression with potential applications in next-generation gene therapies"[8].

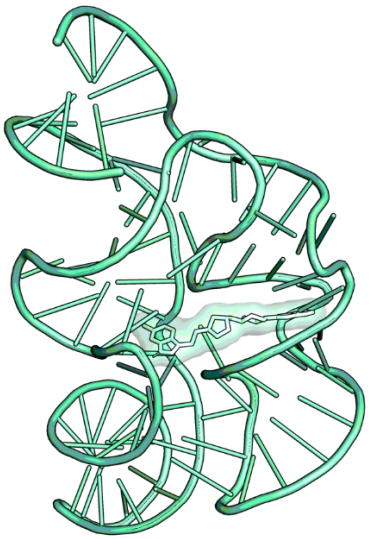

Figure 3: Structural model of the active complex of BI-5232 bound to the native TPP riboswitch[8].

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-5232	BI-5209
MW [Da]	446.5	492.58
TPP WT Riboswitch (K_d) [nM][a]	1.4	299

[a]Assay conditions: SPR binding to native thiM TPP riboswitch.

Surface Plasmon Resonance experiments were carried out at 25 °C on a BiacoreT200 instrument. Immobilization of a single stranded DNA (5'-CGTCGCAGATCGTGTCTTCC[Am C7) to CM5 chips (Cytiva) was performed as described by Liu *et al.*[8] RNA was captured via the single stranded oligonucleotide. The utilized RNA (*in vitro* transcribed and PAGE purified) consists of the natural TPP aptamer sequence from the thiM riboswitch[7], a short linker (U3) and a hybridization sequence complementary to the immobilized DNA (5´ TAATACGACTCACTATAGGAAGACACGATCTGCGACGTTTGCGACTCGGGGTGCCCTTCTGCG TGAAGGCTGAGAAATACCCGTATCACCTGATCTGGATAATGCCAGCGTAGGGAAGTCGC 3´). As a control, a TPP aptamer with an inactivating G40C mutation was used.

Binding assays were performed with a flow rate of 30 µL/min in 10 mM HEPES pH 7.4, 100 mM NaCl, 50 mM KCl, 5 mM MgCl2, 0.05 % (v/v) Tween 20, 2 % (v/v) DMSO. Compounds were tested in a

single cycle experiment at five different concentrations (e.g. 320 nM, 1.6 µM, 8 µM, 40 µM, 200 µM), with 2 min association and a final dissociation time of 15 min. For regeneration, 50 mM EDTA was used. TPP was used at concentrations of 625 pM, 1,25 nM, 2,5 nM, 5 nM and 10 nM).

Sensorgrams were evaluated using Biacore T200 Evaluation Software. The responses at the different concentrations were either fitted using a steady state affinity model or by direct curve fitting using a 1:1 interaction model to determine the KD value as well as kinetic parameters (ka and kd).

In vitro DMPK and CMC parameters

BI-5232 is a soluble compound with good properties, suitable for use in cell assays including moderate permeability and low efflux in the MDCK PGP cell line, and low microsomal clearance in rat and human.

PROBE NAME / NEGATIVE CONTROL	BI-5232	BI-5209
clogP	2.0	1.6
High Throughput Solubility @ pH 6.8 [µg/ml]	>112	-
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	0.1	4.3
CACO efflux ratio	11.8	13.7
MDCK PGP P_{AB} @pH7.4 [*10^{-6} cm/s]	1.7	-
MDCK PGP efflux ratio	0.5	-
Microsomal stability (human/rat) [% Q_H]	<23/<22	<23/-

Negative control

BI-5209, a structurally very close analogue, is available as a negative control.

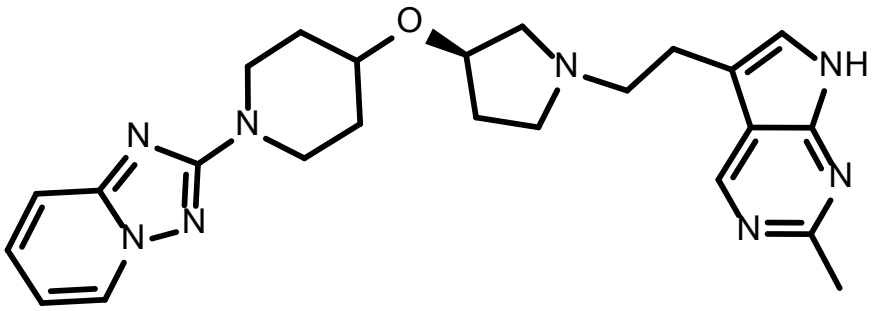

Figure 4: BI-5209, which serves as a negative control.

Selectivity

BI-5232 inhibits MU/H, 5HT2B/H AG, 5HT1A/H, 5HT3/H, M3/H, M2/ and M1H with 55, 62, 64, 77, 86, 86 and 92% @10µM respectively. All other targets measured in the SafetyScreen44 are below 50% inhibition. Compounds are considered selective, if they do not hit any of the measured targets in the SafetyScreen44™ > 50%; panel measured at 10 µM.

SELECTIVITY DATA AVAILABLE PROBE NAME / NEG. CONTROL	BI-5232	BI-5209
SafetyScreen44™ with kind support of eurofins	yes	Ongoing
Invitrogen®	No	No
DiscoverX®	No	No
Dundee	No	No

Supplementary data

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

References

1. Tickner Z. J., Farzan M. Riboswitches for controlled expression of therapeutic transgenes delivered by adeno-associated viral vectors. *Pharmaceuticals*. **2021**, 14 (06), 554. DOI: 10.3390/ph14060554, PubMed.
2. Winkler W., Nahvi A., Breaker R. R. Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression. *Nature*. **2002**, 419 (6910), 952 – 956. DOI: 10.1038/nature01145, PubMed.
3. Panchal V., Brenk R. Riboswitches as drug targets for antibiotics. *Antibiotics*. **2021**, 10 (1), 45. DOI: 10.3390/antibiotics10010045, PubMed.
4. Wachter A. Riboswitch-mediated control of gene expression in eukaryotes. *RNA Biol*. **2010**, 7 (1), 67-76. DOI: 10.4161/rna.7.1.10489, PubMed.
5. Sudarsan N., Barrick J. E., Breaker R. R. Metabolite-binding RNA domains are present in the genes of eukaryotes. *RNA*. **2003**, 9 (6), 644-647. DOI: 10.1261/rna.5090103, PubMed.
6. Serganov A., Polonskaia A., Phan A. T., Breaker R. R., Patel D. J. Structural basis for gene regulation by a thiamine pyrophosphate-sensing riboswitch. *Nature*. **2006**, 441 (7097), 1167 – 1171. DOI: 10.1038/nature04740, PubMed.
7. Edwards T. E., Ferré-D'Amaré A. R. Crystal structures of the thi-box riboswitch bound to thiamine pyrophosphate analogs reveal adaptive RNA-small molecule recognition. *Structure*. **2006**, 14 (9), 1459 – 1468. DOI: 10.1016/j.str.2006.07.008, PubMed.
8. Hucke O., Gottschling D., Schnapp G., Ries B., Kreuz S., Klugmann M., Hedwig V., Spöring M., Scharsich S., Hartig J., Aspnes G. Designing drug-like high affinity ligands for a TPP-orthogonal riboswitch derived from the thiM-aptamer. *ACS Fall* **2023**. https://doi.org/10.1021/scimeetings.3c10304.
9. Liu Y., Wilson W. D. Quantitative analysis of small molecule-nucleic acid interactions with a biosensor surface and surface plasmon resonance detection. *Methods Mol Biol*. **2010**, 613, 1-23. DOI: 10.1007/978-1-60327-418-0_1, PubMed.