

by
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TPP Riboswitch activator

BI-5232



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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

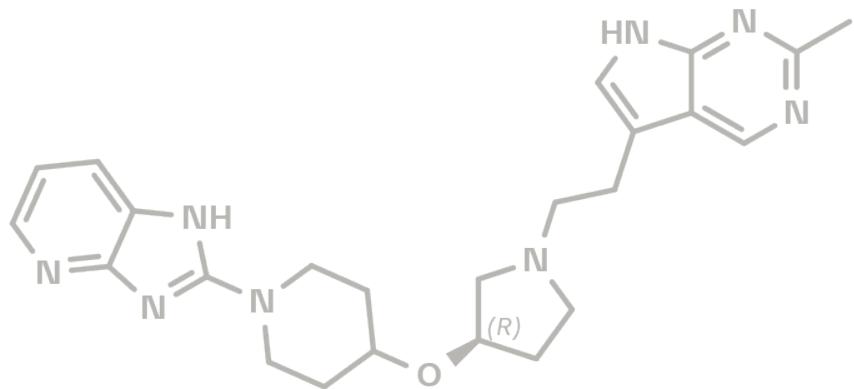


Figure 1: 2D structure of BI-5232, a high affinity activator of the TPP riboswitch. Single enantiomer with stereochemistry as shown.

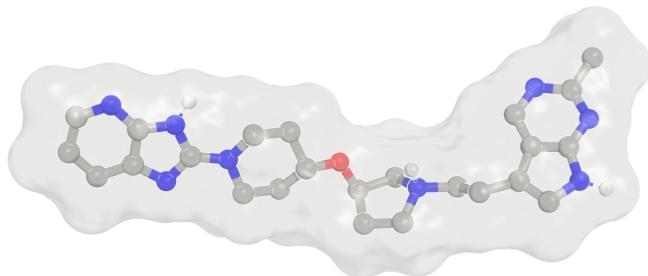


Figure 2: BI-5232, 3D conformation, as observed in the modelled complex with the TPP riboswitch.

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^{1,2}. 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 identified³ and is the most widespread riboswitch in nature, being distributed among archaeabacteria, eubacteria as well as eukaryotes^{4,5,6}. 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 binding⁷. 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^{7,8}. TPP acts as a bridge that connects the two helical regions and locks them in a 'Y-shape'^{7,8}. 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 bind⁹.

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"⁹.

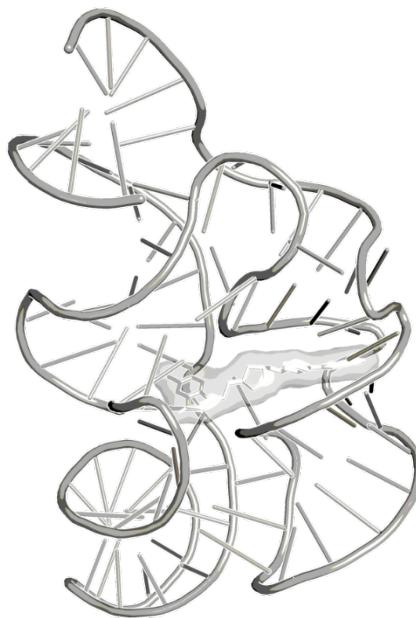


Figure 3: Structural model of the active complex of BI-5232 bound to the native TPP riboswitch⁹.

In vitro activity

PROBE NAME / NEGATIVE CONTROL	BI-5232	BI-5209
MW [Da, free base] ^a	446.6	446.6
TPP WT Riboswitch (K_d) [nM] ^b	1.4	299

^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 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.⁹. 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 riboswitch⁸, a short linker (U3) and a hybridization sequence complementary to the immobilized DNA (5' TAATACGACTCACTATAGGAAGACACGATCTGCGACGTTGCGACTCGGGGTGCCCTTCTGC 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 μ M, 200 μ M), 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 direct curve fitting using a 1:1 interaction model to determine the K_D value as well as kinetic parameters (k_a and k_d).

***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
logD @ pH 11	0.5	1.3
Solubility @ pH 6.8 [μ g/mL]	>112	-
Caco-2 permeability AB @ pH 7.4 [$\times 10^{-6}$ cm/s]	0.1	4.3
Caco-2 efflux ratio	11.8	13.7
Microsomal stability (human/rat) [% QH]	<23 / <22	<23 / -

Negative control

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

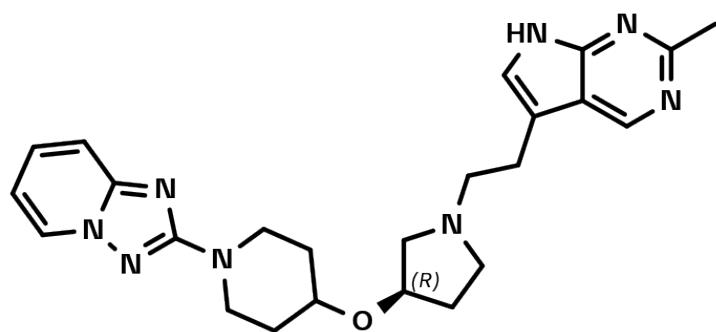


Figure 4: BI-5209, which serves as a negative control.

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.

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 [openMe](#).

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