STEP activator | BI-0314

Table of contents

Summary

BI-0314 is the first allosteric activator for STEP (PTPN5). The compound may be used as starting point for the development of selective STEP activators.

Chemical Structure

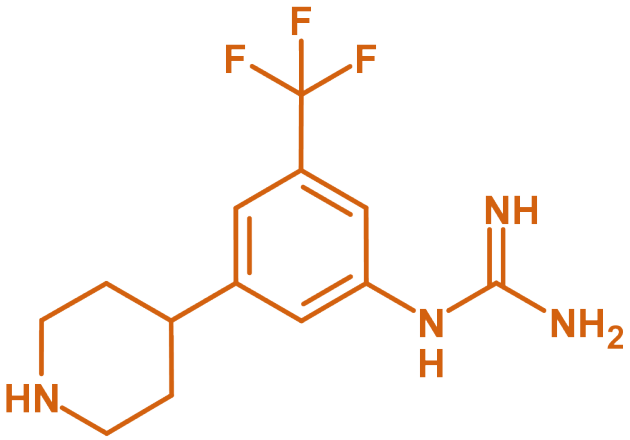

Figure 1: 2-D structure of BI-0314, a STEP activator

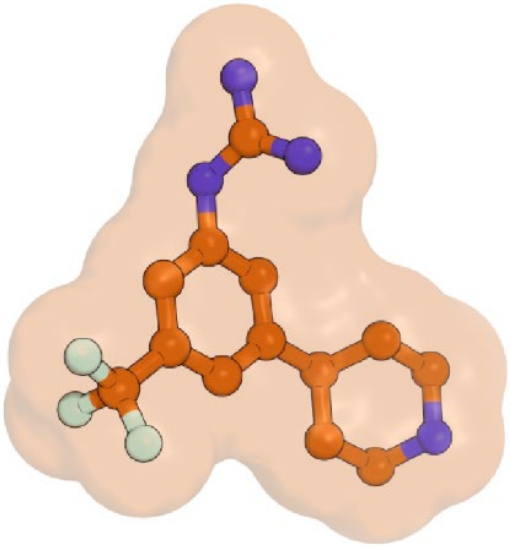

Figure 2: BI-0314, 3D conformation, as observed in complex with STEP (PDB code: 6H8S)

Highlights

BI-0314 is the first allosteric modulator for STEP (STriatal-Enriched Protein Tyrosine Phosphatase). It binds to the active phosphatase domain and upregulates its catalytic activity. Its activity has been demonstrated in enzymatic assays showing an activation of STEP of ~60% at 500 µM using different readouts. The selectivity of BI-0314 was tested against the tyrosine phosphatases PTP1B and TCPTP and no signs of activation were observed at concentrations up to 500 µM.

Target information

STEP is a multi-domain tyrosine phosphatase which exists as two splice variants, the membrane anchored longer isoform STEP61 and the cytosolic STEP46. Both isoforms share the identical kinase interaction motif (KIM) and the protein tyrosine phosphatase (PTP) domain with the phosphatase consensus motif $C(X)_5R$. The KIM domain mediates binding to various target kinases with high affinity, while the PTP domain catalyzes their subsequent dephosphorylation. To avoid developing ligands which potentially suffer from substrate specificity, we preferred targeting the PTP domain over the KIM domain. The PTP domain bears various conserved structural motifs, such as the WPD loop, which is crucial for the catalytic step, as its aspartate (D461) mediates proton transfer to the phosphate leaving group.

To elucidate the mode of action, an X-ray structure with BI-0314 bound STEP has been solved demonstrating remote site binding ~20 Å away from the active phosphatase site. The allosteric binding site could be confirmed also in solution by 15N TROSY NMR. Long range allosteric mechanisms have been confirmed by extensive molecular dynamics simulations. The identification of a druggable allosteric pocket provides new opportunities for the discovery of selective STEP modulators as treatment options for CNS disorders.

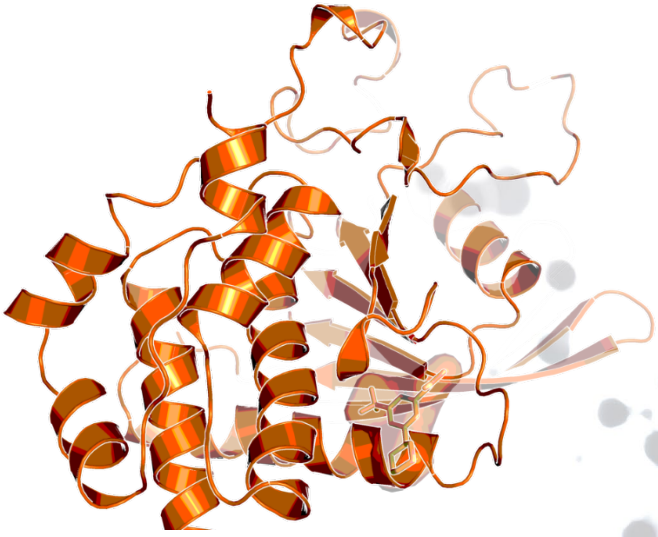

Figure 3: STEP structure and allosteric binding site with bound ligand (orange sticks). PDB code: 6H8S.

In vitro activity

BI-0314 displays an activation of STEP of ~60% at 500µM on the dephosphorylation of a pFYN derived peptide.

PROBE NAME			BI-0314
MW [Da] (Free base)[b]			286.3
Enzyme	**Substrate**	**Assay technology**	**Effect of BI-0134 in assay**
hSTEP46[b]	pFYN-peptide	RapidFire (MS)	Activating 56% ± 5 % at 500 µM, (n=8) 33% ± 12 % at 100 µM, (n=12)
hSTEP46[b]	pFYN-peptide	AlphaLISA	Activating 28% ± 5 % at 100 µM, (n=4)
PTP domain of hSTEP[b]	pFYN-peptide	AlphaLISA	Activating 61% ± 6% at 500 µM, (n=10)
hSTEP46[b]	DiFMUP	Fluorescence	Activating 48% ± 8 % at 1000 µM, (n=3) 27% ± 5 % at 300 µM, (n=3)

[a] for detailed assay conditions see Ref. 3

[b] will be shipped as salt (for MW of the salt and salt form please refer to vail-label).

In vitro DMPK and CMC parameters

Not determined.

In vivo DMPK parameters

Not determined.

In vivo pharmacology

Not determined.

Negative control

Not determined.

Selectivity

The phylogenetically closest enzyme (TCPTP) and the “generic” tyrosine phosphatase PTP1B have been investigated and at concentrations up to 500µM of BI-0314 no signs of activation could be observed. No other panels have been tested.

SELECTIVITY DATA AVAILABLE	BI-207127	BI-7656
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 Xray crystal structure of target in complex with BI-0314 is available (PDB code: 6H8S)

Reference molecule(s)

No other STEP *activators* are described so far. However, there are quite potent orthosteric inhibitors described, which only show moderate selectivity over other phosphatases. (Xu 2014[1], Witten 2017[2])

Supplementary data

Selectivity data can be downloaded free of charge from opnMe.

References

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