STEP activator | BI-0314

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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

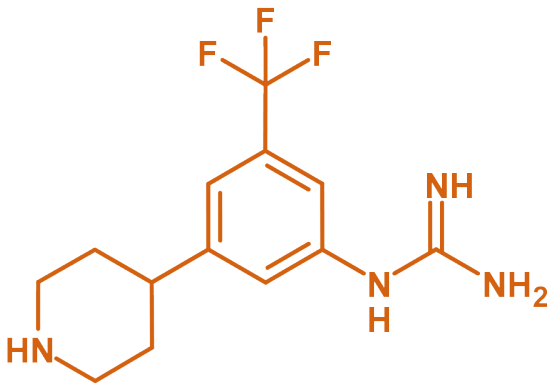

Figure 1: 2-D structure of BI-0314, a STEP activator

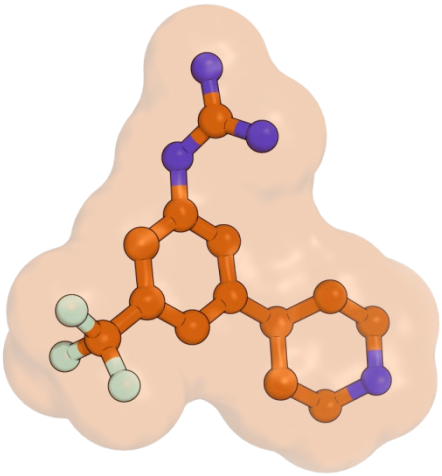

Figure 2: BI-0314, 3D conformation, as observed in complex with STEP (PDB code: 6H8S)

Highlights

With BI-0314 we report the discovery of the first small molecule allosteric modulator for STEP (STriatal-Enriched protein tyrosine Phosphatase), that binds to the phosphatase domain and upregulates the catalytic activity of STEP. Its activity has been demonstrated in enzymatic assays showing an activation by ~30% at 100µM and ~60% at 500µM in complementary readouts and on different STEP constructs. The selectivity against PTP1B and TCPTP has been tested and no

activation at 500µM could be seen. 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 ^{15}N 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.

Target information

STEP is a multi-domain tryrosine 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.

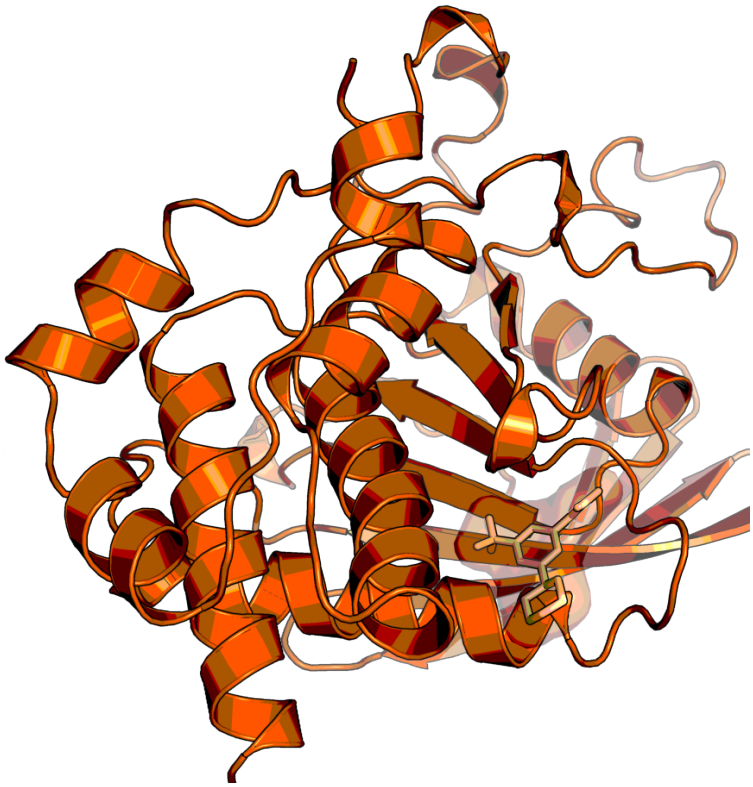

Figure 3: STEP structure and allosteric binding site with bound ligand (orange sticks). PDB code: 6H8S.

In vitro activity

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

In vitro activity

PROBE NAME			BI-0314
MW [Da] (Free base) [b]			286.3
Enzyme	**Substrate**	**Assay technology**	**Effect of BI-0314 in assay**
hSTEP46[b]	pFYN-peptide	AlphaLISA	Activating 56% ± 5 % at 500 µM, (n=8) 33% ± 12 % at 100 µM, (n=12)
hSTEP46[b]	pFYN-peptide	RapidFire (MS)	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 available

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.

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, Witten 2017)

Summary

With BI-0314 the first allosteric activator of tyrosine phosphatases is described. We hope to spark the design of selective allosteric ligands of phosphatases with the disclosure of the X-ray structure, and the elucidation of the mode of action of BI-0314 on STEP.

Supplementary data

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

References

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