Identification of novel PTPN1/PTPN2 inhibitors to target latent HIV reservoirs

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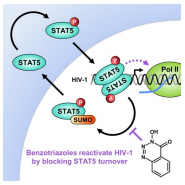
Introduction

3-Hydroxy-1,2,3-Benzotriazin-4(3H)-one (HODHBt) is a latency reversal agent (LRA) that enhances γc-cytokine signaling by increasing phosphorylation and transcriptional activity of STAT5. We have shown that HODHBt increases IL-2 and IL-15 activation of STAT5, promoting reactivation from latency in a primary cell model and cells isolated from people living with HIV[1,2]. Furthermore, we have shown that HODHBt enhances IL-15 mediated NK and CD8T cell effector function[3,4]. Recently, we used cellular-thermal shift assay followed with mass-spectrometry (CETSA-MS) to uncover the HODHBt target. We have found that HODHBt binds and inhibits the catalytic domain of the phosphatases PTPN1 and PTPN2[5]. In spite of the positive properties of this class of compounds towards developing HIV cure approaches, their activity in the high micromolar range hinders further pharmacological development. In here, we aimed to identify novel inhibitors and/or novel scaffold molecules targeting PTPN1/PTPN2 that could be further developed towards HIV cure strategies.



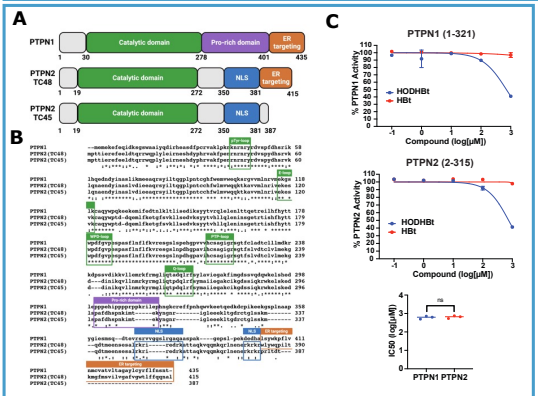


Figure 1. HODHBt targets the catalytic domain of PTPN1 and PTPN2. A. Schematic representation of PTPN1 and PTPN2. **B.** Sequence alignment between PTPN1 and the two PTPN2 isoforms. The catalytic domains retain 98% similarity between proteins. Nuclear localization sequence (NLS) allows for nuclear translocation of PTPN2. **C.** HODHBt directly inhibits the catalytic activity of PTPN1 and PTPN2 using a fluorogenic assay (n=3). Error bars indicate SD. IC50 values calculated for 3 independent experiments.

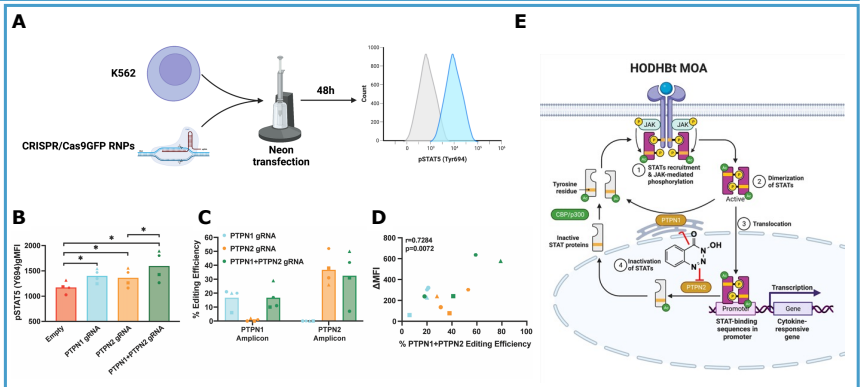


Figure 2. PTPN1 and PTPN2 synergistically control STAT5 phosphorylation. A. Experimental outline of the CRISPR/Cas9 RNP transfection in K562 cells. **B.** pSTAT5 levels in K562 cells after CRISPR/Cas9 knockout of PTPN1, PTPN2, or PTPN1/PTPN2 (n=4). Paired t-test used to calculate p-values (*p<0.05). **C.** Editing efficiencies from the 4 independent experiments for each amplicon measured using T7 Endonuclease digestion. **D.** Correlation between pSTAT5 ΔMFI (guide RNA minus Cas9 alone) for each experiment and combined knockout editing efficiency. Pearson correlation coefficient calculated. **E.** Proposed mechanism of action of HODHBt enhancing cytokine mediated STAT activation.

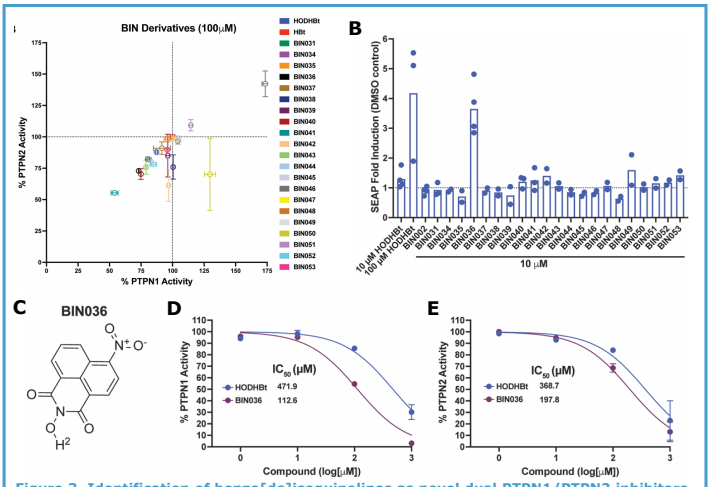


Figure 3. Identification of benzo[de]isoquinolines as novel dual PTPN1/PTPN2 inhibitors. A. Evaluation of the ability of benzo[de]isoquinoline derivatives to inhibit the catalytic domain of PTPN1 and PTPN2 at 100μM. The data represents the mean and SD of one experiment performed in duplicates. **B.** STAT5 transcriptional activity of a panel of benzo[de]isoquinoline derivatives at 10μM in HEK-Blue IL-2 cells calculated as fold induction over DMSO control (n=2-4). **C.** Structure of the benzo[de]isoquinoline analogue BIN036. Comparison of the ability of HODHBt and BIN036 to inhibit the catalytic domain of PTPN1 **(D)** and PTPN2 **(E)** (n=2). IC_{50} indicated. Error bars represent SD.

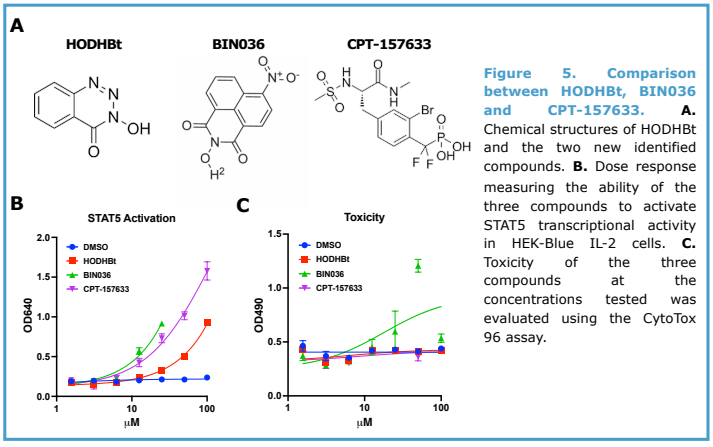


Figure 5. Comparison between HODHBt, BIN036 and CPT-157633. A. Chemical structures of HODHBt and the two new identified compounds. **B.** Dose response measuring the ability of the three compounds to activate STAT5 transcriptional activity in HEK-Blue IL-2 cells. **C.** Toxicity of the three compounds at the concentrations tested was evaluated using the CytoTox 96 assay.

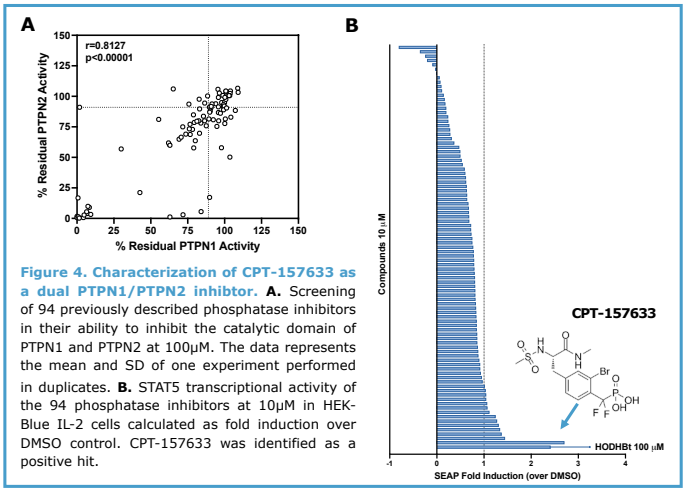


Figure 4. Characterization of CPT-157633 as a dual PTPN1/PTPN2 inhibtor. A. Screening of 94 previously described phosphatase inhibitors in their ability to inhibit the catalytic domain of PTPN1 and PTPN2 at 100μM. The data represents the mean and SD of one experiment performed in duplicates. **B.** STAT5 transcriptional activity of the 94 phosphatase inhibitors at 10μM in HEK-Blue IL-2 cells calculated as fold induction over DMSO control. CPT-157633 was identified as a positive hit.

Conclusions

- ✓ We have identified a new family of compounds derived from benzo[de]isoquinoline, with BIN036 as the lead compound, with lower IC50 against PTPN1 and PTPN2 than our original compound HODHBt.
- ✓ We have identified a previously known phosphatase inhibitor, CPT-157633, with enhanced activity over that of HODHBt
- ✓ Both novel compounds enhance STAT5 transcriptional activity in the low μM range compared with the high μM range of HODHBt
- ✓ Further studies are in progress to evaluate the LRA and enhance immune effector function activities of both compounds.

References

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