Identification of Allosteric Inhibitors of PTP1B through Synergistic Computational and Experimental Approaches

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Overall Goal

Find new inhibitors of protein tyrosine phosphatases (PTPs), using PTP1B as the test enzyme, via both computational and "wet-bench" experimental approaches.

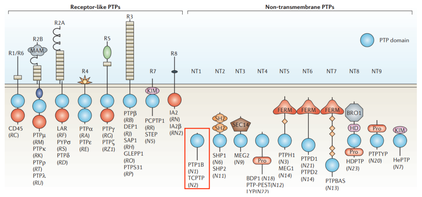


Figure 1. The classical PTPs are divided into receptor-like and non-transmembrane subfamilies [1].

Background

Protein Tyrosine Phosphatase 1B

- An non-transmembrane PTP about 50 kDa in size (435 amino acids).
- A negative regulator of insulin and leptin signaling pathways by dephosphorylating the phospho-tyrosine residues on the hormone receptors [2].
- PTP1B is a validated target for diabetes, obesity, and cancer [2].

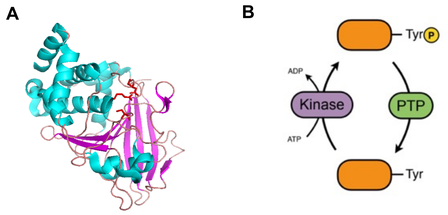


Figure 2. Structure and Function of PTP1B. A) Structure of PTP1B with active sites C215, R221. **B)** Dephosphorylation of tyrosine residue by a phosphatase and addition by a kinase coupled with ATP.

Allosteric Binding Induced Inhibition

- The PTP family is a difficult therapeutic drug target due to the highly conserved mechanism of its active sites. In addition, active site-directed ligands are highly charged and essentially cell impermeable.
- Allosteric binding sites may offer the potential for improved cell selectivity.
- Allosteric inhibitors of PTP's already exist, but identifying them relies on intensive compound screening and prior structural knowledge of enzyme/ligand complexes.
- **Highlights the need for a basic rational approach that can be generalized to a wide range of PTP targets.**

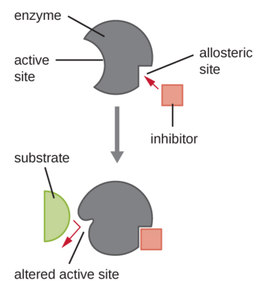


Figure 3. Overview of allosteric inhibition.

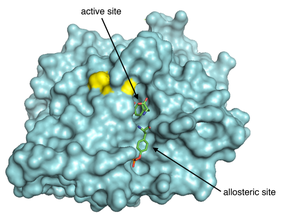
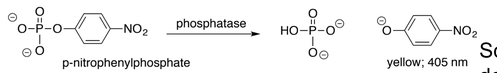


Figure 4. An allosteric site located near the active site of PTP1B.

Enzyme Inhibition Assay



Screening for inhibition was done in 96-well assay plates using p-nitrophenylphosphate as a PTP1B substrate.

Figure 5. PTP1B catalyzes the dephosphorylation of p-nitrophenylphosphate to p-nitrophenol. The absorbance of p-nitrophenol can be monitored at 405nm.

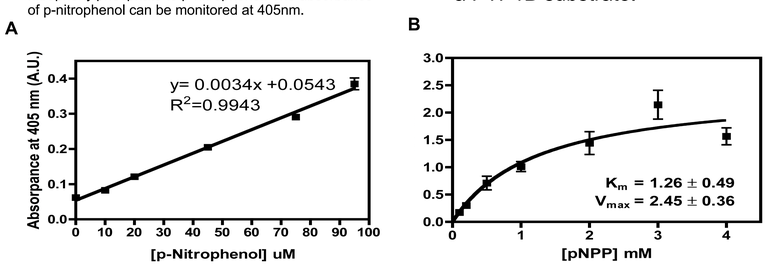


Figure 6. Assay Validation. A) Representative calibration curve of p-nitrophenol used to determine the amount of substrate dephosphorylated during the inhibition assays. **B)** Uninhibited enzyme kinetics of PTP1B.

1st Screen Results

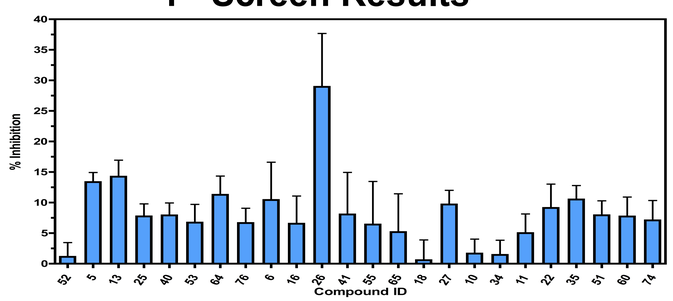


Figure 7. Percent Inhibition of PTP1B. 44 compounds were screened at 100 μM using the single point enzyme inhibition assay (2 mM pNPP and 10 nM PTP1B). Compounds with positive inhibition results are shown.

2nd Screen Results

Compounds with the highest percent inhibitions in the initial single point screen were tested again at 25, 50 and 100 μM to validate their inhibition efficacy.

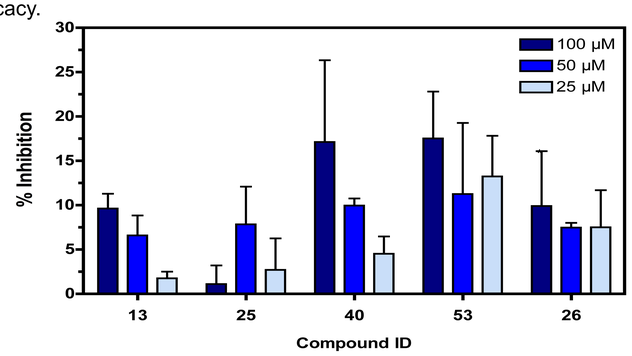


Figure 8. Percent Inhibition of PTP1B. Top compounds from the single point analysis were screened at the indicated concentration values in triplicates (2 mM pNPP and 10 nM PTP1B).

Characterization of Lead Compounds

- Identified compounds 40 and 53 as the lead compounds for further analysis based on the results of the 2nd screen test.
- Compound 53 shows promising dose-dependence inhibition of PTP1B, but compound 40 did not exhibit a clear concentration-dependent inhibition (large error bars).

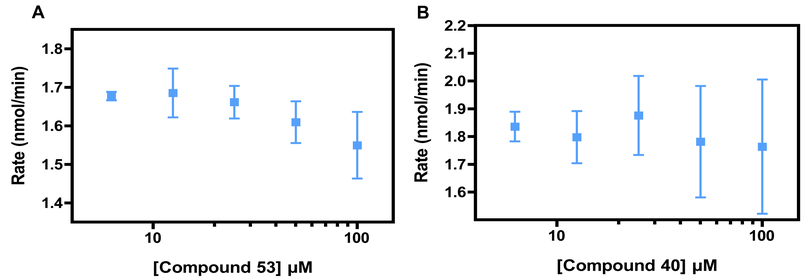


Figure 9. IC_{50} graphs of 10 nM PTP1B with Compound **(A)** 53 and **(B)** 40 at various concentrations.

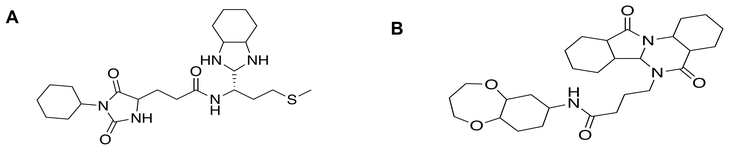


Figure 10. Chemical structures of **(A)** Compound 53 and **(B)** Compound 40.

Future Work

- Test inhibitor binding affinity using thermal shift assay
- General Pipeline:

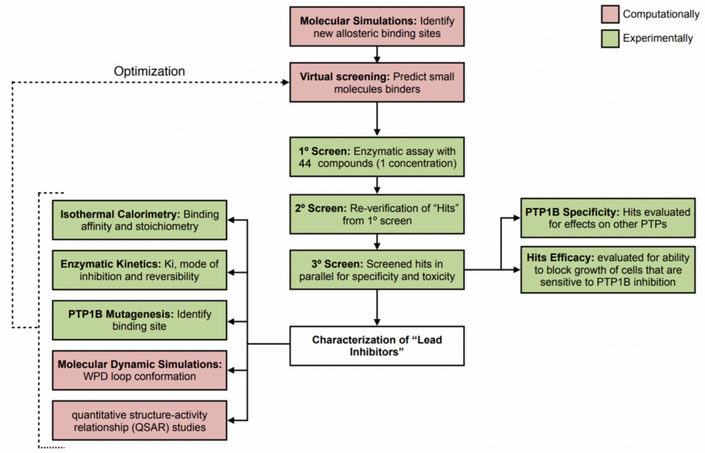


Figure 11. Pipeline diagram for identifying further novel inhibitors.

[1] Tonks, N. K. *Nat. Rev. Mol. Cell Biol.* **2006**, *7* (11), 833–846.
[2] Wiesmann, C. et al. *Nat. Struct. Mol. Biol.* **2004**, *11* (8), 730–737.