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(Research Article)

Small Molecule Inhibitors of PTP1B and TCPTP

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ABSTRACT

Fragment based drug design is a new approach in identifying the initial chemical starting point for drug discovery programs. Fragment based drug design allows screening of a substantial number of compounds usually hundred - thousand compounds. It identifies fragments which binds specifically but with low affinity in the range of 0.1-10mM.The small sized fragments make the subsequent optimization is relatively easier to build the molecule by exploring the chemical space in the binding pocket.The virtual screening study of small molecule compounds from a chemical library was carried out and selected few molecules as inhibitors of PTP1B/TCPTP. These molecules were tested by in-vitro biochemical assay and were inhibiting both PTP1B and TCPTP.

Key Words: PTP1B(Protein Tyrosine Phosphatase), TCPTP(T-cell PTP), pNPP(Para Nitro Phenol Pyrophosphate), FBDD(Fragment Based Drug Discovery).

INTRODUCTION

Protein tyrosine phosphatase (PTP1B) is an intracellular enzyme which acts on phosphorylated tyrosine substrates and dephosphorylate them, a key mechanism in counteracting protein kinases during signal transduction to regulate many cellular processes likecell growth, differentiation, metabolism and cell apoptosis[1-4]. PTP1B is a non receptor intracellular phosphatase enzyme is implicated as a key negative regulator of insulin and leptin signaling pathway[5-7]. PTP1B dephosphorylates specific tyrosine residues on insulin receptor and on insulin receptor substrate proteins.[5,8] It was reported that PTP1B deficient mice have improved glycemic control and resistance to diet induced obesity.[9-10] Recent studies have shown that PTP1B will also play a role in tumorigenesis.Thus PTP1B inhibition may be attractive target for treating type II diabetes and obesity and cancer.[11] TCPTP is an intracellular phosphatase protein expresses ubiquitously and is a homologous protein of PTP1B shares around 72% homology with PTP1B in catalytic site thus making selective inhibitor is a more complex task.

The most critical part in making selective inhibitor is to specifically inhibit only PTP1B to avoid unwanted side effects as this protein involved in several physiological and pathological processes including Src kinase activation[12], antagonizing signaling by EGFR[13], transformation by Neu oncogene[14] and p^{130cas} Crk-associated substarte. These results together suggest that potent selective inhibitor is an important prerequisite for PTP drug discovery. The other significant factor which is equally complicated to developing selective PTP1B inhibitor to TCPTP is their highly homologous catalytic sites. Since these catalytic sites are highly polar nature ,compounds targeting these sites will have cell permeability issues. The PTP1B core catalytic site is made up of loops(WPD loop, Q loop and pTyr loops) each will have specific function to carry out during dephosphorylation of the substrate tyrosine residue.The discovery of second aryl phosphate binding site[15] adjacent to the

active site in PTP1B demonstrated the potential for multiple site recognition of substrates by PTPs.

We have carried out virtual screening study on in-house chemical library compounds(~1500) to identify PTP1B small molecule inhibitor for the fragment based drug discovery.These low molecule inhibitor were later tested in-vitro by pNPPassay to check the inhibition to PTP1B,few compounds showed inhibition to PTP1B[16] and later tested with TCPTP.These compounds also inhibited TCPTP owing to its highly similar catalytic sites architecture, one compound(AU-008) showed nearly nearly 3 fold selective to PTP1B over TCPTP.

MATERIALS AND METHODS

PTP1B (1-321) and TCPTP (1-314) catalytic domains were cloned, expressed and purified as previously described.[17] The recombinant plasmids were transformed with E.Coli BL21 DE3 competent cells and grown till OD_{600} reaches 0.6 and then induced PTP1B cells with 0.2mM IPTG for 16hrs at 18°C where as TCPTP with 0.5mM IPTG for 16hrs at 25°C.The cells were harvested after induction and then lysed by sonication. The soluble recombinant proteins were purified by affinity chromatography (NI-NTA fig.1a and b) followed by Gelfiltartion chromatography (Superdex-75 fig. 2a and b) from Amersham .The proteins were 95% pure after gel filtration and were stored at-80°C.

Biochemical Assay

The virtual screening study of in-house compounds (~1500) from Aurigene Discovery Technologies Ltd was performed for PTP1B/TCPTP inhibitors and short listed 50 compounds for in-vitro pNPP assay to check the inhibition.The in-vitro assays were carried out using pNPP(Para Nitro Phenol Pyrophosphate) as a substrate at room temperature. Para-nitrophenyl phosphate (pNPP) is a chromogenic substrate for most of the phosphatases like alkaline phosphatase,acid phosphatase, serine/threonine phosphatase ,tyrosine phosphatase etc. During the reaction the phosphatase acts on pNPP substrate in alkaline condition yields para-nitrophenol,which is intense yellow colour can be measured at 405nm on a spectrophotometer.

The kinetic parameters for PTP1B and TCPTP catalyzed hydrolysis of pNPP were done using the buffer which contain 25mMTrispH:7.5, 75mM, NaCl, 0.1%BSA, 0.1mM DTT in a reaction volume of 50µl at room temperature. The initial OD405 values of pNPP at different substrate concentrations for every 5mins were recorded after adding respective enzymes in the 96 well plates. The initial velocities were obtained by plotting OD_{405} values against time. The Km values of pNPP for PTP1B and TCPTP were deduced by fitting initial rates to substrate concentrations using prism 3.0(Graph pad prism software). The short listed 50 compounds from virtual screening were tested for inhibition to both PTP1B and TCPTP using pNPP as a substrate at its Km values i.e. 0.8mM and 1.5mM for PTP1B and TCPTP respectively.

RESULTS AND DISCUSSION

All the 50 compounds were tested for inhibition to PTP1B as well as TCPTP proteins by pNPP assay. Only 3 compounds (AU-008,AU-247 and AU-2525) showed 80% inhibition to PTP1B and TCPTP at around100-300µM concentrations of the compounds. The three compounds IC_{50} values were determined by adding compounds at different concentrations ranging from 0.1µM to 10mM and the reactions were started by adding enzymes and allowed for 30 minutes at room temperature. The reactions were stopped by adding 1N NaOH and the absorbance was measured at 405nm using micro plate reader (Spectramax-190).The IC_{50} values of the 3 compounds for both the proteins were deduced by fitting OD_{405} values to a sigmoidal dose response equation using prism 3.0 (Graphpad software) and were presented in the table-1.

The compounds which showed inhibition to both the proteins have IC_{50} values in the micro molar range owing to their low molecular weights. These molecules can be taken as a starting pharmacophore for developing a selective potent inhibitor by exploring sites in the vicinity of binding pockets. The compound AU-008 has shown nearly 3 fold selective for PTP1B (67µM) over TCPTP (180 µM) can be taken as a hit molecule for the fragment based drug discovery approach to build potent selective inhibitor PTP1B over other cellular PTPs. The IC50 graphs of the 3 compunds are shown in fig 3a,3b and3c.

CONCLUSION

Since the objective of this study is to identify low molecular weight inhibitors to PTP1B/TCPTP for FBDD approach.Since the SBDD strategy is much more effective than HTS as in the former case it is possible to identify specific small molecular compounds with low affinity.These low molecular fragments are combined or optimized to generate lead compounds. These low affinity initial fragments identified can be further build up into larger complex compounds that target additional interactions in the active site of the protein. We have identified 3 compounds from short listed inhibitors by virtual screening have shown inhibition in micro molar range, out of which compound AU-008 has shown nearly 3 fold selectivity to PTP1B over TCPTP, can be taken as

lead identification for fragment based drug design (FBDD)approach for developing potent inhibitors to PTP1B/TCPTP or even selective inhibitor of PTP1B over homologous TCPTP.

Table-1: IC_{50} values of the compounds AU-008, AU-247 and AU-2525 for both PTP1B and TCPTP

Compound	PTP1B(µM)	TCPTP(µM)
AU-008	67	180
AU-247	210	174
AU-2525	55	36

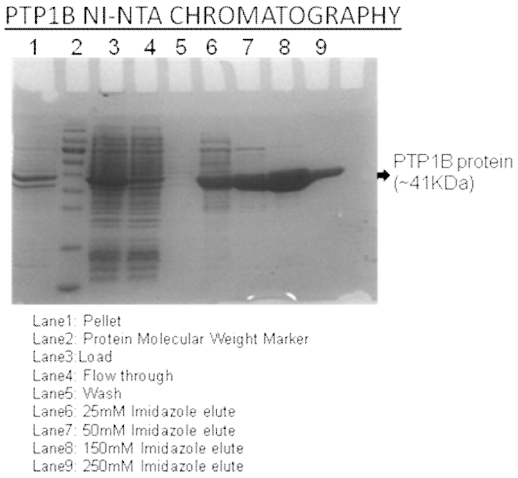

Fig.1a: The NI-NTA affinity chromatographyof PTP1B

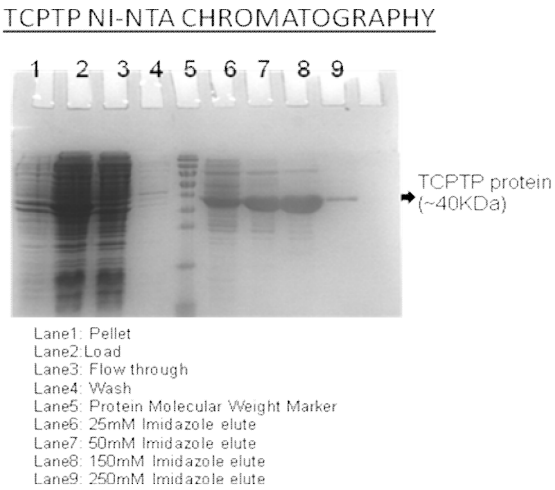

Fig.1b: The NI-NTA affinity chromatography of TCPCP

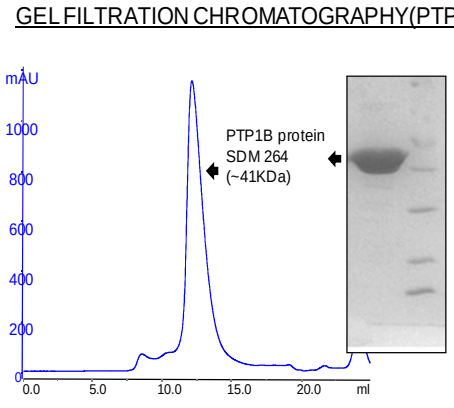

Fig.2a: Gel filtration chromatography of PTP1B protein

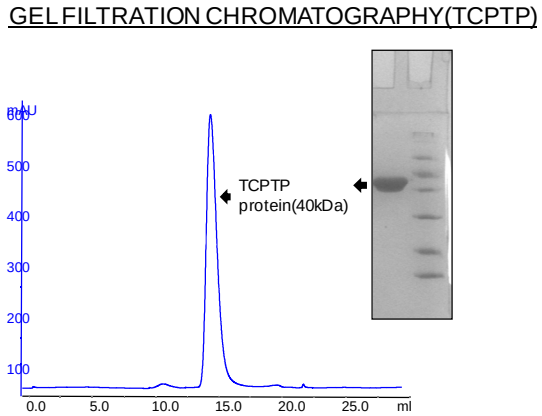

Fig. 2a: Gel filtration chromatography of TCPTP protein.

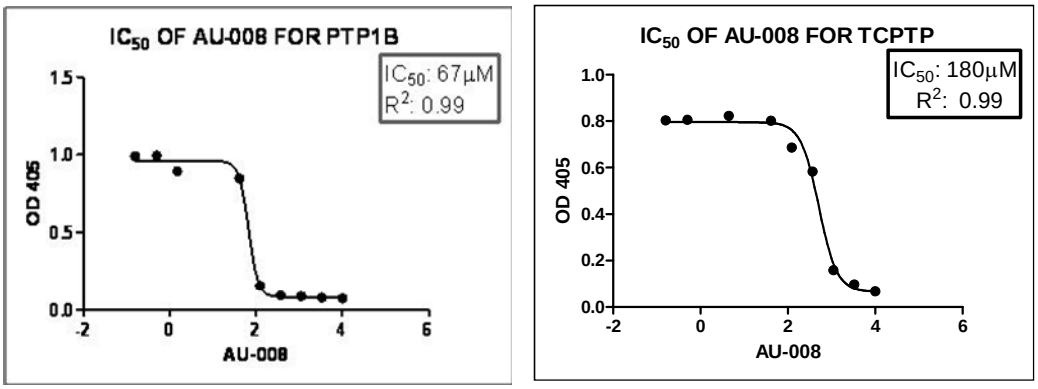

Fig.3a: IC_{50} values of AU-008 for PTP1B and TCPTP

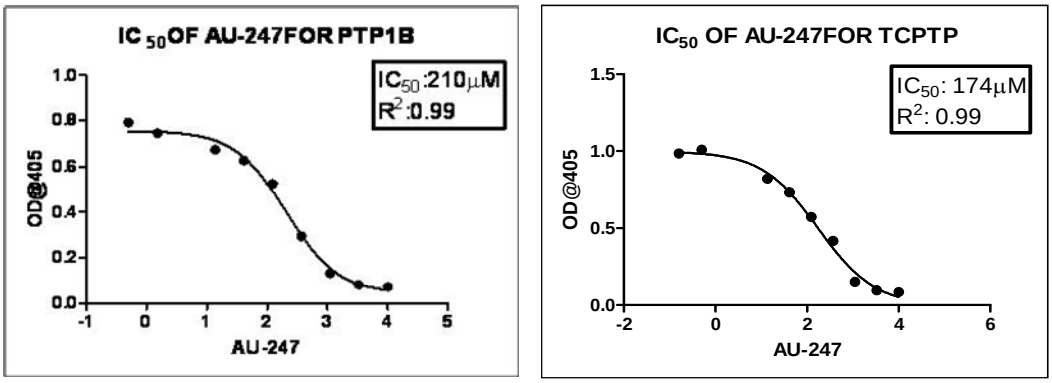

Fig.3b: IC_{50} values of AU-247 for PTP1B and TCPTP

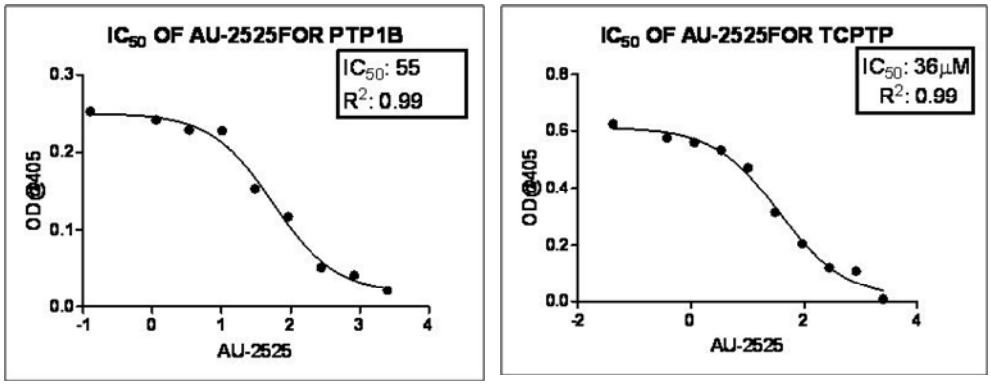

Fig.3c: IC_{50} values of AU-2525 for PTP1B and TCPTP

REFERENCES

1. Hunter T., Signaling-2000 and Beyond. Cell 2000; 100: 113–27.
2. Tonks NK, Neel BG, Combinatorial control of the specificity of protein tyrosine phosphatases, Curr. Opin. Cell Biol., 2001; 13: 182–95.
3. Kappert K, Peters KG, Bohmer FD, Ostman A., Tyrosine phosphatases in vessel wall signaling, Cardio Vasc Res., 2005; 65: 587–98.
4. Stoker AW, Protein tyrosine phosphatases and signaling, J. Endocrinol., 2005; 185: 19–33.
5. Johnson TO, Ermolieff J, Jirousek MR, Protein tyrosine phosphatase 1B inhibitors for diabetes, Nat Rev Drug Discov, 2002; 1: 696–709.
6. Liu G, Trevillyan JM, Protein tyrosine phosphatase 1B as a target for the treatment of impaired glucose tolerance and type II diabetes, Curr Opin Investig Drugs, 2002; 3: 1608–16.

7. Tobin JF, Tam S., Recent advances in the development of small molecule inhibitors of PTP1B for the treatment of insulin resistance and type 2 diabetes, Curr. Opin. Drug. Discov. Devel., 2002; 5: 500–12.
8. Kennedy BP, Ramachandran C., Protein tyrosine phosphatase-1B in diabetes, Biochem Pharmacol, 2000; 60: 877–83.
9. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, *et al*., Increased insulin sensitivity and obesity resistance in mice Lacking the protein tyrosine phosphatase 1B gene, Science, 1999; 283:1544–8.
10. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, *et al*., Increased energy expenditure decreased adiposity and Tissues specific insulin sensitivity in protein - tyrosine phosphatase 1B deficient mice, Mol. Cell. Biol., 2000; 20: 5479–89.
11. Lessard L, Stuible M, Tremblay ML., The two faces of PTP1B in cancer, Biochim. Biophys. Acta., 2010; 1804: 613–9.
12. Bjorge JD, Pang A, Fujita DJ, Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines, J. Biol. Chem., 2000**,** 275:41439-41446
13. Liu, F, and Chernoff, Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor, J. Biochem. J.,1997, 327, 139–145.
14. Wiener, J. R., Kerns, B. J. M., Harvey, E. L., Conaway, M. R., Iglehart, J. D.,Berchuck, A., and Bast, R. C., Jr. Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression, J. Natl. Cancer Inst.,1994, 86, 372–378.
15. Puius, Y. A., Zhao, Y., Sullivan, M., Lawrence, D. S., Almo, S. C., and Zhang, Z.Y. Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: A paradigm for inhibitor design, Proc. Natl. Acad. Sci. U. S. A. ,1997,94, 13420–13425.
16. Sekhar Reddy M.V.V.V., Chakshusmathi G., Lakshminarasu M., Novel small molecule inhibitor of PTP1B, International Journal of Pharma and Bio Sciences, 2012,3(2), 412-417.

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