***Research Article***

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# DEVELOPING SELECTIVE INHIBITORS OF PTP1B OVER TCPTP

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## ABSTRACT

Insulin resistance is the primary cause for the type-2 diabetes, affected by more than 50million people in India. PTP1B is well characterized negative regulator of insulin receptor which suppress the insulin action by dephosphorylating tyrosine residues on (insulin receptor) IR.Developing selective inhibitors of PTP1B over other cellular PTPs is challenging task since the homology among PTPs is very high particularly TCPTP which shares nearly 80% homology to PTP1B (catalytic domains). Because of this high degree of sequence and structural homology within the catalytic domains of PTP1B and TCPTP no small molecular inhibitor has been reported till date shown great selectivity to PTP1B over TCPTP. In this study we have made few small molecule inhibitors based on Indole derivatives to address the selectivity issues by targeting Site A Site B catalytic sites of both PTP1B and TCPTP.

**Key words:** Diabetes, Insulin, Insulin receptor, Tcell PTPase, Protein tyrosine phosphotases, dephosphorylate phosphotyrosines.

## INTRODUCTION:

Protein tyrosine phosphotases (PTPases) are enzymes which dephosphorylate phosphotyrosines and act in accordance with (protein tyrosine kinases) PTKase which phosphorylate tyrosine residues of target proteins.The balanced action of the two types of enzymes are very crucial in regulating vital Cellular processes like growth, proliferation, survival or apoptosis etc. PTP1B is a non-receptor protein tyrosine phosphotase enzyme of large PTPase family which has been best validated target

For type-2 diabetes and obesity[1]. Biochemical, genetic evidence suggested that PTP1B plays a key role in regulating body weight and glucose homeostasis.[2&3] These studies indicates that inhibiting ptp1b is an effective strategy for alleviating major metabolic syndromes .Tcell PTPase (TCPTP) is highly homologous to PTP1B which implicated in regulating T-cell activation.[4] so developing selective inhibitor to PTP1B without antagonizing TCPTP is highly desired for combating type-2 diabetes and obesity.Any imbalance in this dynamic reversible phosphorylation by PTKs and dephosphorylation by PTPs result in development of human diseases like cancer and diabetes.[5-6]

But addressing selective inhibitor to PTP1B over TCPTP is more difficult task owing to two reasons. One is that it's high catalytic domains homology to TCPTP and another one is that the high polar nature of the catalytic site, inhibitors which developed by targeting this active catalytic site have poor pharmacophore properties. The catalytic active site of PTPases comprises of signature motif HCXXGXXR which consists of Cys (215) residue is crucial for catalysis of dephosphorylation of substrat's phospho tyrosine residue. The signature motif is surrounded by loops namely WPD loop, Q loop and pTyr loops which aid in mediating the catalysis. Since the catalytic sites of PTP1B and TCPTP were identical in the active site sub pockets, search for regions which are in the close vicinity of catalytic site were Explored. Extensive research in this direction yielded two sites other than catalytic site were identified i.e. non catalytic phospho tyrosine binding site (Site B: R24, R254) and allosteric site (Site C: Lys41 and Arg47)[7a&7b.]The sequence comparison of these sites among PTPases(including TCPTP) has shown that these regions share less homology and could be targeted for the selective inhibitors. It has been reported that TCPTP knockdown mice were died within 3-5 weeks after birth from the defects of hematopoiesis and immune functions,[8] thus developing a selective PTP1B inhibitor is a prerequisite obligation.

In this study we have cloned, expressed and purified catalytic domains of PTP1B and TCPTP and have been used for screening compounds.Indole-3-acetamide amide derivatives of nearly 8 compounds were synthesized and their purity and molecular weights were verified and confirmed by NMR and LCMS.These compounds were screened against PTP1B and TCPTP to check whether any of these compounds showing selective inhibition to PTP1B over TCPTP.

**MATERIALS AND METHODS:**

**Cloning:**

The catalytic domains of PTP1B and TCPTP were cloned and expressed as reported previously with few modifications.[9&10]The PTP1B (1-321) and TCPTP (1-314) catalytic domains were amplified by PCR using c-DNA commercial clones obtained

from open Biosystems (Cat.No: MHS1011-76533 & MHS4768-99609427 respectively) as templates. The custom made primers were used for the amplification of the genes and both constructs were cloned in to Pet28a plasmid. The PTP1B gene was cloned using Sal I and Hind III enzymes and TCPTP was cloned using BamHI and XhoI restriction enzymes in to Pet28a . The recombinant clones were confirmed by restriction digestion and DNA sequencing analysis.

**Expression and purification:**

The recombinant plasmids of PTP1B (1-321) and TCPTP (1-314) were transformed in to E.coli. BL 21 DE3 cells (Novagen.Cat.No:70235-3).The starter cultures of the both the constructs were inoculated each separately in to 1.0L LB media and allowed to grow at 37°C till $OD_{600}$ reaches 0.8 and then induced with 0.2 mM IPTG. The PTP1B protein was expressed at 18°C and TCPTP at 25°C for 18 Hrs at 150rpm.

The induced cultures were processed independently and were pelleted at 4000 rpm for 45 mins. and the cells were resuspended in lysis buffer(50mMTris pH:8.0, 200mM NaCl,0.5Mm EDTA ,10% Glycerol and 1mM DTT including protease inhibitor cocktail).The cells were lysed by sonication using sonicator( from sonodyne).The cell lysates were centrifuged at 12000rpm and the supernatants of both the constructs containing soluble recombinant proteins were incubated separately with NI-NTA metal affinity resin at 10(protein):1(resin).Then the resin-protein mixtures were separately poured in to two columns and washed thoroughly with wash buffer B(50mMTris pH:8.0, 200mM NaCl,0.5Mm EDTA ,10% Glycerol).Then the proteins from the affinity resins were eluted by washing the resins in batch mode by increasing imidazole concentration in the wash buffer B.The eluted respective proteins were checked on the SDSPAGE.(Fig 1a &1b)and in both the cases 150mM imidazole elute has more protein and were loaded separately on Gel filtration chromatography and the proteins eluted from suoerdex-75(Amersham) were checked on the SDSPAGE(Fig.2a &2b). The monomer proteins after superdex-75 gel filtration chromatography were more than 95 % pure and were used for in vitro assays.

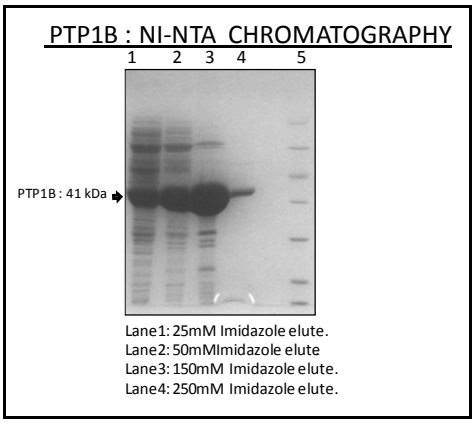


Fig.1a: Ni-NTA purification eluted samples of PTP1B on SDSPAGE

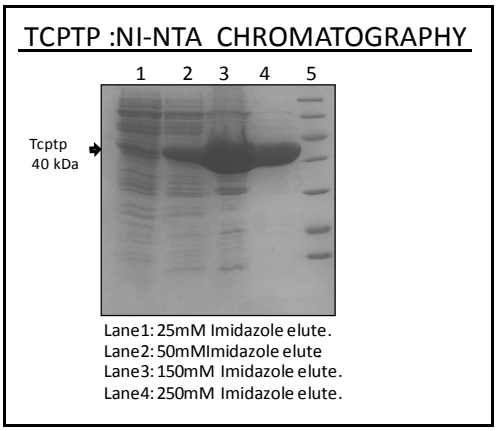


Fig.1b: Ni-NTA purification eluted samples of TCPTP on SDSPAGE

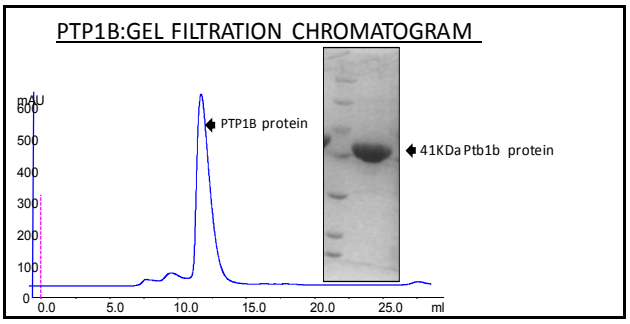


Fig.2a: Gel filtration chromatography sample of PTP1B on SDSPAGE

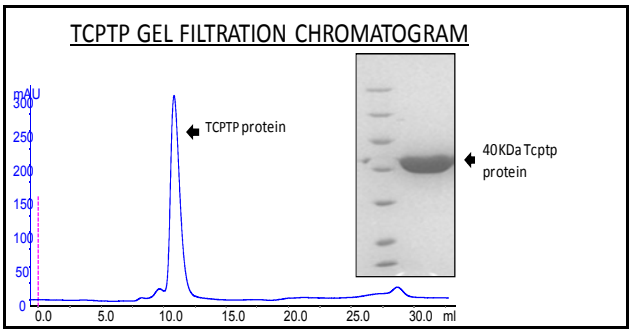


Fig.2b: Gel filtration chromatography sample of TCPTP on SDSPAGE

## RESULTS AND DISCUSSION:

**Biochemical Assay**:

The enzymatic activities of PTP1B catalytic domain were determined by hydrolysis of (Para Nitro Phenol Pyrophosphate) pNPP at room temperature. Para-nitrophenyl phosphate is a chromogenic substrate for most of the phosphotases including tyrosine phosphatases. The reaction yields pNP (para-nitophenol) which becomes an intense yellow soluble product under alkaline conditions. The absorbance can be measured at 405 nm on a spectrophotometer. The Km value of pNPP for PTP1B and TCPTP catalytic domain were determined using assay buffer (25mMTrispH:7.5, 75mM, NaCl, 0.1%BSA, 0.1mM DTT) and was found to be 800µM and 1500 µM respectively.

The following Indole-3-acetamide amide derivatives (Table 1 & Fig3) were made in Aurigene Discovery technologies and were tested for inhibition to PTP1B and TCPTP.

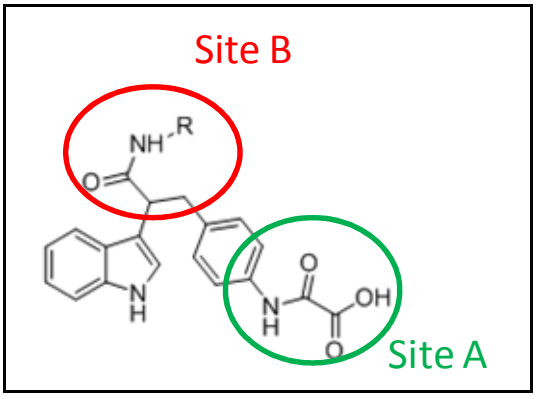


Fig.3: Core structure of Indole-3-acetamide amide derivatives

| COMPOUND | R | IC 50 VALUES(µM) | |
|---|---|---|---|
| | | PTP1B | TCPTP |
| 14a | 4-F Ph | 105 | 20 |
| 14b | 3-CF3, 5-OMe Ph | 166 | 62 |
| 14c | 3,6-Di-OMe Ph | 441 | 231 |
| 14d | 2,4-Di-OMe Ph | 281 | 78 |
| 14e | 3-Py | 365 | 161 |
| 14f | Cy hexyl | 461 | 206 |
| 14g | Ch2 Ph | 183 | 48 |
| 14h | CH2,CH2 Ph | 110 | 56 |

Table1: Indole-3-acetamide amide derivatives and their $IC_{50}$ for PTP1B and TCPTP. The $IC_{50}$ values determination of the compounds were done in a 96 well flat bottom plate for 1 Hr at room temperature in a final volume of 50µl. The reactions were stopped by adding 1M NaoH and the $OD_{405}$ were measured by using spectrophotometer.The $IC_{50}$ values of all the compounds were deduced by fitting initial rates of p- nitrophenol production to a sigmoidal dose response equation using prism 3.0(Graph pad software).The indole derivative compounds synthesized for this study have not shown any significant selective inhibition to PTP1B over TCPTP. The binding modes of all the derived compounds were analyzed by virtual screening analysis using GOLD version 5.1(data not shown)[11] and were occupying Site A and Site B sites in both the proteins and binding in similar fashion.

**CONCLUSION:**

The virtual screening analysis has shown that none of the compounds were showing any selectivity as the binding pattern of these indole based derivative compounds occupying Site A and Site B in identical fashion and the selectivity could be increased either by substituting the core Indole scaffold with other derivatives or increasing the molecular weight of the compounds by reaching Site C site/ allosteric site [12&13] of the respective proteins.

**REFERENCES:**


1. Zhang Z-Y. Curr. Opin. Chem. Biol 2001; 5:416–423. [PubMed: 11470605]
2. Elchelby M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A,Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP. Science 1999;283:1544–1548. [PubMed: 10066179]
3. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker- Krongrad A, Shulman GI, Neel BG, Kahn BB. Mol. Cell. Biol, 2000;20:5479–5489. [PubMed: 10891488]

4. You-Ten, K. E.; Muise, E. S.; Itie´ , A.; Michaliszyn, E.;Wagner, J.; Jothy, S.; Lapp, W. S.; Tremblay, M. L. J. Exp.Med. 1997, 186, 683.
5. Hunter, T. (1997) *Cell* **88,** 333–346

6. Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.;Collins, S.; Lee Loy, A.; Normandin, D.; Cheng, A.;Himms- Hagen, J.; Chan, C.-C.; Ramachandran, C.;Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. Science1999, 283, 1544–1548.
7. (a) Puius, Y. A.; Zhao, Y.; Sullivan, M.; Lawrence, D. S.;Almo, S. C.; Zhang, Z.-Y. Proc. Acad. Sci. U.S.A. 1997, 94,1342.
(b) Shen, K.; Keng, Y.-F.; Wu, L.; Guo, X.-L.; Lawrence,D. S.; Zhang, Z.-Y. J. Biol. Chem. 2001, 276, 47311.
8. You-Ten, K. E., Muise, E. S., Itie, A., Michaliszyn, E., Wagner, J., Jothy, S., Lapp, W. S., and Tremblay, M. L. (1997) J. Exp. Med. 186, 683–693
9. Barford, D., Keller, J. C., Flint, A. J., and Tonks, N. K. (1994) *J. Mol. Biol.*239, 726–730
10. Lars Fogh Iversen, Karin Bach Møller,Anja K. Pedersen,Günther H. Peters *May 31, 2002 The Journal of Biological* Chemistry*,* 277, 19982-19990.

11. GOLD Suite v5.0.1, The Cambridge Crystallographic Data Centre 12 Union Road, Cambridge, CB2 1EZ, UK.

12. Puius, Y. A., Zhao, Y., Sullivan, M., Lawrence, D. S., Almo, S., and Zhang, Z.-Y. (1997) Prot. Natl. Acad. Sci. U. S. A. 94, 13420–13425
13. Jia, Z., Ye, Q., Dinaut, A. N., Wang, Q., Waddleton, D., Payette, P., Ramachandran,C., Kennedy, B., Hum, G., and Taylor, S. D. (2001) J. Med.Chem. 44, 4584–4594