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Computer-aided, Rational Design of Potent and Selective Inhibitors of Protein Tyrosine Phosphatase 1B(PTP 1B), a Key Enzyme in the Insulin Signaling Pathway and Novel Therapeutic Target for Obesity and Type 2 Diabetes Mellitus

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Summary

Protein Tyrosine Phosphatase 1B (PTP1B) has been shown tc be a negative regulator of insulin signaling by dephosphorylating key tyrosine residues within the regulatory domain of the â-subunit of the insulin receptor. Recent gene knockout studies in mice have shown these mice to have increased insulin sensitivity and improved glucose tolerance. Furthermore, these mice also exhibited a resistance to diet induced obesity. Inhibitors of PTP1B would have the potential of enhancing insulin action by prolonging the phosphorylated state of the insulin receptor. In addition, recent clinical studies have shown that the haplotype ACTTCAG0 of the PTPN1 gene, which encodes PTP1B, is a major risk contributor to type 2 diabetes mellitus (T2DM). Thus, there is compelling evidence that small molecule inhibitors of PTP1B may be effective in treating insulin resistance at an early stage. Using an *in silico* structure–based approach, we have designed a potent and selective, small peptide inhibitor of PTP1B. The designed peptide is found to satisfy

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Lipinski's rule of 5 for its suitability as a drug. Therefore, it can be considered to be a suitable lead compound for the development of new drugs in treating insulin resistance at an early stage thereby leading to a prevention strategy for T2DM and obesity.

Key words: Insulin signaling, Insulin resistance, PTP 1B, Structure based drug design.

Introduction

The insulin signaling pathway (Fig. 1) is critical for the regulation of intracellular glucose levels and the avoidance of diabetes. Binding of insulin to the insulin receptor leads to autophosphorylation of the â- subunits and to tyrosine phosphorylation of insulin receptor substrates (IRS-1). IRS-1 activates phosphoinositide 3-kinase (PI3K). This, in turn, activates phosphate-dependent kinase (PDK-1) that subsequently activates PKC, resulting in the translocation of the glucose transporter (GLUT4) fron cytoplasmic vescicles to the membrane, for glucose uptake.

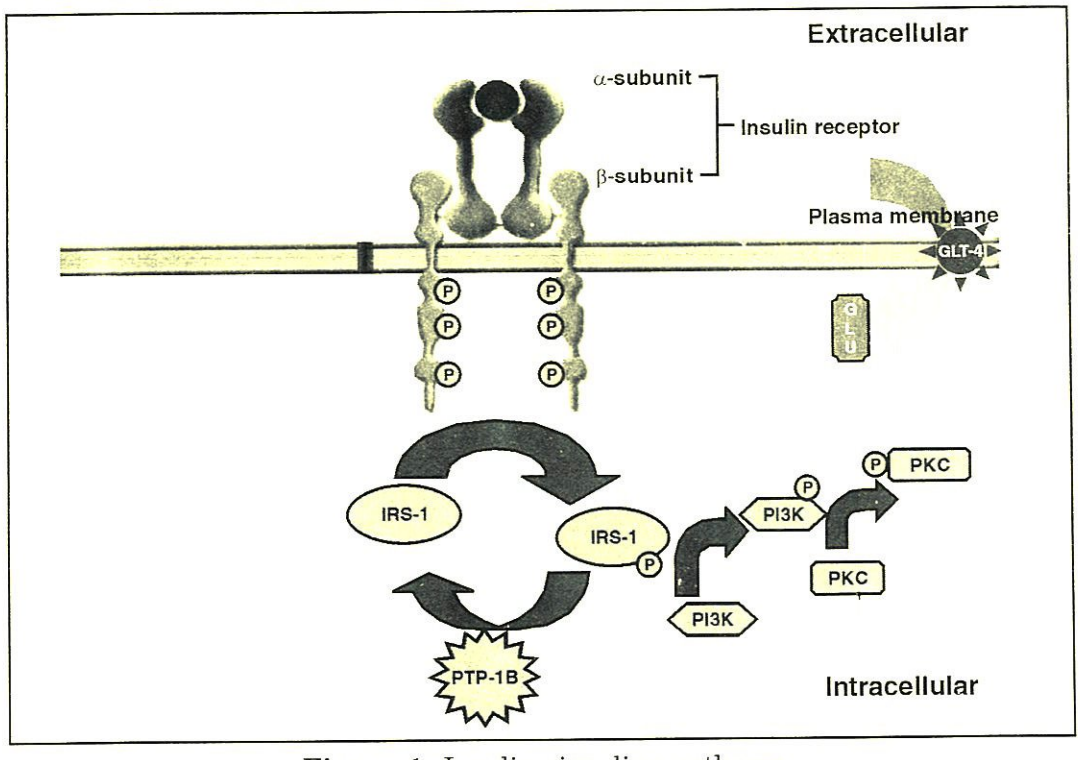

Figure 1: Insulin signaling pathway

Insulin resistance, underlying T2DM, occurs when normal circulating levels of the hormone or its action are insufficient to regulate these processes appropriately. Thus, insulin resistance is a defect in signal transduction.

The maintenance of metabolic control, which depends upon the insulin signal transduction, also requires termination of the insulin signal at a certain stage. The termination is brought about by specific phosphatases which dephosphorylate tyrosine residues in IRS-1. One such phosphatase is Protein Tyrosine Phosphatase 1B (PTP1B). Inhibitors of PTP1B would have the potential of prolonging the phosphorylated state of IRS-1, thereby enhancing the downstream metabolic events in the insulin signaling cascade.

Studies in human subjects as well as gene knockout studies in mice lend substantial support to this hypothesis. PTP1B is encoded by the PTPN1 gene which is located in the genomic region that has been linked to type 2 Diabetes Mellitus (T2DM) in multiple genetic studies. In a recent study (1) undertaken to investigate the association of PTPN1 gene polymorphism with insulin sensitivity (Si) and fasting plasma glucose respectively, it was found that eight SNP haplotype analyses showed that the haplotype ACTTCAG0 was significantly associated with lower Si (greater insulin resistance) and high fasting glucose, while the haplotype CTCCTGT0 was significantly associated with lower insulin resistance and lower fasting glucose. The same two haplotypes have, in another study (2) been shown to be associated with T2DM risk and protection respectively, and that the haplotype ACTTCAG0 was a major risk contributor to T2DM. These studies, together with earlier experimental studies in which mice lacking the PTP1B gene were found to exhibit increased insulin sensitivity and improved glucose tolerance, as well as resistance to diet-induced obesity (3, 4), strongly suggest that selective, small molecule inhibitors of PTP1B may be effective in treating insulin resistance at an early stage thereby leading to a prevention strategy for T2DM and obesity.

In this work, a computer-based approach to design such inhibitors is presented. As compared to conventional methods for drug discovery, computer-based methods have a distinct advantage of speeding up the drug discovery process through the use of sophisticated and accurate molecular modeling approaches (5), and the availability of high-resolution structures of known inhibitors (ligands) bound to protein targets. The use of such knowledge at the molecular level to design more effective and selective ligands constitutes the structure-based drug design process. This process can be either what is termed as 'combinatorial' or 'rational'. An example of the combinatorial approach is illustrated in Fig. 2. Starting from an initial, known 'lead' compound, various permutations of the functional groups are generated and each of them is checked for its binding

to the target. In contrast, the rational approach is based on examining the details of the binding site and of the interactions between the target and the known inhibitor, and then designing a more potent inhibitor which fits better into the binding site, as illustrated in Fig. 3, in the modeled complex of the target and the inhibitor.

The modeling of the target-ligand complex involves two main concepts: Docking and Scoring. Docking refers to the manipulation (translation and rotation) of the ligand in the binding site so as to maximize the interaction energy between the target and the ligand. The main components of the interactions of the interaction energy are the electrostatic, non-bonded and hydrogen-bonding terms. The interaction energy is also the basis of the scoring function, which gives a good estimate of the potency of the inhibitor (the potency is expressed as either IC_{50}, the inhibitory concentration for 50% inhibition, or as K_d, the dissociation constant).

We now proceed to outline the steps in designing a novel, potent and selective inhibitor of PTP1B (6).

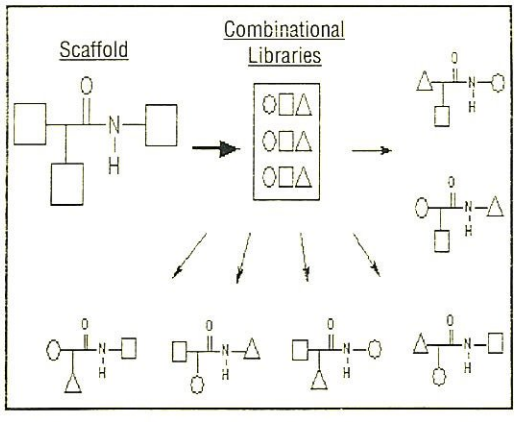

Figure 2: Combinatorial Approach

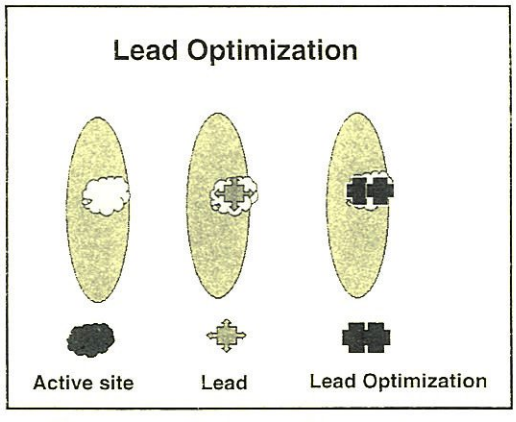

Figure 3: Rational Drug Design

Materials and Methods

The starting point for the modeling is the X-ray crystal structure of the complex of PTP1B with the diaryloxaminic acid based inhibitor, compound 23 (7) [PDB code: 1NNY] (8,9), which is amongst the most potent inhibitors known to date (K_d = 22nM). The overall structure of PTP1B is shown in Fig. 4. The crystal structures of PTP1B complexes with several inhibitors have been determined. These reveal that, in addition to the phosphotyrosine binding site (catalytic site), there are two other binding sites (Fig. 4). Inhibitors that are seen to bind to the catalytic site plus one of the other two binding sites found

to be highly potent with activities in the nanomolar range. Therefore, it stands to reason that an inhibitor that has interactions with all the three binding sites would be even more potent. Our aim in this work is to design a small peptide inhibitor which has (i) interactions with all the three binding sites, (ii) potency comparable to that of the known inhibitors and (iii) is selective for PTP1B as compared to the closely related protein tyrosine phosphatases such as TCPTP, PTP-LAR and calcineurin, a potent serine/threonine phosphatase.

The molecular modeling studies were carried out using the molecular modeling software package SYBYL 6.81 (Tripos Inc.) running on a Silicon Graphics O2 Workstation. Docking of ligands to PTP1B was done with the version 3.0 of the program AutoDock (10).

Results and Discussion

1. *Modeling of the complex of PTP1B with a known diaryloxaminic acid based inhibitor [Compound 23(7)].*

The modeling methodology was first tested on a known inhibitor by first extracting the ligand from the crystal

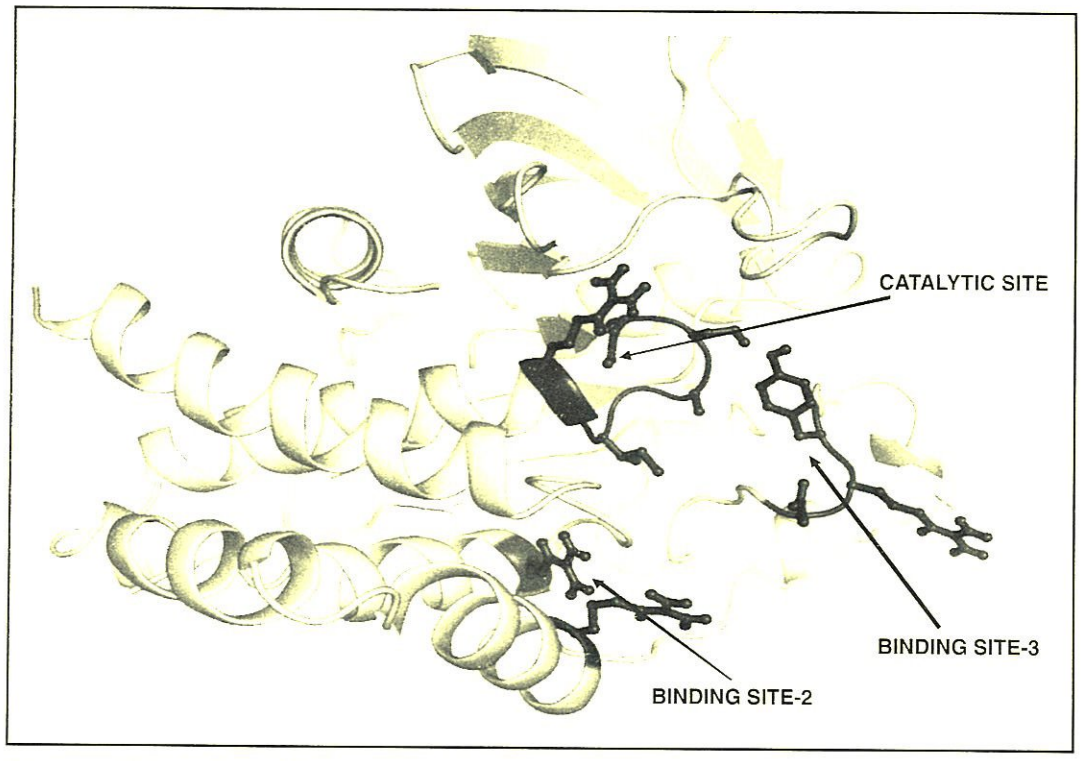

Figure 4: X-ray Crystal Structure of PTP1B. The active site residues are shown in black, ball-and-stick rendering. [All graphical figures generated using PyMOL v0.99 (11)]

structure, and then docking it in the region encompassing the three binding sites of PTP1B. It can be seen from Fig. 5 that the docked position overlaps very well with the crystal structure position of the ligand. In addition, the activity (K_d value) predicted by AutoDock 3.0 is 21nM, which agrees very well with the experimentally observed value of 22nM. Thus the modeling methodology has been validated for the known case, and it can now be used for designing and predicting the binding and activity of a novel peptide inhibitor.

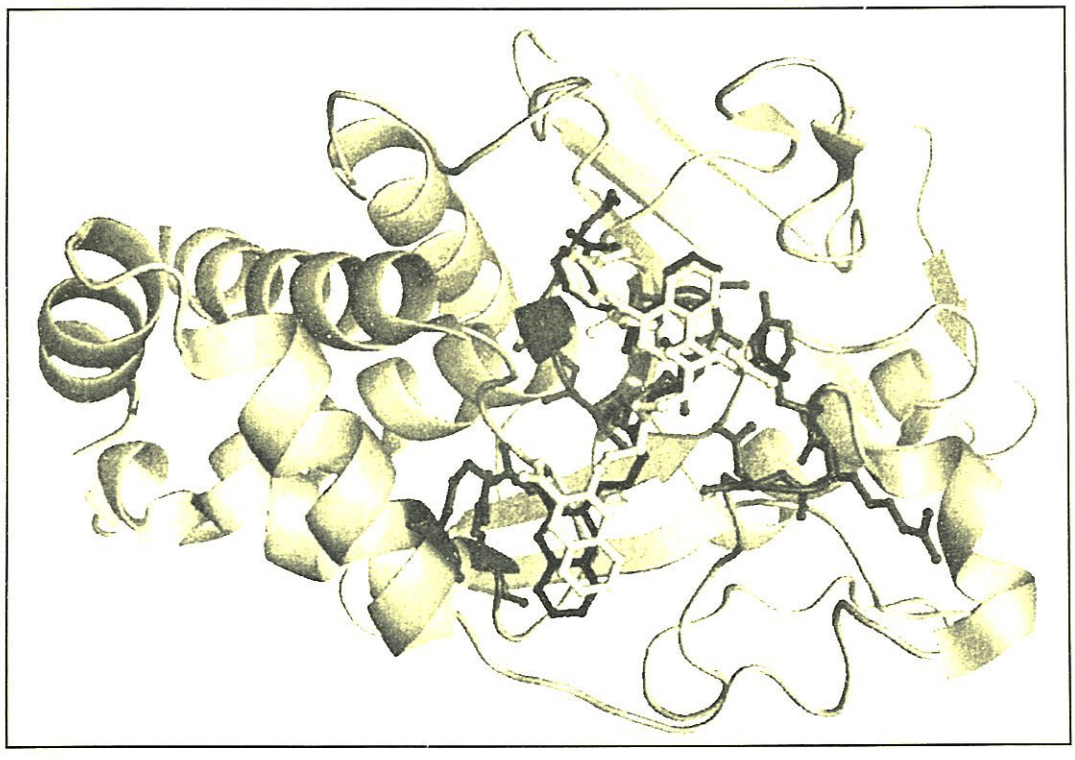

Figure 5: Superimposition of Crystal (Black) and Docked (White) positions of the Known Inhibitor

2. *Design of the peptide inhibitor and modeling of the PTP1B-inhibitor complex*

Kinetic, peptide binding and crystallographic studies on peptides containing phosphotyrosine (pTyr) led to the consensus substrate Asp/Glu-pTyr-pTyr-Lys/Arg for PTP1B (12). This peptide sequence contains both positively and negatively charged residues. Also, almost all of the known potent inhibitors contain aromatic rings. The above observations suggest that the designed

peptide should be a tetrapeptide with an aromatic residue, two charged residues and a Pro residue for constraining the conformation. Several tetrapeptide sequences with this composition were designed, their most probable conformations were determined, and each of them was docked with PTP1B. Fig.6 shows the modeled complex of PTP1B and one of the peptides, Tyr-Lys-Pro-Asp. It can be seen that the designed tetrapeptide has interactions with all the three binding sites. The predicted K_d value of 1.03 nM confirms that the peptide is a potent inhibitor of PTP1B, with a potency comparable with that of the most potent known inhibitors.

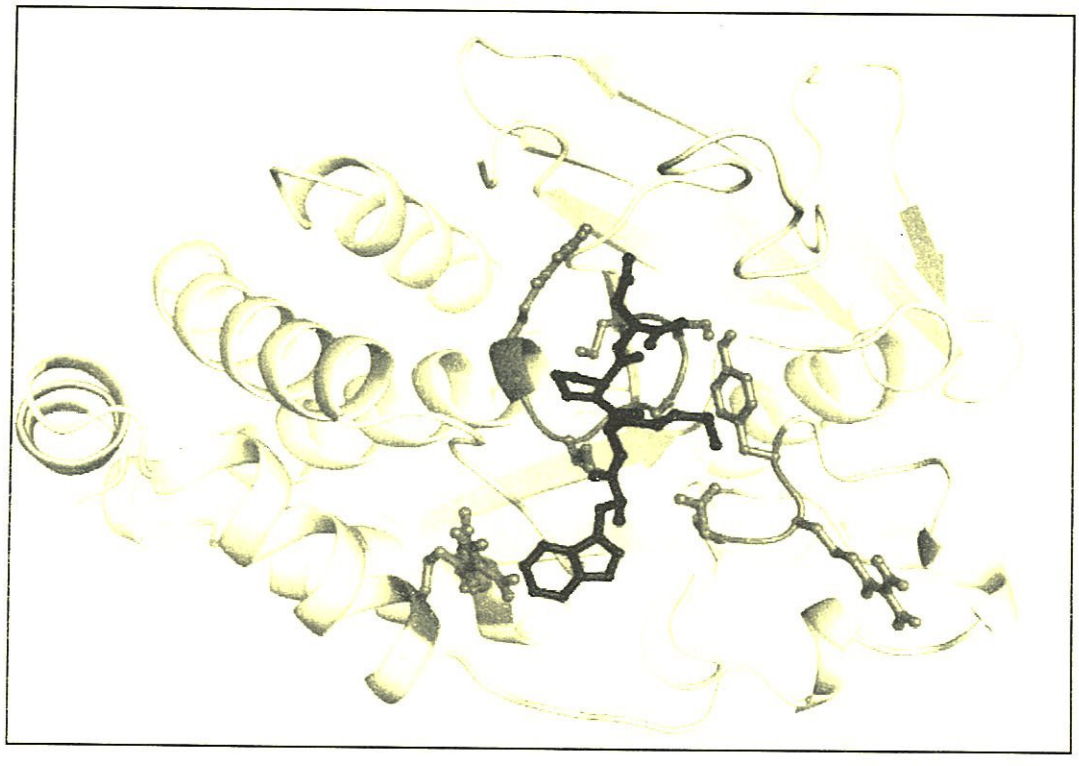

Figure 6: Final docked position of the Designed Inhibitor (shown in black stick rendering).

3. *Selectivity against closely related PTPases*

The next crucial test of the designed inhibitor is to check whether it is selective for PTP1B as compared to other, closely related phosphatases such as T-cell protein tyrosine phosphatase (TCPTP), PTP-LAR and calcineurin.

The X-ray crystal structures of these phosphatases are known, the PDB codes

being 1L8K (13), 1LAR (14) and 1U32 (15) respectively. Docking studies with each of them were carried out and the results are given in Table 1. It can be seen from the Table that the designed peptide inhibitor is selective for PTP1B. More significantly, it is 800-fold selective over TCPTP (K_d = 0.83μM). This is much higher than the best selectivity of 10-fold achieved so far with existing inhibitors (16).

Table I

Selectivity of the designed inhibitor

	Target	Activity (M)	Selectivity Ratio
1	PTP1B	1.03 X10^{-9}	1.0000
2	TCPTP	8.34 X10^{-7}	0.0012
3	LAR	7.16 X10^{-7}	0.0014
4	Calcineurin	1.64 X10^{-7}	0.0062

4. *Suitability of the peptide as a drug*

The designed peptide is seen to be both active and selective for PTP1B. It can, therefore, be considered as a suitable 'lead compound' for the design of a new class of PTP1B inhibitors. For the inhibitor to be a suitable drug, it needs to satisfy pharmacokinetic criteria of Absorption, Distribution, Metabolism and Excretion (ADME properties). These properties need to be determined experimentally. In the absence of experimental data, a useful 'rule of thumb' is Lipinski's rule of 5 (Table II). The designed inhibitor with a molecular weight of 526 Da, 5 hydrogen bond donors and 5 hydrogen bond acceptors, satisfies four of the five conditions. LogP could not be measured as the facilities do not exist in our laboratory.

Table II

Lipinski's "rule of 5"

(named because of its emphasis on the number 5 and multiples of 5), which predicts that poor absorption and permeability of potential drug candidates will occur if

- there are more than 5 hydrogen-bond donors (expressed as the sum of –OHs and –NHs),
- the molecular weight is more than 500,
- the logP is more than 5, or
- there are more than 10 hydrogen-bond acceptors (expressed as the sum of nitrogens and oxygens).

Conclusion

Using an *in silico* structure–based approach, we have designed a potent and selective, small peptide inhibitor of PTP1B, a key enzyme in the insulin signaling pathway. The designed peptide is found to satisfy Lipinski's rule of 5 for its suitability as a drug. Therefore, it can be considered to be a suitable lead compound for the development of new drugs in treating insulin resistance at an early stage thereby leading to a prevention strategy for T2DM and obesity.

A similar approach has been used by us earlier in designing peptide inhibitors of HIV-1 integrase (17) and HIV-1 reverse transcriptase (18).

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