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Novel synthetic molecule inhibits Protein Tyrosine Phosphatase 1B (PTP1B) for anti-hyperglycemia: Investigation by Molecular Docking Approach

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ABSTRACT

Synthetic molecule (RBMS-01) was investigated as an inhibitor of human PTP1B in an attempt to explain its anti-hyperglycemic activity. The investigation included molecular docking experiments to fit the synthetic molecule within the binding pocket of PTP1B. This novel compound was found to readily fit within the binding pocket of h-PTP1B in a low energy orientation characterized with optimal electrostatic attractive interactions bridging the quinoline positively charged nitrogen atom of the synthetic molecule. Then, the comparative studies with other natural compounds were analyzed. Our findings strongly suggest that PTP1B inhibition is at least one of the reasons for the reported anti-hyperglycemic activities of the novel synthetic molecule.

Key words: PTP1B, anti-hyperglycemic activity, Catalytic Site, quinoline derivative

INTRODUCTION

PTP1B is localized to the cytoplasmic face of the endoplasmic reticulum and is expressed ubiquitously, including in the classical insulin-targeted tissues such as liver, muscle and fat [1]. Mounting evidence from biochemical, genetic and pharmacological studies support a role for PTP1B as a negative regulator in both insulin and leptin signaling[2][3]. PTP1B can associate with and dephosphorylate activated insulin receptor (IR) or insulin receptor substrates (IRS) [4][5] Overexpression of PTP1B in cell cultures decreases insulin-stimulated phosphorylation of IR and/ or IRS-1, whereas reduction in the level of PTP1B, by antisense oligonucleotides or neutralizing antibodies, augments insulin initiated signalling [6][7]. Analyses of quantitative trait loci and mutations in the gene encoding PTP1B in humans support the notion that aberrant expression of PTP1B can contribute to diabetes and obesity [8][9][10]. Mice that lack PTP1B display enhanced sensitivity to insulin, with increased or prolonged tyrosine phosphorylation of IR in muscle and liver [11][12]. This is unexpected because insulin is also an anabolic factor, and increased insulin sensitivity can result in increased weight gain. PTP1B was subsequently shown to bind and dephosphorylate JAK2, which is downstream of leptin receptor[13][14]. Inhibiting neuronal PTP1B would require drugs that penetrate the blood–brain barrier. Consistent with the above results, antisense-based oligonucleotides that target PTP1B have shown efficacy in type 2 diabetes and have entered phase 2 clinical trials [15][16]. In addition, small-molecule inhibitors of PTP1B can work synergistically with insulin to increase insulin signalling and augment insulin-stimulated glucose uptake [17]. Moreover, pretreatment of leptin-resistant rats with a potent and selective PTP1B inhibitor results in a marked improvement in leptin-dependent suppression of food intake [18][19]. Collectively, these biochemical, genetic and pharmacological studies provide strong proof-of-concept, validating the notion that inhibition of PTP1B could address both diabetes and obesity and making PTP1B an exciting target for drug development.

Several PTP1B inhibitors have been developed to date using a structure-based design approach [20][21]. Amongst these, one of the most active compounds is 6, 7, 8, 9-Tetrahydro-2h-11-Oxa-2, 4, 10-Triaza-Benzo [B] Fluoren-1-One (Fig. 2). The crystal structure of PTP1B with the inhibitors reveals that in addition to the phosphotyrosine binding site (catalytic site) (residues Arg221). Inhibitors that bind to both the sites are found to be highly potent with activities in the nanomolar range. Recently, a second binding site (Site 2) (residues Tyr46-Asp48) was also found to contribute to the potency and selectivity of inhibitors [22][23]. All the inhibitors that have been developed so far are either non-peptidic or peptidomimetic in nature.

Our aim is to work to design a small novel molecule having more interactions with the mentioned sites, which has interactions with all the mentioned binding sites, has potency comparable with that of the known inhibitors and is selective for PTP1B as compared to the closely related PTP's.

MATERIALS AND METHODS

The starting point for the modeling studies was the crystal structure of the complex of PTP1B with a quinoline based inhibitor [24] (PDB 1D 1G7F). The selected structure is of the best 3D resolution (1.80Å) compared to other available h-PTP 1B structures. The complex was prepared by the module protein preparation wizard, where hydrogens were added automatically and refinement of the structure was also done. Since water molecules were removed for the protein-ligand interactions, then bond orders were re-assigned. The structure was minimized to a Root mean square deviation 0.30A.

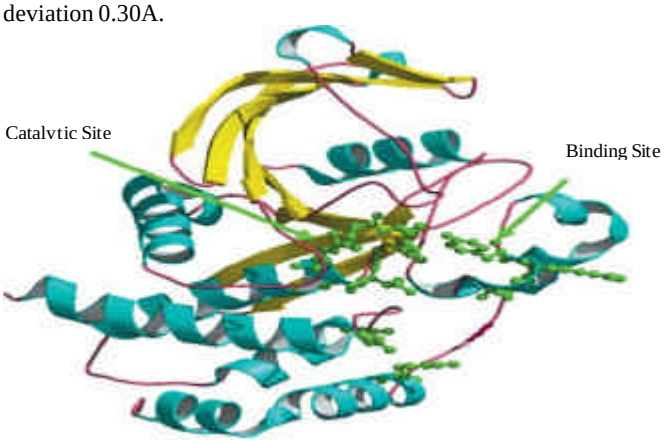

Fig. 1 Overall structure of the catalytic domain of PTP1B. The secondary structure elements are shown in Blue (helices), yellow (beta strands) and pink (loops and turns). The 2 binding sites are shown in green ball-and-stick rendering. The above given figure was produced using Pymol.

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Ligand Preparation

Ligands were obtained from Interbioscreen Ltd., Russia. It is a synthetic molecule with various functional groups. The ligand (Fig. 2) was prepared using ligprep module of the Maestro application. The ligand were prepared and assigned the appropriate bond orders manually. Each ligand was prepared manually with a full minimization with force field of the parameters using OPLS (Optimized potential for liquid simulations[25]] to eliminate bond length and angles biased from the crystal structure. Here the multiple structure for each ligand were produced with different combinations, so the protonation states ie., ionization states were exist to involve in any physiological condition. This operation can be performed using ionizer in Ligprep. Here the protein functions in the pH range of 7. One or more forms of different conformations sites were produced to interact more strongly with the binding site related. Here the concept discussed with possible tautomeric activity of the particular inhibitor.

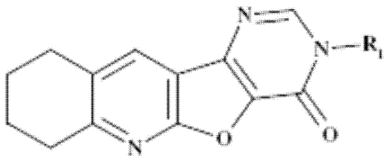
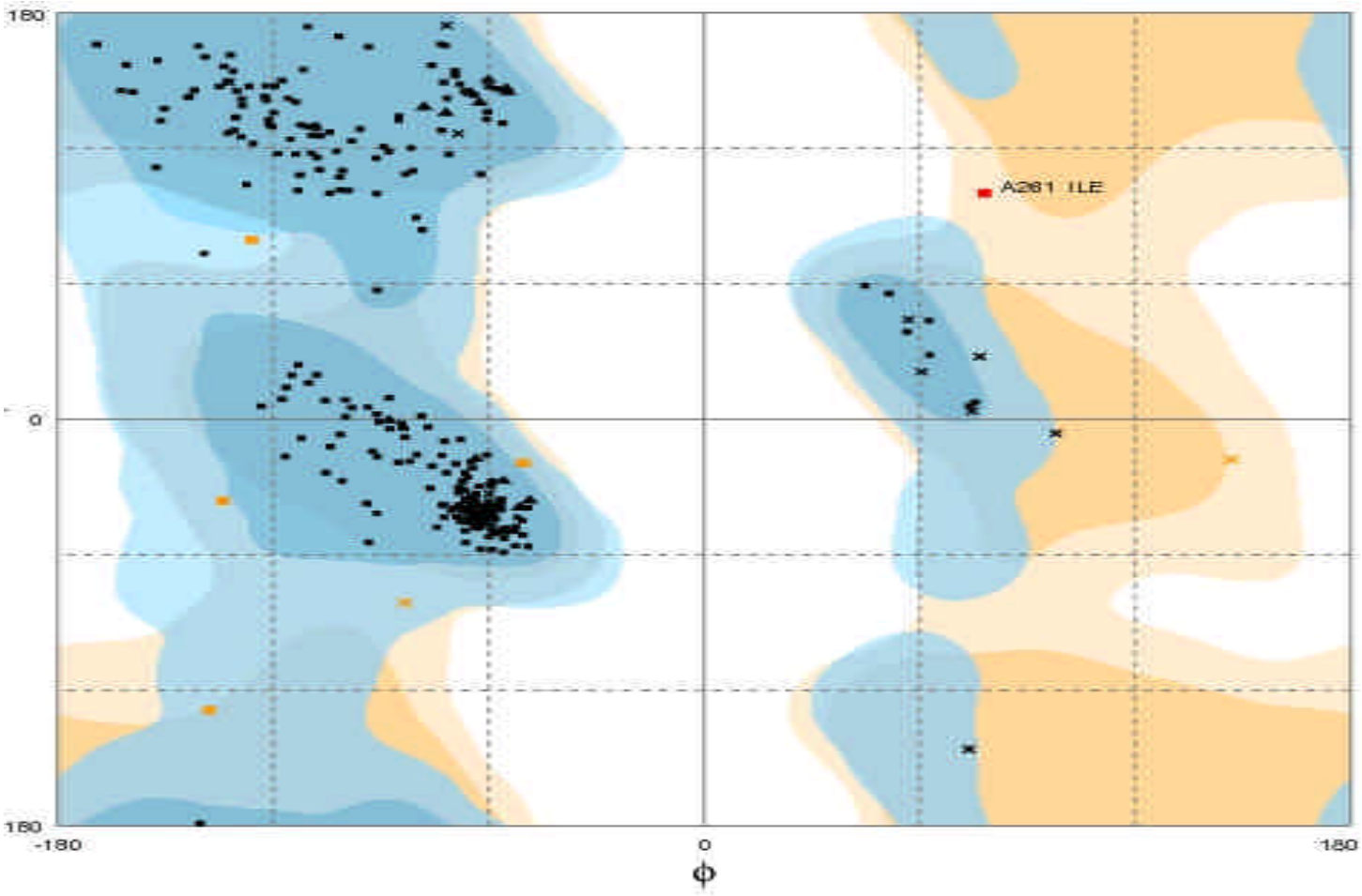


Fig 2. Structure of RBMS-01

Ramachandran Plot



Number of residues in favoured region (~98.0% expected) : 288 (97.6%)
Number of residues in allowed region (~2.0% expected) : 6 (2.0%)
Number of residues in outlier region : 1 (0.3%)

Fig. 3 Ramachandran Plot Analysis

Ligand docking parameters

Macromolecule and ligand were used as the initial coordinates for docking process. The docking can be used in two approaches like a. ligand is flexible and receptor is rigid. Then, ligand is rigid and receptor is flexible. Here, the rigid option is used for ligand docking. Here, the GLIDE docking approach is used[26].

The ligand docking was the receptor grid generation; for that purpose we have used the protein tyrosine Phosphatase 1B structure complexed with peptide substrate. During the grid generation, no vander Waals radius sampling was done and the partial charge cutoff has been taken as 0.25 [28] and no constraints were applied. The binding site of macromolecules taken according to the literature for all the other ligands for docking. The ligand docking calculations were done in the High Throughput virtual screening

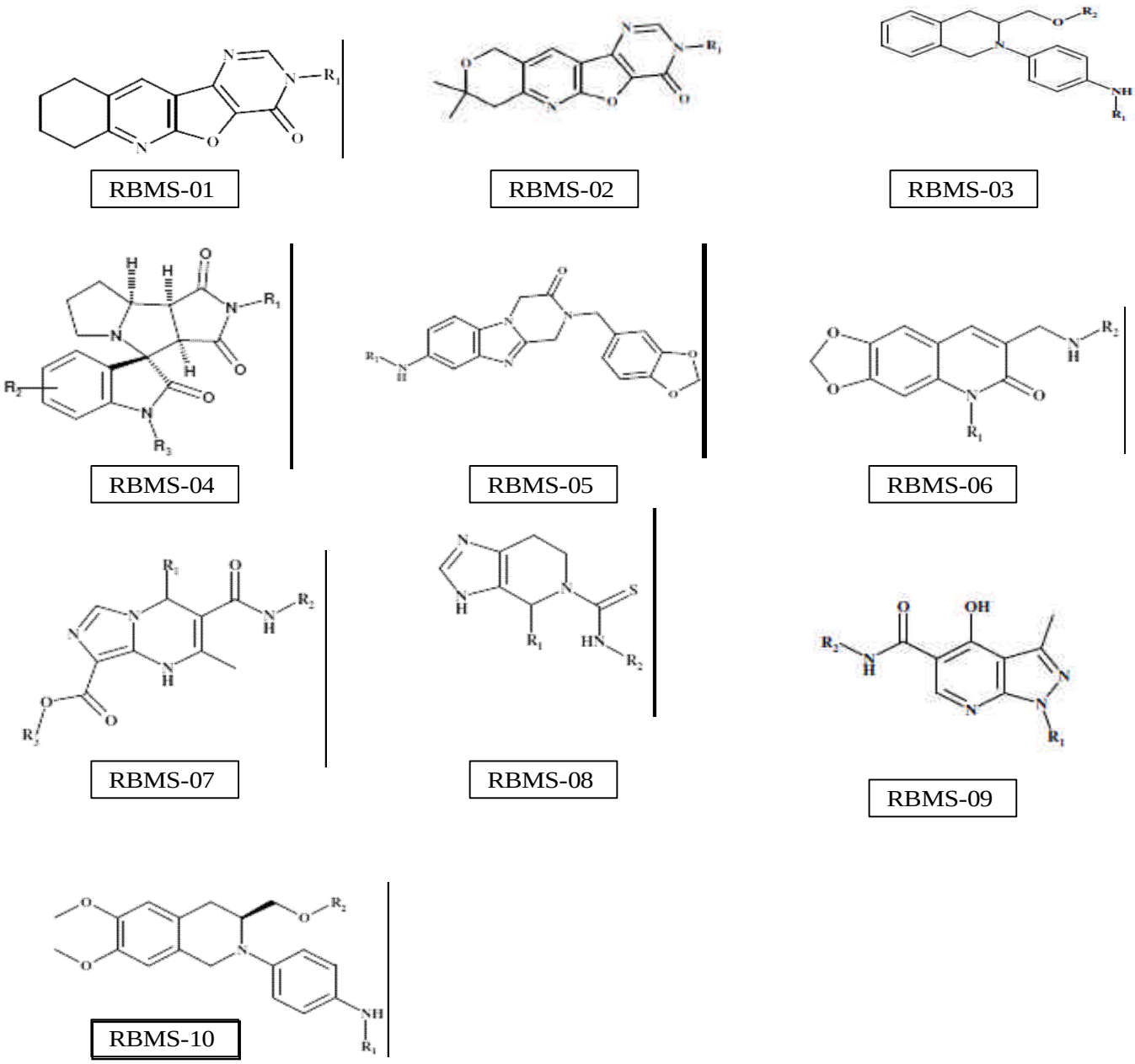


Fig 4. Chemical structure of different phosphatase inhibitors in their minimized positions.The structure were generated using Chemsketch (versions ACD Chemsketch 12.0)

(HTVS) mode of GLIDE. During the docking process the ligands are flexible and receptor treated as rigid. All the inhibitors were passed through a scaling factor of 0.8 and partial charge cutoff of 0.15.

Table 1. Result of Vander Waals, Electrostatic, Docking score and Their energy values after HTVS, GLIDE docking.

Inhibitor	Evdw	Ecoul	Docking Score	Energy
RBMS-01	-14.54	-30.17	-8.34	-41.54
RBMS-02	-14.27	-25.63	-6.18	-22.05
RBMS-03	-14.87	-24.78	-7.03	-21.99
RBMS-04	-12.04	-21.57	-7.02	-24.75
RBMS-05	-8.12	-37.64	-7.02	-33.82
RBMS-06	-9.86	-27.41	-6.01	-29.18
RBMS-07	-7.65	-32.92	-5.99	-32.10
RBMS-08	-7.13	-27.62	-5.99	-26.42
RBMS-09	-14.15	-22.84	-6.94	-33.75
RBMS-10	-13.82	-34.18	-6.85	-23.47

Virtual Screening

The GLIDE docking methods is applied to various inhibitors to build an affinity model to the Protein tyrosine Phosphatase 1B. That results free energy of binding for the phosphatase. The training sets of different inhibitors are generated by scoring functions. It was characterized by orientations and Hydrogen bond positions. According the energy values, the synthetic molecule of RBMS-01 which shows the better activity comparatively. It results in the form of low RMS deviations (0.30) indicated. The cavity energy term is very small; it indicates that there is a low energy penalty when the ligand is buried in the cavity. This observation shows that inhibition of the ligand is depends on the various conformations with

the rigid type of docking. In the best docked result, the synthetic molecule, named RBMS-01 has a best interaction with Arg, Cys, Lys, Tyr with protein tyrosine Phosphatase 1B. The values were given in table 1, which mentioned above. The mentioned structures were given above.

Induced Fit Docking

The ligands were docked into binding site of the receptor where the receptor is rigid and the ligand is free to move, that has been filtered out from many compounds using Virtual screening [27]. The protein tyrosine Phosphatase 1B shows the critical hinging and displays more conformations, may allows side chain modelling. The conformations show that many alterations in the receptor and it is more closely conforms and fit to the ligands. The purpose of docking is to find the affinity between the macromolecular and ligand complex, where it appears it results binding affinity. Then, it shows the binding between the ligands into a rigid receptor where assumes the correct one with low energy values. The purpose of using this method to eliminate the steric clashes, then the appropriate interaction will be resulted. The IFD docking was performed from least energy after series of filtration from HTVS and standard precision using GLIDE docking. The synthetic compound, RBMS-01 was continued; it recorded as -41.54 Kcal as least energy. This reflects by sampling of the receptor degree of freedom and a minimization of the receptor-inhibitor complex for many different receptor poses and it is attempted to identify low free energy conformation of the each complex.

Modeling of the Complex of PTP1B with a synthetic molecule

The Schrodinger V9.0 methodology was first tested on a known inhibitor, which was docked in the region encompassing the two binding sites of PTP1B, after identifying the ligand from the database. Thus the Schrodinger V9.0 tool used for producing the better results that are consistent with the observations.

Pharmacokinetic predictions of the best fit molecules

The ligands with the comparable scores with other molecules were sbjected to predict pharmacokinetic properties using the QikProp module of the software. QikProp settings determine which molecules are flagged as being dissimilar to other 95% of the known drugs. Predicted significant ADME properties such as permeability through the predicted log IC50 value for blockage of K+ channels (QPlogHERG), QikProp predicted gut-blood barrier and no violations of Lipinski's rule of five are reported here. The predicted property of docked compound described here.

Table 2 ADMET properties of RBMS-01

Contents	Values
Compound Name	RBMS-01
Molecular Weight	241.249
Octanol/Water	1.824
Log IC_{50}	-3.874
Lipinski's violations	Nil
QlogP MDCK	332

RESULTS AND DISCUSSIONS.

Functional Inference

It can be seen that the positions of the N- and C-termini and of K2 and P3 remain stable. The N-terminus and K2, which were initially hydrogen-bonded to D48 of the enzyme (Fig. 5) continue to form hydrogen bonds with D48 throughout the simulation. This is also the case with the C-terminus which remains hydrogen bonded to Y46. The backbone of the ligand, which was allowed to vary in the simulation, is also seen to remain fairly stable with hydrogen-bonding to residues S216 and A217 being retained throughout.

A Gln262Ala mutant allowed the study of the mechanism of hydrolysis of the Cys215–phosphate intermediate. Gln262 is involved in the activation

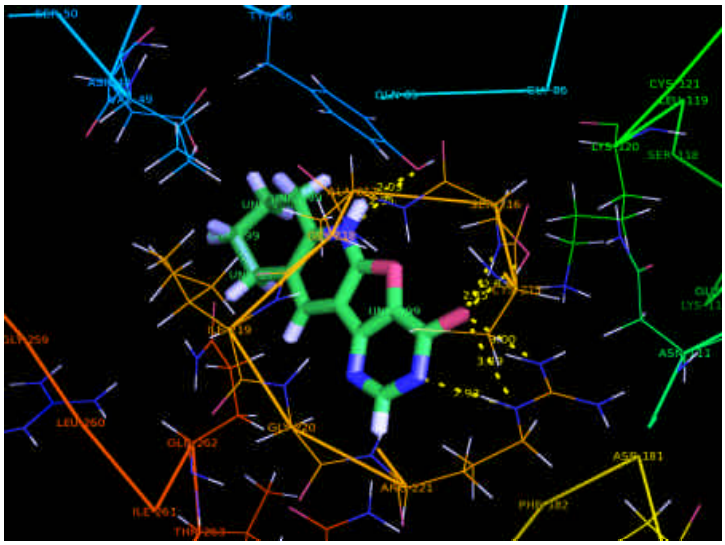


Fig. 5 represents the binding of parent (RBMS-01) with PTP1B (1g7f). Identified through the distance using Pymol molecular visualization tool.

of a water molecule for nucleophilic attack on the cysteinyl–phosphate intermediate. The alanine mutant allows crystallization of this normally transient intermediate

Fig.6 represents the binding of parent (RBMS-01) with PTP1B) 1g7f. It identifies through the binding complex with amino acid residues. It was represented through Chimera tool. The Trp 46 and Asp 48 have targeted towards the binding site and 215 – 221 were targeted towards the catalytic site.

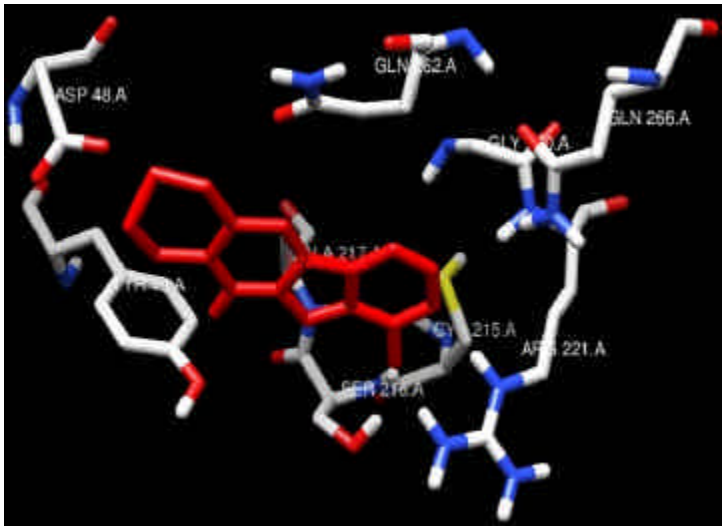


Fig.6 represents the binding of parent (RBMS-01) with PTP1B) 1g7f. It identifies through the binding complex with amino acid residues. It was represented through Chimera tool. The Trp 46 and Asp 48 have targeted towards the binding site and 215 – 221 were targeted towards the catalytic site.

Derivatives of the parent molecule

The above mentioned compound produces the good docking and energy scores comparatively. The R1 group can be synthezed in the 2nd position of the benzene ring. The –NH group shares with the dimethyl group and another is ketone group with heterocyclic compound. The second substituted ring shows the better activity.

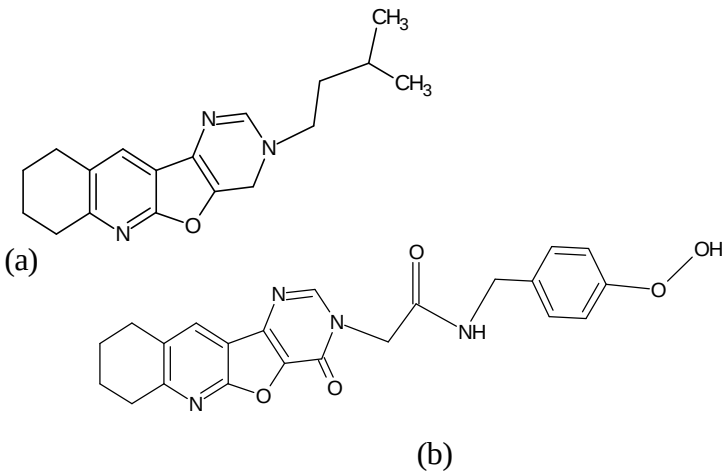


Fig 7a. RBMS-01 (der 1) b. RBMS-01 (der-2)

Table: 3 Provides information about the methyl group derived in the 2nd position of Amino Group of the RBMS-01 compound. Where, the RBMS-02 shows a better energy value.

Compound	HTVS	
	Energy	Gscore
RBMS-01-DER1	-36.82	-5.75
RBMS-01-DER2	-28.11	-4.81

Design of the Inhibitor and Modeling of the PTP1B Inhibitor Complex

On the basis of crystallographic, kinetic, and binding studies involving phosphotyrosine (pTyr), it is a consensus substrate sequence for PTP1B (13). In addition, almost all of the known, potent inhibitors contain aromatic rings. We, therefore, designed a potential inhibitor. Each of this ligand was docked after a conformational energy search as described in the methods and the most suitable one (in terms of the docking energy and the interactions) was found. The final docked position of the designed inhibitor is shown in Figure 8 and Fig 9, and the list of contacting residues (up to 4Å). It can be seen from the Figure that the inhibitor makes hydrogen bonds with residues Ser216, Ala217, and Arg221 in the catalytic site, with Tyr46 and Asp48 in binding site 3, and has hydrophobic interactions (up to 4 Å) with Arg24 in the binding. Thus the designed synthetic molecule has potency comparable with that of the most potent known inhibitors. This loop also contains the active-site Nucleophile Cys215. Other loops bearing invariant residues form the sides of the catalytic cleft and contribute to catalysis and substrate recognition (Asp)181, (Phe)182, Tyr46, (Val)49, (Lys)120, 116 and (Gln)262). Further, it records the Glide energy value as -39.81 Kcal/mol. By considering all the parameters and sites involved, the derived compound shows more effective than the parent compound.

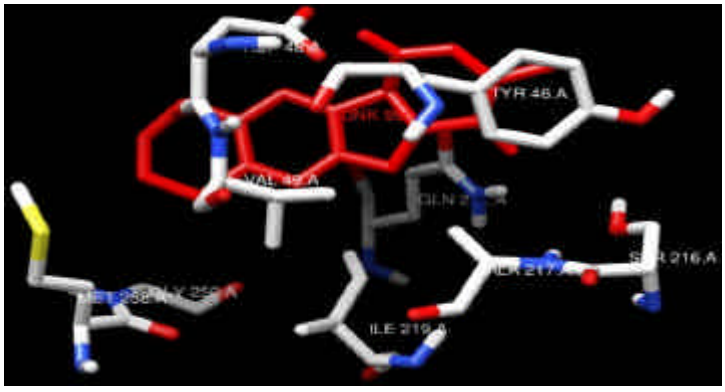


Fig 8. shows an interaction of derived compound with PTP1B inhibitor, Cys215 – Arg 221 makes more important interaction to produce an effective activity.

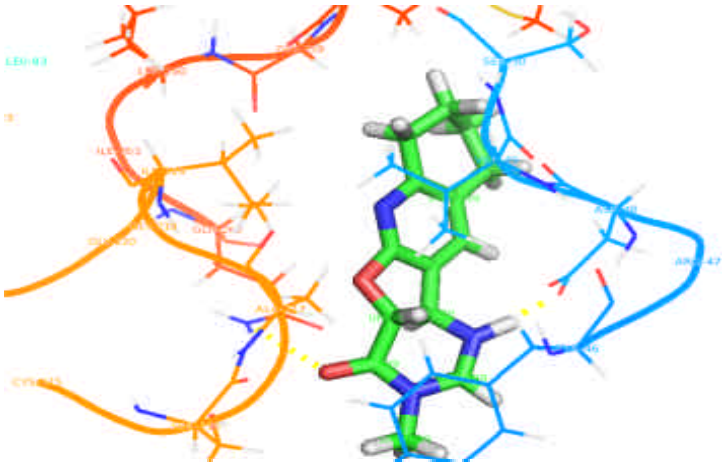


Fig 9. It shows an interaction of derived molecule with PTP1B using PYMOL. It describes that the distance between the molecules were computed. The 116, 120 and Ser 216 having an interaction.

CONCLUSION

Mounting evidence from biochemical, genetic and small molecule studies have established PTP1B as an outstanding drug target for the treatment of diabetes and obesity. Recent studies have revealed that it is highly feasible to achieve potency and selectivity in PTP1B inhibitor development. In addition, several strategies are being explored to improve the bioavailability of PTP1B inhibitors. It is probable that potent and selective PTP1B inhibitors with optimal pharmacological properties will emerge in the not too distant future. Further, an evaluation of these compounds through this approach shows better drug-like properties and further validated as potential treatments for an anti-hyperglycemic activity.

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