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Docking of oxalyl aryl amino benzoic acid derivatives into PTP1B

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Abstract:

Protein Tyrosine Phosphatases (PTPs) that function as negative regulators of the insulin signaling cascade have been identified as novel targets for the therapeutic enhancement of insulin action in insulin resistant disease states. Reducing Protein Tyrosine Phosphatase1B (PTP1B) abundance not only enhances insulin sensitivity and improves glucose metabolism but also protects against obesity induced by high fat feeding. PTP1B inhibitors such as Formylchromone derivatives, 1, 2-Naphthoquinone derivatives and Oxalyl aryl amino benzoic derivatives may eventually find an important clinical role as insulin sensitizers in the management of Type-II Diabetes and metabolic syndrome. We have carried out docking of modified oxalyl aryl amino benzoic acid derivatives into three dimensional structure of PTP1B using BioMed CAChe 6.1. These compounds exhibit good selectivity for PTP1B over most of phosphatases in selectivity panel such as SHP-2, LAR, CD45 and TCPTP found in literature. This series of compounds identified the amino acid residues such as Gly220 and Arg221 are important for achieving specificity via H-bonding interactions. Lipophilic side chain of methionine in modified oxalyl aryl amino benzoic acid derivative [1b (a_2, b_2, c_1, d)] lies in closer vicinity of hydrophobic region of protein consisted of Meth258 and Phe52 in comparison to active ligand. Docking Score in [1b (a_2, b2, c_1, d)] is -131.740Kcal/mol much better than active ligand score -98.584Kcal/mol. This information can be exploited to design PTP1B specific inhibitors.

Keywords: drug design; docking; oxalyl aryl amino benzoic acid derivatives; PTP1B

Background:

Type-II Diabetes mellitus is the most common form of diabetes. In this case, patients can still produce insulin, but they do not produce enough or their bodies can't use it properly. At present, there are about 125 million people affected by the disease globally. It has been projected by the year 2010; the no. of persons with diabetes will double. [**1**] Reversible protein phosphorylation is the predominant strategy used to control the activity of proteins in eukaryotic cells. Approximately 30% of 10,000 proteins in a typical mammalian cell are thought to be phosphorylated. **[2]** Many cellular functions could be artificially manipulated if one could exogenously control the activity of protein kinases and phosphatases. **[3]** This has led to intense interest in identifying small molecules capable of inhibiting action of specific kinases. **[4]** or phosphatases. **[5, 6]** Major goal of new therapies for Type-II Diabetes is to potentiate action of insulin. One of key proteins involved in insulin signaling is insulin receptor. When insulin binds to its receptor, changes in intracellular confirmation of receptor result in o-phosphorylation of specific tyrosine residues. **[7]** This serves as first step in insulin signaling and it is followed by a cascade of intracellular events that mediate the physiological effects of insulin. **[8]** There is compelling evidence that protein tyrosine phosphatase 1B is primarily responsible for dephosphorylation of insulin receptor and therefore acts to downregulate insulin signaling. **[9-13]** A PTP1B inhibitor would be expected to increase the half life of phosphorylated insulin receptor and enhance the effects of insulin. The development of PTP1B inhibitors began in early 1990 and continues today. **[14, 15]** Formylchromone derivatives **[16]**, 1, 2-Naphthoquinone derivatives **[17]** and Oxalyl aryl amino benzoic acid derivatives **[18]** have been identified as potent inhibitors for PTP1B. In an effort to develop a small, potent and selective PTP1B inhibitor, we used iterative structure based drug design to identify and optimize a lead compound.

Methodology:

Crystal structure of PTP1B

The crystal structure of PTP1B (PDB-ID: 1NZ7) was retrieved from protein data bank (http://www.rcsb.org) and its coordinates were selected for docking studies by BioMed CAChe 6.1. This crystal structure of PTP1B contains 2563atoms. Before docking H-atoms were added to protein for correct ionization and tautomeric states of amino acid residues such as Asp, Ser, Glu, Arg, His and also bond types were corrected for HET groups.

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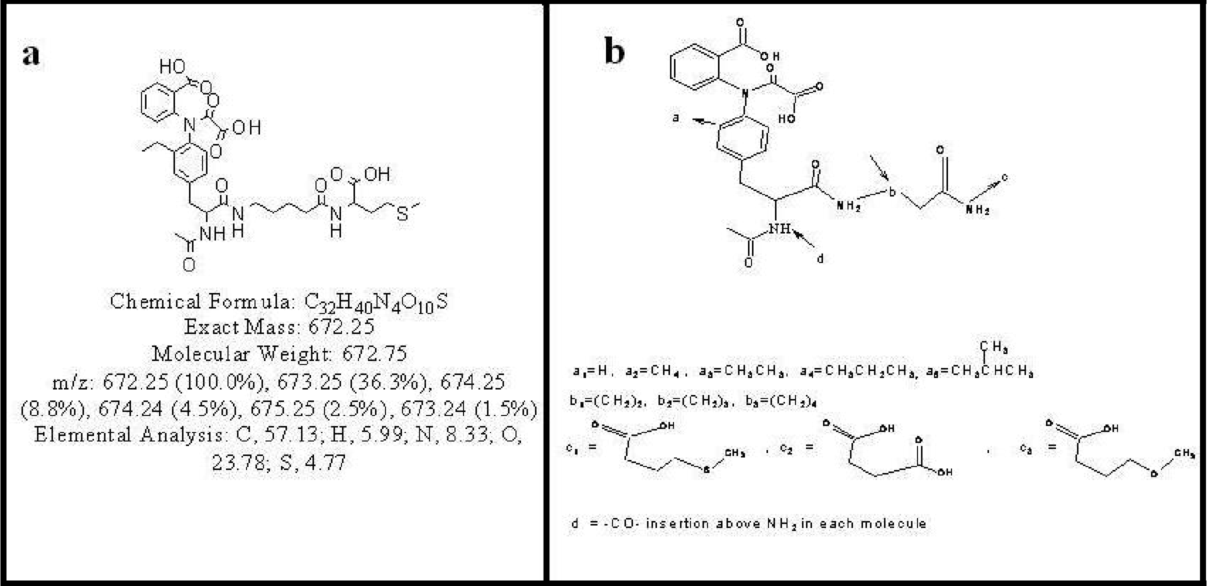

Figure 1: (a) active compound; (b) modified active compound.

Active site analysis
The active site was analyzed by selecting neighbors within 3Å around ligand. The active site contains Arg24, Ser28, Tyr46, Asp48, Val49, lys120. Ser216, Ala217, Gly218, Meth258, Gly259, Gln262, Thr263 and Gln266.

Selection of docking molecules
A set of 63 inhibitors as shown in Figure 1b were designed in Chem office software from most active oxalyl aryl amino benzoic acid derivative found from literature **[18]** whose structure is shown in Figure 1a. The active molecule was modified from position a, b, c and d as shown in Figure 1b.

Molecular modeling
Phosphatase1B inhibitors were built and energy minimized on Biomed CAChe. For calculating energy BioMed CAChe provides classical mechanics, quantum mechanics and includes solvent effects of water by using COSMO (Conductor-like screening Model, which approximates the dielectric screening energy of a solvent by the method of image charges). The geometry optimization was carried out for each modified molecule by solving Schrodinger equation using the MOPAC6 (Molecular Orbital Package, which contains a method for modeling the effect of highly polar solvents) and semi empirical AM1 Hamiltonian.

Docking by superposition
The fastest and easiest method for docking a ligand into active site is to superimpose the ligand on to a bound ligand already in the active site and then delete the bound ligand. The modified ligand was superimposed on already docked ligand (active molecule) in crystal structure of PTP1B and then already docked ligand was removed and then H-bonds were calculated among modified ligand and PTP1B.

BioMed CAChe docking score
Docking score was carried out in project leader of BioMed CAChe in which active site of PTP1B was fixed but ligand was flexible. Cache docking score performs force fields which includes terms as bond stretching, angle bending, torsional, and nonbonded interactions such as Vander Waals and Hydrogen bond interactions. The molecule based scoring function is made up of four components 1. Protein-ligand H-bond energy (external H-bond). 2. Protein-ligand Vander Waals energy (external vdw) 3. Ligand internal Vander waals energy (internal vdw) 4. Ligand intramolecular H-bond energy (internal H-bond). Docking Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Results and discussion:
The docking of modified oxalyl aryl amino benzoic acid derivatives into active site of PTP1B was carried out using Cache 6.1. The final docking score in Kcal/mol for each docking experiment was calculated. The docking evaluations were made on basis of H-bond and docking score. The molecules having improved docking score and H-bonding interactions are given below:

CACheScore fitness function for active molecule
From docking of active molecule (Figure 1a) into active site of PTP1B, we observed five H-bonds with protein amino acid residues that are Gly220, Ala217, Asp48, Gln262 and Arg221, as indicated in Table 1a (supplementary material). Active molecule entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The docking score was found to be -98.584Kcal/mol.

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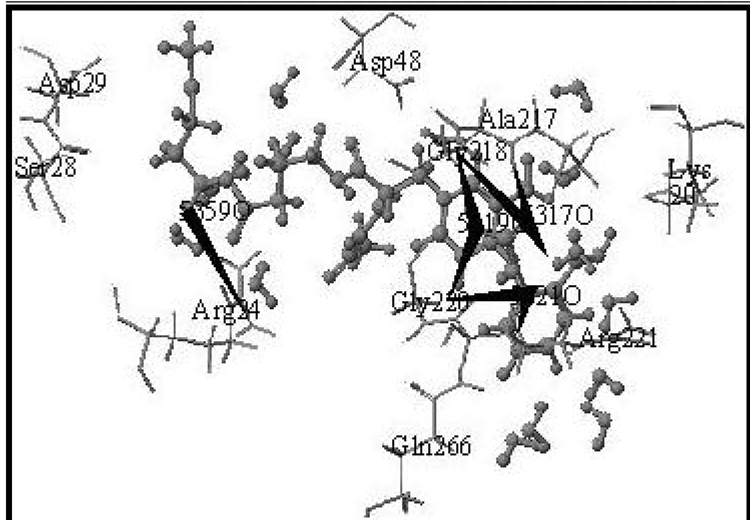

Figure 2: Cache score based interactions of molecule [1b (a_2, b_2, c_1, d)] docked into active site of PTP1B

CACheScore fitness function for modified active molecules

Three H-bonds were observed with protein amino acid residue Arg24 only, as indicated in Table 1a (supplementary material) when [1b (a_2, b_1, c_3, d)] molecule was docked into active site of PTP1B. The docking score was found to be -130.261Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 0.8523.

Four H-bonds were observed with protein amino acid residues that are Ala217, Arg221, Gly220 and Arg24 as indicated in Table 1a (supplementary material) when [1b(a_3, b_2, c_2, d)] molecule was docked into active site of PTP1B. The docking score was found to be -130.596Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 0.7418.

Seven H-bonds were observed with protein amino acid residues that are Ala217, Arg221, Gly220, Gly218 and Arg24 as indicated in Figure 2 and Table 1a (supplementary material) when [1b(a_2, b_2, c_1, d)] molecule was docked into active site of PTP1B.

The docking score was found to be -131.740Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 1.0956.

Two H-bonds were observed with protein amino acid residue Ala217 only as indicated in Table 1b (supplementary material) when [1b (a_3, b_2, c_1, d)] molecule was docked into active site of PTP1B, The docking score was found to be -122.297Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 0.5276.

Four H-bonds were observed with protein amino acid residues that are Ala217, Ile219, Gly220 and Gln262 as indicated in Table 1b (supplementary material) when [1b(a_4, b_2, c_3, d)] molecule was docked into active site of PTP1B. The docking score was found to be -126.602Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 0.6264.

Three H-bonds were observed with protein amino acid residues that are Gly220, Ile219 and Asp48 as indicated in Table 1b (supplementary material) when [1b(a_2, b_3, c_2, d)] molecule was docked into active site of PTP1B. The docking score was found to be -121.306Kcal/mol. Modified inhibitor entered into hydrophobic region of protein comprising amino acid residues indicated in Table 2 (supplementary material). The best RMSD was found to be 0.7942.

In active molecule five H-bonds have been observed whereas in modified molecule i.e. [1b (a_2, b_2, c_1 d)] seven H-bonds have been observed and hydrophobic region has also expanded in modified molecule comprising amino acid residues such as Asp29, Gly259, Arg254, Cys226, Arg79, Glu76, Phe52, Met253 and Val253.But active molecule contains only three amino acid residues such as Arg24, Arg254 and Arg257 in hydrophobic region. Hence it has been observed modified ligand leads to stronger interaction with PTP1B than active molecule found from literature. Further, from our docking studies, it has been shown that the diamides of alanine linker forms H-bond with Asp48 in active ligand but not in [1b (a_2, b_2, c_1, d)]. WPD loop remains open in both active ligand and [1b (a_2, b_2, c_1, d)]. Oxamic acid group in [1b (a_2, b_2, c_1, d)] shows

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stronger interactions with Ala217, Arg221, Gly220 and Gly218 in comparison to active ligand which shows interaction only with Gly220 and Ala217. The carboxylic acid of methonine in [1b (a_2, b_2, c_1, d)] interacts with Arg24 but not in active ligand. Lipophilic side chain of methionine in [1b (a_2, b_2, $c_{1,\ d}$)] lies in closer vicinity of hydrophobic region of protein consisted of Meth258 and Phe52 in comparison to active ligand. Docking Score in [1b (a_2, b_2, c_1, d)] is -131.740Kcal/mol which is much better than active ligand score -98.584Kcal/mol.

Conclusion:
A detailed docking analysis of modified oxalyl aryl amino benzoic acid derivatives into active site of PTP1B has been studied in the present work, to identify the inhibitor binding position and affinity to PTP1B using BioMed CAChe 6.1. On the basis of improved H-bonding and docking score than the active compound found in literature it has been concluded that modified oxalyl aryl amino benzoic acid derivative [1b(a_2, b_2, c_1, d)] molecule can proved to be better inhibitor than the active ligand found in literature.

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Supplementary material

Table 1a: List of hydrogen bonding interactions between modified inhibitors and PTP1B

Molecule		**Cache Score**		
Mol name	No. of H bonds	Protein residue atom	Ligand atom	H bond distance A°
Active Ligand	5	Gly220 (NH)	2304 (H)	1.684
		Ala217 (NH)	2302 (O)	1.649
		Asp48 (NH)	2585 (O)	1.930
		Gln262 (NH)	2339 (O)	2.017
		Arg221 (NH)	2315 (O)	1.908
[1b(a_2, b_1, c_3, d)]	3	Arg24 (NH)	5383 (H)	1.990
a_2=CH_4		Arg24 (NH)	5357 (O)	1.926
b_1=$(CH_2)_2$		Arg221 (NH)	5330 (O)	2.160
c_3=				
d=-CO-				
[1b(a_3, b_2, c_2, d)]	4	Ala217 (NH)	5317 (O)	1.364
a_3=CH_3CH_3		Arg221 (NH)	5330 (O)	2.162
b_2=$(CH_2)_3$		Gly220 (NH)	5319 (O)	2.193
c_2 =		Arg24 (NH)	5384 (O)	2.125
d=-CO-				
[1b(a_2, b_2, c_1, d)]	7	Ala217 (NH)	5317 (O)	2.119
a_2=CH_4		Arg221 (NH)	5321 (O)	1.094
b_2=$(CH_2)_3$		Gly220 (NH)	5321 (O)	2.106
c_1=		Gly220 (NH)	5319 (O)	2.067
d=-CO-		Gly218 (NH)	5319 (O)	1.461
		Gly218 (NH)	5317 (O)	1.144
		Arg24 (NH)	5359 (O)	2.171

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Table 1b: List of hydrogen bonding interactions between modified inhibitors and PTP1B

Molecule	**Cache Score**			
Mol name	No. of H bonds	Protein residue atom	Ligand atom A°	H bond distance
[1b(a_3, b_2, c_1, d)] a_3=CH_3CH_3 b_2=$(CH_2)_3$ c_1= d=-CO-	2	Ala217 (NH)	5319 (O)	2.094
		Ala217 (NH)	5317 (O)	2.109
[1b(a_4, b_2, c_3, d)] a_4=$CH_3CH_2CH_3$ b_2=CH_3CH_3 c_3= d=-CO-	4	Ala217 (NH)	5317 (O)	1.323
		Ile219 (NH)	5319 (O)	2.178
		Gly220 (NH)	5319 (O)	1.639
		Gln262 (NH)	5354 (O)	1.834
[1b(a_2, b_3, c_2, d)] a_2=CH_3 b_3= $(CH_2)_4$ c_2= d=-CO-	3	Gly220 (NH)	5319 (O)	1.119
		Ile219 (NH)	5319 (O)	1.978
		Asp48 (CO)	5378 (H)	1.857

Table 2: List of amino acid residues contributing to the hydrophobic pocket in docking of modified inhibitors into PTP1B

Modified inhibitor	**Hydrophobic pocket of PTP1B**
Active Ligand	Arg 254, Arg24 and arg257
[1b(a_2, b_1, c_3, d)]	Lys237, Met253, Leu260,Ala217 Arg199, Val184, Phe191, Gly183 and Pro185
[1b(a_3, b_2, c_2, d)]	Glu76, Val249, Arg254, Lys237, Ile261, Thr263, Phe191, Leu195 and Arg199
[1b (a_2, b_2, c_1, d)]	Asp29, Gly259, Arg254, Cys226, Arg79, Glu76, Phe52, Met253 and Val213
[1b (a_3, b_2, c_1, d)]	Met253, Gln78, Met74 and Leu260
[1b (a_4, b_2, c_3, d)]	Ser28, Phe52, Leu260, Tyr81 and Met74
[1b (a_2, b_3, c_2, d)]	Arg254, Glu75, Cys226, Tyr81 Phe182, Thr224, Gly259 and Gln262

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