International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-3, 2016 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Molecular Docking and Dynamic Studies of Bioactive Compounds from *Triticum Aestivum*(L.) Against Obesity Enzymes

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Manuscript No: IJPRS/V5/I3/00110, Received On: 09/07/2016, Accepted On: 20/07/2016

ABSTRACT

In this work, we hypothesize that the reason could be because of the inhibition of obesity associated enzymes by the Wg derived phytochemicals. Molecular docking was used to explore the efficacy of Wg components to inhibit the key enzymes related with obesity; pancreatic lipase and Protein tyrosin phosphatase 1B. Autodock4.0 molecular docking software that applies Lamarckian Genetic Algorithm was used. The ligand structures were retrieved from pdbe database. Based on this analysis, it has been found compound I- 1-(4,6-dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea and compound II- 2,2-dimethyl-7-propyl-chroman-3-ol could be used to reduce fat absorption in obese persons. The compounds were screened for inhibition of PTP 1B and Pancreatic lipase protein, a lipid biomarker, by molecular docking and dynamic studies. Both the compound I and II may be a potent inhibitor of PTP 1B because it exhibited minimum binding -6.06 and -5.56 kJ mol−1 and docking 35.97 and 84.50 uM energy, respectively. For PL, the compounds show -5.79 and -5.57 kJ mol−1 as binding energy. Molecular dynamics studies revealed that Wg compounds had minimum potential energy with the target protein. In order to understand the mechanism of ligand binding and to identify potent PTP 1B and PL inhibitors, a study involving molecular docking and virtual screening have been performed. It can be concluded that these phytochemicals or their derivatives can be used for further in-vitro and in-vivo studies to design valuable drugs.

KEYWORDS

Wheat grass, Obesity, PL, PTP 1B, Autodock Bilayer

INTRODUCTION

Obesity is a non communicable disease coordinated not only with life threatening diseases such as diabetes, cancer and cardiovascular diseases, but also one of the factors for brain dysfunction and primary stage of dementia[1]. At present, in global wide 312 million are characterized as obese and more than 1.1 billion people are overweight[2].

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"Obesity as an abnormal or excessive fat accumulation harmful to human health" defined by World Health Organization (WHO). Researchers are being urged to find long term relief for weight management[3,4]. Imbalance of energy homeostasis leads many metabolic disorders, is becoming most challenging problem in biomedical field. Studies of body weight control in molecular level, is giving more idea for development of novel drugs for new targets in therapeutic interventions[5].

Nature has given lot of treasures to humans. In that, natural medicines are very important,

because of endless chances for its innovative drug therapy. Plants occupy half the life in living organism. It can be used both pure compounds and standardised extracts[6]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect.

Wheat (*Triticum aestivum*) germinated over a period of 6-10 days is called wheatgrass (Wg), because of its rich source of vitamins, antioxidants and minerals also familiar as "living food". "Wheatgrass" refers to the young grass of the common monocot wheat plant *Triticum aestivum*. Its consumption in the Western world began in the 1930s. Today, wheatgrass is quickly becoming one of the most widely used supplemental health foods and is available in many health food stores as fresh produce, tablets, frozen juice, and powder. It is believed to have antioxidant enzymes like Superoxide Dismutase and Cytochrome Oxidase. It contains rich source of chlorophyll that is known to be responsible for deactivating the metabolic activation of carcinogens. In India, wheatgrass is consumed either tablet or as a juice to keep up good health. Wg juice is known to cure healing properties in many degenerative diseases and also very effective in the treatment of thalassemia, distal ulcerative colitis, and benefits other parts of the body[7,8].

In line with folk sayings, the health benefits of wheatgrass may include improved digestion, blood pressure reduction, and heavy metal detoxification from the bloodstream, immune system modulation, and gout alleviation. Several papers have indicated that wheatgrass has anti-tumor activities[9] and anti-oxidant properties[10]. In addition, wheatgrass may help prevent some disorders, including diabetes and heart disease[11].

The development of natural products for the treatment of obesity is a challenging task, which can be launched faster and cheaper than conventional single-entity pharmaceuticals[12]. In this context, since dietary lipids represent the major source of unwanted calories, the inhibition of fat digestion is an interesting approach for reducing fat absorption[13]. Orlistat is the only authorised anti-obesity drug in Europe and has been shown to act through inhibition of pancreatic lipase (PL), which is a key enzyme for the digestion of dietary triglycerides[14]. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the leptin and insulin signaling pathways. Coordinated tyrosine phosphorylation is essential for signaling pathways regulated by insulin and leptin. Obesity are characterized by resistance to hormones leptin. PTP1B has been found to be a major regulator of body fat stores, energy balance, and insulin sensitivity in vivo. Pancreatic acinar cells secrete pancreatic lipase, an important enzyme of pancreatic juice responsible for digestion of dietary triglycerides in the small intestine. Pancreatic lipase is a common target for anti-obesity drug research. Pancreatic lipase (triacylglycerol acyl hydrolase) plays an essential role in the digestion of triacylglycerols. Lipase inhibitors may affect the amount of fat absorbed, yet they do not block the absorption of a particular type of fat.

Protein–ligand docking is a molecular modelling technique. The goal of protein–ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme. Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates[15].

Drug design, sometimes referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. This type of modelling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target

is known as structure based drug design. In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed[16].

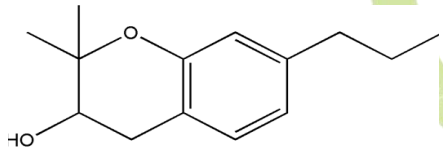
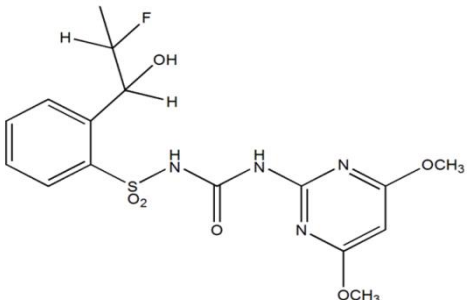
The aim of this computational study is to find the potency of the isolated compounds to treat metabolic disorder -Obesity. To process this study we have chosen two important drug targets (PTP 1B and PL) protein/enzyme/receptor to study the quorum sending mechanism using computational biology tools.

MATERIAL AND METHODS

Compound Details

Retrieval of protein and its preparation

Drug target protein 1: Crystal Structure of the human PTP1B catalytic domain of uniprot number P18031 with unique (http://www.rcsb.org/) protein data bank ID: 1JF7 with 298 residues length of 33% helical (10 helices; 99 residues) 21% beta sheet (13 strands; 65 residues) and 2.2Å x-ray diffraction resolution is retrieved from pdbe database (www.ebi.ac.uk/pdbe). Similarly, for drug target protein 2: Structure of the pancreatic lipase-colipase complex of uniprot number: P16233 with unique (http://www.rcsb.org/) protein data bank ID: 1N8S with 449 residues length of 22% helical (13 helices; 99 residues) 29% beta sheet (26 strands; 133 residues) and 3.04 Å x-ray diffraction resolution is retrieved from pdbe database (www.ebi.ac.uk/pdbe).

Structure of the compound	Mol. composition	Mol. wt	No of atoms
Compound I: 2,2-dimethyl-7-propyl-chroman-3-ol 	C: 0.763, H: 0.092, O: 0.145	220.314	16
Compound II: 1-(4,6-Dimethoxy-pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea 	C: 0.464, H: 0.046, F: 0.046, N: 0.135, O: 0.232, S: 0.077	414.42	47

The common method of auto dock protein preparation is followed by adding and applying the hydrogen bonds and kollamaan charges respectively. Finally, the structure is saved in .pdb for docking.

Auto Grid Calculation

AutoGrid is a program that pre-calculates grid maps of interaction energies for various atom types, such as aliphatic carbons, aromatic carbons, hydrogen-bonding oxygens, and so on, with a macromolecule such as a protein, DNA or RNA.

These grid maps are then used by AutoDock docking calculations to determine the total interaction energy for a ligand with a macromolecule.

Doing this pre-calculation saves a lot of time during the docking, primarily because we do not have to update non-bonded lists during the calculation. Also, what was a calculation with order N-squared complexity is reduced to one that is order N, where N is the number of atoms interacting.

The process of rigid docking was carried out for drug targets with isolated compound in 3D grid centre for drug target 1(Refer above for the name) of 19.38 X 12.611 Y 46.434 Z co-ordinates with grid spacing of 0.553 in 46x42x46 grid points.

However, for the drug target 1, the flexible residue are provided from the reference structure PNU177836 that already bound with the protein as an inhibitor (http://www.rcsb.org/pdb/explore/ explore.do?structureId=1JF7) grid was calculated. Similarly, for drug target 2 (Refer above for the name) of 38.33X 22.27 Y 74.51 Z co-ordinates with grid spacing of 0.503 in equal 50 x 50x 50 grid points, since there is no observation of any bound structure, hence initially study was carried out with or list at the current used for treatment for obesity is docked and the residues are identified and the same grid point is kept constant for isolated compound docking.

The Secondary Structure of Human Ptp1b and Pancreatic Lipasecatalytic Domain

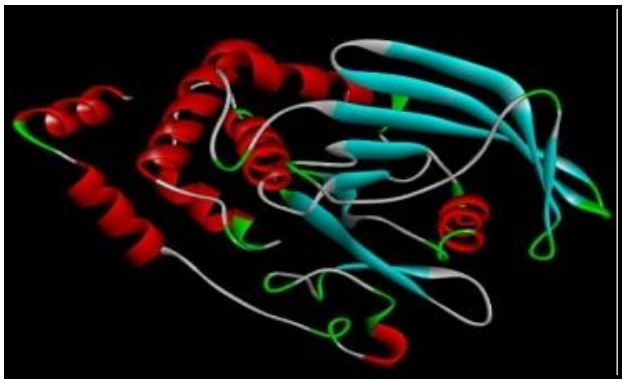


Figure 1: PTP 1B

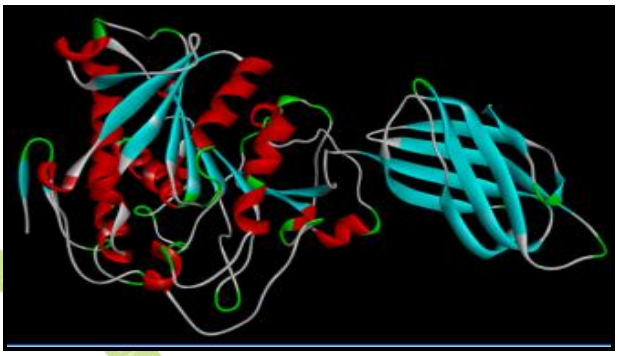


Figure 2: Pancreatic lipase

Molecular docking

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand—protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking is processed in autodock 4.0v in linux environment with genetic algorithm and lamarckian default setting of 150 generations and 10 runs for each protein and compound.

RESULTS AND DISCUSSION

Docking Analysis In the study, oriented towards the design and development of effective drug against obesity, we determined the best wheat grass components that could serve as lead molecules for drug design.

Obesity is a multi factorial disease characterized by an excessive weight to height proportion owing to enlarged fat deposition that is attributed to a higher calorie intake as compared to the energy expenditure. Pancreatic lipase (PL) plays

a key role in the efficient digestion of dietary fats by hydrolysing them into mono-acylglycerol and fatty acids, thus minimising the intestinal absorption of triglycerides[17]. This enzyme has been widely used for determination of the potential efficacy of natural products as anti-obesity agents[18].

The docking of *Triticum aestivum* compounds namely 2,2-dimethyl-7-propyl-chroman-3-ol and 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea (fig) into active site of PTP1B and PL were carried out using Autodock tool 4.2. The protein, PTP 1B and PL was targeted against compound I and compound II.

Given the three-dimensional structure (Fig 1 & 2) of a target receptor molecule usually a protein; chemical compounds having potential affinity toward sit are designed rationally, with the aid of computational methods. Detailed bioinformatics analysis offers a convenient methodology for efficient *in silico* preliminary analysis of possible function of new drug. The target protein and inhibitors were geometrically optimized. The isolated compounds used as inhibitors were docked against active site of the target protein using Auto Dock which gives an insight into the binding modes for the inhibitors.

Compound I When docked in the SER-ARG-ALA catalytic pocket of PTP 1B, the result shows a binding energy value of -6.06 kcal/mol and Ki value of 35.97 μm. (Table1). While Compound II (ASP-ALA-GLN) show binding energy value and ki with values -5.56 and 84.50 kcal/mol respectively.

Compound I When docked in the CYS catalytic pocket of PL, the result shows a binding energy value of -5.79 kcal/mol and Ki value of 57.09 uM. (Table2). While Compound II show binding energy value and ki with values -5.57 and 82.08 kcal/mol respectively. According to the inhibition constant values, Compound I and II shows lower binding energy.

Lower binding energy of the ligands indicates better inhibition affinity and thus low Ki value. From the docking analysis it is observed that the both two compounds could be used for the inhibition of obesity. A docking analysis of plant derived compounds into active site of PTP1B has been studied in the present work, to identify the inhibitor binding position and affinity to PTP1B using Autodock tool[19].

Wheatgrass compounds leads to stronger inhibition with PTP1B and PL. According to the report, amentoflavone, naturally occurring bioflavonoids derived from *Selaginella tamariscina*, inhibited activity of PTP1B[20]. Chalcone derivatives are proven to have the anti-pancreatic lipase, applying into obesity treatment[21].

For the purpose of selecting some of wheat grass derivatives by using docking model running on computer, this research is hoped to find out potential anti obesity compounds which are able to resist pancreatic lipase and PTP 1B activities. Based on docking outcomes, it is found that isolated compounds with many hydroxy or amino substituents not only lead to good cohesion results but also be capable of creating bonds with some critical amino acids. Among the derivatives used in the docking model, some of them were studied for many other effects such as anti-inflammatory, anti-bacterial, anti- tumor abilities (S19, S14, S18)[22,23].

A new point of this research is that in the list of used compounds, there are some derivatives containing two hydroxy substituents at meta-position (resorcinol derivatives). These derivatives bring about favourable docking results[21].

Receptor-Ligand Interaction –Drug Target 1 (1JF7)

The isolated compound I and II docked with PTP 1B.The various energy parameters of intermolecular energy , electrostatic energy and vanderwals energy and binding mode with specific residues of flexible docking is shown in the figure 3 & 9 for compound1 .The interaction with active site amino acid residues in for compound is stated in table 3 & 4.

Table 1: Drug target 1 receptor -ligand interaction with isolated compound its free energy binding and inhibition constant, ki

Receptor and Ligand	Compound Name	Amino acid Binding	Distance in Å	Free Energy of Binding kcal/mol	Inhibition Constant, Ki
Structure of the human PTP1B catalytic domain	2,2-dimethyl-7-propyl-chroman-3-ol	SER B:216	3.3	-6.06	35.97 uM
		ARG B:221	4.6		
		ALA B:217	4.1		
	1-(4,6-Dimethoxy-pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea	ASP B:48	3.8	-5.56	84.50 uM
		ALA B:217	3.7		
		GLN B:262	5.7		

Table 2: Drug target 2 receptor -ligand interaction with isolated compound its free energy binding and inhibition constant, ki

Receptor and Ligand	Compound Name	Amino acid Binding	Distance in Å	Free Energy of Binding kcal/mol	Inhibition Constant, Ki
Structure of the Pancreatic Lipase	2,2-dimethyl-7-propyl-chroman-3-ol	CYS A:304	4.4	-5.79	57.09 uM
	1-(4,6-Dimethoxy-pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea	ASN A:425	3.9	-5.57	82.08uM
			3.8		
			4.9		

Receptor-Ligand Interaction–Drug Target 2 (1N8S)

The isolated compound I and II docked with drug PL. The various energy parameters of intermolecular energy , electrostatic energy and vanderwals energy and binding mode with specific residues of flexible docking is shown in the figure 4 & 10 for. The interaction with active site amino acid residues in for compound is stated in table 3 & 4.

Binding of Compound I with Cavity of Active Site of PTP 1B and PL Protein

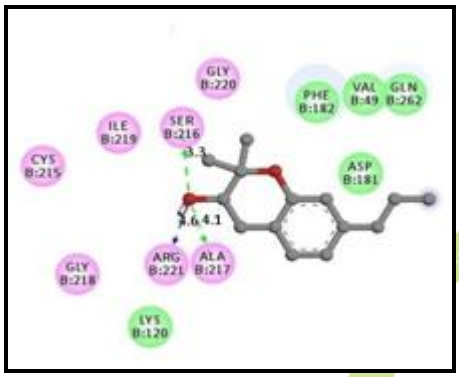


Figure 3: PTP 1B

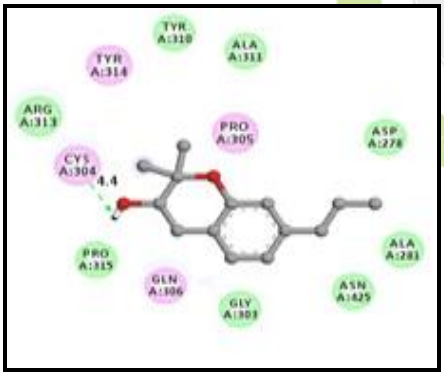


Figure 4: Pancreatic Lipase

Table 3: Energy parameters of best docking for compound I

Parameters	PTP 1B	Pancreatic Lipase
Final intermolecular energy	-6.96 kcal/mol	-6.68 kcal/mol
vdW + Hbond + desolv Energy	-6.85 kcal/mol	-6.62 kcal/mol
Electrostatic Energy	-0.11 kcal/mol	-0.07 kcal/mol
Final Total Internal Energy	-0.87 kcal/mol	-0.84 kcal/mol
Torsional Free Energy	+0.89 kcal/mol	+0.89 kcal/mol
Unbound System's Energy	-0.87 kcal/mol	-0.84 kcal/mol

3d Structure of Interaction of Compound I with PTP 1B and Pancreatic Lipase

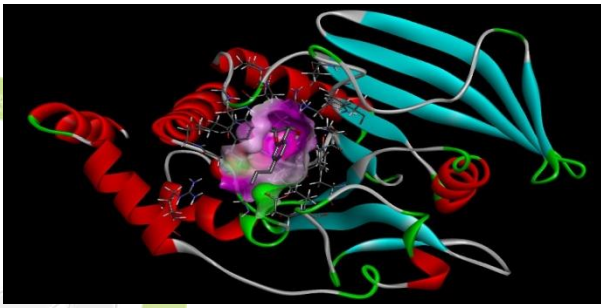


Figure 5: PTP 1B

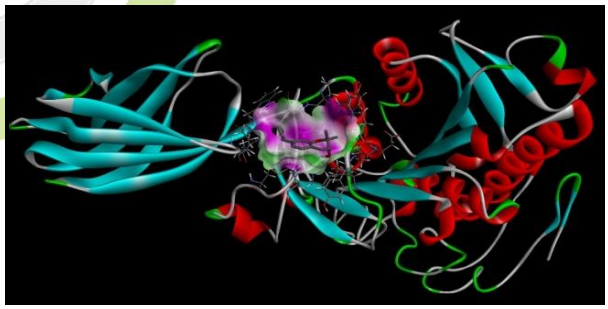


Figure 6: Pancreatic Lipase

3D Structure of Interaction of Compound II with PTP 1B and Pancreatic Lipase

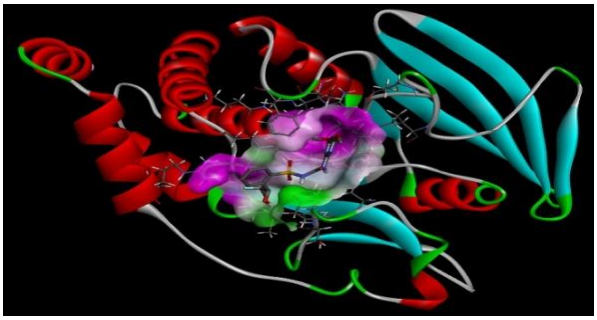


Figure 7: PTP 1B

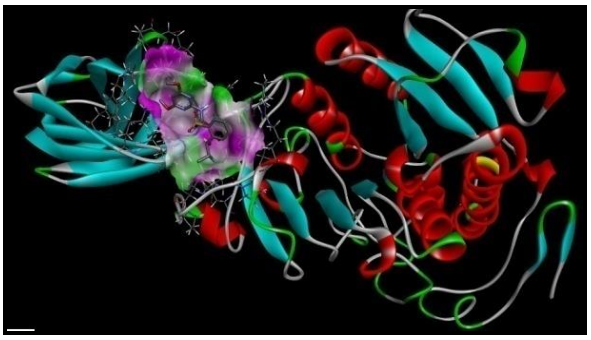


Figure 8: Pancreatic Lipase

Binding of Compound II with Cavity of Active Site of PTP 1B and PL Protein

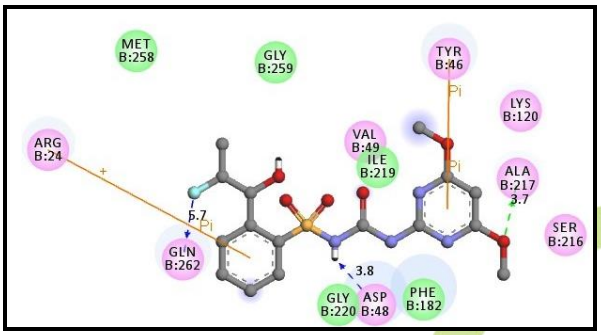


Figure 9: PTP 1B

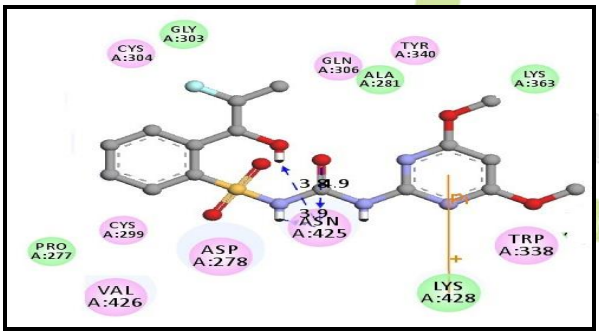


Figure 10: Pancreatic Lipase

Table 4: Energy Parameters of Best Docking for Compound II

Parameters	PTP 1B	Pancreatic Lipase
Final intermolecular energy	-7.94 kcal/mol	-7.96 kcal/mol
vdW + Hbond + desolv Energy	-7.77 kcal/mol	-6.77 kcal/mol
Electrostatic Energy	-0.18 kcal/mol	-1.19 kcal/mol
Final Total Internal Energy	-0.24 kcal/mol	-1.91 kcal/mol
Torsional Free Energy	+2.39 kcal/mol	+2.39 kcal/mol
Unbound System's Energy	-0.24 kcal/mol	-1.91 kcal/mol

CONCLUSION

From this study it was concluded that, isolated compound has potency to bind with active amino acid residues of PTP1B catalytic domain .Similarly, compounds have potency to bind with active amino acid residues of PTP 1B shows three interaction, whereas with PL, the isolated compounds shows one amino acid residue interaction Thus, the isolated compound has potency of obesity activity and can serve as a drug candidate. The supportive Ki values help the compounds to improve the activity in wet lab to prove the same. Thus, further study, as need to be carried out *in vitro* and *in vivo*.

ACKNOWLEDGEMENT

The author gratefully thank to the Department of Biochemistry, school of biosciences, Periyar University, Salem-11 for providing infrastructure facility to carry out the research.

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