COMPUTATIONAL STUDIES OF GAMMA-ORYZANOL ON HUMAN PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) AND ITS ROLE AS ANTI DIABETIC AGENT

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Abstract

Diabetes mellitus and diabetes insipidus (DI) are distinct disorders characterized by disrupted fluid and glucose regulation, respectively. DI is caused by a deficiency of or insensitivity to antidiuretic hormone (ADH), whereas diabetes mellitus involves impaired insulin function, with Type 2 diabetes mellitus (T2DM) being the most prevalent form globally. T2DM is associated with insulin resistance and is linked to complications in glucose homeostasis. Protein Tyrosine Phosphatase 1B (PTP1B) has emerged as a crucial negative regulator of insulin signaling and a validated therapeutic target for T2DM. Inhibition of PTP1B enhances insulin sensitivity and may improve glycemic control.

Keywords: PTP1B Inhibitors, Shikonin, Gamma-Oryzanol, Phytoconstituents

1. Introduction

Diabetes Insipidus

The DI is a condition characterized by excessive thirst and excretion of large amounts of severely diluted urine, with reduction of fluid intake having no effect on the latter. There are several different types of DI, each with a different cause. The most common type in humans is central DI, caused by a deficiency of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH). The second common type of DI is nephrogenic diabetes insipidus, which is caused by an insensitivity of the kidneys to ADH. It can also be an iatrogenic artifact of drug use.

The incidence of diabetes insipidus in the general population is 3 in 100, 000. [3] The name refers to the inability to retain fluid (diabetes = siphon) and the lack of sugar in the urine (insipidus = tasteless). The DI mainly caused by either defect in ADH production or defect in the kidneys' response to ADH. There are several forms of Diabetes insipidus (DI) which are as follows.

Type I - Insulin Dependent Diabetes Mellitus (IDDM) Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children or childhood-onset diabetes, and insulin-dependent diabetes mellitus (IDDM).

Type II - Non Insulin Dependent Diabetes Mellitus (NIDDM)

Serum levels of insulin remains elevated. Type 2 diabetes is a chronic, lifelong disease that results when the body’s insulin does not work effectively. It is adult-onset diabetes. Obesity-related diabetes and non-insulin-dependent diabetes mellitus (NIDDM). It is a metabolic disorder that is characterized by high blood glucose in the context of insulin

resistance and relative insulin deficiency. Type II or non-insulin-dependent diabetes tends to develop gradually in later life. It is also known as “maturity” or “adult-onset” diabetes. Type II accounts for 90 percent of all U.S diabetic population. Caucasians, African-North Americans, and aboriginal North Americans, Indians are especially prone to it. Many people with type II have none of the usual symptoms and therefore remain oblivious to the problem for years, until complications begin to appear. Type II is not “mild” diabetes just because routine insulin injections aren’t needed to sustain life. The strongest factors or causes of type II diabetes are family history of diabetes and obesity and due to a combination of lifestyle and genetic factors. Recently, intra uterine growth restriction (IUGR) or prenatal under nutrition (macro and micro nutrient) was identified as another probable factor.

Gestational diabetes

It resembles type 2 diabetes, involving a combination of relatively inadequate insulin secretion and responsiveness. Occurs in 2-5 % of all pregnancies and may improve or disappear after delivery. During pregnancy, all women produce vasopressinase in the placenta, which breaks down ADH. Gestational Diabetes is thought to occur with excessive vasopressinase production.

Gestational diabetes develops in some women during pregnancy and then disappears after they give birth. There’s a connection between gestational diabetes and the onset of type II in later life. Pregnancy raises a woman’s chances of developing diabetes by 16 percent after she turns 40. Fasting plasma level ≥ 7.0 mmol/L(126 mg/dL)

Plasma glucose ≥ 11.1mmol/L (200 mg/dL).

Symptoms of hyperglycemia and causal plasma glucose ≥ 11.1 mmol/L(200 mg/dL)

People with fasting glucose levels from 100 to 125 mg/Dl (5.6 to 6.9 mmol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/Dl (7.8 mmol/L) but not over 200 mg/Dl

Gestational diabetes affects 2 to 5 percent of pregnancies in Canada. In some pregnant women, it develops for the first time. Two things may trigger diabetes: weight gain and the production of certain hormones which alter the way insulin works. A baby born to a diabetic mother, although very large may have immature organs and suffer the complications of prematurity. If blood sugar levels cannot be normalized, complications may arise during pregnancy. Gestational diabetes shows up in the last half of the pregnancy, from the 24th week onward.

Symptoms

Excessive urination and extreme thirst (especially for cold water and sometimes ice or ice water) are typical for DI. Symptoms of diabetes insipidus are quite similar to those of untreated diabetes mellitus, with the distinction that the urine does not contain glucose and there is no hyperglycemia (elevated blood glucose). Blurred vision is a rarity. Signs of dehydration may also appear in some individuals since the body cannot conserve much (if any) of the water it takes in.

The extreme urination continues throughout the day and the night. In children, DI can interfere with appetite, eating, weight gain, and growth as well. They may present with fever, vomiting, or diarrhea. Adults with untreated DI may remain healthy for decades as long as enough water is consumed to offset the urinary losses. However, there is a continuous risk of dehydration and loss of potassium.

Diagnosis

(11.1 mmol/L) are considered to have impaired glucose tolerance.

The protein tyrosine phosphatase 1B (PTP1B)

The process of tyrosine phosphorylation and dephosphorylation is the basic mechanism of cell growth and differentiation, and the balance of this process was maintained by protein tyrosine phosphatase (PTP) and protein tyrosine kinase (PTK). PTPs are superfamily of receptor-like and non-transmembrane proteins, whose members are

highly specific, tighly regulated and important modulators of cellular signal initiation and termination. Protein tyrosine phosphatase 1B (PTP1B) is a key member of the family and also a negative regulator in insulin signal transduction and a potential targets for treatment of type 2 diabetes mellitus. Therefore, small-molecule PTP1B inhibitors have broad application prospects in the treatment of type 2 diabetes. Here, we briefly introduce composition of PTP1B, and then focus on the role of PTP1B in insulin signaling of type 2 diabetes mellitus and also highlight the research progress of PTP1B inhibitors used in therapy of type 2 diabetes mellitus recently.

PTP1B is specifically expressed in various human tissues interacted with other members of the PTPs family. In addition, PTP1B functionare regulated by several post-translational medications such as oxidation, nitrosyaltion, sulfyhydration, sumoylation, phosphorylation and proteolytic cleavage. The diverse modifications illustrated the dynamic regulation of this enzyme and its ability to modulate numerous signaling pathways likely in a cell/tissue- and stimulus-dependent manner, with high specificity and precision. PTP1B, as a potential target for type 2 diabetes and obesity, have expanded out of PTP1B gene cDNA span.

The PTP1B inhibitors

PTP1B is an intracellular PTP, involved in the negative regulation of the insulin as well as leptin signaling. PTP1B has emerged as a validated therapeutic target for the treatment of type 2 diabetes and related metabolic abnormalities. PTP1B has been inhibited experimentally using a variety of mechanisms and chemical entities. PTP1B inhibitors could potentially improve insulin resistance and normalize plasma glucose and insulin level without inducing hypoglycemia. Recent years, many pharmaceutical companies have developed various PTP1B inhibitors as drug candidates for therapy of Type 2 diabetes in clinical trials, including ertiprotafib, ISIS 113715, ISIS-PTP1BRx, and trodusquemine. In addition, some new synthetic PTP1B inhibitors were reported such as Thiazolidinediones, Benzofuran and benzothiophene biphenyls, Vanadium complexes and Amino benzoic acid. Thiazolidinediones (TZDs) are commonly known as glitazones that share a common molecular scaffold: 2, 4-TZDs and can correct hyperglycemia by enhancing insulin sensitivity in target tissues and were shown to improve glycemic control by ameliorating insulin resistance in both peripheral tissues and liver in type 2 diabetic patients.

1.3 Gamma-oryzanol

The wonder compound Gamma-oryzanol seems to be a mixture of lipids derived from fat fraction of rice (Oryza sativa) barn thus a popular name of rice bran oil. Rice bran obtained during milling of rice is gaining commercial importance in the world as it has many beneficial nutritive and biological effects. Rice bran oil (RBO) can be extracted from rice bran by solvent extraction using food grade n-hexane. The crude rice bran oil obtained in the solvent extraction process is subjected to either chemical refining or physical refining to meet the specifications of edible grade vegetable oil.

Review of Literature

The NCBI literature database PubMed was searched for finding the literature featuring research citing the relatedness of human protein tyrosine phosphatase 1B and diabetes mellitus. A few literature featuring their story and the use of potential inhibitors were also reported.

Diabetes mellitus is a multifactorial disease that affects both developing and developed countries and is a major public health concern. Many synthetic drugs are available in the market, which counteracts the associated pathologies. However, due to the propensity of side effects, there is an unmet need for the investigation of safe and effective drugs. This research aims to find a novel phytoconstituent having diminished action on blood glucose levels with the least side effects. Shikonin is a naturally occurring naphthoquinone dying pigment obtained by the roots of the Boraginaceae family. Besides its use as pigments, it can be used as an antimicrobial, anti-inflammatory, and anti-tumor agent. This research aimed to hypothesize the physicochemical and

phytochemical properties of Shikonin's in silico interaction with protein tyrosine phosphate 1B, as well as it's in vitro studies, in order to determine its potential anti-diabetic impact. To do so, molecular docking experiments with target proteins were conducted to assess their anti-diabetic ability. Analyzing associations with corresponding amino acids revealed the significant molecular interactions between Shikonin and diabetes-related target proteins. In silico pharmacokinetics and toxicity profile of Shikonin using ADMET Descriptor, Toxicity Prediction, and Calculate Molecular Properties tools from Biovia Discovery Studio v4.5. Filter by Lipinski and Veber Rule's module from Biovia Discovery Studio v4.5 was applied to assess the drug-likeness of Shikonin. The in vitro studies exposed that Shikonin shows an inhibitory potential against the PTP1B with an IC50 value of 15.51 μM. The kinetics studies revealed that it has a competitive inhibitory effect (Ki = 7.5 M) on the enzyme system, which could be useful in the production of preventive and therapeutic agents. The findings of this research suggested that the Shikonin could be used as an anti-diabetic agent and can be used as a novel source for drug delivery.

Protein tyrosine phosphatase 1B is a very promising target for the treatment of metabolic disorders such as type II diabetes mellitus. Although it was validated as a promising target for this disease more than 30 years ago, as yet there is no drug in advanced clinical trials, and its biochemical mechanism and functions are still being studied. In the present study, based on our experience generating PTP1B inhibitors, we have developed and implemented a scaffold-hopping approach to vary the pyrrole ring of the pyrrolo[1,2- a]quinoxaline core, supported by extensive computational techniques aimed to explain the molecular interaction with PTP1B. Using a combination of docking, molecular dynamics and end-point free-energy calculations, we have rationally designed a hypothesis for new PTP1B inhibitors, supporting their recognition mechanism at a molecular level. After the design phase, we were able to easily synthesize proposed candidates and their evaluation against PTP1B was found to be in good concordance with our predictions. Moreover, the best candidates exhibited glucose uptake increments in cellulo modell, thus confirming their utility for PTP1B inhibition and validating this approach for inhibitors design and molecules thus obtained.

PTP1B is identified as the insulin signaling pathway downregulator; involved in pancreatic β-cell apoptosis. Further, it associates in regulating multiple pathways in diabetes mellitus; kindled us to identify the binding affinity of bioactives from Cymbopogon citratus by targeting PTP1B and identify the probably associated with it; further identifying the probable pathways involved in diabetes mellitus. In this regard, ChEBI database was used to retrieve bio-actives from C. citrates and 3D structures for the same were obtained from the PubChem database. The energy of bioactives was minimized and converted into ligand and the docking was carried using autodock 4.0 against PTP1B. Further, multiple characters of bio-actives like drug-likeness score, ADMET profile, probable adverse effects, and boiled egg model for bioavailability were also studied. Swertiajaponin was predicted for the highest drug-likeness score i.e. 0.26. However, swertiajaponin was predicted with the highest probable side effect of nephrotoxicity with pharmacological activity of 0.478. Similarly, swertiajaponin was predicted for the highest binding affinity with PTP1B with the binding energy of - 8.3 kcal/mol. Likewise, KEGG identified 80 pathways associated with PTP1B modulation in which 7 pathways were involved in diabetes mellitus in which FoxO signaling pathway was predicted to have the least false discovery rate by modulating 7 genes. Swertiajaponin could act as the potent inhibitor of PTP1B; scored highest druglikeness score but possessed minimum GIT absorptivity; further, PTP1B was identified to be linked with multiple pathways that are concerned with diabetes mellitus.

Type 2 diabetes mellitus (DM) is a complex chronic disorder and a major global health problem. Insulin resistance is the primary detectable abnormality and the main

characteristic feature in individuals with type 2 DM. Protein tyrosine phosphatase 1B (PTP1B) is a key negative regulator of the insulin signaling pathway, which dephosphorylates insulin receptor and insulin receptor substrates, suppressing the insulin signaling cascade. Therefore, the inhibition of PTP1B has become a potential strategy in the management of type 2 DM. In this study, a library of 22 pyrazoles was evaluated here for the first time against human PTP1B activity, using a microanalysis screening system. The results showed that 5-(2-hydroxyphenyl)-3-{2-[3-(4-nitrophenyl)-1,2,3,4-tetrahydronaphthyl]}-1-phenylpyrazole 20 and 3-(2-hydroxyphenyl)-5-{2-[3-(4-methoxyphenyl)]naphthyl}pyrazole 22 excelled as the most potent inhibitors of PTP1B, through noncompetitive inhibition mechanism. These findings suggest that the presence of additional benzene rings as functional groups in the pyrazole moiety increases the ability of pyrazoles to inhibit PTP1B. The most active compounds showed selectivity over the homologous T-cell protein tyrosine phosphatase (TCPTP). Molecular docking analyses were performed and revealed a particular contact signature involving residues like TYR46, ASP48, PHE182, TYR46, ALA217 and ILE219. This study represents a significant beginning for the design of novel PTP1B inhibitors.

Diabetes mellitus (DM) is a complex disease which currently affects more than 460 million people and is one of the leading cause of death worldwide. Its development implies numerous metabolic dysfunctions and the onset of hyperglycaemia-induced chronic complications. Multiple ligands can be rationally designed for the treatment of multifactorial diseases, such as DM, with the precise aim of simultaneously controlling multiple pathogenic mechanisms related to the disease and providing a more effective and safer therapeutic treatment compared to combinations of selective drugs. Starting from our previous findings that highlighted the possibility to target both aldose reductase (AR) and protein tyrosine phosphatase 1B (PTP1B), two enzymes strictly implicated in the development of DM and its complications, we synthesised 3-(5-arylidene-4-oxothiazolidin-3-yl)propanoic acids and analogous 2-butenoic acid derivatives, with the aim of balancing the effectiveness of dual AR/PTP1B inhibitors which we had identified as designed multiple ligands (DMLs). Out of the tested compounds, 4f exhibited well-balanced AR/PTP1B inhibitory effects at low micromolar concentrations, along with interesting insulin-sensitizing activity in murine C2C12 cell cultures. The SARs here highlighted along with their rationalization by in silico docking experiments into both target enzymes provide further insights into this class of inhibitors for their development as potential DML antidiabetic candidates.

Among the 2-arylbenzofuran derivatives isolated from Morus alba, the farnesylated 2-arylbenzofuran is a rarer constituent. The derivative has been reported to exert anti-obesity effect; however, its inhibitory effect on protein tyrosine phosphatase 1B (PTP1B) has not been investigated. In the previous study, the presence of the farnesyl group in the structure of 2-arylbenzofurans was found to have positive influences on their pancreatic lipase inhibitory activity. In the present study, we have confirmed the authenticity of the notation based on the PTP1B inhibitory activity of farnesylated 2-arylbenzofurans. Specifically, two farnesylated 2-arylbenzofurans [morusalfurans B (2) and C (3)] showed strong inhibitory effects on PTP1B with IC50 values of 8.92 and 7.26 μM, respectively, which was significantly higher than that of the positive controls [sodium orthovanadate (IC50 = 15.10 μM) and ursolic acid (IC50 = 11.34 μM)]. Besides, two 2-arylbenzofurans [morusalfurans A (1) and F (6)], one flavonoid [morusalnol B (9)], and one geranylated stilbene [morusibene A (11)] exhibited PTP1B inhibition with IC50 values ranging from 11.02 to 26.56 μM. Kinetic studies revealed compounds 2, 3, 6, and 11 as mixed type PTP1B inhibitors, while 1 and 9 are known as noncompetitive. Molecular docking simulations demonstrated that these active compounds can bind with the respective catalytic or/and allosteric sites of PTP1B with negative binding energies and the results are in accordance with that of the kinetic studies. To the best of our knowledge,

this is the first time, the PTP1B inhibitory activity of eleven compounds (1-11), as well as the mechanism of action underlying the effects on PTP1B enzyme of the active compounds, were investigated. In vitro and in silico results suggest that the farnesylated 2-arylbenzofurans from M. alba may potentially be utilized as an effective treatment therapy for type 2 diabetes mellitus and its associated complications.

All over the world, diabetes mellitus type 2 has spread as a problematic pandemic. Despite currently available treatments, approved drugs still show undesirable side effects and loss of efficacy or target symptoms instead of causes. Protein tyrosine phosphatase 1B (PTP1B), since its discovery, has emerged as a very promising target against this disease. Although the information regarding the enzyme is immense, little is known about the selectivity between this enzyme and its closest homologue, lymphocyte T tyrosine phosphatase (TCPTP), which is responsible for complicated side effects. In this study, on the basis of different computational approaches, we are able to highlight the importance of a phenylalanine residue located in PTP1B, but not in TCPTP, as a crucial hotspot that causes selectivity and stability for the whole ligand bound system. These results not only allow to explain the selectivity determinants of PTP1B but also provide a useful guide for the design of new allosteric inhibitors.

Aim and Objectives Computational studies of gamma-oryzanol on human protein tyrosine phosphatase 1B (PTP1B) and its role as anti diabetic agent

Molecular modeling of human protein tyrosine phosphatase 1B canonical and its natural variants sequences

Selection of best models and their evaluation

Structural analysis of the models and their binding site characterization

Molecular docking studies of gamma-oryzanol compounds on the models

Prediction of gamma-oryzanol as potential inhibitor agonist of protein tyrosine phosphatase 1B

Plan of Work

Molecular modeling of human protein tyrosine phosphatase 1B canonical and its natural variants sequences

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Materials and Methods

The human protein tyrosine phosphatase 1B receptor and its natural variants

The UniProtKB knowledge base (https://www.uniprot.org/) was searched with keyword "ptp1b" resulting 22 reviewed and 20 unreviewed entry. After searching in 22 reviewed entries about 6 entries were reported for humans from which the human protein tyrosine phosphatase 1B (PTP1B) was selected with accession id of P18031. It's a 435 amino acid residue long protein with two reported natural variants with single mutations for varinat1 (G381S) and varinat2 (P387L). The protein sequences of canonical and two natural variants were downloaded in .fasta format.

The Gamma-oryzanol compounds from PubChem

The NCBI PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was searched with the keyword "gamma Oryzanol" 8 compound entries, 65 substances entries, 141 literature entries and 77 patent entries. Out of eight compound entries the four compounds with PubChem CIDs (5282164, 51346127, 91971120, 134689750) were downloaded in 3D .sdf format and converted to .pdb format for further analysis. They were also renamed as compound1, compound2, compound3 and compound4 for ease in docking studies. A list of physio-chemical properties of these four compounds is given in table-1 and the their 3D structural views are shown in figure-1. All four

compounds have similar physio-chemical chemical properties with little variations in their structures.

Table-1: The physio-chemical properties of selected compounds

Sl. No.	Property name	CID: 5282164		CID: 51346127	CID: 91971120	CID: 134689750
1	Molecular Weight	602.9		602.9	602.9	602.9
2	XLogP	12.1		12.1	12.1	12.1
3	Hydrogen bond donor count	1		1	1	1
4	Hydrogen bond acceptor count	4		4	4	4
5	Rotatable bond count	9		9	9	9

Methods

5.2.1 Molecular modelling of human PTP1B receptor and its natural variants

Prior to model building the .fasta format sequences of canonical and two natural variants were converted to .ali format. This conversion is crucial because the molecular modelling program Modeller 10.1 recognizes protein sequences in this format.

5.2.1.1 Database similarity searching and selection of suitable templates

It seems to be the first and vital step in molecular modelling as quality of the model to be built mostly rely on the quality of the template selected for the purpose and their alignment. Out of three sequences the canonical sequence was selected for searching of templates using NCBI BLASTp program. The whole canonical sequence was put in fasta format in the given enter Query Sequence box with blank Query subrange parameters and no Job Title. The Protein Data Bank proteins (PDB) database was selected from Choose Search Set with the blastp algorithm from Program Selection. The Algorithm parameters were adjusted as Max target sequence of 10, Expect threshold of 0.05, Word size of 3, Scoring matrix as BLOSUM62, Gap Costs as (Existence of 11, Extension of 1) with Conditional compositional score matrix adjustment.

After BLAST searching the top 10 templates were ranked by three important scores like Query Coverage, E-value and Percent Identity as shown in table-2. These three scores help in selection of suitable templates out of all templates in the result. Out of 10 templates the third template 5T19 was selected as suitable template for model building and its structure was downloaded in .pdb format.

Table-2: The BLASTp search result showing templates and scores

Sl. No.	Templates PDB IDs and chain	Query Coverage	E value	Percentage of Identity
1	1A5Y_A	75%	0.00	98.79

2	1NL9_A	73%	0.00	100.00
3	5T19_A	73%	0.00	100.00
4	6W30_A	73%	0.00	100.00
5	7KEN_A	74%	0.00	99.69
6	4I8N_A	73%	0.00	100.00
7	6CWU_A	73%	0.00	99.69
8	6CWV_A	73%	0.00	99.69
9	1L8G_A	73%	0.00	99.38
10	3QKP_A	73%	0.00	99.69

5.2.1.3 Alignment of target sequence with template

The alignment of target sequence (ptp1b.ali) with the template structure (5t19.pdb) was done by editing the information in the align2d.py file (mentioned below) and running the file in Modeller. The two files generated after alignment were ptp1b-5t19.ali and ptp1b-5t19.pap which represent the alignment of the both target and template sequences and structural information respectively.

```
from modeller import *

env = environ()

aln = alignment(env)

mdl = model(env, file='5t19', model_segment=('FIRST:A','LAST:A'))

aln.append_model(mdl, align_codes='5t19', atom_files='5t19.pdb')

aln.append(file='pptp1b.ali', align_codes='ptp1b')

aln.align2d()

aln.write(file='ptp1b-5t19.ali', alignment_format='PIR')

aln.write(file='ptp1b-5t19.pap', alignment_format='PAP')
```

5.2.1.4 Building of models

The target sequence in ptp1b.ali format, template structure in 5t19.pdb format and their alignment file ptp1b-5t19.ali were put model_single.py file (mentioned below) with two scoring methods as (DOPE and GA341) and run in Modeller so as to generate about 10 homology models.

```
from modeller import *

from modeller.automodel import *

#from modeller import soap_protein_od

env = environ()

a = automodel(env, alnfile='ptp1b-5t19.ali', knowns='5t19', sequence='ptp1b',

              assess_methods=(assess.DOPE, assess.GA341))

a.starting_model = 1

a.ending_model = 10

a.make()
```

The models for varinat1 and variant2 were also built with the template 5T19 and the

methods followed was as mentioned for canonical model building.

5.2.1.5 Evaluation of models using PROCHECK

After building of models for canonical and two variants the best models were selected with the lowest readings of DOPE score and evaluated for stereo chemical accuracy of the models using PROCHECK program of ERRAT server (https://servicesn.mbi.ucla.edu/ERRAT/) . The models were evaluated by uploading the best models in .pdb formats and predicting Ramachandran Plots.

5.2.2 Molecular docking studies of gamma-oryzanol on the human PTP1B models

5.2.2.1 Preparation of gamma-oryzanol compounds

The four gamma-oryzanol compounds (5282164, 51346127, 91971120, 134689750) were prepared in AutoDockTools by opening their .pdb format files individually. During preparation the compounds were added with Gasteiger chares, polar hydrogns and selecting all 10 rotatable bonds. The selection of rotatable bonds is important as it makes the compounds flexible so that it can easily accommodated in the receptor active sites. Then they were saved in .pdbqt format for further docking studies in AutoDock VINA.

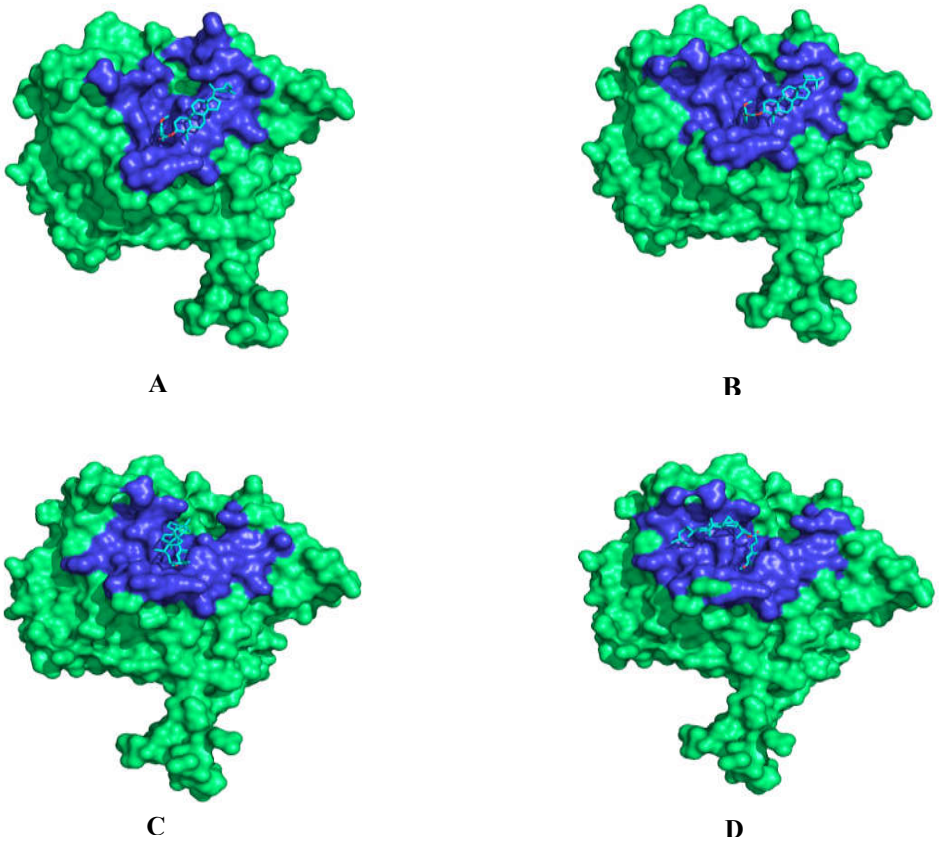
5.2.2.2 Preparation of PTP1B receptor models

The three PTP1B receptor models (canonical, variant1, variant2) were imported to AutoDockTools in .pdb format and prepared for docking. During preparation the receptor models were added with the polar hydrogens and saved in .pdbqt format for further analysis.

5.2.2.3 Preparation of grid box for docking

Prior to preparation of grid box the template 5T19 structure was analyzed for the selection of reported co-crystal ligand 73U. The amino acid residues lining 5Å area of the 73U were selected for defining the active site as shown in figure-2. The selected residues were fetched in all three prepared receptor models and the grid boxes were prepared containing these residues. After preparation of grid boxes the grid parameters were saved for their inclusion in the configuration file.

Figure-4: The alignment of PTP1B models with the template A: canonical model, B: variant1 model and C: varinat2 model



6.2 Discussions

6.2.1 Molecular docking of gamma-oryzanol compounds on PTP1B canonical model

The docking analysis is not confined to achievement of suitable docking scores by different conformation poses rather it must show the number of hydrogen formed between the residue atoms and their corresponding ligand atoms. It also important to mention the number of hydrophobic residues lining the active site where the conformation poses are accommodated.

Out of 36 conformation poses of four gamma-oryzanol compounds on PTP1B canonical model only 24 poses were observed to have at least one hydrogen bond with the residues lying in the active site. The residues involved in such hydrogen bond formation were Tyr46, Lys120, Phe182, Ala217 and Arg221. The detailed explanation of hydrogen bond interaction is mentioned in table-12 and the ligand interaction diagrams for for the best poses are shown in figure-6.

Table-12: Hydrogen bond interaction of gamma-oryzanol compounds on PTP1B canonical model

Sl. No.	Compound poses	Docking score	Ligand atom involved	Residue involved	H-bond in Å
1	5282164_1	-7.6	O44	Ala217	3.3
2	5282164_2	-7.4	O42 O44 O44	Arg221 Arg221 Phe182	3.4 3.4 3.4
3	5282164_4	-6.9	O42 O44 O44	Arg221 Arg221 Phe182	3.4 3.3 3.4
4	5282164_5	-6.9	O44	Phe182	3.4
5	5282164_6	-6.9	O42 O44	Arg221 Arg221	3.4 3.4
6	5282164_7	-6.9	O44	Arg221	2.7
7	5282164_8	-6.8	O44	Ala217	3.3
8	5282164_9	-6.8	O42	Tyr46	3.6
9	51346127_1	-7.8	O44	Ala217	3.3
10	51346127_2	-7.6	O42	Tyr46	3.2
11	51346127_3	-7.5	O44	Arg221	3.3
12	51346127_4	-7.5	O44	Arg221	3.5
13	51346127_7	-7.1	O42 O44	Arg221 Arg221	3.4 3.2
14	51346127_8	-7.0	O42 O44	Arg221 Phe182	3.3 3.4
15	51346127_9	-7.0	O44	Ala217	3.4
16	91971120_1	-7.6	O42 O44	Arg221 Arg221	3.4 3.3
17	91971120_2	-7.5	O42 O44	Arg221 Phe182	3.3 3.4
18	91971120_3	-7.5	O42 O44	Arg221 Arg221	3.3 3.3
19	91971120_4	-7.3	O42	Arg221	3.3

			O44	Arg221	3.4
20	91971120_5	-7.2	O42 O44	Arg221 Phe182	3.6 3.3
21	91971120_6	-7.0	O42 O44	Arg221 Arg221	3.3 3.4
22	91971120_7	-6.8	O44	Ala217	3.4
23	91971120_8	-6.8	O33	Lys120	3.5
24	91971120_9	-6.7	O42 O44	Arg221 Arg221	3.5 3.4

Conclusion

In the current work we report the 3D computational models of human protein tyrosine phosphatase 1B canonical and its two natural variants. Although there are many crystal structures for protein tyrosine phosphatase 1B are reported in PDB we have done computational modelling of canonical and natural variants with the reliable structural information from PDB. Again we also report gamma-oryzanol as potential inhibitor for human protein tyrosine phosphatase 1B receptor in the light of many inhibitor reported in literature and used the same binding site reported for inhibitor like 73U. Our finding are quite generous and we found gamma-oryzanol suitable to be accommodated in the designated active sites of the designed models with good docking scores (ranging -7.0 to -8.0 kcal/mol) and important interactions with some conserved amino acid residues. This study needs further computational analysis and in vitro validation.

References

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