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Identification of cost effective drug through *in-silico* approach of *Catharanthus roseus* plant ligand-receptor docking for Type 2 Diabetes NIDDM.

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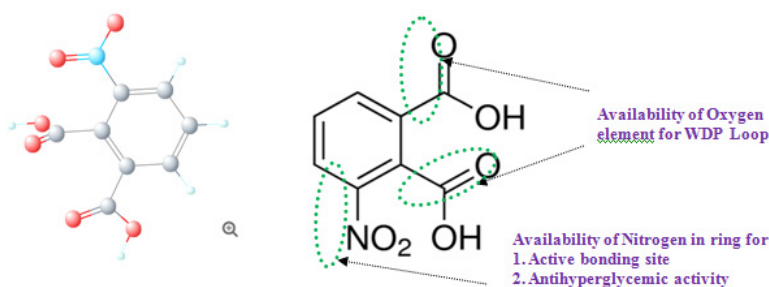
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Abstract: Objective:-Diabetes type2 is common disease found in every age-segment of human beings. This study was proposed to design cost effective drug through herbal medicinal plant *Catharanthus roseus* and its parts, directly or indirectly being used for diabetes treatment.

Methodology: - GCMS is conducted on alcoholic stem extraction to identify the different alkaloids substances. The chemical structure of these alkaloids was identified through pubchem database. PTB1B is a novel target for type 2 diabetes and its 3D structure designed by target sequence. The blast of target sequence was performed with PDB to get template 1NL9 which is 100% identical with target sequence. As a result, 1NL9 resolution is found at least 2.0 Angstroms and its *R*-factor is not greater than 20.0, hence this structure is considered as a model or a receptor. Docking of these chemical structure of alkaloids against protein-tyrosine phosphatase1B(PTP1B)is done through Autodock4.2 software. The present study involves computation of 21 compound to identify the least energy molecules. These molecules follows the Lipinski's rule and assist the drug development avoiding expensive post clinical experiments. This process provides the actual active compound for drug discovery.

Conclusion: - It is anticipated that 3-Nitrophthalic Acid has least energy molecule and it fulfills the Lipinski rules of five, hence it could inhibit the PTP1B and improve insulin action for recovery of Type-2 Diabetes. Such studies reduce the time and costs involved in drug discovery process and have no adverse effects on the environment.



Keywords: PTP1B, 1NL9, Docking, *Catharanthus roseus*. Type 2 Diabetes

1. Introduction

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus:

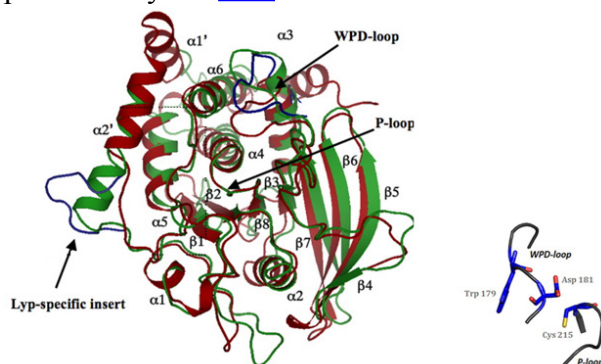
- Type 1 DM results from the body's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown^[5].
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly^[5]. As the disease progresses a lack of insulin may also develop. This form was previously referred to as "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise.
- Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level^[5].

Introduction of new drugs and novel therapeutic solutions is a long and costly process (Myers and Baker 2001; DiMasi *et al.*, 2003)^{[6][13]}. In this direction to recover or overcome from chronic diseases the drug discovery was a tiresome task in past, as lot of practical experiments were based on many chemical reactions, various methodology like laboratory work, directly usage of herbs as a medicines etc. Also, these are time consuming and need dedicated manpower's for a single discovery of drug in spite of that outcome was very less. As a revolution of computer software's, simulators and automation technology made the drug discovery cost and time effective. Imitating the common biological terms *in vivo* and *in vitro*, the term *in silico* refers to performing experiments using computers. Although the

historical origin of this term is not clear, it is safe to assume that *in silico* is a reference to the chemical element Silicon (Si), a key component of computer chips.

Several treatments and remedies are available for diabetes but still aggressive research is going on.

Catharanthus roseus (*Vinca rosea*) is the indirectly used for treatment of diabetes. Its extracts like flower, leaf, seeds, stem, roots, are used to combat diabetes. Previous research studies show that it has some important alkaloids, flavonoids, phenolics & steroids which can minimize the effect of diabetes. These chemical compounds lowers the insulin resistance in muscle and also reduce glucose produced by the liver.



WPD loop

Loop contains Asp181 and Arg224 which maximizes hydrophobic interactions.

Secondary phosphate site

Catalytically inactive, and provides weaker binding interactions compared to the primary site.

Per research studies, we found some important properties of inhibitors. The properties like (i) For basic antihyperglycemic activity Nitrogen containing ring required; (ii) Molecule should contain phosphate like moiety that is able to bind

at catalytic side of enzyme (iii) Oxygen required for hydrophobic interaction in WPD loop; (iv) Ring nitrogen required for selectivity at active binding site *i.e.* in the YRD motif (Rohan V, Trupti *et al.*, 2011)^[24]

PTP1B (protein-tyrosine phosphatase 1B) is a non-transmembrane enzyme that is found on the endoplasmic reticulum (ER). Its significance stems from being a negative regulator of insulin as well as leptin signaling. The PTP1B dephosphorylates, or in other words removes a phosphate group from the insulin receptor, IR, and also its primary substrates, which are called the Insulin Receptor Substrate proteins (IRS proteins). PTP1B in leptin, removes the phosphate from the tyrosine kinase, JAK2, which is called Janus kinase 2. More recently, it has been found to be a contributing factor in the onset of tumors, and has been linked more directly with breast cancer. It is also considered a potential drug target as its inhibition may lead to a stop in type 2 diabetes, obesity, and some forms of cancer.

Protein tyrosine phosphatase 1B (PTP1B) is an effective target for the treatment of both type 2 diabetes and obesity however, targeting PTP1B for drug discovery is challenging because of the highly conserved and positively charged active-site pocket. Tremendous progress has been made in the development of potent and selective PTP1B inhibitors that engage both the active site and no catalytic sites. Several strategies are being pursued to improve the pharmacological properties of PTP1B inhibitors^[24]. Protein Tyrosine Phosphatase 1B (PTP1B) is increasing importance in the pathophysiology of insulin resistance in diabetes mellitus but also a drug target for the management of insulin resistant status such as obesity and type 2 diabetes mellitus (Radhika R *et al.*, 2012)^[23]. Also PTP1B is involved in the down regulation of insulin and leptin signalling. Thus, inhibitors of PTP1B have potential as therapeutics for treating Type

II diabetes and obesity^[24]. Target identification and prediction of novel drugs In-silico methods have been of great importance (A Wadood *et al.*, 2013)^[1]. There is compelling evidence that 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 (Rao GS *et al.*, 2006)^[22].

2. Natural PTP 1B inhibitors

2.1 Phenolics: Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached^[4]. According to their structural characteristics, the phenolics discussed below are classified into seven groups, including flavonoids, bromophenol, phenolic acid, phenolics containing furan or pyran rings, coumarins, lignans and miscellaneous phenolics^[3].

2.2 Flavonoids: Flavonoids are polyphenolic compounds comprised of 15 carbons, with two aromatic rings connected by a three-carbon bridge. They are the most numerous natural products and exist extensively in nature. Flavonoids include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Their potential beneficial effects, such as antiviral, antitumor, antiplatelet, anti-inflammatory and antioxidant activities, greatly interest chemists and pharmacologists. For example, just between January 2007 and December 2009, 796 new naturally occurring flavonoids were isolated from various natural resources^[3].

3.0 Material and Methodology: -

3.1 Plant material:

Powdered and weighed plant materials (Flowers, leaves, stem and roots) each sample 5 gm were taken in 100mL Erlenmeyer flasks containing distilled water 250mL and 0.1 mL sulphuric acid in 99.9 distilled water was added to

it and shake with spatula till 30 minute. Mixture was macerated for 3-4 hrs and boiled gently for 25 minutes. Heavy magnesium oxide 12.5g was added to the mixture and again boiled gently for 20 minutes. It was cooled at room temperature and an equal amount of distilled water was added to make up for loss of distilled water during boiling. Alcohol(10mL) was added to remove the mucus. Mixture was filtered through Whatman filter paper (Kogan *et al*, 1953)^[12]. Filtrate was evaporated to dryness *in vacuo*, reconstituted in distill water for further analysis.

3.1.1 Identification of *C. Roseus* inhibition capability :-Antimicrobial activity had been tested all four dried sample against the microbes like bacteria and fungi. I got maximum inhibition zone in stem extract of *Catharanthus roseus*.

3.1.2 Preparation of GCMS Sample: Dried stem alkaloid extract dissolved in 10mg/ml ethanol and take 3 to 3.5 ml sample for GCMS analysis.

3.1.3 Protein Data Bank(PDB): The structure of 1NL9 which is an essential target for anti-diabetic drug design was retrieved from Protein Data Bank (1NL9.pdb).

3.1.4 Pubchem: The PubChem Compound Database contains validated chemical depiction information provided to describe substances in PubChem Substance. Structures stored within PubChem Compounds are pre-clustered and cross-referenced by identity and similarity groups.

3.1.5. Marvin Sketch: Marvin Sketch is an advanced Java based chemical editor for drawing and editing chemical structures, queries and reactions. The changes in the ligand structures to produce its analogues were made using Marvin Sketch.

3.1.6 AutodockTools

In this step, all bound waters, ligands and cofactors were removed from the proteins. The macromolecule was checked for polar hydrogens, partial atomic Kollman charges were assigned, and then atomic solvation parameters were allotted. Torsion bonds of the inhibitors were selected and defined. Secondly, the three dimensional grid box was created by Auto Grid algorithm to evaluate the binding energies on the macromolecule coordinates. The grid maps representing the intact ligand in the actual docking target site were calculated with Auto Grid. The results of the Autodock tools were viewed in the Accelrys Discovery Studio Visualiser 3.5.

3.2 Methodology: Following methods are used in *in-silico* drug design research and those are discussed as below.

3.2.1 Homology modelling: -

Homology modelling, is also recognized as comparative modelling of protein and it is a method that allows to generate an unknown atomic-resolution model of the “target” protein from its amino acid sequence and an experimental three-dimensional (3D) structure of a related homologous protein (the “template”). Homology modelling involves the recognition of one or more identified protein structures probably to show resemblance with the structure of the query sequence, and on the making of an alignment that maps residues in the query sequence to residues in the template sequence. It has been reported that the protein structures are more conserved than protein sequences amongst homologues, but sequences have less than 20% sequence identity and can have very different structures (Kaczanowski *et al*, 2010)^[12]. The proteins which are related with evolution have similar sequences and naturally occurring homologous proteins have similar protein structure. It has been revealed through the research that the evolutionarily protein three

dimensional structure is more conserved than expected because of the sequence conservation to generate a structural model of the target using the sequence alignment and template structure. Since the protein structures are more conserved than DNA sequences, detectable levels of sequence similarity usually involve substantial structural similarity. Six Bioinformatics software tools are used to generate the 3D structure of the target on the basis of the known 3D structures of templates. SWISS-model repository is a database of protein structures created with homology modelling.

3.2.2 Molecular docking (Interaction networks):-

In the field of molecular modelling docking it is a technique which envisages the favoured orientation of one molecule to a second, when bound to each other to form a stable complex (Soloman AK,2008)^[26]. Molecular docking denotes ligand binding to its receptor or target protein. Molecular docking is used to recognize and optimize drug candidates by examining and modelling molecular interactions between ligand and target macromolecules. Molecular docking are used to generate multiple ligand conformations and orientations and the most appropriate ones are selected. There are several molecular docking tools available that includes ArgusDock, DOCK, FRED, eHITS, AutoDock. Molecular modelling involves scoring methods that are used to rank the affinity of ligands to bind to the active site of a receptor. In virtual high-throughput screening, compounds are docked into the active site and then scored to determine which one is more likely to bind tightly to the target macromolecule.

3.2.3 Virtual high-throughput screening: -

Virtual screening is a computational technique where large libraries of compounds are evaluated for their potential to bind specific sites on target

molecules such as proteins, and well-matched compounds tested. The research in the drug discovery process involves virtual screening (VS) which is a computational method used for the rapid exploration of large libraries of chemical structures to identify those structures that are most likely to bind to a drug target, usually a protein receptor or enzyme. Virtual screening plays a vital role in the drug discovery process^[7]. The term “virtual screening” is relatively new as compared to the more general and old concept of database searching (Walters, et al.,1998)^[29] define virtual screening as “automatically evaluating very large libraries of compounds” using a computer program. It is clear from above definition that VS has been a numbers game at large scale and it is focusing to find out answers of questions like how can we screen down the huge chemical space of over 10 possible compounds to a practicable number that can be synthesized, purchased, and tested. Although filtering the whole chemical universe might be an interesting question, more practical VS scenarios focus on designing and optimizing targeted combinatorial libraries and enriching libraries of available compounds from in-house compound repositories or vendor offerings. It is less expensive than High-Throughput Screening, Faster than conventional screening, scanning a large number of potential drugs like molecules in very little time. HTS itself is a trial and error approach but can be better complemented by virtual screening.

3.2.4 Quantitative structure activity relationship (QSAR): -

Quantitative structure-activity relationships (QSAR) methods are used to show a relationship of structural and or property descriptors of compounds with their biological activities. These descriptors explaining the properties like steric, topologic, electronic and hydrophobic of numerous molecules, have been determined through empirical methods and only more

recently by computational methods (Suh *et al.*, 2002)^[27].

3.2.5 Implementation Steps :

COMPUTER AIDED DRUG DESIGN

1. Retrieval of protein sequence from NCBI and run Blast against PDB database.
2. If we get more sequence identity near 100% then it has to be modeled through different 3D structure prediction tool.
3. Validation is carried out by PDBsum search.
4. Pubchem database for retrieval of inhibitor of PTP1B. We will search identity/similarity of inhibitor on >95% similarity.
5. 3D structure of Template 1NL9 was used as a Receptor for docking. Open in Discovery studio and delete heteroatom, water and Ligand groups.
6. Take PDBsum and fill PDB code click on Ligplot where amino acid bind and take x,y,z coordinate.
7. If ligand is available, we will perform Pharmacophore feature screening. If no free ligand, then will perform target based screening.
8. Perform docking by Autodock 4.2 between receptor and ligand. First we should prepare ligand and prepare protein for docking. Analyze the docking Result. We will select the least energy molecules because it has highest binding affinity as a novel drug to treat diabetes.

3.2.7 Lipinski's rule of five

The ability to predict the pharmacological properties of compounds based on their structure is important. There are specific rules that apply to predict activity. *Lipinski's Rule of Five* is a refinement of drug-likeness and is used to predict whether a chemical compound will have pharmacological or biological activity as an orally active drug in humans^[2]. This rule was

formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small, lipophilic molecule. The Lipinski "Rule of Five" states that compounds are likely to have good absorption and permeation in biological systems and are more likely to be successful drug candidates if they meet the following criteria:

- Five or fewer hydrogen-bond donors
- Ten or fewer hydrogen-bond acceptors
- Molecular weight less than or equal to 500
- Calculated logP less than or equal to 5

Compound classes that are substrates for biological transporters are exceptions to the rule. The molecular docking studies and Lipinski's rules facilitate drug development avoiding expensive post clinical experiments.

Target Sequence of Protein Tyrosin Phosphatase 433aa in length retrieved from NCBI.

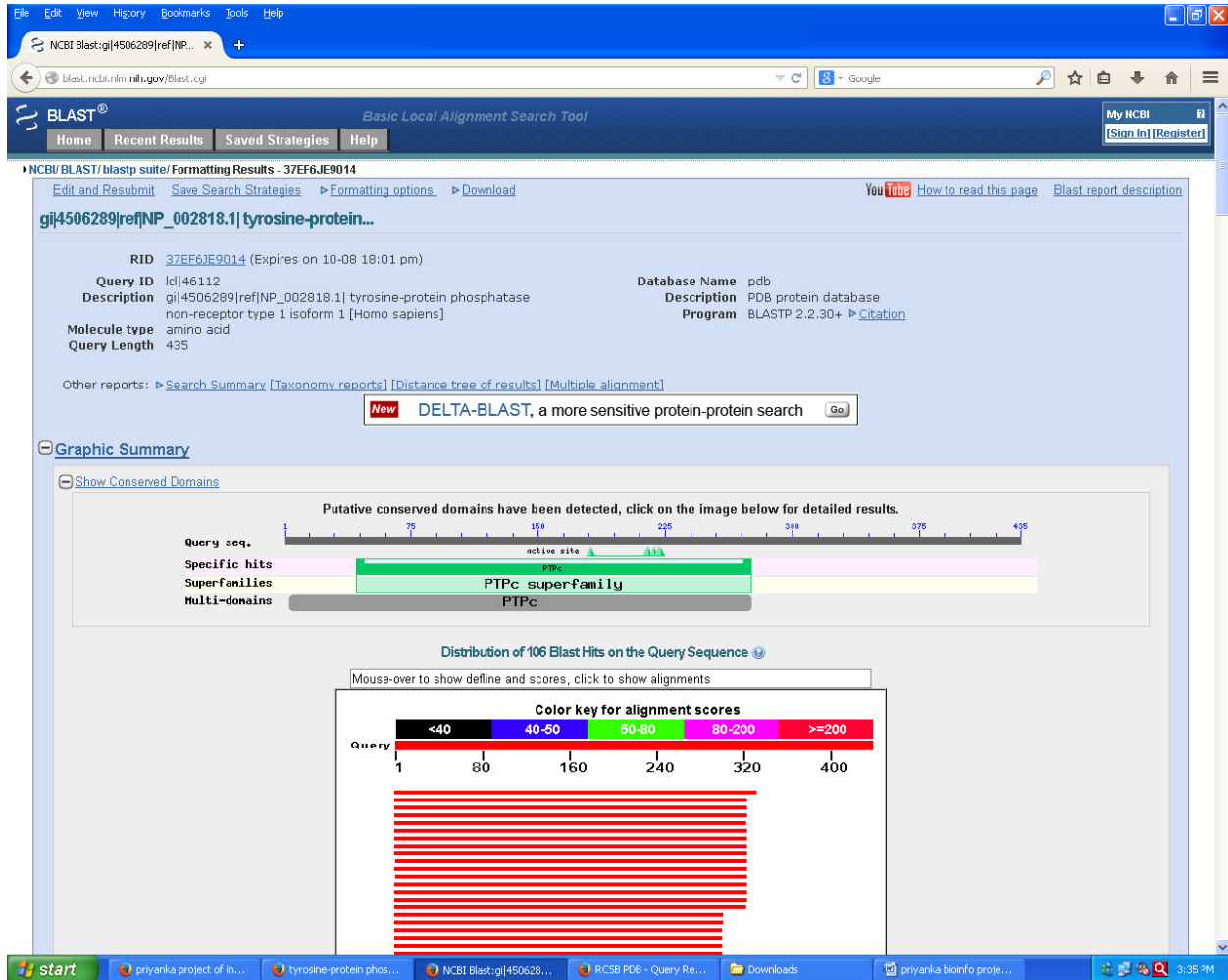
>gi|4506289|ref|NP_002818.1| tyrosine-protein phosphatase non-receptor type 1 isoform-1[Homo sapiens]

MEMEKEFEQIDKSGSWAAIYQDIRHEASD-FPCRVAKLPKNKNRNRDVSFPDHS-RIKLHQEDNDYINASLIKMEEAQRSY-ILTQGPLPNTCGHFWEMVWEQKSRGV-VMLNRVMEKGLKCAQYWPQKEEKE-MIFEDTNLKLTLISEDIKSYTTRQLELEN-LTTQETREILHFHYTTWPDFGVPESPAS-FLNFLFKVRESGSLSPHEGPPVVHCSAGI-GRSGTFCLADTCLLLMDKRKDPSSVDIK-KVLLEMRKFRMGLIQTADQLRFSYLA-VIEGAKFIMGDSSVQDQWKELSHE-DLEPPPEHIPPPRPPKRILEPHNGKCREFFPNHQWVKEETQEDKDCPIKEEKG-SPLNAAPYGIEMSQDTEVRSRVVGGSLR-GAQAASPAKGEPSLPEKDEDHALS YWKP-FLVNMCVATVLTAGAYLCYRFLFNSNT

4.2 Validation of 3D structure by using

4. Figures and Tables

4.1 Figure:-



Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Protein Tyrosine Phosphatase 1b, Cysteine/Phosphate Intermediate (Homo sapiens)	684	684	75%	0.0	99%	IABY_A
Chain A, Potent, Selective Protein Tyrosine Phosphatase 1b Inhibitor Compound 12 Using A Linked-Fragment Strategy (Homo sapiens)	672	672	73%	0.0	100%	INB9_A
Chain A, Crystal Structure Of Ptp1b Complexed With 7-(1-(1-dioxo-1h-benzodisothiazol-3-yl)methyl)-2-(oxalyl-amino)-4,7-dihydro-5h-benzofurazan (Homo sapiens)	671	671	73%	0.0	99%	1L8Q_A
Chain A, Protein Tyrosine Phosphatase 1b - Asp191 Mutant With Open Wedge Loop (Homo sapiens)	670	670	73%	0.0	99%	2Q9P_A
Chain A, Crystal Structure Of Protein Tyrosine Phosphatase 1b In Complex With An Inhibitor 14-(2s)-2-(1,3-benzoxazol-2-yl)-2-(4-fluorophenyl)-2-methylpropanoic acid (Homo sapiens)	671	671	73%	0.0	100%	4B8L_A
Chain A, Crystal Structure Of Protein Tyrosine Phosphatase 1b Complexed With Acetyl-D,L-Boa-Pho-L-L-P-g-G-L (Homo sapiens)	670	670	73%	0.0	99%	1E8L_A
Chain A, Human Ptp1b Catalytic Domain Complexed With Trp (Homo sapiens)	669	669	73%	0.0	99%	1B2C_A
Chain A, Ptp1b With The Catalytic Cysteine Oxidized To Sulfonic Acid (Homo sapiens)	669	669	73%	0.0	99%	1O6O_A
Chain A, Structure Of Peptide Inhibitor Complex Of Ptp1b (Homo sapiens)	670	670	73%	0.0	99%	3ZMP_A
Chain A, Structural Basis For Inhibition Of Protein Tyrosine Phosphatase 1b By Isothiazolidinone Heterocyclic Phosphonate Mimetics (Homo sapiens)	669	669	73%	0.0	100%	2CMA_A
Chain A, Crystal Structure Of Protein Tyrosine Phosphatase 1b Complexed With Phosphatidylcholine-Containing Hexa-Peptide (Gadent-2b) (Homo sapiens)	669	669	73%	0.0	99%	1FTU_A
Chain A, Nitrate In The Active Site Of Ptp1b Is A Putative Mimetic Of The Transition State (Homo sapiens)	670	670	73%	0.0	100%	4BQ_A
Chain A, Crystal Structure Of The S-Nitrosylated Cys215 Of Ptp1b (Homo sapiens)	668	668	73%	0.0	99%	3E0V_A
Chain A, Crystal Structure Of Protein Tyrosine Phosphatase 1b Complexed With Phosphatidylcholine (Homo sapiens)	668	668	73%	0.0	99%	1FTV_A
Chain A, Crystal Structure Of Protein Tyrosine Phosphatase 1b Complexed With Two Bis(Para-Phosphonohexyl)dimethane (Bapm) Molecules (Homo sapiens)	667	667	73%	0.0	99%	1AAX_A
Chain A, Human Protein Tyrosine Phosphatase 1b, C215A, S218A Mutant (Homo sapiens)	667	667	73%	0.0	99%	3ZVZ_A
Chain A, Crystal Structure Of Ptp1b With Bicyclic Thiophene Inhibitor (Homo sapiens)	627	627	68%	0.0	100%	2A9R_A
Chain A, Crystal Structure Of Ptp1b Inhibitor Complex (Homo sapiens)	625	625	68%	0.0	99%	2HT7_A
Chain A, Human Ptp1b Catalytic Domain Complexed With Fru179326 (Homo sapiens)	625	625	68%	0.0	100%	1J7D_A
Chain A, The Structure Of Phosphotyrosine Phosphatase 1b In Complex With Compound 2 (Homo sapiens)	625	625	68%	0.0	100%	1J6L_A

4.1.1 A Screen showing Protein BLAST Result of Target Sequence with PDB Database.



4.1.2 A Screen showing structure of the Template 1NL9

Its resolution of at least 2.0 Angstroms and *R*-factor no greater than 20.0. So, we selected this structure as a model or receptor.



1. ps² Model



2. PHYRE Model



3. Swiss-Model



4. Raptor X Model



5. CPH Model



6. 3D-Jigsaw Model

Procheck

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and *R*-factor no greater than 20.0 a good quality model would be expected to have over 90% in the most favored regions [A, B, L]. So ps² model is best model.

4.2.1 Tables: - Listing of 3D structure models with Favored regions.

From this table ps² and Phyre2 is best 3D structure model of protein because percentage of residues incoreregion more than 90%. Four general classes of inhibitors of PTP1B: Difluoromethylene, phosphonates, 2-carbomethoxy -benzoic acids, 2-oxalylaminobenzoic acids and lipophilic compounds. The docking studies were performed for the downloaded inhibitor from pubchem database with 1NL9 receptor and the results were compared with the ligand 989 322(A) present within the receptor. bond interactions with receptor. All bound waters, ligands and cofactors were removed from the proteins for docking. Arg 221(A), Gly 220(A), Ile219(A), Ala 217, Ser216(A), Asp48(A) are active site present in 1NL9 receptor. The docked complexes of the designed compounds along with the ligand receptor poses have designed compounds were found to display good binding affinity to the receptor. Green dotted line indicates H-bond.

4.2.1 Table:

Model name along with favoured regions

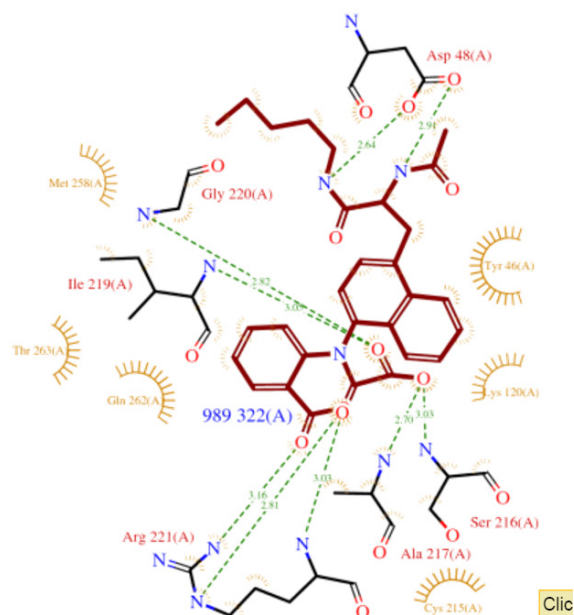
Sr.	Model Name	% Favored regions
1	ps ²	95.70%
2	PHYRE2	90.30%
3	Swiss-Model	88.30%
4	Raptor X	88.30%
5	CPH	87.80%

6	3D-Jigsaw	84.60%
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4.2.2 Table:

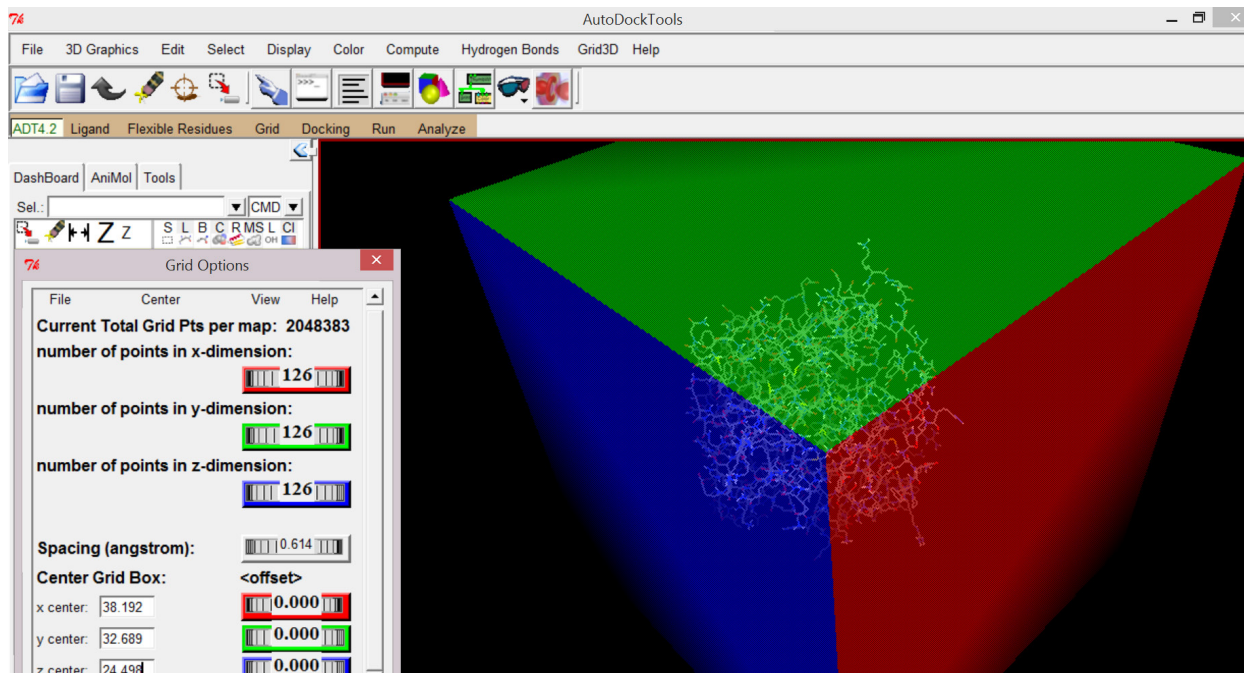
Co ordinate calculation of active site

Active site Ser 216(A)	X	Y	Z
N	38.928	31.231	25.045
O	37.456	34.148	23.951
X,Y,Z co-ordinates	38.192	32.689	24.498



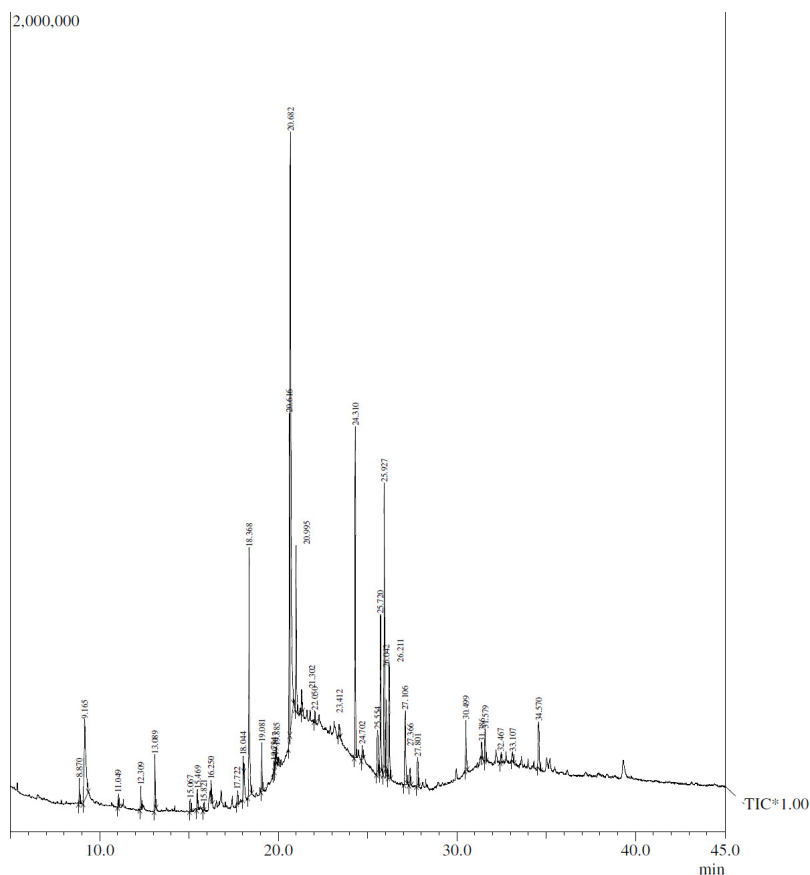
LIGPLOT of interactions involving ligand

Grid file is the imaginary box, within the range of volume where the given ligand search for best possible binding characteristics with lowest energy and high affinity. We have set the 3D space where in the search process for better binding conformation has to be attained. 126 is the maximum given by Autogrid and any spatial modification required are to be done (Shivakant tripathi, 2013). Docking algorithm based on the tetrahedral grid model of proteins allows a more precise description of shape complementarity (P.



4.1.3 A Screen Shot of Grid Preparation in Receptor

Graph4.3:- GCMS Profile of Alkaloids Stem



Peak#	R. Time	Area%	Compound Name	Structure
	8.870	0.54	1-Dodecanol	
	9.165	5.96	4-Hydroxy-4-Phenyl-3-Buten-2-One	
	11.049	0.26	Phenol, 3,5-Di-Tert-Butyl-	
	12.309	0.53	1-Hexadecanol \$\$ N-Cetyl Alcohol \$\$ N-Hexadecan-1-Ol	
	13.089	1.41	3-Methyldiphenylamine \$\$ Benzenamine, 3-Methyl-N-Phenyl	
	15.067	0.33	Benzene, 1,1'-(1,2-Cyclobutenediyl)Bis-, Trans- \$\$ Trans-1,2-Diphenylcyclobutane	
	15.469	0.56	Bromoacetic Acid, Pentadecyl Ester \$\$ Pentadecyl Bromoacetate #	
	18.044	0.69	2-Methyl-E,E-3,13-Octadecadien-1-Ol \$\$ (3E,13Z)-2-Methyl-3,13-Octadecadien-1-Ol #	
	18.368	7.68	Ethyl Pentadecanoate	
	19.081	1.32	N-Pentadecanoic Acid, Trimethylsilyl Ester	
	19.885	0.54	2-Buten-1-On, 3-Amino-1-Phenyl-4,4,4- Trifluoro	
	20.616	8.78	Linoleic Acid Ethyl Ester \$\$ Ethyl Linoleate \$\$ 9,12-Octadecadienoic Acid (Z,Z)-, Ethyl Ester	
	20.682	22.72	Oelsaeure	
	20.995	4.64	Heptadecanoic Acid, Ethyl Ester \$\$ Ethyl Heptadecanoate \$\$ Einescs	
	21.302	0.71	11-Trans-Octadecenoic Acid, Trimethylsilyl Ester	
	23.412	0.36	9-Octadecenamide, (Z)- \$\$ Adogen 73 \$\$ Oleamide	
	24.310	8.85	1,2-Propanediol, 3-Benzyloxy-1,2- Diacetyl- \$\$ 3-Benzyloxy-1,2-Diacetyl-1,2- Propanediol	

25.554	1.47	1,2-Benzenedicarboxylic Acid, 3-Nitro- 3-Nitrophthalic Acid	
25.927	9.26	(E)-3,3-Diphenyl-4-Hexenoic Acid	
26.211	3.89	S - (1 , 3 - D i p h e n y l b u t y l) Dimethylthiocarbamate	
27.106	2.93	5-(O-Benzamido-N-Bnezoylanilino) Tropolone	
27.366	0.60	2,4-DIBENZOYL-2,4-DIAMINO- NITROBENZOL \$\$ N-[5- (BENZOYLAMINO)-2-NITROPHENYL] BENZAMIDE	
30.499	1.56	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl- ,Squalene	
31.579	0.98	Cholest-5-En-3-Ol (3.Beta.)- Carbonochloridate \$\$ Cholesterol, Chloroformate	

Chitra,2011)^[19].

Results and discussion: -

Drug Likeliness Analysis using Lipinski

Rule:- Chemical name of 21 alkaloids are identified from GCMS of *Catharanthus roseus* extractions. Docking of modified structures are done with Autodock 4.2 tool considering PTP1B as receptor to identify their energy values. Docking allows one to virtually screen the number of compounds and predict the strongest binders based on scoring functions. It calculates how the receptor and the ligand fit together and dock to each other well. It has been observed that only 12 ligands docking successfully responded by tool, rest showed infinite energy. In this study compounds like 3-Nitrophthalic Acid, (E)-3,3-Diphenyl-4-Hexenoic Acid,

5-(O-Benzamido-N-bnezoylanilino) tropolone, trans-1,2 Diphenyl cyclobutane can be used as anti-diabetic. These compounds are docked with PTP1B to produce different least energy values and these values are -7.3, -6.38, -6.31 and -6.28 (Kcal/mol) respectively.

The receptor and ligand are well fitted to each other to calculate least energy. A traditional method to evaluate drug-likeness is to check compliance of “Lipinski’s Rule of Five” against with compound as shown in table 4.2.4, which covers the numbers of hydrophilic groups, molecular weight and hydrophobicity. We have taken structures from Pubchem of all ligands and observed that 3-Nitrophthalic Acid having Nitrogen in ring for active binding sites as well as antihyperglycemic activity. Also, it has Oxygen element for hydrophobic interaction in

4.2.4 Table: - Ligand results and its drug Likeliness Analysis Using Lipinski rule

S.No	Ligand Name	Ligand results through Autodock tool				Drug Likeliness Analysis using Lipinski Rule of Five				
		Min. Energy of binding (Kcal/mol)	Reference RMSD Value (A0)	Cluster RMSD Value (A0)	No. of torsion in Ligand	Molecular Weight <500 (g/mol)	Hydrogen Bond Donor <5	Hydrogen Bond Acceptor <10	Xlog P Value <5	Rule of Five
1	3-Nitrophthalic Acid	-7.3	49.94	0	5	211.12	2	6	0.7	4
2	(E)-3,3-Diphenyl-4-Hexenoic Acid	-6.38	45.63	0	9	338.39	1	4	4.6	4
3	5-(O-Benzamido-N-Bnezoylanilino) Tropolone	-6.31	69.92	0	6	436.45	2	4	4.6	4
4	Trans-1,2-Diphenylcyclobutane	-6.28	53.05	0	2	208.29	0	0	4.6	4
5	3-Methyldiphenylamine	-5.44	49.59	0	2	183.24	1	1	3.8	4
6	4-Hydroxy-4-Phenyl-3-Buten-2-One	-4.9	54.18	0	3	162.18	1	2	2	4
7	Phenol, 3,5-Di-Tert-Butyl-	-4.82	50.77	0	3	206.32	1	1	4.9	4
8	2-Buten-1-On, 3-Amino-1-Phenyl-4,4,4-Trifluoro	-4.25	52.51	0	4	215.17	1	5	2.6	4
9	Cholest-5-En-3-Ol (3.Beta.)-Carbonochloridate \$\$ Cholesterol	-6.64	44.14	0	7	386.65	1	1	8.7	3
10	9-Octadecenamide, (Z)-\$\$ Adogen 73	-3.64	39.27	0	15	281.47	1	1	6.6	3
11	1-Dodecanol	-2.98	51.05	0	11	186.33	1	1	5.1	3
12	1-Hexadecanol	-2.69	51.25	0	15	242.44	1	1	7.3	3

WDP loop as shown in figure 4.3.2. It covers almost all conditions which are highly required for anti-diabetic inhibitor. The 3-Nitrophthalic Acid can be taken as good anti diabetic agents as it is small hydrophilic compound, having good binding affinity with receptor enzyme protein and having least energy among all others. It is a precursor of 3-amino benzoic acid which is similar to 2-oxalylaminobenzoic acids, general

classes of inhibitors of PTP1B. It will inhibit the PTP1B and improve the insulin action. Also one compound Cholest-5-En-3-Ol (3.Beta.)-Carbonochloridate having 2nd least energy but it follow “Lipinski rule of five” except ‘Log_p’ value, hence It could also be consider for drug discovery. Calculation of Lipinski’s properties showed that most of the compounds followed all criteria. Such studies reduce the time and

costs involved in drug discovery process to further lead the laboratory work. and have no adverse effect on the environment.

Protein-protein dockin basically depends on the calculation of energy minimization and calculations of root mean square-distance(RMSD)(Thanigaivelan *et al.*, 2010)^[27]. The molecular docking studies and Lipinski's rules facilitate drug development avoiding expensive post clinical experiments(P.Lalitha *et al.*,2011)^[20].While the seminal work of Hansch and Fujita (1964) on the statistical relationships between the molecular structure and a specific chemical or biological property^[9] (Quantitative structure-activity relationships) initiated the application of modern data mining and statistical techniques such as the virtual ligand screening (Oprea and Matter, 2004)^[18] and the virtual affinity profiling (O'Connor and Roth, 2005; Paolini *et al.*, 2006)^{[17][21]}, biophysical (Jones and Woodhall, 2005; Graupner and Gutkin,2009)^[8]^[10] and neurochemical network models (Noori and Jäger, 2010; Noori, 2012; Noori *et al.*, 2012)^{[14][15][16]}mainly apply deterministic dynamical systems to identify drug-induced alterations of electrophysiological and or neurochemical network characteristics.

Conclusion:-. Docking and by applying “Lipinski Rule of Five” on ligands we can conclude that maximum compounds extracted from *Catharanthus roseus* are having Antimicrobial attributes. In which 3-Nitrophthalic Acid compound may be a anti diabetic agent as it satisfying most of the conditions, hence the above studies supports the identification of least energy molecule compound which inhibited the PTP1B and it could improve the insulin action. Further seminal work on the statistical relationships between the molecular structure and a specific chemical or biological property work can be initiated for drug discover through data mining and statistical techniques. In such way, these

all factors are very helpful for categorization and identification of new bio products & their toxicity prediction in human body. Such studies reduce the time and costs involved in drug discovery process and have no adverse effect on the environment.

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