EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Special Issue-4 (October-2017)

Journal home page: http://www.ijcrar.com

In Silico Molecular Modeling and Docking Studies of *Catharanthus roseus* derived Alkaloids against *GLUT4*

R. Padmini*, K. Reshma and K. Sangeetha

Department of Biochemistry and Bioinformatics & Clinical Trial Management, Dr. MGR Janaki College of Arts and Science, Adyar, Chennai, Tamil Nadu, India

Corresponding author

KEYWORDS

Type 2 Diabetes, GLUT4, Homology modeling, *Catharanthus roseus*, AutoDock

A B S T R A C T

Diabetes is a complex disease associated with genetic and environmental factors. Type 2 Diabetes is the most common form of Diabetes which has become an emerging issue in developing countries which affects approximately 70% of the patients. Glucose transporter-4 (GLUT4) is the most important glucose transporters which plays a significant role in the progression of Type 2 Diabetes. Knowledge of the three dimensional structure of Glut4 will provide the capacity to understand the mechanism of glucose transport and its regulation. Homology modeling was performed using MODELLER9.14 to predict the three dimensional structure of Glut4. *Catharanthus roseus* belongs to the family apocynaceae and it has various medicinal properties. The plant has a rich source of alkaloids which is responsible for its medicinal purposes. The bioactive compounds present in the leaves shows antidiabetic activity. The interaction studies of the compounds such as cathovaline, vindoline, yohimbine, vindolinine, and lochnerine present in the leaves of *Catharanthus roseus* and modeled structure of Glut4 was studied using AutoDock. Among these compounds cathovaline, vindolinine and lochnerine show least binding energy with favorable Hydrogen bond interactions. This study paves the way for understanding the mechanism of these bioactive compounds against Glut4 protein and it can act as a potential anti-diabetic agent in future studies.

Introduction

Diabetes is evolving as one of the most fatal diseases due to impaired glucose metabolism. It is a complex disease associated with various metabolic disorders. The main feature of which is chronic hyperglycemia which is due to insufficient action of insulin (Yutaka *et al.*, 2010).

The impaired metabolism of glucose, lipids, and proteins in diabetes produces alterations in macro and micro-vascular circulation that are associated with the five classic complications of the disease, i.e., retinopathy, neuropathy, nephropathy, cardiovascular complications, and delayed

wound-healing. (Mealey BL., 2006) There are two primary forms of diabetes, Insulin dependent Diabetes mellitus (Type 1)and Non-Insulin dependent Diabetes mellitus (Type 2) (Yanling Wu*et al.,* 2014). It is difficult to establish the prevalence in the general population, which has been estimated at 1- 6% according to the diagnostic criteria used. Approximately 90% of cases correspond to patients with non-insulin dependent Type-2 Diabetes (Mealey BL., 2000).Type 2 Diabetes mellitus is a complex metabolic disorder of heterogeneous etiology with social, behavioural and environmental risk factors unmasking the effects of genetic susceptibility. (Kiess W *et al.,* 2003).

Glucose transporter-4(Glut4) is a 12 transmembrane, 509-aminoacid protein containing a large cytoplasmic loop between transmembrane (Birnbaum M.J., 1989). Glut4, the predominant Insulin responsive glucose transporter isoform, plays a key role in the process of transporting extracellular glucose into Insulin sensitive cells. It exist only in skeletal muscle and adipose tissues which are responsible for 50% to 80% glucose transportation in the body. After being transported into Insulin sensitive cells, the glucose is decomposed or synthesized to glycogen to maintain normal glucose tolerance (Chang L *et al.,* 2004). In adipose cells, Glut4 are constantly recycling between intracellular compartments and the plasma membrane. In the basal state the majority of the Glut4 is located in an intracellular tubulo-vesicular compartment referred to as the Glut4 storage compartment (GSC).Insulin induces an increase in the rate of exocytosis of Glut4 containing vesicles, resulting in a rapid shift in the steady state distribution of Glut4 to the plasma membrane.The Glut4 glucose transporter is thus a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis(Shaohui H *et al.,* 2007).

Catharanthus roseus is tropical/subtropical plant that spread throughout the world. (Karthikeyan B *et al.,* 2008.) The plant belongs to the family of Apocynaceae. The colour of the flower is purple, pink and white. (Padua *et al.,* 1999) It has different names such as Tapak dara, Kemuning china, Periwinkle, Ainkati, Nityakalyani, Sadaphul, Chatilla etc.

The plant and its parts as many activities such as anticancer, antidiabetic, antibacterial, hypolipidemic, antioxidant, antihypertensive, wound healing activity, antihelminthic, cytotoxic activity etc. The plant have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions.The phytoconstituents of *Catharanthus roseus* are alkaloid,glycoside,carbhohydrate,flavonoids, tannin,saponins,protein,aminoacid and the *Catharanthus roseus* leaves contain tannin, alkaloids, flavonoids. The plant contains about 130 alkaloids. The several alkaloids are reserpine, ajmalcine, raubasin, catharanthine and vindoline. The compounds present in leaves are vindolidine, vindolicine, vinblastine, vincristine, leurosine, yohimbine, ibogaine etc. (EM Sutrisna 2015).

In molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. (Lengauer and Rarey, 1996). Protein-ligand docking aims to predict and rank the structures arising from the

association between the ligand and the target protein of known 3D structure (Abdelouahab Chikhi, 2008).

Materials and Methods

Uniprot

Uniprot is a freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological functions of proteins derived from the research literature. Uniprot provides databases such as the Uniprot knowledgebase (UniProtKB), the Uniprot reference clusters (UniRef) and the Uniprot Archive (UniParc) (www.uniprot.org/). The primary sequence of Glut4 (solute carrier family 2, facilitate glucose transporter member 4) with an accession number P14672 is retrieved from Uniprot.

BLASTP

BLAST for Basic Local Alignment Search Tool is an algorithm for computing primary biological sequence information. Such as the amino -acid sequence of proteins or the nucleotides of DNA sequence. A Blast search enables a researcher to compare a query sequence with a library or databases of sequences and identity library sequences that resemble the query sequences above a certain threshold. In BlastP, the primary sequence of Glut4 has been given and searched against protein databank for the template.

MODELLER

MODELLER 9.14 is used for homology or comparative modeling of protein in three-dimensional structure. The user provides an alignment of a sequence to be modeled with known related structure and MODELLER automatically calculates a model containing all non-hydrogn atoms. Comparative modeling consists of four main steps (1) Fold assignment that identifies overall similarity between the target and atleast one known template structure (2) Alignment of the target sequences and the template (3) Building a model based on the alignment with the chosen template and (4) Predicting the accuracy of the model. The target sequence and 3D structure template (5EQG) has been given as input. The three dimensional structure of Glut4 protein structure is obtained using MODELLER program.

Procheck

The Procheck suite of programs provides a detailed check on the stereochemistry of a protein structure. Its outputs comprise a number of plots in PostScript format and a comprehensive residue-by-residue listing. The Procheck programs are useful for assessing the quality not only of protein structures in the process of being solved but also of existing structures and of those being modeled on known structures. The Ramachandran plot provides an easy way to view the distribution of torsion angles in a protein structure.

Pubchem

Pubchem (https://pubchem.ncbi.n/m.nih.gov) is a public respository for information on chemical substances and their biological activities. Chemical entities in Pubchem substances records that have known structure are validated converted into a standardized form and imported into Pubchem compound. Pubchem consist of three inter-linked databases, substance, compound and bio Assay. The 2D structure

compounds of *Catharanthus roseus* such as cathovaline, vindoline, yohimbine, vindolinine, lochnerine are obtained from Pubchem.

Acd Chemsketch

Acd/chemsketch is a molecular modeling program used to create and modify images of chemical structure and it is a software that allows molecules and molecular models displayed in two and three dimensions to understand the structure of chemical bonds and the nature of the functional groups.The 2D structure compounds of *Catharanthus roseus* are converted into 3D structure and saved in MDL mol format.

Open Babel

OpenBabel is an open collaborative project allowing anyone to search, convert, analyze or store data from molecular modeling, chemistry, solid-state materials, biochemistry or related areas.

It is available for Windows, Unix, Linux, Macos and Android. It is free and open source software released under a GNV general public licenes (GPL).The mol format of 3D structure compounds are converted into pdb format using open babel.

Autodock

Autodock is a molecular modeling simulation software. It is especially effective for protein-ligand docking. Autodock is a widely used docking program developed at the scripps research institute. Application of Autodock requires several separate pre-docking steps example: ligand preparation, receptor preparation and grid map calculations before the actual docking process can take place. Before the docking runs, Autodock Tools (ADT) was used to add polar hydrogen to the prepared receptor. The three dimensional affinity and electrostatic grid box were generate to cover the entire active site using autogrid.

Pymol

Pymol is computer software, a molecules visualization systems created by warren Lyford Dclano. It is user sponsered, open-source software released under the python license. The docking results are viewed and analyzed using Pymol.

Results and Discussion

Homology Modeling

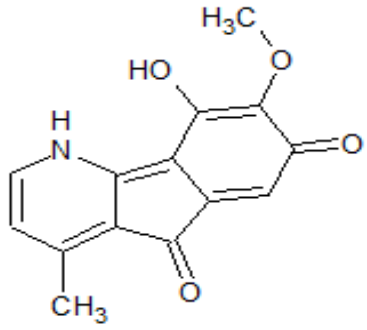
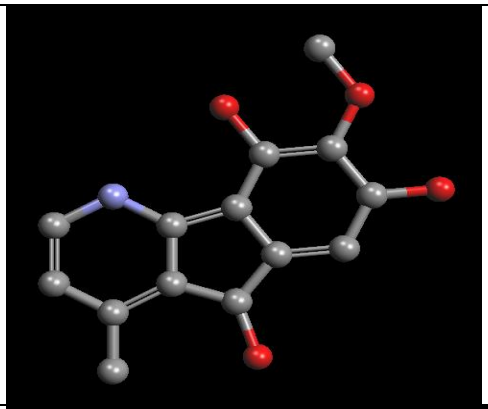
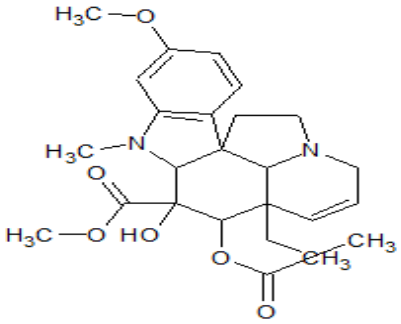
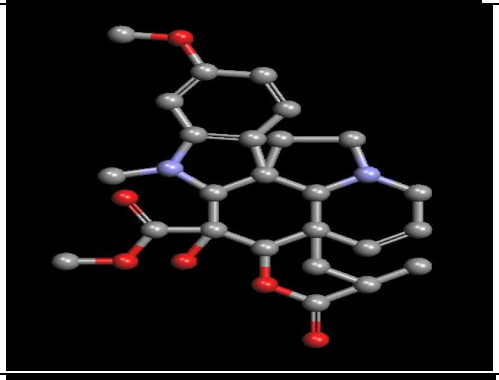
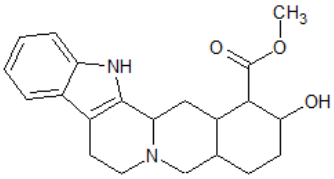
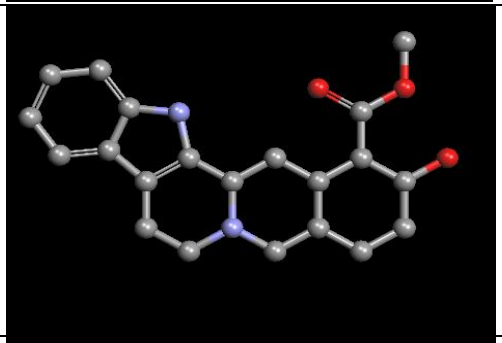
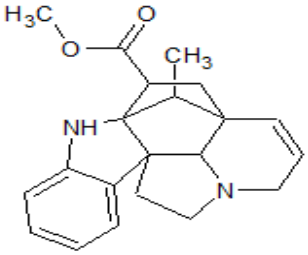
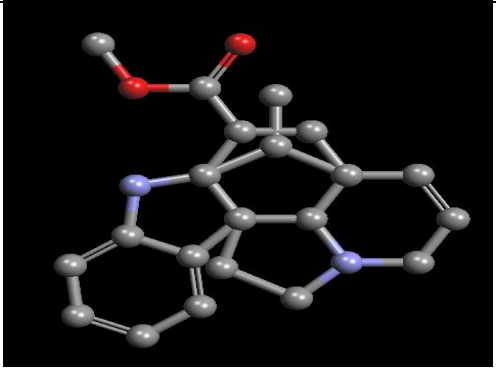
The Primary Sequence of Glut4 to be modeled is given in BlastP and searched against Protein Data Bank to identify the template. The structure with the higher sequence similarity to the modeled sequence has been selected as a template.The template for the Homology Modeling MFS transporter in complex with inhibitor (2~{S})-3-(4-fluorophenyl)-2-[2-(3-hydroxyphenyl) ethanoylamino]-~{N}-[(1~{S})-1-phenylethyl]propanamide (5EQG.PDB) was Identified (Fig 2). Using the template which is downloaded from PDB and the primary sequence, the Glut4 structure is generated by the MODELLER (9.14) program.

Structure Validation

Protein Structure validation is done to evaluate the Glut4 protein which is modeled in Modeller. The Procheck checks the stereochemical quality of a protein structure. Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. The coloring/shading on the plot represents different regions labelling of residues in disallowed regions can be

switched off or alternatively can be extended into other region (Fig.3). The results of Ramachandran plot of residues with favourable regions are shown in (Fig.4).

Table.1 2D and 3D Structure of Ligands

COMPOUNDS	2D STRUCTURE	3D STRUCTURE
CATHOVALINE		
VINDOLINE		
YOHIMBINE		
VINDOLININE		

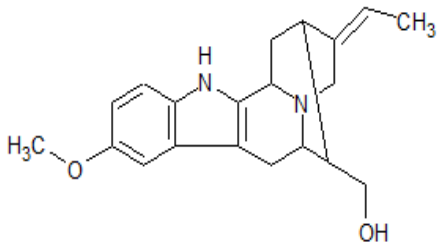
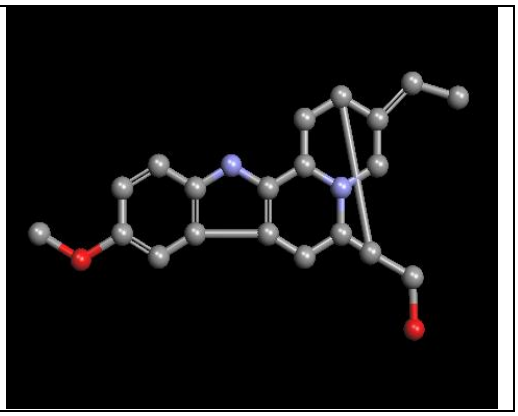
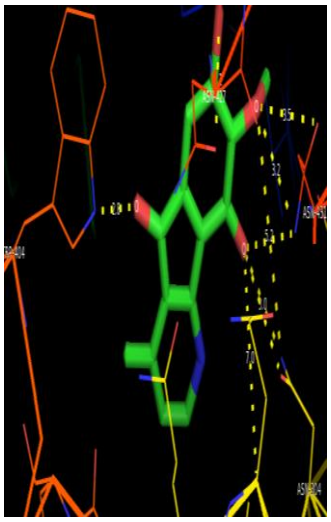
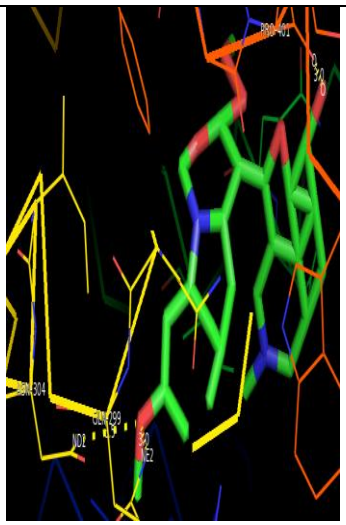
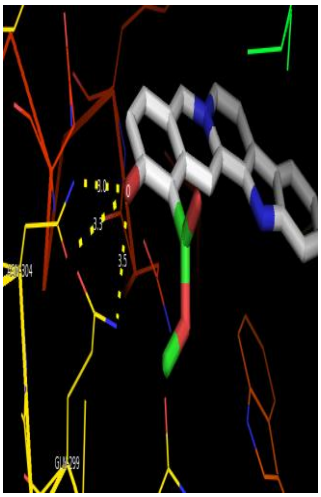
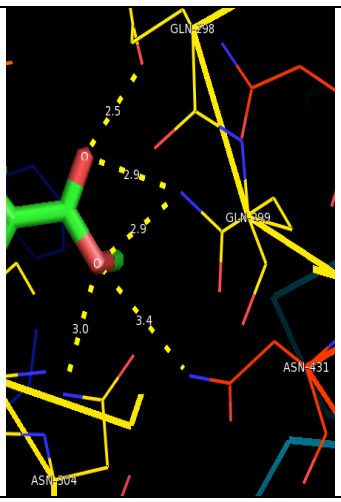
LOCHNERINE		

Table.2 Molecular Properties of the Ligands

COMPOUNDS	MOLECULAR WEIGHT	HYDROGEN BOND DONOR	HYDROGEN BOND ACCEPTOR	NUMBER OF ATOMS
CATHOVALINE	257.245	2	5	19
VINDOLINE	456.539	1	8	33
YOHIMBINE	354.45	2	4	26
VINDOLININE	336.435	1	4	25
LOCHNERINE	324.424	2	3	24

Table.3 Hydrogen Bond Interaction between Glut4 and Bioactive constituents of *Catharanthus roseus*

COMPOUND	INTERACTION	BINDING ENERGY	HYDROGEN BOND	KEY RESIDUE	DISTANCE
CATHOVALINE		-6.37	2	TRP 404 ASN 427 ASN 431 ASN 304	2.8 3.1 3.5,3.2 5.2,3.0

VINDOLINE		-5.39	1	ASN 304 GLN 299 PRO 401	3.5 3.0 3.0
YOHIMBINE		-8.0	1	ASN 304 GLN 299	3.3,3.0 3.5
VINDOLININE		-8.27	2	GLN 298 GLN 299 ASN 431 ASN 304	2.5 2.9 3.4 3.0

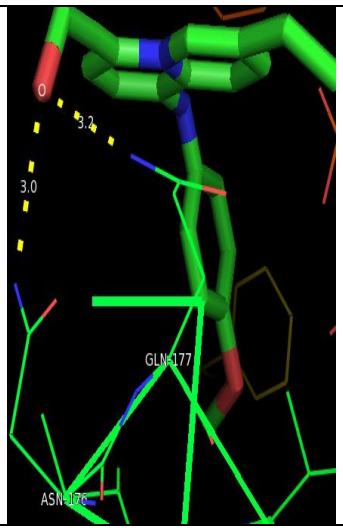
LOCHNERINE		-7.58	2	ASN 176 GLN 177	3.0 3.2

Fig.1 *Catharanthus roseus* leaves

Fig.2 Homologous template identification in BLAST

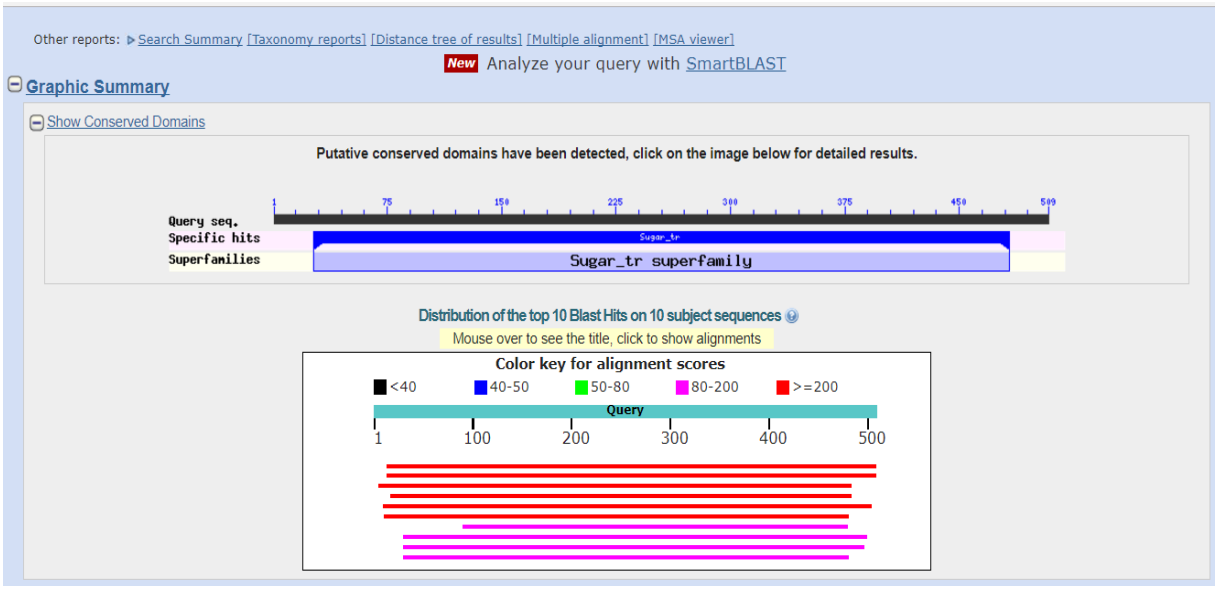


Fig.3 Ramachandran plot

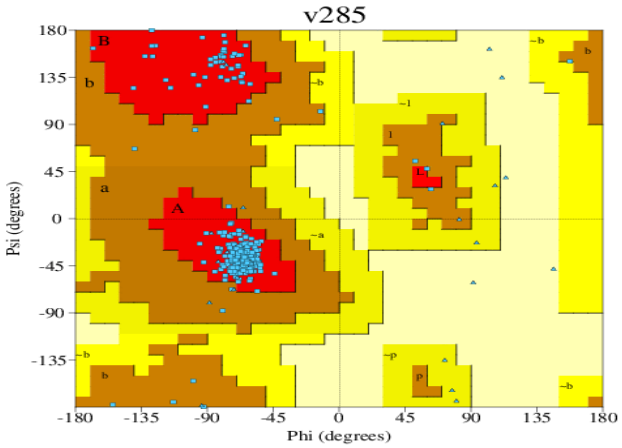


Fig.4 PROCHECK Statistics for Glut4 protein

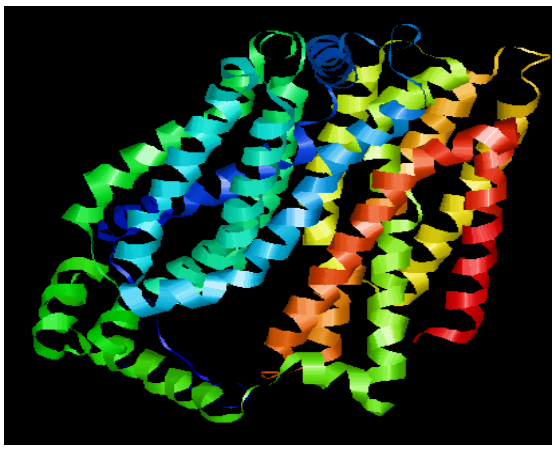
PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	382	97.0%
Additional allowed regions	[a,b,l,p]	12	3.0%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		394	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		52	
Proline residues		27	
Total number of residues		475	

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 favoured regions [A,B,L].

Fig.5 Crystal structure of Glut4



Ligand-Structure Preparation

The 2D structure compounds of *Catharanthus roseus* such as cathovaline, lochnerine, vindoline, yohimbine, vindolinine, were obtained from Pubchem Database.The 2D structure of compounds are converted into 3D structure of compounds using Chemsketch and saved in MDLmol format.The MDLmol format of 3D structure of compounds are converted into Pdb format using OpenBabel. The 2D and 3D structure of ligands are shown in Table.1 and Molecular properties of ligands are shown in Table.2

Molecular Docking Studies

Molecular Docking is an effective and competent tool for *Insilico* screening.Molecular docking may be defined as an optimization problem which would describe the best-fit orientation of ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or molecules.In modern drug designing molecular docking is routinely used for understanding drug-receptor interaction.Molecular docking were done to determine the interaction between the ligands and the Glut4 Protein.AutoDock Tools was used to prepare, run and analyze the docking simulations. Kollman charges and Polar hydogen were added to the receptor for the preparation of protein in docking simulation. In Autdock, the file is saved in Pdbqt format. Autodock uses a grid -based method to allow rapid evaluation of the binding energy of trial conformations. Auto Grid Boxes (X, Y, Z coordinates 126*126*126) were determined. The primary method for conformational searching is a Lamarckian genetic Alogrithm. The Docking results show the interactions between the compounds and the Glut4 protein. After the successful completion of docking the results are obtained from docking were analyzed using Pymol. Before Visualizing,the Pdbqt format obtained from Autodock is converted into Pdb format using OpenBabel.The best Binding mode that fitted with the binding site cavity were checked. The protein-ligand hydrogen interactions were also viewed and the atomic distances were measured. The Interaction between the ligands from *Catharanthus roseus* and Glut4 protein along with Hydrogen bond, Binding energy and distance were given in Table3.In this study, the compounds cathovaline, vindolinine, lochnerine has many interactions with least Binding energy.

Molecular docking provides a comprehensive insight into molecular mechanisms of biological processes. Influence of molecular docking is highly experienced in the field of structure based drug discovery, wherein docking is vital in validating novel lead compounds. In this study, the Molecular docking were performed between the protein Glut4 and the compounds such as cathovaline, vindoline, yohimbine, vindolinine, lochnerine of *Catharanthus roseus.* Among these compounds cathovaline, vindolinine, lochnerine had least Binding energy and Hydrogen bond Interactions. Further *in vitro* and *in vivo* analysis are required to understand the molecular mechanisms of these compounds to act as an antidiabetic agent with better medicinal effects.

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How to cite this article:

Padmini R., K. Reshma and Sangeetha K. 2017. In Silico Molecular Modeling and Docking Studies of *Catharanthus roseus* derived Alkaloids against *GLUT4*. *Int.J.Curr.Res.Aca.Rev.* Special Issue-4: 48-58.