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Molecular Docking studies on Andrographis echioides as a target for Diabetes Mellitus – An Insilco study

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Abstract

Diabetes mellitus is the group of metabolic diseases and now it is the third leading cause of death in humans. In the current study designing valuable antidiabetic drug was explored by using molecular docking techniques. Andrographis echioides is a unique medicinal plant of Acanthaceae family. It is habitually found in Tamilnadu, Kerala and Srilanka. The present study attempts to begin a relationship involving ethnopharmacological claims and bioactive constituents are in Andrographis echioides against all possible targets for diabetes during molecular docking for the dynamic goal. The process of molecular docking involves study of different bonding modes of one ligand with energetic cavities of target receptors protein aldose reductase (AR), and insulin receptor (IR) with help of docking software. It evaluates how small molecules called ligands (flavonoids) and the target macromolecule (aldose reductase enzyme) fit together. Both the receptors were docked with 17 bioactive compounds of Andrographis Echioides, From the outcome of docking score values on different receptors for antidiabetic activity, it has been found is that constituents, namely, 3, 4 Altrosan showed the preeminent docking results on about all the receptors, while the most important results were observed on AR. Insilco molecular docking provides essential indicator that can be used to plan new molecules with enhanced activity. The docking results have known enhanced insights into the progress of better aldose reductase inhibitor to treat diabetes associated secondary complications

Keywords: *Diabetes, Bioinformatics, Molecular Docking, Aldose reductase, Insulin receptor, Andrographis echioides.*

1. INTRODUCTION

Diabetes mellitus is a hereditary disorder of various aetiology characterized by chronic hyperglycemia with disorders of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and insulin resistance. According to the International Diabetes Federation, the global majority of diabetes is always on the rise with more or less 382 million in 2013 and it may raise to 591 million by 2035 [1].Andrographis echioides (Acanthaceae) which is usually known as 'false water willow' is an herb usually originates all over India. The plants from genus Andrographis are used in various disorders. It has a wide variety of pharmacological action such as antibacterial activity, anticancer, anti-inflammatory and antidiabetic of this plant scientifically [2]. Generally, molecular docking is one of the most ideal methodologies in designing the structure-based drug like molecules. Docking has predicted the conformation of ligand molecule to suitable target binding site of protein based on the several interactions like hydrogen bonding, steric etc. On the basis of these interactions a score is developed and with the help this score one can screen the ligand molecule as potent one [3].

Identifying the correct target for the management of diabetes is one of the hotspots of do research in the past few years. The targets used by scientists are Dipeptidyl peptidase IV (DPP IV), Glycogen phosphorylase, Protein Tyrosine Phosphatase 1-Beta (PTP-1B), Glucokinase, Peroxisome Proliferator-activated Receptor (PPAR) -γ etc. Protein – Ligand docking studies have been broadly employed for structural based drug designing against Diabetes mellitus. Ganugapati et al have explored docking studies of green tea flavonoids through Auto Dock 4.0 and Argus lab 4.0.1., and found that epicatechin acts as a strong insulin receptor activator [4].

With the fast development in biological and chemical information, docking has been significantly reshaping research and increase pathways in drug applicant identification. Using computational techniques in drug innovation and progress is usually valued in terms of execution, time and money. Molecular docking is a competent tool for new micro molecule drugs discovery for targeting protein [5].

Aldose reductase belongs to aldoketoreductases super family. It is the first-rate limiting enzyme in polyol pathway and reduces glucose to sorbitol by utilizing NADPH as a cofactor. Sorbitol dehydrogenase is the enzyme responsible for the conversion of sorbitol into fructose [6]. The polyol pathway represents a small way of glucose utilization, accounting for less than 3% of glucose utilization. However, in the occurrence of high glucose, the activity of this pathway is increased and could represent up to 30% of total glucose consumption [7]. Abnormal activation of the polyol pathway during diabetes leads to increase of osmotically active sorbitol leading to osmotic as well as oxidative stress, resulting in tissue damage [8]. Though, the inhibition of aldose reductase has basic approach to the prevention and treatment of diabetic complications and a potential target for drug design [9].

Drug action is triggered when the binding of small molecule to the receptor protein isfully done. Such protein-ligand contact is similar to the lock-and-key principle, in which the lock encodes the protein and the key is group with the ligand. The major active force for binding appears to be hydrophobic interaction whose specificity is however controlled by hydrogen bonding interactions [10]. Therefore, in the present study the species Andrographis is known to have antidiabetic property [11].Hence phytocompounds from this species were selected and further investigated for its binding efficiency to estimate the best fit molecule using Chemsketch.

The 3D structure of insulin like growth factor receptor is generated by homology modeling and has chosen this protein to design a specific and effective inhibitor by structure-based drug designing. At present a number of drugs whose progress was greatly influenced by or based on structure-based design and screening strategies. This data signifies that after further optimization process these possible leads can produce a tough inhibitor for the insulin like growth factor 1 receptor. The insulin receptor (IR) is a transmembrane receptor that is activated by insulin, IGF-I (insulin-like growth factor I), IGF-II (insulin-like growth factor II) and belongs to the large class of tyrosine kinase receptors (Ward et al., 2010). Metabolically, the insulin receptor plays a key role in the regulation of glucose homeostasis; a useful process that under deteriorate conditions may result in a range of clinical manifestations including diabetes and cancer [12].

The high accumulation of sorbitol causes diabetic problems such as retinopathy, angiopathy, nephropathy, and cataracts. Therefore, the catalyst of the first step in the polyol pathway would be the efficient therapeutic target for the treatment of long-term diabetic complications. Treatment of diabetic complications with inhibitors of aldose reductase is biochemically attractive because the aldose reductase-initiated accumulation of sorbitol, and its resulting pathology, only appears to be vital under non physiological conditions of hyperglycemia [13].

The 3D structure of proteins is even and preserved than primary structure (amino acid sequence). Homology modeling has proven as the method of option to generate reliable 3D model of a protein from its amino acid sequence. At present, in silico methods and tools haveprovided many advantages over time strong wet-lab experiments on characterization of proteins (PDB). The main aim of this study is to confirm the ethnopharmacological knowledge of Andrographis echioides with the help of modern computer aided drug designing tools and to develop safe and more reliable management for diabetes.

2. Materials and Methods

2.1 Ligand preparation

The 3-D structures for the phytochemical compounds were obtained from Pubchem and chemspider database, and a Virtual library was developed. Each structure was energy minimized using CHARM force field and Momany-Rone electrostatic charge was assigned to all the structures, followed by generation of conformers by using Boltzmann jump method. These conformations were used as starting conformation to perform docking [14].

2.2 Protein

The structure of aldose reductase complexed with peptide substrate was obtained from PDB data bank (PDB Code: 1IEI). The resolution factor is 1.45A° and the method of incorporation is X-ray diffraction method. The minimization of the receptors was done using Swiss PDB viewer and also, the active site residue was identified. Using the control panel of this standalone software, the ligand molecules attached to the receptor were selected. All the residues surrounding the ligand which comes in 8.0A° were identified and selected. Using Argus lab, ligand molecules present were removed and final preparation was done by removing water molecules and adding H-atoms.

2.3 Docking software

Autodock 4.0 has the ability to predict the interaction of small molecule with moleculartargets with reasonable accuracy and speed. Autodock performs the docking of ligand to a set of grids (pre calculated by autogrid) describing the target protein. The energy grid was built within a cubic box and docking was performed based on Lamarckian genetic algorithm [15].

2.4 ADMET

The ADMET functionality of Discovery studio 2.5 estimates the aqueous solubility of a set of ligands, blood brain barrier penetration, cytochrome p450 [CYP450] 2D6 inhibition, hepatotoxicity, human intestine absorption [HIA] and plasma protein binding in a single stretch. It also accesses a broad range of toxicity measures for a set of ligands [16].

3. Results

This research has helped to identify the compounds present in plant of A. echioides, till now an investigated plant. Seventeen chemical constituents have been notorious from of this plant. The presence of a variety of bioactive compounds justifies the use of this plant ailment by habitual practitioners. So, it is suggested as a plant of Phyto pharmaceutical importance. The auto dock score and the re-rank scores of the most excellent poses for each of the docking studies of A.echioides with ten bio active compound structures of AR, and their average is presented in Table 1.

Fig 2 shows that the Aldosereductase has been docked with streptozotocin by using with ligand fit algorithm. It has been docked with active site of the receptor which forms 5 hydrogen bonds with the dock score of 21.547 and ligand internal energy of -1.455. The compounds identified in A. echioides hydroalcoholic extract by GCMS were then docked using docking software. The energy values obtained for each receptor using auto dock 4.0. The binding energy between plant constituents and aldose reductase ranged from -6.38 to -10.08 KJ/Mol and with insulin receptor (Fig 3), docking energy ranged from -3.70 to -7.55 KJ/Mol. In Fig 4 and Fig 5 exemplifies docked create of aldose reductase enzyme with the active site clearly demonstrated the binding positions of the ligand with the enzyme.

Fig 6 illustrates that the Docking of aldosereductase has been performed with the ligand fit algorithm. The receptor has forming 4 hydrogen bonds with the phytochemical compound 3,4 Altrosan present in the plant extract at the active site with the dock score of 27.787 KJ/Mol and ligand internal energy of -5.967 KJ/Mol is high. Since, 3,4 Altroson (Table 1) complex hadbinding energy -10.08 KJ/Mol and 4 hydrogen bond interactions Trp 20, Tyr 48, His110, Trp111. Which have effective binding when compared to streptozotocin and standard ligand glibenclamide.

Chromatographic mass spectral studies and identified 17 compounds. All the identified compounds were subjected to docking studies against aldose reductase and insulin receptor compared with standard, Glibencilamide. The binding energy among plant constituents and aldose reductase ranged from -5.94 to -10.08 KJ/Mol. The binding energy of Glibencilamide against aldose reductase was found to be -9.62K KJ/Mol are high, (Fig 7) and 4 hydrogen bond interactions Trp 20, Tyr 48, His110, Trp 111 (Table 1). Comparatively 3,4, Altrosan complex is better than Glibencilamide complex based on higher binding energy and thegreater number of hydrogen bonds.

In view of these (Fig 8) the use of in silico methods to predict ADMET properties is intended as a first step in this direction to analyze the novel chemical entities to prevent wasting time on lead candidates that would be toxic or metabolized by the body into an inactive form and unable to cross membranes and the results of such analysis are herein reported biplot.

In silico ADMET properties such as ADMET BBB level, absorption, aqueous solubility, hepatotoxicity, AlogP98, CYP2D6 and PSA were studied for the standard compounds from standard data set and test compounds from test data set. An ADMET model was generated that predicts the human intestinal absorption (HIA) after oral administration of the inhibitors tested. The intestinal absorption model includes 95% and 99% confidence ellipses in the ADMET_PSA_2D and ADMET_AlogP98 plane (Figure 8). There are four prediction levels for the absorption of compounds as good (0), moderate (1), poor (2) and very poor (3). These levelsare defined by the 95% (red line) and 99% (green line) confidence ellipsoids (Figure 8). The upper limit of PSA_2D value for the 95% confidence ellipsoid is at 131.62, while the upper limit of PSA_2D value for the 99% confidence ellipsoid is at 148.12 (Figure 8). Based on the in silico ADMET analysis it was found that the test compound (Pubchem ID: 9874248) content the ADMET descriptors criteria at the optimal level among the test compounds tested whereas all the compounds from the standard dataset fulfilled the ADMET descriptors criteria. The constituents of A.echioidesanalyzed by docking results clearly indicated that 3, 4 Altrosan was got maximum activity even greater than the standard compound, whereas all other identified constituents also supported its aldose reductase inhibition activity. The interaction of 3, 4 Altrosan with aldose reductase is depicted
(Fig 6).

4. Discussion

Diabetes mellitus consists of a range of dysfunctions characterized by hyperglycemia and resulting from the mixture of resistance to insulin action, insufficient insulin secretion, and also improper glucagon secretion, important to chronic hyperglycemia [17].
The traditional medicinal system has plenty of opportunities, which are still needed to be explored till date for the treatment of many ailments [18]. if one can use the modern computational chemistry tools for exploring the potential of the traditional medicinal system, and then astonishing results can be received. The related studies have been taken away in the past by many scientists where bioactive compounds are docked on particular receptor to estimate its affinity [19].
The docking poses were ranked according to their docking scores and both the ranked list of docked ligands based on their binding energy with the enzyme and their related binding posesdocked pose of aldose reductase enzyme with the ligands butein and epalrestat; it clearly confirmed the binding positions of the ligand with the enzyme [20].
Different flavonoids were selected for the in-silicodocking studies. Lead optimization of the selected compounds was done by computation of drug likeness properties. The drug likeness scores of the compounds were evaluated with the help of Lipinski's rule. The docking studies were performed by the use of AutoDock4.0. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that higher possibility of the compound has higher activity.
Docking studies were performed using software in order to evaluate the inhibitory effects of A.echioidesagainst aldose reductase, an enzyme involved in diabetic complications. Human aldose reductase protein was chosen as the target protein due to their vital role in regulation of blood glucose concentration. Docking studies and binding free energy calculations revealed that 3,4 Altrosan has maximum interaction energy (-10.08 kcal/mol) and Methoprene with the least interaction energy (-5.12 kcal/mol) as compared to the other investigated ligands.
The previous study was to discover the potent compound with antidiabetic activity in the identified constituents of Helicteresisorausing docking method. The docking studies were performed using two receptors, aldose reductase and insulin receptor proteins. Both the receptors were docked with 15 plant constituents and yohimbine was found to be the effective constituent as it docked well to the active sites of both the receptors. From the docking results obtained it was clear that all the constituents having superior activity with yohimbine as the best one [21].

Most of the drugs of synthetic Aldose reductase (AR) inhibitors were withdrawn from the market because of undesirable side effects and poor pharmacokinetic properties [22]. Now we are looking for safe as well as effective pharmacophore for developing better AR inhibitors. The earlier study has reported that, specific terpenoids entirely isolated from E. antiquorumare successfully in use. Diabetes has been corroborated by several researchers. Fidarestat was used as a standard aldose reductase inhibitor [23]. It is evidenced from the data obtained that the binding energy of all the antidiabetic ligands possesses significant aldose reductase inhibitory activities which are comparable with the standard, fidarestat. Several reports are available in the literature regarding the aldose reductase inhibitory activity of various phytochemicals derived from traditionally important medicinal plants [24].

The previous study shows, the ligand-protein inverse docking simulation technique was performed with 11synthetic ligands thiazolidinediones derivatives with basic α, β- unsaturated ketone and thiazolidine-2,4-dione moieties reported to be having aldose reductase inhibitory activity by using MVD program [25].
Further, the results of the Insilcofindings evidenced that lead molecules are capable of significantly reducing the intracellular sorbitol accumulation, which has been implicated in the pathogenesis of late-onset diabetic complications like retinopathy, neuropathy and nephropathy. Further studies are in progress to evaluate the effect of these phytochemicals on the activities of other relevant receptors involved in the secondary complications of diabetes mellitus.
The 3,4 Altrosan drug-likeliness calculated (Fig 8), according to (ADME-T) algorithm which examine the capability of compounds for transported across the intestinal epithelium, they probably have high affinity binding to the plasma proteins, and may be through the blood-brain barrier, and it necessary for ability drug transported throughout the body. Taken together, it has been found 3, 4 Altosan was most active compound that has more energy bonds and interactionproperties. So, we suggest that 3, 4 Altosan may has a potential of diabetic effect. In silico ADMET analysis was done to predict the ADMET properties of the standard drugs and test compounds. This might provide insights to develop new drugs to target the diabetes, aldose reductase of 3, 4 altrosan.
The study was to identify the potent compound with antidiabetic activity in the identified constituents of A.echioidesusing docking method. From the docking results obtained it was clear that all the constituents having good activity with 3, 4 Altrosan is the best one.

5. Conclusion

Structure-based drug design tool is an effective route of drug discovery. From this, an idea to get the alternative for the existing drugs which have adverse effect after a prolonged period. Natural inhibitory compounds have no adverse effects with highest binding energy and affinity to bind the molecular markers.
The present study helped to identify the potent bioactive constituent present in the A. echioides, attributing aldose reductase inhibitory activity, as 3, 4 altrosan among all other constituents. This result clearly demonstrates that the approach used in the study is successful in finding novel antidiabetic compounds from plant. Also, the study states and confirms the importance of small molecules from plant, their use in enhancing protein-ligand interaction studies, in silico and provide vital clues that can be used to design new molecules with improved activity. These results clearly indicate that the A. echioides showed excellent binding interactions with aldose reductase enzyme than the standard. This in silico studies is actually an added advantage to screen the aldose reductase enzyme inhibition. And we have demonstrated the binding interactions between the bioactive compounds with Aldose reductase using Autodock.The results also indicate that the significant antidiabetic properties of these bioactive compounds may attribute to its aldose reductase inhibitory effects

6. Tables

Table.1. The experimental docking results of present studied Streptozotocin induced with Andrographis echioides as well as standard drug Glibencilamide values for binding energy, interacting residues, number of hydrogen bonds and bond length.

Proteins	Drugs/ Ligands	Binding energy (KJ/mol)	Interaction residues	Number Hydrogen bonds	Bonds leng Å
1IEI	3,4-Altrosan	-10.8	Trp20, Tyr4 His110,Trp111	4	2.4, 2.8, 3.0,2
	n-Decanoic acid	-6.15	Tyr48, Trp111	2	4.8, 4.5
	2-Cyclopentene-1-Undeconicacid (+)-	-7.82	Tyr48, His110, Trp111	3	3.9, 3.5, 3.1
	2,5-Cyclohexadiene-1,4 dione, 2,5 dihydroxy-methyl-6-(1-meethylethyl)-	-6.56	Tyr48, His110,	3	3.7, 3.2, 4.2
	4-Hydrazono-5-hydroxy imino-4,5,6,7 -tetrahyc benzo furazan	-5.94	Trp111	1	3.9
	Vitamin E	-6.17	Tyr48, Trp111	2	4.7, 4.4
	3-Hexadecyloxycarbon 5-(2-hydroxyethyl)-4-methylimidazolium ion	-8.55	Tyr48, His110, Trp111	3	3.3, 3.0, 3.2
	(-)-3-á-Acetoxy-5-etienic acid	-6.25	His110, Trp111	2	3.6, 3.1
	Methoprene	-5.12	Tyr48 Trp20, Tyr48,	1	4.8
	Glibencilamide	-9.62	His110, Trp111	4	3.1, 2.9, 3.2,2

7. Figures

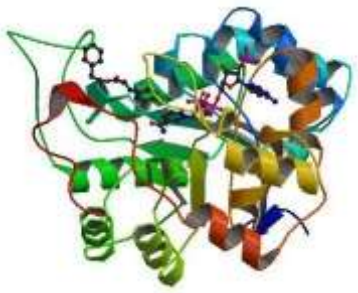

Figure 1: Aldose reductase complexed with a phenolic compound

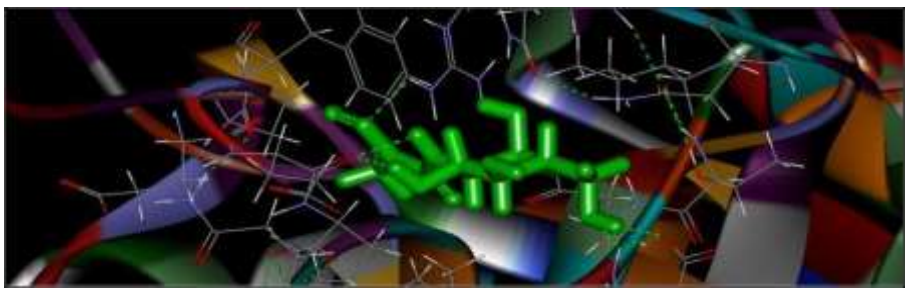

Figure 2 Interaction of Streptozotocin with Aldose reductase

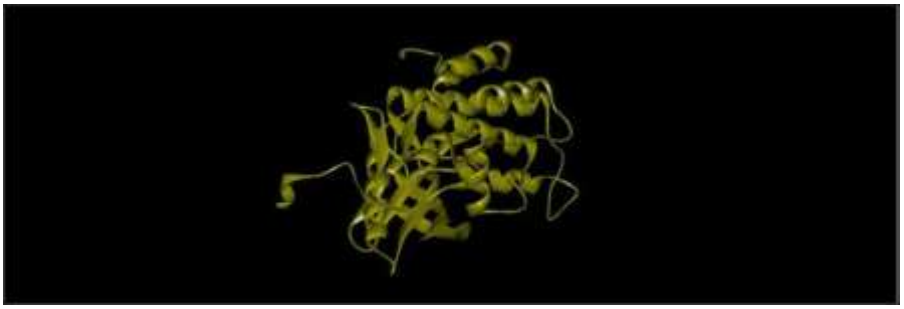

Figure 3 Insulin Receptor3 (IR3)

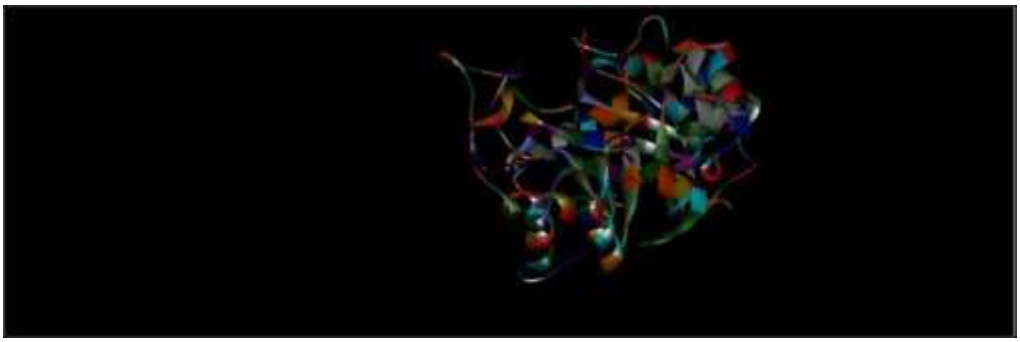

Figure 4 Aldose reductase

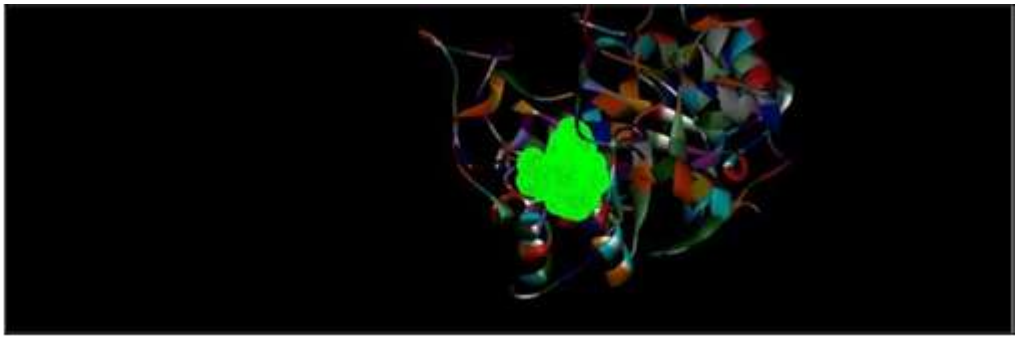

Figure 5 Aldosereductase enzyme - Active site

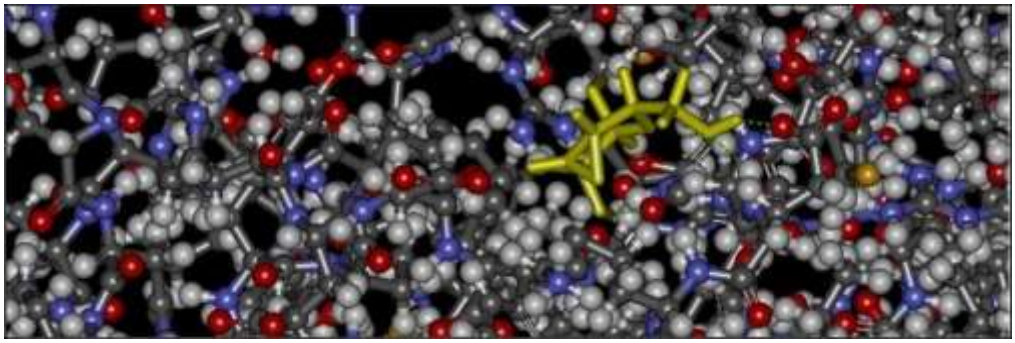

Figure 6 Aldose reductase with 3,4-Altrosan

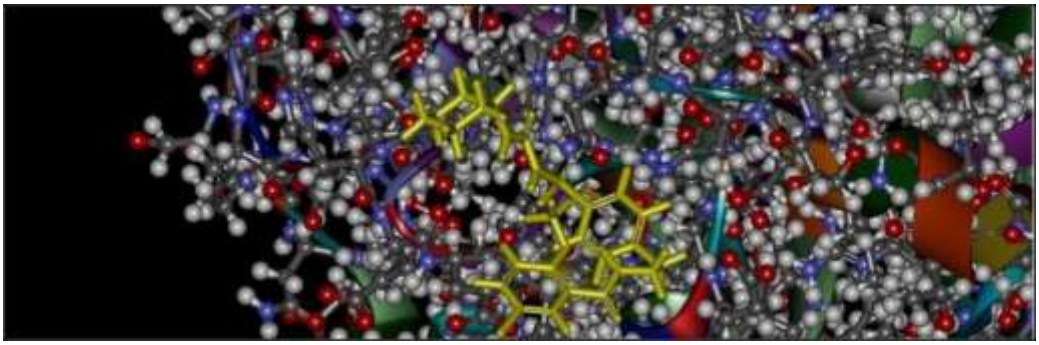

Figure 7 Aldose reductase with Glibencilamide

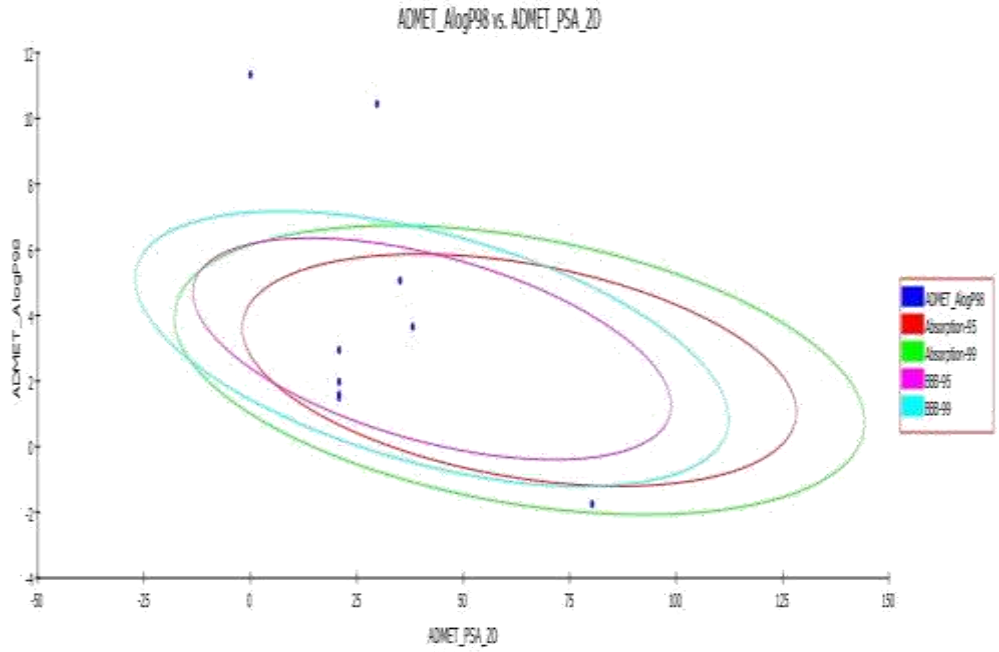

Figure8 ADMET PROPERTIES OF BIPLOTMAP

Plot of Polar Surface Area (PSA) vs. Log P for a standard and test set showing the 95% and 99% confidence limit ellipses corresponding to the Blood Brain Barrier and Intestinal Absorption models.

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