THE COMPUTER AIDED DRUG DESIGNING FOR THE DIABETIC TARGET: ALDOSE REDUCTASE

DALAL H.*

Department of Biotechnology, Junagadh Agricultural University, Junagadh- 362 015, Gujarat, India.
*Corresponding Author: Email- dalalhina@gmail.com

Abstract- Glucose, a sugar, is vital to our health because it is the main source of energy for the cells that make up muscles and other tissues. It is also our brain's main source of fuel. Diabetes mellitus refers to a group of diseases that affect how the body uses blood glucose. To regulate the uptake of glucose from the blood into most cells of the body, beta cells (β-cells), found in the islets of Langerhans in the pancreas, releases a hormone called insulin. Diabetes mellitus is a condition which results from either the body's failure to produce enough insulin or when the cells fail to respond to insulin properly. Aldose reductase, an enzyme, located in the eye (cornea, retina, lens), kidney, myelin sheath, and also in other tissues was discovered as a new target, not previously to be linked to diabetes. The enzyme can be inhibited by Aldose Reductase (AR) inhibitors, being studied as a potential treatment to prevent eye, nerve and kidney damage in people with diabetes. Keeping that in mind present study is aimed to design better analogues of Tolrestat, which may have better binding with AR and again can perform function against diabetes. As a first step in addressing this issue, combinatorial library has been generated using SMI-LIB and fragmentation was done using fragmentor in J-Chem package and as a result of that five fragments were obtained. The biggest and core fragment was selected as scaffold for SMI-LIB for further library generation. These molecules were utilized for virtual screening of all drug like molecules using pharmacophoric properties, chemical fingerprints and molecular docking. After analyzing the docking results it was found that the molecule TOL727 thus achieved after the *in-silico* processing in computer aided drug design have the least docking energy as compared to the original Tolrestat molecule. As a conclusion this ligand can be used for further testing in lab and if found with good activity can be suggested as a better lead molecule for aldose reductase.

Keywords- Diabetes, Aldose Reductase, Aldose Reductase inhibitors, Tolrestat

Introduction

Diabetes is neither a disease nor it is contagious still this metabolic disorder is threatening the well being of millions of people around the globe and devastating the physical, social and economics welfare of virtually every country in the world. It has been known to man since the time of ancient civilization. The ancient Greeks, Egyptians, Babylonians, Partisans, Chinese and Indians, knew it. They identified it with the sweet taste of urine [1]. Diabetes mellitus is a common disease in the United States. It is estimated that over 16 million Americans are already caught with diabetes, and 5.4 million diabetics are not aware of the existing disease. Diabetes prevalence has increased steadily in the last half of this century and will continue rising among U.S. population. It is believed to be one of the main criterions for deaths in United States, every year. This diabetes information hub projects on the necessary steps and precautions to control and eradicate diabetes, completely [2]. Diabetes is a metabolic disorder where in human body does not produce or properly uses insulin, a hormone that is required to convert sugar, starches, and other food into energy. Diabetes mellitus is characterized by constant high levels of blood glucose (sugar). Human body has to maintain the blood glucose level at a very narrow range,

which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood, for the production of energy. Glucose entering into the cells is metabolized in part to its sugar alcohol form sorbitol via NADPH dependent oxidoreductase known as the enyme Aldose Reductase (also known as aldehyde reductase) in non-insulin dependent tissues [3,4]. AR is a broad-specificity aldo-keto reductase with wide species and tissue distribution [5-7]. However the physiological role of AR (EC 1.1.1.21) under euglycemic conditions is still a matter of debate and remains unclear, the enzyme is believed to be of primary importance in the development of severe degenerative complications of diabetes mellitus. According to Therapeutic Targets Database, AR is a successful target for diabetic complications (TTD target ID: TTDS00115).

AR and Its Inhibition in the Control of Diabetic Complications

There is a wealth of experimental information that has been accumulated on polyol pathway and the complications of diabetes over the past four decades but the question still remains whether it is an important player in retinopathy and other complications of human diabetes. The cells of the retina, kidney and nervous tissues are

insulin-independent unlike most of the other cells which require the action of insulin for glucose to gain entry into them. So glucose moves freely across the cell membrane in those special cells, regardless of the action of insulin. Cells uses glucose for energy and the unused glucose which hasn't been used for energy will enter the polyol pathway, making it active on the elevation of intracellular glucose levels [8,9]. AR, generally used as the first and rate-limiting enzyme in the polyol pathway catalyzes the conversion of D-glucose to D-sorbitol [3,10]. AR is associated with the the NADPH-dependent reduction of a wide variety of carbonyl-containing compounds to their corresponding alcohols with a broad range of catalytic efficiencies. AR reduces aldehydes generated by reactive oxygen species (ROS) to inactive alcohols, and glucose to sorbitol, using NADPH as a co-factor [11]; the second step is the oxidation of sorbitol to fructose catalyzed by sorbitol dehydrogenase (SDH) using NAD^+ as a cofactor. Oxidation of sorbitol to fructose by SDH using NAD^+ as a co-factor leads to the redox state change and a cascade of interrelated metabolic and signaling imbalances which may alter the function of a cell adversely [10].

Under normal physiological conditions AR participates in osmoregulation [12], but under hyperglycaemic conditions it contributes to the onset and development of severe complications in diabetes [13]. Concentrations of glucose are elevated in diabetic patients and AR has been believed to be responsible for diabetic complications including a number of organs including lens, retina, Schwann cells of peripheral nerves, placenta and red blood cells [14-17]. Also, Sorbitol production is markedly enhanced by hyperglycemia in experimental diabetes models. The accumulation of sorbitol within the cell results in rise of cell osmolality and a decrease of intracellular myo-inositol, leading to decrease in NA-K-ATPase activity [18]. In cells where AR activity is sufficient to deplete reduced glutathione (GSH), oxidative stress is augmented. These biological phenomena have been connected to a to a range of diabetic complications [19] and thus, AR has been received considerable attention as a promising target for the treatment of diabetic complications and has become the focus of various drug design projects [20].

AR: Primary Structure

The primary structure of AR is first determined for the rat lens enzyme as a small monomeric protein composed of 315 amino acid residues [21,22] The structural similarities with another NADPH-dependent oxidoreductase: human liver aldehyde reductase (EC 1.1.1.2) [23] and to ρ-crystallin, a major lens protein of frog *Rana pipiens* [24] were also demonstrated. On the basis of degree of similarity, related structures and same evolutionary origins, AR, aldehyde reductase and p-crystallin are suggested to be the members of a superfamily of related proteins, namely aldo-keto reductase superfamily. Strong sequence similarity also been found with prostaglandin F synthase [25], 2,5-diketo-D-gluconate reductase [26], chlordecone reductase [27], and a yeast protein encoded by GCY gene [28].

AR: Tertiary Structure

The Crystallographic structures for AR have been determined for porcine AR holoenzyme [11,29] and human ARs [17,30]. AR folds into a β/α-barrel structural motif composed of eight parallel β strands with a large hydrophobic active site [31]. The coenzyme NADPH is surrounded by the conserved hydrophilic residues of the AR active site in the bottom of a deep hydrophobic cleft [32], located in the center of the barrel core. Adjacent strands are connected

by eight peripheral α-helical segments running anti-parallel to the β sheet [30]. The NADPH cofactor is situated at the top of the β/α barrel, with the nicotinamide ring projects down in the center of the barrel and pyrophosphate straddling the barrel lip [4]. Previous findings suggest that many compounds with diverse chemical structures that have been shown to be complexed with AR holoenzyme such as zopolrestat [30], sorbinil and tolrestat [32] can interact with the enzyme in different conformations. Since the AR enzyme retains flexibility in its tertiary structure and differences in selectivity among aldose and aldehyde reductases enzyme inhibitors, the approaches to predict the rigid inhibitor binding site of AR can not be taken further [33]. This specific selectivity has been attributed to the interaction of the inhibitors with the enzyme molecule, the conformational change of which leads to the opening of a specificity pocket upon binding with AR inhibitiors. Depending upon the inhibitor, the active site of AR adapts itself to bind tightly to different inhibitors and the enzyme change its shape through different conformational changes of the same residues. This pocket binds inhibitors that are more effective against AR than against aldehyde reductase [32]. To effectively block the enhanced flux of glucose through polyol pathway, the inhibitor needs to be specific for AR and devoid of intercalating into other structurally related proteins coexisting in the "target" organs of diabetic complications [10]. Thus, In the present study, AR has been selected to design new and effective drug like molecule which can be used in future to cure Diabetes Mellitus more effectively and efficiently [2].

AR Inhibitors: Potential Targets for the Prevention of Diabetic Complications

Although so many drugs are known for diabetes which work through different mechanisms with different side effects are also available such as Glipizide and Repaglinide. Glipizide may cause headache, dizziness, diarrhea, and gastric problems as side effect. Skin rashes can occur and cause itching, hives, or a diffuse measles-like rash. Rare but serious side effects include hepatitis, jaundice, and a low sodium concentration. Glipizide may also cause hypoglycemia. The risk of hypoglycemia increases when Glipizide is combined with other glucose reducing agents. The side effect of repaglinide drugs is hunger, nausea, tiredness, perspiration, headache, heart palpitations, numbness around the mouth, tingling in the fingers, tremors, muscle weakness, blurred vision, cold temperature, excessive yawning, irritability, confusion, or loss of consciousness [34]. So keeping these problems in vision, different groups are working on novel targets that will solve existing problems and provide a better life for diabetic patients.

Inhibiting AR would provide a way of avoiding the complications of diabetes, and identifying inhibitors is, therefore, an important pharmaceutical goal. AR inhibitors have therefore been noted as possible pharmacotherapeutic agents for the treatment of diabetic complications. AR inhibitors are a class of drugs being studied as a way to prevent eye and nerve damage in people with diabetes [1]. AR inhibitors can be divided into four main categories according to their structures. The first category includes substituted acetic acids, which further can be divided into different subclasses such as 1,3-dioxo-1H-benz[de]isoquinolines, for example - alrestatin [35], which was the first AR inhibitor found to be orally active, naphtalene derivatives such as tolrestat [36], which is the drug of interest in the present study and has been intensively studied, including in large scale trials [37], 3,4-dihydro-4-oxophtalazines such as ponalrestat [38] and zopolrestat [39], and by rhodanines such as epalrestat [40].

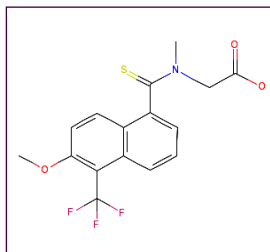## Important Characteristics of Tolrestat Drug

Generic Name: Tolrestat.

Synonym: Alredase; N-(6-Methoxythio-5-(trifluoromethyl)-1-naphthoyl)sarcosine; Tolrestatum; Glycine; N-((6-methoxy-5-(trifluoromethyl)-1-naphthalenyl)thioxomethyl)-N-methyl [36].

Accession No: EXPT03082.

Chemical IUPAC name: TOLRESTAT.

Chemical Formula: C16H14N1O3F3S1

Chemical Structure:

Molecular Weight: 357.347 g/mol.

State: Solid

LogP/Hydrophobicity: 3.764.

Drug Type: Experimental drug.

Drug Category: AR Inhibitor.

Smile String: COc1ccc2c(cccc2c1C(F)(F)F)C(=S)N(C)CC(O)=O

The second category are spirohydantoins, the most well known of which are sorbinil [41]. The third category are flavonoids such as quercetin, observed to have aldose reductase-inhibitory activity [42]. The fourth category of AR inhibitors includes phenylsulfonyl-nitromethane derivatives such as ICI 215918 [43] and ZD5522 [44].

The role of AR and the potential of its inhibitors as therapeutic agents targeted at chronic diabetic complications had been widely studied [19,45]. Animal studies with AR inhibitors were very promising. In diabetic rats treatment for five month with an AR inhibitor minimized albuminuria and glomerular basement membrane thickening [46,47]. AR inhibitors in this study improved nerve conduction velocity and prevented diabetic cataracts; however, there was no improvement or prevention of retinopathy in diabetic dogs treated for 5 years [48].

Tolrestat: The Compound of Interest

Tolrestat (An International Nonproprietary Nam, AY-27773) chemical name- N-[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]- N-methylglycine has been discovered as a potent, orally active AR inhibitor [36] which was approved for the control of certain diabetic complications [49]. Among all the inhibitors of AR, tolrestat and sorbinil are, pharmaceutically, the most well-studied AR inhibitors [32]. AR inhibitors (tolrestat or sorbinil) or antisense AR mRNA also prevented hyperproliferation of cultured rat aortic SMCs induced by high glucose. Cell cycle progression in the presence of high glucose was blocked by tolrestat [50] which induced a G0-G1 phase growth arrest.

Evidence of Effectiveness of Tolrestat Drug in Prevention of Diabetic Complications

The 'late complications' of diabetes mellitus, i.e., nephropathy, neuropathy and retinopathy are firmly rooted in inadequate control of blood glucose: hyperglycaemia. Hyperglycaemia causes elevated cytosolic glucose and/or rates of glucose metabolism i.e.,

'hyperglysolia,' within cells of vulnerable tissues [51]. AR activity increases as glucose concentration rise in diabetes in those tissues that are not insulin sensitive, which includes the lenses, peripheral nerve and glomerulus. When too much sorbitol becomes trapped in the eye or nerve cells, it can cause cataracts (clouding of the eye lens) or damage nerve cells in the eyes of people with diabetes. This can cause an eye disorder known as diabetic retinopathy. Excess sorbitol may also be involved in a common nerve disease known as diabetic neuropathy and a common kidney disease called diabetic nephropathy. Many AR inhibitors have been developed as drug candidates but virtually all have failed although some are commercially available in several countries. The efficacy of tolrestat in the treatment of diabetic retinopathy, neuropathy and nephropathy in the animal and human clinical trials has been assessed in various studies.

Diabetic Neuropathy

Excess sorbitol may also cause a form of damage to nerves throughout the body that is known as diabetic neuropathy [Fig-1]. However, experts are not sure of the exact cause of this disease. Tolrestat have been undergoing extensive clinical investigation for the treatment of diabetic complications including polyneuropathy [52-57]. In one animal trial, the effects of tolrestat and placebo in patients with subclinical diabetic neuropathy were compared and found that at 12 months, nerve function significantly improved in patients receiving tolrestat and deteriorated in patients taking placebo. Tolrestat may be useful in the primary prevention of diabetic neuropathy [52].
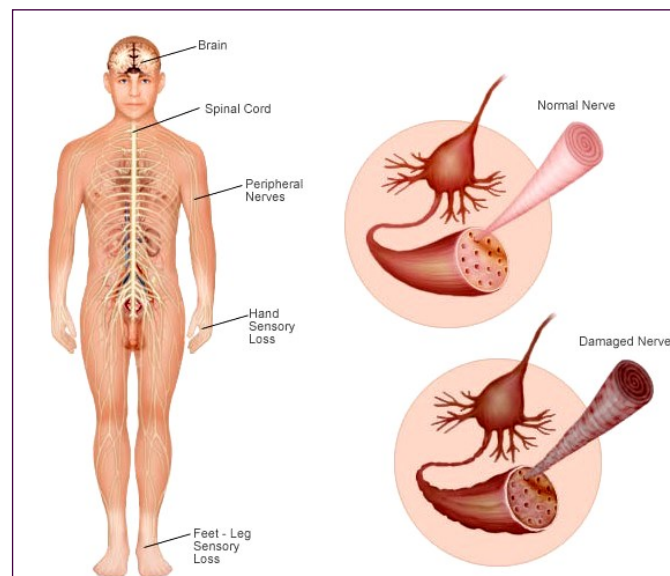
Fig. 1- Diabetic neuropathy: It is a nerve complication of diabetes caused by high blood sugar levels. Over time, high blood sugar levels can permanently damage nerve fibres everywhere in the body and therefore, it can affect all organs and systems. Half of all diabetic patients suffer from some form of diabetic neuropathy (*Source: HealthTree.com*).

In one study with human trials, the effects of discontinuing tolrestat, an AR inhibitor, on peripheral sensorimotor diabetic neuropathy were studied and the data indicate that withdrawal from long-term treatment with tolrestat has a detrimental effect on several measures of diabetic neuropathy, whereas continuation of treatment is associated with stabilization of these measures [53]. In

another study, the efficacy and safety of tolrestat, were evaluated in 21 diabetic patients with symptomatic diabetic peripheral sensorimotor polyneuropathy in a 24-week, open-label clinical trial. The patients received single daily oral doses of tolrestat (200 mg). In this study, the overall response to tolrestat was rated as good or excellent in 95% of the patients by both the investigator and the patients. Adverse effects and clinical laboratory abnormalities were few and not clinically significant. Thus tolrestat (200 to 400 mg daily) appears to be safe and effective in improving the neuropathic symptoms in diabetic patients with mild-to-moderate peripheral sensorimotor polyneuropathy [54].

Tolrestat have also been shown to reverse mild diabetic neuropathy. The progression of mild diabetic autonomic and peripheral neuropathy may be halted or even reversed by pharmacologic intervention with the aldose-reductase inhibitor tolrestat [55]. Tolrestat has also been found later to be non toxic and beneficial in the treatment of painful neuropathy by the same group. The major side effect was an elevation of liver enzymes, which returned to normal after the discontinuation of the drug [52]. The long-term effects of tolrestat on symptomatic diabetic sensory ploneuropathy were also shown to be promising [56]. All the mentioned studies have shown that the tolrestat is a promising and useful drug in the treatment of the peripheral diabetic neuropathy in addition to attainment of a better glycemia's control [57].

Diabetic Retinopathy

People with diabetes have problems related to insulin, which sometimes allow the amount of sorbitol in a person's eye to build up excessively. This can lead to cataracts, a clouding of the eye lens that impairs vision. AR can also damage the nerve cells of the eyes. This can cause a disorder of the eyes' blood vessels known as diabetic retinopathy [Fig-2]. Diabetic cataract formation follows an increase in sugars in the lens. The excess sugar within the lens is reduced by AR to its alcohol, but the lens capsule is relatively impermeable to sugar alcohols [58]. Because of the excess sugar alcohol (polyol), the lens imbibes water, causing osmotic imbalance [59].
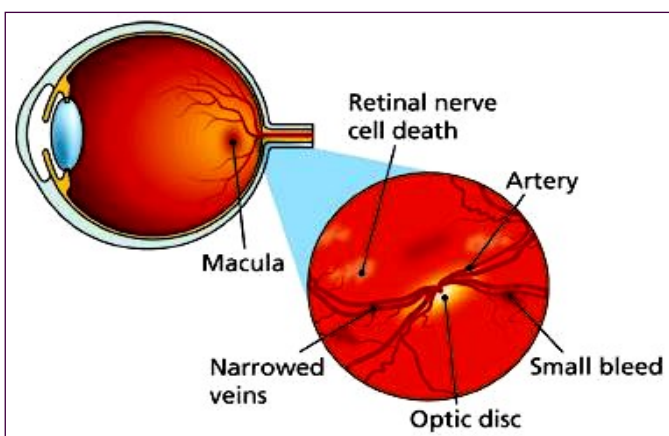
Fig. 2- Changes to the retina during Diabetic retinopathy (Adopted from Diabetes UK (*Source: www.diabetes.org.uk*) .

Eventually, increased sodium and decreased potassium levels and decrease glutathione levels lead to cataract formation. Studies have shown that the topical administration of tolrestat have been shown to be effective for the prevention of ocular changes in diabetes mellitus, thus preventing the cataract in rats [58, 60]. Treatment with

orally administered tolrestat prevented essentially all of the vessel abnormalities in another study on rats [61]. Studies with Human trials investigating the effects of AR inhibition with Tolrestat on diabetic retinopathy noticed that hard exudates, intraretinal hemorrhages and focal fluorescein leakage increased on average in the placebo and decreased in the tolrestat group of patients, indicating the positive and promising effects of tolrestat in case of Diabetic retinopathy [62]. All these data indicates that Tolrestat may help prevent retinopathy or delay its progression.

Diabetic Nephropathy

In addition, the accumulation of sorbitol or other polyols may play a role in the development or progression of the kidney disease diabetic nephropathy, according to the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK). Studies on animals have indicated that suppression of AR might promote kidney function, and research on people with insulin-dependent diabetes have shown that ARIs lower the glomerular filtration rate and benefit those with proteinuria [59]. However, the effects of AR inhibitors on diabetic nephropathy are controversial. In one study 20 patients were treated with tolrestat (200 mg po qd) or placebo for 6 months. Tolrestat reversed glomerular hyperfilteration, with glomerular filteration rate (GFR) decreasing from 156 to 125 ml/min. (p<0.001). Urinary albumin excretion decreased from 197 to 158 mg/day (p<0.001) [63]. In another study of 16 diabetic patients were treated for 12 month with tolrestat (200 mg/day) or ascorbic acid (500 mg bid). Tolrestat had no effect on proteinuria after 9 month [64]. On the other hand, another study determined the ability of tolrestat to intervene in the further progression of already established urinary albumin excretion of streptozotocin-diabetic female rats. Diabetic rats were grouped as low-urinary albumin excretion (0.2-1.0 mg albumin/day) or high-urinary albumin excretion (1.9-5.9 mg albumin/day), at which time tolrestat intervention (25 mg/kg per day) was begun for half of the diabetic rats in each urinary albumin excretion group After six months of treatment tolrestat caused a significant reduction in the urinary albumin excretion rate of the low-urinary albumin excretion group only. The diabetes-induced rise of total urinary protein in both groups was significantly reduced by tolrestat. It showed the efficacy of tolrestat to reduce the progression of urinary albumin excretion and retinal basement membrane thickening in long-term diabetic rats [65].

Despite of the fact that Tolrestat have been withdrawn from the market (by Wyeth in 1997, after failing Phase III trial in the U.S) because of the risk of severe liver toxicity and death, it is still approved to be marketed in Europe, and is still a widely studied AR inhibitor because of its positive results in Diabetic complications as described in the previous section. Thus the present study is aimed to design the better analogues of Tolrestat and hence Tolrestat is being selected to design novel drug like molecules which may have better binding with AR.

Material and Methods

The Recovery of Drug Molecule from DrugBank

DrugBank is a richly annotated resource that combines detailed drug data with comprehensive drug target and drug action information. Since its first release in 2006, DrugBank has been widely used to facilitate *in silico* drug target discovery, drug design, drug docking or screening, drug metabolism prediction, drug interaction prediction and general pharmaceutical education. DrugBank is able

to provide detailed, up-to-date, quantitative, analytic or molecular-scale information about drugs, drug targets and the biological or physiological consequences of drug actions. As a chemically oriented drug database, DrugBank is able to provide many built-in tools for viewing, sorting, searching and extracting text, image, sequence or structure data [66].

Fragmentation

Tolrestat was fragmented to different fragments using RECAP method in molecular operating Environment. The purpose of RECAP Analysis is to fragment every molecule in a database according to simple Retro synthetic Analysis rules and collect statistics on the resulting fragments. RECAP Analysis fragments a molecule by breaking certain bonds that are estimated to be those that can be reformed by common reliable chemistry. Each resulting fragment is assigned a unique extended SMILE. When the Tolrestat drug is fragmented by using the RECAP Analysis then five fragments are obtained. Form these resulted smile fragments, the appropriate smile fragment has been selected as scaffold for Combinatorial Library Design.

Selection of the Scaffold

The core group containing fragment is selected as the scaffold for designing the combinatrorial library using SmiLib software [67].

Combinatorial Library Generation

The use of Combinatorial Chemistry synthetic methods allow a very large number of molecules to be synthesized much more rapidly and at lower cost than traditional synthetic chemistry. The aim of Library Design is to reduce the number of molecules, which need to be made without decreasing the diversity of the library. This has the potential of finding leads more rapidly because a smaller number of molecules are tested by avoiding molecules, which are very similar. The virtual library is constructed by functionalizing central molecular fragments called *scaffolds.*

SmiLib V2.0 software also generates the combinatorial library. SmiLib is a platform independent command line and graphical user inferface software tool designed to rapidly create combinatorial libraries [67] SmiLib deals with three classes of molecules: scaffolds, linkers and building blocks. Linkers function as junctions between scaffolds and building blocks. The Linker is represented as [A][R]. [A] represents the attachment points in scaffold and [R] for R-groups [Table-1]. The resulting molecule is a fusion product of its single molecule components. Thus SmiLib concatenates scaffolds with linkers and those with building blocks.

Filtration of the Combinatorial Library Molecules: Using ADME TOX FILTER

Filtration is required for finding the Toxic and Non-Toxic Compound (s). For filtration the FAF (ADMEtox) [68] has been used. This tool is an attempt to have a database of molecules that have physical properties and chemical functionality consistent with known drugs/leads/hits. Common filtering protocols are variations of Lipinski s rule-of-five (potential for oral bioavailability) and/or filters that include a limit on the number of rotatable bonds, on the polar surface area, on logP (P = calculated octanol/water partition coefficient) or also compounds containing specific chemical substructures associated with poor chemical stability or toxicity and sometimes that attempt to predict drug metabolism [69] [Table-2]. The molecular library in SMILE format is uploaded in ADMEtox.

Table 1- The scaffold molecule used and examples for some of the Building Blocks; The entire combinatorial library is enumerated by exhaustively cycling through all combinations of R-groups at every attachment point on every scaffold. The virtual library is written to an output database.

Scaffold	C([R2])N(C([R1])C(O([R3]))=O)C(=S)c1ccccc1
Linker	[A][R]
Building Blocks	CCCN[A]
	CCC(N[A])C=O
	c1cc(ccc1)C[A]
	CCCC[A]
	C(CCC)(N[A])C
	CC(C)C[A]
	CCC[A]
	c1cc(ccc1O)C[A]
	CC[A]
	CCC[A]
	CCCC[A]
	C(CC)(N[A])C
	c12ccccc1ccc(c2)C[A]
	C1CCC(CC1)C[A]
	[A]C(C)(C)C
	C(C)(C)C[A]
	[A]c1cc(ccc1NC)C
	[A]c1cc(ccc1OCC)C
	[A]c1cc(ccc1OCC)C
	[A]c1cc(ccc1I)C
	[A]c1cc(ccc1OC)C
	c1cc(ccc1OC)C[A]

Table 2- The Lipinski's rule of five filtering tool parameters set for filtration of the Combinatorial Library Molecules

Parameter	Range
Toxic atoms Filter	Filter the toxic atoms
Molecular weight	200-500
Hydrogen bond donors	5
Hydrogen bond acceptors	10
LogP	<5

The results obtained are then separated for toxic and non toxic molecules by using a Perl script. This will generate the list of toxic and non toxic compounds in separate folders in the bin the non toxic compounds are taken are subjected to cleaning and optimization. After filtration, the non-toxic compound are cleaned and optimized by the use of MarvinView and these results are used for further process docking. The process of cleaning and optimization removes the strains from the molecules and give the molecules a 3 dimensional orientation.

The Process of Virtual Screening by ScreenMD

ScreenMD performs fast virtual screening of large compound libraries using molecular descriptor sets. Virtual screening aims to find compounds that exhibit required chemical, structural, pharmacological or other properties [70]. Such properties are represented as molecular descriptor sets and these descriptor sets are compared against each other by calculating a dissimilarity score between them. Thus the goal of the screening procedure is often expressed as an allowed maximal dissimilarity score, structures with a dissimilarity score below such predefined threshold are accepted by the screening process, while others are rejected. Those that are accepted forms the hit set. As a generic definition, a molecular descriptor is simply a set of values associated with a two or three di-

mensional molecular structure. It includes chemical (topological) fingerprints, 2-dimensional pharmacophore fingerprints, reaction fingerprint and structural keys of molecular structures. Some examples are LogP, LogD, BCUT, HDon, HAcc, PF, CF etc. ScreenMD is done on command line and then checked for the presence of Z-Axis and then converted it to mol2 format and splitted with the help of Perl script.

Auto Dock/Docking

Further step is docking of the non- toxic ligands after filtration and 1AH3 (known receptor for Tolrestat), by using the Auto Dock program in EXOME™ is an integrated, multi-user work environment for Bioinformatics research, data management and training [W2]. It has Bioinformatics software suites required by high-end Biological Research labs. From suites for large genome analysis, to automated drug design. Using EXOME™-Horizon the user can carry out analyses too complex resource intensive for other platforms. Auto Dock is a computational molecular docking tool in EXOME™-Horizon as a part of the Structure Analysis Tools, which can be used for predicting whether one molecule will bind to another, usually a protein.

In the EXOME™-Horizon docking processes are completed in following steps,

- Macromolecules preparation
- Ligand preparation
- Autodocking
- Analysis

In macromolecules preparation step, 1AH3 protein was splitted into different chains. From that, chain A (which has the known active site for ligand binding) was selected from PDB and H- atoms, partial charges and solvent were added. Before going to Auto Dock the resulted non-toxic SMILEs are converted into MOL2 (since the accepted formats for ligand in Auto Dock are only PDB, MOL2 and PDBQ) using another tool in EXOME™-Horizon called BABEL (Molecular Format Conversion Tool). In ligand preparation step MOL2 format of non-toxic Tolrestat drug like library is prepared after adding H- atoms and Partial Charges. Additional parameters were also added in the ligand preparation such as- merge charges of non-polar H Atoms, Atom connectivity to Atom records, information in mol2 file to define root and active torsion was used, rings for aromaticity were checked, cut-off angle between adjacent atoms in aromatic rings were set. Then the prepared 1AH3 Receptor and molecules are docked by using the Grid parameters (X= 59.222 Y= 32.518 Z= 93.627). The result of docking process that is done with the default parameters that are calculated by the Auto Dock program, are usually false. To avoid this, the X,Y,Z co-ordinates of the chain A is obtained with the help of Active Site Finding Tool, acsite and co-ordinates finding tool called PASS. Along with the Center of Grid Map, the following parameters were also used for Docking. Docking is performed using the finest algorithm called Lamarckian Genetic Algorithm.

Parameters	Values
No of Grid Points	X=60, Y=60, Z=60
Spacing of Grids	0.375

Results and Discussion

In order to generate combinatorial library, SMI-LIB software is used. The fragmentation was done using fragmentor in J-Chem package and as a result of that five fragments were obtained. The biggest

and core fragment was selected as scaffold [Fig-3] for SMI-LIB for further library generation. As a result, using same scaffold, 10648 compounds were obtained, which reduced to 1361 after filtration in FAF filter. This program filters lead like molecules on the basis of Lipinski's rule. These molecules were utilized for virtual screening. ScreenMD performs fast virtual screening of large compound libraries using molecular descriptor sets. After the step of screening, 16 molecules were obtained, which were further used as a ligand in the docking step. The ligand molecules generated as a result of Combinatorial library synthesis and Virtual screening that have been docked with the receptor AR and a single molecule TOL727 [Fig-4] was resulted which has least docking energy as compared to original Tolrestat molecule [Table-3] is achieved. When docked with AR (1AH3) only one molecule in combinatorial library gave better binding energies as compared to Tolrestat [Table-3], [Fig-3].
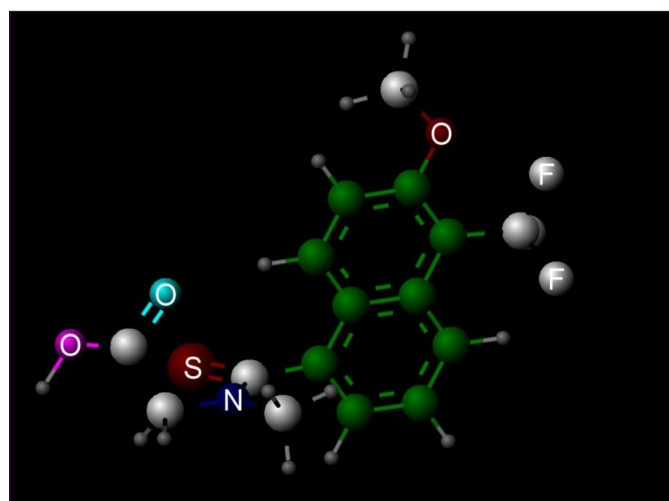

Fig. 3- Scaffold of Tolrestat used in combinatorial library synthesis

Table 3- The results of molecular docking performed in the Exome Horizon Tool : Comparison of pharmacophoric properties and binding energies for TOL727 and Tolrestat.

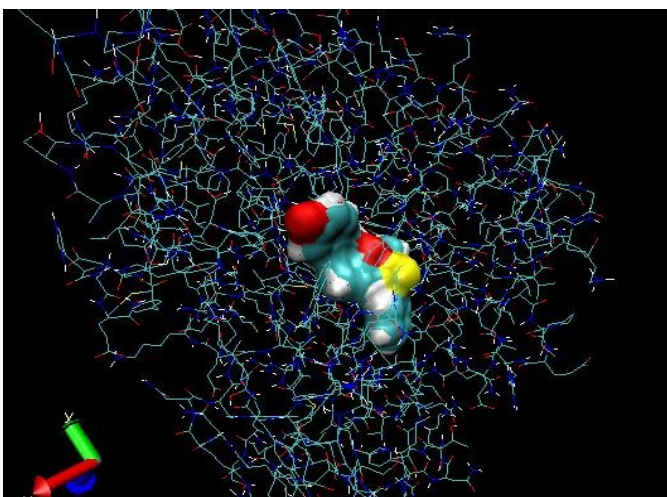| Molecular ID	Tolrestat	TOL727
Chemical Formula	$C_{16}H_{14}N_1O_3F_3S_1$	
Docking Energy (D)	-10.84	-11.91
Aromatic (r)	10	6
Hydrophobic (h)	9	15
HBD (d)	2	1
HBA (a)	3	4
HBA/HBD (a/d)	1	-

On comparison it was found that numbers of hydrophobic groups are more in most of the ligands as compared to Tolrestat. While in case of other molecules, number of hydrophobic groups is approximately same to the number of aromatic groups, which also takes part in hydrophobic interactions. [Fig-4] shows the only molecule having less binding energy receptor bound ligand with AR (1AH3). This clearly shows that conformation adopted by all other ligands is totally different as compared to original ligand. It was found that Tolrestat and the result from SmiLib are almost similar in structure and also in docked state and also their comformation is totally different as compared to original ligand for AR 1AH3. As a conclusion Tolrestat shows very good binding with AR and number of analogues of Tolrestat can be further studies for designing better drugs for diabetes. The final ligand found (TOL 727) have been shown
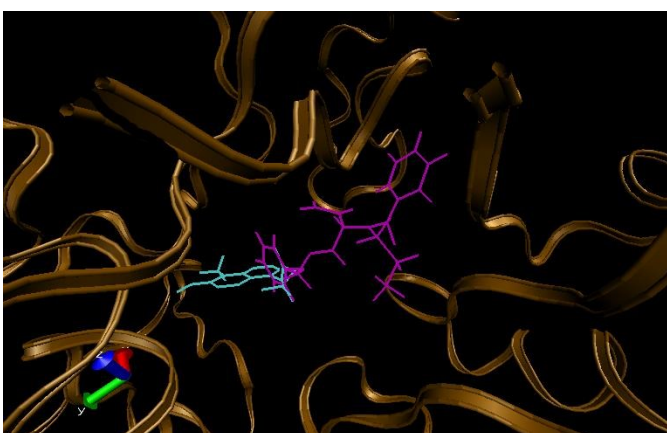
docked with the active site of 1AH3 molecule in its best confor-mation [Fig-5]. Comparison of bindings in the molecular surface view of TOL 727 and Tolrestat in the 1AH3 chain has also been done [Fig-6],[Fig-7],[Fig-8],[Fig-9].
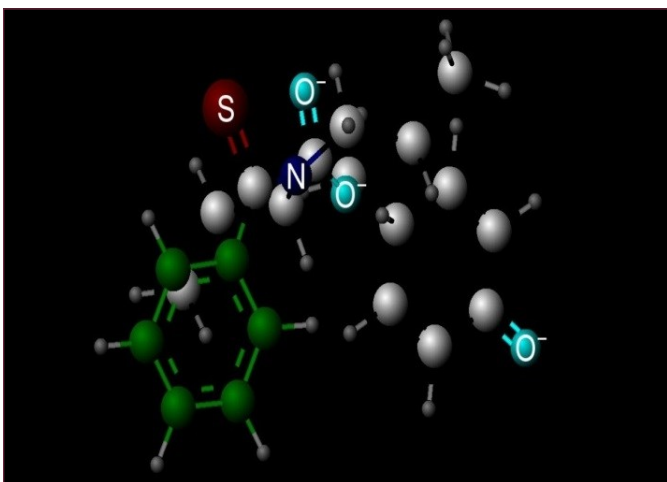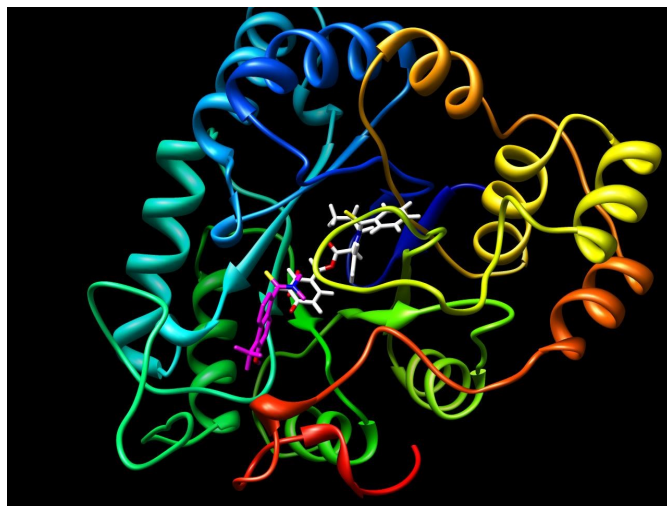

**Fig. 4-** Molecular structure of TOL 727

Fig. 5- The final ligand (TOL 727) found in its best confirmation docked with the active site of 1AH3 molecule.

Fig. 6- The molecular surface view depicting the docked state of TOL 727 in Purple and Tolrestat in Cyan in the 1AH3 chain shown in Ochre. 1AH3 is displayed as Ribbons while the ligands are shown as Sticks.
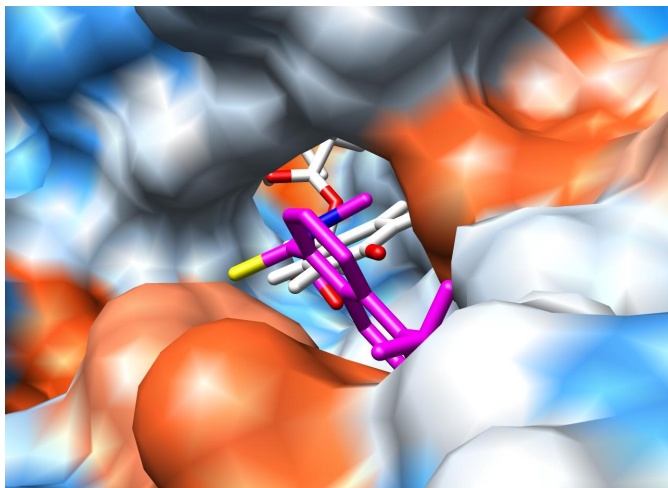

Fig. 7- The molecular surface view depicting the docked state of TOL727 and Tolrestat (in Purple) in the 1AH3 chain. 1AH3 is dis-played as Cartoons while the ligands are shown as Sticks.
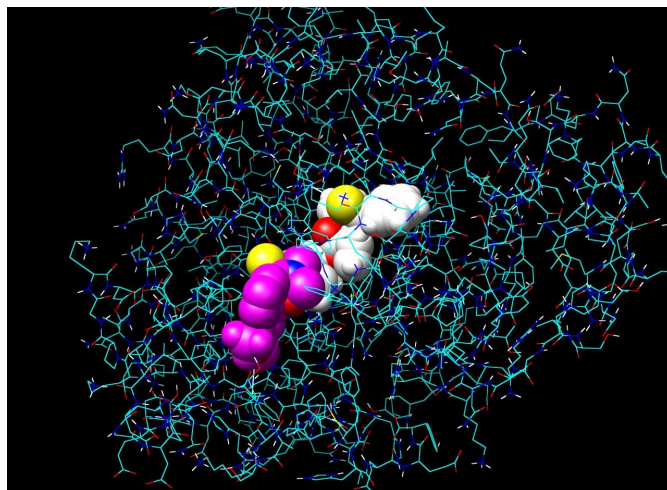
Fig. 8- The molecular surface view depicting the docked state of TOL727 and Tolrestat (in Purple) in the 1AH3 chain shown in Cyan. 1AH3 is displayed as Sticks while the ligands are shown with their surface drawn.

The molecule TOL727 thus achieved after the *in silico* processing in computer aided drug design is having the least docking energy as compared to the original Tolrestat molecule. This might be due the presence of more number of hydrophobic interactions in between ligand and receptor. The crystal structures of the porcine AR holo-enzyme and its complexes with the inhibitors Tolrestat and sorbinil have already been solved and it was found that and binding of the inhibitors was found to involve two contact zones in the active site: first, a recognition region for hydrogen-bond acceptors near the coenzyme, with three centers, including the anionic site; and sec-ond, a hydrophobic contact zone in the active-site cleft, which in the case of Tolrestat includes the specificity pocket. It was concluded that the active site of AR adapts itself to bind tightly to different in-hibitors; this happens both upon binding to the inhibitor`s hydro-philic heads, and at the hydrophobic and specificity pockets of AR, which can change their shape through different conformational changes of the same residues [32]. This flexibility could explain the large variety of possible substrates of AR (W1). Same hypothesis

had also been analyzed in this study and it was found that TOL727 binds in a different mode in the same binding site with more hydrophobic contacts [Fig-4]. Although number of hydrogen bonds formed in between Tolrestat and TOL727 is different, still TOL727 have higher binding energies as compared to Tolrestat. As a conclusion this ligand can be used for further testing in lab and if found with good activity can be suggested as a better lead molecule for AR.

Fig. 9- The molecular surface view depicting the docked state of TOL727 and Tolrestat (in Purple) in the 1AH3 chain shown in Cyan. 1AH3 is displayed as surface drawn on charge basis while the ligands are shown as Sticks. Most negatively charged portions are colored in Blue and most positively charged portions are colored in Red. Hydrophobic regions are displayed as White.

Conclusion

Ongoing work indicates that AR is a critical component of intracellular signaling, and inhibition of the enzyme prevents high glucose-, cytokine-, or growth factor-induced activation of protein kinase C. Thus, treatment with AR inhibitors prevents vascular smooth muscle cell growth and endothelial cell apoptosis in culture and inflammation and rest enosis *in vivo*. Keeping that in mind this study has designed to generate the better analogues of Tolrestat, which may have better binding with AR and again could be used for the treatment of diabetic complications. After analyzing the docking results it was found that the molecule TOL727, achieved after the insilico processing in computer aided drug design have the least docking energy as compared to the original Tolrestat molecule. As a conclusion this ligand can be used for further testing in lab and if found with good activity can be suggested as a better lead molecule for AR.

Abbreviations Used

AR : Aldose Reductase

NADPH : Nicotinamide Adenine Dinucleotide Phosphate

TTD: Therapeutic Targets Database

ROS: Reactive Oxygen Species

SDH: Sorbitol dehydrogenase

GSH: glutathione

EC : Enzyme Commission number

NIDDK: National Institute for Diabetes and Digestive and Kidney Diseases

ARI: Aldose Reductase Inhibitor

GFR: Glomerular Filteration Rate

ADME-tox: Absorption, Distribution, Metabolism, and Excretion - Toxicity

FAF: Free ADME-Tox Filtering

PDB - Protein Data Bank

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