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RESEARCH ARTICLE

SNP BASED DRUG INTERACTION STUDY ON THE KEY ENZYMES OF POLYOL PATHWAY

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ABSTRACT

Diabetes Mellitus is a chronic health disorder. It is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. One of the complications of Diabetes Mellitus results in activation of Polyol pathway. Activation of the Polyol pathway results in a decrease of reduced NADP+ and oxidized NAD+; these are necessary cofactors in redox reactions throughout the body. Aldose reductase and sorbitol dehydrogenase are the enzymes of Polyol pathway. Excessive activation of the Polyol pathway increases intracellular and extracellular sorbitol concentrations, increased concentrations of reactive oxygen species and decreased concentrations of nitric oxide and glutathione. Each of these imbalances can damage cells; in diabetes there are several acting together. Drug designing study was carried out concentrating on the enzymes aldose reductase and sorbitol dehydrogenase of Polyol pathway, the drugs selected showed interaction with aldose reductase but showed no interaction with sorbitol dehydrogenase. So the study was concentrated mainly with aldose reductase (rate limiting enzyme). From the results it was concluded that five lead compounds namely

- *Metformine,*
- *1-(4-methylphenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine*
- *1-(4-flurophenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine*
- *1-(4-chlorophenyl)sulfonyl-3-(3 methylbutyl)urea*
- *1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol showed interaction with the enzyme aldose reductase of Polyol pathway also when their SNP(single nucleotide polymorphism) amino acids were mutated so these compounds were selected as lead compounds from which effective drugs could be built and can be used to restrict the activation of the pathway.*

Key words: *Hyperglycemia, polyolpathway, aldose reductase, sorbitol dehydrogenase, snp, five lead compounds, Metformine, diamine, urea, propan-2-ol,*

INTRODUCTION

Drug Designing

A drug, broadly speaking, is any substance that, when absorbed into the body of a living organism, alters normal bodily function. Most specifically defined as "a chemical substance used in the treatment, cure, prevention, or diagnosis of disease is used to otherwise enhance physical or mental well-being".

Drug discovery and development is an intense, lengthy and an interdisciplinary endeavor. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical in vitro and in vivo studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development.

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Traditionally, drugs were discovered by synthesizing compounds in a time-consuming multi-step processes against a battery of in vivo biological screens and further investigating the promising candidates for their pharmacokinetic properties, metabolism and potential toxicity. Such a development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, *de novo* design, in vitro, insilico ADMET screening and structure-based drug design.

In-silico drug design

In-silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today nd forms the core of structure-based drug design. High-performance computing, data management software and Internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into workable knowledge in modern day drug discovery process. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost.

Diabetes Mellitus

Diabetes mellitus is a chronic health disorder. The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.

Diabetes is due to one of two mechanisms:

1. Inadequate production of insulin (which is made by the pancreas and lowers blood glucose) or
2. Inadequate sensitivity of cells to the action of insulin.

The two main types of diabetes correspond to these two mechanisms and are called insulin dependent (type 1) and non-insulin dependent (type 2) diabetes. In type 1 diabetes there is no insulin or not enough of it. In type 2 diabetes, there is generally enough insulin but the cells upon it should act are not normally sensitive to its action.

The word "diabetes" is borrowed from the Greek word meaning "a siphon." The 2nd-century A.D. Greek physician, Aretus the Cappadocian, named the condition "diabetes." He explained that patients with it had polyuria and "passed water like a siphon."

Types of diabetes

Four related classifications of diabetes have been identified:

- Type 1: Results from body's failure to produce insulin.
- Type 1: Results from a condition in which the body fails to use insulin properly, combined with relative insulin deficiency.
- Gestational diabetes: Pregnant women who have never had diabetes before but who have high blood sugar (glucose) levels during pregnancy are said to have gestational diabetes. Gestational diabetes affects about 4% of all pregnant women and is usually of type 2.
- Pre-diabetes: It is a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes.

Effects of diabetes

The effects of diabetes mellitus include:

- Long term damage- this includes progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, and features of autonomic dysfunction, including sexual dysfunction.
- Dysfunction – endothelial dysfunction, adipocyte dysfunction, pancreatic dysfunction, lower urinary tract dysfunction in women, immunoregulatory dysfunctions, and sexual dysfunctions.
- Failure of various organs- eyes, kidneys, nerves, heart, and blood vessels.

Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.

Characteristic symptoms of diabetes

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and,

in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made.

Diabetic complications

Diabetes and its treatments can cause many complications.

- Acute complications including – hypoglycemia, ketoacidosis, or nonketotic hyperosmolar coma may occur if the disease is not adequately controlled.
- Serious long-term complications include- cardiovascular disease, chronic renal failure, retinal damage- which can lead to blindness, several types of nerve damage, and microvascular damage.

Polyol Pathway

The polyol pathway is also called as Sorbitol-aldose reductase pathway, the polyol pathway appears to be implicated in diabetic complications, especially in microvascular damage to the retina, kidney, and nerves.

Pathway

Glucose is a highly reactive compound, and it must be metabolized or it will find tissues in the body to react with. Cells use glucose for energy, though unused glucose enters the polyol pathway where aldose reductase reduces it to sorbitol. This reaction oxidizes NADPH to NADP+. Sorbitol dehydrogenase can then oxidize sorbitol to fructose, which also produces NADH from NAD+. Hexokinase can return the molecule to the glycolysis pathway by phosphorylating fructose to form fructose-6-phosphate. However, in uncontrolled diabetics who have high blood glucose - more than the glycolysis pathway can handle - the reaction's mass balance ultimately favors the production of sorbitol.

The polyol pathway is a two-step metabolic pathway in which glucose is reduced to sorbitol, which is then converted to fructose. It is one of the most attractive candidate mechanisms to explain, at least in part, the cellular toxicity of diabetic hyperglycemia because (i) it becomes active when intracellular glucose concentrations are elevated, (ii) the two enzymes are present in human tissues and organs that are sites of diabetic complications, and (iii) the products of the pathway and the altered balance of cofactors generate the types of cellular stress that occur at the sites of diabetic complications.

Results of activation of Polyol Pathway

Activation of the polyol pathway results in a decrease of reduced NADP+ and oxidized NAD+; these are necessary cofactors in redox reactions throughout the body. The decreased concentration of these cofactors leads to decreased synthesis of reduced glutathione, nitric oxide, myo-inositol, and taurine. Myo-inositol is particularly required for the normal function of nerves. Sorbitol may also glycate nitrogens on proteins, such as collagen, and the products of these glycations are referred-to as AGEs - advanced glycation endproducts. AGEs are thought to cause disease in the human body, one effect of which is mediated by RAGE (receptor for advanced glycation endproducts) and the ensuing inflammatory responses induced. They are seen in the hemoglobin A1C tests performed on known diabetics to assess their levels of glucose control.

Excessive activation of the polyol pathway increases intracellular and extracellular sorbitol concentrations, increased concentrations of reactive oxygen species and decreased concentrations of nitric oxide and glutathione. Each of these imbalances can damage cells; in diabetes there are several acting together. It has not been conclusively determined that activating the polyol pathway damages microvasculature.

Thus excessive activation of the polyol pathway leads to increased levels of sorbitol and reactive oxygen molecules and decreased levels of nitric oxide and glutathione, as well as increased osmotic stresses on the cell membrane. Any one of these elements alone can promote cell damage, but here we have several acting together

Enzymes of polyol pathway

Two enzymes are involved in the pathway

1. Aldose reductase(the rate limiting enzyme)
2. Sorbitol dehydrogenase

Aldose reductase

Aldose reductase (or aldehyde reductase) is an enzyme in carbohydrate metabolism that converts glucose to sorbitol. Specific reactions catalyzed by this enzyme include:

- glucose+ NADPH + H^+ →sorbitol + $NADP^+$
- galactose+ NADPH + H^+→ galactitol + $NADP^+$

Function of aldose reductase-

- ✓ The aldose reductase reaction, in particular the sorbitol produced, is important for the function of various organs in the body.

- ✓ For example, it is generally used as the first step in a synthesis of fructose from glucose;
- ✓ The second step is the oxidation of sorbitol to fructose catalyzed by sorbitol dehydrogenase.
- ✓ The main pathway from glucose to fructose (glycolysis) involves phosphorylation of glucose by hexokinase to form glucose 6-phosphate, followed by isomerization to fructose 6-phosphate and hydrolysis of the phosphate, but the sorbitol pathway is useful because it does not require the input of energy in the form of ATP
- ✓ Seminal vesicles: Fructose produced from sorbitol is used by the sperm cells.
- ✓ Liver: Fructose produced from sorbitol can be used as an energy source for glycolysis and glyconeogenesis.

Role in diabetes

In a hyperglycemic state, the affinity of aldose reductase for glucose rises, causing much sorbitol to accumulate, and using much more NADPH, leaving less NADPH for other processes of cellular metabolism. Thus aldose reductase is long been believed to be responsible for diabetic complications involving a number of organs. Many aldose reductase inhibitors have been developed as drug candidates but virtually all have failed although some are commercially available in several countries.

Sorbitol dehydrogenase

Sorbitol dehydrogenase also known as SORD is a protein which in humans is encoded by the SORD gene. It is involved in converting sorbitol, the sugar alcohol form of glucose, into fructose. Together with aldose reductase, it provides a way for the body to produce fructose from glucose without using ATP.

Its reaction is

$$\text{Sorbitol} + NAD^+ + H^+ \rightarrow \text{fructose} + NADH$$

A zinc ion is also involved in catalysis. Organs that use it most frequently include the liver and seminal vesicle; it is found in all kinds of organisms from bacteria to humans. A secondary use is the metabolism of dietary sorbitol, though sorbitol is known not to absorb well in the intestine as its related compounds glucose and fructose, and is usually found in quite small amounts in the diet anyway (except when used as an artificial sweetener).

In this study SNP based drug interaction study was carried out on the key enzymes of Polyol pathway; a complication of Diabetes Mellitus, using Bioinformatics tools.

MATERIALS

Bioinformatics Tools & Databases

The tools and databases used in the present study are,

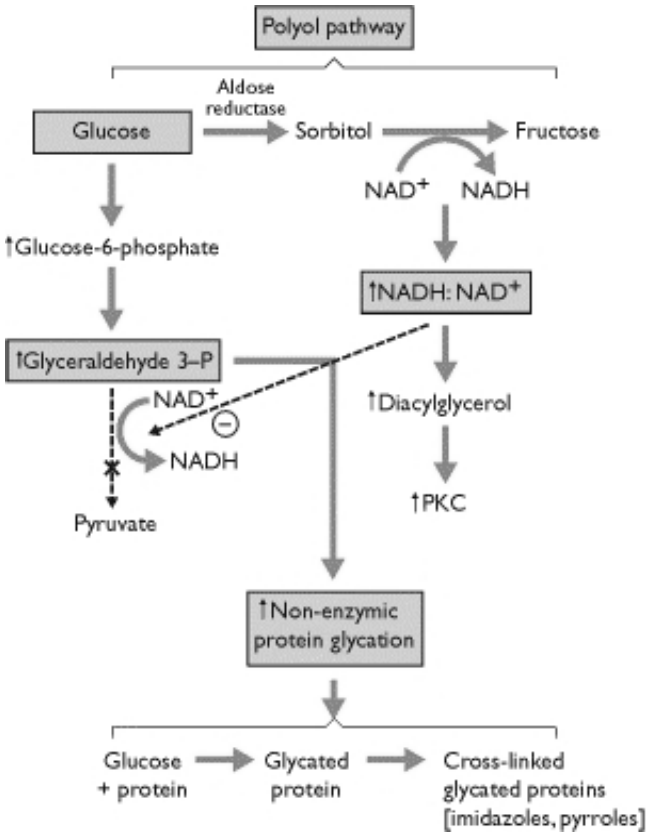
- ❖ NCBI
- ❖ PIR
- ❖ PDB
- ❖ DRUG BANK
- ❖ SWISS PDB VIEWER
- ❖ HEX
- ❖ ZINC DATABASE
- ❖ NCI ENHANCED BROWSER
- ❖ MOLECULAR FORMATS CONVERTER
- ❖ CLUSTALW
- ❖ SWISSPROT
- ❖ PREADMET

RESULT AND DISCUSSSION

Disease selection

The disease selected is "**Diabetes Mellitus and its Complications due to Polyol Pathway"**. The Polyol pathway is found to be implicated in diabetic complications, especially in microvascular damage to the retina, kidney, and nerves.

a. The enzymes involved in this pathway are Aldose reductase and Sorbitol dehydrogenase.
b. Enzyme Aldose reductase is rate limiting enzyme.



The study is concentrated on the enzymes of polyol pathway, aldose reductase and sorbitol dehydrogenase. Drugs and analogs are selected and are interacted with these enzymes using various bioinformatics tools. The most interactive drugs and analogs are noted.

Enzymes of study: Aldose reductase and Sorbitol dehydrogenase

PDB IDs of the enzymes aldose reductase and sorbitol dehydrogenase were retrieved from the database PIR.

- Enzyme name aldose reductase or sorbitol dehydrogenase is typed in text search box of PIR home page.
- The retrieved page contains all the information of all the organisms, to specifically retrieve human enzyme details; the display option is made unique to human.
- That is, protein name is selected and the name of the enzyme is typed. And organism name in selected and human is typed. In the display option PDB ID is also selected as to retrieve PDB IDs.
- Thus PIR gives the PDB IDs of the given enzyme aldose reductase or sorbitol dehydrogenase.

Thus the interaction site amino acids are retrieved.

- This procedure is followed for each and every PDB IDs thus retrieved for both the enzymes, aldose reductase and sorbitol dehydrogenase.
- And the interaction site amino acids are noted down.
- All the amino acids of all the IDs are compared and common amino acids in the interaction site are noted separately for the further study.
- The list of amino acids for every Id is listed below.

Interaction site amino acids thus retrieved for aldose reductase are

PDB IDS	Interaction site amino acids of aldose reductase
1T41	Thr266, Arg267, Glu272, Val265,Ser264, Asn273, Lys263, Thr20, Trp21, Tyr49, Ser214, Leu213, Gly214, Asp44, Ser211, His111, Trp112, Ser160, Gln183, Asn160
1US0	His164, Asn161, Ser160, Gln183, Trp112, His111, Pro212, Gly214, Ser211, Leu213, Ser215, Lys263, Asn273, Ser264, Glu272, Arg269, Val265, Thr266, Asp44, Trp21, Thr20, Tyr49, Lys22, Lys95, Glu52, Asp99, Asn53, Glu54
1EF3	Arg269, Thr266, Asn273, Val265, Ser264, Leu213, Lys263, Ser215, Thr20, Trp21, Ser211, Asp44, Tyr49, Gln184, Leu301, His111, Ser160, Asn161, Trp112, Glu272, Asn273
1PWM	Gln184, Ser160, Asn161, Asp44, His111, TRp112, Ser211, Leu212, Asn273, Glu272, Arg269, Thr266, Val265, Ser264, Gly214, Lys263, Ser215, Thr20, Trp20, Leu301, Lys22, Tyr49.
1ADS	Asn61, Gln184, Ser160, Ser211, Leu213, Ser215, Asn273, Lys263, Asp44, Glu271. Trp21, Thr20, Arg269, Ser264, Thr266, Val265, Lys22.

1MAR	Atoms missing, no amino acids retrieved
1IEI	Thr266, Val265, Lys22, Thr20, Trp21, Ser264, Lys263, Arg269, Ser215, Glu272, Asn273, Ser211, Leu211, Leu213, Tyr49, Asp44, Lys78, His111, Trp112, Ser160, Gln184
1AZ1	Glu272, Asn273, Arg269, Leu213, Ser214, Ser211, Thr266, Ser264, Lys263, Val265, Asp43, Thr20, Lys22, Trp21, Asn161, Ala300, Leu311, Leu302, Ser160, Trp112, His111, Tyr49
1ABN	Atoms missing, no amino acids retrieved
1X98	Glu271, Asn273, Leu213, Gly214, Arg269, Ser211, Gln184, Ser215, Lys263, Ser264, Thr266, Asn161, Ser160, Asp44, Val265, Trp21. Trp112, His111, Lys22, Tyr49, Thr20.
1PWL	Ser160, His11, Trp112, Gln184, Asn161, Asp44, Tyr49, Thr20, Trp21, Ser211, Lys263, Lys22, Asn273, Ser264, Val265, Leu213,Ser215, Glu271, Arg269, Thr266, Gly214.
1XGD	No ligand bound
1Z3N	Thr266, Arg269, Glu271, Val265, Ser264, Asn273, Lys263, Lys22, Thr20, Trp21, Asp44, Leu213, Tyr49, Ser211, His111, Gln184, Ser160, Trp112, Asn161
1AZ2	Glu271, Asn273, Thr266, Val265, Lys263, Ser211, Tyr220, Thr20, Trp21, Asp44, Asn160, Ser160, His111, Trp112
1X97	Glu271, Asn273, Arg269, Thr266, Leu213, Ser264, Val265, Pro211, Lys263, Gly220, Ser211, Thr20, Asp44, Trp21, Lys22, Gln183, Ser160, Asn161, His111, Tyr49
1T40	Glu272, Arg269, Thr266, Asn273, Ser264, Lys263, Leu213, Ser215, Lys22, Thr20, Trp21, Ser211, Asp44, Tyr49, Gln184, His111, Ser160, Asn161, Trp112
1X96	Thr266, Val265, Arg269, Glu271, Ser264, Lys22, Tyr49, Thr20, Trp21, Lys263, Ser215, Leu213, Asp44, Ser211, His111, Trp112, Ser160, Asn161, Gln184
1Z89	Gln184, Ser159, Asn161, Trp112, His111, Asp44, Asn273, Ser211, Leu213, Gly214, Glu272, Ser215, Lys263, Thr20, Trp21, tyr49, Ser264, Arg269, Lys22, Val265, Thr266

Thus the common amino acids in interaction site of aldose reductase are

From the above list, thus found common amino acids from all the IDs of aldose reductase are His111, Trp112, Thr267, Glu272, Val265, Ser264, Asn273, Lys263, Thr20, Trp21, Tyr49, Ser215, Leu213, Asp44, Ser211, Ser160, Gln184, Asn161, Lys22, and Gly214.

Interaction site amino acids thus retrieved for sorbitol dehydrogenase are

PDB IDs	Interaction site amino acids of sorbitol dehydrogenase
1PL6	Arg209, Asp204, Pro183, Leu275, Ile184, Val273, Cys45, Glu156, Val297, Arg299, Pro183, Ile184, Val237, Leu275.
1PL7	Arg209, Asp204, Leu275, Pro183, Ile184, Val273, Val296, Glu156, ARG299
1PL8	Glu156, Pro183, Arg299, Ile184, Val297, Val273, Leu275, Arg209, Asp204

Thus the common amino acids in interaction site of sorbitol dehydrogenase are

From the above list, thus found common amino acids from all the IDs of sorbitol dehydrogenase are Arg209, Asp204, Pro183, Ile184, Val273, Val297, Arg299, Arg209, Leu275.

Thus retrieved common amino acids are used in further study for comparison purpose. The next step is the selection of drugs. These drugs are interacted with the enzymes and interaction site amino acids are noted.

Drug selection

The Drugs for the disease "Diabetes mellitus and its complications due to Polyol pathway" is searched through Drugbank and Internet explorer.

Thus selected some of the drugs are

Drugs	
1. Acetohexamide	11. Nadolol
2. Aspirin	12. Oxprenolol
3. Buformin	13. Phenelzine
4. Chlorpropamide	14. Pindolol
5. Clofibrate	15. Pioglitazone
6. Fidarestat	16. Repaglinide
7. Gliclazid	17. Rosiglitazone
8. Glimepiride	18. Sitagliptin
9. Metoprolol	19. Sunlindac
10. Metformin	20.Timolol

These are the selected drugs, which are interacted with the enzymes, and the amino acids in the interaction site are noted

Interaction study: Enzymes (Aldose reductase and sorbitol dehydrogenase) with the drugs.
The drugs were interacted with the enzymes and the amino acids in the interaction site were noted down. This process was done using the program "Hex".

For example: Aldose reductase and Acetohexamide.

- Hex program is opened.
- Loading receptor.
  File→ open receptor→ pathname of receptor
  Before loading the drug, the ligand that is found interacting in the enzyme structure is removed using Swiss PDB viewer.
- Loading drug (ligand)
  File→ open ligand→ pathname of ligand
- Coloring receptor and ligand
  Controls→ Molecule→ Receptor→ color chart→ select color
  Controls→ Molecule→ Ligand → color chart→ select color
- Matching noted
  Controls → Matching→ Activate
- Docking
  Controls→ Docking→ Activate
  On completion of docking, the image is saved as a PDB file.
  This PDB file is opened in Swiss PDB viewer and the amino acids in the interaction site are noted.

List of drugs and interaction site amino acids

SL. No	Drugs	Interaction site amino acids
Aldose reductase		
1.	Acetohexamide	Asn161
2.	Aspirin	Lys195, Glu194
3.	Buformin	Pro212, Cys187
4.	Chlorpropamide	Glu314
5.	Clofibrate	Gln193
6.	Fidarestat	Glu194
7.	Gliclazid	His111, Cys299
8.	Glimepiride	Lys22
9.	Metoprolol	His42
10.	Metformin	His42, His111
11.	Nadolol	Arg297
12.	Oxprenolol	Asn8
13.	Phenelzine	Ile43
14.	Pindolol	Ser211
15.	Pioglitazone	Arg297
16.	Repaglinide	Arg297
17.	Rosiglitazone	Asn161, Leu196, Lys195
18.	Sitagliptin	Oxt315
19.	Sunlindac	Tyr49
20.	Timolol	Arg218
Sorbitol dehydrogenase		
Drugs showed no interaction with the enzyme so no amino acids were retrieved.		

These were the interaction sites amino acids retrieved from drug interaction. This information is used for comparison purpose in the further study.

To enrich the study various analogs for these drugs were searched (with Zinc and NCI Database) and their interactions with the enzymes were also noted. This would provide with various other similar compounds that interact with these enzymes.

Analog Selection
Analogs were searched using Zinc and NCI enhanced database browser.

From Zinc database:

- The query for the database was given in the smile format.
- Smile formats of each and every drug is present in Drugbank, these are saved.

- The online page of zinc database is opened; the smile of each drug is copied and pasted on to the query box.
- On clicking the search box, various related similar compounds are retrieved.
- From these various compounds, 10 compounds are selected for each drug without violating the Lipinski's rule,

"Lipinski's" rule: Christopher A. Lipinski formulated a so-called **rule of five** as criteria for oral bioavailability. These four "rules" are common characteristics found in most drugs available today. The rule of five derives its name from the fact that the relevant cutoffs are all multiples of five:

- not more than 5 hydrogen bond donors (OH and NH groups)
- no more the 10 hydrogen bond acceptors (notably N and O)
- molecular weight under 500
- LogP under 5

- The PDB format of the analogs thus selected was saved for further study.

From NCI enhanced database browser:

- The query for the database was given in the smile format.
- Smile formats of each and every drug is present in Drugbank, these are saved.
- The online page of NCI enhanced database browser is opened; the smile of each drug is copied and pasted on to the query box.
- On clicking the search box, various related similar compounds are retrieved.
- From these various compounds, 10 compounds are selected for each drug without violating the Lipinski's rule.

The analogs are searched either from zinc or from NCI enhanced database browser. 180 drug analogs were selected were interacted with the enzymes and their interactions with the enzymes was noted.

If there were any interaction, the interaction site amino acids were noted.

The interaction process was carried out with the help of program "Hex". The steps involved were similar to that done during drug analysis.

The interaction site amino acids were noted using "Swiss PDB Viewer". The steps involved were similar to that done during drug analysis.

Some of the analogs showed interaction during the process, their interaction was noted and also the interaction site amino acids were noted

List of analogs and their interaction site amino acids with the enzymes

Interaction site amino acids of aldose reductase with analogs					
Analogs	**Zinc ID**	**Interaction site amino acids**	**Analogs**	**Zinc ID**	**Interaction site amino acids**
1.	146412	Glu194, Asn293	**22.**	5162173	Ala300
2.	146413	Trp112, Cys299	**23.**	5352882	His111
3.	146414	Trp21, His111	**24.**	3848	Tyr49
4.	203637	Val48	**25.**	9355	Glu315
5.	203639	His111, Trp21	**26.**	27810	Lys195
6.	1530579	Arg297, Lys195, Glu194,	**27.**	27812	Lys195, Glu194
7.	1530580	Trp220, Arg297	**28.**	4354	Asn257, Ile205
8.	406544	Cys299	**29.**	56646	Leu194
9.	1618706	Trp112	**30.**	984510	Arg297
10.	1650339	Asn161	**31.**	984511	Arg297
11.	1708272	Asn161, Gln193, Arg297	**32.**	1314340	Asn161
12.	1726559	His111	**33.**	1314341	Val206
13.	162651	Gln193	**34.**	984514	Lys195
14.	538438	Ser215	**35.**	968327	Arg297
15.	2486	Trp21, Val 48	**36.**	1482946	Asn161, Tyr210
16.	605064	Lys21, Asn161	**37.**	968328	Lys263
17.	523925	Lys78	**38.**	968330	Asn293
18.	523926	Trp112	**39.**	1489478	Trp112
19.	537791	Ser215, Leu213	**40.**	4614003	Leu213, Ser215
20.	1530567	His111	**41.**	2176	Trp21
21.	403609	Glu314, Gln193, Lys195			
Interaction site amino acids of sorbitol dehydrogenase with analogs					
Analogs showed no interaction with the enzyme so no amino acids were retrieved					

Out of 180 analogs, 41 analogs were found to interact with the enzyme aldose reductase. But among these analogs no one interacted with the enzyme sorbitol dehydrogenase.

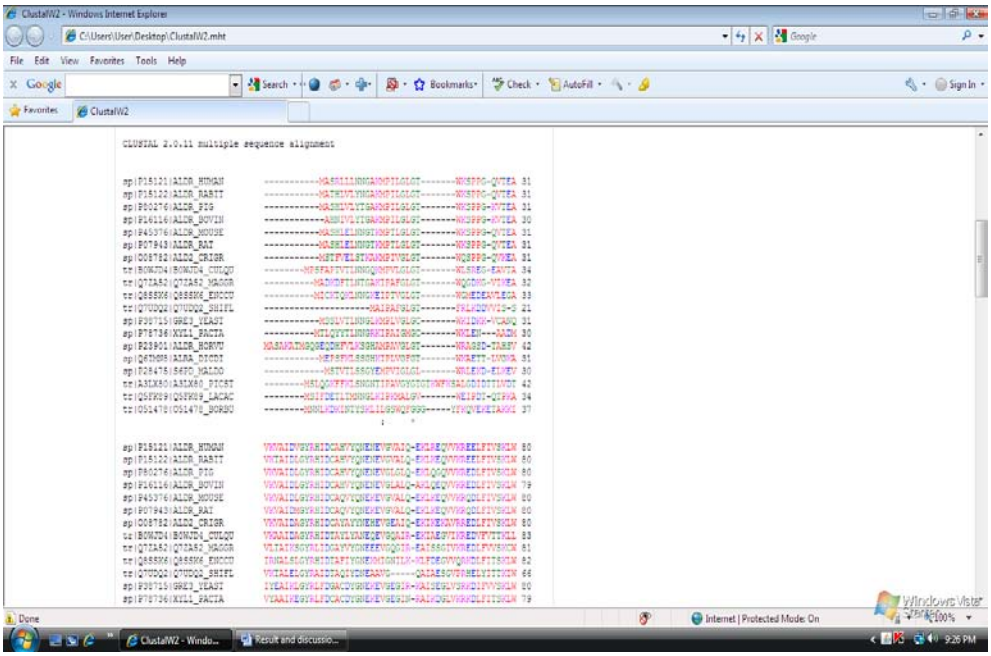
This information was also used for the purpose of comparison in the further study. It was noted that the drugs and analogs showed no interaction with the enzyme sorbitol dehydrogenase so the enzyme sorbitol dehydrogenase was excluded from further study.

It was noted that sorbitol dehydrogenase showed no interaction with the selected drugs or the analogs, so the enzyme sorbitol dehydrogenase was excluded from further study. Now the study is concentrated only with the enzyme aldose reductase.

Conserved amino acid study: For the enzyme aldose reductase

For comparison purpose conserved amino acids from multiple sequences were also gathered,

- Conserved sequences are similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures or polymeric carbohydrates across species or within different molecules produced by the same organism.
- Conservation of protein structures is indicated by the presence of functionally equivalent, though not necessarily identical, amino acid residues and structures between analogous parts of proteins.
- The genetic sequences of the enzyme aldose reductase of multiple organisms or the amino acid sequence of proteins from several species is screened.
    - This process is done using ClustalW.
    - 18 sequences of aldose reductase were selected from PIR, their FASTA sequences were retrieved.
    - Retrieved FASTA sequences were saved.
    - Saved FASTA sequences were then uploaded to clustalW or copied on to the query box.
    - This retrieves the conserved amino acids of the sequences.



Thus retrieved conserved amino acids are

Gly19, Gly39, Asp44, Ala46, Tyr49, Lys78, Leu97, Tyr104, Asp105, His111, Pro113, Gly152, Gly158, and Glu184. This information is also used for further comparison.

The required information's for the purpose of comparison in the study were retrieved; now the enzyme of interest is studied to gather the important amino acids in it, like active site, substrate-binding site and modified sites.

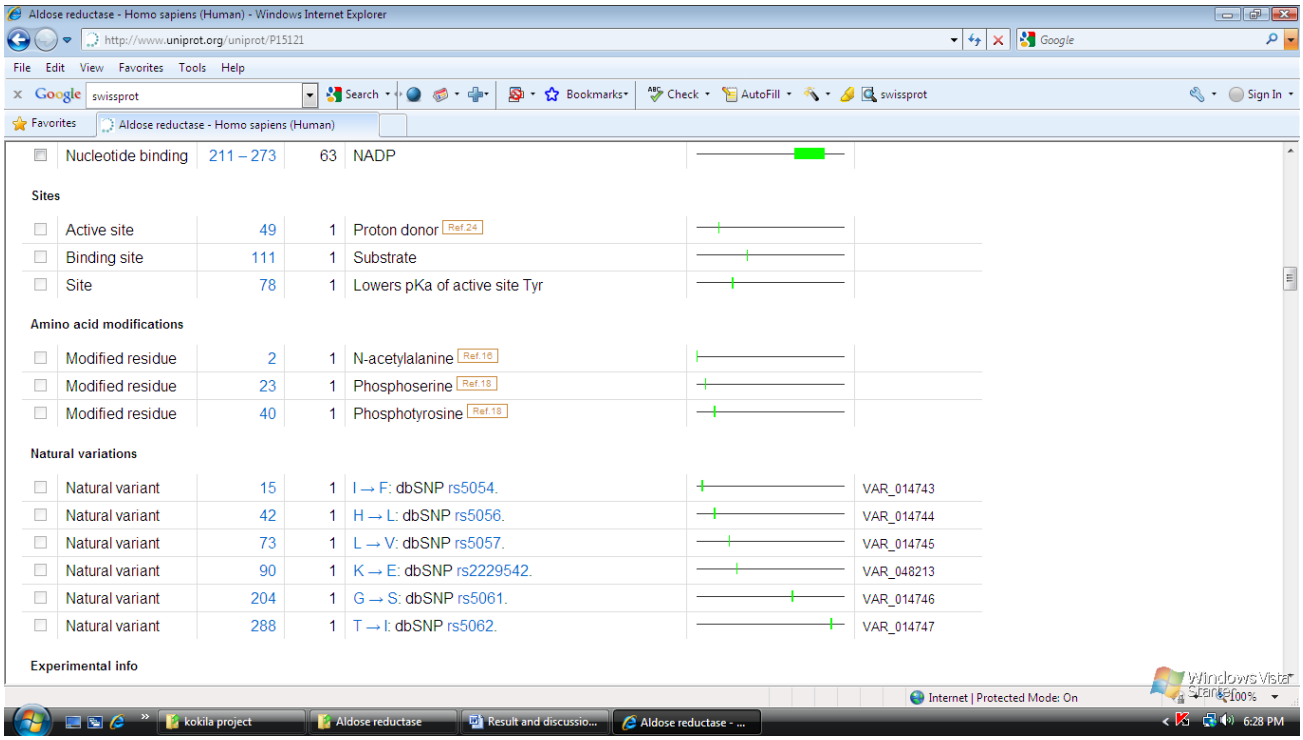
Enzyme study: study of active site, substrate binding site, modified site and natural variant amino acids.

Aldose reductase

Information retrieved from Swiss Prot.

S.N	Feature key	Position	Length	Descriptions
Sites				
1.	Active site	49	1	Proton donor
2.	Binding site	111	1	Substrate

3.	Site	78	1	Lowers pKa of active site Tyr
Amino acid modifications				
1.	Modified residues	2	1	N-acetylalanine
2.	Modified residues	23	1	Phosphoserine
3.	Modified residues	40	1	Phosphotyrosine
Natural Variations				
1.	Natural variant	15	1	I→F: dbSNP
2.	Natural variant	42	1	H→L: dbSNP
3.	Natural variant	73	1	L→V: dbSNP
4.	Natural variant	90	1	K→E: dbSNP
5.	Natural variant	204	1	G→S: dbSNP
6.	Natural variant	288	1	T→I: dbSNP
Experimental Info				
1.	Mutagenesis	44	1	D→N: Reduced Enzymatic activity
2,	Mutagenesis	49	1	Y→F: Complete loss of enzymatic activity
3.	Mutagenesis	78	1	K→M: Reduced Enzymatic activity
4.	Mutagenesis	111	1	H→N: Reduced Enzymatic activity



Comparison of this information with all the retrieved results showed that His42-dbSNP (Natural variant) and His111-Substrate binding site are found as important amino acids that are found both in interaction site of enzyme, interaction site amino acids of drugs and enzyme & interaction site amino acids of analogs and enzyme. His111 is also found as one of the amino acid in the conserved amino acid in multiple sequence alignment.

Thus the drugs and analogs showing interaction with His42 and His111 are taken for further study.

His42 of the enzyme was found interacting with 2 drugs, namely

- Metformin and
- Metoprolol

His111 of the enzyme was found interacting with 7 compounds

- Gliclazid
- Metformin
- 1-(4-methylphenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine [Zinc ID: 146414]
- 1-(4-flurophenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine [Zinc ID: 203639]
- 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea [Zinc ID: 1726559]
- 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol [Zinc ID: 1530567]
- (2S)-1-(tert-butylamino-3-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]propan-2-ol [Zinc ID: 5352882]

It is noted that interaction of the drugs and analogs is found with His at two sites 42 and 111. So these sites are selected for further study. That is, to retrieve most unique drugs and analogs these two sites were point mutated.

- His42 is an SNP, if the drugs or analogs showing interaction with the enzyme even when the site is mutated are most effective.
- His111 is in substrate binding site, the drugs or analogs showing interaction with the enzyme even after mutation are of most importance.

In the next process point mutation is carried out so that the His could be replaced with the possible amino acids and the interaction between the enzyme and the lead compounds could be identified even when the SNP site was modified.

Point Mutation

In this process,

- First the codon for the amino acid (Histidine) is identified.
- Various possibilities of point mutation are noted.

Codon for Histidine is **CAC**

Possibilities of point mutations and their corresponding amino acids are

SL.No	Codon	Mutation site	Possibilities of mutation	Retrieved codon	Amino acid for codon
1.	CAC	First nucleotide-CAC	G	GAC	Asp
			A	AAC	Asn
			U	UAC	Tyr
2.	CAC	Second nucleotide-CAC	G	CGC	Arg
			U	CUC	Leu
			C	CCC	Pro
3.	CAC	Third nucleotide-CAC	A	CAA	Gln
			G	CAG	Gln
			U	CAU	His

Thus the amino acids that can be replaced are Asp, Asn, Tyr, Arg, Leu, Pro, and Gln.

The process of point mutation is carried out using Swiss PDB viewer.

- First His at the position 42 is replaced with these amino acids.
- Second His at the position 111 is replaced with these amino acids.
- Retrieved enzyme structures are saved, and each and every structure are interacted with the selected drugs and analogs and most interactive drugs and analogs from them are selected.

Process of point mutation:

- The PDB file of the enzyme aldose reductase is opened on Swiss PDB viewer.
  File → Open PDB file → Pathname
- The ligand in the enzyme is deleted
  Wind → Control panel → Enzyme and ligand colored differently → ligand residues are selected → build → Remove selected residues
- Mutation
  Color His42 differently using control panel→ Mutat e → Click His42 → Select the amino acid to be replaced → Save structure as PDB file.
- This process is carried out for replacement of all the amino acids. And are saved. Selected drugs and analogs are interacted with these structures using the program HEX and most interactive drugs and analogs are selected.
- For mutation in the site His111 the same process is followed.

Result obtained after mutation at the site His42.

Drugs that interacted with the site His42 were Metformin and Metoprolol. These drugs were interacted with the mutated structure of the enzyme at the site His42 using HEX. This was done to find the most interactive drug.

Interaction study gives this result

Replaced amino acid at the site His42	Interaction	
	Metformin	Metoprolol
Arg	Cys200, Gln193	-
Asp	Cys200	-
Pro	Cys200, Gln193	-

Gln	Cys200, Gln193	-
Asn	Cys200	-
Leu	Cys200, Gln193	-
Tyr	Cys200	-

Thus from the above result it was found that the drug Metformin is interactive.

Drugs and analogs that interacted with the site His111 of the enzyme were

- Gliclazide
- Metformin
- 1-(4-methylphenyl)-1,3,5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine [Zinc ID: 146414]
- 1-(4-flurophenyl)-1,3,5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine [Zinc ID: 203639]
- 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea [Zinc ID: 1726559]
- 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol [Zinc ID: 1530567]
- (2S)-1-(tert-butylamino-3- [4-[2-(cyclopropylmethoxy) ethyl] phenoxy] propan-2-ol [Zinc ID: 5352882]

So these drugs and analogs were interacted with the mutated structure of the enzyme at the site His111.

Interaction Study for Drugs

1. **Drug 1**(Gliclazide)
2. **Drug 2**(Metformin)

Result for interaction study with the drugs

Replaced amino acid at the site His111	**Interaction**	
	Drug 1 (Gliclazide)	**Drug 2 (Metformin)**
Arg	-	Cys200, Gln193
Asp	Asp111	Cys200, Gln193
Pro	-	Cys200
Gln	-	Cys200
Asn	Asn111	Cys200
Leu	-	Cys200
Tyr	-	Cys199

Thus it is found that Drug 2(Metformin) is more interactive, so only this drug is selected for further study.

Interaction Study for Analogs

1. **Analog 1**,1-(4-methylphenyl)-1,3,5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine [Zinc ID: 146414]
2. **Analog 2**,1-(4-flurophenyl)-1,3,5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine [Zinc ID: 203639]
3. **Analog 3**, 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea [Zinc ID: 1726559]
4. **Analog 4**, 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol [Zinc ID: 1530567]
5. **Analog 5**, (2S)-1-(tert-butylamino-3- [4-[2-(cyclopropylmethoxy) ethyl] phenoxy] propan-2-ol [Zinc ID: 5352882]

The interaction study with analogs showed this result

Replaced amino acid at the site His111	**Interaction**				
	Analog 1	**Analog 2**	**Analog 3**	**Analog 4**	**Analog5**
Arg	Trp21, Tyr49	Tyr49	Arg111	Thr141	Thr141
Asp	-	Tyr49	Tyr210	Ser211,Cys299	-
Pro	Trp21	Trp21	Cys299	Asp111	Asp111
Gln	Asn161,Gln111	-	Cys299	Gln111	Gln111
Asn	Trp21	Trp21	Tyr210	Tyr210	Lys22
Leu	Trp112	Trp112	Cys299	Leu79,Cys299	-
Tyr	Trp112	Trp112	Tyr210	Lys22	-

Thus it is found that analogs 1, 2, 3 & 4 are more interactive, so these analogs are selected for further study.

The lead compounds thus obtained from the interaction study after mutation at the site His42 and His111 are

- Metformin
- 1-(4-methylphenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine [Zinc ID: 146414]
- 1-(4-flurophenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine [Zinc ID: 203639]
- 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea [Zinc ID: 1726559]
- 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol [Zinc ID: 1530567]

Thus selected drugs and analogs are subjected to PreADMET study, so that the effectiveness of the drugs and analogs can be judged and ranked.

DISCUSSION

Diabetes Mellitus (DM) is present in more than 165 million individuals worldwide and has increasingly become a significant health concern, especially regarding vascular and cardiac disease. DM can impair vascular integrity and alter cardiac output that eventually diminishes the capacity of sensitive regions of the brain, leading to functional impairment and dementia. (Kenneth Maiese, 2008).

Diabetes mellitus is an independent risk factor for cardiovascular disease and is also associated with increased susceptibility to cardiovascular complications. It has been suggested that alterations in glucose metabolism and glucose flux via the aldose reductase pathway make the diabetic heart more sensitive to ischemic-reperfusion injury. (Akula Annapurna *et al*, 2008).

A pathway from glucose via sorbitol (polyol pathway) bypasses the control points of hexokinase and phosphofructokinase in glucose metabolism. It also may produce glycerol, linking the bypass to lipid synthesis. (Jonathan Jeffery and Hans Jornvallt, 1983).

The polyol pathway, which comprises the enzymes aldose reductase and sorbitol dehydrogenase, is recognized to play a major role in the pathogenesis of diabetic complications. (Kicic E, Palmer TN, 1994). Diabetes-induced changes in retinal metabolism and function have been linked to increased aldose reductase activity. (Obrosova IG *et al*, 2001).

Activation of polyol pathway due to increased aldose reductase (ALR2) activity has been implicated in the development of diabetic complications including diabetic retinopathy (DR), a leading cause of blindness. However, the relationship between hyperglycemia-induced activation of polyol pathway in retina and DR is still uncertain. (G. Bhanuprakash Reddy *et al*, 2008).

In the present study, many drugs and their analogs retrieved from Zinc database were interacted with the enzymes of Polyol pathway, which is with aldose reductase and sorbitol dehydrogenase. It was found that most of the drugs and analogs were interacting with the enzyme aldose reductase, but failed to interact with sorbitol dehydrogenase. So the study was concentrated only with the enzyme aldose reductase. Also it is noted that aldose reductase is the rate limiting enzyme of Polyol pathway more importance is not given to sorbitol dehydrogenase.

Sorbitol dehydrogenase (SDH; NAD+ oxidoreductase, EC (1.1.1.14), is a member of the polyol pathway, which is important in the development of such diabetic complications as cataract, neuropathy, retinopathy and nephropathy but the role of SDH in diabetic conditions has been almost ignored. (Ayumu HOSHI *et al*, 1996)

As the inhibition of aldose reductase blocks the formation of sorbitol, as such the conversion of sorbitol by sorbitol dehydrogenase is of not much importance. The role of sorbitol dehydrogenase is almost ignored in the diabetic complications.

In the present study the selected drugs and analogs were docked with the enzyme aldose reductase, from them 5 were selected as lead compounds. These lead compounds were then subjected to PreADMET testing to predict their absorption, distribution, metabolism, excretion, toxicity and to rank the drugs.

Thus only 5 compounds were selected as lead compounds from which most effective drugs could be built to treat the disease diabetic complication due to polyol pathway activation as the result of the enzyme aldose reductase. Thus found

lead compounds are as follows and were ranked according with PreADMET study, 1-(4-methylphenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine is ranked first. 1-(4-flurophenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine is ranked second. 1-(4-chlorophenyl) sulfonyl-3-(3 methyl butyl) urea is ranked third. Metformin is ranked fourth. 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol is ranked fifth.

Thus in the present study "SNP based drug interaction study on the key enzymes of Polyol pathway: a complication of Diabetes Mellitus", 5 compounds were selected as lead compounds and were ranked. These lead compounds thus can be used to build an effective drug to interact with the enzyme aldose reductase and help in curing the diabetic complications.

CONCLUSION

The selected disease was "Diabetes mellitus and its complication due to Polyol pathway". Diabetes mellitus is a chronic health disorder. Diabetes and its treatments can cause many complications, hypoglycemia, ketoacidosis, or nonketotic hyperosmolar coma, cardiovascular disease, chronic renal failure, retinal damage- which can lead to blindness, several types of nerve damage, and microvascular damage. Glucose is a highly reactive compound, and it must be metabolized or it will find tissues in the body to react with. Cells use glucose for energy, though unused glucose enters the polyol pathway where aldose reductase reduces it to sorbitol.

The enzymes of Polyol pathway are aldose reductase and sorbitol dehydrogenase, this study is concentrated on these enzymes. It was found that sorbitol dehydrogenase showed no interaction with the drugs and analogs selected. So the study was concentrated mainly on aldose reductase. The interaction site amino acids of the enzymes and drugs and analogs were studied and compared. On the basis of it the common amino acids were point mutated and again interaction was noted. In this manner the most interactive drugs and analogs were selected. Thus selected drugs were ranked with the help of PreADMET study.

Five compounds were selected as lead compounds and were ranked. 1-(4-methylphenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine was ranked first. 1-(4-flurophenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine was ranked second. 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea was ranked third. Metformin was ranked fourth 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol is ranked fifth. Most of the lead compounds fail in toxicity testing but five of these compounds are qualified in the PreADMET study, particularly also toxicity testing. So these five lead compounds can be used for building more effective drugs to interact with the enzyme aldose reductase and help in the treatment of disease "Diabetes mellitus and its complication due to Polyol pathway".

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