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RESEARCH ARTICLE

STUDY ON THE FIVE LEAD COMPOUNDS OF INHIBITING POLYOLPATHWAY ENZYME SDH

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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 confirmed with PreADMET analysis.*

Keywords: *Hyperglycemia, polyolpathway, aldose reductase, sorbitol dehydrogenase, snp, five lead compounds, Metformine, diamine, urea, propan-2-ol, PreADMET analysis.*

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 and 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.

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).

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

PreADMET study

The lead drugs are subjected to PreADMET study; this gives the information about the absorption, distribution, metabolism, excretion and toxicity of the lead candidates. This helps to select the most effective compound and helps to rank the lead drugs.

1) Metformin

PreADMET result and interpretation

Descriptors

SL.No	Descriptors	Count
1.	No. total atoms	20
2.	Molecular weight	129.164400
3.	Molecular formula	C4H11N5
4.	Formal charge	1
5.	Single bonds	17
6.	No .rotatable bonds	0
7.	No .aromatic bonds	0

8.	No .H-bond acceptors	1
9.	No .H-bond donors	3
10.	No .rigid bonds	19
11.	No .rings	0

Druglikeness prediction

SL.No	Rules	Result	Inference
1.	CMC like rule	Not Qualified	Drug not under qualifying range
	CMC like rule violation fields	Molecular_weight, AMolRef	
	CMC like rule violations	2	
2.	Lead like rule	Violated	Drug not under qualifying range
	Lead like rule violation fields	SKLogD_value	
	Lead like rule violations	1	
3.	MDDR like rule	Mid-structure	Structure of other ranges
	MDDR like rule violation fields	No _Rings N0_Rotatable_bonds	
	MDDR like rule violations	2	
4.	Rule of five	Suitable	Suitable with better solubility and permeability
	Rule of five violation fields	-	
	Rule of five violations	0	
5.	WDI like rule	Out of 90% cut off	lead like lead
	WDI like rule violation fields	Balaban_index_JX	
	WDI like rule violations	1	

ADME prediction

SL.No	ADME	Values	Inference
Absorption			
1.	Human intestinal absorption (HIA, %)	45.666887	Moderately absorbed compounds
2.	In vitro Caco-2 cell permeability (nm/sec)	21.0831	Middle permeability
3.	In vitro MDCK cell permeability (nm/sec)	64.3012	Middle permeability
4.	In vitro skin permeability (logKp, cm/hour)	-3.24138	Falls within the cut off set for skin permeability
Distribution			
1.	In vitro plasma protein binding (%)	3.957658	Chemicals weakly bound
2.	In vivo blood- brain barrier penetration (C.brain/C.blood)	0.227225	CNS - Inactive compounds, Middle absorption to CNS

Toxicity prediction

SL.No	Toxicity	Value	Inference
Ames Test			
1.	Ames TA100 (+S9)	Negative	Most of the result is predicted negative, so the lead is suitable
2.	Ames TA100 (-S9)	Negative	
3.	Ames TA1535 (+S9)	Negative	
4.	Ames TA1535 (-S9)	Negative	
5.	Ames TA98 (+S9)	Positive	
6.	Ames TA98 (-S9)	Negative	
7.	Ames test	Mutagen	
Carcinogenicity			
1.	Mouse	Positive	Likely to be carcinogenic
2.	Rat	Negative	

Discussion

- 3 rules out of 5 have been qualified in Druglikeness testing.
- In ADME testing, moderate permeability is shown in absorption, and the drug is well distributed and has middle absorption to CNS.
- In toxicity testing, most of the results are predicted negative, so the lead is suitable but it may be carcinogenic.

- This compound thus can act as a lead that can be used to build an effective drug to interact with aldose reductase and can be used for the treatment of diabetic complications.

2) 1-(4-methylphenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine

PreADMET result and interpretation

Descriptors

SL. No	Descriptors	Count
1.	No. total atoms	41
2.	Molecular weight	271.364
3.	Molecular formula	C15H21N5
4.	Formal charge	1
5.	Single bonds	35
6.	No .rotatable bonds	0
7.	No .aromatic bonds	6
8.	No .H-bond acceptors	1
9.	No .H-bond donors	3
10.	No .rigid bonds	43
11.	No .rings	3

Druglikeness prediction

SL. No	Rules	Result	Inference
1.	CMC like rule	Qualified	Qualified with CMC clean
	CMC like rule violation fields	-	
	CMC like rule violations	0	
2.	Lead like rule	Suitable if its binding affinity is greater than 0.1microM	Leadlike Leads
	Lead like rule violation fields	-	
	Lead like rule violations	0	
3.	MDDR like rule	Mid-structure	Structures of other ranges
	MDDR like rule violation fields	N0_Rotatable_bonds	
	MDDR like rule violations	1	
4.	Rule of five	Suitable	Suitable with better solubility and permeability
	Rule of five violation fields	-	
	Rule of five violations	0	
5.	WDI like rule	In90% cutoff	Lead like drug
	WDI like rule violation fields	-	
	WDI like rule violations	0	

ADME prediction

SL.No	ADME	Values	Inference
Absorption			
1.	Human intestinal absorption (HIA, %)	94.454967	Well absorbed compounds
2.	In vitro Caco-2 cell permeability (nm/sec)	21.9347	Middle permeability
3.	In vitro MDCK cell permeability (nm/sec)	17.366	Low permeability
4.	In vitro skin permeability (logKp, cm/hour)	-2.70312	Falls within the cut off set for skin permeability
Distribution			
1.	In vitro plasma protein binding (%)	66.858247	Chemicals weakly bound
2.	In vivo blood- brain barrier penetration (C.brain/C.blood)	0.992455	CNS - Inactive compounds, Middle absorption to CNS

Toxicity prediction

SL. No	Toxicity	Value	Inference
Ames Test			
1.	Ames TA100 (+S9)	Positive	Most of the result is predicted negative, so the lead is suitable
2.	Ames TA100 (-S9)	Negative	
3.	Ames TA1535 (+S9)	Negative	
4.	Ames TA1535 (-S9)	Negative	
5.	Ames TA98 (+S9)	Negative	
6.	Ames TA98 (-S9)	Negative	
7.	Ames test	Mutagen	
Carcinogenicity			
1.	Mouse	Negative	Likely to be carcinogenic
2.	Rat	Positive	

Discussion

- All the 5 rules have been qualified in Druglikeness testing. The inference shows that the compound can act as a Leadlike drug.
- In ADME testing, moderate permeability is shown in absorption, and the drug is well distributed with middle absorption to CNS.
- In toxicity testing, most of the result is predicted negative, so the lead is suitable but it may be carcinogenic.
- So this compound can be used as a lead drug to build an effective drug.

3) 1-(4-flurophenyl)-1,3,5-triazaspiro[5-5]undeca-2,4-diene-2,4-diamine

PreADMET result and interpretation

Descriptors

SL. No	Descriptors	Count
1.	No. total atoms	38
2.	Molecular weight	275.328
3.	Molecular formula	C14H18FN5
4.	Formal charge	1
5.	Single bonds	32
6.	No .rotatable bonds	0
7.	No .aromatic bonds	6
8.	No .H-bond acceptors	1
9.	No .H-bond donors	3
10.	No .rigid bonds	40
11.	No .rings	3

Druglikeness prediction

SL. No	Rules	Result	Inference
1.	CMC like rule	Qualified	Qualified with CMC clean
	CMC like rule violation fields	-	
	CMC like rule violations	0	
2.	Lead like rule	Suitable if its binding affinity is greater than 0.1microM	Leadlike Leads
	Lead like rule violation fields	-	
	Lead like rule violations	0	
3.	MDDR like rule	Mid-structure	Structures of other ranges
	MDDR like rule violation fields	N0_Rotatable_bonds	
	MDDR like rule violations	1	
4.	Rule of five	Suitable	Suitable with better
	Rule of five violation fields	-	

	Rule of five violations	0	solubility and permeability
5.	WDI like rule	In90% cutoff	Lead like drug
	WDI like rule violation fields	-	
	WDI like rule violations	0	

ADME prediction

SL.No	ADME	Values	Inference
Absorption			
1.	Human intestinal absorption (HIA, %)	94.166952	Well absorbed compounds
2.	In vitro Caco-2 cell permeability (nm/sec)	0.287429	Low permeability
3.	In vitro MDCK cell permeability (nm/sec)	25.8238	Middle permeability
4.	In vitro skin permeability (logKp, cm/hour)	-3.21428	Falls within the cut off set for skin permeability
Distribution			
1.	In vitro plasma protein binding (%)	60.444895	Chemicals weakly bound
2.	In vivo blood- brain barrier penetration (C.brain/C.blood)	0.85961	CNS - Inactive compounds, Middle absorption to CNS

Toxicity prediction

SL.No	Toxicity	Value	Inference
Ames Test			
1.	Ames TA100 (+S9)	Positive	Some of the result is predicted negative, so the lead may be suitable
2.	Ames TA100 (-S9)	Positive	
3.	Ames TA1535 (+S9)	Negative	
4.	Ames TA1535 (-S9)	Negative	
5.	Ames TA98 (+S9)	Negative	
6.	Ames TA98 (-S9)	Negative	
7.	Ames test	Mutagen	
Carcinogenicity			
1.	Mouse	Negative	Likely to be carcinogenic
2.	Rat	Positive	

Discussion

- All the 5 rules have been qualified in Druglikeness testing. The inference shows that the compound can act as a lead drug.
- In ADME testing, moderate permeability is shown in absorption, and the drug is well distributed with middle absorption to CNS.
- In toxicity testing, some of the results are predicted negative, so the lead may be suitable but it may be carcinogenic.
- So this compound can be used as a lead drug to build an effective drug.

4) 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea

PreADMET result and interpretation

Descriptors

SL.No	Descriptors	Count
1.	No. total atoms	36
2.	Molecular weight	304.7
3.	Molecular formula	C12H17ClN2O3S
4.	Formal charge	-1
5.	Single bonds	27
6.	No .rotatable bonds	0
7.	No .aromatic bonds	6
8.	No .H-bond acceptors	3
9.	No .H-bond donors	1
10.	No .rigid bonds	36
11.	No .rings	1

Druglikeness prediction

SL.No	Rules	Result	Inference
1.	CMC like rule	Qualified	Qualified with CMC clean
	CMC like rule violation fields	-	
	CMC like rule violations	0	
2.	Lead like rule	Suitable if its binding affinity is greater than 0.1microM	Leadlike Leads
	Lead like rule violation fields	-	
	Lead like rule violations	0	
3.	MDDR like rule	Mid-structure	Structures of other ranges
	MDDR like rule violation fields	N0_Rotatable_bonds	
	MDDR like rule violations	2	
4.	Rule of five	Suitable	Suitable with better solubility and permeability
	Rule of five violation fields	-	
	Rule of five violations	0	
5.	WDI like rule	In90% cutoff	Lead like drug
	WDI like rule violation fields	-	
	WDI like rule violations	0	

ADME prediction

SL.No	ADME	Values	Inference
Absorption			
1.	Human intestinal absorption (HIA, %)	96.887772	Well absorbed compounds
2.	In vitro Caco-2 cell permeability (nm/sec)	4.83608	Middle permeability
3.	In vitro MDCK cell permeability (nm/sec)	28.2892	Middle permeability
4.	In vitro skin permeability (logKp, cm/hour)	-1.88807	Falls within the cut off set for skin permeability
Distribution			
1.	In vitro plasma protein binding (%)	92.615810	Chemicals strongly bound
2.	In vivo blood- brain barrier penetration (C.brain/C.blood)	0.942478	CNS - Inactive compounds, Middle absorption to CNS

Toxicity prediction

SL.No	Toxicity	Value	Inference
Ames Test			
1.	Ames TA100 (+S9)	Positive	Some of the result is predicted negative, so the lead may be suitable
2.	Ames TA100 (-S9)	Negative	
3.	Ames TA1535 (+S9)	Negative	
4.	Ames TA1535 (-S9)	Negative	
5.	Ames TA98 (+S9)	Positive	
6.	Ames TA98 (-S9)	Negative	
7.	Ames test	Mutagen	
Carcinogenicity			
1.	Mouse	Negative	Not carcinogenic
2.	Rat	Negative	

Discussion

- All the 5 rules have been qualified in Druglikeness testing. The interference shows that the compound can act as a lead drug.
- In ADME testing, moderate permeability is shown in absorption, and the drug is strongly bound so not well distributed and has middle absorption to CNS.

- In toxicity testing, some of the results are predicted negative, so the lead may be suitable but it is not carcinogenic.
- So this compound can be used as a lead drug to build an effective drug.

5) 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol
PreADMET result and interpretation

Descriptors

SL.No	Descriptors	Count
1.	No. total atoms	52
2.	Molecular weight	308.439
3.	Molecular formula	C18H30NO3
4.	Formal charge	1
5.	Single bonds	47
6.	No .rotatable bonds	0
7.	No .aromatic bonds	6
8.	No .H-bond acceptors	3
9.	No .H-bond donors	2
10.	No .rigid bonds	53
11.	No .rings	2

Druglikeness prediction

SL.No	Rules	Result	Inference
1.	CMC like rule	Failed	Drug not under qualifying range
	CMC like rule violation fields	-	
	CMC like rule violations	0	
2.	Lead like rule	Violated	Drug not under qualifying range
	Lead like rule violation fields	1	
	Lead like rule violations	SKLogD_value	
3.	MDDR like rule	Mid-structure	Structure of other ranges
	MDDR like rule violation fields	No _Rings N0_Rotatable_bonds	
	MDDR like rule violations	2	
4.	Rule of five	Suitable	Suitable with better solubility and permeability
	Rule of five violation fields	-	
	Rule of five violations	0	
5.	WDI like rule	Failed	Drug not under qualifying range
	WDI like rule violation fields	Kier_alpha_03	
	WDI like rule violations	1	

ADME prediction

SL.No	ADME	Values	Inference
Absorption			
1.	Human intestinal absorption (HIA, %)	88.732485	Well absorbed compounds
2.	In vitro Caco-2 cell permeability (nm/sec)	22.0829	Middle permeability
3.	In vitro MDCK cell permeability (nm/sec)	120.253	Middle permeability
4.	In vitro skin permeability (logKp, cm/hour)	-3.96929	Falls within the cut off set for skin permeability
Distribution			
1.	In vitro plasma protein binding (%)	21.066528	Chemicals weakly bound
2.	In vivo blood- brain barrier penetration (C.brain/C.blood)	0.631041	CNS - Inactive compounds, Middle absorption to CNS

Toxicity prediction

SL. No	Toxicity	Value	Inference
Ames Test			
1.	Ames TA100 (+S9)	Negative	Most of the result is
2.	Ames TA100 (-S9)	Negative	
3.	Ames TA1535 (+S9)	Negative	

4.	Ames TA1535 (-S9)	Negative	predicted negative, so the lead is suitable
5.	Ames TA98 (+S9)	Positive	
6.	Ames TA98 (-S9)	Negative	
7.	Ames test	Mutagen	
Carcinogenicity			
1.	Mouse	Positive	Likely to be carcinogenic
2.	Rat	Negative	

Discussion

- 2 rules out of 5 are been qualified in Druglikeness testing. The interference shows that the compound can't be taken as a lead drug.
- In ADME testing, moderate permeability is shown in absorption, and the drug is well distributed with middle absorption to CNS.
- In toxicity testing, most of the results are predicted negative, so the lead may be suitable and it may be carcinogenic.
- This compound can act as a lead compound, but is of the least compound.

Overall inference of PreADMET study for all the selected lead compounds

1. 1-(4-methylphenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine
2. 1-(4-flurophenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine
3. 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea
4. 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol
5. Metformin

Lead compounds	PreADMET study				
	Druglikeness				
	CMC like rule	Lead like rule	MDDR like rule	Rule of Five	WDI like rule
1.	✓	✓	✓	✓	✓
2.	✓	✓	✓	✓	✓
3.	✓	✓	✓	✓	✓
4.	-	-	✓	✓	-
5.	-	-	✓	✓	✓

Lead compounds	PreADMET study			
	ADME prediction		Toxicity	
	Absorption	Distribution	Ames test	Carcinogenicity
1.	✓	✓	✓	Likely to be
2.	✓	✓	✓	Likely to be
3.	✓	✓	✓	-
4.	✓	✓	✓	Likely to be
5.	✓	✓	✓	Likely to be

Result

According to PreADMET testing, its result and interpretation the lead compounds are ranked as follows

- 1-(4-chlorophenyl) sulfonyl-3- (3 methyl butyl) urea is ranked first.
- 1-(4-methylphenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine is ranked second.
- 1-(4-flurophenyl)-1, 3, 5-triazaspiro [5-5] undeca-2, 4-diene-2, 4-diamine is ranked third.
- Metformin is ranked fourth.
- 1-[4-[2-(cyclopropylmethoxy) ethyl] phenoxy]-3-(isopropyl amino) propan-2-ol is ranked fifth.

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".

REFERENCES

1. Akula Annapurna PhD, Siva Reddy Challa MPharm, Gomedhikam J Prakash PhD, Routhu Kasi Viswanath, (2008). Therapeutic potential of sulindac against ischemiareperfusion-induced myocardial infarction in diabetic and nondiabetic rats. *Exp Clin Cardiol* ; 13(2):66-70.
2. Ayumu Hoshi, Motoko Takahashi, Junichi Fujii, Theingi Myint, Hideaki Kaneto, Keiichiro Suzuki, Yoshimitsu Yamasaki, Takenobu Kamada and Naoyuki Taniguchi, (1996). Glycation and inactivation of sorbitol dehydrogenase in normal and diabetic rats *Biochem. J.* (1996)*318*, 119±123 (Printed in Great Britain).
3. G. Bhanuprakash Reddy, 1 A. Satyanarayana,1 N. Balakrishna,1 Radha Ayyagari,2 M. Padma,3 K. Viswanath,3 J. Mark Petrash4, (2008). Erythrocyte aldose reductase activity and sorbitol levels in diabetic retinopathy *Molecular Vision* 2008; 14:593-601.
4. Jonathan Jeffery, John Chesters', Colin Mills' Peter J. Sadler2 and Hans Jornvall3, (1984). Sorbitol dehydrogenase is a zinc enzyme. *The EMBO Journal* vol.3 no.2 pp.357-360.
5. Kenneth Maiese, (2008). Diabetic stress: new triumphs and challenges to maintain vascular longevity. *Expert Rev Cardiovasc Ther. March; 6(3): 281–284.*
6. Kicic E, Palmer TN, (1994). Is sorbitol dehydrogenase gene expression affected by streptozotocin-diabetes in the rat? *Biochim. Biophys. Acta PMID: 8204669.*
7. Obrosova IG, Stevens MJ, Lang HJ, (2001). Diabetes-induced shanges in retinal NAD-redox status: pharmacological modulation and implications for pathogenesis of diabetic retinopathy. *Pharmacology, PMID: 11287819.*

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