SCREENING OF NOVEL DRUGS AGAINST ALPHA-GLUCOSIDASE, A KEY ENZYME IN DIABETES

A Thesis submitted in partial fulfilment of the requirements for the degree of

Bachelor of Technology

In

Biotechnology

By

NEHA GOYAL (110BT0538)

Under the guidance of

Prof. NANDINI SARKAR

Department of Biotechnology & Medical Engineering
National Institute Of Technology Rourkela

CERTIFICATE

This is to certify that the thesis entitled**, "Screening of novel drugs against alpha-glucosidase,a key enzyme in diabetes"** submitted by **Neha Goyal(110BT0538)** in partial fulfilment of the requirements for the award of **Bachelor of Technology Degree** in **Biotechnology** at National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Place: Rourkela

Date: 12/05/2014

Supervisor

Prof.Nandini Sarkar

ACKNOWLEDGEMENT

I would like to express my sincere and hearty gratitude to my guide **Prof. Nandini Sarkar** , Biotechnology and Medical Engineering Department for their invaluable guidance and constant encouragement through the entire duration of my work and helping me accomplish my project successfully.

I would also like to thank **Prof. Shubhankar Paul, Mr. Kazavali, Miss Ritika Chauhan, Mr. Sarath Chandra** for assisting me and guiding me throughout the project. I also thank all the faculty members of the department for providing consultation and access to resources. I also express my profound gratitude to my parents and friends for their blessings and support without which this task could have never been completed.

Place: Rourkela

Date: 11/05/2014

NehaGoyal

110BT0538

ABSTRACT

Diabetes is a metabolic disease which is very common in today's generation and patient suffering from diabetes either has faulty cells which do not respond to insulin properly or has inadequate production of insulin in the body due to which glucose level in the blood rises which leads to frequent urination, Patient becomes increasingly thirsty (Polidipsia) and hungry (polyphagia). There are mainly three types of diabetes TYPE1, TYPE2 and GESTATIONAL but type is the most common one. In type 2 Diabetes Mellitus patient's body does not produce enough insulin for proper functioning or the cells do not react to the insulin properly. Obese and overweight people have more chances of developing type2 diabetes mellitus. According to age also risk of Diabetes Mellitus increases. As we get older chances of having type 2 diabetes mellitus increases. Researchers from university of eidenburgh have reported that men whose testerone levels are low have higher chances of having Diabetes Mellitus Type 2 because Testerone levels are directly linked to insulin resistance. Approximately 90% of all the cases worldwide are of Diabetes Mellitus type2.

TABLE OF CONTENTS

Contents

List of Figures

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Diabetes Mellitus is a metabolic disease in which a person has high level of blood sugar. Many symptoms are produced due to high blood sugar like Increased thrust, frequent urination, Increased Hunger . diabetes can cause many complications like nonketotic hypersmolar coma and diabetic ketoacidosis, Heart disease, Kidney failure if it is not treated on time [1]. Main cause of high blood sugar level is alpha glucosidase enzyme, it breaks down starch and disaccharide to glucose [2]. Maltase is also a similar kind of enzyme that cleaves maltose is functionally equivalent. Alpha Glycosidase inhibitors are anti-diabetic drugs used for diabetes mellitus type 2 which prevents the digestion of carbohydrate .

There are three types of diabetes type1, type2 and gestational diabetes. In Type1 Diabetes body does not produce insulin, It is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas which leads to insulin deficiency. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin. In type2 cells fail to use insulin properly and in Gestational Diabetes Pregnant women are highly affected because women have high levels of glucose in their blood and they are unable to produce sufficient insulin to transport whole glucose into their cells which results in progressively rising levels of glucose [3].

Alpha Glucosidase leads to high production of glucose in the body hence by targeting this enzyme and identifying its inhibitors excess glucose production can be controlled [4].

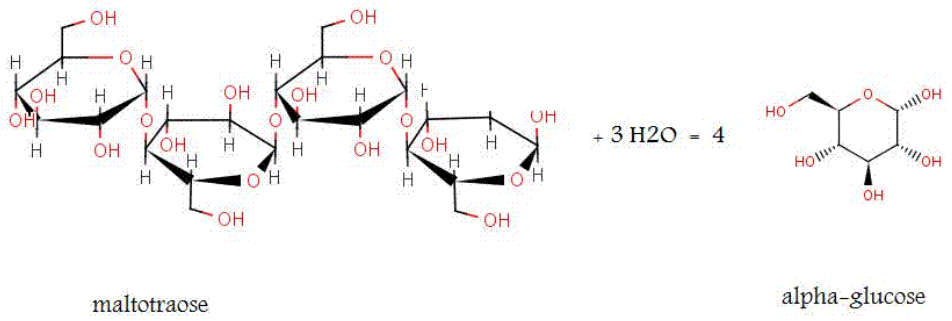


Figure1: Alpha-Glucosidase catalyzed action.

Classification of diabetes mellitus

(American Diabetes Association 1998)

1. Type 1: ß-cell destruction usually leading to absolute insulin deficiency.
2. Type 2: Variation from insulin resistance and increased insulin levels to a dominant defect in insulin secretion with insulin resistance.
3. >40 well-defined types of diabetes .
4. Gestational diabetes:

   Any degree of glucose intolerance first noted in pregnancy.

Figure 2 : classification of diabetes mellitus.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Diabetes Mellitus

Diabetes Mellitus is a metabolism disorder which means most of the food which we consume for energy is broken down into glucose and glucose is a form of sugar in the blood, Glucose is principle source of fuel of our body but it cannot enter our cells without insulin being present. Insulin is a hormone which is produced by the pancreas [5]. After taking food, Pancreas automatically releases an adequate quantity of insulin so that glucose present in blood can move to cells, as soon as it enters the cells blood-glucose level drops.

A person with diabetes has the condition called hyperglycemia in which quantity of glucose in blood is too elevated and the reason is either the body does not produce enough insulin, produces no insulin or cells do not respond properly to the insulin. In this situation instead of providing essential energy glucose passes out of the body in urine [6].

Diabetes mellitus has been associated with depression, it is more common in adults with type 2 diabetes mellitus (T2DM) as compared to those without. Both micro- and macro vascular diabetic complications are associated with depression .Less glycemic control in T2DM patients could lead to more complications of diabetes and such patients are more likely to develop depression. More research is required in this area to determine the exact relationship between depression and T2DM and to unfold the mystery of mechanism behind this. The number of adults with diabetes worldwide is predicted to almost double over the next 25 years, from approximately 171 million in 2000 to 366 million by 2030. Diabetes mellitus is rapidly getting close to what could be referred to as epidemic proportions. In every 10 seconds, diabetes causes

one death. It is also associated with cardiovascular disease (CVD), adult-onset blindness as as well as and renal failure. Nonetheless, diabetes can be kept under control through small investments, and can be wholly prevented through interventions that are simple and cost-effective.

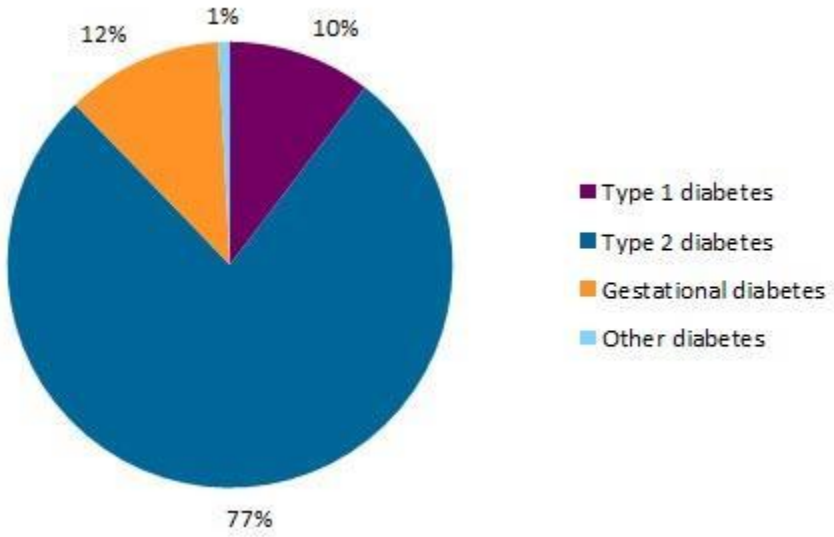


Figure 3 : Diabetes distribution among population.

2.1.1. SYMPTOMS OF DIABETES

- Polydipsia

- polyphegia
- Lethargy
- Stupor
- Blurred vision
- Smell of acetone
- Weight loss
- Kussmaul breathing (hyper-ventillation)
- Nausea
- Vomiting
- Abdominal pain

2.1.2. CAUSES OF DIABETES

Lifestyle and environmental factors are 2 major factors responsible for diabetes especially in adults but main causes are listed below.

- Inherited Traits or hereditary : A person can inherit diabetes due to some genes which passes from one generation to another but it depends upon closeness of blood relationship if mother is diabetic, the risk is 2 to 3% and if father is diabetic the risk is more than the previous case and if both the parents are diabetic then the child has much greater risk for diabetes.
- Age : Increased age gives more possibility than in younger age. Diabetes may occur at any age, but 80% of cases occur after 50 year, incidences increase with the age factor.

- Malnutrition Related Diabetes (poor diet) : low protein and fiber intake, Improper nutrition, high intake of refined products can be the major reasons for developing diabetes.
- Fat and obesity Distribution : overweight means increased insulin resistance means if body fat is more than 30%, BMI 25+, waist grith 40 inches in males or 35 inches in women.
- Sedentary Lifestyle : People with this type of lifestyle are more prone to diabetes, compared to those who exercise regularly.
- Stress **:** emotional disturbance or physical injury is frequently blamed as the initial cause of the disease.
- Drug Induced: Olanzapine (Zyprexa), risperidone (Risperdal), quetiapine (Seroquel),ziprasidone (Geodon), Clozapine (Clozaril) can induce this lethal disease.
- Infection : Some of the strephylococci is supposed to be responsible factor for infection in pancreas.
- Sex : Diabetes is commonly seen in elderly males and females but those females with multiple pregnancy or suffering from (PCOS) Polycystic Ovarian Syndrome are more vulnerable.
- Hypertension**:** According to mant studies it has been reported that there is direct relation between high systolic pressure and diabetes.
- Lipoproteins and serum lipids: High triglyceride and cholesterol level in the blood is related to high blood sugars but in some cases studies had suggested that risk is involved even with low HDL levels in circulating blood.

2.1.3 ROLE OF ALPHA-GLUCOSIDASE IN DIABETES

In Diabetes Mellitus glucose level in blood rises extremely which causes many adverse effects to patient. Alpha Glucosidase is an enzyme which is the main reason for this and hence by using its inhibitors we can inhibit the excess glucose production.

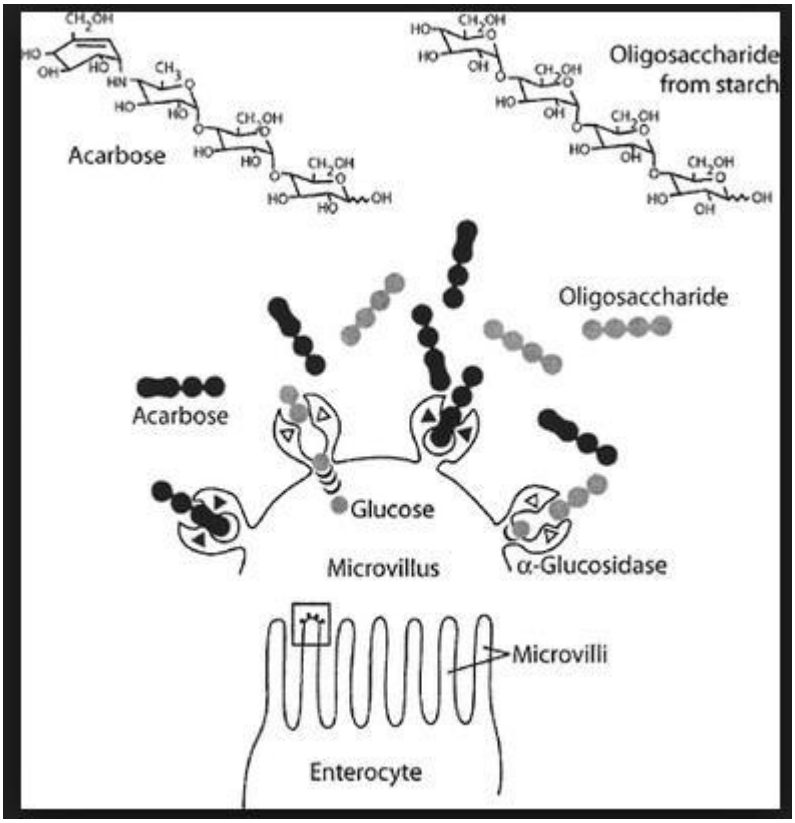


Figure 4: Pathway of alpha-glucosidase

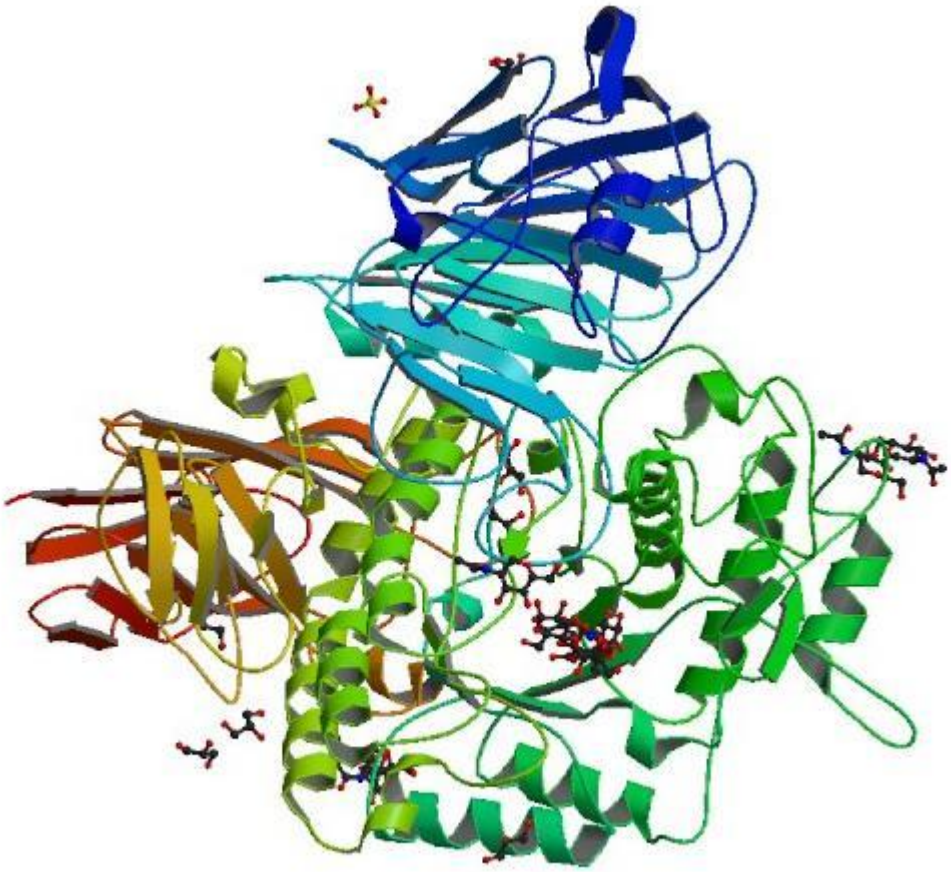


Figure 5 :

Biological Assembly Image for 2QMJ

Crystral Structure of the N-terminal Subunit of Human Maltase-Glucoamylase in Complex with Acarbose

Protein chains are colored from the N-terminal to the C-terminal using a rainbow (spectral) color gradient

2.2 ROLE OF ALPHA-GLUCOSIDASE INHIBITORS IN DIABETES MELLITUS

Alpha-Glucosidase inhibitors play a key role in suppressing elevated glucose concentration in blood. Good inhibitors are acarbose, voglibose and migilol which are already available in the market and use as drugs.

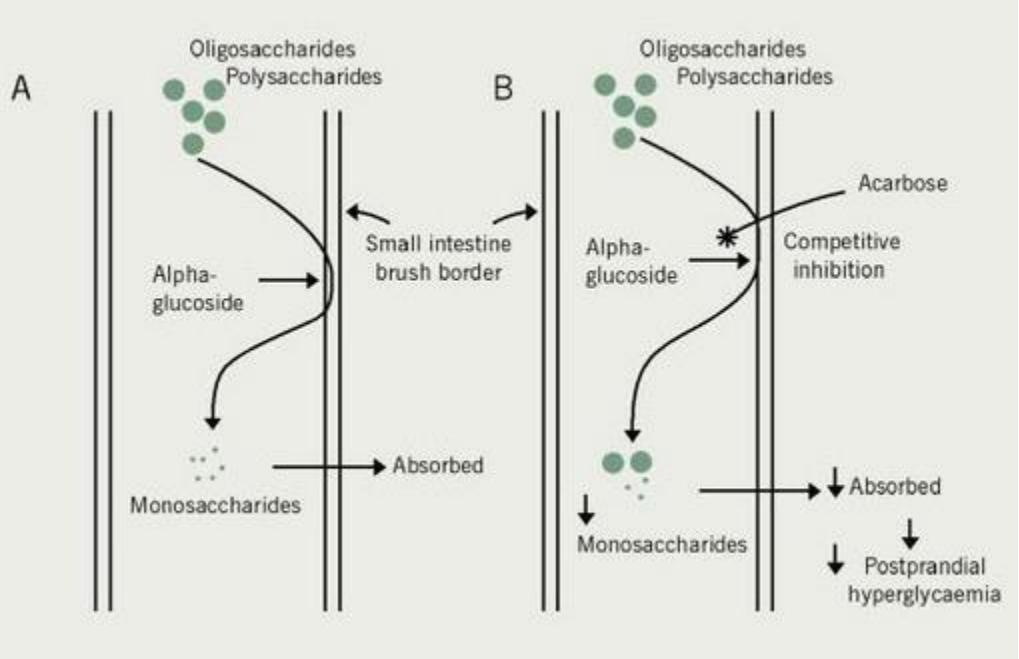


Figure 6 : Role of Alpha Glucosidase inhibitors.

CHAPTER 3

OBJECTIVES

3 OBJECTIVES

- Screening of synthetic as well as natural compounds to find a novel drug.
- Finding the best drug based on binding energy calculations, toxicity prediction and drug likeness.
- Identification of active sites in the target enzyme.
- Screening of ligands based on their binding at the active site of target enzyme.
- Docking of selected ligand and target to determine the binding energy of interaction..
- Analysis of the docking result for identification of best inhibitor molecule.

CHAPTER 4

PLAN OF WORK

PLAN OF WORK

- . Selection of the target enzyme based on its metabolic pathways.
- Finding active sites of target enzyme by uniprot as well as castp.
- Selection of appropriate inhibitors from natural molecule database as well as synthetic compounds.
- Docking of ligands and target enzyme by using ATODOCK.
- Selection of best inhibitor on the basis of binding energy.
- Toxicity prediction of the best inhibitor so that we can propose it as a drug to be used.

CHAPTER 5

MATERIALS AND METHODS

5 MATERIALS AND METHODS

5.1 MATERIALS:

- **Requirement of files:**

  - PDB file of Alpha-Glucosidase (PDB ID 2QMJ).
  - PDB file of ligand molecule. \
  - PDBQT file of ligand and Alpha-Glucosidase

- **Requirement of Software's:**

  - Autodock
  - Pro-drg
  - Argus Lab
  - chimera

- **Requirement of online server's:**

  - http://www.rcsb.org/
  - http://www.swissdock.ch/
  - http://www.uniprot.org/
  - http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg

METHODS:

In this Insilco based screening, we have selected Alpha-Glucosidase (PDB ID 2QMJ) which breaks down starch and disaccharides to glucose and is responsible for main cause Diabetes.

5.2 Preparation of protein molecule:

Alpha-Glucosidase (PDB ID2QMJ) were downloaded from the protein data bank (http://www.rcsb.org/pdb) [7], already complexed with the inhibitors. Alpha-Glucosidase is human

intestinal maltase-glucoamylase made up of single chain A, 870 long amino acid sequence protein. Then this PDB file of Alpha-Glucosidase is opened in chimera 1.6.1 where the inhibitor molecule that is complexed with Alpha Glocosidase was removed and we get pure form of alpha glycosidase. Then pdb file of this molecule was saved for further use in docking process.

Selection of ligand molecule:

Ligand molecule was selected from (http://www.msdiscovery.com/natprod.html) containing 800 chemical entities obtained from different natural sources and from available drugs in the market..

5.2.1 Docking:

In this study we have used MGL TOOLS for docking.

5.2.2 Inputs

In Autodock server we have to follow some steps to access docking. The sample files can be directly uploaded into the form [8].

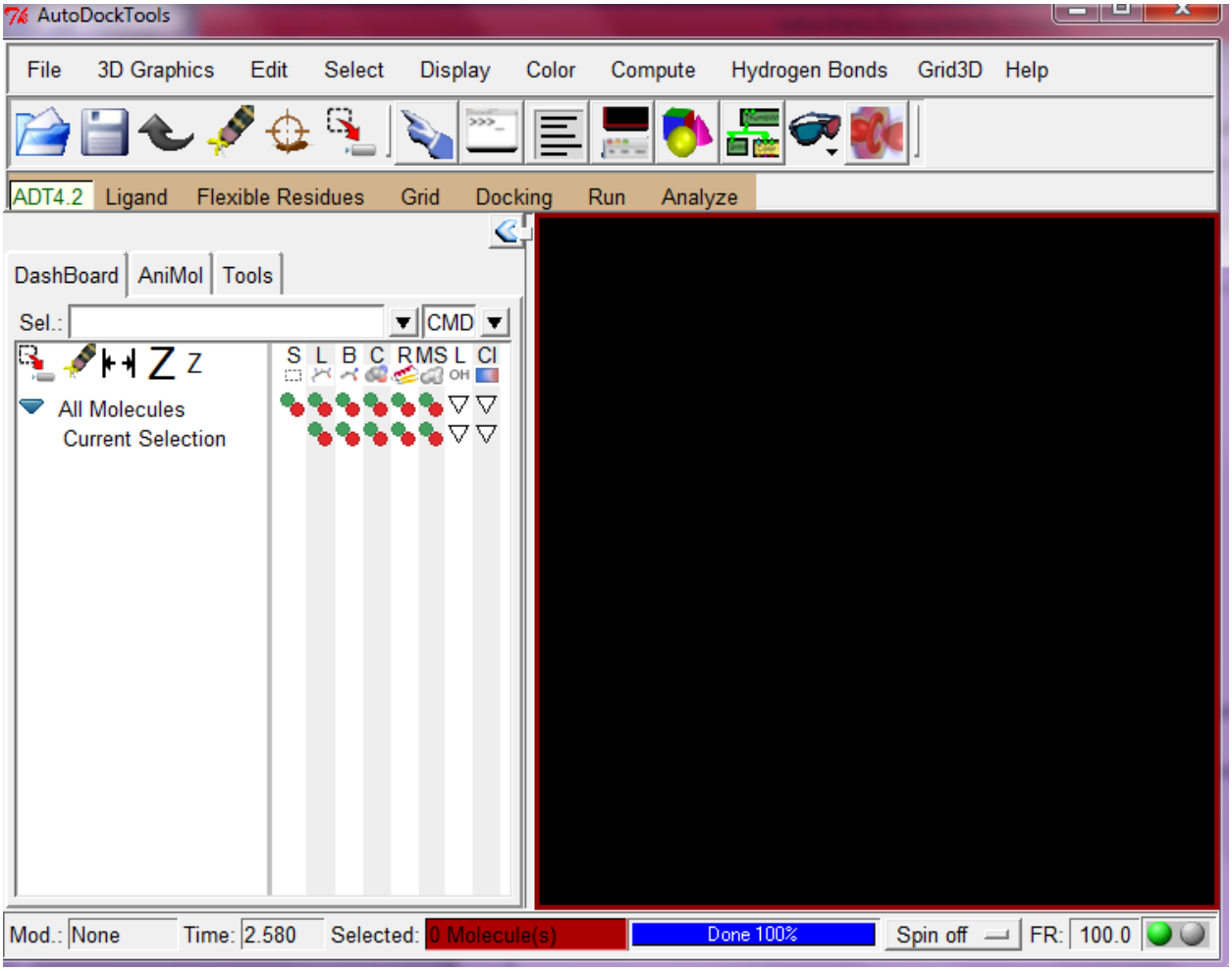


Figure 7: Autodock

5.2.3 Selection of target protein:

Target protein was loaded by just providing the PDB ID or protein FASTA sequence or by URL or by uploading as the PDB files that was saved earlier after processed in chimera. Before

uploading the target protein we have to take care of Active sites which we can get from uniprot or CASTP [9] [10].

5.2.4 Selection of ligand molecule:

Ligand molecule can be selected by uploading the PDB file of ligand molecule.

5.2.5 Outputs:

When docking is completed then we get a text file of docking energy,according to the value we can find whether ligand chosen can be a effective inhibitor or not.

5.2.6 Analysis and active site prediction:

The active site/ binding site of Alpha-Glucosidase was predicted by using uniprot or CASTP [8].

Sites

	Active site	529	1	Nucleophile By similarity
	Active site	532	1	By similarity
	Active site	1420	1	Nucleophile By similarity
	Active site	1423	1	By similarity
	Active site	1526	1	Proton donor By similarity

Figure 8 : Prediction of active sites in uniprot.

5.2.7 Structural analysis of docked molecule:

Binding energy obtained after docking can be compared with already available drug in the market and we can carry out further tests to check its toxicity and drug likeliness.

```
mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.4      0.000      0.000
   2         -6.8      2.595      3.285
   3         -6.3      2.672      5.373
   4         -6.3      3.615      5.852
   5         -6.3      2.453      5.248
   6         -6.1      2.465      4.187
   7         -5.9      1.589      2.143
   8         -5.8      2.609      4.884
   9         -5.6      2.586      4.343
Writing output ... done.
```

Figure 9: Binding energy result.

CHAPTER 6

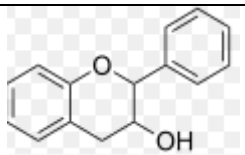
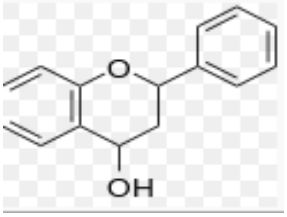
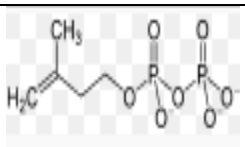
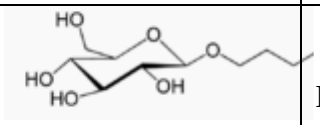
RESULTS

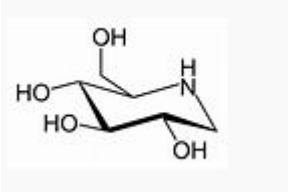
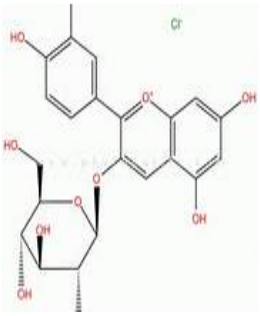
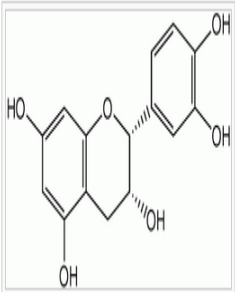
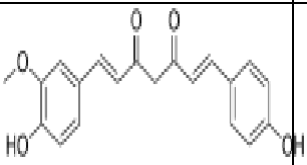
- **RESULTS**

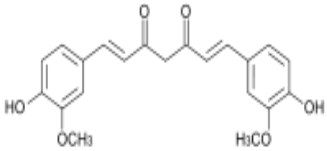
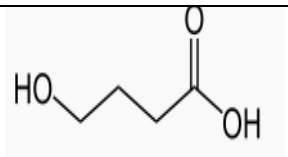
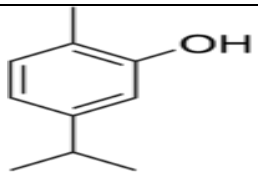
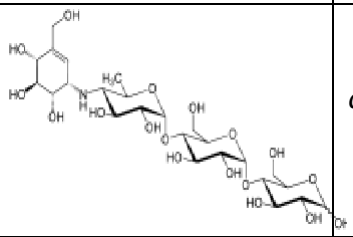
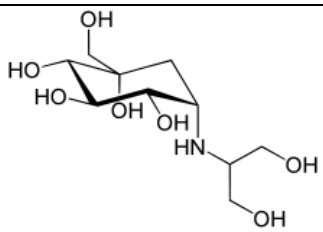
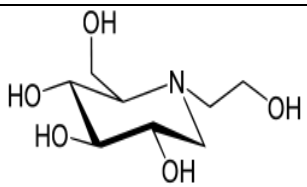
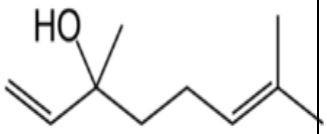
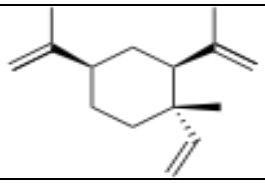
In this study Alpha Glucosidase (PDB ID 2QMJ) was docked with 80 different natural as well as synthetic compounds. Based on the binding energy we got the best inhibitor of Alpha-Glucosidase.

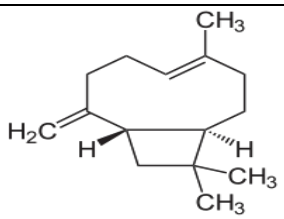
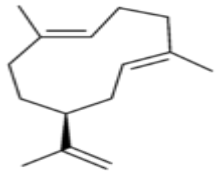
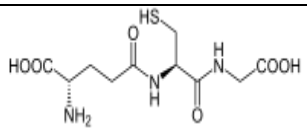
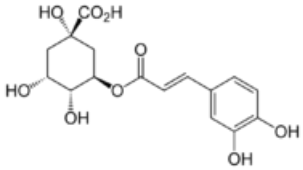
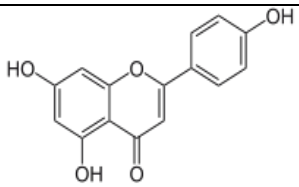
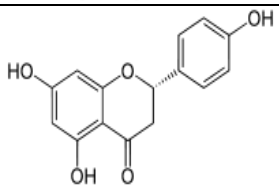
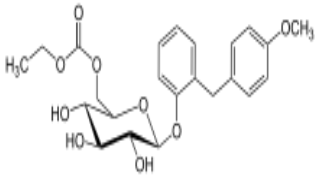
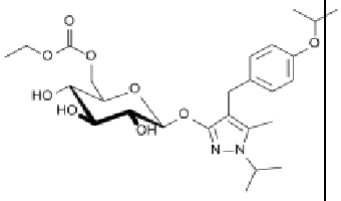
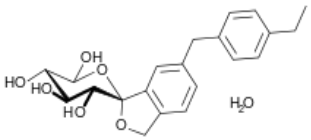
6.1. BEST POTENTIAL INHIBITORS BASED ON ENERGY CALCULATIONS OF THEIR INTERACTIONS.

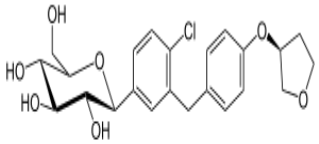
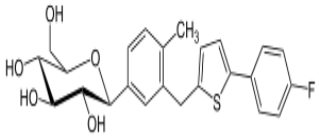
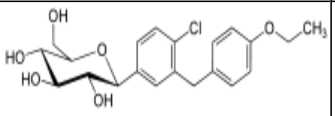
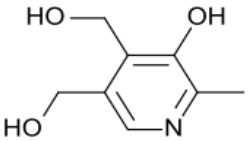
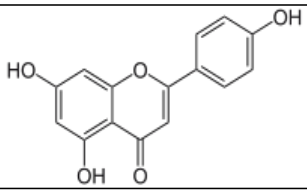
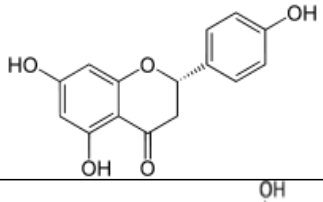
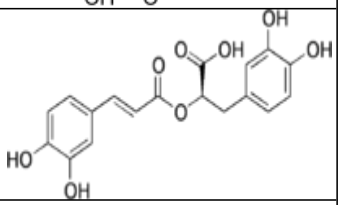
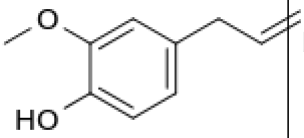
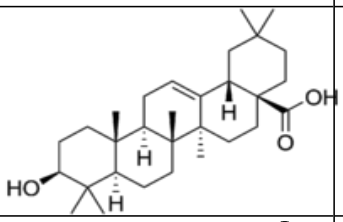
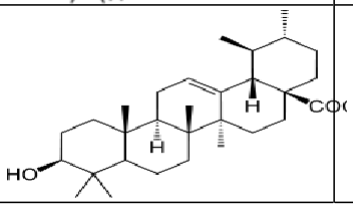
Table 1 shows the structure properties and docking statics of top 44 compounds.

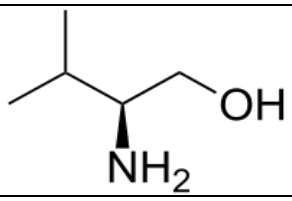
Sr.no.	Name of compound	Structure	Source	Mol. wt.	Binding energy
1.	Flavan-3-ol		Numerous plant essential oil	148.2065	-7.1
2.	Flavan-4-ol		Numerous plant essential oil	148.2065	-6.8
3.	Terpenoid isopentyl pyrophosphate		Streptomyces species	428.3993	-5.5
4.	Decyl-glcoside		METHYL ORSELLINATE	182.1777	-6.1

5.	1-deoxy nojirimycin		Cyanidin-3-galactoside	182.1777	-5.9
6.	Cyaniding-3-galactoside			182.1777	-6.0
7.	Proanthrocyanidins		Jasminium spp and Vicia faba	212.19	-5.8
8.	Demethoxycurcumin		Persea spp turmeic	338.35	--6.1

9	Bis-Demethoxycurcumin		*streptomyces tenebrarius, saccharomyces porispora hiltus*	368.38	-6.2
10	3b-acetoxy-16b-hydroxy-butylinic acid		From natural sources.	224.2154	- 5.6
11	carvacrol		catabolism of ornithine essential oil of *Origanum vulgare*, oil of thyme, oil obtained from pepperwort, wild bergamot	150.217	- 5.2
12	ACARBOSE		*actinoplanes spp*	645.605	+20.82
13.	voglibose		*micromonospora species*	267.28	-9.54
14.	miglitol		honey & plant exudates excreted by the kidneys	207.224	-7.68
15.	linalool		widespread in nature Lauraceae, laurels,cinnamon, rosewood, and Rutaceae, citrus fruits	154.25	- 5.4
16.	Beta-elemene		widespread in plants and animals	204.35	-5.6

17.	Beta-caryophyllene		clove oil, the oil from the stems and flowers of *Syzygium aromaticum*(cloves)	204.36	-6.2
18.	Germacrene D		plant species essential oil of the red deadnettle	204.35	-6.4
19.	Glutathione		synthesized in the body from the amino acids L-cysteine, L-glutamic acid, andglycine	307.32	-6.2
20.	Chlorogenic acid		Potatoes, bamboo, peach and prunes, green coffee bean extract	354.31	- 5.8
21.	Apigenin		fruits and vegetables, but parsley, celery and chamomi le tea	270.24	- 6.0
22.	Naringenin		grapefruits, oranges and tomatoes	272.257	-6.1
23.	Sergliflozin etabonate		From plants	448.463	- 7.2
24.	Remogliflozin etabonate		From plants	522.586	-7.5
35.	Tofogliflozin		From plants	404.45	- 7.6

26.	empagliflozin		proximal tubules of nephronic components in the kidneys	450.91	-7.0
27.	canagliflozin		From natural plants	444.52	- 7.8
28.	dapagliflozin		From natural plants	408.873	-7.7
29.	pyridoxine		Fish, soybeans, avocado, lima beans, chicken, bananas, cauliflower, green peppers	169.18	- 6.8
30.	Apigenin		parsley, celery and chamomile tea	270.24	-6.4
31.	Naringenin		grapefruits, oranges and tomatoes (skin)	272.257	- 6.6
33.	Rosmarinic acid		mono- and dicotyledonous angiosperms	360.31	-7.1
34.	Eugenol		cloveoil, nutmeg,cinnamon,basil bay leaf	164.20	-6.4
35.	Oleanolic acid		*Rosa woodsii*, *Prosopis glandulosa, Hyptis capitata*	456.70	-5.8
36.	Ursolic acid		*Mirabilis jalapa*	456.70	- 6.2

37.	Valinol		amino acid valine	103.16	-7.2

CHAPTER 7

DISCUSSION

DISCUSSIONS:

- Diabetes Mellitus disease is a common metabolic disorder leads to alteration of glucose concentration in blood because of inadequate insulin formation in the body or faulty cells which do not respond properly. The symptom of Diabetes Mellitus generally appears in the age group 45-55 but now a days any age group people can get this disease. The Diabetes pathological hallmarks consist of Alpha-Glucosidase enzyme which converts oligosaccharides to monosaccharides and hence there is an increase in glucose level in the blood. The drugs those are accessible for the treatments Diabetes Mellitus have limitations like they have low usefulness and can cause diverse effects to eyes and heart. The enzymes alpha-glucosidase is an effector target molecule because by blocking it we can get good inhibitors.

CHAPTER 8

CONCLUSION

8 CONCLUSIONS

Diabetes is a metabolic disease which affects the kidney,heart and other parts of the body. In diabetes body inslin production is inappropriate or cells do not respond to insulin properly. In this study virtual screening of compound obtained from synthetic as well as natural product database is done by using docking study with Alpha Glucosidase concluded that the compound having highest binding energy and bind to or near the active site can block the enzyme to bind with the substrate and can work as a potential inhibitors of alpha glucosidase for the treatment of Diabetes. Docking analysis has concluded involvement of hydrogen and hydrophobic interactions between the target enzyme and potential inhibitor molecule.. Finally based on the binding energy and toxicity study we have found Voglibose,Casuarine as best synthetic drugs and Flavan-3-ol to be the top natural potential inhibitors against alpha Glucosidase.

CHAPTER 9

FUTURE WORK

9 FUTURE WORKS

In the current state of Insilco, each work is first checked by Insilco designing or Virtual screening then invitro and invivo investigation is done. The future work of this Insilco study will be to do molecular dynamic simulation of the Alpha Glucosidase inhibitors compound to study their behavior in real system. In vitro and in vivo validation will be is must to propose the selected inhibitors as a potential drug candidate for inhibition of alpha glucosidase.

CHAPTER 10

REFERENCES

1. Seshasai, S., et al., *Diabetes mellitus, fasting glucose, and risk of cause-specific death.* The New England journal of medicine, 2011. **364**(9): p. 829-841.
2. Khanna, R., et al., *P. 17.8 The co-formulation of pharmacological chaperone AT2220 with recombinant human acid alpha-glucosidase improves enzyme uptake and glycogen reduction in a mouse model of Pompe disease.* Neuromuscular Disorders, 2013. **23**(9): p. 828-829.
3. Batch, B.C., M.E. Cox, and L.F. Lien, *Physiology of Diabetes Mellitus and Types of Insulin*, in *Glycemic Control in the Hospitalized Patient*. 2011, Springer. p. 1-6.
4. NG, L.T., C.-H. Su, and M.-N. Lai, *Alpha-glucosidase inhibitor*. 2012, Google Patents.
5. East, H., et al., *About secondary causes of diabetes mellitus.* Journal of the Mississippi State Medical Association, 2012. **53**(11): p. 380-383.
6. Adisakwattana, S. and B. Chanathong, *Alpha-glucosidase inhibitory activity and lipid-lowering mechanisms of Moringa oleifera leaf extract.* Eur Rev Med Pharmacol Sci, 2011. **15**(7): p. 803-8.
7. Rose, P.W., et al., *The RCSB Protein Data Bank: redesigned web site and web services.* Nucleic acids research, 2011. **39**(suppl 1): p. D392-D401.
8. Rambabu, R., S.R. Peri, and A.R. Allam, *Computational analysis and function prediction of a hypothetical protein 1RW0.* Int J Computat Bioinfo and In Silico Model, 2012. **1**(5): p. 58-62.
9. Cui, X., A. Yeliseev, and R. Liu, *Ligand interaction, binding site and G protein activation of the mu opioid receptor.* European journal of pharmacology, 2013. **702**(1): p. 309-315.
10. Consortium, U., *Update on activities at the Universal Protein Resource (UniProt) in 2013.* Nucleic acids research, 2013. **41**(D1): p. D43-D47.