Design and Synthesis of Nateglinide Derivative as Potential Antidiabetic Agents

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This is to assure that this written submission in my project report entitled "Design and Synthesis of Nateglinide Derivatives as Potential Anti-diabeticAgents"represents original ideas in my own words and where other ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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Zakari Umar Ya'u

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This is certified that the work described in this project report entitled "Design, Synthesis and Evaluation of Nateglinide Derivatives as Potential Anti-Diabetic Agents" has been carried out by Zakari Umar Ya'u at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

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Zakari Umar Ya'u

ABSTRACT:

Diabetes mellitus is a group of metabolic disorder which is associated with elevated blood sugar level. Worldwide, it is one of the major health concern and related dead. Although diabetes mellitus incidence rate is lower in the developing countries while in contrast to the developed countries, which is widely believed to be related to the newly emergence and adapted lifestyles. Progression of diabetes mellitus mainly related to beta cells and their receptors in the pancreas. Current treatment includes: Administration of some oral hypoglycemic agents, injections as well as inhalable. Focus nowadays is more on theproduction of hypoglycemic agent which will balance the sugar level instead of that which will decrease it to an unprecedented fatal level.

In-silico molecular docking analysis is proven to be a valuable tool in drug discovery and development and we employ it to identify most potent potential modified structures of the anti-diabetic agent in the treatment of diabetes. Based on structural features of nateglinide, new pharmacophore of choice is designed with the conformational restricted structure to nateglinide. The novel designed ligands posses two electron deficient rings linked with the heterocyclic linker. The designed ligands were studied through molecular docking at AutoDockvina interface. The propose structures where synthesis by coupling reaction from amino acid and naphthoic acid and there alpha amylase activity in vitro where evaluated respectively. Some of them showed comparative good binding affinity and inhibit alpha amylase at appreciable concentration as to nateglinide and acarbose. This may serve as a potential beta cells receptors agonist and alpha amylase inhibitors and could emerge successful agent in the management of type 2 diabetes mellitus.

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CHAPTER 1 INTRODUCTION

1.0 Introduction

Ancient Egyptian document from c.1500 BCE described diabetes as one of the metabolic diseases, mentioning it as "too great emptying of the urine"[1]. Type 1 diabetes is believed to be the early described case of diabetes. Almost at the same time as the Egyptian, the Indian physician implicates the disease and categorized it as Madhumeha which means honey urine due to the urine ability to attract ants[2]. The Appolonius of Memphis coined the word "diabetes" (to pass through) in the year 2030 BCE[3]. During the period of the Roman Empire, diabetes was regard as rare one, with Galen mentioning that he had seen only dual cases at thetime of his career. This may be evident due to the ancient life-style and diet, or because the symptoms were clinically observed at the advanced stage of the disorder. The diarrhea of the urine (diarrhea urinosa) was the name giving to the disease by Galen[4]. Aretaeus of Cappadocia (2nd or early 3rd century CE), detailed reference work provides the earliest surviving manuscript[5]. The concept of diabetes he described reflects the beliefs of the "Pneumatic School" which described sign and cause of the disorder as attributed to coldness and moisture.

What is diabetes? The condition in which processing of food material to produce energy is reduced or terminated is known as diabetes. Food ingested into our body is metabolized to glucose for the provision of energy in the body. A hormone called insulin is produced by the beta-cells in the pancreas that enable the utilization of glucose for energy. Absent, insufficient or insensitivity to insulin means a person is diabetic. As such, sugar gets accumulated in the blood. This is the reason why on common ground, diabetes is referred to as "sugar". Severe health issues like cardiac, glaucoma, failure of the kidney, and the cause of amputation. It is considered as a most deadly disease in the United State is diabetes.

1.1 Types of Diabetes

Type 1: Type 1 diabetes, which depends on insulin and is known as IDDM and also as juvenile onset diabetes mellitus due to the fact it occurs mostly in children. 5-10% of all diagnose patient account to this type of diabetes.

Type 2: Type 2 diabetes which is earlier known or refers to as non-insulin dependent diabetes mellitus (NIDDM). Most cases are found in adult also known as adult onset diabetes mellitus. 90-95% of diagnosed patients are having type two diabetic types. Family history, obesity and old age are most common risk factor of type 2 diabetes mellitus.[6,7]

Gestational Diabetes this is related to type of diabetes which is as a result of pregnancy due to intolerance of glucose.[6]

Other types of diabetes: this happens due to special kind of medical condition like pancreatic disease and some administered medication. An example of such type of diabetes can be maturity onset diabetic of the youth.

1.2 What are the symptoms of diabetes? Diabetic checked up should be done to wave away any doubt by a subject suspecting it. A subject suspecting diabetes when diagnosing may found to possess some or none of the symptoms associated with diabetes: urinating frequently, persistent thirst, unprecedented weight loss and extreme hunger, blown or loss of vision, loss of sensitivity in the foot or hand, weak muscles, non-moisturized skin, nausea, wound heal very slowly, pains in the abdomen and so on.[8]

1.3 Causes of Diabetes

There are multiple causes of diabetes. Both the types of diabetes have its cause. The following are the causes for diabetes:

Type 1 diabetes

- *Genetic susceptibility*: Hereditary plays an important part in determining who has and is going to develop diabetes. Many genes, as well as interaction among genes,is thought to influence susceptibility to and protection from type 1 diabetes. There are certain gene variants that carry instructions for making proteins called Human Leukocyte Antigen (HLA) the protein produced by this will determine if one can have diabetes.[1,5]
- *Autoimmune of beta cells*: White blood cells called T-cells attack and destroy beta cells, diabetes is diagnosed when most of the beta cells are destroyed so patients need insulin for survival.[7,8]
- *Environmental Factors*: Factors such as food, viruses, toxins, may play a role in the development of diabetes but still the development mechanism due to these factors not understood.

Type 2 diabetes

Some factors in causes of type 1 diabetes such as genetic susceptibility can cause type 2 diabetes.

- *Insulin resistance: a* conditionin which the body's muscle, fat, and liver cells don't use insulin effectively.[5,11]

- *Obesity and physical Inactivity:* An imbalance between caloric intake and physical activity can lead to obesity, which causes insulin resistance.[11]

1.4 Epidemiology of diabetes mellitus

Disease complication with regard to diabetes are enormousand reaching unprecedented high in almost all countries, influenced by the hike in a number of people with obesity couple with the unhealthy living. In the year 2013, 380million patientsare estimated to be diabetic, which is predicted to reach up to 592million in next to 2035. Classification of diabetes etiologically is currently accepted worldwide. Type 1 and 2 are the main categories in which more than 85% of diabetic subjects are been diagnosed with type 2.[11]

1.5 Treatment for Type 2 diabetes: treatment can be through healthy diet, burning off excess sugar via exercise, easy access to glucose testing equipment at home, oral hypoglycemic drug and to a most severe extent, insulin. Statistics shows that only 40% of patient with type 2 diabetes depend on direct insulin supplement.

Can diabetes be prevented? Various research and statistic studies provide sufficient information about reducing the risk of diabetes via physical exercise and diet regulation.

Is there a cure for diabetes? So far, no total cure for diabetes is ever documented, but recently some breakthrough is recorded like bête-cell replacement and regeneration but with limited success.[12, 13]

Table 1: Different levels of blood glucose in normal and diabetic conditions[14]

SAMPLE	**NORMAL**	***DIABETES***
Fasting blood sugar	80 – 90 mg/dl	126mg/dl and above
Random blood sugar	80 – 139 mg/dl	200 mg/dl and above
2 Hrs glucose tolerance test	80 – 139 mg/dl	200mg/dl and above

1.6 Medication used for the management of type two[13,15,16]

Type 2 diabetes drugs are administered basically, orally or by injections.

- ✓ **Oral Hypoglycemic**

Table 2: Oral anti-diabetic drugs

S.NO	*CLASS*	DRUG
1	Insulin secretagogues:Sulphonylureas (bind to the SUR1 subunits and blocks the ATP-sensitive K^+ channel	1st generation: Chlorpropamide, Acetohexamide, Tolazamide, Tolbutamide 2nd generation: Glipizide, Glyburide,Glimepiride,
2.	Insulin sensitizers Biguanides (activation AMP kinase)	Metformin
3.	Thiazolidinedione: ($PPAR_y$agonist)	Pioglitazone, Rosiglitazone
4.	Alpha-glucosidase inhibitors	Acarbose, Miglitol
5.	Non-sulfonylureas: Meglitinides (block ATP-sensitive K^+ Channel)	Repaglinide(prandine), Nateglinide(Starlix)
6.	PPAR alpha agonist	Fibrates /rexinoids
7.	Protein tyrosine kinase inhibitor	CLX 0300/0301/ 0900/0901

- ✓ **Injectable**

Amylin Mimetic

Amylin mimetic includes pramlintide; helps to decrease the blood glucose made by the liver, helps also to slow the food breakdown in the stomach and intestines and this slows down the blood glucose.[17]

GLP-1Receptor Agonist

Such as albiglutide, dulaglutide, exenatide, liraglutide; it has all the 3 functions that are

- Help the pancreas to make glucose.
- Decrease the amount of glucose in the liver.
- Slows the breakdown of food in the stomach and the intestine.[18]

INSULINS Such as detemir, glargine, glulisine human lispro; replace the insulin made naturally by the body in the pancreas, helps to decrease the amounts of glucose made by your liver and helps to move glucose from the bloodstream into your muscle and fats where glucose is used for glucose.

- ✓ **INHALABLE**

Such as insulin which is mainly known as AFREZZA which is human insulin.[19, 20]

1.7 Beta cells as a molecular target

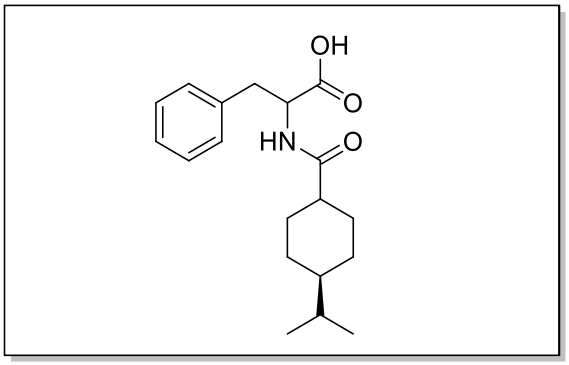
Beta cells are a type of cell in the pancreas located in the so-called islets of Langerhans. Theymakeup 65-80% of the cells in the islets. The Islets diameter is about 50 to 300 micrometers. They are composed of several types of cells. 70% of beta cells are at least, found in the core islet are localized. Alpha cells are the cells been surrounded that glucagon is been secreted, somatostatin is been secreted by assmaller number of delta cells, while pancreatic polypeptide is been secreted by F-cells. Extracellular space provides the medium in which all cells communicate with each other. This pattern results in the secretion of substances from a living cell which alters the receiver cells function. A very good example is the suppression of glucagon secretion as a result of insulin secretion. Bundles of neurovascular nerves enter sympathetic, parasympathetic and arterioles through the central beta cells.[21]

CHAPTER 2 REVIEW OF LITERATURE

2.0 Drug of interest: Nateglinide and acarbose.

2.1 Nateglinide: Many synthetic anti-diabetic drugs are in circulation for the type 2 diabetes treatment "non-insulin dependent diabetes mellitus" (NIDDM), which Nateglinide with the trade name "Starlix" happens to be one of the synthetic anti-diabetic drugs. The above-mentioned drug "Nateglinide" is named chemically as N-(trans-4-isopropylcyclohexyl-carbonyl)-D-phenylalanine. The melting point of around 129-130°C, which physically appears as a white to gray powder. Soluble in awide range of solvent like ether, chloroform, methanol, and ethanol while partially soluble in octanol and acetonitrile and completely insoluble in water.[12]

Chemical structure of Nateglinide



Molecular formula: $C_{19}H_{27}NO_3$

Molecular weight: 317.0

Storage condition: store at +4°C.The product is stored under desiccating conditions. 12months is the maximum storage time.

2.2 Clinical pharmacological action of drug

Mechanism of action: The drug in question belongs to the member of Meglitinide class of glucose-lowering drugs. The insulin release stimulating effect of Nateglinide in the beta-pancreas of the islets of Langerhans in the digestive system characterizedits ability to lower blood glucose. It inhibits the ATP-dependent potassium channels to achieve it hypoglycemic properties in the beta-cell membrane. The voltage-gated calcium channels are caused to open by the depolarization of the beta-cells. Insulin secretion occurs as a result of flowing in (influx) of calcium which induces fusion of insulin-containing vesicles with themembrane of the cell. Nateglinide was succeeded/refined/developed by "Novartis" (a Swiss Pharmaceutical Company).

It is optically active, hence it exists both in D&L isomeric form. 1.6mg/kg of D-Nateglinide ingested orally lowered the blood glucose by approximately 20% and when compare with it L-isomer, 100mg/kg is required for same activity.[12,22]

2.3 **Pharmacokinetics of drug**

Absorption: immediately before ameal, nateglinide is administered orally which is fast absorbed with mean peak plasma drug concentration (C_{max}) occurring generally within a span of an hour (T_{max}) after intake. Appreciable bioavailability of 73% approximately is estimated. The lengthen absorption of nateglinide, remain the same when dosing after or with ameal. But the rate (T_{max}) of the absorption is declined which directly affect the plasma concentration (C_{max}) during the progressing stage.[22]

Distribution: Data shows based on the administration of nateglinide intravenously (IV). Ten liters of nateglinide is the estimated quantity distributed in a normal subject. The drug in question bound extensively to the serum albumin (protein), and glycoprotein to a minute amount. Independent of drug concentration binding to some extends with serum protein over the test range of 0.1-10microg/ml.[22]

Metabolism: The biotransformation of nateglinide is catalyzed by the enzyme mix-function oxidase system antecedent to its removal. Hydroxylation is the major pathway for biotransformation provided by the conjugation of glucuronide. Nateglinide has less potent antidiabetic activity than it metabolite. The parent molecule is nateglinide which almost the same potency as the minor metabolite (isoprene). Isoenzymes P450, CYP2C9 (70%), and CYP3A4 (30%) predominantly metabolized nateglinide.[23]

Excretion: The metabolite and nateglinide itself are completely and rapidly expelled from the subject after ingested orally. ¾ of the drug in question that is been administered is observed in the urine after dosing at 6 hours interval. Overall, 10% of the drug metabolite was found in the faces, 73% of the metabolite in the urine while 16% excreted as it is (parent compound). Average excretion half-life, via multiple dosing to about 240mg thrice a day for a week, shows no significant accumulation of the drug in the system[22].

2.4 Drug Action on Special Population

Geriatric: The properties of nateglinide such as pharmacokinetics are not influenced by age. Hence dose adjustment is not necessary in the case of the elderly diabetic patient.

Gender: The relevancy of nateglinide related to gender differences is not significant; hence dose adjustment, in this case, is not necessary.[24]

Race: analyzing the pharmacokinetics of nateglinide on black, Caucasian, and other ethnic origins show insignificant influence on the pharmacokinetics of nateglinide.

Renal Impairment: type 2 diabetic patient with moderate to severe renal complications when compared to a person shows similar efficiency in clearance. Drug exposure is reduced to a minimum level in patient with type 2 diabetes and renal dysfunction.[22]

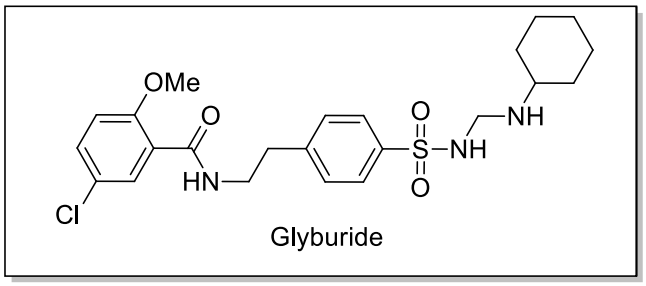
Hepatic Impairment: Increment in overall exposure to nateglinide is observed by 30% in the insufficient mild hepatic non-diabetic patient when compare to a healthy subject. Proper measures should be considered in the case of apatient with the chronic liver disease.

2.5 Drug Interactions

The assessment of nateglinide (Starlix) metabolism outside the living cell (in vitro) suggest the isoenzyme CYP2C9 and cytochrome P450 metabolized up 70% of nateglinide and to a lower extent 30% by CYP3A4.

Glyburide

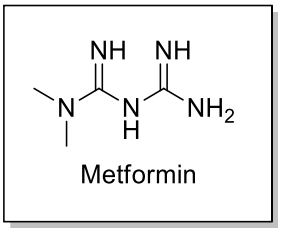
*Glyburide**:*** unorganized number of dose overlap study, aperson suffering from type 2 type diabetes mellitus are been giving 120mg of nateglinide thrice a day before taking meals in thespan of 1 day cooperated with 10mg of glyburide. The pharmacokinetics relevancy clinically was not significant.[24]



Glyburide

Metformin

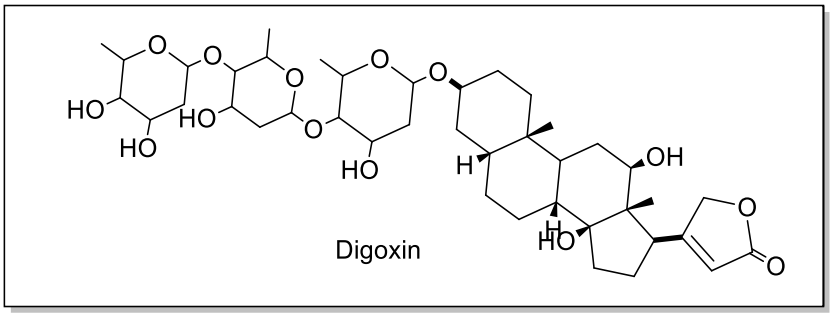
Metformin: giving 500mg of metforminthrice a day together with 120mg of nateglinide (Starlix) in an empty stomach to diabetic patient, the pharmacokinetic relevancy clinically were not significant.[25]



Metformin

Digoxin

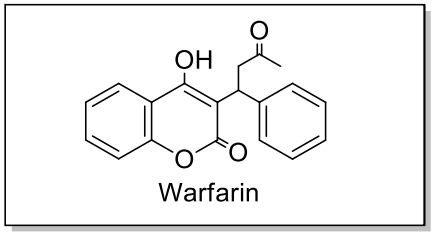
Digoxin: Giving 1mg of digoxin together with 120mg of nateglinide (Starlix) once in an empty stomach to a non-diabetic subject. The pharmacokinetic relevancy clinically was notsignificant.[26]



Digoxin

Warfarin

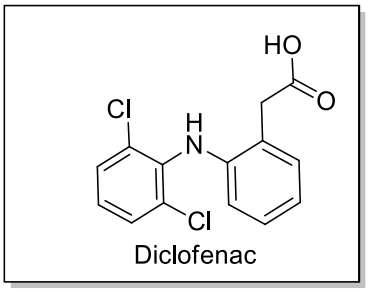
Warfarin: Giving 30mg of warfarin together with 120mg of nateglinide (Starlix) thrice in an empty stomach to a non-diabetic subject for four days. The pharmacokinetic alteration was insignificant and does not affect the prothrombin.[27]



Warfarin

Diclofenac

Diclofenac: Giving 75mg of diclofenac together with 120mg of nateglinide (Starlix) during breakfast and lunch to a non-diabetic person. Changes detected in either of the drug components pharmacokinetics are insignificant.[28]



Diclofenac

2.6 Acarbose[29]

Patent: expired.

Generic name: acarbose.

Most common brand name: Precose, Glucobay, Prandase.

Route of administration: orally.

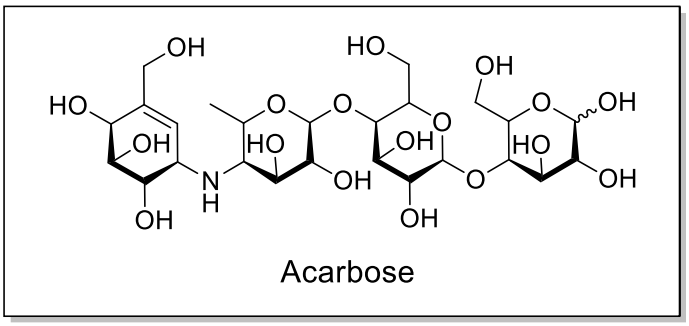
Biological half-life: 2hours.

Bioavailability: extremely law.

Excretion: renal.

Formula: $C_{25}H_{43}NO_{18}$.

Molecular weight: 645.61g.mol^{-1}.



Acarbose

Acarbose is an anti-diabetic drug use to treat type 2 diabetes mellitus and pre-diabetes in some countries. Known as Glucobay in Europe and China (Bayer AG), Precose in North America (Bayer pharmaceuticals), and in Canada as Prandase (Bayer AG). It's affordable and well known in China unlike in the USA which is otherwise. A physician explains the use in the US as a limited one because its potency is not enough to justify it cause of diarrhea and flatulent with regard to it side effect. However, it's considered safe, effective, and well conform to a large group of Asian patient with Type 2 diabetes as suggested by recent studies.[30]

Uses

With exercise and proper diets, acarbose controls the metabolism of starch which is indirectly controlling the blood sugar level as well as type 2 diabetes. Slowing down the breaking or metabolism of starch is what acarbose does from the intake of food so as to reduce the rate at which sugar level in the blood rises immediately after meal. The good thing about acarbose is that, it does not have much negative effect drug interaction in treating diabetes due to the reason that it does not act directly on insulin, hence it can be giving in combination with other anti-diabetic drugs without expecting much negative drug interaction.[31]

How to use acarbose

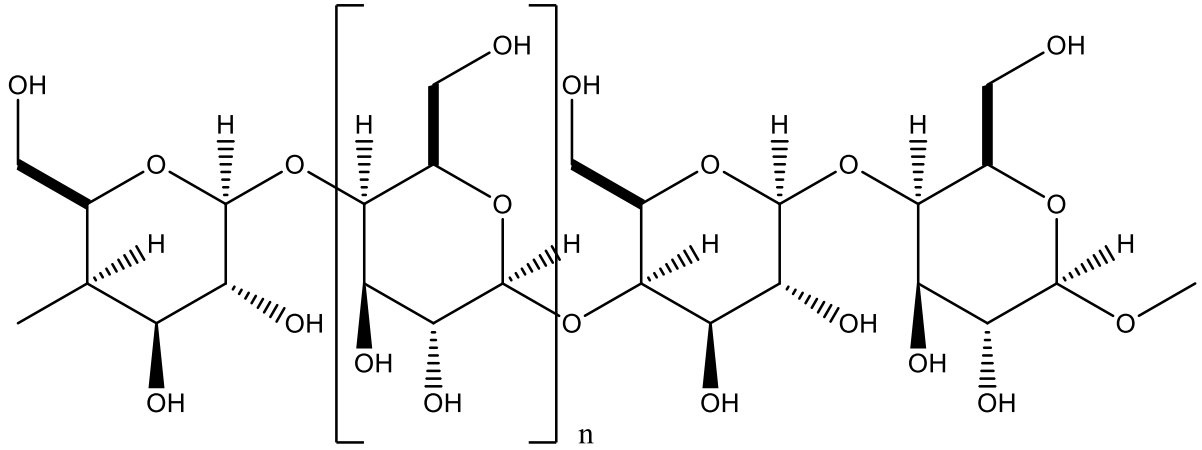
Initially, it is taken 3 times a day through oral route with the first meal you are about to chew or as prescribed by the doctor. Parameters, like a medical condition, weight of the patient, and the his\her body response to therapy determines the dosage. Gradual increase in the dosage of the drug in a particular patient can lead to a more effective dose. 300mg is the maximum dose one can administer per day. To maximize it benefit, regular proper diet, exercise and check-up is required.[32]

2.7 Alpha-amylase enzymes and its inhibitors

A new therapeutic approach or drug design target in the treatment of type 2 diabetes mellitus and obesity is alpha amylase enzyme inhibition; since this enzyme is responsible for the digestion of carbohydrates to small absorbable molecules or monosaccharides; inhibition of this enzyme will prevent from carbohydrates' digestion and further absorption; so the blood sugar level will remain as such and will not raise. Alpha-amylase inhibitors are generally divided into two classes: proteinaceous inhibitors and non-proteinaceous inhibitors.[33]

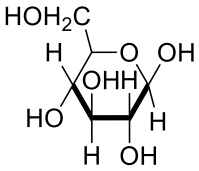
Alpha-amylase enzymes can't function in the absence of calcium, hence they are considered to be calcium metalloenzymes. In as much as there are many digestive enzymes in humans, pancreatic alpha-amylase happens to be a significant one. The enzymes alpha-amylase is responsible for the breakdown of starch which is a polysaccharide to maltose a monosaccharide molecule. The major source of energy which are sugars and carbohydrates in animal storage entity. 1,4-a-D-Glucan glucanohydrolase; EC 3.2 is the approved name for alpha amylase by the commission of enzyme nomenclature. Starch is hydrolyzed to maltose by the enzymes alpha-amylase. Starch has been a complex molecule found mostly or made by bacteria or plant from there anabolic pathways. The structure of amylase, the glucose molecule is simply bonded

between the first carbon in the ring and the fourth carbon of the preceded ring. Oxygen is the linker group.[34, 35]



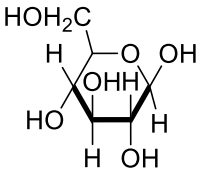
Amylose

Glucose is the building block of starch. Note that a single oxygen bond left 2 hydrogens and oxygen most have been lost in the formation.

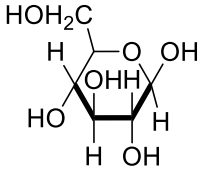


Glucose

Free glucose or pair of free maltose generate in the similar pattern by the bond breaking, a water molecule is added to generate the ‘OH’ at carbon 1 1nd 2. This is known as hydrolysis.



Alpha-D-Glucose



Alpha-D-Glucose

A large molecule like starch can’t cross the blood-brain barrier with ease like glucose and other smaller units, therefore the alpha amylase enzymes facilitate the cleavage of large molecules into thesmaller unit in order to achieve a smooth assimilation of the molecules. Excess conversion of starch to sugar, lead to increase blood sugar level which gives way to the activity of insulin. The insulin mediates various cells in the human body to metabolize the excess sugar moieties and stored it as glycogen. This process (cycle) continues in a healthy person and in contrasts to the

above mention alpha-amylase enzymes and the insulin mediation activity in a healthy person, excess activity of alpha amylase enzymes coupled with insulin deficiency or resistance to insulin, blood glucose level rises to a certain unprecedented level which will subsequently lead to hyperglycemia. Several studies have taken place in this aspect to control hyperglycemia in relation to the inhibition of alpha-amylase enzymes in the pancreas due to undigested carbohydrate in the colon with symptoms such as bacterial fermentation like flatulence and diarrhea.[36, 37]

Some plants families such as Cereals and Leguminosae family generate alpha amylase inhibitors for the purpose of defense against pests and pathogens. The alpha-amylase inhibitors which produce by plants can inhibit endogenousalpha-amylases, insect alpha-amylases and mammalian alpha-amylases. Bothe proteinaceous and non-proteinaceousalpha-amylase inhibitors are available in plants. Proteinaceousalpha-amylase inhibitors are classified into six classes according to their similarity in sequences and three-dimensional structures; Knottin type, Kunitz-like, cereal type, γthionin-like, thaumatin-like and lectin-like. These inhibitors are used in many agricultural, clinical and industrial processes. In the medical fields, the inhibitors are useful to treat diabetes mellitus.

2.8. Recent advancement on beta cells receptor agonist

Islet transplantation: Islet transplantation is the best islet substitution treatment as such, accomplishing much better glycemic control than day by day insulin infusions. The primary effective trial of human islet transplantation was accounted for in 1990, reestablishing normoglycemia in 5 out of 9 diabetic patients for more than 100 days. The majority of the early trials of human islet transplantation neglected to maintain normoglycemia in the islet beneficiaries for over 1 year. In 2000, Shapiro and partners reported a without steroid convention of human islet transplantation, which was hitherto alluded as the Edmonton convention, and amazingly accomplished insulin-freedom in 7 patients for a middle term of 11.9 months.[38,39] The Edmonton convention extraordinarily enhanced the islet transplantation and was recently adjusted as the brilliant standard by islet transplant revolves far and wide. Up to 2012, more than 1000 patients got pancreatic islet transplantation. In 6 chose transplantation focuses, more than half of patients remained insulin-autonomous for over 5 years taking after islet transplantation[31].

Beta Cell Regeneration: Human beta cells don't increment all through lifetime unless amid a couple of special cases including embryonic improvement, pregnancy, T2D, et cetera. Every one

of these situations has been under close examination to recognize a potential beta cell-particular mitogenic figure. Many variables, including glucagon like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), theinsulin-like development component 1 (IGF1), epidermal development consider (EGF), hepatocyte development calculates (HGF), serotonin, prolactin, placental lactogen, etc, have been screened however restricted achievement has met so far.26 In the interim, a hot verbal confrontation about the wellsprings of beta cell multiplication, whether they are from the replication/extension of prior beta cells or recovery from begetter cells or different sorts of cells (separation or trans-differentiation), is continuous in the scholarly community. Knowing the wellsprings of beta cell multiplication might be of basic significance in finding the right system to renew beta cell misfortune to treat diabetes.[37, 38]

CHAPTER 3: OBJECTIVES

The research project aimed to identify a potential alpha-amylase inhibitor. The research plan involves structurally modified compounds are desined based on anti-diabetic drugs nateglinide similar to peptide-like proteinaceous alpha-amylase inhibitor naturally found in amaranth, wheat, barley, ragi etc.

A successful inhibition of this enzyme serves as an anti-diabetic agent in the management of type 2 diabetes mellitus.

The specific objectives of the project are:

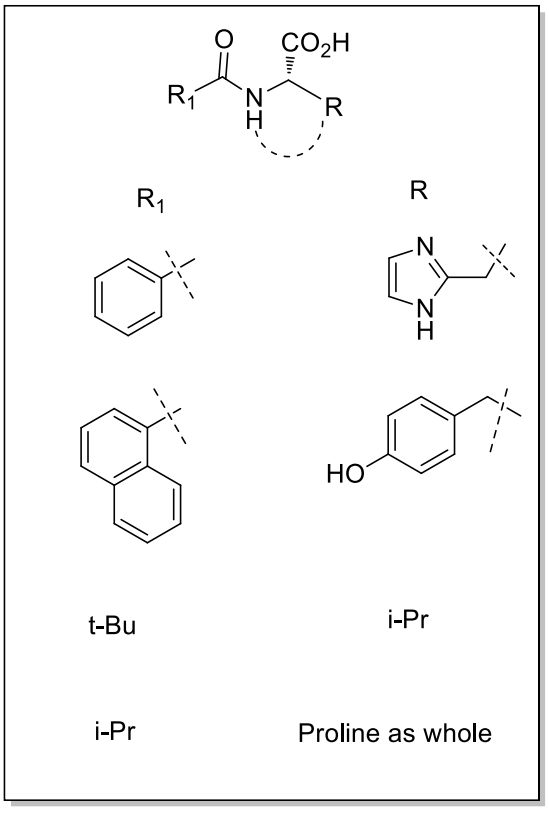
- The design of structurally modified peptide IMP for α-amylase inhibitor.
- Study the binding interaction of designed molecules by molecular docking and identifying the most potent compounds.
- Synthesis of a most potent compound as identified through molecular docking.
- Characterization of synthesized compounds through spectroscopy and biological evaluation.
- Alpha-amylase inhibition studies.

CHAPTER 4: WORK PLAN

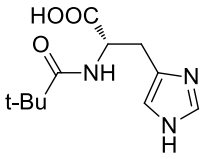
To develop and synthesize a new anti-diabetes agent, it is necessary to have the knowledge of the binding interactions of existing drugs with the respective protein cell receptors. By taking into consideration of various aspects of ligand-receptor interactions, new scaffolds will be designed and synthesis in other to develop most potent alpha-amylase inhibitor as a successful anti-diabetic drug.

4.1 Ligand preparation: Database of various ligands will be prepared and geometry, as well as energy, will be minimized through ChemDraw. All the optimized ligands will be saved in pdb format.[40]

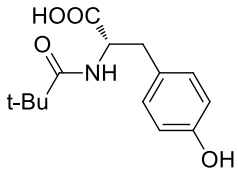
4.2 Protein preparation: Protein structures will be downloaded from protein data bank and prepared prior to docking in order to add hydrogen atoms, optimize hydrogen bonds, remove or delete the ligand next to water molecule.[41]



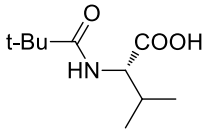
Proposed Nateglinide derivatives



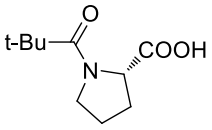
(*S*)-3-(1*H*-imidazol-4-yl)-2-pivalamidopropanoic acid



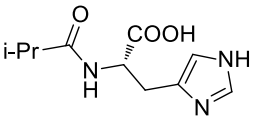
(*S*)-3-(4-hydroxyphenyl)-2-pivalamidopropanoic acid



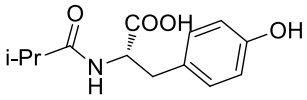
(S)-3-methyl-2-pivalamidobutanoic acid



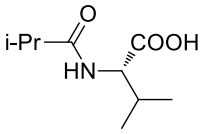
(*S*)-1-pivaloylpyrrolidine-2-carboxylic acid



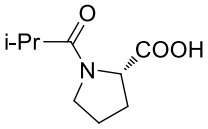
(*S*)-3-(1*H*-imidazol-4-yl)-2-isobutyramidopropanoic acid



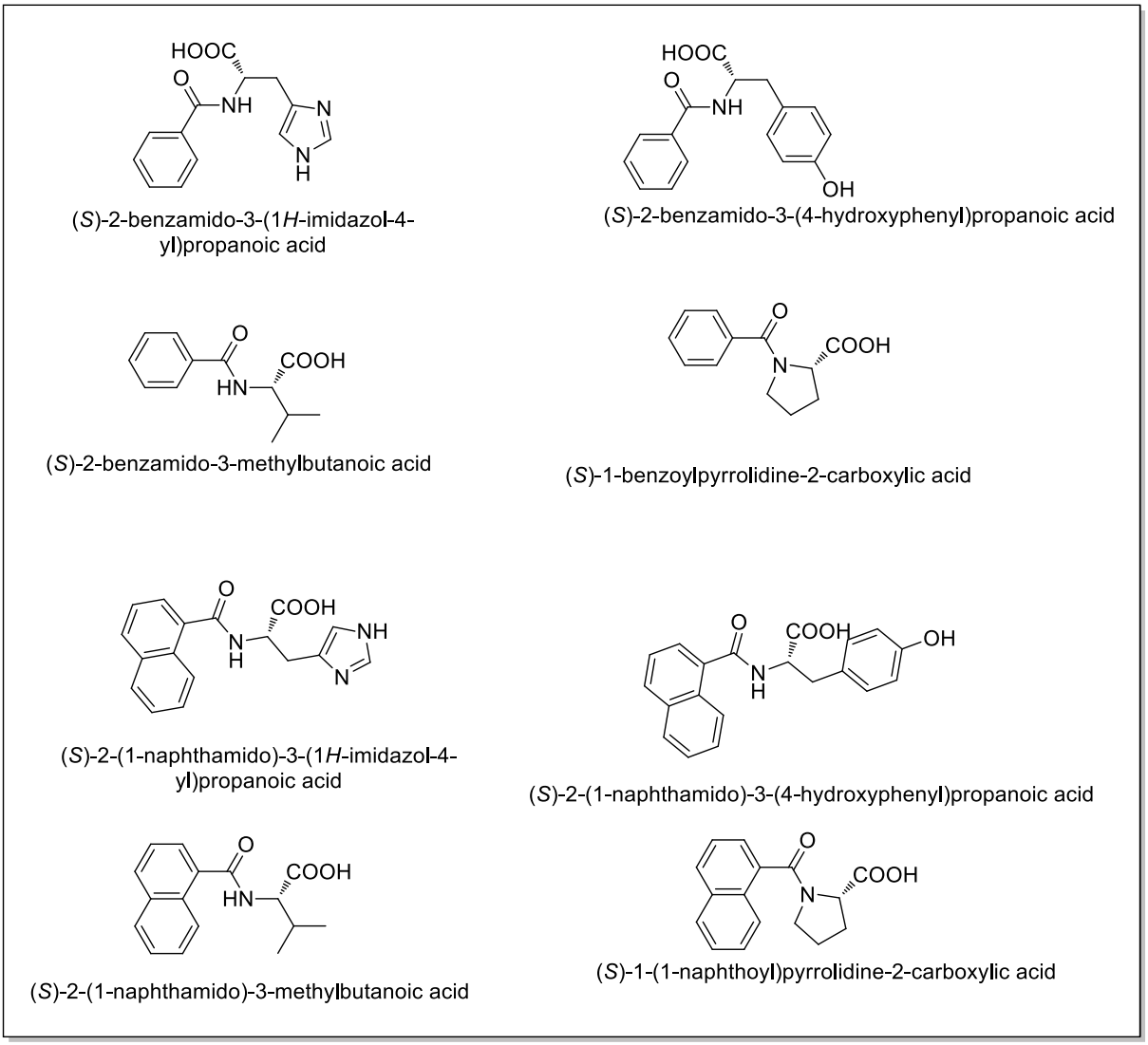
(*S*)-3-(4-hydroxyphenyl)-2-isobutyramidopropanoic acid



(S)-2-isobutyramido-3-methylbutanoic acid

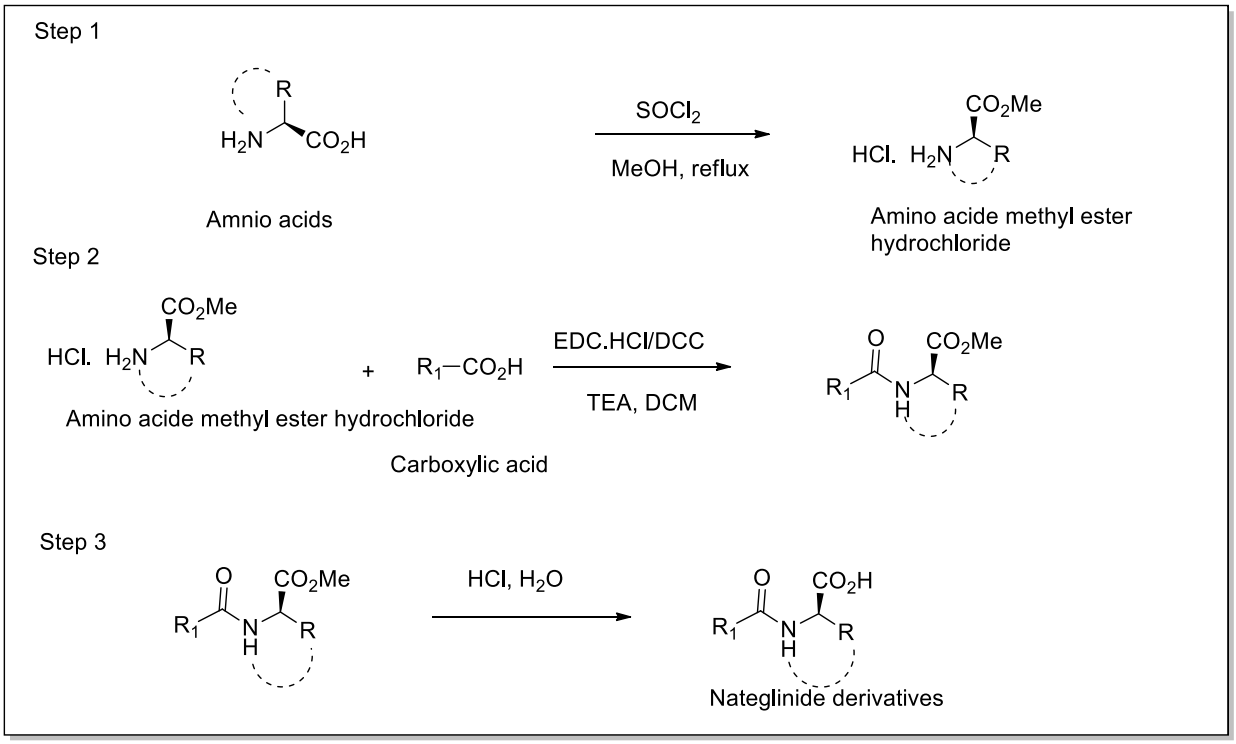


(*S*)-1-isobutyrylpyrrolidine-2-carboxylic acid



The best selected ligand will be synthesized by coupling reaction using amino acids and carboxylic acids as per the skeletal (Scheme 1).

4.3 Synthetic scheme:



Scheme 1: Proposed synthetic scheme for nateglinide derivatives

4.4 Alpha amylase inhibitory activity test

This is test is done in order to assess the anti-diabetic activity of the designed ligand. As a matter of fact, alpha amylase is known to be the main cause of the breakdown of large molecule of carbohydrate to a smaller ones, as such inhibiting this enzymes will serve as a basis for the treatment of type 2 diabetes.

CHAPTER 5: RESULTS & DISCUSSION

5.0 Molecular modeling: The structure of nateglinide derivatives in complex form shows bent conformation for nateglinide, due to hydrogen bonding interactions. Therefore, it generated an idea to synthesize conformational restricted model with similar structural properties to investigate the anti-diabetic activity. With our current interest on alpha amylase inhibitors in the treatment of diabetes, we designed conformational restricted nateglinide series 1, 2, 3 and 4 derivatives with various bioisosteric replacements as shown in the table below. The designed compounds were drawn in 2 and 3D structure by using ChemDraw and geometric repulsion energy was minimized by using molecular mechanics method.All geometry minimized structure were then converted or transformed into readable .pdb format by in Autodock-vina software (ADT).[42, 43]

Molecular docking: To determine most potentially active ligands towards AR modulator by using Autodock-vina. All designed molecules were prepared for docking purpose as discussed below:

5.1 Preparation of Protein (4gqr): From the File menu, choose Read Molecule, highlight the PDB file for your protein, and click Open or, right click on Python Molecular Viewer (PMV) Molecules at the bottom of the window and choose the protein pdb file. Also fix any problems with the PDB files, such as missing bonds or atoms, and remove extraneous structures such as water molecules. Before beginning this section, inspect the PDB file to learn what such structures may be present. We want to keep only the protein and such cofactors as may be bound to it naturally. And then save it as pdbqt file (figure 1).[43]

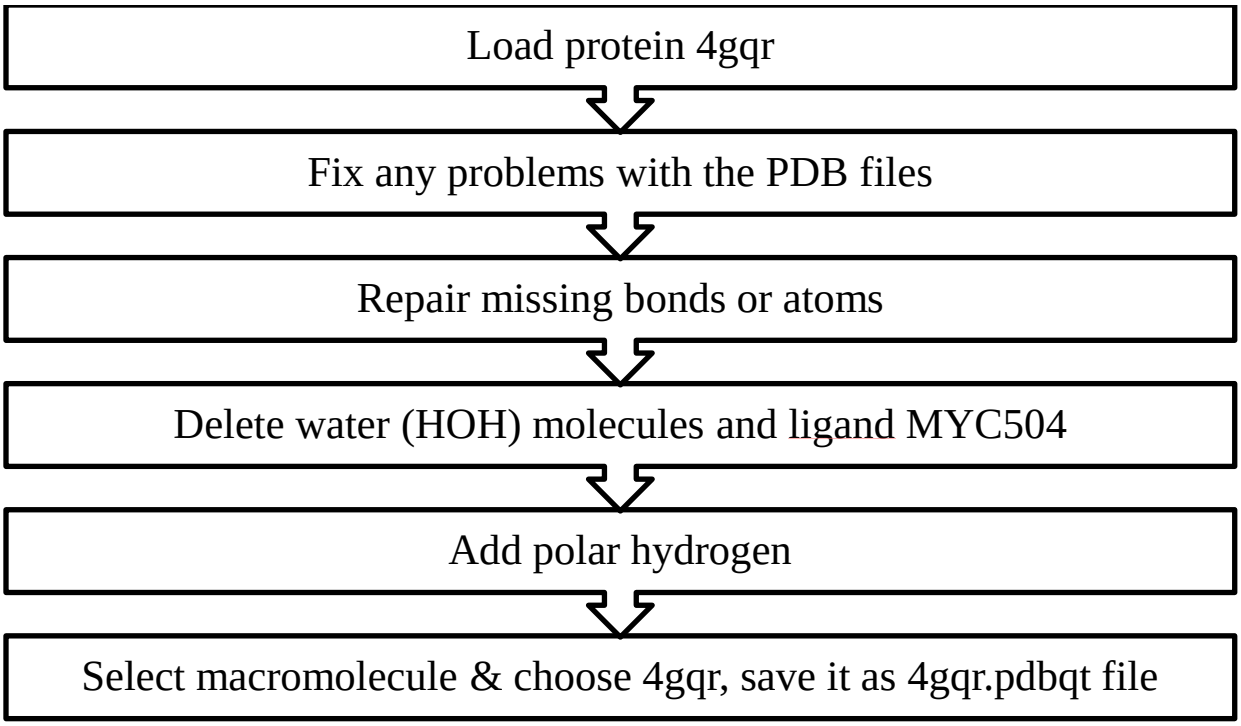


Figure 1: Schematic flowchart for protein preparation

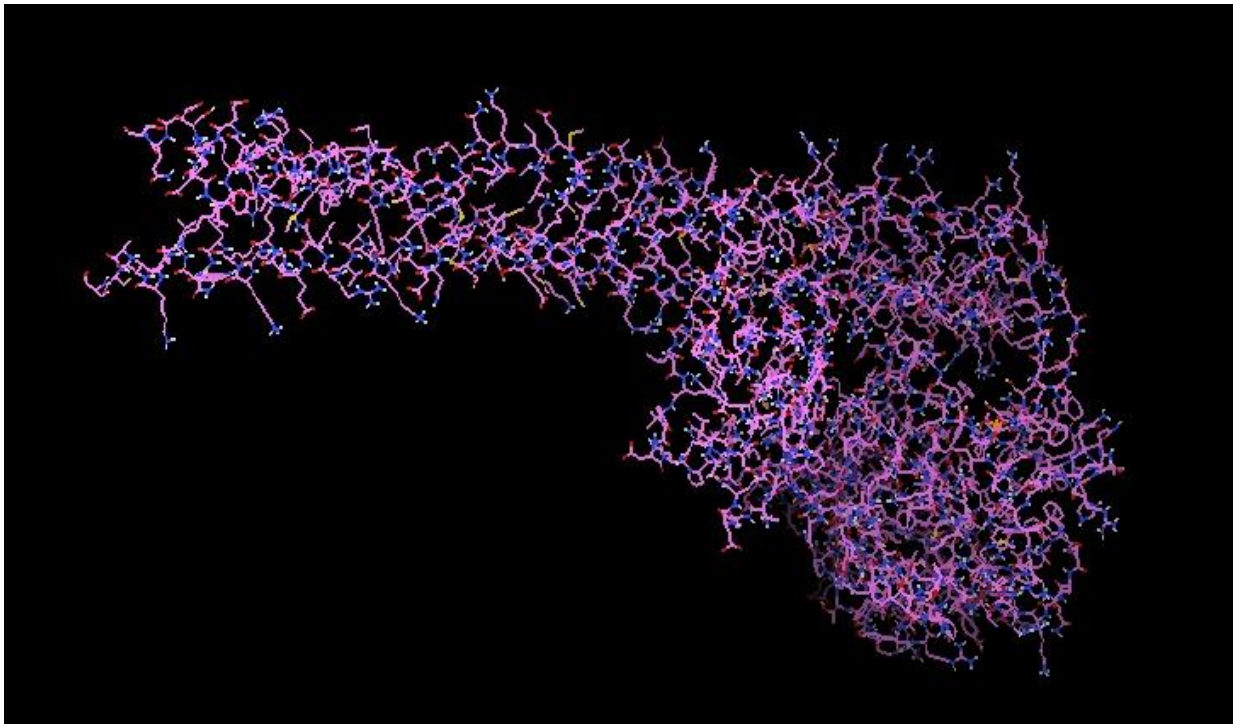


Figure 2: Prepared protein

5.2 Preparation of Ligand : Go to the ligand on the menu bar, then click on input molecule and then open the ligand and select pdb files. Then choose the file containing the ligand, and click Open. Then a message will be pop up on screen as:[43]

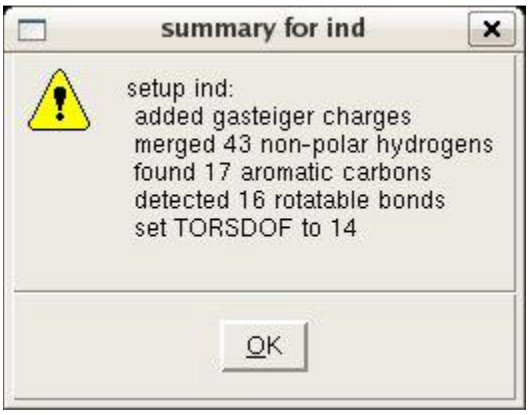


Figure 3. Pop up message while loading the protein

On the menu bar, select Ligand -> Torsion Tree -> Detect Root. A small dot will appear, marking the choice.

Next, select Ligand -> Torsion Tree -> Choose Torsions. The Torsion Count widget appears.

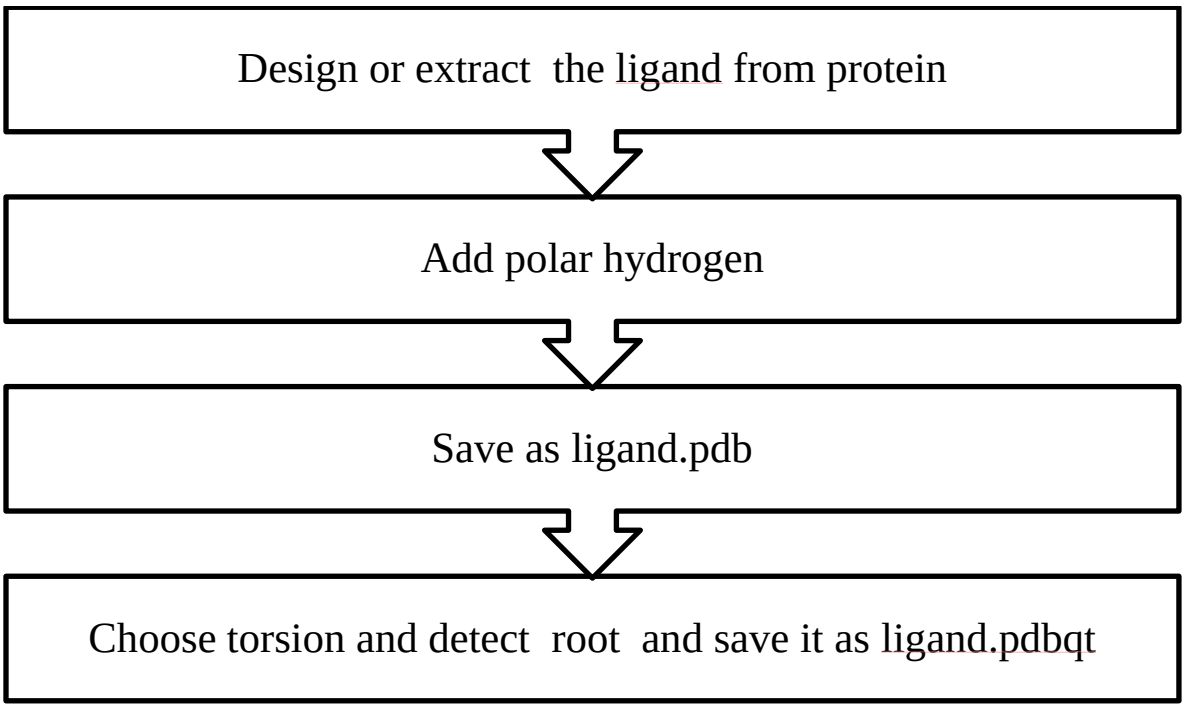


Figure 4: Schematic flowchart for ligand preparation

After manipulating torsions, press was done. Display now looks like as shown below:

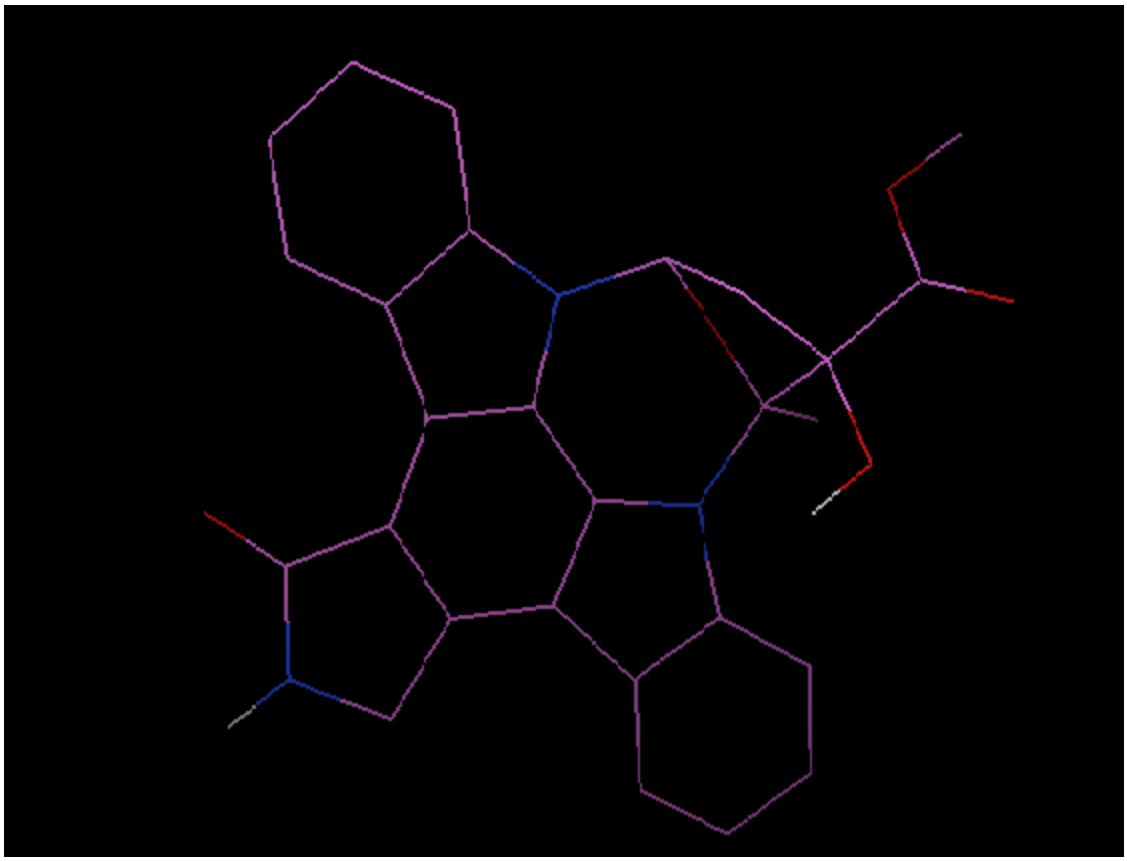


Figure 5: Prepared ligand

5.3 Docking and Validation of Protein: Load ligand.pdbqt file and set it as map type by choosing ligand. After this centralize ligand by setting grid box and then save it by close saving current. Then save the protein as pdbqt file and then prepare aconfiguration file and save it as conf.txt, then analyze the docking results in command prompt as shown below:[44]

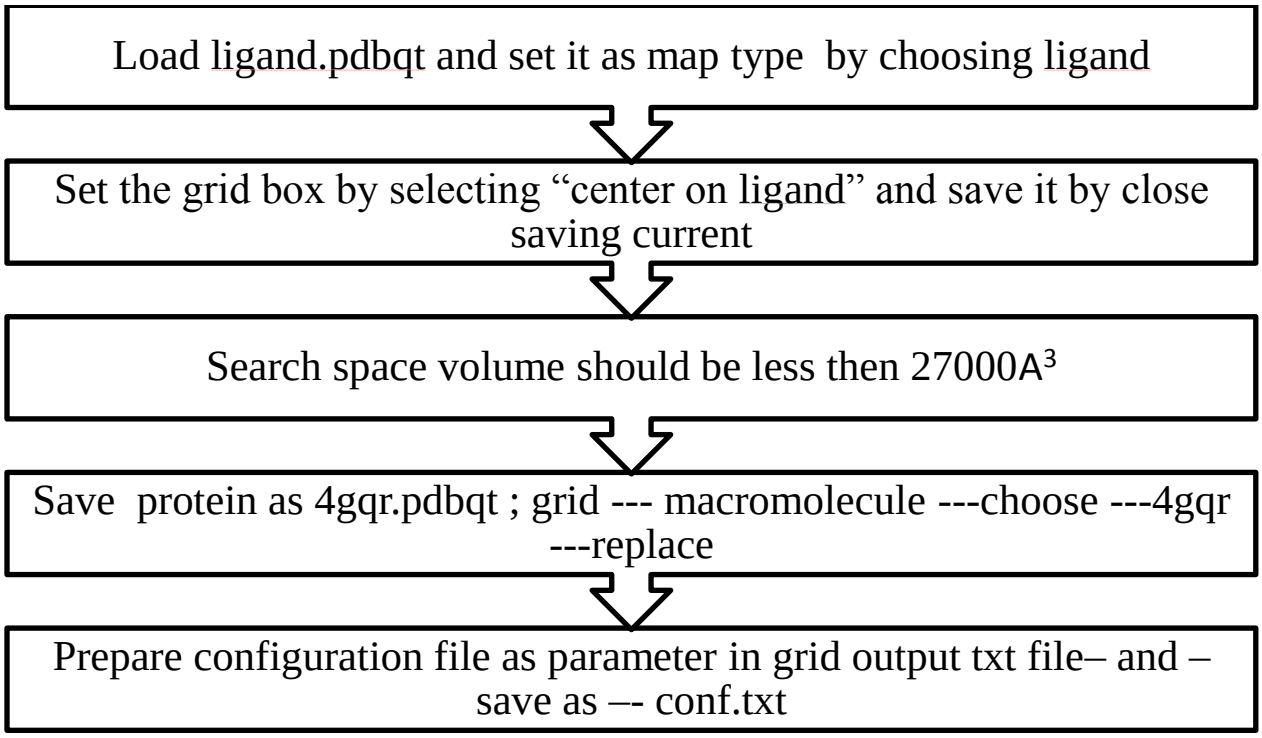


Figure 6: Schematic flow chart for preparation of grid for docking

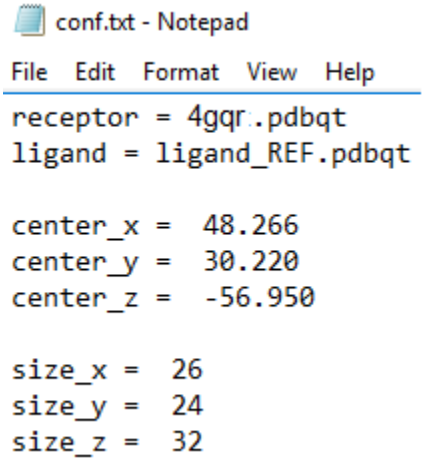


Figure 7.Preparation of configuration file for docking

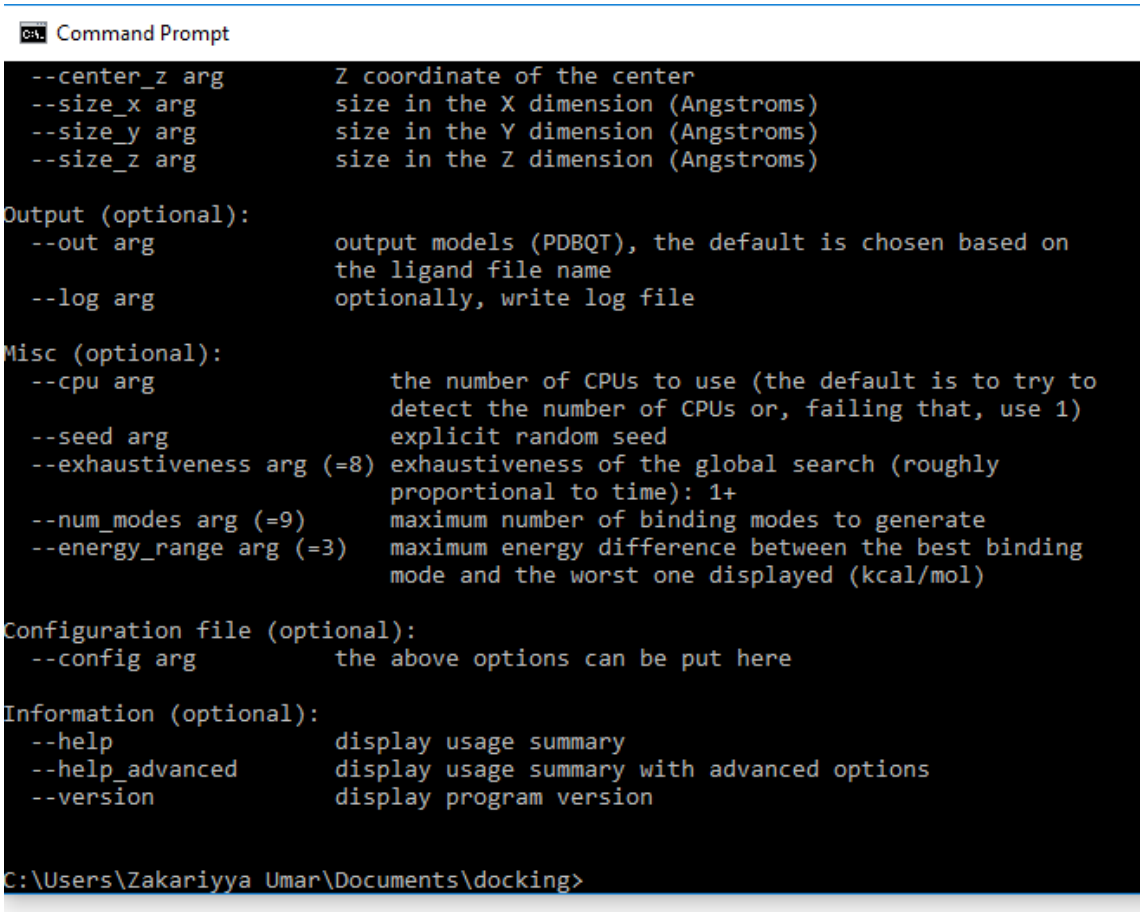


Figure 8: Step 1 docking in command prompt

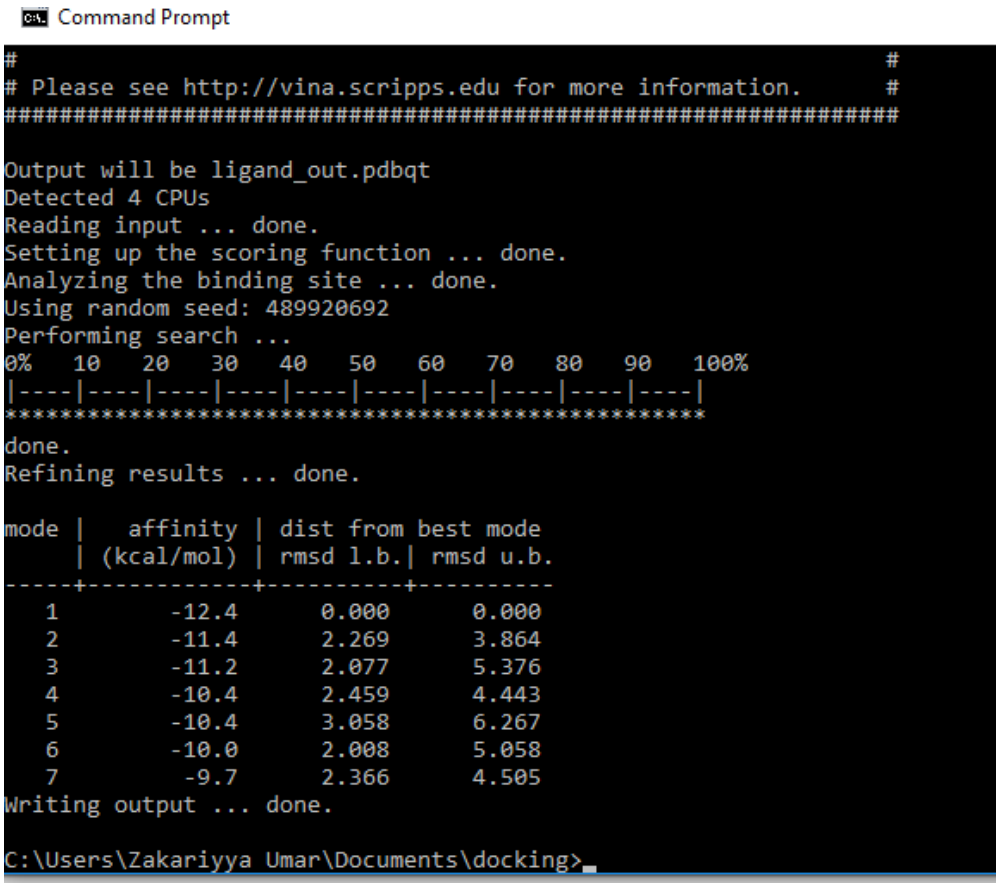


Figure 9: Step 2 docking in command prompt

5.5 Interaction of ligand generated from it respective protein 4gqr

MYC504ligand with 4gqr alpha protein

MYC504 is amino acid next to water molecule in the list of amino acid in 4gqr protein structurewhich is an alpha type protein. The docking score was reaffirmed and reference grid for the first series of the ligand was formed.

Docking of various designed ligands :16 designed molecules (ligand) from the series were docked and their results are given as such in table 3.

Designed ligands of nateglinide derivatives.

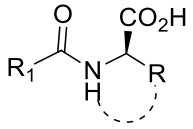
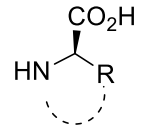
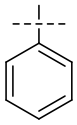
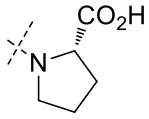
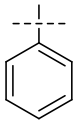
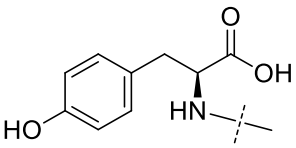
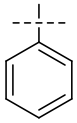
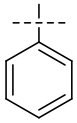
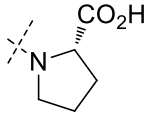
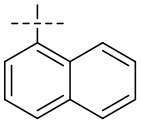
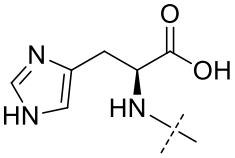
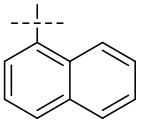
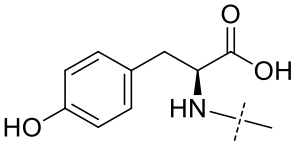
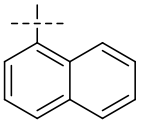
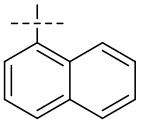
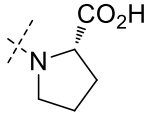
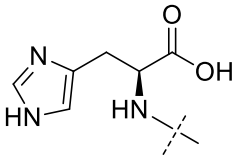
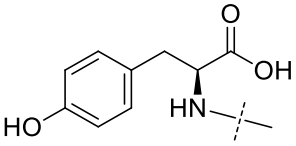
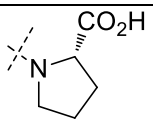
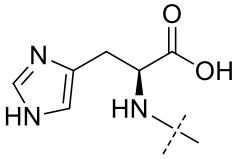
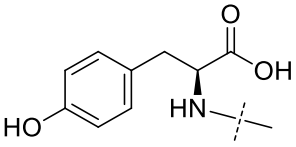
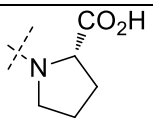


Table 3: Docking using 4ggr (alpha-amylase) protein

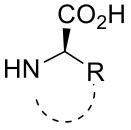
S.No	Code	R_1		Affinity score
1	Zu1			-6.5
2	Zu2			-7.0
3	Zu3		i-Pr	-6.2
4	Zu4			-7.1
5	Zu5			-7.4
6	Zu6			-7.8
7	Zu7		i-Pr	7.8
8	Zu8			-7.7

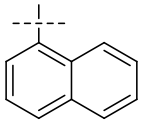
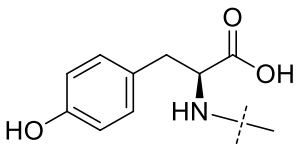
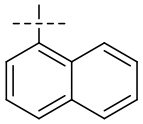
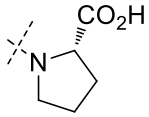
9	Zu9	t-Bu		-5.9
10	Zu10	t-Bu		-6.6
11	Zu11	t-Bu	i-Pr	-5.5
12	Zu12	t-Bu		-5.9
13	Zu13	I-Pr		-5.7
14	Zu14	i-Pr		-6.6
15	Zu15	i-Pr	i-Pr	-5.6
16	Zu16	i-Pr		-5.7
17	Nateglinide	---	---	-7.4
18	4gqr-ligand			-8.0
19	Acarbose			-7.0

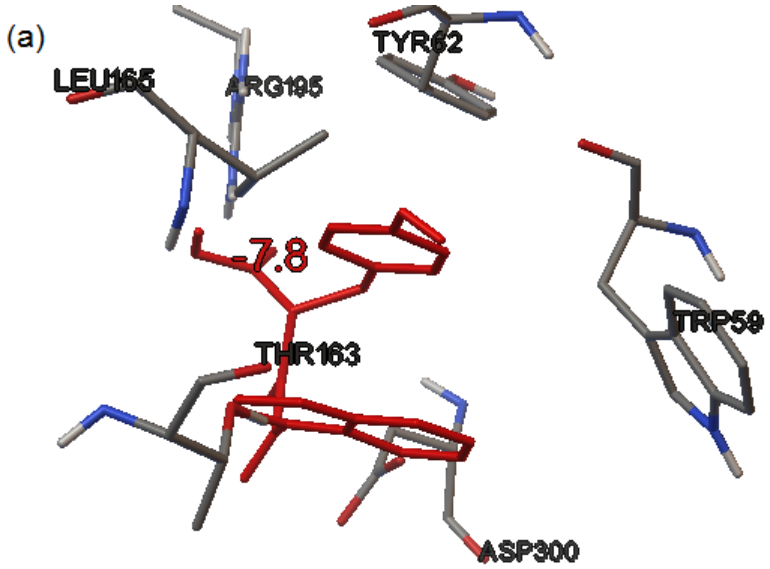
Most potentially active compounds on the basis of their affinity score were identified. The close contacts have shown for the most potent compounds or ligands in figure

5.6 Binding affinities of the most potent and feasible compounds:

Table 4: 4gqr protein

S.NO.	Code	R_1		

1	Zu6			-7.8
2	Zu8			-7.7



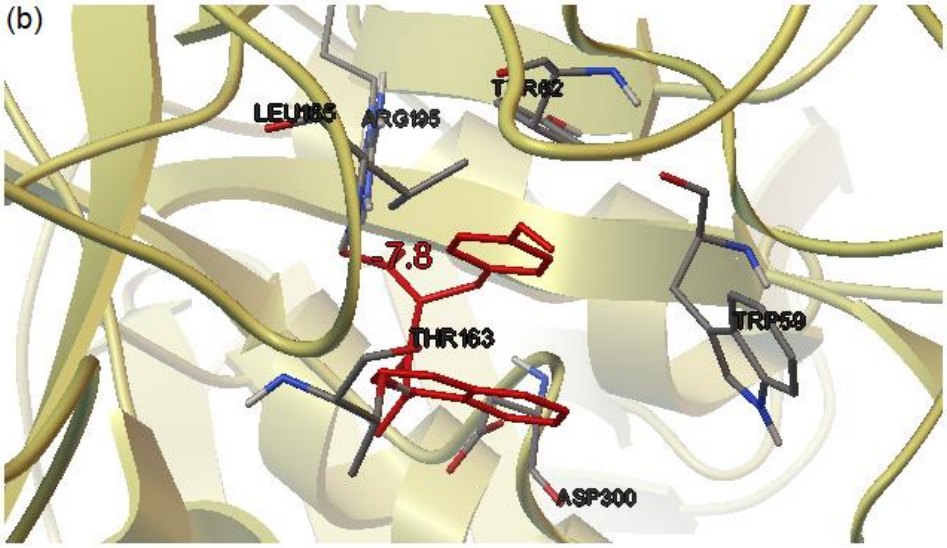


Figure 10. Overlay of docked ligand with 4gqr protein (a) Stick and line model (b) Zu6 in ball and stick model, 4gqr protein in ribbon structure.

The interaction between the best ligand Zu6 and protein was analyzed which is depicted in figure 10. It showed that the ligand has one hydrogen bond with Arg195 residue at hydroxyl of carboxylic acid. Naphthalene part of ligand is buried in hydrophobic region of binding site.

5.7 CHEMISTRY AND SYNTHESIS: the procedures to obtain the designed ligand and their intermediate are discussed below:

5.7.1 Synthesis amino acid methyl ester: to a stirred suspension of methanol ($2cm^3$ $mmol^{-1}$) and 1 eqv of the amino acid, a drop wise addition of 1.5 eqv thionyl chloride at 0^0C. The reaction warm up at room temperature and stirred for 24hrs, after which the solution is concentrated in vacou to give the required amino acid methyl ester hydrochloride. The reaction completion is check by thin layer chromatography (TLC) and characterized by spectral analysis to confirm the product.[45]

Table 5: Synthesis of amino acid methyl esters

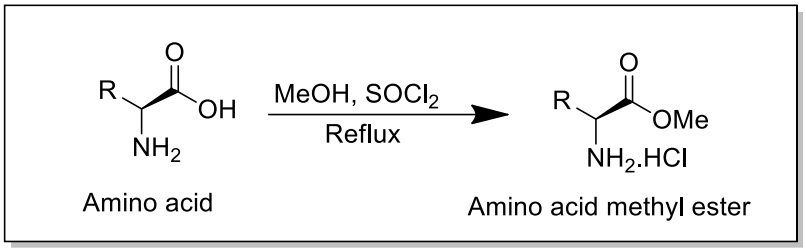
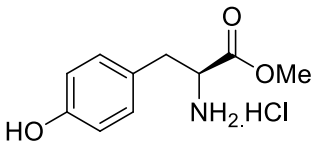
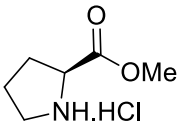


Table 5: Synthesis 1

Entry	Compound	Yield (%)
1.		63
2.		64

5.7.2 Coupling of amino acid methyl ester with aromatic acid: 2ml of DMF and DCC (125mg, 0.65mmol) and HOBt (75m, 0.65mmol) was added to 154mg, 0.65mmol of the amino acid methyl ester HCl, and the organic acid which is then cool down to 0^0C. The whole mixture was allowed to warm up at room temperate then stirred overnight. The solution was concentrated in vacuum. The reaction completion is checked using TLC. The product is then confirmed using spectral analysis methods.[46]

Table 6: Coupling of amino acid methyl ester with aromatic acid

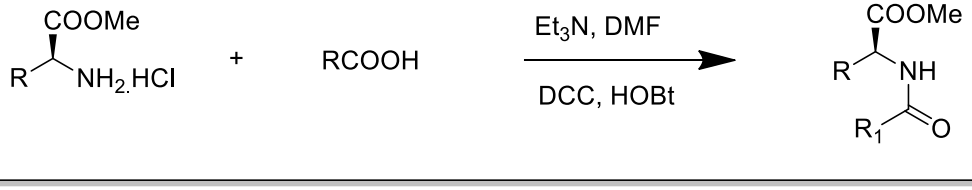
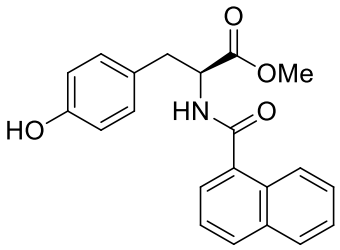
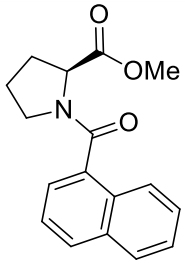


Table 6: Synthesis 2

Entry	Compound	Yield (%)
1.		39
2.		52

5.7.3 Hydrolysis of Synthesis of (*S*)-2-(1-naphthamido)-3-(4-hydroxyphenyl)propanoic acid:

in this case, the ester of the amino acid peptide is hydrolyzed to it corresponding amino acid by addition of 1M lithium hydroxide monohydrate in (1:1) THF/H_2O to the peptide of the amino acid methyl ester at 0^0C and stirred for 5mins. Then, it was stirred at room temperature for 1hr. The solution was extracted with ether to removes the impurities and the un-reacted materials. The aqueous layer was collected, and acidified to approximately 2 pH then extract again with ether. The organic layer is collected and dried with anhydrous sodium sulphate. The dried organic layer is the concentrated in vacuum. The reaction completion is checked using TLC and the product is confirmed using spectral analysis.[47]

Table 7: Hydrolysis of peptide.

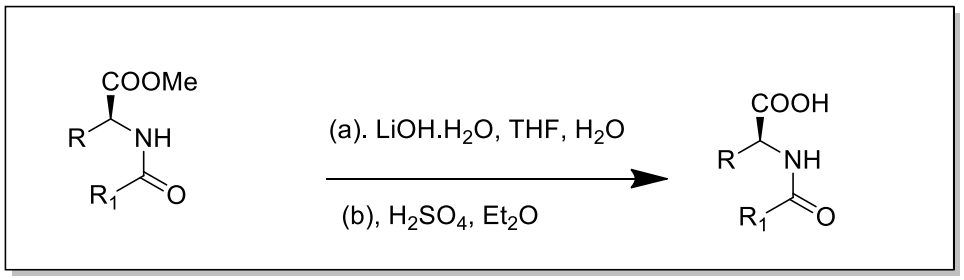
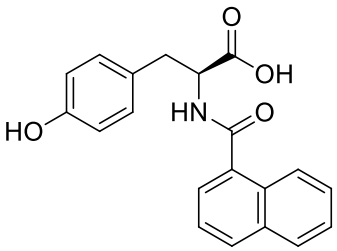
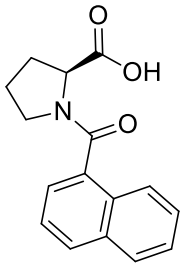
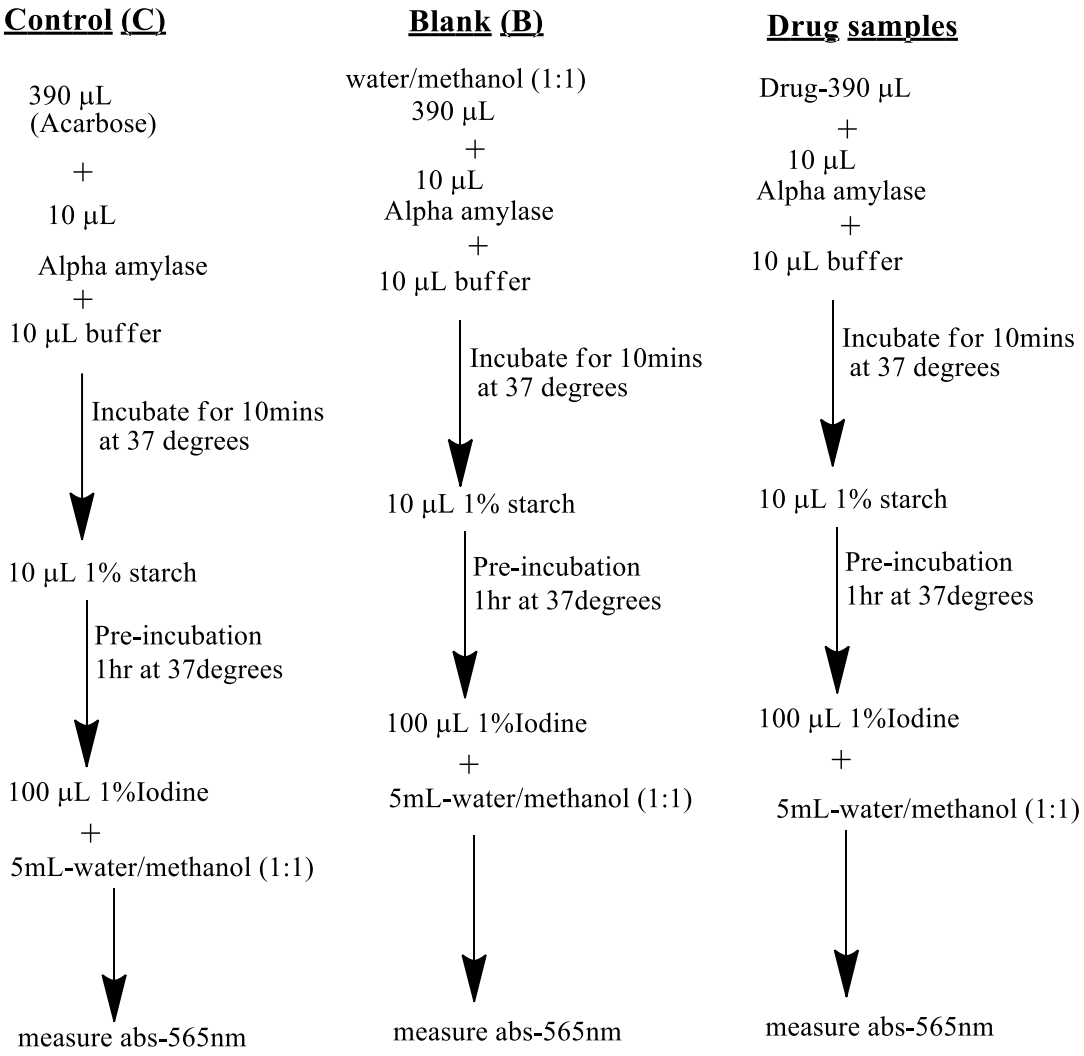


Table 7: Synthesis 3

Entry	Compound	Yield (%)
1.	 Zu6	48
2.	 Zu8	64

5.8 Alpha amylase inhibition result

Alpha amylase inhibition was measured in % by the using the method as described in flow chart below.[48]

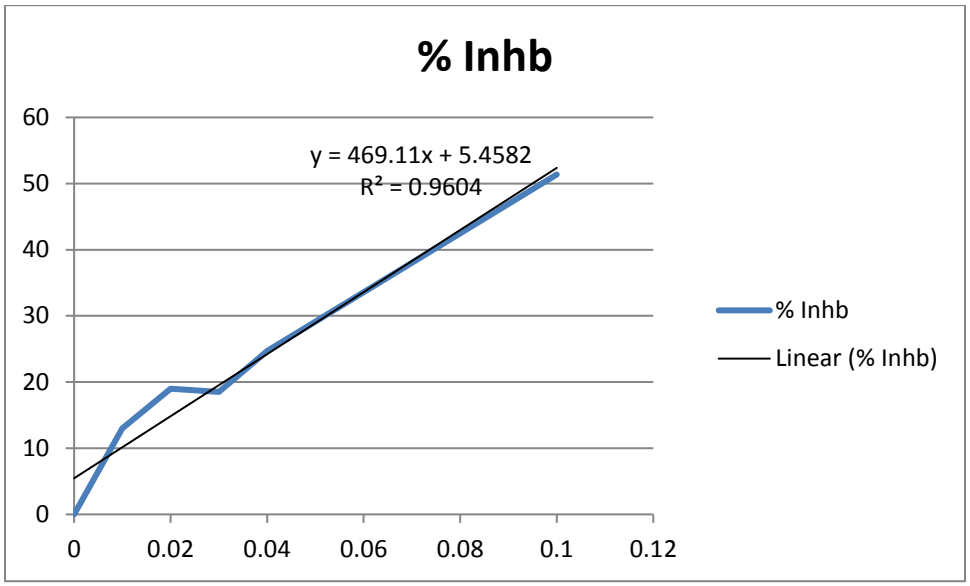


1. **Alpha amylase inhibition assayof (*S*)-2-(1-naphthamido)-3-(4-hydroxyphenyl)propanoic acid (Zu6).**

Table 8: absorbance and % inhibition of alpha amylase by Zu6

s.no	Conc.	Absorbance[a]	% inhibition
1	0.01	0.0168	13%
2	0.02	0.0177	19%
3	0.03	0.0176	18.5%
4	0.04	0.0185	24.7%
5	0.1	0.0224	51.37%

[a]average of triplicate



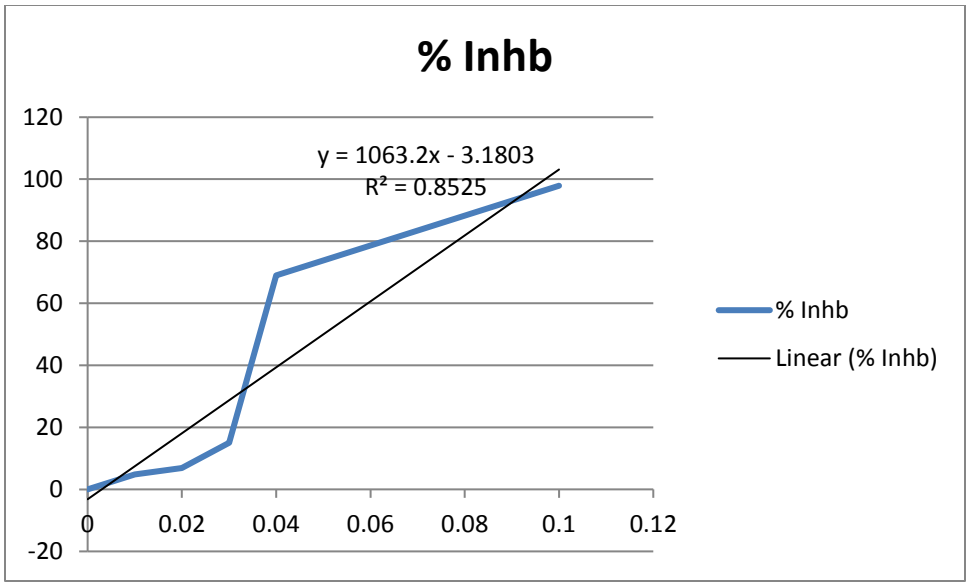
IC_{50}=95µg/mL

Figure 11. % inhibition of alpha amylase by Zu6

2. **Alpha amylase inhibition assayof (*S*)-1-(1-naphthoyl)pyrrolidine-2-carboxylic acid (Zu8)**

Table 9: Absorbance and % inhibition of alpha amylase by Zu8.

s.no	Conc.	Absorbance	% inhibition
1	0.01	0.0156	4.8%
2	0.02	0.0159	6.85%
3	0.03	0.0171	15%
4	0.04	0.0250	69.2%
5	0.1	0.0292	97.9%



IC_{50}=50µg/ml

Figure 12.% inhibition of alpha amylase by Zu8

As per molecular docking both the synthesized compounds Zu6 and Zu8 showed good inhibition of alpha amylase enzyme. The $IC_{50}$of both ligand obtain above 95 µg/ml, and50µg/ml respectively, found to have appreciable alpha amylase inhibition activity when compare to acarbose that is having IC_{50} = 83.23 µg/ml.

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CHAPTER 6: EXPERIMENTAL WORK

Table 10: LIST OF CHEMICALS

S.No	Chemical name	Company name
1	L-Tyrosine	Loba chemicals
2	L-Proline	Loba chemicals
3	Methanol	Loba chemicals
4	Thionyl chloride	Loba chemicals
5	HOBt	Qualigens
6	DCC	Loba chemicals
7	DMF	CDH
8	Triethylamine	Loba chemicals
9	Naphthoicacid	Sigma Aldrich
10	Benzoic acid	Loba chemicals
11	H_2SO_4	Avantor
12	LiOH. H_2O	Thomas baker
13	THF	Loba chemicals
14	Ether	Loba chemicals

Table 11: LIST OF INSTRUMENTS

S.No	Instruments	Company name
1	FT-IR spectrophotometer	Shemadzu
2	Heating mentle	Navyug scientific works
3	Hot air oven	Navyug scientific works
4	Magnetic stirrer	Remi sales
5	Mass spectrophotometer	WATERS, Q-TOF micromas
6	NMR spectrometer	Brukeravance
7	Refrigerator	Kelvinator

8	Rotary evaporator	Popular traders
9	U.V chamber	Popular traders
10	Micro pipette	

6.1 Chemical synthesis:

400MHZ spectra at the ^{1}H-NMR were recorded on the BruckerAvance. DMSO is used as the solvent while the internal standard used is TMS. "δ" given in (Hz) is the chemical shift for ^{1}H. "s", "d", and "m" are the abbreviation use to denote singlet, doublet and multiplet. The completion of the reaction was monitored by TLC (Merck). The solvent used in the reaction were removed under reduced pressure with the help of commercial grade reagent rotary evaporator. Further purification of the solvent was not required.

6.1.1 Synthesis of amino acid methyl ester hydrochloride

Carefully addition of 1.5 equiv. of thionyl chloride drop wise to a stirred suspension of 1.0 equiv. of amino acid in 2ml, 21mmol at 0-8^{0}C. The reaction condition was allowed at room temperature and reflux for 24hrs, at the completion of the reaction, the solvent used is evaporated under reduced pressure to give the amino acid methyl ester hydrochloride in quantitative yield.

(*S*)-methyl 2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (Table 5, entry 1): white crystalline solid, 63% yield, M.P= 146^{0}C, IR(cm^{-1}) = 1056.06 (C-O), 2087.05 (C=O), 2900 (C-H), 3336.0 (N-H).

(*S*)-methyl pyrrolidine-2-carboxylate hydrochloride (Table 5, entry 2): white crystalline solid, 64% yield, M.P= 73^{0}C, IR (cm^{-1}) = 1087.89 (C-O), 1745.64 (C=O), 3001 (C-H), 3328.28 (N-H).

6.1.2 Synthesis of N-peptide of organic acid with amino acid methyl ester

13 of 170mg, 1.3mmol of the amino acid methyl ester were suspended in DMF placed on ice-bath. 265mg, 1.3mmol of DCC and 166.5mg, 1.25mmol of HOBt were suspended as well. The suspended solution was stirred for 1hr at 0^{0}C and 9 of 250mg, 1.05mmol of the acid were added. The suspended solution was stirred at room temperature for 24hrs. The ppt were filtered out and concentrated. The reaction completion was checked using TLC and characterized using spectral analysis.

(*S*)-methyl 2-(1-naphthamido)-3-(4-hydroxyphenyl)propanoate (Table 6, entry 1): white crystalline solid, 39% yield, M.P= 260^0C, IR (cm^{-1}) = 1094.64 (C-O), 2853.78 (N-H), 2924.18 (C-H)

(*S*)-methyl 1-(1-naphthoyl)pyrrolidine-2-carboxylate (Table 6, entry 2):white crystalline solid, M.P=94^0C, 52% yield,IR (cm^{-1}) = 1087.89 (C-O), 1745.64 (C=O), 3328.28 (N-H).

6.1.3 Hydrolysis of the amino acid peptide

0.01mol solution of the amino acid peptide in (1:1, 36mL) of THF-H_2O, 36mg of $LiOH.H_2O$ is added at 0^0C. The solution was stirred for 1hr at room temperature. After reflux, the solution was extracted with ether to remove the impurities and then acidify to 3.5pH with H_2SO_4 to remove the impurities and un-reacted materials. The solution is again extracted with ether. The organic layer is dried over anhydrous Na_2SO_3 and then concentrated in vacou. The reaction completion was checked using TLC and characterized using spectral analysis.

(*S*)-2-(1-naphthamido)-3-(4-hydroxyphenyl)propanoic acid (Zu6, Table 7, entry 1): white crystalline solid, 48% yield, M.P= 249^0C; IR (cm^{-1}) = 1041.60 (C-O), 1631.83 (C=O), 2983.01 (C-H), 3426.66 (N-H); ^{1}H-NMR: 2.51-2.53 (m, 1H), 2.51-2.91 (m, 1H), 4.31-4.41 (m, 1H), 5.80 (bs, 2H, NH, OH), 6.66-6.67 (m, 2H), 6.68-6.71 (m, 2H), 7.01-7.04 (m, 2H), 7.12 (d, J =8Hz, 1H), 7.45-7.46 (m, 2H), 7.47-7.49 (m, 2H), 7.81-7.91 (m, 2H).

(*S*)-1-(1-naphthoyl)pyrrolidine-2-carboxylic acid (Zu8, table 7, entry 2): white crystalline solid, 64% yield, M.P= 81^0C; IR (cm^{-1}) = 1066.67 (C-O), 1620.28 (C=O), 3132.50 (C-H); ^{1}H-NMR (400 MHz, DMSO): 1.67-1.74 (m, 1H),1.87-1.92 (m, 1H), 2.03-2.08 (m, 1H), 2.96-3.03 (m, 1H), 3.12-3.18 (m, 1H), 3.84 -3.88 (m, 1H), 7.43-7.55 (m, 3h), 7.87-7.91 (m, 1H), 8.01 (d, J = 8 Hz, 1H), 8.07 (d, j = 8 Hz, 1H), 8.80 (d, J = 8 Hz, 1H); ^{13}C-NMR: 23.64, 28.73, 45.22, 60.34, 124.83, 125.55, 126.08, 127.43, 128.15, 128.52, 129.69, 130.68, 132.60, 133.44, 168.82, 172.20.

6.2 Alpha-amylase Inhibition

α-Amylase activity was performed using the starch-iodine method. 10 µL of α-amylase solution having concentration 0.025 mg/mL was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentrations of extract. After incubation at 37 ℃ for 10 min, 100 µL of the 1% starch solution was added and re-incubated for 1 h.After re-incubation 0.1 mL of 1% iodine solution was added and further diluted with 5 mL distilled water. The absorbance was taken at 565 nm. [48]

$$\text{Inhibition}(\%) = \frac{(\text{Abs. of sample} - \text{Abs. of control})}{(\text{Abs. of blank} - \text{Abs. of control})} \times 100$$

where, Absorbance of the sample(test sample,α-amylase,starch), Absorbance of blank (no α-amylase), and Absorbance of control (no starch)

Inhibition of alpha amylase enzymes to check for anti-diabetics properties of the synthesis ligand:

Reagent requirements

- 1% starch solution
- 1% iodine solution
- Phosphate buffer- sodium hydrogen orthophosphate and disodium hydrogen orthophosphate
- 0.006M NaCl
- Alpha-amylase 0.1g in 400ml
- Acarbose
- Synthetic drug sample 0.001g in 10ml (W/M 1:1)

Inhibition centration (IC_{50}): It is the concentration of the drugs at which 50% inhibition take place.

CHAPTER 7: SUMMARY

Diabetes is in a top list of the most deadly metabolic disease as a result of high blood sugar. High blood sugar results in hyperglycemia (diabetes). It is broadly classified into type 1 and type 2 diabetes. Different type of drugs with different pharmacological strategies have used so far, from insulin sensitizers, insulin enhances and secretagogues and insulin agonist etc.

This research work was focused on structural modification of nateglinide, by assuming that peptidic could nature serves as alpha –amylase inhibitors similar to the naturally occurring proteinaceous alpha-amylase inhibitor found in amaranth**,** Wheat, barley, ragietc.Based on the recent advancement in the field of science, specifically in the area of medicine, it is not a popular way to synthesize a drug randomly and the checking and assessing the biological activities of the drugs. Such practice results in enamors waste of time and resources which are uncalled. Hence, a more successful and reliable approach is designed in collaboration with pharmaceutical chemist and bio-informatic scientists to predict accurately the biological activities of a drug using a software like CADD, AutoDockVina, Python Molecular Viewer and so on.Using a mapping pattern, we modify a nateglinide molecule which results in a series of sixteen new molecules and further using the alpha-amylase protein 4gqr, the docking study was performed in AutoDock vina. Based on the binding affinity best two molecules Zu6 and Zu8 were selected and synthesized. The alpha-amylase inhibition assay was performed which showed good activity. The IC_{50} of the synthesis drugs are found to be reliable with reference acarbose, a well-known alpha amylase inhibitor.In future further studies are required to establish the potential of these compounds by studying in-vivo as well as dose and safety.

CHAPTER 8: REFERENCES

1. Guaraldi, F.; Pasquali, R. Diabetes: From Ancient Egypt to the 18th Century. *J Assoc Physicians India*. **2015,***63*,128.
2. Pitchai, D.; Manikkam, R.; Rajendran. SR.; Pitchai, G. Database on pharmacophore analysis of active principles, from medicinal plants. *Bioinformation*. **2010**, *5*, 43-5.
3. Rodrigo, S. "The Sugar That You Eat, The Sugar That Make You Sick"-The Flight Of DiabetesT. HubPages. 28-7-2015.
4. Kevin V. Ergil et al. Ancient Healing: Unlocking the Mysteries of Health and Healing Through the Ages. Copyright 1997 by Publications International Ltd. Chapter on Greek & Roman Medicine, pp 68 – 98.
5. Valenti, G.; Tamma, G. History of Diabetes Insipidus. *G ItalNefrol*. **2016**, *33*, S66.
6. Villarroel, C.; Salinas, A.; López, P.; Kohen, P.; Rencoret, G.; Devoto, L.; Codner. E. Pregestational type 2 diabetes and gestational diabetes exhibit different sexual steroid profiles during pregnancy. *Gynecol. Endocrinol*. **2016**, 1-6, dx.doi.org/10.1080/09513590.2016.1248933.
7. Momeni, Javid, F.; Simbar, M.; Dolatian, M.; Alavi, Majd H.; Mahmoodi, Z. A Comparative Study and Pyogenic Liver Abscess: A Nationwide Population Study. *Curr Med Res Opin.***2016**, DOI:10.1080/03007995.2016.1266312.
8. Tseng, C.W.; Chen, T.Y.; Lin, C.L.; Liang, J.A. Association Between Chronic Pancreatitis and Progenic Liver Abscess: A Nationwide Population Study. *Curr. Med. Res. Opin.***2017**. *33*, 205-210.
9. Ziegler, S.; Beisel, C.; Sutter, K.; Griesbeck, M.; Hildebrandt, H.; Hagen, S.; Dittmer, U.;Altfeld, M. Human pDCs display sex-specific differences in type I interferon subtypes and interferon α/β receptor expression. *Eur J Immunol*. **2016**,*47*, 251-256.
10. Szondy, Z.; Pallai, A. Transmembrane TNF-alpha reverse signaling leading to TGF-beta production is selectively activated by TNF targeting molecules: therapeutic implications. *Pharmacol Res*. **2016**, *115*,31170-31177.
11. Higgins, V.; Piercy, J.; Roughley, A.; Milligan, G.; Leith, A.; Siddall, J.; Benford, M.Trends in medication use in patients with type 2 diabetes mellitus: a long-term view of real world treatment between 2000 and 2015. *Diabetes Metab. Syndr. Obes.***2016,***9*, 371-380.

12. Hemmingsen, B.; Sonne, D,P.; Metzendorf, M,I.; Richter, B. Insulin secretagogues forPrevention or delay of type 2 diabetes mellitus and its associated complications in persons at increased risk for the development of type 2 diabetes mellitus. *Cochrane Database Syst Rev*. **2016**, DOI: 10.1002/14651858.CD012151.
13. Hsia, D.S.; Grove, O.; Cefalu, W,T. An update on sodium-glucose co-transporter-2inhibitors for the treatment of diabetes mellitus. *CurrOpinEndocrinol Diabetes Obes.***2016**, *24*, 73-79.
14. Yılmaz, S.; Yumusak, E.; Burulday, V. Changes of normal appearing optic nerve head On diffusion-weighted imaging in patients with diabetic retinopathy. *Clin.Imaging*. **2016,** *4.* 60-63.
15. Caballero, A.E. Long-term studies of treatments for type 2 diabetes. *PostgradMed*. **2017,** *129*,352-365.
16. Ghosh, S.; Trivedi, S.; Sanyal, D.; Modi, K,D.; Kharb, S. Teneligliptin real-worldefficacy assessment of type 2 diabetes mellitus patients in India (TREAT-INDIAstudy). *Diabetes Metab Syndr Obes*. **2016.** *9*,347-353.
17. Bower, R,L.; Hay, D,L. Amylin structure-function relationships and receptorpharmacology: implications for amylin mimetic drug development. *Br J Pharmacol.***2016**. *173,* 1883-1898.
18. Nonogaki, K.; Kaji, T.; Yamazaki, T.; Murakami, M. Pharmacologic stimulation of central GLP-1 receptors has opposite effects on the alterations of plasma FGF21 levels induced by feeding and fasting. *Neurosci Lett.***2016**. *12*, 14-17.
19. Visentin, R.; Giegerich, C.; Jäger, R.; Dahmen, R.; Boss, A.; Grant, M.; Dalla.; Man, C.; Cobelli, C.; Klabunde, T. Improving Efficacy of Inhaled Technosphere Insulin (Afrezza) by Postmeal Dosing: In-silico Clinical Trial with the University of Virginia/Padova Type 1 Diabetes Simulator. *Diabetes Technol Ther*. **2016,** *8*, 574-585.
20. McGill, J,B.; Ahn, D.; Edelman, S,V.; Kilpatrick, C,R.; Santos.; Cavaiola, T. Making Insulin Accessible: Does Inhaled Insulin Fill an Unmet Need? *AdvTher*.**2016.** *33*,1267-1278.
21. Green, A,D.; Vasu, S.; McClenaghan, N,H.; Flatt, P,R. Implanting 1.1B4 human β-cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice. *World J Diabetes***. 2016**. *7,* 523-533.

22. Kudo-Fujimaki, K.; Hirose, T.; Yoshihara, T.; Sato, F.; Someya, Y.; Ohmura, C.; Kanazawa, A.; Fujitani, Y.; Watada, H. Efficacy and safety of nateglinide plus vildagliptin combination therapy compared with switching to vildagliptin in type 2 diabetes patients inadequately controlled with nateglinide. *J. Diabetes Investig*. **2014**, *4*, 400-409.
23. Vanhove, T.; Remijsen, Q.; Kuypers, D.; Gillard, P. Drug-drug interactions between immunosuppressants and antidiabetic drugs in the treatment of post-transplant diabetes mellitus. *Transplant Rev (Orlando)*. **2016**, .doi.org/10.1016/j.trre.2016.09.001.
24. Han X, Chiang C, Leonard CE, Bilker WB, Brensinger CM, Li L, Hennessy S. Biomedical Informatics Approaches to Identifying Drug-Drug Interactions:Application to Insulin Secretagogues. *Epidemiology***2017**, *28*, 459-468.
25. Wairkar, S.; Gaud, R. Co-Amorphous Combination of Nateglinide-Metformin Hydrochloride for Dissolution Enhancement. *AAPS PharmSciTech*. **2016,***17*, 673-681.
26. Scheen, A,J. Drug interactions of clinical importance with antihyperglycaemic agents: an update. *Drug Saf*. **2005**, *7*, 601-631.
27. Anderson, D,M.; Shelley, S.; Crick, N.; Buraglio, M. No effect of the novel antidiabetic agent nateglinide on the pharmacokinetics and anticoagulant properties of warfarin in healthy volunteers. *J ClinPharmacol*. **2002**, *42*, 1358-1365.
28. Anderson, D,M.; Shelley, S.; Crick, N.; Buraglio, M. A 3-way crossover study to evaluate the pharmacokinetic interaction between nateglinide and diclofenac in healthy volunteers. *Int J ClinPharmacolTher*. **2002,***40*, 457-464.
29. Shani M, Lustman A, Vinker S. Diabetes medication persistence, different medications have different persistence rates. *Prim Care Diabetes***2017,**doi: 10.1016/j.pcd.2017.03.006. [Epub ahead of print] PubMed PMID: 28420583.
30. Wickramaratne, M.N., Punchihewa, J.C., Wickramaratne, D.B. In-vitro alpha amylase inhibitory activity of the leaf extracts of Adenantherapavonina. *BMC Complement Altern Med*. **2016,** *16*, 466.
31. Zahra, S.; Elham, R.; Mohamad, T.G.; Mohamad, A.G. Effect of Metformin, Acarbose and Their Combination on the Serum Visfatin Level in Nicotinamide/Streptozocin-Induced Type 2 Diabetic Rats. *Iran Red Crescent Med J*.**2016**, *18*, e23814. doi: 10.5812/ircmj.23814.

32. Chiasson, J.L., Josse, R.G., Hunt, J.A., Palmason, C., Rodger, N.W., Ross, S.A., et al. The efficacy of acarbose in the treatment of patients with non-insulin-dependent diabetes mellitus. A multicenter controlled clinical trial. *Ann Intern Med* **1994**, *121,* 928-935.
33. Agarwal, P.; Gupta, R. Alpha-amylase inhibition can treat diabetes mellitus, *Research and Reviews Journal of Medical and health science*, **2016**, *5*, 1-8.
34. Semaan, D.G.;Igoli, J.O.; Young, L.; Marrero, E.; Gray, A.I. Rowan, E.G. In vitro anti-diabetic activity of flavonoids and pheophytins from AllophyluscominiaSw .on PTP1B, DPPIV, alpha-glucosidase and alpha-amylase enzymes. *J Ethnopharmacol.***2017,***203,* 39-46.
35. Martinez-Gonzalez, A.I.;Díaz-Sánchez, Á.G.; Rosa, L.A.; Vargas-Requena, C.L.;Bustos-Jaimes, I.; Alvarez-Parrilla, A.E. Polyphenolic Compounds and Digestive Enzymes: In Vitro Non-Covalent Interactions. *Molecules* **2017,** *22*,pii: E669. doi: 10.3390/molecules22040669.
36. Moriguchi-Mori K, Higashio H, Isobe K, Kumagai M, Sasaki K, Satoh Y, Kuji A, Saino T. P2Y purinoceptors mediate ATP-induced changes in intracellular calcium and amylase release in acinar cells of mouse parotid glands. *Biomed Res.***2016**, *37,* 37-49.
37. Kandandapani S, Tan CY, Shuib AS, Tayyab S. Influence of Buffer Composition and Calcium Chloride on GdnHCl Denaturation of Bacillus licheniformis α-Amylase. *Protein PeptLett.* **2016**; *23,* 537-543.
38. Green, A,D.; Vasu, S.; McClenaghan, N,H.; Flatt, P.R. Implanting 1.1B4 human β-cell pseudoislets improves glycaemic control in diabetic severe combined immune deficient mice. *World J Diabetes*. **2016,** *7*, 523-533.
39. Schive, S.W.; Foss, A.; Sahraoui, A.; Kloster-Jensen, K.; Hafsahl, G.; Kvalhei, G.;Lundgren, T.; von Zur-Mühlen, B.; Felldin, M.; Rafael, E.; Lempinen, M.; Korsgren, O.;

    Jenssen, T,G.; Mishra, V.; Scholz, H. Cost and clinical outcome of islettransplantation in Norway 2010-2015. *Clin Transplant*. **2017**, *3,* e12871.
40. Energy minimizations were performed MM2 Interface program on ChemBio3DUltra 12.0, and structures were drawn by ChemBioDraw Ultra 12.0 (CambridgeSoft).
41. Khatik, G. L. Diabetes mellitus: Recent advancement in ppar agonists as therapeutic agents. *Int. J. PharmaBioSci.* **2015**, 6, 46-69.

42. Trott, O.; Olson, A.J. AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *J. Compt. Chem.* **2010**, *31*, 455-461.
43. Kaur, P., Khatik, G.L. Identification of novel 5-styryl- 1,2,4-oxadiazole/Triazole derivatives as the potential antiandrogens. through molecular docking study. *Int. J. Pharm. Pharm. Sci.***2016**, *8*, 72-77.
44. Chaurasiya S, Kaur P, Nayak SK, Khatik GL. Virtual screening for identification of novel potent EGFR inhibitors through AutodockVina molecular modeling software. *J. Chem. Pharm. Res.* **2016**; 8:353-60.
45. Steven D. B; Stephen, G. D; Simon Jones and Hitesh J.S. Asymmetric alkylations using SuperQuat auxiliaries—an investigation into the synthesis and stability of enolates derived from 5,5-disubstituted oxazolidin-2-ones. *J. Chem. Soc., Perkin Trans.1*.**1999.** 387-398.
46. Catry MA, Madder A. Synthesis of functionalised nucleosides for incorporation In to nucleic acid-based serine protease mimics. *Molecules* **2007**, *12*, 114-129.
47. Dahiya R, Kumar A, Yadav R. Synthesis and biological activity of peptide derivatives of iodoquinazolinones/nitroimidazoles. *Molecules* **2008**,*13*, :958-976.
48. Sudha, P., Zinjarde, S. S., Bhargava, S. Y., & Kumar, A. R. Potent a-amylase inhibitory activity of Indian Ayurvedic medicinal plants, *BMC Complementary and Alternative Medicine* **2011,** *11*, 5