Design, synthesis and evaluation of chalcone derived alpha amylase inhibitors as potent antidiabetic agents

Dissertation II

Master of Science (Pharmaceutical Chemistry)

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December, 2017.

Statement by the candidate

This is to submit that this written submission in my project report entitled "Design, synthesis and evaluation of chalcone derived alpha amylase inhibitors as potent antidiabetic agents" represents original ideas in my own words and where others' 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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Roqia Bashary

Forwarded Through

Dr. Gopal Lal Khatik

Associate Professor

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The work described in this project report entitled "Design, synthesis and evaluation of chalcone derived alpha amylase inhibitors as potent antidiabetic agents" has been carried out by Roqia Bashary under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Research Supervisor:

Dr. Gopal Lal Khatik

Date: 12/2/2017

Place: Lovely professional university

Certificate by School

This is certified that the work described in this project report entitled "Design, synthesis and evaluation of chalcone derived alpha amylase inhibitors as potent antidiabetic agents" has been carried out by Roqia Bashary at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

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CHAPTER 1: INTRODUCTION

1. Introduction

The most common metabolic disorderliness among the millions of people around the world is diabetes mellitus. Studies have shown that the prevalence of the disease is growing, from a worldwide of 221 million persons in 2010 to a 300 million persons in 2030[1]. Two types of diabetes mellitus are there: type 1 and type 2, type 1 is due to immunological defects of pancreatic beta cells while type 2 kind of diabetes mellitus is due to a deficiency in insulin secretion or insulin resistance [2]. Majority of patients (~90%) are suffering from type 2 diabetes. Both genetic and environmental factors are involved in the emersion of diabetes mellitus.

Due to the multiplicity of factors which are involved in the appearance of diabetes mellitus its management is difficult. Much more efforts are needed to be considered in treatment of diabetes mellitus type 2 while insulin might be sufficient in contrast to type 1 diabetes mellitus [1]. Insulin and its newly preparations, sulphonylureas, biguanides, meglitinides, thiazolidinediones, alpha – glucosidase inhibitors, incretins and guar gum are the most commonly used antidiabetic agents [3]. Due to several side effects or drawbacks associated with the commercially available antidiabetic agents such as hypoglycemia or low blood sugar, upset stomach, nasopharyngitis, and etc [4], new therapeutic approaches are needed to be considered.

1.1 Treatment

1.1.1 Insulin

Insulin is one type of hormone which is produced by beta cells of pancreas which is responsible for regulation as well as metabolism of carbohydrates, fats, and proteins. It is also responsible for regulation of blood glucose level by inhibiting of glucose production and excretion into the blood by liver. Therefore, insulin is the most commonly used medication to treat high blood glucose level in diabetes patients.

Generally, insulin is getting degraded inside gastrointestinal tract that's why it is always available in the form of injectable for subcutaneous, intravenous and intramuscular administration [4].

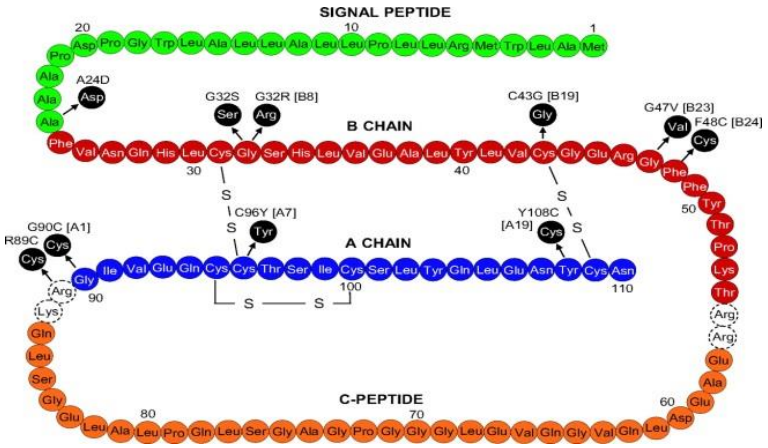


Figure 1: Insulin structure

http://www.pnas.org/content/104/38/15040/F2.expansion.html

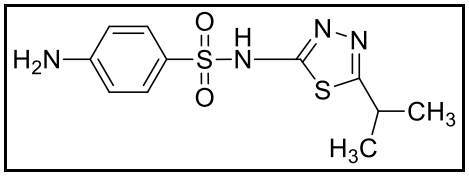
1.1.2 Oral antidiabetic agents

As we have already discussed that the main drawback with insulin and its preparations are their degradation inside gastrointestinal tract so we cannot formulate them as oral dosage forms. This has created incentives to design orally active antidiabetic agents.

A large number of compounds which are related to different chemical classes has already been designed and formulated as oral antidiabetic agents. They are as follow:

1.1.2.1 Sulphonylureas

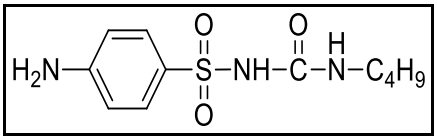
Sulphonylureas discovery was random; when in 1942, M. Janbon and his colleagues were observing for antimicrobial properties of some sulphonamides, they also observed that the compound 5-isopropyl-2-sulphonylamido-1,3,4-thiadiazole [figure 2(1)] caused hypoglycemia in experimental animals.



(1)

Figure 2: 5-isopropyl-2-sulphonylamido-1, 3, 4-thiadiazole

As we see this structure contains arylsulphonylthiourea moiety which led to the synthesis of a number of sulphonylureas. The very first clinically useful compound belongs to this group became 1-butyl-3-sulphonyl urea (carbutamide) [figure 2(2)].

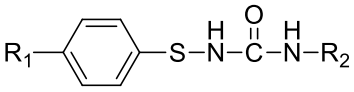


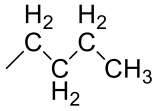
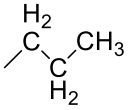
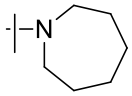
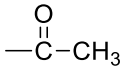
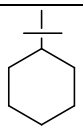
(2)

Figure 2: Carbutamide

Due to the adverse effects on bone marrow which produced by this medicine hence, it was withdrawn later, but it opened the way for researchers to design and synthesize its beneficial structural analogues as oral antidiabetics. As the amino group in fourth position is responsible for bacteriostatic activity of sulphonamides, so it was replaced by other moieties in the same position to prevent anti-bacterial activity but the other nitrogen of the urea group carried different types of substituents. Tolbutamide (3), Chlorpropamide(4), Tolazamide(5) and Acetohexamide(6) formed the first generation of orally active hypoglycemic agents (table1).

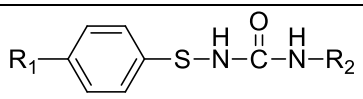
Table 1: First generation of sulphonylureas as active hypoglycemic agents

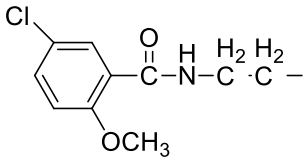
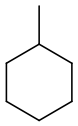
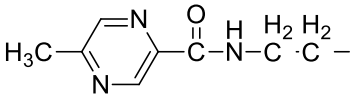
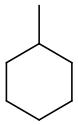
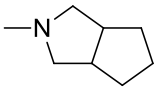
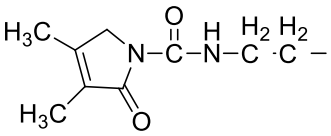
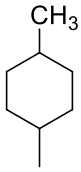
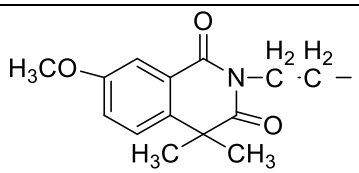
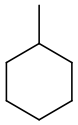


S No	Name	R^1	R^2
1	Tolbutamide(3)	$-CH_3$	
2	Chlorpropamide(4)	$-Cl$	
3	Tolazamide(5)	$-CH_3$	
4	Acetohexamide(6)		

The second generation of sulphonylureas Glibenclamide, Glipizide, Gliclazide, Glimipiride and Gliquidone are more potent as compare to the first generation (table2).

Table 2: Second generation of sulphonylureas as hypoglycemic agents

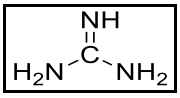


S No	Name	R^1	R^2
1	Glibenclamide(7)		
2	Glipizide(8)		
3	Gliclazide(9)	—CH_3	
4	Glimepiride(10)		
5	Gliquidone(11)		

Sulphonylureas can only be used if patient has somewhat functional pancreas because the mechanism of action for both generation of sulphonylureas is stimulation of insulin secretion from βcells of pancreases, and also it can reduce hepatic clearance of insulin and further increasing in level of insulin[5].

1.1.2.2 Biguanides

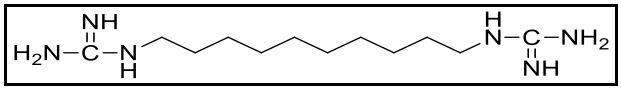
Guanidine (figure3a) is a compound which can be found in plant sources and has been identified that it possesses the property to reduce the blood sugar level, but along with toxicity for the clinical use.



(12)

Figure 3(a): Guanidine

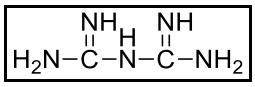
In order to overcome this problem polymethylenediguanides have been synthesized, wherein two guanidine residues are linked together by a polymethylene chain of 10-12 carbons which are called synthalins. The decamethylene derivative whose structure has shown below was 150 times more potent than that of guanidine.



(13)

Figure 3(b): Decamethylene derivative

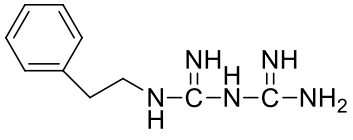
Kidney and liver damage were the main drawbacks for this compound and its structural analogues. Later, in 1957 biguanides (a structure where two amidino compound are linked through a common nitrogen [figure3-(14)]were prepared and taested[6].

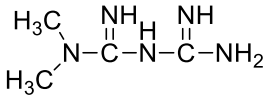
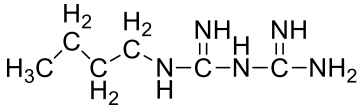
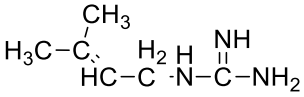


(14)

Figure 3(c): Biguanide

Table3: Biguanide derivatives

S No	Name	Chemical structure
1	Phenformin(15)	

2	Metformin(16)	
3	Butformin(17)	
4	Isoamylene guanidine(18)	

1.1.2.3 Non Sulphonylureas (Meglitinides)

These are not derivatives of sulphonylureas but they are acting via same way as sulphonylureas derivatives, means these are short-acting insulin secretagogue and acting on the same β-cell receptors.

Their main differences along with sulphonylureas are: Their short acting and lacking sulphuric acid moiety so can be used in allergic patients to sulfa drugs.

Glinide is the synonymous word for this group, two most useful drugs which are falling in this category are: Repaglinide is a derivative of benzoic acid and Nateglinide is a derivative of D-phenylalanine [6](figure 4).

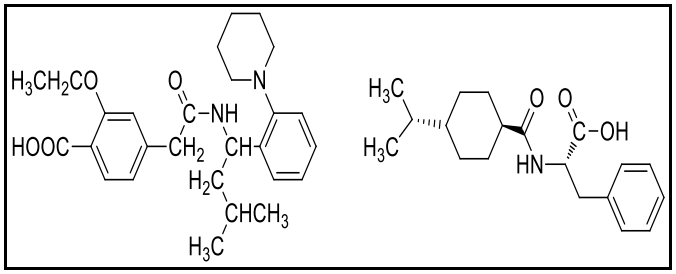


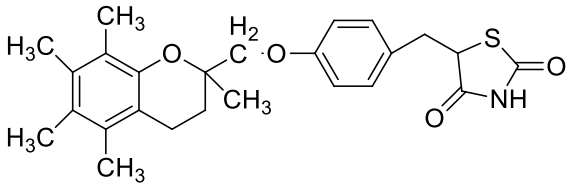
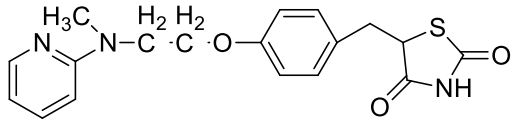
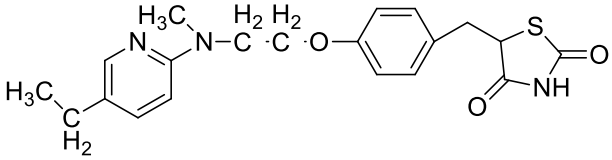
Figure 4: Repaglinide (19), and Nateglinide (20)

1.1.2.4 Thiazolidinediones

As the name suggests these are the derivatives of hydrogenated heterocyclic ring thiazolidinedione. The very first drug for this class was Troglitazone which was introduced in 1997 as "insulin sensitizer". These are the PPARs peroxisome proliferator-activated receptorγ agonists lead in glucose uptake in muscle and reduced endogenous glucose production [4,6].

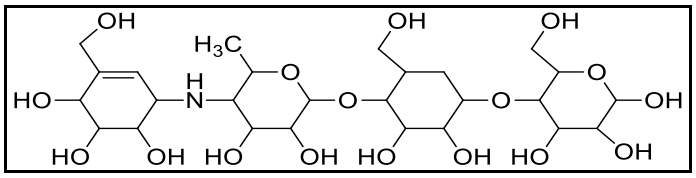
Table 4: Thiazolidinedione derivatives

S NO	Name	structure

1	Troglitazone(21)	
2	Rosiglatazone(22)	
3	Pioglitazone(23)	

1.1.2.5 Alpha-Glucosidase inhibitors

Alpha-glucosidase is one type of enzyme which is located in the small intestine and plays a role in the breaking down of complex carbohydrates like starch from diet to small or simple monosaccharides like glucose. Alpha-glucosidase inhibitors target this enzyme and prevent the catabolism of starch, lead to slow carbohydrate absorption into blood circulation so they can cause the reduction of the postprandial glucose peak, so the drug should be administrated at the starting of the meal. Since they do not have any effect on glucose secretion, therefore, they do not have any hypoglycemic activity. The oligosaccharide of a microbial source which is known as acarbose is a useful alpha-Glucosidase inhibitor(figure5) [3,7].



(24)

Figure 5: Acarbose

1.1.3 Newer anti-diabetic agents

1.1.3.1 Salsalate:

It is a salicylic acid (salicylate) derivative which belongs to Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). It is effective in both type 2 diabetic patients and obese adults without any diabetes as well. Its mechanism of action is the inhibition and release of prostaglandins and making the insulin work better. Many side effects associated with it which are the loss of hearing, difficulty in breathing and swallowing, shortness of breath and horseness.

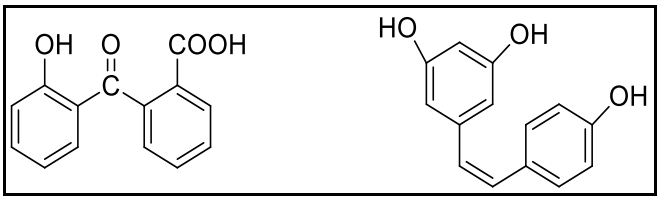


Figure 6: Salsalate (25), and Cis-resveratrol (26)

1.1.3.2 Resveratrol:

Resveratrol is a newer anti diabetic agent which is also effective in acting against cancer, inflammation, and cardiovascular disease. Studies have shown that resveratrol can act as good anti-diabetic agent in reducing blood sugar level and also beneficial in controlling hyperlipidemia [8].

1.1.4 Molecular targets for antidiabetic drugs

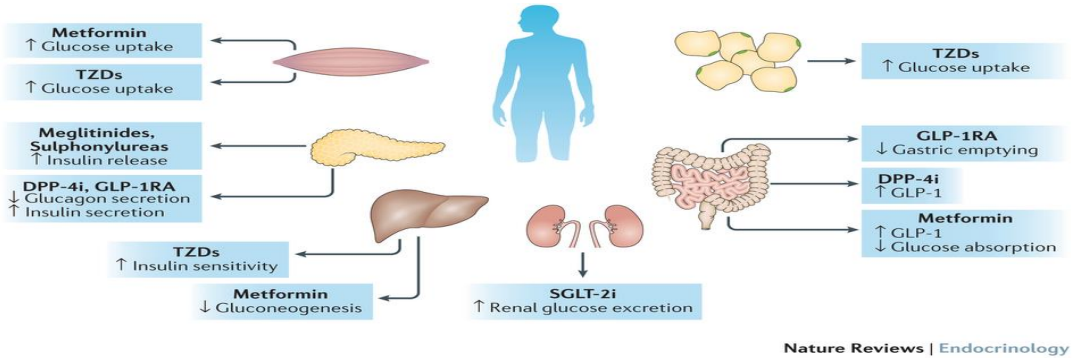


Figure7: Molecular targets for anti-diabetic drugs

http://www.nature.com/nrendo/journal/v12/n6/abs/nrendo.2016.51.html

1.1.4.1 Peroxisome Proliferator Activated Receptors (PPARs):-

Peroxisome Proliferator-Activated Receptors are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. Peroxisome Proliferator-Activated Receptors are three types which encoded by separate genes.

They are PPARα, PPARβ/δ and PPARγ. Peroxisome – proliferator-activated receptor gamma (PPARγ) is a transcription factor activated by thiazolidinediones (table 4).

1.1.4.2 PPARα/γ:

They can cause hypoglycemia and hypolipidemia in the type 2 diabetic patients. PPARα/γ dual agonist is supposed to provide additive and possibly synergistic effects. The very first PPARα/γ dual agonist was of KRP-297 (MK-767), a TZD derivative that was reported to bind PPARα and PPARγ. Others are tesaglitazar (AZ-242), ragaglitazar and muraglitazar [8].

1.1.4.3 Glucagon like peptide –1(GLP-1) receptor:

Glucagon like peptide –1(GLP-1) hormone is a neuropeptide and an incretin derived from the transcription product of the proglucagon gene.

It is a very potent hypoglycemic hormone that stimulates the beta cells of pancreas in order to release insulin in response to rising glucose level and also inhibits glucagon release. This leads to lowering blood glucose level and hypoglycemia. It can also enhance the insulin sensitivity in both alpha and beta cells and increase beta cells mass and insulin expression. GLP-1 agonists which are also known as incretin mimetics and insulin secretagogues are such as Sulphonylureas and Meglitinides and newer insulin secretagogues such as Exenatide, Liraglutide, and Lixisenatide. The later ones have lower risk of hypoglycemia as compared to older ones [9].

1.1.4.4. β3- Adrenoreceptor:

One of the subtypes of the superfamily of G- protein coupled receptors (GPCRs) is β3-Adrenoreceptor, activation of these receptors lead to lipolysis stimulation and energy along with oxygen consumption in adipose tissues and skeletal muscle. Two compounds; SR – 58611(sanofi – synthelabo) and TAK – 677 (Takeda) which are under phase 2 clinical trials are available as selective β3- Adrenoreceptor agonists. In some certain types of fat cells βadrenergic receptor (β3AR), function in a manner contrary to that of adrenergic system means their activation leads to the losses of metabolic energy as the form of heat of or energy [8].

1.1.4.5. α- Lipoic Acid

One of the most important co-factor in several mitochondrial enzyme complexes during oxidative metabolism of glucose and cellular energy production is α- Lipoic Acid (LA).

Structurally it is a fatty acid of eight carbons which is synthesized in trace amount in microorganism like bacteria and human [8].

1.1.4.6. Liver selective glucocorticoid

Liver selective glucocorticoid inhibitors can be used as blood sugar lowering agent, because glucocorticoids are antagonists of insulin so they increase the blood sugar level by inhibition of glucose wastage from the body and also increasing of the glucose production by liver cells and its output. So in order to cure the diabetes mellitus type 2, design and synthesis of liver selective glucocorticoid antagonists will be a powerful and useful approach. According to reports Mifepristone is the compound which inhibits liver glucocorticoids thus, insulin will not be antagonized by glucocorticoids[8].

1.1.4.7. Dipeptidy1 Peptidase IV:

Dipeptidy1 Peptidase IV enzyme plays a major role in the metabolism of glucose. It has a no of functions such as:

a. Inactivation of incretin release.

b. Stimulation glucagon release

Dipeptidy1 Peptidase IV inhibitors such as Sitagliptin lower the blood glucose level by inhibition of it[10].

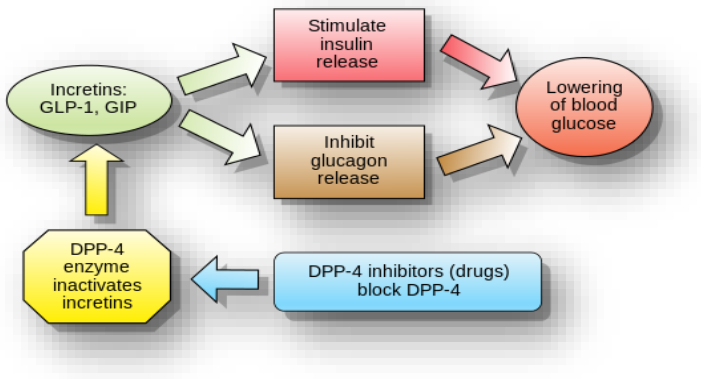


Figure8: mechanism of action of Dipeptidy1 Peptidase IV inhibitors

https://upload.wikimedia.org/wikipedia/commons/6/64/Incretins_and_DPP_4_inhibitors.svg

1.1.4.8 GPR-19 (G-protein coupled receptor 119):

In human, this receptor is encoded by GPR119 gene which is predominantly expressed in human and rodent pancreas and GIT system. According to reports which are available the activation of this receptor may cause a reduction in food intake and body weight gain in rats.

It also has shown the regulatory properties of insulin and incretin secretion. GPR-19 agonists have been suggested as a novel treatment for the obesity and diabetes [11].

1.1.4.9. GPR-40(G-protein coupled receptor 119):

It is also known as free fatty acid receptor-1 which belongs to a class of G-protein coupled receptors. It is expressed in pancreas and to a less extent in brain. This receptor is activated by medium to long chain fatty acids. It also stimulates the insulin secretion, for this reason, it is a drug target design for the synthesis of antidiabetic agents [12].

1.1.4.10. S-GLT2

This protein is a member of the Sodium glucose co-transporter family. This transporter is available in kidneys and its function is the reabsorption of glucose from urine to blood so by inhibition of it, we inhibit the glucose reabsorption and further blood glucose level rising [13].

1.1.4.11. Alpha amylase:

One type of enzyme which is available in the brush border of the small intestine is alpha-amylase (figure9); means it is a membrane-bound enzyme. Its function is the breakdown of polysaccharides from dietary complex to monosaccharides which can be absorbed. As we know most of the polysaccharides such as starch in their straight chain have the α(1-4) glycosidic linkage; alpha-amylase enzyme mainly targets this linkage and lead to the breakdown of carbohydrates.

So by inhibition of alpha-amylase enzyme, the polysaccharides cannot break and there will not be any further release of monosaccharides such as glucose. Its inhibitors reduce the glucose levels that can occur after a meal, showing the speed of conversion of starch to monosaccharides [14]. Inhibition of the enzyme activity will be lowering glucose absorption by the small intestine and also will be controlling the elevation of glucose levels. This would then allow more undigested starch to make it to the colon.

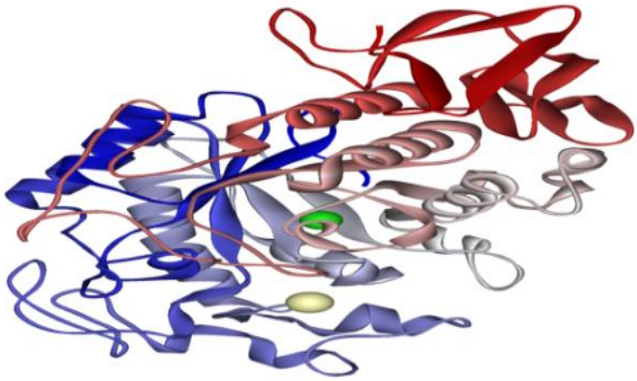


Figure 9: Human salivary alpha-amylase structure

https://en.wikipedia.org/wiki/Alpha-amylase#/media/File:Salivary_alpha-amylase_1SMD.png

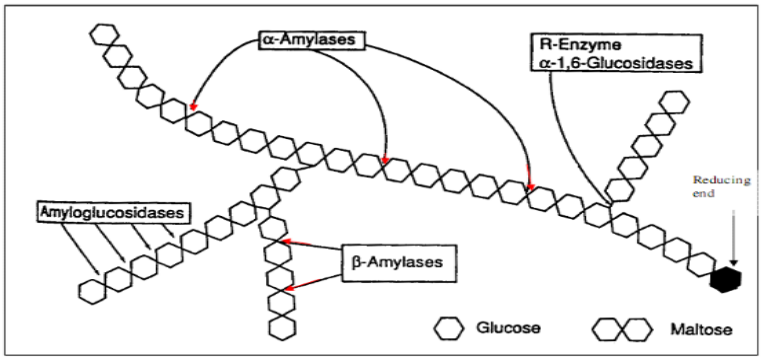


Figure10: mechanism of action of alpha-amylase

http://www.memoireonline.com/08/13/7254/m_Evaluation-of-the-hypoglycemichypolipidemic-and-anti-alpha-amylase-effects-of-extracts-of-the-twig19.html

CHAPTER 2: REVIEW OF LITERATURE

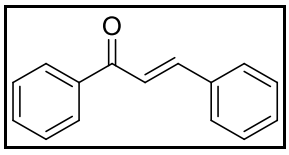
2.1. Alpha amylase inhibitors

A new therapeutic approach or drug design target in the management 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 [1415]. Alpha-amylase inhibitors are generally divided into two classes: 1. proteinaceous inhibitors, 2. Non-proteinaceous inhibitors.

2.1.1. Non proteinaceous inhibitors

2.1.1.1. Chalcone

Chalcone(benzylidine acetophenone or phenyl styryl ketone) is an α, β- unsaturated carbonyl compound with two phenyl rings, due to which it is also known as 1,3-diphenyl-prop-2-en -1-one. It is a part of the very important class of natural products, flavonoid family which possesses a wide range of biological activities. Studies have shown that by modifications in the structure of chalcones and introduction of other moieties a number of useful and effective derivatives with pharmacological activities which can be used for treatment of cancer, inflammation, microbial infections, diabetes, and etc., with improved potency and lesser toxicity can be achieved. In the auwer's synthesis of flavonoids and in the biosynthesis of flavonoids chalcones are as intermediates. There are different methods in order to synthesize chalcones in the laboratories but two important methods are Aldol condensation method and Claisen Schmidt method. According to reports trans_Chalcone, has the mammalian alpha-amylase inhibitory activity [16,17].



(27)

Figure 11: Trans chalcone

2.1.1.2. N-substituted phthalamide derivatives of coumarines

A number of N-substituted phthalamide derivatives of coumarines have been synthesized and evaluated for their alpha-amylase enzyme inactivation. Results indicated that one molecule (31d) was inhibited the alpha-amylase enzyme very much potently as compared to rest seventeen, so this would be an important and useful molecule for the inactivation of alpha-amylase enzyme and diabetes management. While the next molecule (31e), has not shown any alpha amylase enzyme inhibitory activity and two other of them, (31g) and (31i) exhibited supporting activity to alpha-amylase enzyme and potentiated its ability to hydrolyze the polysaccharides [18].

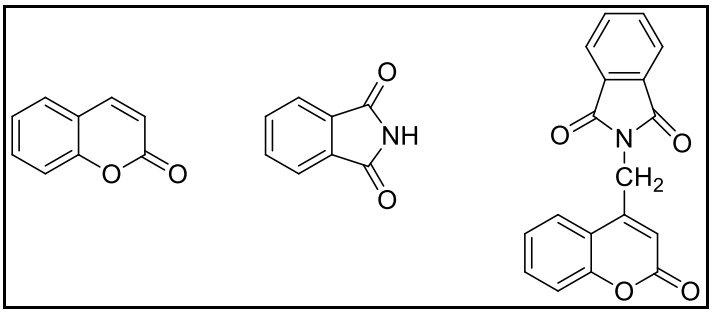
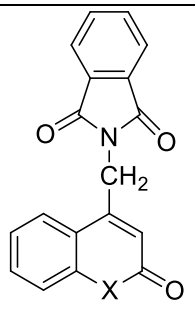


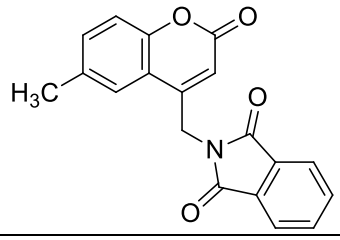
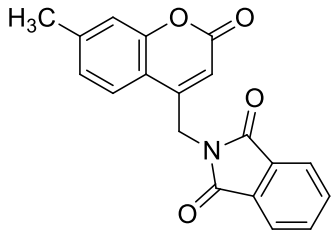
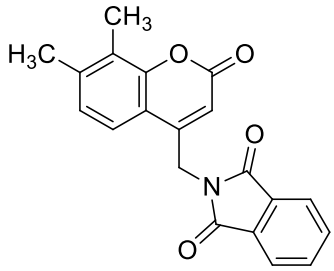
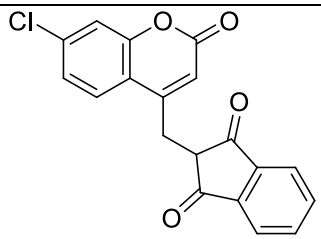
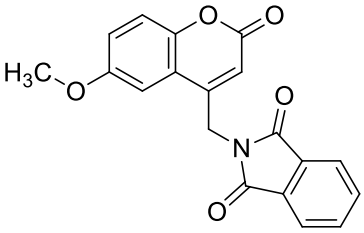
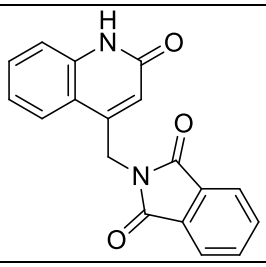
Figure 12: Coumarin(28), Phthalamide(29), and Phthalamidecoumarine(30)

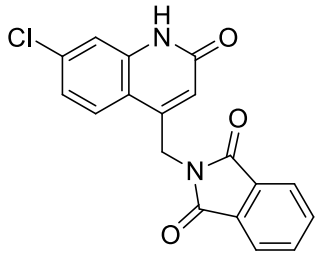
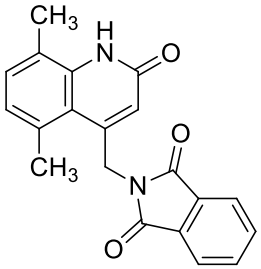
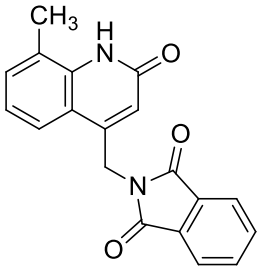
Table5: N-substituted cyclic imide derivatives



(31)

code	structure

31a	
31b	
31c	
31d	
31e	
31f	

31g	
31h	
31i	

2.1.1.3. Zinc oxide nanoparticles

New researches and studies have shown that Zinc oxide nanoparticles at 20 µg/ml concentration, pH=7, and thirty five°C temperature exhibit 49% glucose, which supposed to have same inhibitory activity as acarbose. It is also reported that zinc oxide nanoparticles are not harmful and poisonous to cells up to this particular dose [19].

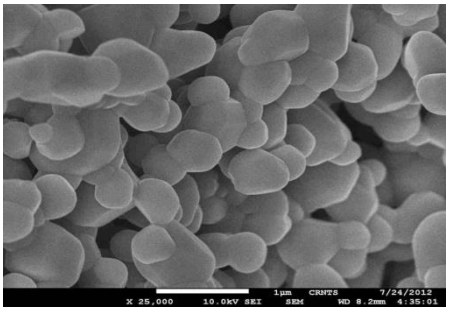
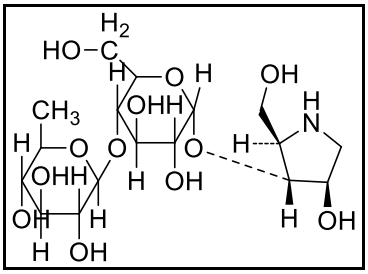


Figure13: zinc oxide nanoparticles

http://www.nanolabs.co.in/zinc-oxide-nanoparticles.html

2.1.1.4. CS-1036

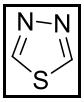
(2R,3R,4R)-4-hydroxy-2-(hydroxymethyl)pyrolidine-3-yl 4-O-(6-deoxy-β-D-Glucopyranosyl)-α-D-glucopyranoside(CS-1036) inhibits both salivary and pancreatic alpha-amylase in the gastrointestinal system and prevents starch digestion and further glucose absorption. The docking studies have shown that the pyrrolidine ring of CS-1036 interacted with active centre of the enzyme and its disaccharide part bounded to the starch binding site of pancreatic amylase [20].



(32)

Figure 14: CS-1036

2.1.1.5. Thiadiazole derivatives

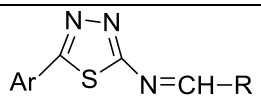


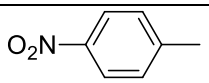
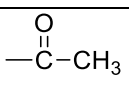
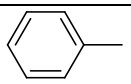
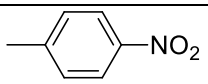
(33)

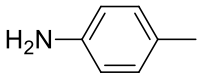
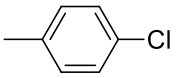
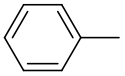
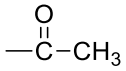
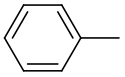
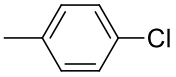
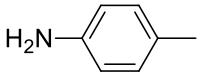
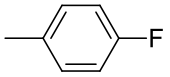
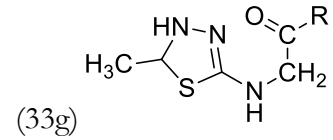
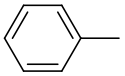
Figure 15: 1, 3, 4 Thaidiazole ring

Studies have shown that out of seven molecules of 1,3,4 thiadiazole derivatives which have been synthesized three were found to have potent anti-diabetic activity and two were found with moderate anti-diabetic activity. TD7 molecule showed significant in-vivo and in-vitro alpha-amylase inhibitory activity. [21].

Table 6: Thaidiazole derivatives



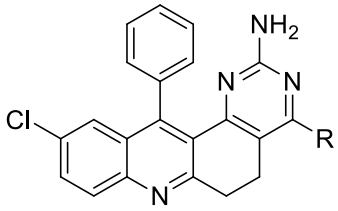
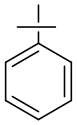
compound	Ar	R
TD1 (33a)		
TD2 (33b)		

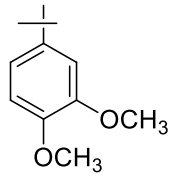
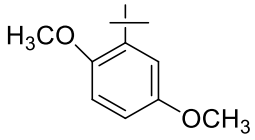
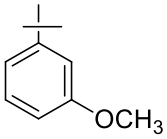
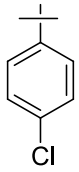
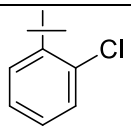
TD3 (33c)		
TD4 (33d)		
TD5 (33e)		
TD6 (33f)		
 (33g)		
TD7 (33h)		

2.1.1.6. Pyrimidine derivatives (Dihydropyrimido[4,5-a]acridin-2-amine analogues)

Among the numerous biological activities of pyrimidine ring, its ability to inactivate alpha-amylase and alpha-Glucosidase enzymes is of interest. 2-amino pyrimidine and acridin amine and its analogues are the target molecules for the synthesis of alpha-amylase inhibitors. In a research, the 10-chloro-4,12-diphenyl-5,6-dihydropyrimido[4,5-a]acridin-2-amine derivatives have been evaluated for their activity against alpha amylase and alpha-glucosidase enzymes. Out of six molecules, compounds (34e) and (34d) have shown good activity against alpha amylase and alpha-glucosidase enzymes as compared to the other analogues [22].

Table 7: 10-chloro-4,12-diphenyl-5,6-dihydropyrimido[4,5-a]acridin-2-amine derivatives

 (34)	
No	**R**
34a	

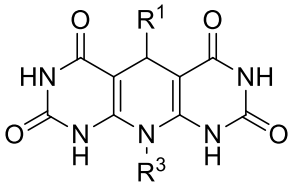
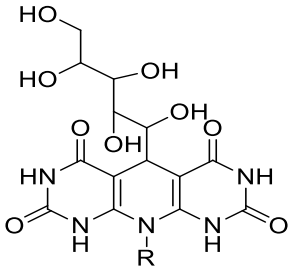
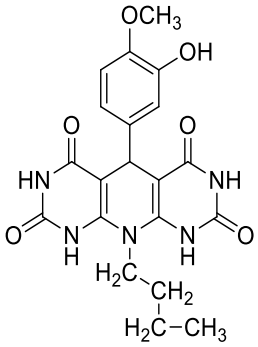
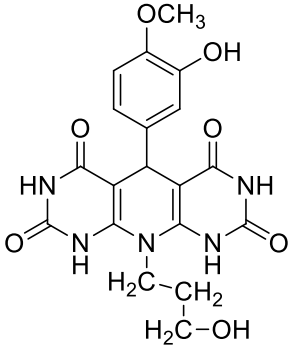
34b	
34c	
34d	
34e	
34f	

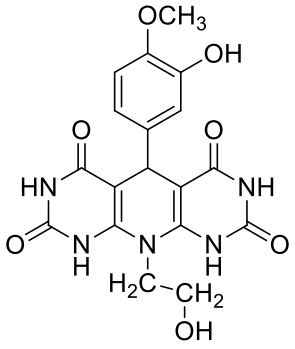
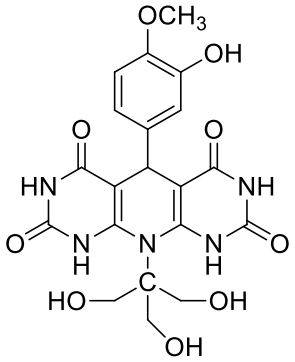
2.1.1.7. Alpha amylase inhibitors based in pyrimidine fused heterocycles

A series of pyrimidine fused heterocyclic; derivatives synthesized and have been examined against mouse/yeast alpha-glucosidase and pancreatic alpha-amylase enzymes. It was observed that the pyrimidine fused heterocycle and substituents at substructure 27 (containing a polyhydroxy chain) and R of the same structure (depends up on selected amine compound) are having a significant effect on enzyme inhibitory properties of developed molecules. Out of eighteen molecules, C14 - C17 evaluated against alpha-amylase enzyme in which C14-C16 exhibited moderate inhibitory activity and C17, revealed weak activity in comparison to acarbose. Table 8 shows the mentioned compounds structures and activity. Overall we can say that the significant properties of pyrimidine fused heterocycle containing compounds are a key in order to synthesize more secured drugs with lower side effects as compare to commercially available antidiabetic agents [23].

Table 8: Pyrimidine fused heterocyles derivatives

S No & code	structure

1	 (35)
2	 (36)
C14	 (37)
C15	 (38)

C16	 (39)
C17	 (40)

2.1.1.8. Gedunin and Azadiradione: Human Alpha-Amylase inhibiting Limonoids from Neem(Azadirachta indica)

Gedunin and Azadiradione were screened for their alpha-amylase inhibitory activity. As per reports, the both molecules possess strong human pancreatic alpha-amylase inhibitory activity which can be lead drug candidates to manage post-prandial hyperglycemia [24].

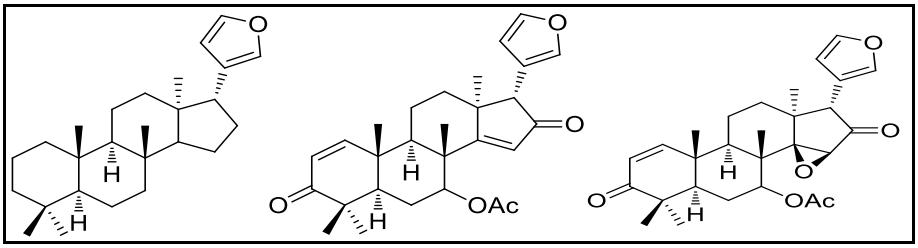


Figure 16: Basic Limonoid skeleton (41), Azadiradione (42), and Gedunin (43)

Note: A number of other compounds such as Trestatins from streptomycis galbus (inhibited mammalian alpha-amylases), ascorbic acid and its derivatives (inhibited malt, bacterial pancreatic and salivary alpha-amylases), pseudo-oligosaccharides such as acarbose, isoacarbose, acarviosine-glucose, hibiscus acid from roselle tea and cyclodextrins(inhibited procine and human pancreatic alph-amylase.) are also under category of non proteinaceous inhibitors [25].

2.1.2. Peptides or proteinaceous inhibitors

Some plants families such as Cereals and Leguminosae generate alpha amylase inhibitors for the purpose of defense against pests and pathogens. The alpha amylase inhibitors which are producing by plants can inhibit engogenous alph-amylases, insect alph-amylases and mammalian alpha-amylases. Bothe peptide based and non-peptide based alpha amylase inhibitors are available in plants. Proteinaceous alpha-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 field the inhibitors are useful to treat diabetes mellitus [25].

Table 9: examples of proteinaceous alpha-amylase inhibitors.

Proteinaceous alpha-amylase inhibitors		
No	**Name**	**example**
1	Knottin type	Amaranth seed
2	Kunitz-like	Cereals such as barley, wheat and rice
3	cereal type	Wheat, barley and ragi
4	γthionin-like	Cowpea and soybean
5	thaumatin-like	Maize and barley
6	lectin-like	Common bean and cowpea

CHAPTER 3: RATIONALE

According to review of literature, chalcone is the scaffold which can inhibit the alpha-amylase enzyme thus, carbohydrates cannot get metabolized and smaller absorbable carbohydrates such as glucose will not be released. On the other hand, docking studies also have shown an affinity of -7.5 of chalcone with alpha-amylase enzyme(figure 17), which is higher than affinity of acarbose(-6.9) an standard alpha-amylase inhibitor.

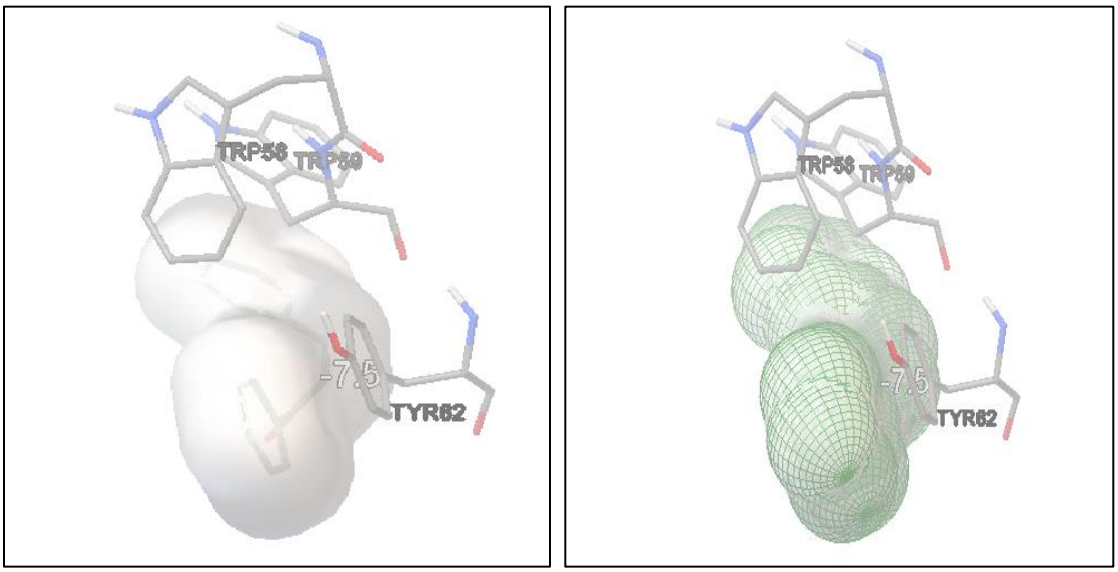


Figure 17: The interaction of chalcone with alpha-amylase

As we see in figure 17 it seems that aromatic ring at β carbon to carbonyl group is having stronger interaction with active site especially with tryptophan 58 and tryptophan 59 residues. This means that with some modifications and introduction of an extra aromatic ring such as aniline at β position of carbonyl group we can achieve much better affinity score (figure 18). As in case of compound CHA1 which is having aniline substituent at β carbon of carbonyl group the affinity has increased to -8.0 (figure 19).

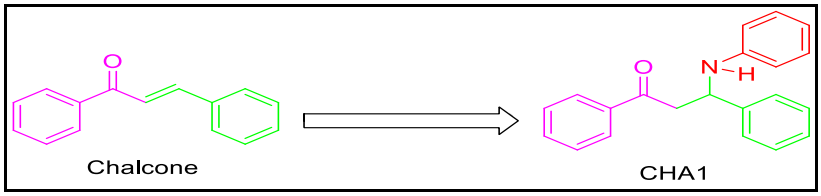


Figure 18: Comparative structure of chalcone and its modified compound CHA1

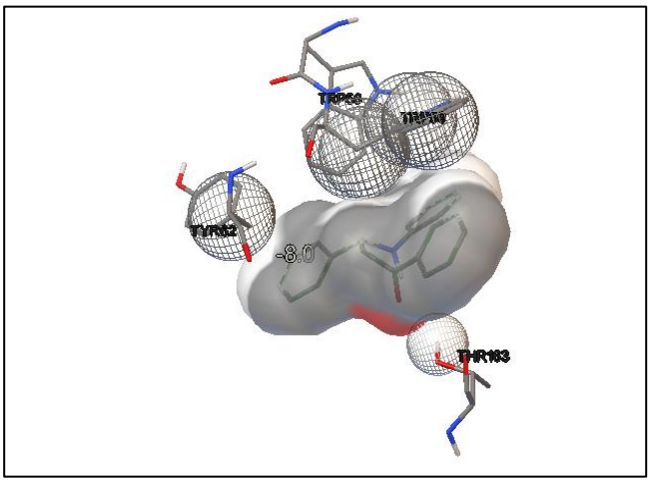


Figure 19: The interaction of CHA1 with alpha-amylase

Inroduction of other substituens such as CH_3, OH, Cl, F, and OCH_3 at different meta and para positions of three aromatic rings will be leading to formation of a number of derivatives with higher affinities (discussed in detail at sixth chapter).According to above assumptions we can conclude that aniline and the other aromatic rings at β carbon are the lipophilic one which can make stronger interactions with active site, α carbon is the linker and aromatic keton is the acidic part (figure 20).

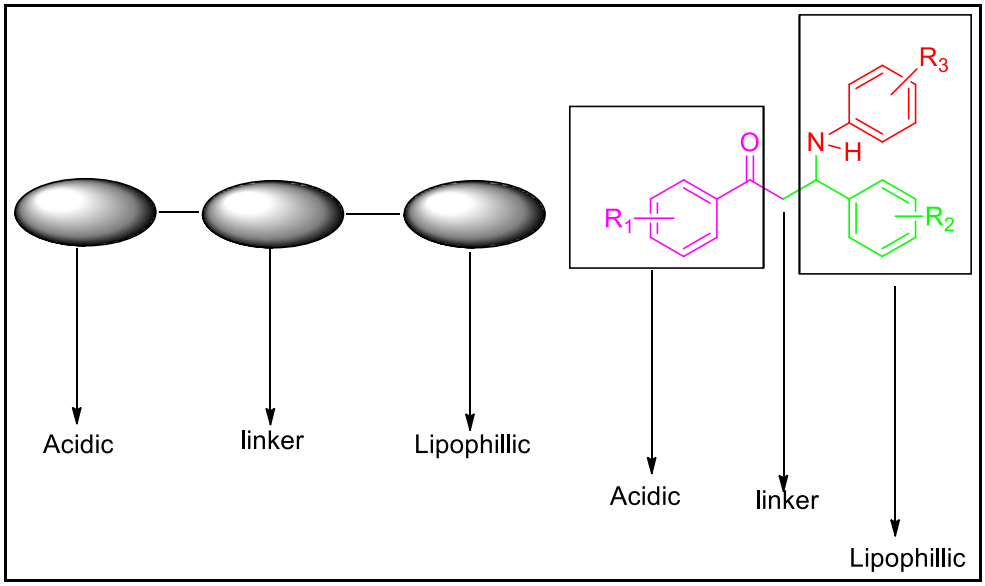


Figure 20: Schematic diagram for designed molecule

CHAPTER 4: OBJECTIVES

This research project aims in identifying a potential antidiabetic agent that inhibits the alpha-amyalse enzyme and could emerge as a successful clinical candidate for the treatment of T2DM.

The specific objectives of the project are:

- Design of chalcone derivatives.
- Study the binding interactions of the cholcone derivative by molecular docking and identify the most potent compounds.
- Synthesis of most potent compound as identified through molecular docking.
- Characterization of synthesized compound through spectroscopy
- *In vitro* alpha amylase inhibition studies.

CHAPTER 5: WORK PLAN

5.1. Docking studies:

To develop new molecules it is necessary to know affinity of the proposed molecules towards our target molecule and from the affinities which we will be obtaining by docking studies we can choose the best molecules and synthesize them.

5.1.1. Ligand preparation:

Database of various ligands will be prepared and their geometry will be optimized through ChemDraw program. All the optimized ligands will be saved in pdb format.

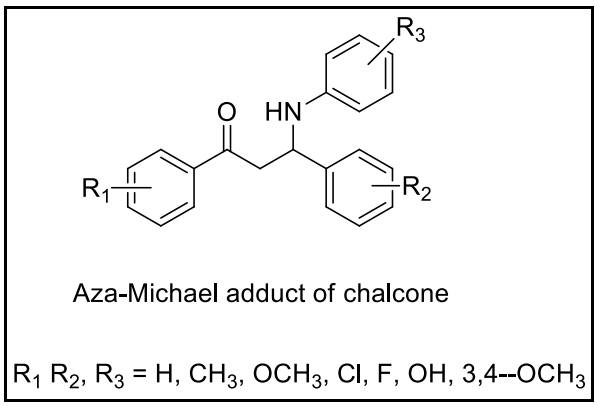


Figure 21: proposed chalcone ligands

5.1.2. Protein preparation:

Protein structures will be downloaded from protein data bank and prepared prior to docking in order to add polar hydrogen atoms, optimize hydrogen bonds, remove atomic clashes, and performing other operations by selecting the protein chain, heteroatoms, ligands and waters present in pdb files.

5.1.3. Docking study:

Setup the docking parameters and start docking calculations by selecting the protein and ligand from the library and by analyzing the interactions between protein and ligand.

5.1.4. Analysis of docking results:

Analysis of results will be carried out by comparison of binding affinity of docked molecules and ligand which we already extracted from protein and standard (acarbose) as well.

5.2. Synthesis of synthetically feasible most potent compounds:

Synthesis of synthetically feasible most potent compounds will be carried out using the scheme depicted below:

Route 1

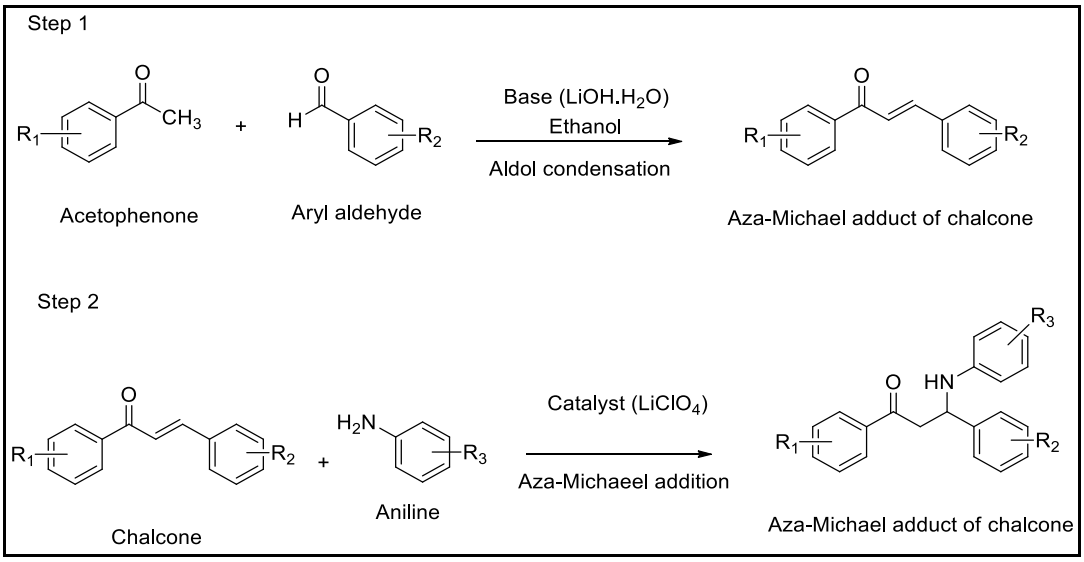


figure 22: Route1 , includes synthesis of chalcone derivatives

Route 1 includes synthesis of chalcone derivatives. It includes two steps, first step is an aldol condensation for synthesis of Trans chalcone (or substituted trans chalcone) by treating acetophenone (or substituted acetophenone) and aryl aldehyde (or substituted aryl aldehyde) using lithium hydroxide as a catalytic base and ethanol as a solvent. Second step is Aza-Michael addition of aniline over synthesized trans chalcone using lithium per chlorate as a catalyst to obtain Aza-Michael adducts of chalcone and substituted derivatives.

Route 2

Multi-component reaction: Mannich reaction

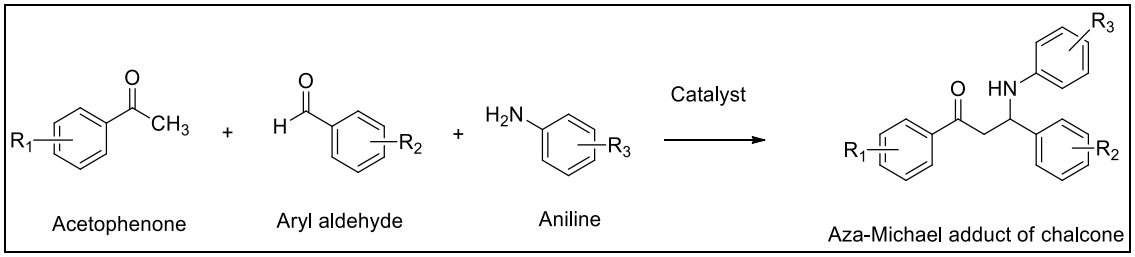


Figure 23: Route 2 of Chalcone derivatives synthesis

Route 2 includes a multi component reaction (Mannich reaction) of acetophenone, aryl aldehyde and aniline using lithium per chlorate as a catalyst to synthesize Aza-Michael adduct of chalcone.

5.3. *In-vitro* alpha amylase inhibition:

After synthesis and characterization of the compounds, we will be evaluating the synthesized compounds activity against alpha-amylase enzyme. As a matter of fact, alpha amylase is known to be the main cause of the breakdown of large molecule of carbohydrate to smaller ones, as such inhibiting this enzymes will serve as a basis for the treatment of type 2 diabetes. Alpha-amylase activity was performed using the starch-iodine method. Procedure is noted below:

- A 0.025 mg/ml solution of alpha-amylase enzyme was prepared.
- A phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) has been also prepared.
- 10 μL of the prepared alpha-amylase solution is mixed with 390 μL phosphate buffer which was already mixed with different synthesized ligands concentration.
- The mixture was incubated at 37 ℃ for 10 minutes.
- From a solution of 1% of starch, 100 μL is added to mixture and again re-incubated for 1 hour.
- After one hour, 0.1 mL of 1% iodine solution was added and further diluted with 5 mL distilled water.
- At 565 nm the absorbance of the mixture has been taken [26].

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

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 6: RESULTS AND DISCUSSION

6.1. Molecular Docking Study:

- Proposed molecules were designed in ChemDraw software.
- Designed molecules have been converted to 3D structures and their geometry was optimized by semiemperical MM2 method.
- All designed 3D structures have been saved as pdb (protein data bank) format which can be further read by autodock software.

Figure 24: Visualization of 3D structure of CHA1

6.1.1. Procedure of conversion of ligand.pdb to ligand.pdbqt

- All 3D structures further hsve been converted to pdbqt format which is favorable for docking with our target (alph-amylase enzyme or protein). In order to achieve this goal the procedure which discussed below has been followed:

➢ Open autodock software.

➢ From the ligand menu, click input, open, select or insert the pdb molecule from relevant folder.

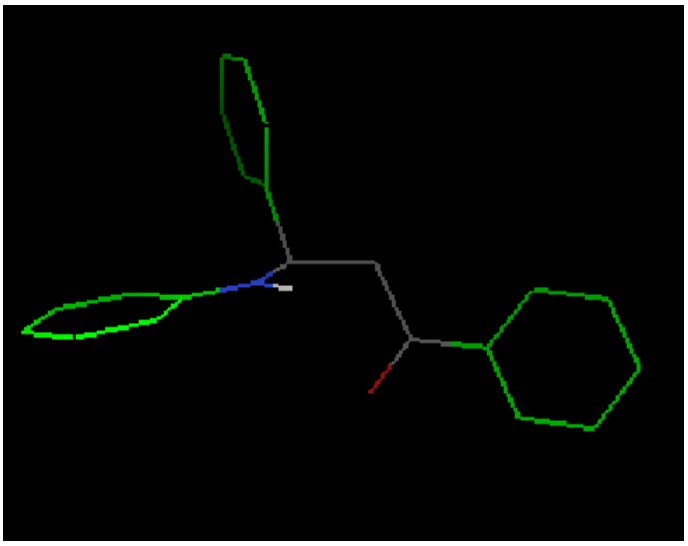


Figure 25: Visualization of CHA1 in autodock software

- Go to ligand menu again, input, choose, click on the name of molecule and then click on "select molecule for autoDock.
- Go to ligand menu, Torsion tree, choose torsion, and done.

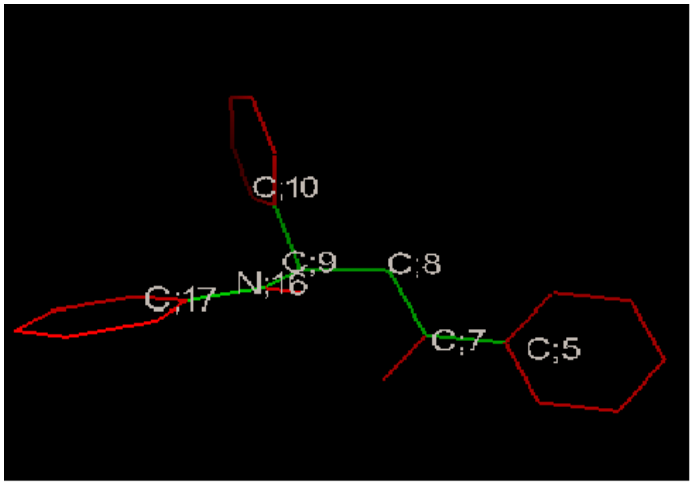


Figure 26: Visualisation of torsions

- Go to ligand menu, torsion tree, detect root.

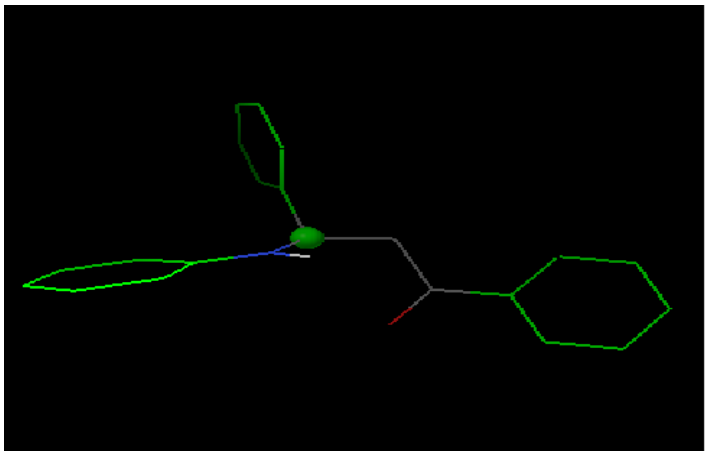


Figure 27: Visualization of root

- Go to ligand menu, output, save as PDBQT, and give the name ligand.pdbqt.

All proposed ligands, ligand from protein and standard molecule should be converted to pdbqt by following the above procedure.

6.1.2. Preparation of protein (4gqr):

- Download the protein 4gqr from the protein data bank site.
- Open AutoDock software.
- Click on file menu, read molecule, select the downloaded protein (4gqr) from download file.

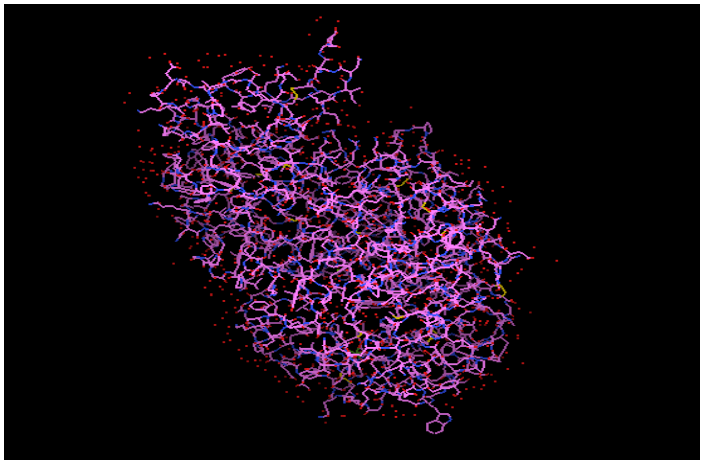


Figure 28: Visualization of the protein (4gqr) structure

- 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.

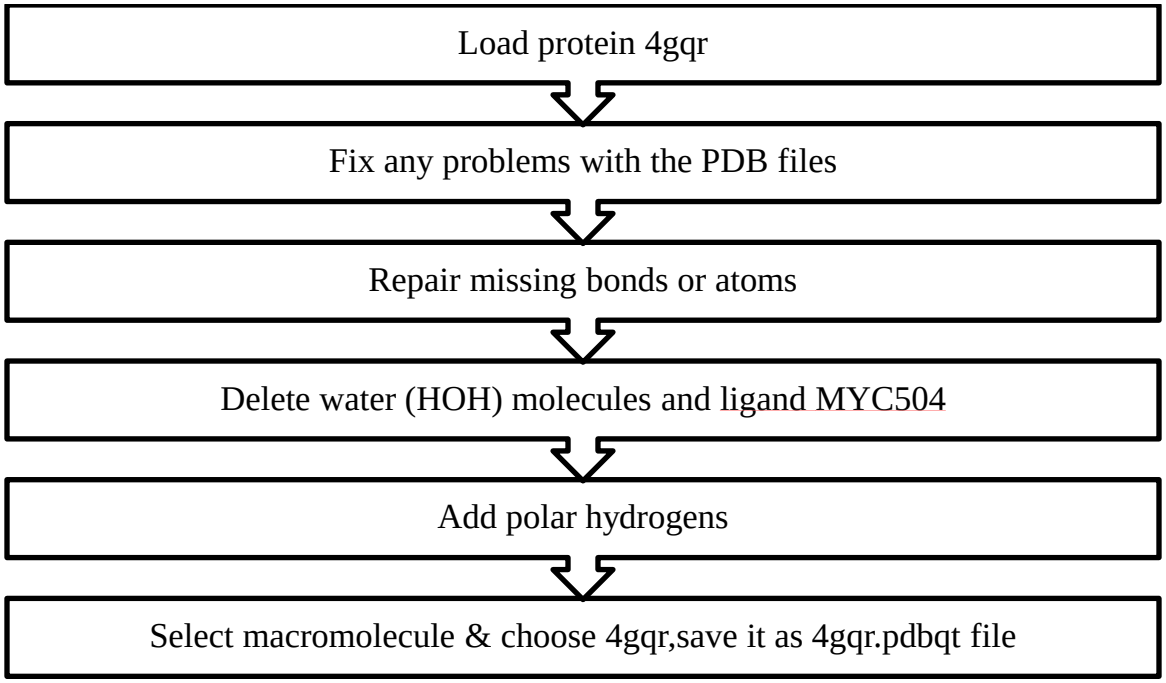


Figure 29: Schematic flowchart for protein preparation

6.1.3. Preparation of ligand from protein

Open AutoDock software, read molecule, insert protein 4gqr, open dashboard for chain A, select all molecules, and deselect the ligand MYC504, delete selected atoms. Add polar hydrogen, and Save it as ligand.pdb. Close the software and again reopen it and from pdb files insert ligand.pdb, choose torsions, detect root and save it as ligand.pdbqt.

Figure 30: Visualization of ligand myc504

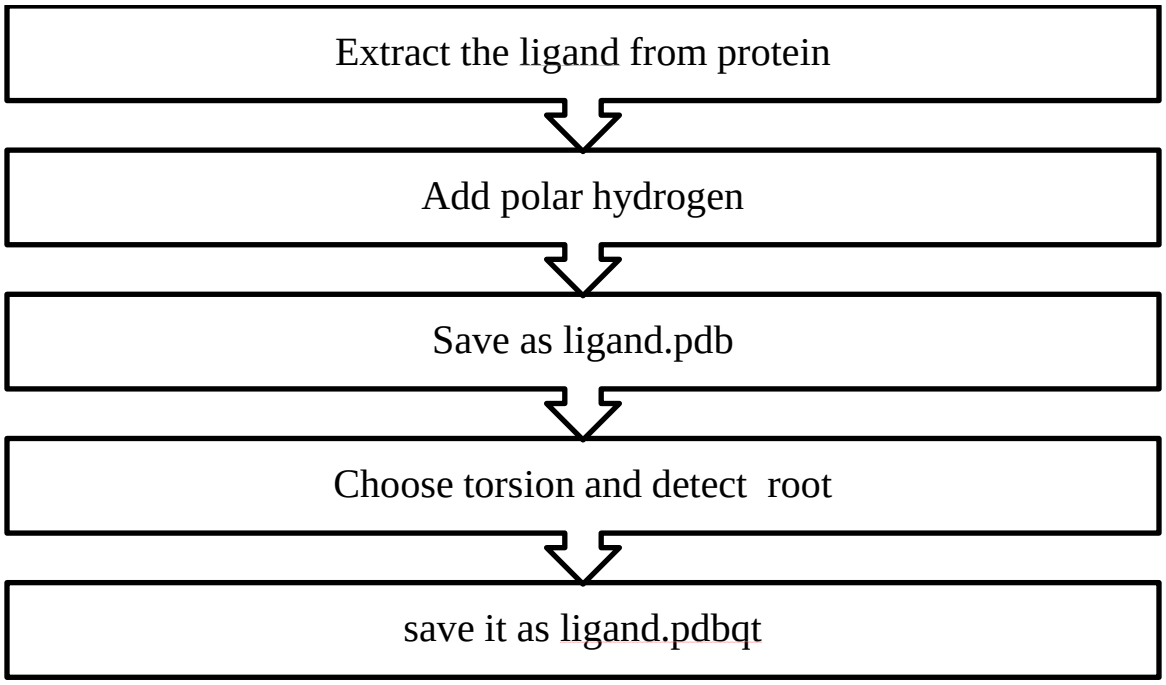


Figure 31: Schematic flowchart for the preparation of ligand from protein

6.1.4. 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 configuration file and save it as conf.txt. Then analyse the docking results in command prompt.

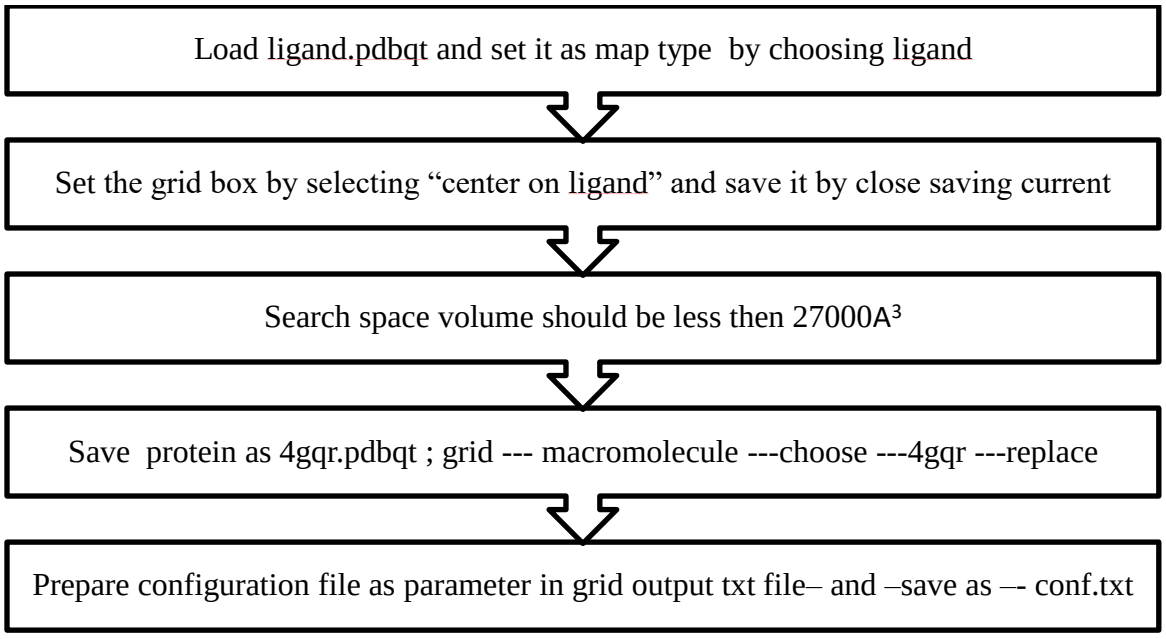


Figure 32: Schematic flow chart for preparation of grid for docking

```
receptor = 4gqr.pdbqt
ligand = ligand.pdbqt

center_x = 13.101
center_y = 14.828
center_z = 39.831

size_x = 40
size_y = 40
size_z = 40
```

Figure 33: Prepared configuration file for docking

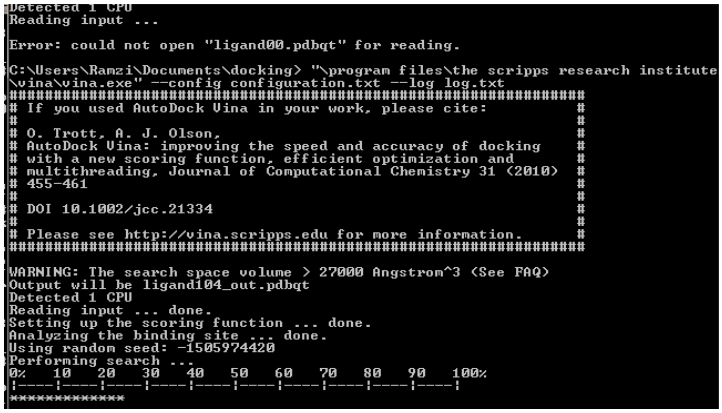
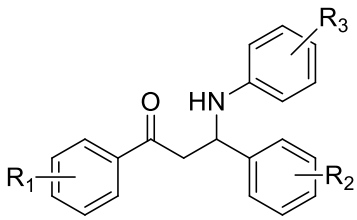


Figure 34: Command prompt

6.1.5. Docking of various designed ligands:

103 molecules of designed chalcone derivatives were docked and their binding affinities were recorded as shown in table 10.

Table 10: designed chalcone derivatives along with their binding affinity score



S no	code	position	R1	R2	R3	Affinity score

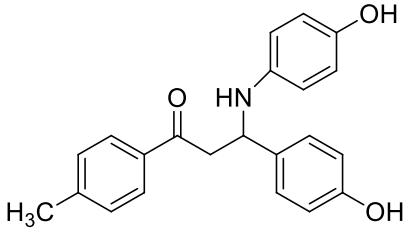
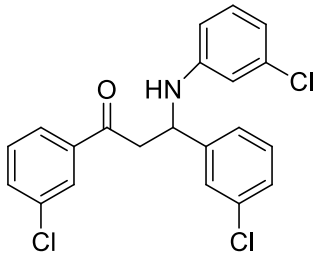
1	CHA1	para	—H	—H	—H	-8.0
2	CHA2	para	—CH_3	—CH_3	—CH_3	-8.0
3	CHA3	para	—OCH_3	—OCH_3	—OCH_3	-8.1
4	CHA4	para	—Cl	—Cl	—Cl	-7.7
5	CHA5	para	—F	—F	—F	-7.8
6	CHA6	para	—OH	—OH	—OH	-7.9
7	CHA7	3,4	—OCH_3	—OCH_3	—OCH_3	-7.9
8	CHA8	para	—CH_3	—H	—H	-7.5
9	CHA9	para	—H	—CH_3	—H	-7.7
10	CHA10	para	—H	—H	—CH_3	-7.4
11	CHA11	para	—OCH_3	—H	—H	-7.4
12	CHA12	para	—H	—OCH_3	—H	-7.4
13	CHA13	para	—H	—H	—OCH_3	-8.3
14	CHA14	para	—Cl	—H	—H	-8.1
15	CHA15	para	—H	—Cl	—H	-7.4
16	CHA16	para	—H	—H	—Cl	-8.2
17	CHA17	para	—F	—H	—H	-7.5
18	CHA18	para	—H	—F	—H	-7.3
19	CHA19	para	—H	—H	—F	-8.2
20	CHA20	para	—OH	—H	—H	-7.5
21	CHA21	para	—H	—OH	—H	-7.6
22	CHA22	para	—H	—H	—OH	-7.6
23	CHA23	Para	—CH_3	—OCH_3	—OCH_3	-8.3
24	CHA24	para	—OCH_3	—CH_3	—OCH_3	-8.2
25	CHA25	Para	—OCH_3	—OCH_3	—CH_3	-7.6
26	CHA26	para	—CH_3	—Cl	—Cl	-7.7
27	CHA27	para	—Cl	—CH_3	—Cl	-8.1
28	CHA28	para	—Cl	—Cl	—CH_3	-7.9
29	CHA29	para	—CH_3	—F	—F	-7.8
30	CHA30	para	—F	—CH_3	—F	-8.0
31	CHA31	para	—F	—F	—CH_3	-7.8
32	CHA32	para	—CH_3	—OH	—OH	-8.6
33	CHA33	para	—OH	—CH_3	—OH	-8.0
34	CHA34	para	—OH	—OH	—CH_3	-7.7
35	CHA35	para	—OCH_3	—Cl	—Cl	-7.8
36	CHA36	para	—Cl	—OCH_3	—Cl	-7.8
37	CHA37	para	—Cl	—Cl	—OCH_3	-8.5
38	CHA38	para	—OCH_3	—F	—F	-8.2
39	CHA39	para	—F	—OCH_3	—F	-7.8
40	CHA40	para	—F	—F	—OCH_3	-8.5
41	CHA41	para	—OCH_3	—OH	—OH	-8.0
42	CHA42	para	—OH	—OCH_3	—OH	-8.0
43	CHA43	para	—OH	—OH	—OCH_3	-8.3
44	CHA44	para	—Cl	—F	—F	-8.3

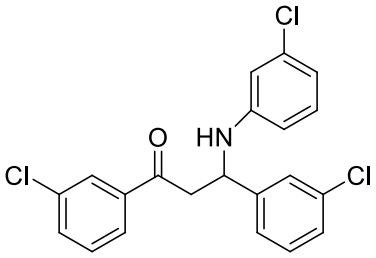
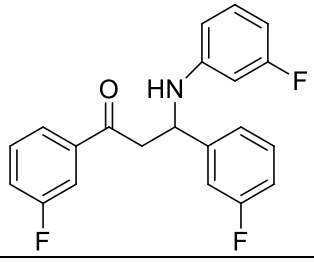
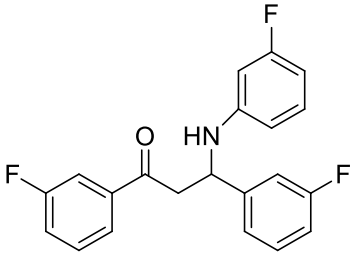
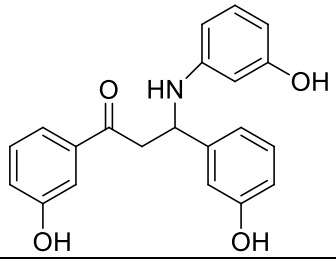
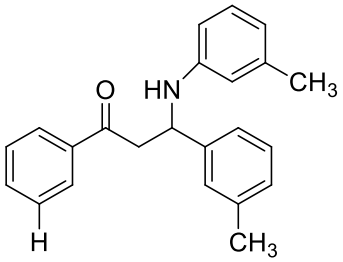
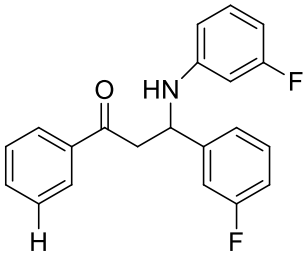
45	CHA45	para	—F	—Cl	—F	-7.9
46	CHA46	para	—F	—F	—Cl	-8.4
47	CHA47	para	—Cl	—OH	—OH	-8.5
48	CHA48	para	—OH	—Cl	—OH	-7.9
49	CHA49	para	—OH	—OH	—Cl	-8.2
50	CHA50	para	—F	—OH	—OH	-8.2
51	CHA51	para	—OH	—F	—OH	-8.0
52	CHA52	para	—OH	—OH	—F	-7.7
53	CHA53	meta	—H	—H	—H	-8.0
54	CHA54	meta	$—CH_3$	$—CH_3$	$—CH_3$	-8.1
55	CHA55	meta	$—CH_3$	$—CH_3$	$—CH_3$	-8.2
56	CHA56	meta	$—OCH_3$	$—OCH_3$	$—OCH_3$	-8.3
57	CHA57	meta	$—OCH_3$	$—OCH_3$	$—OCH_3$	-8.2
58	CHA58	meta	—Cl	—Cl	—Cl	-8.7
59	CHA59	meta	—Cl	—Cl	—Cl	-8.7
60	CHA60	meta	—F	—F	—F	-8.8
61	CHA61	meta	—F	—F	—F	-8.7
62	CHA62	meta	—OH	—OH	—OH	-8.7
63	CHA63	meta	—OH	—OH	—OH	-8.5
64	CHA64	meta	—H	$—CH_3$	$—CH_3$	-8.9
65	CHA65	meta	—H	$—CH_3$	$—CH_3$	-8.0
66	CHA66	meta	$—CH_3$	—H	$—CH_3$	-7.8
67	CHA67	meta	$—CH_3$	$—CH_3$	—H	-8.0
68	CHA68	meta	$—CH_3$	—H	$—CH_3$	-7.7
69	CHA69	meta	$—CH_3$	$—CH_3$	—H	-8.0
70	CHA70	meta	$—OCH_3$	$—OCH_3$	—H	-7.7
71	CHA71	meta	$—OCH_3$	—H	$—OCH_3$	-7.7
72	CHA72	meta	—H	$—OCH_3$	$—OCH_3$	-8.2
73	CHA73	meta	—H	$—OCH_3$	$—OCH_3$	-7.8
74	CHA74	meta	$—OCH_3$	H—	$—OCH_3$	-8.1
75	CHA75	meta	$—OCH_3$	$—OCH_3$	—H	-7.6
76	CHA76	meta	—H	—Cl	—Cl	-8.5
77	CHA77	meta	—Cl	—H	—Cl	-7.7
78	CHA78	meta	—Cl	—Cl	—H	-7.8
79	CHA79	meta	—H	—Cl	—Cl	-8.5
80	CHA80	meta	—Cl	—H	—Cl	-8.4
81	CHA81	meta	—Cl	—Cl	—H	-7.8
82	CHA82	meta	—H	—F	—F	-8.6
83	CHA83	meta	—F	—H	—F	-8.5
84	CHA84	meta	—F	—F	—H	-8.5
85	CHA85	meta	—H	—F	—F	-8.6
86	CHA86	meta	—F	—H	—F	-8.4
87	CHA87	meta	—F	—F	—H	-8.5
88	CHA88	meta	—H	—OH	—OH	-8.6

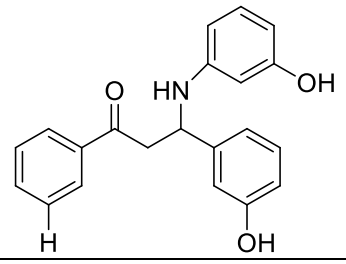
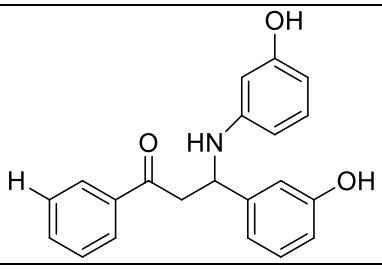
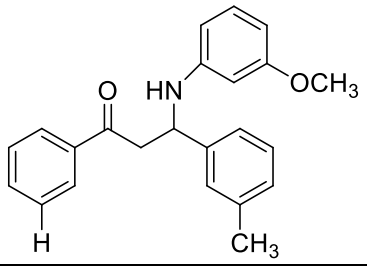
89	CHA89	meta	—OH	—H	—OH	-8.4
90	CHA90	meta	—OH	—OH	—H	-8.5
91	CHA91	meta	—H	—OH	—OH	-8.6
92	CHA92	meta	—OH	—H	—OH	-8.4
93	CHA93	meta	—OH	—OH	—H	-8.4
94	CHA94	meta	—H	$—CH_3$	$—OCH_3$	-8.6
95	CHA95	meta	$—CH_3$	—H	$—OCH_3$	-7.8
96	CHA96	meta	—H	$—CH_3$	$—OCH_3$	-8.6
97	CHA97	meta	$—CH_3$	$—OCH_3$	—H	-7.8
98	CHA98	meta	$—CH_3$	—H	$—OCH_3$	-7.8
99	CHA99	meta	$—CH_3$	$—OCH_3$	—H	-7.8
100	CHA100	meta	$—CH_3$	—Cl	—H	-7.8
101	CHA101	meta	—H	—H	$—CH_3$	-7.7
102	CHA102	meta	—H	$—CH_3$	—H	-8.6
103	CHA103	meta	—H	—OH	$—CH_3$	-8.8
	acarbose					-6.9
	Ligand from protein					-7.8
	Chalcone					-7.0

Those active compounds which are having most potent affinity towards protein 4gqr was identified which depicted in table 11 below.

Table 11: Chalcone derivatives with most potent affinity

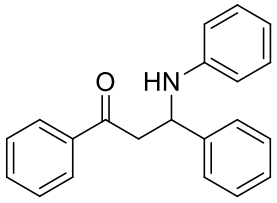
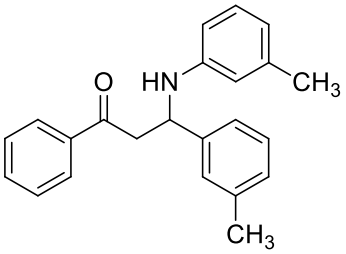
S no	**code**	**position**	**R1**	**R2**	**R3**	**structure**	**Affinity score**
1	CHA 32	para	$—CH_3$	—OH	—OH		-8.6
2	CHA 58	meta	—Cl	—Cl	—Cl		-8.7

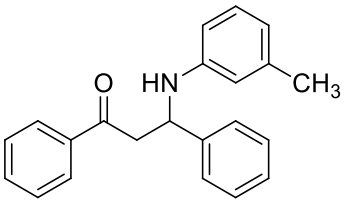
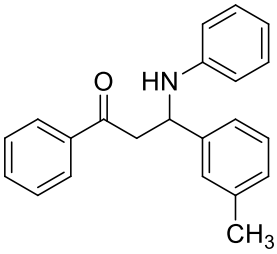
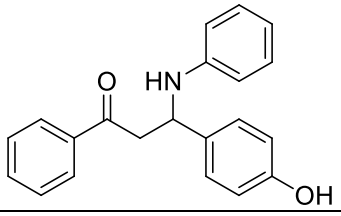
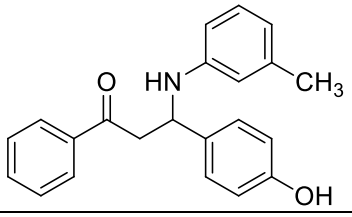
3	CHA 59	meta	—Cl	—Cl	—Cl		-8.7
4	CHA 60	meta	—F	—F	—F		-8.8
5	CHA 61	meta	—F	—F	—F		-8.7
6	CHA 62	meta	—OH	—OH	—OH		-8.7
7	CHA 64	meta	—H	—CH_3	—CH_3		-8.9
8	CHA 82	meta	—H	—F	—F		-8.6

9	CHA 88	meta	—H	—OH	—OH		-8.6
10	CHA 91	meta	—H	—OH	—OH		-8.6
11	CHA 94	meta	—H	—CH_3	—OCH_3		-8.6

Taking in account of availability, cost and feasibility of reaction, some of the chalcone derivative will be synthesized which are shown in table no 12 below:

Table 12: Molecules of interested for synthesis

S no	**code**	**position**	**R1**	**R2**	**R3**	**structure**	**Affinity score**
1	CHA1		—H	—H	—H		-8.0
2	CHA64	meta	—H	—CH_3	—CH_3		-8.9

3	CHA101	meta	—H	—H	—CH_3		-7.7
4	CHA102	meta	—H	—CH_3	—H		-8.6
5	CHA21	para	—H	—OH	—H		-7.6
6	CHA103	Para,meta	—H	—OH	—CH_3		-8.8

As we have seen that the affinity of the CHA64 is the highest affinity -8.9 as compared to chalcone which has shown -7.0 affinity and acarbose with -6.9 affinity score towards the 4gqr protein. This could be the best ligand which can be synthesized and inhibit the alpha-amylase enzyme (figure 32).

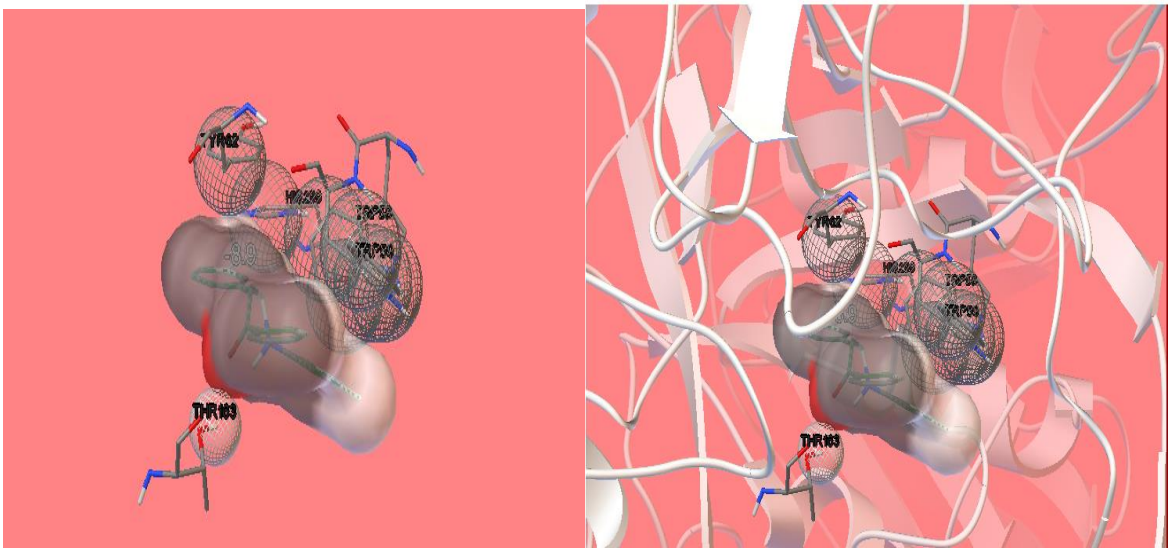


Figure 35: Interaction of best designed ligand(ligand.64) with alpha-amylase

CHAPTER 7: EXPERIMENTAL WORK

7.1 Molecular docking:

For the identification of potentially active ligands, the designed molecules were analyzed by molecular docking using Autodock Vina 1.5.6 software, a molecular docking software [27-32].

For the extraction and preparation of ligands, the desired proteins were downloaded from protein data bank [http://www.rcsb.org/pdb/home/home.do] [33]. The selected protein was validated by the extraction of ligand and docking it in a same manner as actual ligand. For preparation of protein it was reloaded and various problems were fixed such as missing bonds or atoms, and removed extraneous structures like water molecules. Polar hydrogens were added along with the Kollaman charges. After saving the macromolecule (as pdbqt file) the ligand.pdbqt was loaded and set it as map type by choosing ligand and grid box was generated. The compounds were drawn by ChemDraw Ultra and converted to 3D structures. Geometry of all compounds was optimized by semiemperical MM2 method. Molecular docking was performed on optimized structure of protein.

7.2 Chemical synthesis:

The ^{1}H-NMR spectra were recorded at 400 MHz on a Bruker Avance 400 (400MHz) spectrometer in $CDCl_3$ using TMS as an internal standard. The chemical shifts (δ) for ^{1}H are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; m, multiplet. The reactions were monitored by TLC (merck). Evaporation of solvents was performed under reduced pressure using rotator evaporator commercial grade reagents and solvents were used without further purification.

Table 13: LIST OF CHEMICALS

S.No	Chemical name	Company name
1	Benzaldehyde	CDH
2	Acetophenone	CDH

3	Aniline	CDH
4	m-Toluidine	Aldrich
5	4-Hydroxybenzaldehyde	CDH
6	m-Tolualdehyde	Aldrich
7	Ethyl acetate	Renkem
8	Hexane	Renkem
9	NaOH	CDH
10	LiOH. H_2O	Thomas baker
11	Ethanol	Chong Yu High-tech
12	THF	Loba chemicals
13	Ether	Loba chemicals
14	Acetonitrile	Loba chemicals

Table 14: LIST OF INSTRUMENTS

S.No	**Instruments**	**Company name**
1	FT-IR spectrophotometer	Shimadzu
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	Bruker avance
7	Refrigerator	Kelvinator
8	Rotary evaporator	Popular traders
9	U.V chamber	Popular traders
10	Micro pipette	Perfit
11	Digital balance	Contech
12	TLC sheet	Merck

CHAPTER 8: SUMMARY

Diabetes mellitus is one of the most prevalent metabolic disorderliness among the millions of people around the world with an increasing rate. The commercially available antidiabetic agents possess a no of side effects which we have already discussed. Hence, new efforts are needed to be considered.

Alpha-amylase is a good drug design target for the treatment of diabetes mellitus and synthesis of new molecules. Since the enzyme is available in the brush border of the small intestine the drug will remain there as such and there will not be further absorption in the blood and side effects to other organs. Among the different molecules which we have earlier discussed, chalcone is the most preferred molecule for the synthesis of potent antidiabetic agents.

CHAPTER 9: REFERENCES

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