Limonoids: Promising Phytochemicals Targeting PPAR-γ as Antidiabetic Agents

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

The project does not involve any kind of human or animal trial.

Abstract

The peroxisome proliferator activated receptors (PPAR) are members of nuclear receptor supergene family that play a vital role in both glucose and lipid homeostasis. Thiazolidinediones (TZDs) has high affinity to PPAR-γ receptors. But TZD scaffolds in antidiabetic drugs is responsible for fluid retention, weight gain, bone fractures, liver failure, hypoglycemia and allergic skin reaction. Investigators are searching for novel antidiabetic drugs with higher affinity and minimum side effects. Nowadays. phytochemicals are becoming promising compounds for health benefits. In this study, 11 limonoids (non-TZD) nomilin, obacunone, obacunoic acid, ichagin, limonite A- ring lactone, limonin, calamine, cyclocalamin, limonol, limonin and 17-beta-D-glucopyranoside have been analyzed via docking studies. Among them obacunone, limonite-A- ring lactone, nomilin. 7-alpha obacunol and limonol have demonstrated good binding affinity with PPAR-γ receptor that are promising lead antidiabetic compounds.

Keywords: Limonoids, PPAR-γ receptor, Thiazolidinediones, Molecular Docking, Insulin resistance**,** Pharmacokinetic Analysis.

Acknowledgement

First and foremost, All praises to the Almighty Allah Subhanahh Wa Ta”ala who is AR Rahman, Al Hakim blesses His creation with prosperity, wisdom and knowledge. Afterwards, I am grateful to my parents, my family for their support, motivation throughout the journey. At last, I am also thankful to my respected supervisor Dr. Humair Bin Md. Omer for keeping patience on me, helping me to complete the research article.

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List of Acronyms

PKC Protein kinase

CFFA Free fatty acid

IR Insulin resistance

DAG Diacylglycerol

TZD

Thiazolidinediones

DM Diabetes mellitus

JM juxta membrane

Chapter 1

Introduction

Diabetes mellitus (DM) has become a chronic disease known to us around the world. Almost, 3000 years ago, it was informed in Egyptian manuscript. Now slowly, the incidence of growing Diabetes Mellitus is on a large scale in the United States and world- wide. It is a disease of a group of metabolic disorders of carbohydrate raising the blood glucose level (hyperglycemia) in the human body, arresting the production of insulin completely (type -1-
diabetes), or an ineffective response of the cells to insulin, incapability of producing effective quantities of insulin in the body. Various types of factors and conditions are liable to progress the pathogenesis of diabetes. For example: hormonal imbalances, genetics, diet, oxidative stress, lack of physical activity and usage of medications. (Papatheodorou et al., 2018).A great number of people with type 2 diabetes reside in every country and 79% of people with DM are residing in undeveloped countries. Now a days, Gestational diabetes becomes another concern for the pregnant woman taking carbohydrate rich food without involving in activities and it is also fatal to unborn baby for its health-development having risk for cognitive impairment, inattentiveness, impaired working memory and multiple complications in the human body because of metabolic derangement-like muscle breakdown causes wasting proteins, and breakdown of fat acetyl-Co-A which is responsible for producing acetoacetate, B hydroxybutyrate and acetone. These compounds are prime molecules to cause acidosis, the phenomenon is termed "Diabetic ketoacidosis". It results in frequent urination, increased thirst, and flu-like symptoms including nausea, vomiting, dry mouth, abdominal pain, etc. (Dhatariya et al., 2020).

Macrovascular disease or Micro angiopathy are also risky vascular complications for the body. Dysfunctional vascular endothelium occurs in a body by a process known as glycation**.**

Through this process, enormous endogenous chemicals like advanced glycation end products (AGE) accumulate in the body via the complex mallard reaction between the reaction of reducing sugar with albumin (amino acid) and it affects the retina, kidney, heart and peripheral nerves in human organs. (Yamagishi et al., 2012).

To overcome the problem, researchers addressed us with various types of Diabetic drugs like (Sulfonylureas, Glinides, Biguanides, GLP-1 receptor, etc.) having the properties of secreting insulin from the pancreatic beta cell, decreasing hepatic production of glucose, binding with PPARs receptor in muscle, kidney, liver cells and *another possible way to alleviate the disease. However, the questions arising with the use of these* classes of drugs are about pharmacokinetic properties, risk of hypoglycemia, liver toxicity, heart failure issues, and other adverse reactions. Traditional plants can be good resources for finding bioactive (hit) compounds. (Ahmed & Alkali, 2018)

Various types of secondary metabolites (glycosides, alkaloids, terpenes, flavonoids, carotenoids, lignans, saponins) derived from plants exert good anti-diabetic properties have less-side-effects, are effective, and are non-toxic. Terpenes abound in bacteria, fungi, algae, and plants (abundantly in essential oil, resins, waxes, and latex). Terpenes are found in the natural products either hydrocarbon forms or oxygen-containing compounds or oxygen derivatives (alcohols, aldehyde, or ketones) are designated as terpenoids. The traditional plants for example: Salacia Hainanensis Roots of Ficus bengalenoise, Lactuca indicia, and Moutan Cortex are rich in antidiabetic properties (Panigrahy et al., 2021).

Therefore, the aim of the study is in silico studies investigating the terpenoids and their derivatives binding with PPAR–gamma receptor that will result in higher affinity, potency, and efficacy in comparison to the standard drugs.

Objective

The aim of the studies is to investigate the terpenes and their derivatives exploring the novel bioactive compounds with higher affinity with minimum side effects and it is also important to discuss the structure activity relationship analysis for the researchers to realize how different structures of molecule can affect the binding affinity. Therefore, another objective of the study is to discuss structural activity relationship (SAR) analysis of bioactive compounds. Finally, the study of pharmaceutical analysis (ADME) is necessary to ensure the safety, efficacy and quality of the phytochemicals. Pkcsm software is very useful and free software for the study of pharmaceutical analysis (ADME). Therefore, it has been utilized for the prediction of ADME properties of investigating limonoids.

Chapter 2:

Function of Insulin Receptor

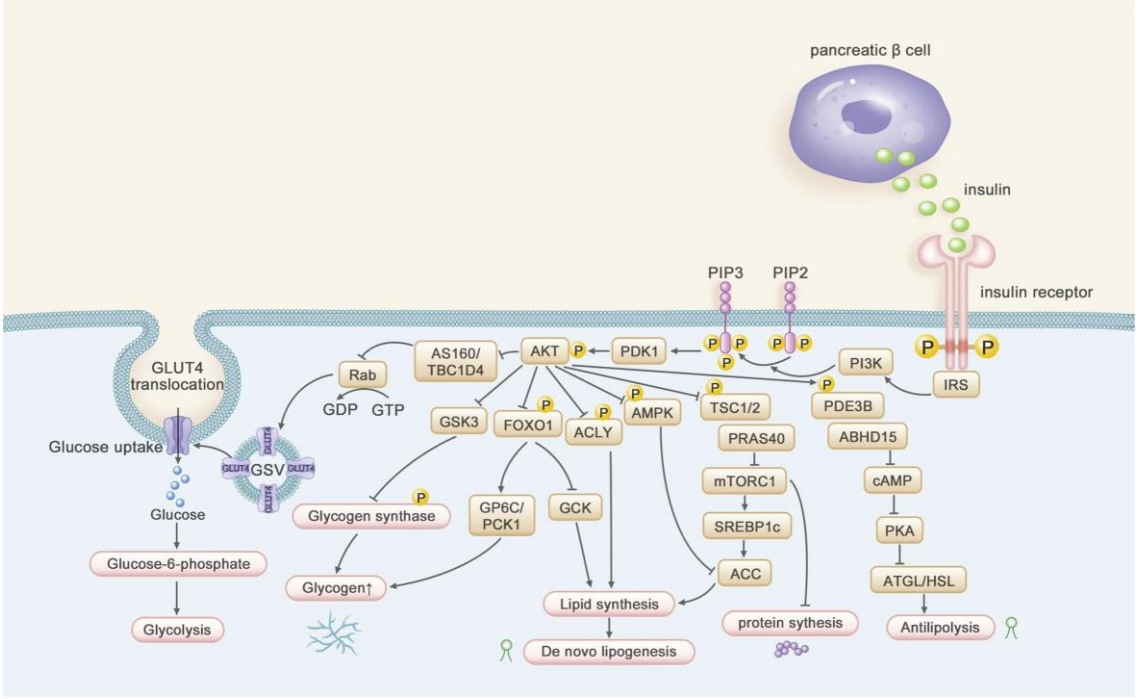


Figure 1:Function of insulin receptor with its signaling network. (Li et al., 2022).

Insulin is an anabolic hormone. After taking carbohydrate-rich meals, insulin is released from the pancreatic beta cell and promotes an anabolic reaction. Currently, insulin also stimulates different kinds of carbohydrate-consuming cells. For example: adipocytes, Liver, and skeletal muscle cells. These kinds of cells abound with insulin receptors (IR). To activate the intracellular signaling pathway, it is essential for Insulin receptor substrate -1 (IRS-1) to connect in the juxta membrane (JM) domain of insulin receptor (IR). The IRS -1 consists of three domains divided into pleckstrin homology (PM), phosphotyrosine–binding (PTB) domain, and C – terminal. The consequence of phosphorylation of IRS -1 in the PH domain, and PTB domain induces the degradation of IRS -1 proteins or dissociation of IRS-1 proteins from the juxta membrane (JM) domain of IR and halts the intracellular signaling pathway. On the other hand, the phosphorylation of Ser-residues at -COOH terminal of IRS-1by IRK –

kinases enzymes in insulin receptor (IR) interrupt the downstream effectors to bind with IRS-1 and abates the negative feedback mechanism of insulin signaling pathway by promoting PI3K/AKT signal transduction (Boura-Halfon & Zick, 2009).

2.1 PI3K/AKT Signaling Pathway

Insulin connects to IR by occurring the dimerization reaction and causing intracellular auto-phosphorylation of Sr/Thr residues in IRS-1. The phosphorylated complex molecule binds with phosphatidylinositol-3-OH kinase (PI3k) and activates PI3k to convert phosphatidylinositol-4,5 bisphosphate (PIP2) into phosphatidylinositol-3,4,5 -triphosphate molecule (PIP3) inducing phosphorylation. The PIP3 and AKT (Protein kinase B) molecules bind with phosphoinositide-dependent kinase -1(PDK1) enzymes and induce phosphorylation of ser/ Thr residues in Akt. (Li et al., 2022).

However phosphorylated AKT plays an important role in biological sciences. This protein kinase B (AKT) has a great impact on glycogen synthase-kinase 3 (GSK3) inducing hepatic glycogen synthesis, also like the Fork head box O1 (FOXO1) inducing gluconeogenesis (Li et al., 2022).

2.2 AKT/AS 160 pathway and Rab protein function

Rab protein is known as small guanine triphosphatases (GTPases).The functions of this pathway is the budding, transportation and this pathway is also very crucial for GLUT4 translocation.

Chapter 3

Mechanisms of Insulin Resistance

3.1 The glucose-fatty acid cycle (The Randle cycle)

In 1963, Randle et al. hypothesized that Free fatty acid (FFA), presence of carnitine palmitoyl transferase -1 (CPT-1) enzyme induces beta-oxidation in mitochondria. Flavin Adenine Dinucleotide (FAD), Nicotinamide Adenine Dinucleotide (NAD) are the prime co-enzymes that play an important role as oxidoreductase enzymes to oxidize FFA into mitochondrial Acetyl-COA. At first, the fatty acid becomes activated, the coenzyme (Co-A) molecules help to produce fatty acyl–CoA which proceeds beta-oxidation pathway. FAD induces oxidation in fatty acyl Co-A results in FADH2 and unsaturated fatty acid. (enoyl co-A). The enoyl Co A hydratase induces hydration (addition of water) in unsaturated fatty acid yields an alcohol (-OH), L-beta hydroxy acyl Co A. At presence of beta hydroxy acyl, Co-A dehydrogenase enzyme. It becomes oxidized results in beta–keto acyl-CoA and NADH+H. Finally, beta-Ketoacyl-CoA reacts with Coenzyme A (CoA-SH) in the presence of thiolase enzyme results in a key molecule (acetyl Co-A) in cellular metabolism.

Firstly, the acetyl Co-A enters TCA cycle and increases forming citrate molecules inside the cell which inhibits the activity of phosphofructokinase enzyme (PFK-1) along with the transformation of F-6-P to the acetyl-CoA. Secondly, NADH, from TCA cycle, through inhibiting pyruvate-dehydrogenase enzyme (PDH) regulates negative feedback mechanism which occurs gluconeogenesis, So the level of glucose increases inside the cells. Thirdly, Glucose-6-phosphate is another reason for accumulating glucose inside the cell through inhibiting hexokinase enzyme activity results in stopping glycogen synthesis. Therefore, no

more glucose can enter cells from blood due to an increase in the glucose level inside the cell induces T2DM (Lee et al., 2022).

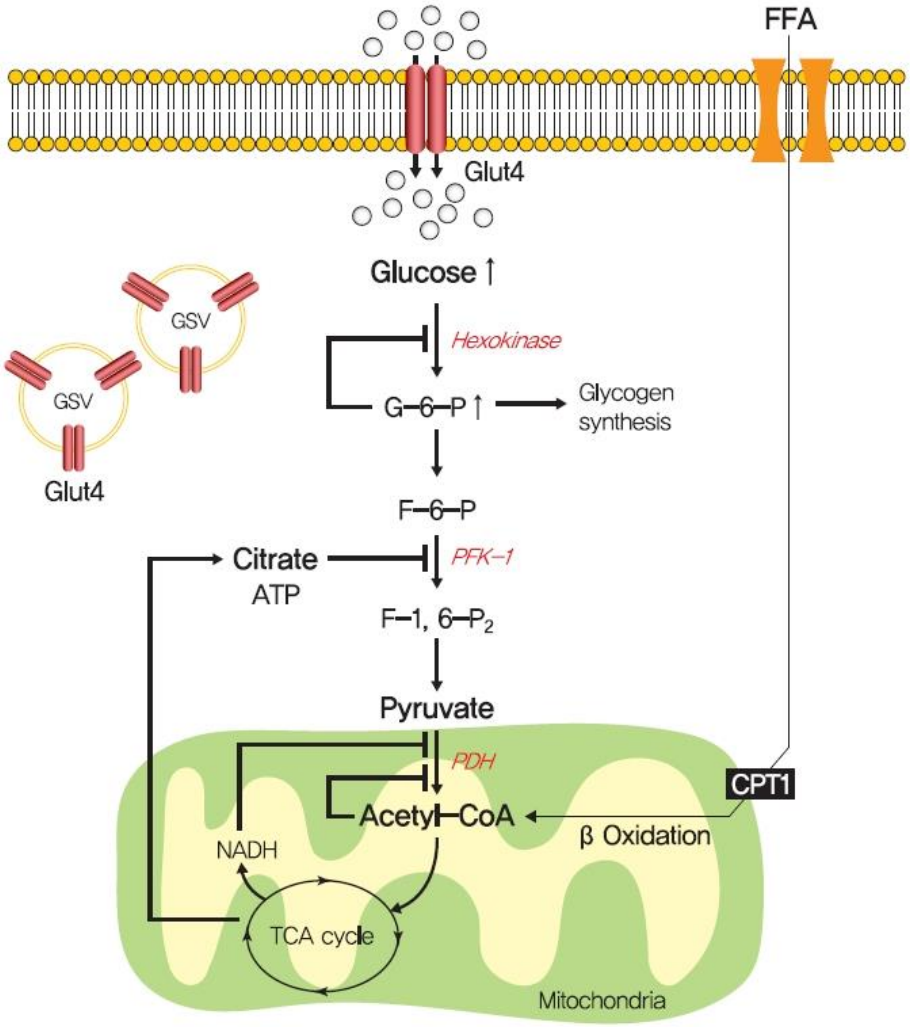


Figure 2: The glucose- fatty acid cycle hypothesis in insulin resistance proposed by Randle et al. 1963 in the skeletal muscle. The cycle increases the accumulation of glucose in the cells and inhibits Glut4 receiving glucose from blood (Lee et al., 2022*).*

3.2 Hexosamine Biosynthesis Pathway

Thirty years ago, Marshall et al. first reported that O-GlcNAcylation modulates insulin sensitivity. The generation of O-GlcNAcylation occurs due to accumulation of the high level of glucose and free- fatty acid (FFA) in the cell. Lee SH, et al. (2021), documents that extra glucose in the cell follows the hexosamine biosynthesis pathway responsible for the

development of insulin resistance. According to this pathway, firstly, glucose transfers into glucose-6-phosphate at the presence of hexokinase enzyme. Then, glucose-6-phosphate co GlcNAcylation produces. The O-GlcNAcylation molecules could affect epigenetic regulation, enzyme activity and normal transcription process and inhibit insulin signaling. (Lee et al., 2022).

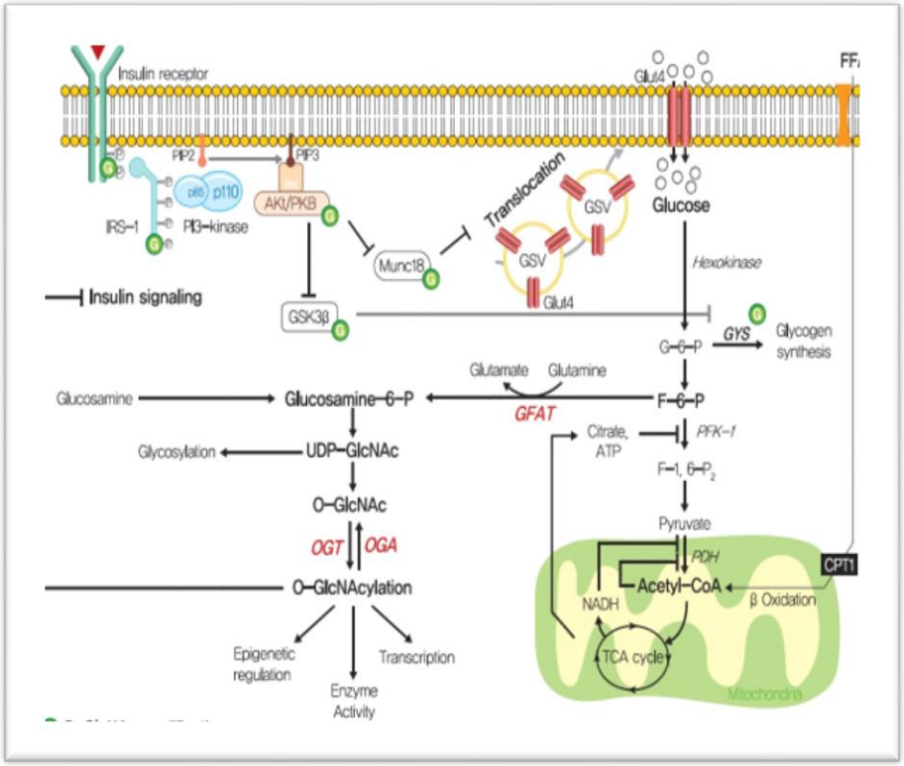


Figure 3: Accumulation of glucose and free fatty acids in the cell introduces. Hexosamine biosynthesis pathway which develops inhibiting insulin signaling.(Lee et al.2022).

3.3 Kennedy Pathway/De novo Lipogenesis Pathway

In glycolysis process, glucose converts into glycerol-3-phosphate and free fatty acid (FFA) metabolized by beta oxidation process into Acyl CoA (a thiol ester). The thiol ester plays an important role in esterifying Glycerol-3-phosphate to yield lysophosphatidic acid (LPA) in the presence of G3P acyltransferase enzyme. The esterification reaction takes place in the whole de novo lipogenesis process at the presence of transferase and hydrolases enzymes and yield

sn-1,2 diacylglycerols (DAG) and lipid molecules like tricyclic glycerol (TAG). (Lee et al., 2022).

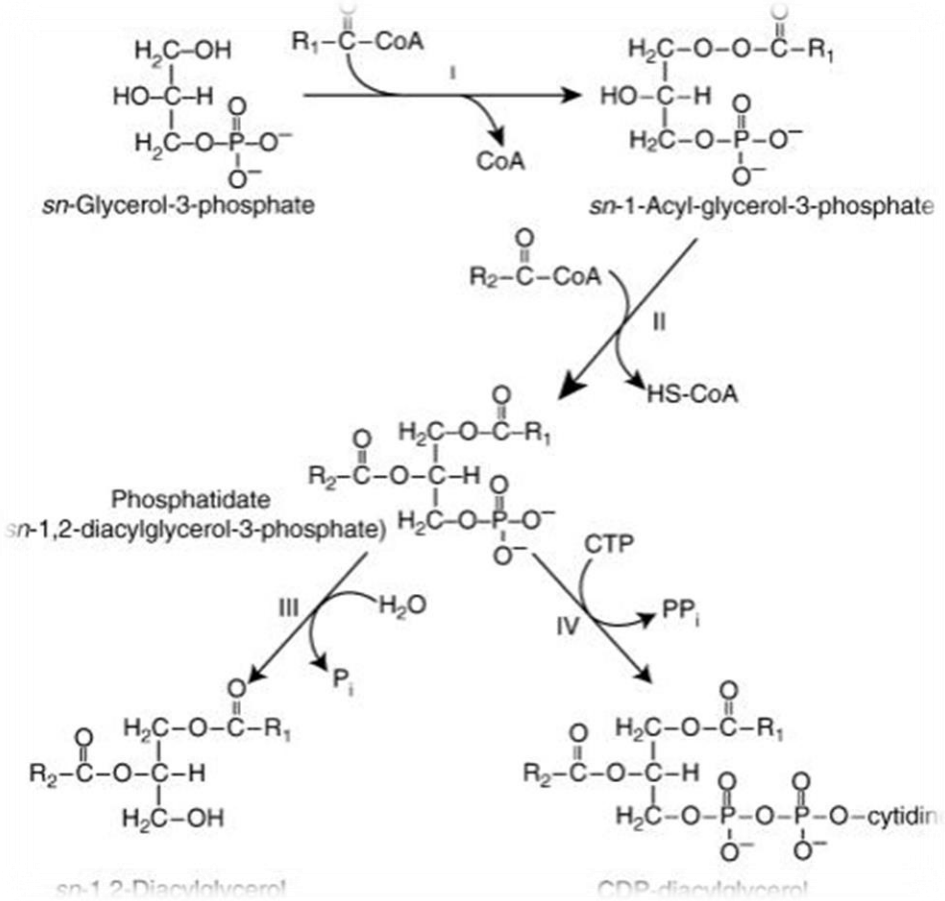


Figure 4: Lipogenesis process in human body because of accumulating high glucose and free fatty acid in body (Lee et al., 2022).

3.4 Diacylglycerols (DAG)

The DAG hypothesis of lipid induced insulin resistance happens during the activation of protein kinase C (PKC) results from accumulating DAG molecules within insulin sensitivity tissues. Three types of PKC are available grouped into conventional, novel and atypical. Lee S.H. et al. (2021) documented that novel PKC (nPKC) has greater affinity to DAG molecules. DAG (sn-1,2 DAG) is the molecule that accumulates in plasma membrane and generates a complex molecule binding with phosphatidyllinositol-4,5–bisphosphate (PIP2). After that, the complex molecule translocates novel protein kinase C (nPKC) which induces the inhibition of IRTK tyrosine kinase activity through the phosphorylation of the kinase protein at Thr 1160 and halt insulin signaling pathway (Lee et al., 2022).

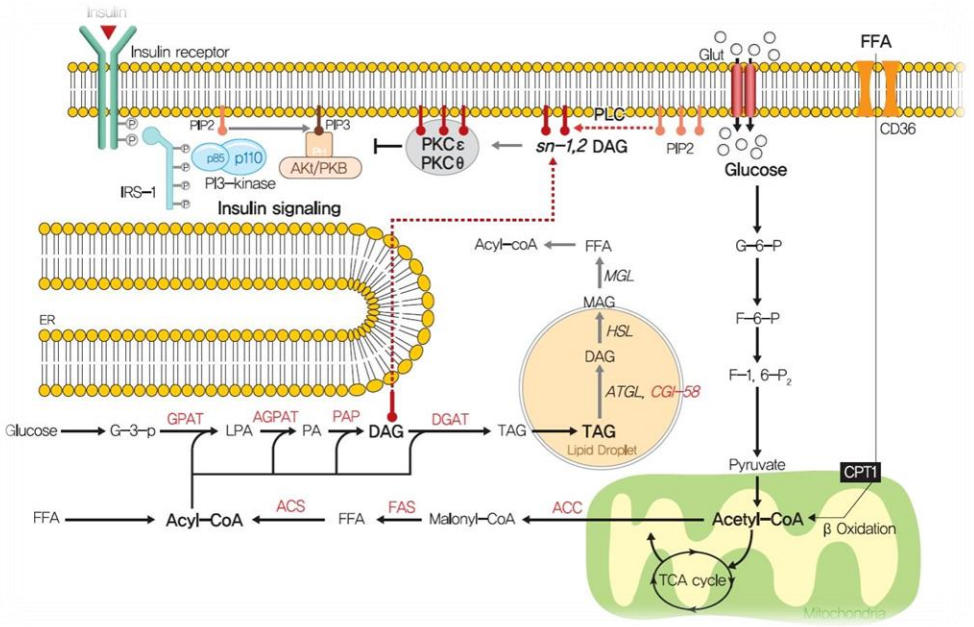


Figure 5*:* Insulin resistance induced by accumulating Diacylglycerols (DAG) . (Lee et al., 2022)

Notably, the translocation and activation of nPKC is dependent on stereoisomers, sn-1,2 DAG molecules which are only responsible for inhibiting insulin signaling and hepatic steatosis. High PKC levels have been documented in the membrane fraction of soleus muscles of

Diabetic rats and treatment with an antigens oligonucleotide (ASO) elicits effectual result to protect the HFD feeding rats and increase insulin sensitivity.

3.5 Endoplasmic (ER) and Oxidative Stress

Lee SH, et al. (2021), documented that Endoplasmic stress is another reason to induce T2DM. ER stress has been considered to induce insulin resistance in pancreatic-beta cells and liver. Excess nourished condition, accumulating of misfolded proteins induce in endoplasmic reticulum causes ER stress and enhance unfolded protein response (UPR). Multiple responses induce ER stress activates PKR like ER -kinase (PERK), the kinase enzyme induces phosphorylation of alpha-subunit of translation initiation factor (elF2) which reduces translation of proteins and activates apoptosis pathway (Lee et al., 2022).

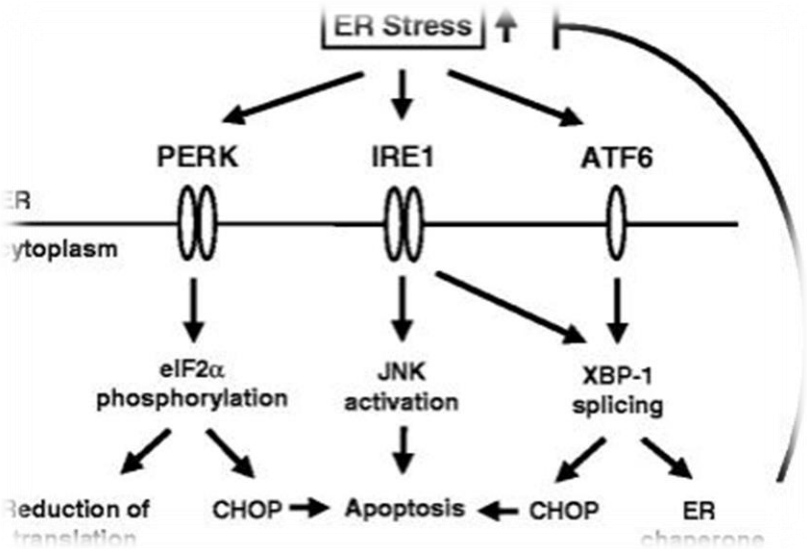


Figure 6: Insulin resistance induced by JNK ACTIVATION (Lee et al., 2022).

ER stress also develops C-Jun-N-terminal -kinase (JNK) activity. JNK is a kinase enzyme phosphorylates IRS-1 proteins and disturbs PI3K/AKT insulin signaling induces T2DM.

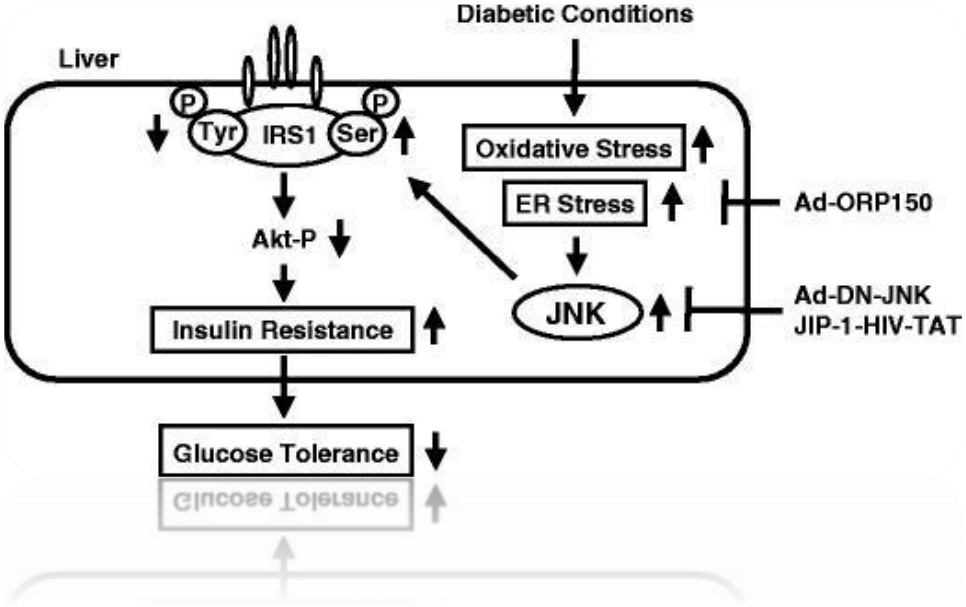


Figure 7: Insulin resistance by oxidative and ER stress (Lee et al., 2022).

Oxidative stress: Oxidative stress also promotes JNK activity. JNK phosphorylates serine 307 of IRS1 on a large scale. Therefore, it also increases insulin resistance and decreases glucose tolerance (Lee et al., 2022).

Chapter 4

Complication Of Diabetes

Diabetic ketoacidosis (DKA): Hyperglycemia can develop because of three processes. The processes are promoted gluconeogenesis, glycogenolysis**,** impaired glucose utilization by peripheral tissues**.** Impaired glucose utilization increases glucose level in human body. By lipogenesis process, the glucose converts into free fatty acid (FFA). The FFAs are oxidized to ketoacids. The two major ketoacids are beta-hydroxybutyrate and acetoacetate. High ketone levels cause an osmotic diuresis leading to hypovolemia, decreased glomerular filtration rate and worsening hyperglycemia (Dhatariya et al., 2020).

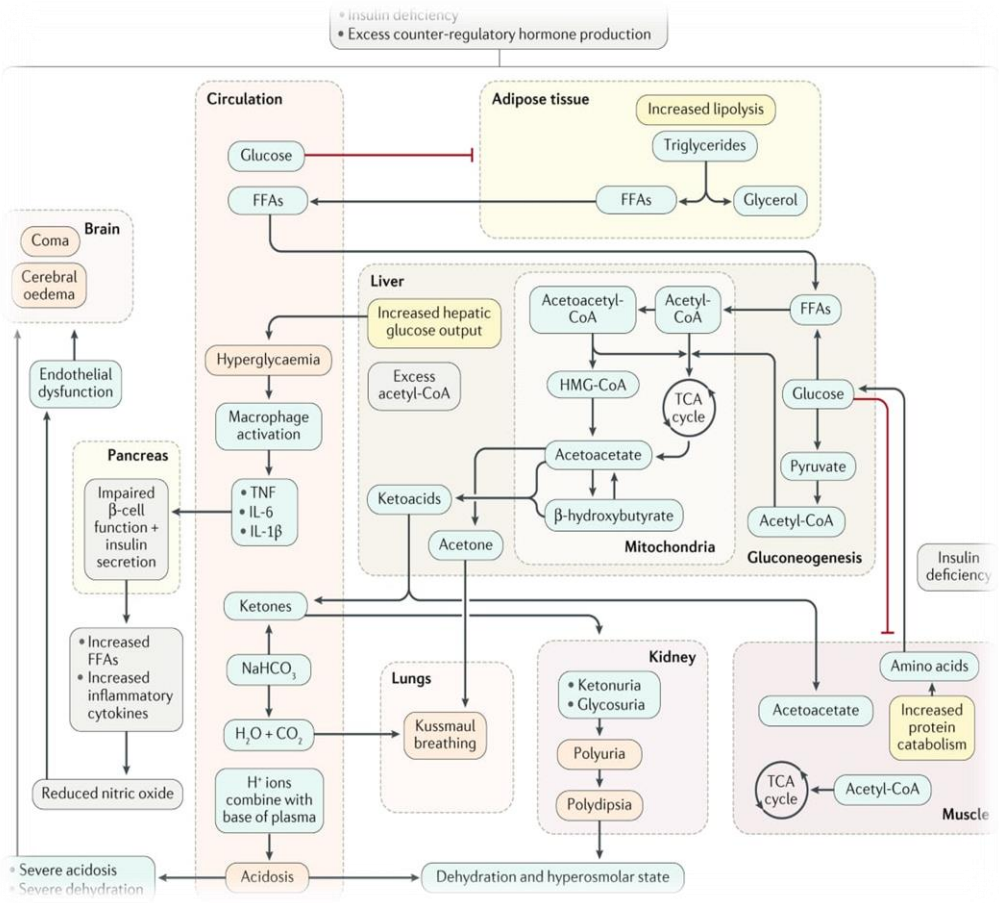


Figure 8: Diabetic complications in multiple vital organs of human body (Pancreas, Brain, kidney) (Dhatariya et al., 2020).

Impairing pancreatic beta cell and neuron function: Hyperglycemia induces macrophage activation and releases pro-inflammatory cytokines (tumor necrosis factor, Interleukin 6, interleukin -1beta). The pro-inflammatory cytokines impair beta cell function and insulin secretion. Furthermore, hyperglycemia produces C-reactive protein which reacts with nitric oxide and generate reactive free radicals. Therefore, endothelial dysfunction takes place and it injures the brain tissue (Dhatariya et al., 2020).

Inducing abnormal breathing pattern: Hyperglycemia due to accumulate glucose and free fatty acid (FFA) in the liver result in producing Ketonuria and Glycosuria which occur polyuria and polydipsia in the body. Therefore, dehydration and hyperosmolar state become the ordinary complication among the T2DM patients. The complication also develops high anion gap in plasma along with increasing the ketoacids level which leads to decrease serum bicarbonate concentration results in evolving carbon-di-oxide (CO2) in blood circulation.

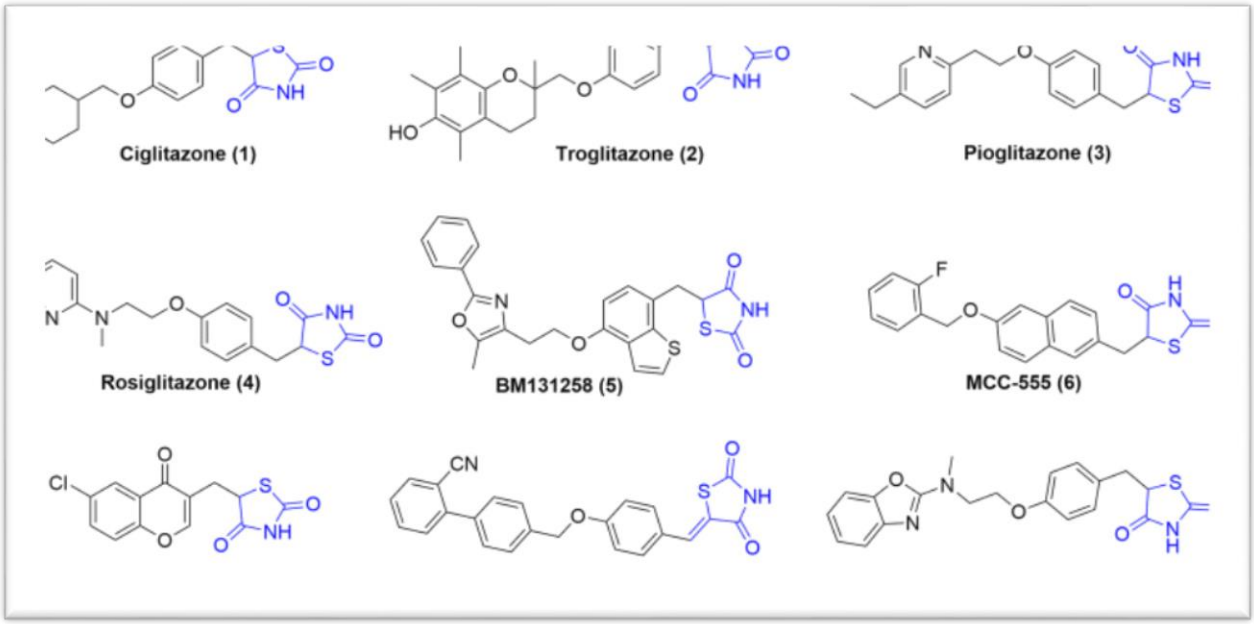
Kussmaul breathing: Kussmaul breathing is an abnormal breathing problem. Patients suffering from T2DM suffocates and experience the Kussmaul breathing due to the circulation of high CO2 in blood. Kussmaul breathing is a sign of medical emergency (Dhatariya et al., 2020).

Damaging human vital organs: During hyperosmolar, hyperglycemic state (HHS), The blood becomes more concentrated than normal. So, the state draws water out of the body's organs including the brain. It causes confusion, delirium, hallucinations, dry mouth, extreme thirst, frequent urination, blurred vision, weakness, paralysis and at last, it leads to the coma or death of the cell (Dhatariya et al., 2020).

Chapter 5

Thiazolidinediones As Antidiabetic Drugs

Thiazolidinediones is named to “glitazones” administrated as oral antidiabetic drugs after proper diet in order to control high blood sugar level in people suffering from type 2 diabetes mellitus (T2DM). Glitazones are recognized as insulin sensitizers due to help the body to sensitize the presence of insulin in blood by increasing the production of insulin receptor in carbohydrate-consuming cells like kidney, muscle and nerve cells. Thiazolidinediones (TZDs) is considered as a privileged scaffold for the improvement of anti-diabetic drugs particularly for PPAR- gamma receptors. Rosiglitazone, pioglitazone, troglitazone, ciglitazone and their derivatives are the prime PPAR gamma agonists having thiazolidinediones scaffold with potential antidiabetic action (Sameeh et al., 2022).



Generalized Structure Active Relationship (SAR) of Thiazolidinediones (TZDS)

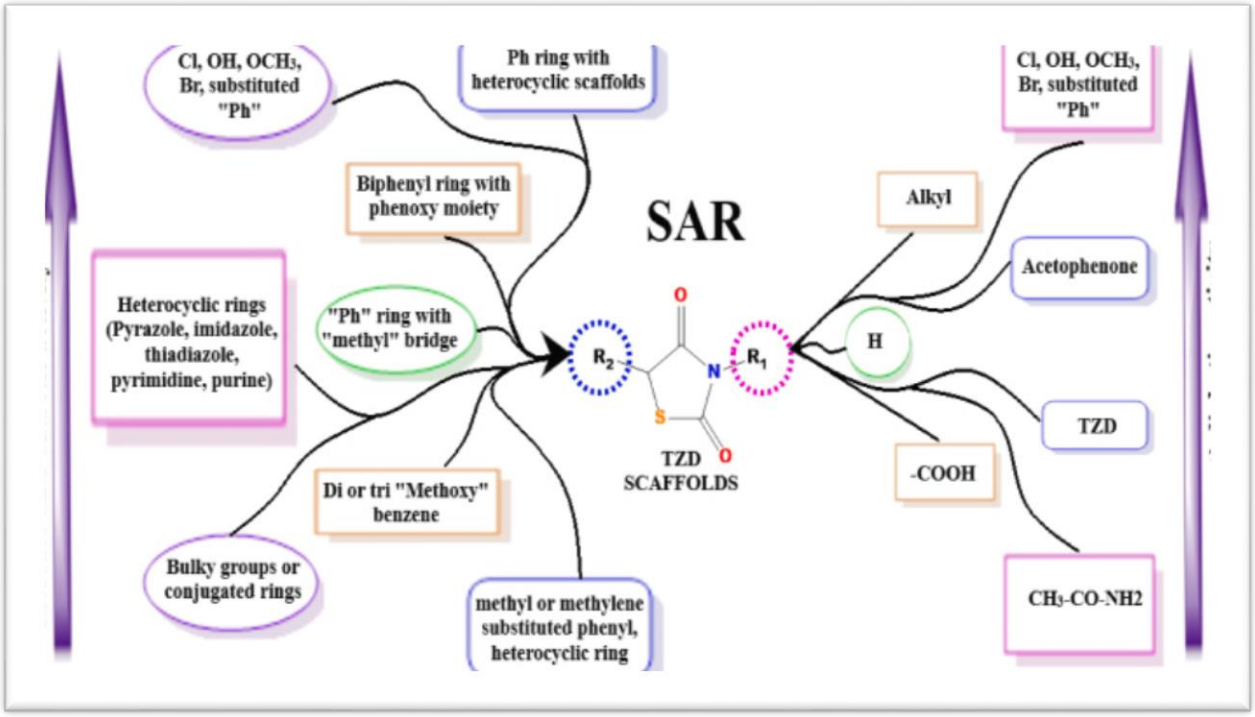


Figure 9: SAR Of TZD SCAFFOLD followed by researchers to rationalize the novel anti - hyperglycemic drugs (Basak et al., 2024).

The investigation of SAR allows multiple bioactive compounds of thiazolidinediones. The SAR enables the researchers to find out new drugs with minimizing side effects, adverse effects, toxic effects and multiple complications. It helps to invent the more potency, efficacy drug as well.

- Attaching hydrogen (H) with nitrogen (N) presenting in the 5th position of TZD increases the anti-diabetic activity.
- Displacement of hydrogen with alkyl group in R1 increases the TZD activity.

- Addition of 4 –bromoacetophenone, Electron donating group (EDG) –OH, -CH3, acetyl and carboxyl functional group in 3rd position in TZD elicits Anti-diabetic activity.
- Introducing halogen atom, furyl methylene group and arylidene group at the 5th position of TZD increase the activity.
- Benzylidene derivatives at the 5th position (R2) increase hypoglycemic action.
- Incorporation of Biphenyl ring with phenoxy moiety at the 5th position of TZD (R2) elevates the activity (Basak et al., 2024).

5.1 Potential Novel Derivatives of Thiazolidine-2,4-dione

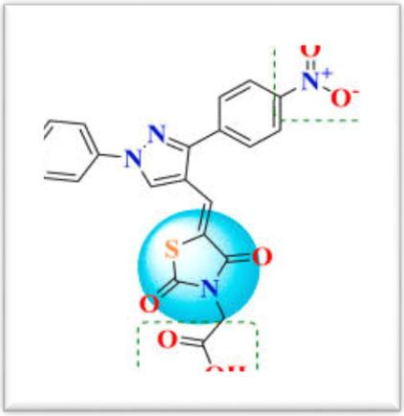


Figure 10: Novel TZD compound (Bansal et al., 2019)

Bansal et al,2019 synthesized fourteen novel derivatives of thiazolidine -2,4 –dione combined with a pyrazole moiety at the 5th position using four step reaction procedure. Among them, the novel compound (figure 10) demonstrated potential efficacy in case of diabetic patients by reducing blood glucose level notably (Bansal et al., 2019).

The SAR study indicates that the presence of acidic head at the 3rd position of TZD along with the presence of pyrazole ring at the 5th position are responsible to elevate the efficacy the novel compound.

The presence of Electron withdrawing group (EWG) with aryl ring is also accountable to elevate the efficacy of novel compound (Bansal et al., 2019).

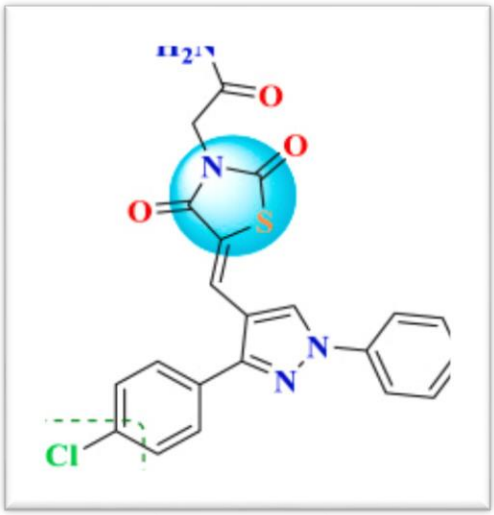


Figure 11: Novel TZD compound (Naim, Alam, Nawaz, et al., 2017*a)*

Mohammad Javed Naim et al.,2017 synthesized amide derivatives connected at 3rd position of TZD. The researcher team selected 11 chemicals from the amide derivatives set because of having good gliding score and they were performed in vivo antidiabetic tests. Among them, four chemicals showed better anti-diabetic activity in vivo test in comparison to Rosiglitazone and pioglitazone. However, the above novel compound shows better binding affinity with SER342, ILE281, pi-pi with ARG 288 and halogen bond with LYS 367.

The analysis of SAR indicated the incorporation of electron withdrawing group (EWG) such as F, Cl and NO2 at the aryl group with pyrazole ring exhibited a greater ability to enhance gene expression and insulin action.

The presence of nitro group at the para position of the aryl ring exhibited greater potency. The potency of the novel compound decreased while adding more than one chloro group on the phenyl ring (Naim et al., 2017a), (Naim, Alam, Ahmad, et al., 2017).

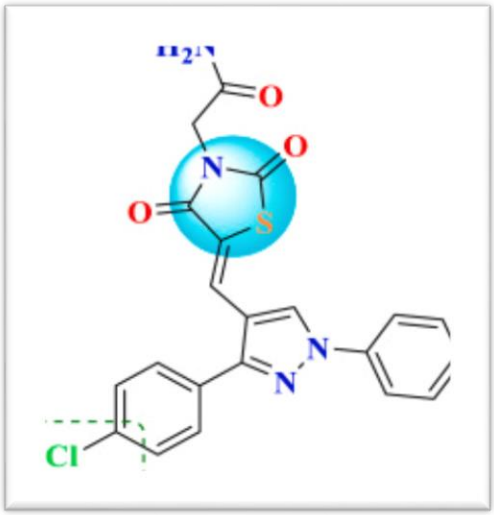


Figure 12: Novel TZD compound (S29 AND S30) (Nazreen et al., 2014)

Nazreen et al.,2015 invented bioactive derivatives combining phenoxy acetic acid with thiazolidinedione exhibited potent PPAR- gamma agonist reducing the blood sugar level in body. In comparison to pioglitazone and rosiglitazone, S-29 and S-30 demonstrated PPAR – gamma transactivation results of 54.21% and 55.41% respectively. In addition, the compounds exhibited higher docking scores than rosiglitazone in the molecular docking studies. Furthermore, the novel molecules have shown no injury or damage in hepatic cells Therefore, the compounds are selected as potential candidates for the development of new antidiabetic drugs (Nazreen et al., 2014).

The analysis of SAR indicates that o-substituted phenoxy acetic acid has higher efficacy in decreasing blood glucose level in comparison to p- substituted phenoxy acetic acid.

The novel compound (S-30) has shown grater efficacy in comparison to S-29. The inclusion of electron withdrawing group (NO2) in the aromatic ring decreased the activity.

The inclusion of methoxy group (-OCH3) in the aromatic ring increased the bioactivity but the more inclusion decreased (Nazreen et al., 2014).

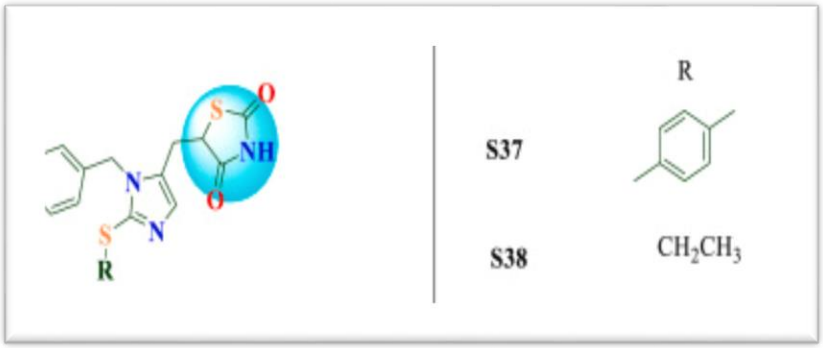


Figure 13: Novel TZD compound (S37 and S38) (Shakour et al., 2021).

Shakour et al., 2021 investigated novel anti-diabetic drugs by synthesizing a library of 13 imidazolylmethyl-l-2,4-thiazolidinediones. In silico, in vivo studies were done in case of the novel drugs. The results demonstrated that all the drugs possess antidiabetic standard drug, pioglitazone. There are no severe adverse effects shown in case of S37. Furthermore, S37 exhibited a substantial decrease the blood glucose level without gaining weight (Shakour et al., 2021).The analysis of SAR indicates that the presence of alkyl group in the imidazole ring in place of benzyl ring diminished the activity of the compound.

S-benzyl ring demonstrated greater efficacy in comparison to S-methyl groups. Furthermore, the presence of ethyl substituent on the S-benzyl ring prolonged the anti-hyperglycemic activity. The existence of fluorine on the both para position of the S-benzyl ring and the para position of N-benzyl ring augmented the bioactivity of the novel drugs (Shakour et al., 2021).

Figure 14: Novel TZD compound (Nazreen et al., 2014)

Nazreen et al.,used hydrogen gas and catalyst (pd/C) to make a notable library of chromone – 2,4 –thiazolidinedione for the rationalization of novel anti-diabetic drugs. It has been confirmed that S39 and S40 are the same effective as pioglitazone in case of lowering blood glucose level. The analysis of the novel compound indicates that Pioglitazone, S-39, S-40 were same because they all have one hydrogen bond with HIS449, one carbon bond with ARG288. At last, the researchers are eyeing on these compounds because of having non-toxic nature. The presence of olefinic bonds in the chromone ring makes the novel S39 and S40 more promising (Nazreen et al., 2014) .

The incorporation of electron withdrawing group (EWG- OCH3, Cl, F, Br and I) in the meta position of the chromone ring in these novel compounds is liable for the augmentation of the anti-diabetic activity (Basak et al., 2024), (Nazreen et al., 2014).

Figure 15: Novel TZD compound (Sameeh et al., 2022)

Sameeh et al., 2022 determines to generate anti-hyperglycemic derivatives by adding an extra TZD fragment in the glitazone class drug (S36). The novel compounds were examined on the

nuclear receptor PPAR- gamma and alpha-amylase. The investigated compounds (S-35 and S36) exhibited the binding ability with CYS 285, GLU 233, SER 299, HIS 323, ASP 300, ARG 195 and other more active sites in PPAR-gamma. The presence of acetyl, carboxyl functional groups enhance the anti-hyperglycemic activity of the compound but the incorporation of acarbose decreased the efficacy. The introduction of K-salt, acetyl chloride and thiadiazol diminished the anti-diabetic activity. The inclusion of extra TZD fragment in the drug prolonged the bioactivity in PPAR- gamma receptor (Sameeh et al., 2022).

Chapter 6

The current status of TZD and Non-TZD classes of drugs

Researchers have left no stone unturned to rationalize the novel anti-hyperglycemic drugs having non- TZD scaffold with potential activation in PPAR–gamma receptors. Because the burning question arises with TZD scaffold in anti–diabetic drugs is fluid retention, weight gain, bone fractures, liver failure, hypoglycemia, allergic skin reaction and so on.

Table 01*:* The current status of TZD and Non-TZD classes of drugs (Soccio et al.,2014).

Serial no	Drug class	Drug name	Current status /Description of the drugs
1.	Classic PPAR gamma agonist (TZD)	Cioglitazone,	The first TZD drug was developed 1983 but it is never used clinically due to severe hepatotoxicity.
		Darglitazone	It has been withdrawn due to hepatotoxicity.
2.	Classic PPAR gamma agonist (TZD)	Rosiglitazone	It was used widely from 1999 to 2007 but the usage of the drug is available in the US still. But in the most countries the drug has withdrawn from the market due to heart attacks
3.	Classic PPAR gamma agonist (TZD)	Rivoglitazone	Not launched in the market
		Englitazone	Not launched in the market
		Netoglitazone	Not launched in the market
		Troglitazone	It has been withdrawn due to hepatotoxicity.
4.	Classic PPAR gamma agonist (TZD)	Lobeglitazone	Available in the market.
		Pioglitazone	Available in the market
5.	Selective PPAR – gamma modulators, (SSPARM)	Balaglitazone	Partial agonist. Fewer side effects are visible in clinical trials. It is not launched in the market.
6	Partial agonists affecting PPAR-gamma Ser 273	MRL-24, SR-1664, GQ-16, Amorfrutin-1	Inhibit the phosphorylation of PPAR –gamma SER-273 and decrease the classical agonism, fewer side effects are visible in case of preclinical studies.
7.	m TOT modulator TZDs	MSDC-0160, MSDC-0602	They are standard drug, pioglitazone derivatives with less classical PPAR- gamma agonism, has effects on mitochondria. However, this class of drugs are in clinical development.

8.	Non- TZD PPAR-gamma agonists	INT-131, GW 1929, MBX - 102	No TZD scaffold is available in these anti-diabetic Drugs. They have exhibited potential PPAR- Gamma agonism and fewer side effects on Preclinical and clinical studies.

Chapter 7

Components of Molecular Docking (Auto Dock Vina)

Molecular docking becomes a promising technique through which small molecules can be docked into the macromolecule to determine the binding affinity for novel drug discovery perspective. Nowadays, flexible docking is the common form of molecular docking. It is a docking mechanism in where both macromolecule (protein) and small molecule (ligand) are considered in flexible form. There are two main components of molecular docking include Search algorithm and Scoring function. Search algorithm is classified into Genetic algorithm, Lamarckian genetic algorithm (LGA), Stochastic search and systematic search. Autodock4 follows genetic algorithm that represents the small molecules as chromosomes and the chromosomes are mutated randomly. It means the small molecules rotate in different angels and generate multiple orientations (poses). Thus, the docking process selects the high scoring pose. After that, the selecting high scoring pose combines with the location of another high scoring pose to generate a new child pose or next generation. The mechanism continues until the high scoring pose is not identified *(Lec55 (1)y, n.d.)*.

On the other hand, AutoDock Vina follows iterative global search algorithm that is the combination of genetic algorithm with local optimization. The local optimization part ensures that the protein is in rigid form and has a defined binding pocket where small molecule mutates to form multiple orientations (poses) for finding out high scoring pose in (Kcal / mol) unit (Pagadala et al., 2017).

After search algorithm, scoring function is one of the main components for molecular docking to calculate the binding affinity (Kcal /mol), to identify the molecular interactions. AutoDock

vina follows empirical function with knowledge-based function which considers Van der Waals (steric interaction), hydrogen bonding, electrostatic interactions, hydrophobic interactions and conformational entropy penalty (Eberhardt et al., 2021).

Table 02: Names of conformational search algorithms & Different types of molecular docking tools based on different scoring function (Ferreira et al, 2015).

Docking program	Developer	Search algorithm	Scoring function	Free for academia?
AutoDock	Scripps Research Institute (CA, USA)	GA, MC	FF	Yes
DOCK	University of California, San Francisco (USA)	IC, EM	FF	Yes
FlexX	BioSolveIT (Sankt Augustin, Germany)	IC	E	No
FRED	OpenEye Scientific Software (NM, USA)	ES	KB	Yes
Glide	Schrödinger (NY, USA)	ES, EM, MC	E	No

Limitations in AutoDock Vina

- Vina is not designed for covalent and metallic bonding.
- It does not consider solvation effects. Water molecule has been removed during docking process.
- Macromolecule (protein) is in rigid form that means the side chains of macromolecule cannot rotate during docking process.
- Large ligands cannot be docked in auto dock vina. So, it is necessary to split the ligands into smaller pieces.
- Vina does not account for electrostatic interactions completely (Alonso et al., 2006).

Chapter 8

Non-TZDS As Anti-diabetic Drugs

TZD possessed drugs like lobeglitazone, pioglitazone are now available in the market due to its high compliance to the diabetic patients suffering from other complications in kidney and heart. Despite the beneficial effects of the conventional drugs, researchers have left no stone unturned to rationalize the novel anti-hyperglycemic drugs having non- TZD scaffold with potential activation in PPAR–gamma receptors. Because the burning question arises with TZD scaffold in anti–diabetic drugs is fluid retention, weight gain, bone fractures, liver failure, hypoglycemia, allergic skin reaction and so on. To overcome the problem, Ahmed H.A and Alkali I. Y. (2018) investigated on some phytochemicals having no TZD scaffold against PPAR-gamma namely diosmetin, syringe Isovitexin, Quercetin, Mangiferin, Plumbagin, Beta-sitosterol, 7-(2-hydroxyethyl)-3 –methyl -8-(1-phenylethylideneaminoamino) purine 2,6 –dione and Lupe-20-ene-3-One against standard TZD drug (Pioglitazone). Based on the findings of the study these phytochemicals, diosmetin, Beta-sitosterol, 7-(2- hydroxyethyl)-3–methyl -8-(1-phenylethylideneaminoamino) purine 2,6–dione and Lupe-20- ene-3-One, Isovitexin, Quercetin, Mangiferin have demonstrated higher affinity against PPAR-gamma receptor as anti-diabetic drugs (Ahmed & Alkali, 2018).

Dhadke H. et. al, (2024) investigated on the inhibitory potential of non-TZD classes drugs namely repaglinide and piperine ligands. The ligands demonstrated acceptable binding energy values ranging from -8.3 Kcal to -9.3 Kcal. The inhibitory potential of non-TZD classes plays more significant rule to treat the abnormalities in PPAR signaling (Dhadke et al., 2024).

Chapter 9

Crystal Structure of The Human Nuclear Receptor PPAR-Gamma

The peroxisome proliferator activated receptors (PPAR) are members of nuclear receptor supergene family play a vital role in both glucose and lipid homeostasis. The PPARs consist of an N-terminal A/B domain, a DNA binding C domain, a D domain and C-terminal ligand binding domain (E/F domain). The promoter (special sequence of DNA) binds to DNA binding C domain of PPARs and ligands bind to (E/F) domain of PPARs. Jonas Uppenbergs et al., 1998 reported that there is total 12 alpha helices and a small beta-sheet consisting of four strands available in ligand binding domain (LBD). They added that the region between helix 1 and helix 3 is called omega loop (LBD) in where more than 20 amino acids residues have been inserted. Helix 2 has been divided into 2a and 2b by beta-strand.

Therefore, 11 amino acids residues have been missed because of high mobility in the region. Because of the separation of helix 2, the groove has been visible between helix 1 and helix 3. Furthermore, they documented that the cavity between helix 1 and beta –sheet is delimited by Phe 226, Pro 227, Ile 296 and Met 329. Another cavity, helix 3,5 10,11 and 12 displayed predominantly polar surfaces created by His 323, Tyr 327, Lys 367, His 449 and Tyr 473 PPAR-gamma nuclear receptor. At last, the ligand binding pocket of apo PPAR-gamma LBD is larger, the accessible of ligands become easy to the surface for both polar and nonpolar molecule (Uppenberg et al., 1998).

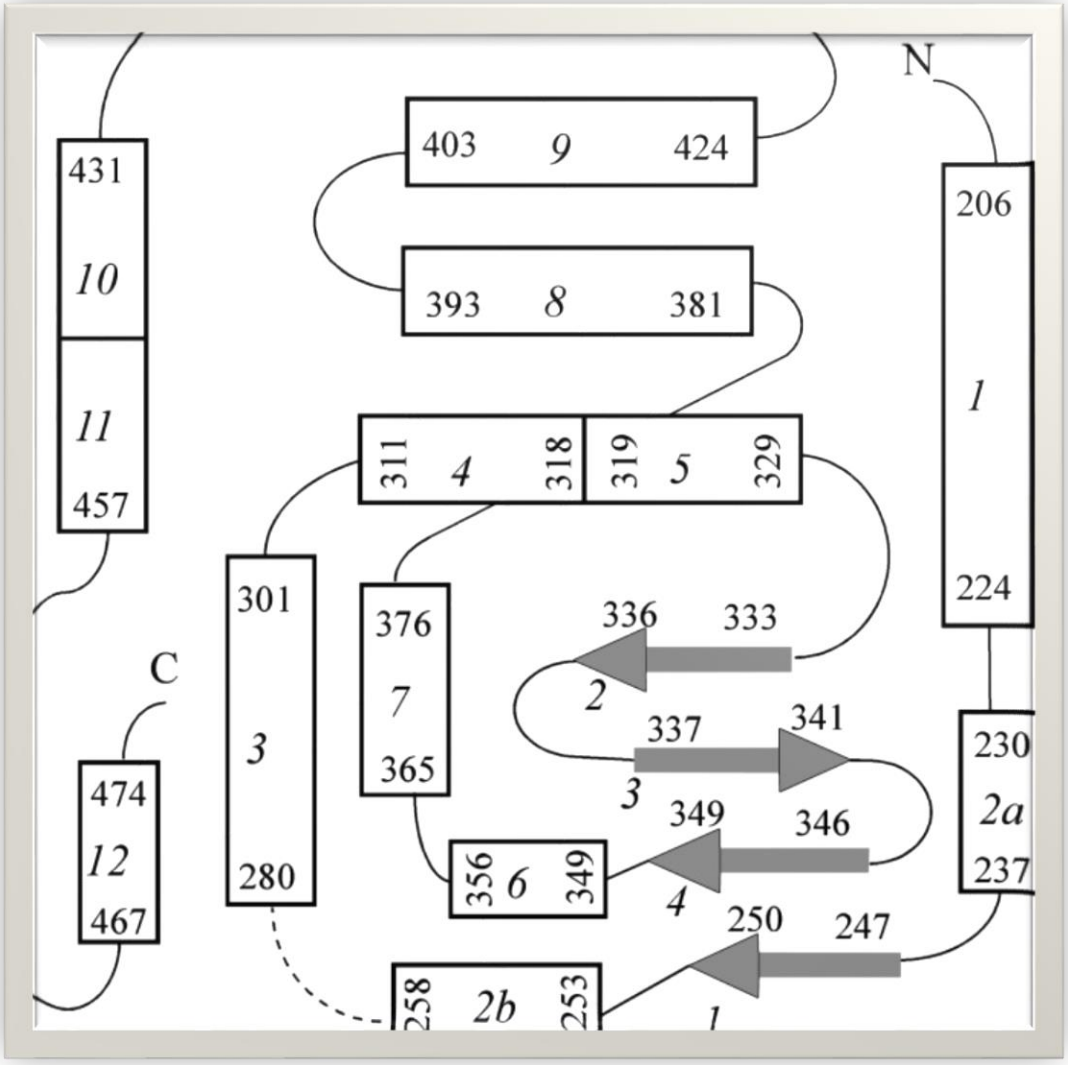


Figure 16: A secondary structure figure of apo-PPAR-gamma LBD. (Uppenberg et al., 1998).

9.1 General Function of PPARs Receptors

Jian Wu et al., (2022) stated that PPAR-γ binds with ligands, after that the complex PPAR-gamma starts translocating to the nucleus and binds with retinoid X receptor alpha (RXR-alpha) in nucleus and forms heterodimer. Then The heterodimer binds with peroxisome proliferator response element (PPRE) of the target gene and expresses the its biological function (lipid metabolism, glucose metabolism, inflammation, cell proliferation, carcinogenesis, drug sensitization). Vladimir V. Sobolev et. al., 2022 documented that the number of PPARs are

highly visible T-cells, macrophages, dendritic cells, B-cells, neutrophils because PPAR-gamma regulates the lipid metabolism in immune cells along with modulating the immune response. Glut, Ldlr, Lrp8, Scarb1 and Vldlr are the prime receptors to control glucose and fatty acids uptakes. The generation of these receptors occurs during the activation of T-cell receptor (Tcr). Because after the activation of Tcr, it activates PPAR-gamma for the immune cells' higher metabolic demands. They also added that under certain conditions, the agonists of PPAR- Gamma are also responsible to induce the apoptosis mechanism in the cells. Moreover, the PPAR- gamma is also potential molecular target for chronic inflammatory disorders include psoriasis (Sauer, 2015).

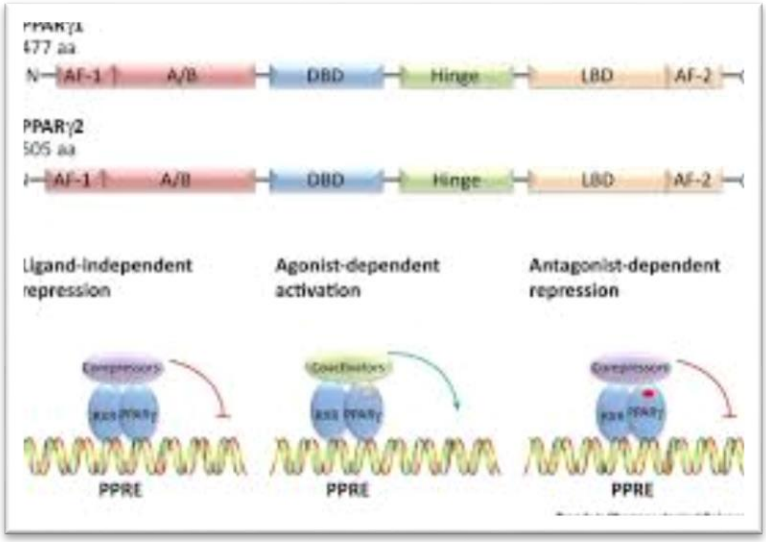


Fig 17: The mechanisms of modulation of PPRA- γ (ligand-independent repression, agonist-dependent activation, antagonist-dependent repression) (Sauer,2015).

In figure 17, it is shown that, Exogenous substances bind with PPAR- γ receptor and induce the conformational change. The changeable structure fits for binding with different kinds of co-activators and co-repressors (endogenous substances). Therefore, the presence of endogenous substances in the body plays a vital role whether transcriptional activity of PPAR-gamma receptors will continue or not (Sauer, 2015).

9.2 Killing Cancer Cells by Starvation

All kinds of cells in the body depend on energy. But cancer cells need more energy in comparison to the normal cells. By blocking the PPAR-gamma receptors (Antagonist dependent repression), it is possible to halt the activity of transport protein (GLUT1) that helps cells to absorb glucose in the cells. Therefore, the cancer cells will suffer from starvation due to break down the homeostasis of glucose and lipid in the cancer cell and the condition activate the suicide program (Apoptosis, Autophagy) which leads to the death of the cancer cells (*Killing Cancer Cells By Starvation (3)*, n.d.).

Chapter 10

Methodology

10.1 Protein Preparation

3D crystal structure of protein (PDB ID: 1FM6) was downloaded from protein data bank (PDB). There are multiple chains available in the macromolecule such as retinoic acid receptor RXR-alpha, peroxisome proliferator activated receptor gamma, steroid receptor co-activator molecules. Apart from these molecules, there are some ligands (9 cis-retinoic acid) that have been removed in the protein preparation. As the standard drug is rosiglitazone that binds in the PPAR-gamma chain. Therefore, all the unnecessary chains, molecules and water molecules have been removed using discovery studio. Then the file has been converted into PDB-file. After that, using (Auto-dock-Tools-1.5.6), the PDB file has been updated through repairing missing residues, adding polar hydrogens and charges. So, the PDB file has been transferred into PDBQT file for rigid-flexible docking and the file was saved.

10.2 Ligand Preparation

The ligands were imported using PubChem and Zinc15 database. The ligands are (nomilin, obacunone, obacunoic acid, ichagin, limonite –A-ring lactone, limonin) that were downloaded in SDF 3D format. After that, the SDF format been transferred into PDB file through Avogadro and has been sure that the hydrogen atom is available in all ligands. At last, the PDB file has been transferred into PDBQT file through AutoDock tools and the file was saved.

10.3 Molecular Modeling

Autodock vina has been used to perform the docking simulations. The grid points in X, Y and Z dimensions were set at 30*30*30. The grid center X, Y and Z were placed in the active site of ligand binding domain of PPAR-gamma and the reference drug was rosiglitazone. Command

prompt was used to find out docking results and the results (poses) were ranked in the order of increasing binding affinity in (Kcal/mol) unit.

10.4 Molecular docking studies

11 limonoids namely (nomilin, obacunone, obacunoic acid, ichagin, limonite –A-ring lactone, limonin, calamine, cyclocalamin, limonol, limonin -17-beta-D-glucopyranoside) have been analyzed for docking studies.

Chapter 11:

Molecular docking results & non-bond interaction with 2D image:

The figure 18 demonstrates the molecular docking results of phytochemicals synthesized in the limonin biosynthesis pathway in the citrus fruits.

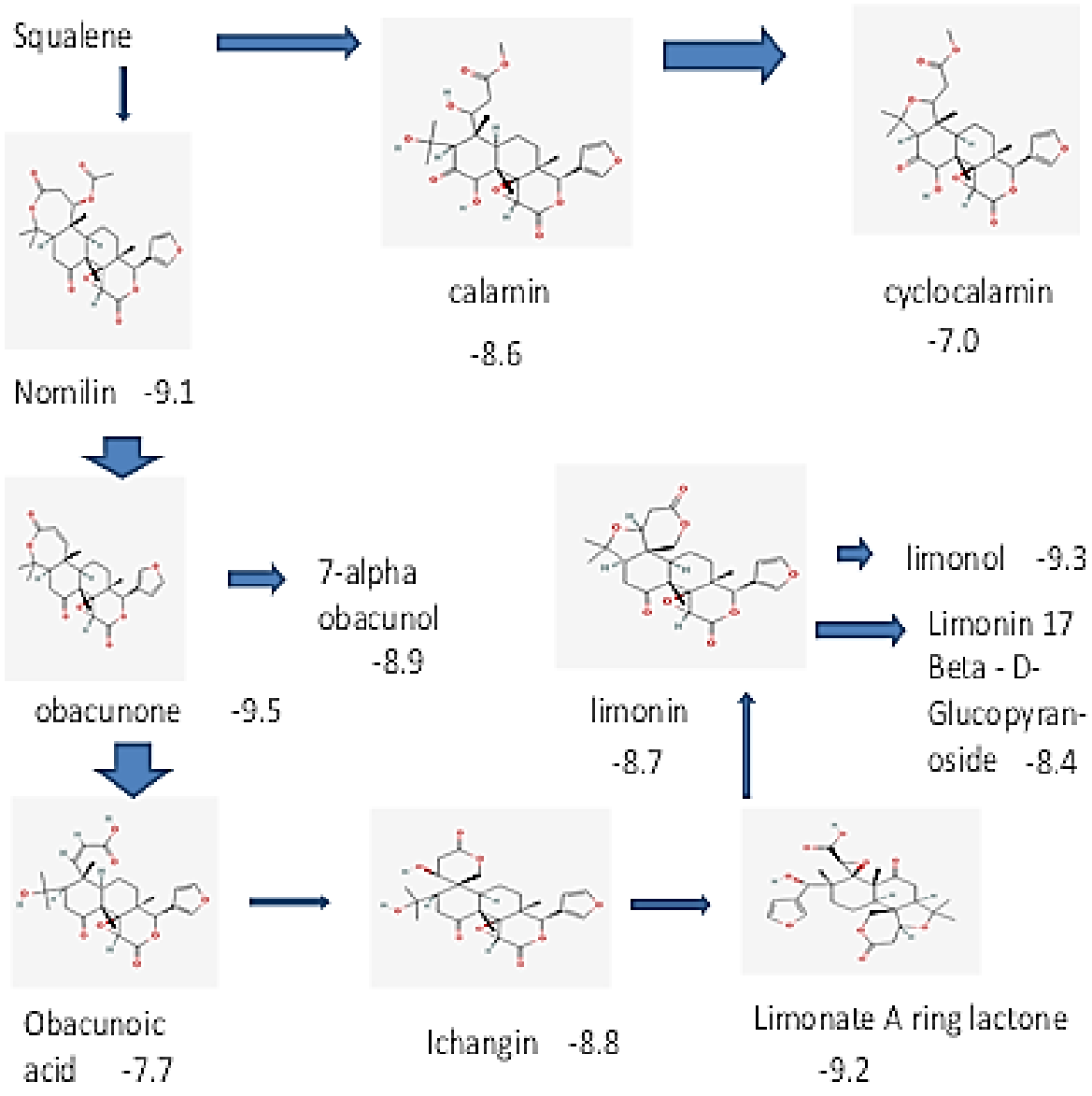


Figure 18: Limonin Biosynthesis pathway with binding energy in (kcal/mole) unit.

Table 3: The table demonstrates the molecular docking results of investigating limonoids.

Limonoids	Docking result (kcal/mole)
Nomilin	−9.1
Obacunone	−9.5
Obacunoic Acid	−7.7
Ichangin	−8.8
Limonate-A-ring lactone	−9.2
Limonin	−8.7
Calamin	−8.6
Cyclocalamin	−7.0
Limonol	−9.3
Limonin 17 Beta- D-Glucopyranoside	−8.4
Herkinorin	−9.6

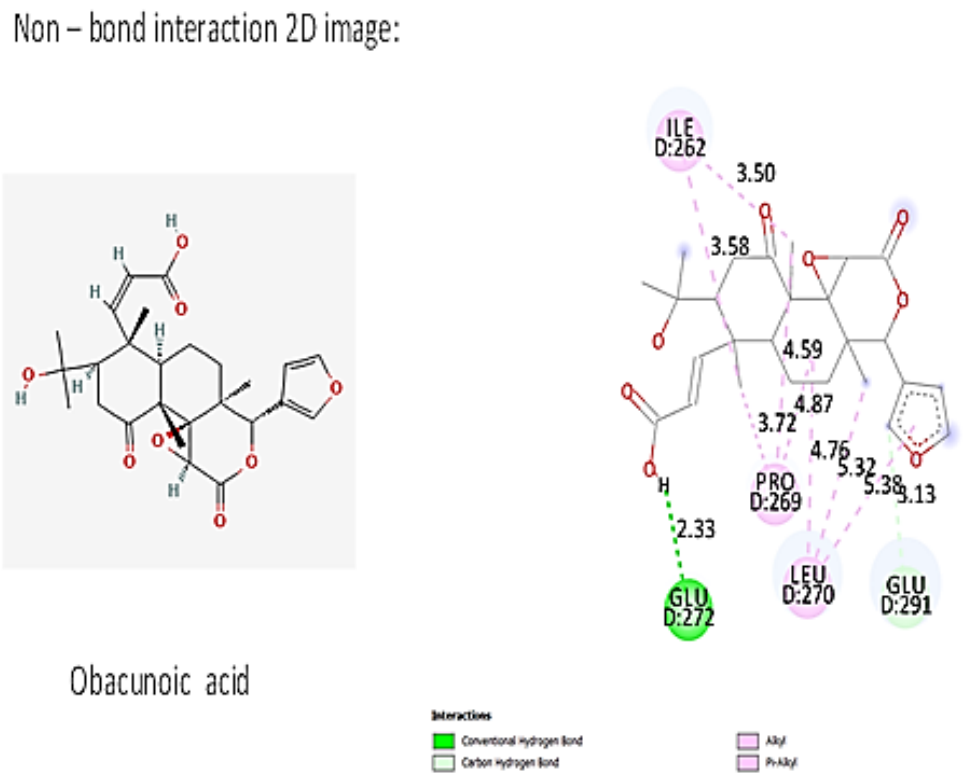
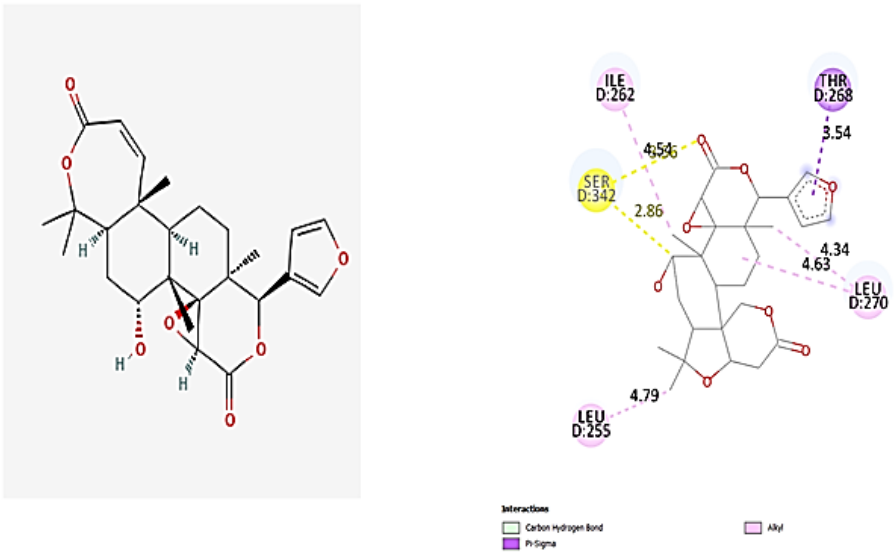


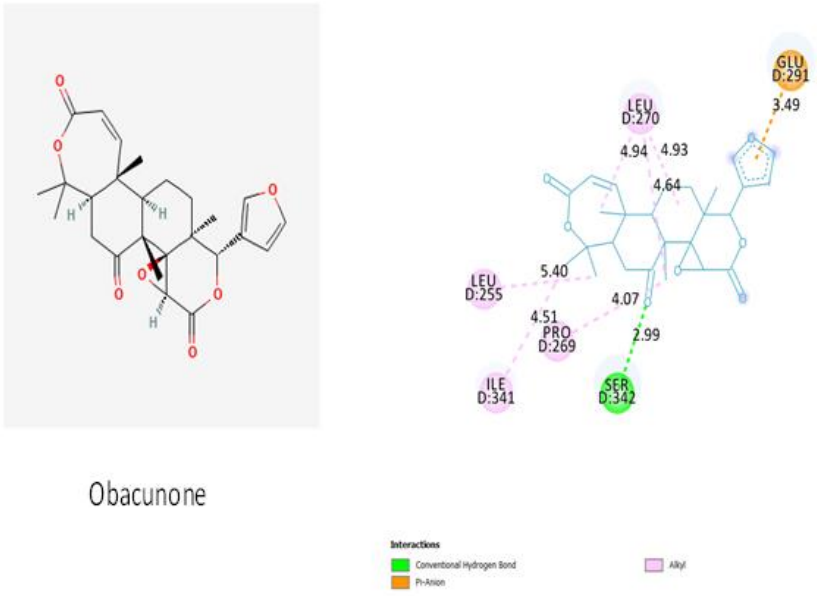
Figure: 19 The non-bond interaction and distance between Obacunoic acid and the residues of PPAR-γ receptor. There are different types of bonds visible with difference colors like

conventional hydrogen bond with green (2.33 angstrom), carbon hydrogen bond, hydrophobic bond (alkyl, pi-alkyl bond)



7 alpha -Obacunol

Figure: 20 The non-bond interaction and distance between 7-alpha-obacunol and the residues of PPAR-γ receptor. There are different types of bonds visible with difference colors like carbon, hydrogen bond, hydrophobic bond (alkyl, pi-sigma)



Obacunone

Figure: 21 The non-bond interaction and distance between obacunone and the residues of PPAR-γ receptor. There are different types of bonds visible with difference colors like

conventional hydrogen bond in green color (2.99 angstrom), electrostatic bond with furan ring (3.49 Å) and hydrophobic bonds with alkyl groups.

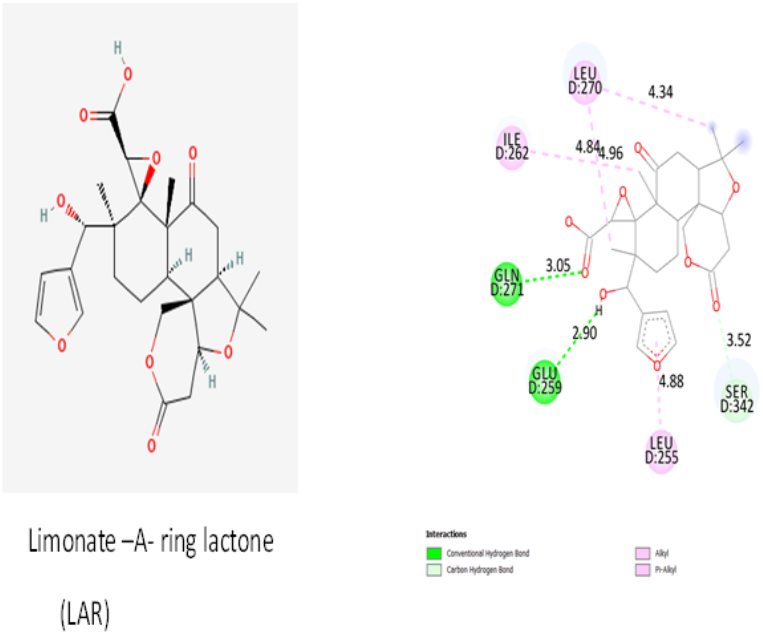


Figure: 22 The non-bond interaction and distance between LAR and the residues of PPAR-γ receptor. Two conventional hydrogen bonds are visible in green colors with optimal distance. One carbon hydrogen is also visible. And the rest of bonds are hydrophobic bonds (alkyl, pi-alkyl). Pi-alkyl bond is formed between furan ring and (LEU D: 255).

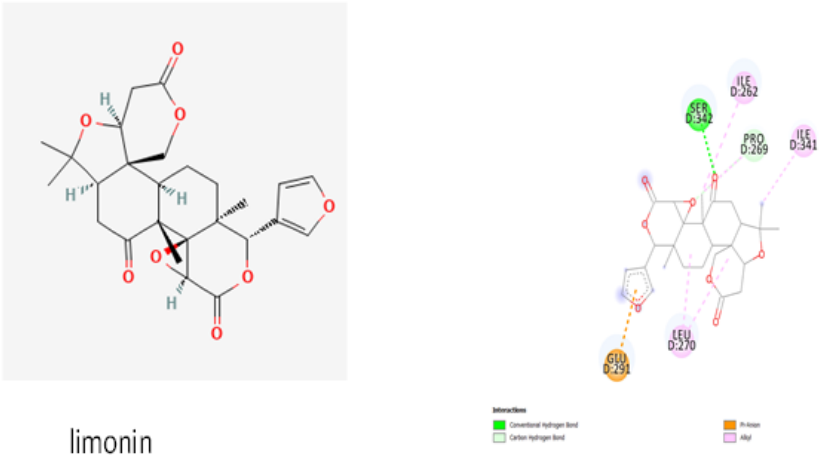


Figure: 23 The non-bond interaction and distance between limonin and the residues of PPAR-γ receptor. One electrostatic bond is formed between furan ring and GLU D: 291. One

conventional hydrogen bond is visible. One carbon-hydrogen bond is also visible and the rests are hydrophobic bonds.

Pharmacokinetic Analysis:

Absorption:

Table 4 has demonstrated the absorption profile of different types of investigating Limonoids (phytochemicals) namely obacunone, obacunoic acid, 7-alpha –Obacunol, limonin, limonate-A-Ring Lactone. The estimated values of water solubility, intestinal absorption, skin permeability, P-glycoprotein Inhibitor-I, P-glycoprotein Inhibitor-II has been included in the absorption profile.

Table 4: Absorption profile of different types of investigating Limonoids.

Phytochemical	WATER Solubility	Intestinal Absorption	Skin Permeability	P-glycoprotein Inhibitor-I	P-glycoprotein Inhibitor-II
Obacunone	-4.5	100	-2.995	No	No
Obacunoic acid	-3.36	68.463	-2.735	No	No
7-alpha Obacunol	-4.633	98.612	-3.061	No	No
Limonin	-4.379	100	-2.852	Yes	No
Limonate-A-Ring Lactone	-3.223	81.306	-2.735	No	No

Distribution:

Table 5 has demonstrated the distribution profile of different types of investigating Limonoids (phytochemicals) namely obacunone, obacunoic acid, 7-alpha –Obacunol, limonin, limonate-A-Ring Lactone. The estimated values of BBB Permeability, CNS Permeability has been included in the distribution profile.

Table5: Distribution profile of different types of investigating Limonoids.

Table 5 has demonstrated the absorption profile of different types of investigating Limonoids (phytochemicals) namely obacunone, obacunoic acid, 7-alpha –Obacunol, limonin, limonate-A-Ring Lactone. The estimated values of CYP 2D6 substrate, CYP 3A4 substrate, CYP 1A2 inhibitor,CYP 2C19 inhibitor, CYP 3A4 inhibitor has been included in the metabolism profile.

Phytochemical name	BBB Permeability	CNS Permeability
Obacunone	-0.656	**-2.981**
Obacunoic acid	**-0.819**	**-3.113**
7-alpha - Obacunol	**-0.541**	**-2.998**
Limonin	**-0.841**	**-2.983**
Limonate-A-Ring Lactone	**-0.811**	**-3.22**

Metabolism:

Table 6 has demonstrated the metabolism profile of different types of investigating Limonoids (phytochemicals) namely obacunone, obacunoic acid, 7-alpha –Obacunol, limonin, limonate-A-Ring Lactone. The estimated values of CYP 2D6 Substrate, CYP 3A4 substrate, CYP 1A2 inhibitor, CYP 2C19 inhibitor, CYP 3A4 inhibitor has been included in the metabolism profile.

Table 6: Metabolism profile of different types of investigating Limonoids

PHYTOCHEML NAME	**CYP 2D6 SUBSTRATE**	**CYP 3A4 SUBSTRATE**	**CYP**1A2 **Inhibitor**	**CYP 2C19 Inhibitor**	**CYP 3A4 Inhibitor**
Obacunone	No	No	No	No	Yes
Obacunoic acid	No	Yes	No	No	No
7-alpha Obacunol	No	Yes	No	No	Yes
Limonin	No	Yes	No	No	No
Limonate-A-Ring Lactone	No	Yes	No	No	No

Toxicity

Table 6 has demonstrated the toxicity profile of different types of investigating Limonoids (phytochemicals) namely obacunone, obacunoic acid, 7-alpha –Obacunol, limonin, limonate-A-Ring Lactone. The estimated values of total clearance, herg-I inhibitor, herg-II inhibitor, hepatotoxicity, skin sensitization has been included in the toxicity profile.

Table 7: Toxicity profile of different types of investigating Limonoids.

Phytochemical Name	**Total Clearance**	**Herg-I Inhibitor**	**Herg-II Inhibitor**	**Hepatotoxicity**	**Skin Sensitization**
Obacunone	0.159	No	No	No	No
Obacunoic Acid	0.249	No	No	Yes	No
7-alpha - Obacunol	0.198	No	No	Yes	No
Limonin	0.092	No	No	No	No

Limonate-A-Ring Lactone	0.191	No	No	Yes	No

Chapter: 12

Discussion

Acetyl – coA plays a significant role as central hub by linking the glycolysis and citric acid cycle, acts as a precursor of multiple primary metabolic products (fatty acids), secondary metabolites (fats, waxes, tetracyclines, anthraquinones, terpenes, steroids). The molecules of terpenes are constructed of multiple isoprene (iso-C5) units. In isoprene unit, there are available of multiple carbon-carbon double bonds which help the isoprene units to induce the addition reactions, the hyperconjugation effects, the resonance of electrons in epoxysqualene molecule leading to limonin biosynthesis pathway in the presence of cytochrome P450 enzymes in the citrus fruits. Nomilin, Obacunone, Obacunoic acid, Ichagin, Limonate –A-ring lactone, Limonin are the prime phytochemicals in the biosynthesis pathway. These are the phytochemicals known as Limonoids**.** Generally, limonoids contain multiple rings, furan ring with highly oxygenated. They abound with oxygen related functional group like keto group, aldehyde group, epoxy group, carboxylic group and alkyl group. These oxygenated functional groups provide conventional hydrogen bond, carbon hydrogen bond to interact with the active site of the PPAR-GAMMA receptor. Furan ring is very essential oxygenated ring for obacunone, limonin, limonite –A-ring lactone (LAR),7-alpha obacunol to provide electrostatic and hydrophobic bond. Therefore, the four phytochemicals have demonstrated high binding affinity. Conventional hydrogen bond is also important for higher binding affinity. For example: limonite-A-ring lactone. Carbon hydrogen bond, hydrophobic bond are also fruitful

for higher binding affinity. The order of bond strength - electrostatic bond > conventional hydrogen bond > carbon-hydrogen bond > hydrophobic bond. But steric-clash may weaken the binding affinity of obacunoic acid with PPAR-gamma receptor. In the case of obacunoic acid, there are multiple bonds formed like conventional hydrogen bond, carbon hydrogen bond, hydrophobic bond. But steric clash between PRO D:269 and LEU D:270 residues may weaken the binding affinity. Therefore, the bond- lengths between LEU D:270 and furan ring, alkyl group have increased and it is also noted that there is no electrostatic bond formed in the acidic molecule. Most of the bonds are hydrophobic bonds in the obacunoic acid.

Drug distribution analysis confirms that there is no Blood Brain Barrier (BBB) permeability

issues have been detected in the pkcsm software. So, the compounds have no side or adverse effects in the internal brain.

Drug Metabolism is classified into two parts are phase I reactions and phase II reactions. The prime molecule of phase I reaction is cytochrome P450 (CYP450). Multiple isoforms are available in the human body namely CYP 1A2, CYP 2C9, CYP 2C19, CYP3A4 etc. Drug metabolism analysis tells us that all the investigating limonoids are CYP 3A4 substrate. It must be noted that 7-alpha – obacunol & obacunone are CYP 3A4 inhibitor. So, the two compounds may interfere the metabolism of CYP 3A4 related drugs. Therefore, the accumulation of drugs in the body can increase and it can result in drug toxicity.

Drug absorption refers to the movement of the drugs into the internal parts of the human body through the bloodstream after administration. The absorption analysis of the limonoids through pkcsm software indicates that the investigating limonoids are lipophilic drugs. Therefore, the intestinal absorption of limonoids is very high.

The toxicity analysis informs that 7-alpha – obacunol, obacunoic acid and Limonate-A-Ring Lactone are risky for patients who are suffering from hepatitis, cirrhosis, fatty liver diseases, liver cancer & acute liver failure.

Chapter: 13

Conclusion

Based on this silico studies, it is confirmed that obacunone, limonite-A- ring lactone, nomilin. 7-alpha obacunol, limonol, these five non-TZD drug class (limonoids) has demonstrated good binding affinity with PPAR-γ receptor. It must be noted that the nuclear receptors are connected with cardiovascular disease, cancer, inflammatory, kidney problems, neuro problems and so on. It has been confirmed based on molecular docking studies the investigating phytochemicals have demonstrated notable binding affinity. but the burning question is "which mechanism will be followed by these phytochemicals"? The answer might be ligand-independent repression, agonist-dependent activation, antagonist-dependent repression. By blocking the PPAR- γ receptors (Antagonist dependent repression), it is possible to halt the activity of transport protein (GLUT1) that helps cells to absorb glucose in the cancer cells. Therefore, these phytochemicals might be considered as more potent, safer carcinopreventive agents than conventional chemotherapeutic agents. At this moment, the researchers should focus on the vitro, vivo, animal studies to identify the real functions of the non-TZD drugs for well-being of human world.

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