“PHARMACOPHORE MODELLING AND VIRTUAL SCREENING OF PPAR GAMMA AGONIST FOR THE TREATMENT OF DIABETES”

A PROJECT SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfilment of the requirements for the degree of

Bachelor of Pharmacy

BY

DESAI KATHAN K. (16BPH039)

Semester VIII

UNDER THE GUIDANCE OF

Guide:

DR. JIGNA SHAH

Co-guide:

DR. HARDIK BHATT

INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
SARKHEJ-GANDHINAGAR HIGHWAY
AHMEDABAD-382481 GUJARAT,
INDIA

APRIL 2020

CERTIFICATE

This is to certify that ***"PHARMACOPHORE MODELLING AND VIRTUAL SCREENING OF PPAR GAMMA AGONIST FOR THE TREATMENT OF DIABETES"*** *is the bonafide work carried out by* ***KATHAN DESAI****(16BPH039), B. Pharm semester VIII under our guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2019-2020. This work is up to my satisfaction.*

Guide:

DR. JIGNA SHAH
M. Pharm., Ph.D.,
Professor and Head,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Co-Guide:

DR. HADIK BHATT
M. Pharm, Ph.D., MRSC
Associate Professor and Head,
Department of Pharm. Chem.,
Institute of Pharmacy,
Nirma University

Prof. Manjunath Ghate
M. Pharm., Ph.D.,
Director, Institute of Pharmacy,
Nirma University
Ahmedabad

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KATHAN DESAI (16BPH039),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Guide:

DR. JIGNA SHAH
M. Pharm., Ph.D.,
Professor and Head,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

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Sarkhej - Gandhinagar Highway
Ahmedabad-382481
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Department of Pharmacology,
Institute of Pharmacy,
Nirma University

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DR. HADIK BHATT
M. Pharm, Ph.D., MRSC
Associate professor and Head,
Department of Pharm. Chem.,
Institute of Pharmacy,
Nirma University

Date: 05 / 05 /2020

DECLARATION

I, ***KATHAN DESAI (16BPH039),*** *student of VIII Semester of B. Pharm. at Institute of Pharmacy, Nirma University, hereby declare that my project entitled* ***"PHARMACOPHORE MODELLING AND VIRTUAL SCREENING OF PPAR GAMMA AGONIST FOR THE TREATMENT OF DIABETES"*** *is a result of culmination of my sincere efforts. I declare that the submitted project is done solely by me and to the best of my knowledge, no such work is done by any other person for the award of degree or diploma or for any other means. I also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.*

KATHAN DESAI (16BPH039),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 05 /05 / 2020___

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KATHAN DESAI (16BPH039),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 05 /05 / 2020

ACKNOWLEDGEMENT

This succeeding work presented under this thesis is on the topic of "PHARMACOPHORE MODELLING AND VIRTUAL SCREENING OF PPAR GAMMA AGONIST FOR THE TREATMENT OF DIABETES DISEASE". This work was merely possible without the constant support and guidance of DR. HARDIK BHATT and DR. JIGNA SHAH. I would also like to thank DR. MANJUNATH GHATE for helping and also providing world class facilities at INSTITUTE OF PHARMACY, NIRMA UNIVERSITY which mainly made it possible to work with various in-silico software of medicinal chemistry which played a pivotal role in accomplishing the computational work. I would also like to thank KEERTI VISHWAKARMA from the bottom of my heart for always helping me out as and when required.

THANKING YOU!

Yours Sincerely,

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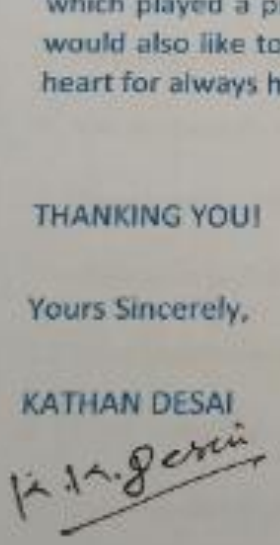

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List of Abbreviations

Sr. number	Abbreviations	Stands for
1	DM	Diabetes Mellitus
2	MODY	Maturity onset Diabetes of the Young
3	OGTT	Oral glucose tolerance test
4	HHS	Hyperosmolar Hyperglycaemic State
5	TNDM	Transient neonatal diabetes mellitus
6	SRC	Steroid receptor coactivator
7	RXR	Retinoid X receptor
8	IRS	Insulin receptor substrates
9	SOCS-3	Suppressor of cytokines signalling-3
10	ACE	Angiotensin converting enzyme
11	GASP	Genetic algorithm similarity program
12	PAI-1	Plasminogen activator inhibitor 1
13	TNF	Tumour necrosis factor
14	RAPID	Randomised pharmacophore identification
15	MOE	Molecular operating environment

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1. ABSTRACT:

As indicated by the documented report of world health organization on diabetes that 422 million people who are suffering from diabetes today worldwide. Out of 4 person 1 is overweight and out of 10 adults 1 is obese person that is having capacity to develop the disease. Insulin hormone is a key to prevent diabetes but, if production of insulin is not as per the requirement then different drugs and mechanisms are needed to produce more insulin and prevent the disease. Despite the use of different drugs which act on different mechanism, PPAR gamma is exceedingly show its effect on diabetes. Under the class of Thiazolidinediones PPAR gamma receptors which act as nuclear receptors helps to prevent the disease by gene transcription process. Rosiglitazone and Pioglitazone are the drugs that act under these PPAR gamma receptor mechanism. For the proper arrangements of ligands various computational techniques are used. Pharmacophore model generation is one of the computational techniques is used for the generation of features and it is called as ligand-based pharmacophore method. After generation of pharmacophore model, molecules are refined and best features are selected for the further process like virtual screening, molecular docking. Compounds having different EC50 values are taken from highest to lowest and from that Pharmacophore model is generated. By using GASP module and DISCOTECH module in the SYBYL software these features are generated. After performing virtual screening, several compounds are generated from that top 10 structures are selected having best QFIT value.

KATHAN DESAI
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY

2. INTRODUCTION:

Diabetes is the term that was firstly documented by the people of Egypt. Diabetes is mainly characterised by loss in the weight and polyuria. This term is later known as Diabetes mellitus (DM), the name was given by Greek physician Aertaeus. In Greek, Diabetes mellitus term can be understood by two different terms. Diabetes means "to pass through" and mellitus is basically the Latin word that is used for Honey (mainly refers to sweetness). It is an important cause of prolonged ill health and from that it is mainly associated with the premature death. Currently Diabetes mellitus has manifested as a Global epidemic because of increase in the obesity ratio of the patient and with that advent of industrialization worldwide. It is very difficult to achieve an accuracy in the measurement of the prevalence of the disease, that is happened due to two main reasons: the collection of data and Standard of that collection are varying widely in the different parts of the world.

Metabolic disorder is a group of certain ailments that occur together, including increasing of heart disease, stroke and Diabetes. In our Human body system, there are different numbers of pathways and systems that functions in synchrony to maintain a physiological state. In this type of metabolic disorder, it reduces mainly the ability of an individual to maintain the level of blood glucose in the blood vessels that results in the number of different minor and major complications.

In Diabetes, metabolic syndrome or disorder is seen by increasing the level of blood glucose level and also abnormal level of Triglycerides and Cholesterol. Diabetes mellitus is a heterogenous group of metabolic disorder which is characterised by hyperglycaemia and high blood glucose concentration (fasting plasma glucose> 7.0mmol/l, OR plasma glucose>11.1mmol/l, 2h after a meal). Blood Glucose is a major source of energy that comes from food sources we eat. Pancreas is an endocrine gland which produces hormone insulin which has major role in converting of food sources into glucose and help glucose to get in to the cells. When it is not possible for pancreas to produce enough insulin as per the requirement of body or not able to use enough insulin, the level of insulin hormones will increase and that leads to increase in blood glucose level and that to Diabetes Mellitus.

When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine that is called as Polyuria, which in turn, results in dehydration, thirst and increase in the drinking that is Polydipsia. Deficiency of insulin causes wastage and decreased in the synthesis of proteins. Another term that is Diabetic Ketoacidosis is an acute emergency and this develops in the absence of insulin that is because of accelerated breakdown of fatty tissues into acetyl-CoA, which in the absence of aerobic metabolism of carbohydrates that is converted to acetoacetate and also in beta-hydroxy butyrate which causes acidosis. If blood glucose level is increased over the long term then possibilities of organ damage and tissue damage would be also increased. That's why it is known as metabolic disorder or endocrine disorder. Treatment that is related with the low blood sugar is more common in people with type-1 and type-2 Diabetes, that is depended on the medication being used for the treatment.

In Hypoglycaemic condition the level of blood glucose is abnormally decreased. It is a very rare situation which can be seen in both, Diabetic and also in Non-diabetic Patients. Due to the Sympathetic activation of Autonomic nervous system, patients may have varying symptoms like sweaty, become agitated, weak and become immobilise and panic. Sometimes in severe condition consciousness can also be altered or can be lost. If it happens, then the patient will lead to coma, and also become epileptic. This situation leads to damage in the brain and results into death of patient. This situation can be occurred by several possibilities like too much exercise, taking too much insulin and incorrectly timed insulin. In the recent incidents of hypoglycaemia that causes both the things, defective glucose counter regulation and hypoglycaemia unawareness. Sometimes decreased in the Insulin level leads to increase in the level of Glucagon and due to that, there is an increase in the level of epinephrine or adrenaline. These are the primary Glucose counter regulatory factors that prevents or mitigate Hypoglycaemia. In most of the Hypoglycaemic cases, Patient is treated with Sugary food and also with the drinks that include sweetness or sugar. Patient with severe Hypoglycaemia can be treated by giving an injection of Glucagon or with Intravenous infusion of Dextrose when the patient is in Unconscious condition.

There is another possibility that is rare but it can be severe called as Hyperosmolar Hyperglycaemic State (HHS), which results of increase the blood glucose level leads to increase in the osmolarity with the significant process of ketoacidosis. When a blood glucose level of any person is reached greater than 300mg/dl or 16mmol/l, leads to withdrawal of water osmotically out of cells and due to that, Kidneys eventually stated the deposition of the glucose in to the Urine. These leads to increase in the osmolarity and also leads to loss of water, condition is called as Hyperosmolar Hyperglycaemic State. If replacement of this fluid is not done, both the effect osmotic effect of high glucose level and loss of water leads to Dehydration. Diabetes coma is a condition which is found in the people who are having Diabetes mellitus (DM). There are different types of Diabetic coma that are identified and explained form which one common term is low level of blood glucose in the patient of Diabetes. Another term is Hyperosmolar nonketotic coma. In this condition, extremely high blood glucose level and Dehydration can able to make the patient unconscious. Due to that a person can suffers a lot with this type of condition.

Another rare autosomal Dominant acquired kind of Diabetes which is known as Maturity onset Diabetes of the Young (MODY). This occurs due to mutation in the one of several single genes that eventually leads to affect the insulin production. In all the cases of different types of Diabetes, it is different from others and it constitutes hardly 1-2% from all. As the name suggests, due to defects in the genes, this disease varies in age at presentation and severity is according due to the specific gee target mutation. The main thing about the MODY is that the people with this type of Diabetes can control it without taking the insulin. In this type of genetic mutation that causes directly damage to the beta cells of pancreas and also cause damage to the function of these beta cells. Also, these beta cells can be damaged by drugs impair insulin secretion and also by some toxins.

As per the World health organization (WHO), different parameters are set as per the level of blood glucose and from that diagnosis is done for the testing of impaired blood glucose level. If level of blood sugar in the body is when reached at 110-125mg/dl, then the patient is considered for having impaired fasting glucose level. People having fasting blood glucose level is 7.8 or greater than that or having blood sugar level below 200mg/dl, then these patients are tested positive for having reduced glucose tolerance. Another test is of Glycated haemoglobin test, better than fasting glucose and mainly used for determining the risk of various disorder like cardiovascular disorder and mainly for determining the death that is associated with any cause. Management of diabetes disease can be done by keeping the level of blood glucose at normal or near normal, but without causing it at low blood sugar level. This can be done by maintain the proper diet and changing in the diet habits and from regular exercise. Another option is that use of proper medication in the right dose and at the right time can achieve the goal of normal blood glucose level in any person.

2.1 TYPES OF DIABETES:

There are many types of diabetes disease and they all are different in different terms. Type-1 diabetes mellitus, Type-2 diabetes mellitus, Gestational Diabetes mellitus and also Neonatal Diabetes mellitus these are all different types of diabetes mellitus that are given below:

TYPE-1 DIABETES:

One of the most critical condition in diabetes is having Type-1 diabetes, also known as Insulin dependent diabetes mellitus. As it often begins with the childhood, also called as juvenile-onset diabetes. Once this type of diabetes has developed in a person then the person requires lifelong treatment. It occurs due to lack of enough insulin production and due to that lack of enough cells of Langerhans and because of that usage of glucose and absorb of glucose by cells that are produced by insulin is less and due to that blood sugar level goes high. Type-1 diabetes can occur at any age. If the person having type-1 diabetes are not taking medications daily, then there will more increase in the blood sugar level. Patients with type -1 diabetes having serum antibodies of different components in the body and also for insulin. Type-1 diabetes is hereditary and 2-5% chances of develop diabetes in the children whose mother or father has type-1 diabetes.

SYMPTOMS OF TYPE-1 DIABETES:

1. Increase in hunger and thirst of a person
2. Tiredness
3. Urination is frequent, fatigue
4. Blurred vision
5. Weight loss without any cause.
6. Upset stomach and vomiting
7. Dry Mouth

DIAGNOSIS AND TREATMENT OF TYPE-1 DIABETES:

If a person having Type-1 diabetes mellitus, then it can be easily checked by measuring the blood glucose level or blood sugar level. In case of not checking of blood glucose for diagnosis, it can be also done by taking urine sample and by testing this urine sample for measurement of glucose level can also be done by this and a person can be diagnosed.

Every person having Type-1 diabetes mellitus has to take insulin shots compulsory for maintaining the level of blood glucose at normal level. Rapid acting insulin shows its effect in 15 minutes and continues to work for 2-4 hours. While in intermediate insulin does not enter into a bloodstream for 2-4 hours after given to a person having type-1 diabetes mellitus. While in short acting insulin, it gets to work about in 30 minutes and it shows its effect. In 2-3 hours, it shows its maximum effect and also keeps working foe 3-6 hours. Long acting insulin, it takes too much time to enter into your blood stream and it lasts about 24 hours. Exercise is an important part for treating this type of Diabetes mellitus. Also checking your blood sugar level before, after meal and treating diabetes accordingly and by all these treatments one can prevents the type-1 diabetes mellitus.

TYPE-2 DIABETES:

Around 90% of people with diabetes having this Type-2 diabetes mellitus, which is commonly known as insulin resistant diabetes. This type diabetes is more common in the young people because they have more chances of obesity. There are stronger genetic chances of type-2 diabetes then type -1. After age of 45, a person having diabetes is most of Type-2 diabetes. In this type of diabetes, pancreas usually creates some amount of insulin but either it is not enough for the body or its usage is not done properly by the cells. So, because of that there is increase in the level of Glucose or sugar level. When body cells don't respond to insulin in the case of obesity so it is called as Insulin resistant diabetes. In case of obese patient, because of insulin resistant pancreas works so hard to produce more insulin but it is still not sufficient for the body to keep blood glucose level at the normal level. So, the level of glucose become abnormal and due to that this type of diabetes occurs.

SYMPTOMS OF TYPE-2 DIABETES:

1. Weight loss - Body will start burning fat and energy due to less insulin availability and because of that weight loss can occur.
2. Blurry vision - High blood sugar level can pull fluid from the lenses of the eyes and results in swelling of the eyes and can cause blurred vision.
3. Increased hunger - Cells are not able to utilize energy and because of that there is not sufficient energy for the cells to utilize and hunger is increased.
4. Infections and sores - Because of poor blood circulations and low nutrition infection and sores can occur.
5. Frequent urination and increased thirst – Due to increase in blood glucose level, body will extract fluid from tissues and thirst is increased and due that urination is frequent.

DIAGNOSIS AND TREATMENT:

Type-2 diabetes mellitus can be verified by different diagnostic testing. Patient with the symptoms of type-2 diabetes can be diagnosed by tests like HbA1c and random plasma glucose test. If HbA1c level is greater than 6.5%, then patient shows the symptoms. Most commonly used test is fasting plasma glucose level, if it is greater than 125mg/dl, it confirms the disorder in that patient. Random plasma glucose is also checked and if it is greater than 200mg/dl, it confirms the disease. If a person does not want to measure the blood glucose level, then it can be done by measuring the urine levels and from that it can be shown that if a person having Type-2 diabetes mellitus or not.

With the usage of medication like first line agents and second line drugs, this type of diabetes can be treated. In first line treatments, Metformin, GLP1RA, SGLT2I are used to treat the disease. Also, Thiazolidinediones are one type of most useful medicaments. While as second-line therapy, DPP4-I, basal insulin therapy and AGI agents are used. In some patients, low vitamin-D level can also increase in the risk of Type-2 diabetes mellitus and in those patients, Diabetes is treated by giving the supplements that gives vitamin-D to body and by that glucose level can be controlled. With the proper diet and exercise one can prevent the risk of diabetes. Aerobics can also help in decrease in HbA1c level and helps in improvement of insulin sensitivity.

GESTATIONAL DIABETES:

Gestational diabetes is basically diagnosed in Pregnancy for the first time because it affects how your body cells use sugar during Pregnancy. So, due to that it affects baby's birth during Pregnancy due to increase in high blood sugar. Controlling these blood sugar can help in maintaining the blood sugar level normal during Pregnancy and also by taking healthy diet and proper nutrition normal blood sugar level can be achieved. It is seen that in woman blood sugar level gets return normal soon after Delivery but if it does not come to normal level due to gestational diabetes it can convert in to Diabetes mellitus. That's the reason you need to check the blood sugar level often in the pregnant woman to keep the level of sugar normal.

SYMPTOMS AND RISK FACTORS OF GESTATIONAL DIABETES:

There is not any kind of symptoms from that you can assure that it is the Gestational diabetes but, frequent urination and thirst in pregnant woman may be the reason of Gestational Diabetes.

- Some common risk Factors of gestational diabetes that can see in woman having Gestational diabetes.
- Overweight and obese patient
- Prediabetes
- Lack of Physical exercise
- Previously delivering a baby having weight more than 9 pounds.
- Excessive birth weight.
- Serious Breathing problems
- Immediate Diabetes in family members.

There are 2 subtypes of gestational diabetes are there, TypeA-1 and TypeA-2. In typeA-1 abnormal oral glucose tolerance test (OGTT), but during fasting and 2 hours after meals normal blood glucose level and diet is sufficient to control glucose levels. Whereas in type A-2, OGTT is compounded by abnormal glucose level. Gestational types of diabetes split up into several subtypes as given below:

- Type-B: Occurs at the age of 20 or older and for less than 10 years duration.
- Type-C: Occurs at the age of 10-19.
- Type-D: Occurs before age of 10 or duration is greater than 20 years.
- Type-E: Overt diabetes mellitus with calcified pelvic vessels.
- Type-F: Diabetic Nephropathy.
- Type-R: It is a type of Proliferative retinopathy.
- Type- RF: Both the Retinopathy and nephropathy.

As per the criteria of National Diabetes Data group, criteria are different for diagnosis purpose of this type of diabetes.

1. Level of fasting blood sugar is 105mg/dl
2. After an hour it reaches to 190mg/dl
3. 165mg/dl reaches after 2 hours.

NEONATAL DIABETES: It is a different type of diabetes mellitus that affects mainly an infant by affecting their body's ability to produce and use of insulin. It is a type of monogenic diabetes that means it is affected by only a single gene and it occurs mainly in the first 6 months of infant's life. If production of insulin and use of these insulins is not enough in an infant, then there is increase in the level of glucagon and due to that glucose accumulation gets increase and this leads to the Neonatal diabetes mellitus. Although it is a very rare kind of disease that occurs in only one in 1000,00 to 5000,00 live births. Sometimes one gets confused between Type-1 diabetes and Neonatal diabetes mellitus. But Type-1 diabetes mellitus occurs after the first 6 months of life and Neonatal diabetes occurs mainly during first 6 months of infant's life. Another term is Transient neonatal diabetes mellitus (TNDM). It is basically a type of neonatal diabetes mellitus but it appears mainly after the infant stage or later in life.

SYMPTOMS OF NEONATAL DIABETES MELLITUS:

1. Thirst and frequent Urination: Polydipsia and Polyuria.
2. Ketoacidosis: It is mainly a diabetic complication that occurs when production of high levels of acids by body in the blood or ketones.
3. Dehydration: Dehydration leads to an increase in thirst, when the blood glucose is elevated in the body.
4. Hyperglycaemia: It occurs due to not use of insulin as much as needed.
5. Hypoglycaemia: It occurs when blood glucose level gets extremely at low level, less than 70mg/dl.
6. Intrauterine Growth restriction: In this condition, unborn baby is smaller than he or she should be. This leads to a risk of certain problems during pregnancy and after birth.

DIAGNOSIS AND TREATMENT OF NEONATAL DIABETES MELLITUS:

There are different types of tests from which identification of NDM can be done. One of them is Fasting plasma glucose test in which measurement of blood glucose after 8 hours when person gone without eat for 8 hours. In genetic testing of NDM, it is done by Uniparental Disomy test in which samples from infant and parents are taken for analysis. Microsatellites markers are used and polymerase chain reaction are also used on the chromosome of interest for doing the test in both the parents and infant to identification of this Uniparental Disomy.

It is mainly treated with oral Sulfonyl urea and glyburides. Insulin therapy is also given to the patients like Long insulin therapy and short acting insulin therapy is given. In long therapy, Insulin-glargine mainly works to lowering the blood glucose level at normal level and it starts work after several hours. Whereas in short- acting insulin, Novolin is used and it takes up to 30 minutes and lasts for 8 hours. Sulfonylurea gives signals the pancreas to release the insulin and it helps to decrease mainly lower in A1C levels by 1-2%.

2.2.1 DIABETES FACTS:

As indicated by the report of World Health Organization in that gauges the new cases of Diabetes and quantity and number of demises by disease (WHO in April 2016find latest prevalence of 2020).

1) 422 million people today are suffering from diabetes worldwide. For past 30 years, diabetes disease has been soaring and become widespread.

2) Currently it is one of the leading causes amongst all the disease from which death occurs. In 2012 Diabetes was the direct cause of death of 2 million people and also in the same year it was an additional cause of death of 2.2 million people at the risk of cardiovascular and other severe disease.

3) In the year of 2015 widespread of diabetes in the people having age above 18 has risen from 4.7 to 8.7. It has soaring in the middle- and lower-income countries more than rich and developed countries.

4) Out of 4 adults, one is overweight and also out of 10 adult people from which more than 1 are obese and at the risk of developing diabetes.

5) Diabetes caused at least USD 780 billion dollars for health expenditures in 2019 that is 10% of all the expense in adults.

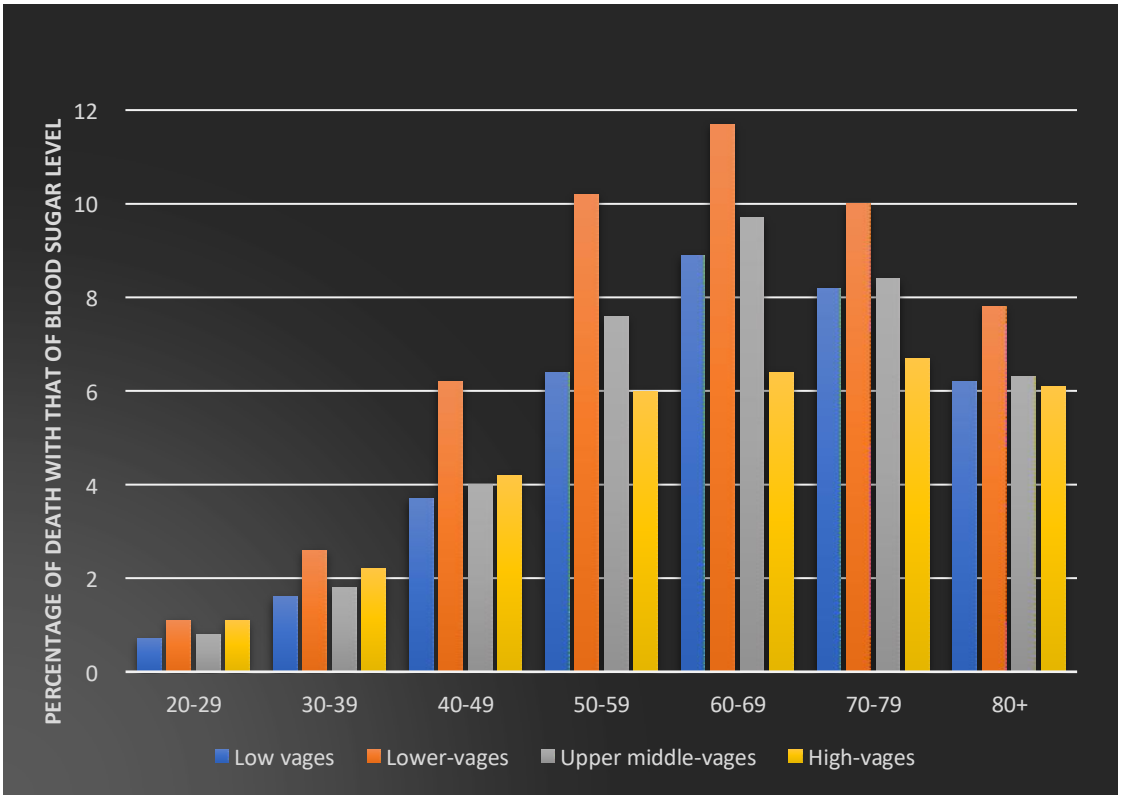


Table 1 Percentage of total death due with that of high blood sugar, by given age and salary (A) Men

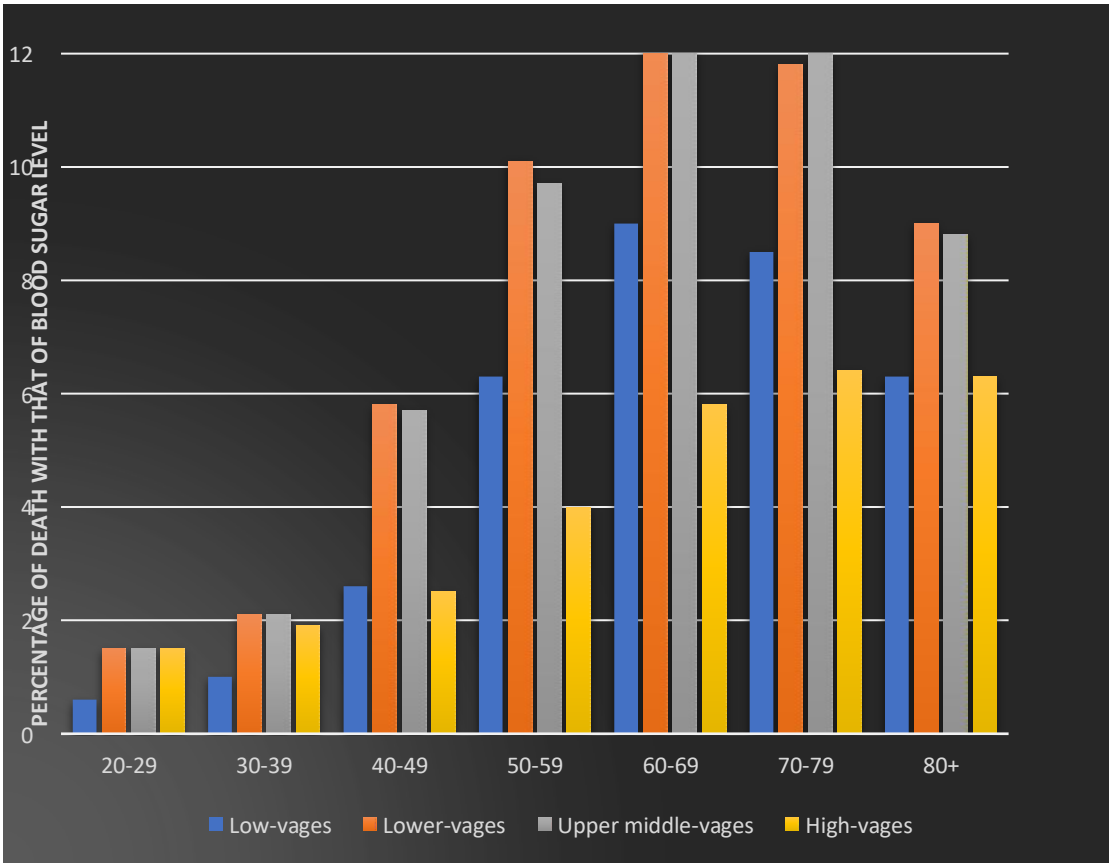


Table 2: Percentage of total deaths with that of high blood sugar, by given age and income (B) Women

6) Around 18,200 adults were diagnosed positive for Type-1 diabetes mellitus and 5800 for Type-2 diabetes mellitus in the year of 2014-15.

7) In United States Diabetes was the 7th leading cause of death based on 83,564 death certificates of diabetes while in 2017 diabetes was mentioned as a cause of death in total 270,702 certificates.

8) In 2018, total expense for diagnosis of diabetes patient in the United states was around 327 billion dollars.

9) The total number of people diagnosed positive for diabetes are 26.8 million out of 34.2 million people and remaining 7.3 million people were undiagnosed.

10) In the year of 2018 percentage of the population having diabetes is 10.5% while in the year of 2019 it has been changed from 10.5% to 18%. It was found that only 35-50% of people who died due to diabetes had listed in the certificates but 10-15% population who were not listed in the certificates.

11) According to National Diabetes and Diabetes Retinopathy survey report released by Ministry of health and welfare Prevalence of diabetes in India has retained at 11.8%in the last four years wherein Male (12%) showed similar percentage of prevalence as female (11.7%).

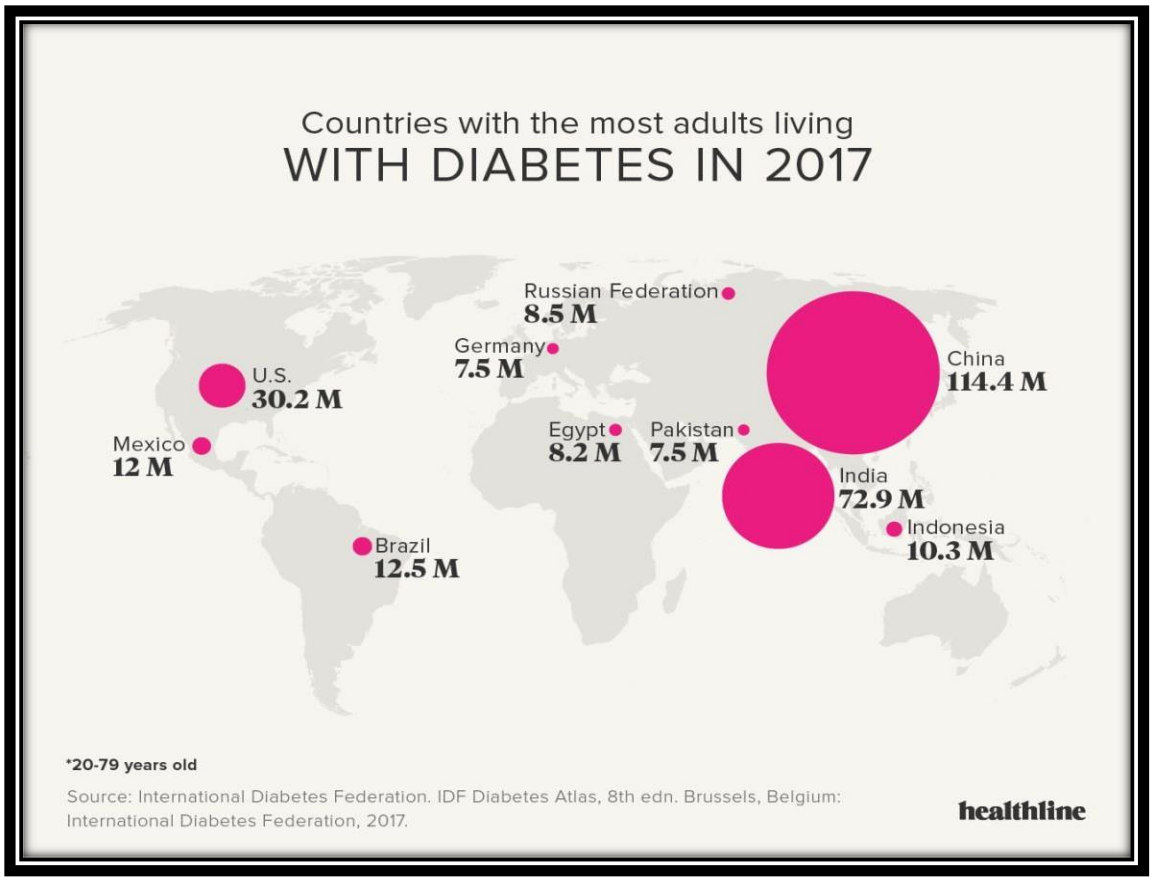


Figure 1: Countries with the most adults living with Diabetes in 2017.

2.2.2 PPAR GAMMA AGONIST AS A POTENT DRUG TARGET:

In nuclear family, Peroxisome proliferator activated receptor (PPAR) are basically transcriptional factors belonging to ligand activated nuclear receptor. On activation by endogenously secreted prostaglandins and fatty acids, they initiate transcription of an array of genes that are involved in energy homeostasis. So, there are basically 3 major types of PPAR receptors, namely PPAR-alpha, PPAR-gamma and PPAR-B\delta. The discovery of PPAR-gamma agonist such as Thiazolidinediones enables the recognition of the mechanism involved in ameliorating adverse effect of chronic disorder such as diabetes.

PPAR receptors were identified in 1990's in rodents and given name after their property of peroxisome proliferation. Major research findings of PPAR-gamma agonist as critical determinants for adipose cell differentiation and site of action of anti-Diabetic Thiazolidinedione drugs. As per the new researches in the field of PPAR gamma agonist for the regulation of obesity and lipid metabolism and possible molecular

determinants of metabolic Disorder, including type-2 diabetes. PPAR have been assigned to subfamily of nuclear receptors that includes Retinoids acid Receptors (RAR) and steroid receptors. PPAR gamma agonist receptors encoded by separating genes that is NR1C3.

PPARs can be activated by mainly 2 different types of ligands, natural ligands and synthetic ligands. Retinoid acid receptors, PPREs are some factors that plays a significant role in achieving transcription mechanism. Gene transcription is achieved by this mechanism.

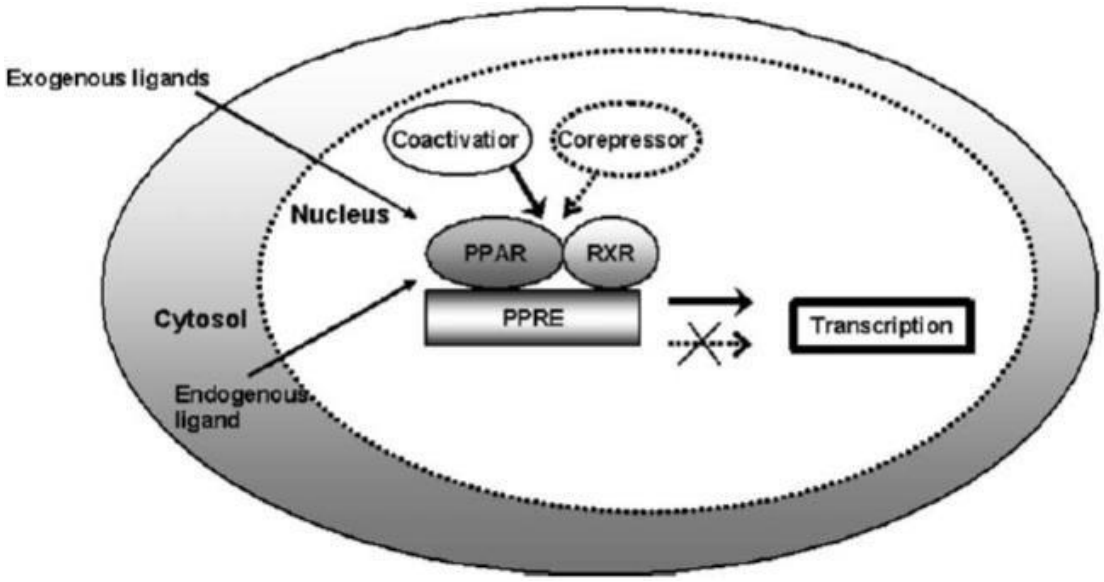


Figure 2: Gene transcription mechanism of PPAR.

Heterodimerisation of PPAR with the retinoid acid receptors and PPREs is mainly possible due to LBD arrangement and subsequently resultant heterodimers binds to PPRE with the recruitment of co-factors. Ability of nuclear receptors is increased due to several proteins that act as co-activators or co-repressor and this leads to suppression of transcription process by doing the possible interaction between nuclear receptors and that of ligand gated manner. In an unliganded state, association of heterodimerised nuclear receptors with multi-components co-activators occurs and that contains mainly histone deacetylase activity like silencing mediators for retinoid and thyroid hormone receptors.

Steroid receptor coactivator (SRC-1) and PPAR binding protein (PBP) are different types of co-activators that contains histone acetylase activity. By this activity, transactivation can be done. DR-1 elements are made by 2 nucleotides with the sequence of AGGTCA mainly separated by a single nucleotide spacer. For PPAR-RXR heterodimer, DR-1 pattern is important, which distinguish it from DR-3 receptors. PPAR gamma receptors basically contains 3 isoforms.

- PPAR gamma 1 & 3 transcripts translate into identical PPAR gamma 1 protein. PPAR gamma -1 is found in a broad tissue almost all tissues.

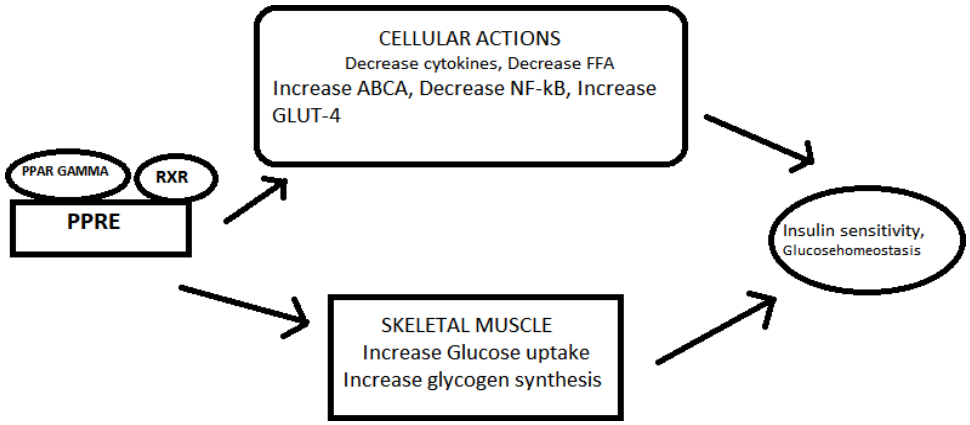


Figure 3: PPAR gamma's gene transcription mechanism and it's biologic effects in different organs.

The basic process of gene transcription by PPAR gamma starts by binding of PPAR gamma receptors with that of ligands, mainly endogenous or exogenous. So, by these binding of ligands with the receptors, ligand bound heterodimerises with retinoid X receptors (RXR), binding of heterodimers with protomer region occurs. With the effect of Co-activators or Co-suppressors, these processes mainly involved the process of transcription of different genes. Insulin sensitivity and adipogenesis are the major mechanisms of these PPAR gamma receptors that results in increase in the improvement of insulin resistance.

2.2.3 PPAR Gamma and insulin sensitivity:

It is indicated that PPAR gamma agonist receptor is an important regulator as a point of maintaining glucose homeostasis. This information comes from discovery that insulin sensitizing diabetes can be controlled by potent insulin sensitizing Thiazolidinedione's. There are basically two possible mechanism that shows that how it can be possible of insulin sensitization by activation of PPAR gamma in adipose tissues and a gene that promotes adipogenesis and fat deposition.

First, PPAR gamma promotes lipid storage in adipose tissues and by these it prevents lipo-toxicity by decrease in plasma lipid profile and accumulation of lipids in muscle and liver. This mechanism involved basically activation of gene encoding molecules that enables lipid storage and the process of lipogenesis by different binding proteins like fatty acids binding proteins (aP2), receptors for lipo-proteins (CD-36), hydrolysis of lipoproteins and lipoprotein lipase. By activation of all these gene encoding molecules partition of lipid molecules or breakdown of lipid molecules are done with increase in triglycerides content in the adipose tissues and decrease level of triglycerides in plasma, liver and muscle and improving insulin sensitivity.

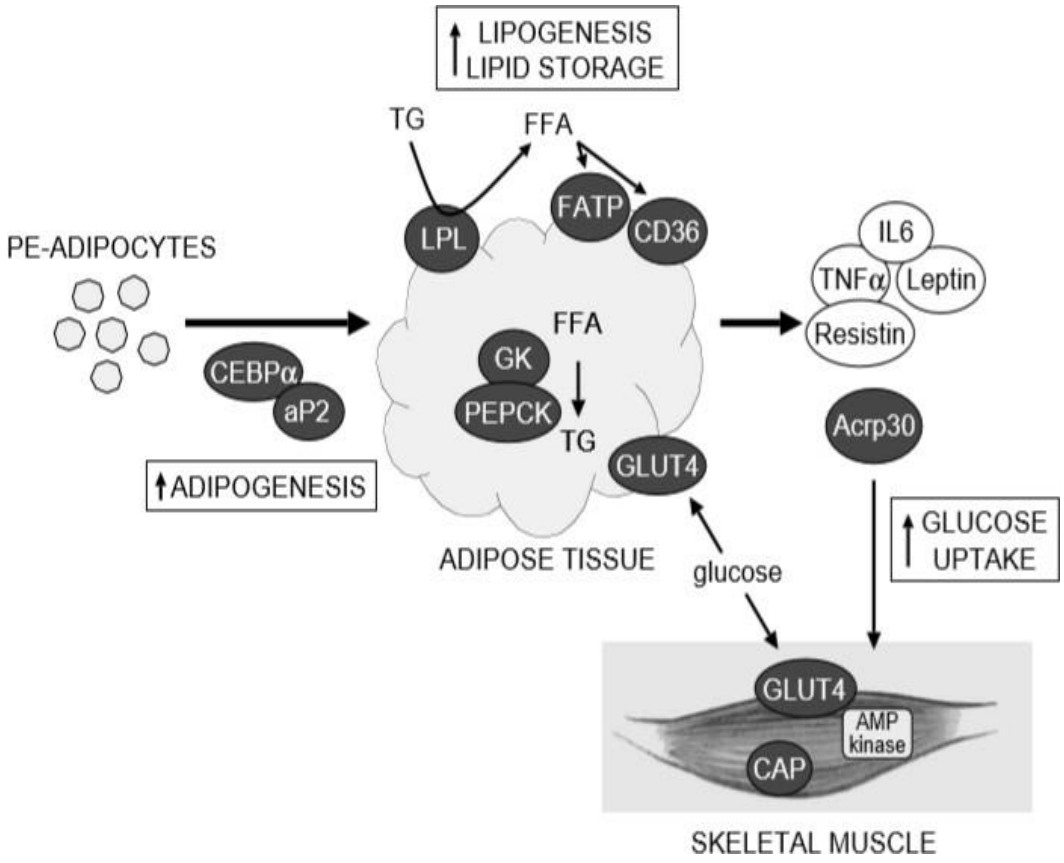


Figure 4: Effect of PPAR gamma activation on insulin sensitivity.

Second type of mechanism is basically the ability of PPAR gamma in control of the endocrine secretion of adipose cells and tissues. As we discussed above PPAR gamma secretes adipokines that promotes insulin sensitivity and also inhibits the expression of tumour necrosis factor-alpha, interlukein-6. So that activation of PPAR gamma increases insulin receptor substrates (IRS) which shows positive rolet in insulin signalling pathway.

An experimental approached is used for the purpose of showing the relationship between PPAR gamma and related insulin sensitivity in various tissues. This experiment is done by using genetically modified mice. In this experiment there is a disruption of homozygous results in death of embryonic on 10^{th} day from abnormal placental development.

Contrary to prediction that has been made based on the action of PPAR gamma agonist, the results of this mice homozygous disruption for PPAR gamma exhibit increased insulin sensitivity. Where in heterozygous state protects against age induced resistance of insulin instead of protecting against high fat feeding induced insulin resistance. That is why 2 different possibilities for heterozygous state has been suggested for improving insulin activity.

First one is PPAR gamma +/- mice have increased serum leptin, which showed improvement in insulin sensitivity. The second one suggests that in the unbound state or in the ground state PPAR gamma supresses insulin action and the decrease the abundance of PPAR gamma in the heterozygous state impairs the suppression of insulin responsive genes results in increase in the insulin sensitivity. Several tissue specific disruptions have been generated providing model to examine function of PPAR gamma. In adipose tissue, PPAR gamma is expressed at higher level whereas at the same time PPAR gamma is expressed at lower level in peripheral tissues. Depending upon the activation of 2 types PPAR gamma tissues, adipocyte derived PPAR gamma and skeletal muscle derived PPAR gamma tissues there is a discussion on how PPAR gamma regulates activity. Adipocyte derived PPAR gamma specific knockouts have impaired insulin sensitivity with hepatic insulin resistance. It can be improved by the treatment with TDZ. So, it suggests that adipocyte derived PPAR gamma is not important for the insulin sensitizing effect of TDZ.

Two mouse models with the disruption of PPAR gamma in skeletal muscle have been taken and generated and show whole body insulin resistance due to decrease in the suppression of the insulin-stimulated hepatic glucose production. But interestingly the effect of thiazolidinediones in these two models show contradictory effect. From 2 one shows improvement insulin sensitivity in high fat diet fed mice which is treated with Rosiglitazone, suggest that PPAR gamma expressed outside skeletal muscle is effective in thiazolidinediones induced insulin sensitization.

In contrast to loss of function mutation, PPAR gamma activity is increased by mutation by impairing its Phosphorylation that has been generated in mouse and results in maintenance of insulin sensitivity in diet induced obesity. In addition to this experiment in mice, also human genetics studies confirm that PPAR gamma agonist improves insulin sensitivity in human. In human PPAR gamma several amino acids have been identified and in that proline to alanine amino acid substitution at the extreme amino terminus is the most common type of mutation that is identified to date, but as we know that its effect on insulin sensitivity is unclear, a meta-analysis of all the data which have published has been done and that supports the hypothesis that alanine amino acids PRO12AL is done and its polymorphism is associated with decreased the risk of Type-2 diabetes.

Several heterozygous mutations in the ligand binding domain of or area of PPAR gamma act in a dominant negative mechanism that include the different types of substitution like Proline to leucine substitution at amino acid number 467 position. The same way it includes the substitutions like valine to methionine at position 290. Now clinical analysis of these mutations reveals that these mutations results in severe insulin resistance and also in Hypertension. Results from the human patients clearly defines a role of PPAR gamma in regulating glucose homeostasis in human.

3 Targeting PPAR gamma for Diabetes:

PPAR gamma is actually most widely expressed in immune cells and also in adipose tissues and in inflammatory cells in a form of monocytes and microphages. Compare to adipose tissues and several PPAR gamma isoforms, expression of PPAR gamma is lesser in liver and also lesser in skeletal muscle. For the differentiation and functioning of brown and white adipocytes, essential of PPAR gamma is required and that leads to accumulation of lipids and fats in these cells.

Due to the specific activation of PPAR gamma, insulin resistance in liver and that of skeletal muscle is reversed by mainly novel class of PPAR gamma agonist, that is Thiazolidinediones. For this use, the first approved drug in the class of thiazolidinediones was Troglitazone. But, due to its hepatotoxicity, it was withdrawn from the market in 2000. This was happened because troglitazone induced CYP3A. Liver damage could be done by CYP43A- dependent metabolism derived potential toxic quinones. So, the available options for these were Rosiglitazone and Pioglitazone. Glycated haemoglobin, fasting post-prandial blood glucose level are reduced by activation of PPAR gamma by Thiazolidinediones. Levels of body circulating insulin in the patient with type-2 diabetes can be decreased by TZDs, for the purpose of improvement in insulin sensitivity. These rosiglitazone and pioglitazone work as both, decreased insulin resistant and increasing in sensitivity and also helps in preservation for that of pancreatic B-cell function. By these both features that are done by TDZs, reducing the incidence of type-2 diabetes mellitus as it is showed by clinical trials on mitigation of type-2 diabetes in people are having higher risk that are treated with Rosiglitazone and Pioglitazone.

while the patients were taking the drugs of different class of PPAR gamma agonist, in the presence of these drugs, insulin sensitivity is increased. whereas there is not any significant evidence of effect of PPAR gamma agonist to decrease the effect of PPAR gamma agonists for the prevention of diabetes disorder when the administration of drugs was no longer. But, due to certain rare adverse effects that are shown with the use of these TDZs, consumption of these TDZs are limited.

In the year of 2007, this TZDs registered its first evidence of the adverse effect that is ischemic cardiovascular events. This occurred due to usage of Rosiglitazone drugs. The alteration in the fluid and electrolyte balance induced by the Rosiglitazone that results in the contribution of cardiovascular disease, such as heart failure and ischemia. Thus, in the year of 2010 several agencies like Food and Drug Administration and the European Medicine Agency, suspended the marketing of this drug Rosiglitazone. On the other hand, Pioglitazone undergoes in meta-analysis and clinical trials indicates the possibilities of ischemic and cardiovascular benefits. Robust evidence of both the drugs mainly shows that both the drugs increase the risk of congestive heart failure and fractures, but weather any meaningful differences exists in the magnitude of risk between the two TDZs in unknown.

Selective modulators such as a new PPAR gamma agonist recently in the market have been proposed for better activity as a drug for type-2 diabetes. For example, potent not-TDZs selective PPAR gamma modulators, INT-131 that affects mainly in reducing plasma glucose level that is depending on dose and this process does not affect body weight and fluid retention. PPAR gamma agonist induced improved effects against metabolic disorders throughout a complex simultaneous modulation of different intracellular signalling pathways. Activation of PPAR gamma affects mainly in the translocation of glucose transporters by increasing its expression. GLUT1 and GLUT4 are both the transporters mainly in adipose cells then reducing consequently glucose plasma level and decrease the amount of glucose in those cells.

In adipose cells, activation of PPAR gamma receptors affects adipose remodelling. As a result of this, differentiation of pre-adipose cells and cell death occurs of adipose cells that are older and some of insulin-resistance adipose cells.

Adding to this, blood levels of adipocytokines are mainly elevated by PPAR gamma, mainly adiponectin protein, mainly these detectable in the plasma with lower concentration of patients having mainly type-2 diabetes. Insulin sensitivity is mainly elevated with that of adiponectin and FFA oxidation is also increased. While in the liver, production of blood glucose is declined. Different experiments are done on this and study shows that stimulation of this target increased FFA uptake of adipose cells and due to that enhanced the use of glycerol for the production of triglycerides type of fatty acids. Thus, it affects mainly on the decreased or reducing release of FFA from that of adipocytes.

In various tissues like tissues of skeletal muscle, tissues of liver and tissues of pancreas lead to an improved and well-maintained hepatic production of glucose and on the other hand utilization of this glucose in skeletal muscle tissues. As we have showed recently the effect of PPAR gamma agonist mainly Pioglitazone that directly effects on the insulin signalling Pathway, in modulating expression and in the different activities of key insulin signalling molecules, including different molecules like IRS-2, AKT, and GSK-3 beta. Transduction of the insulin signal pathways mainly improved by PPAR gamma and by this improvement in intracellular proteins through its improved expression. Facilitation of this phosphorylation of key insulin-signalling molecules is done by above whole process. Although TDZs is mainly affects directly on the insulin signalling pathways by increasing its effect. As per the latest update, insulin-sensitizing capacity of this PPAR gamma ligands by different contributors mainly involves repression of mainly both types of cytokine production, local and systemic.

While on other side nondiabetic obese patients are treated with the drug TDZs is notable and it reduces the cytokines level which is circulating and other types of proinflammatory markers, and with insulin-sensitivity all these effects were associated. As per the suggestion that hepatic activation of PPAR gamma receptors is associated with that of declined expression of suppressor of cytokines signalling-3(SOCS-3), it is a cytokine signalling inhibitors, which has been supposed to act mainly to be a crucial

linkage between inflammation and liver insulin resistance. Insulin signalling is modulated by SOCS-3 by associating directly to the insulin receptors and promotion of IRS-2 degradation. This SOCS-3 at the same time take participated in a classical negative feedback loop that is mainly used in the modulation of cytokine-mediated signalling Pathways.

Other term Demonstrated that Pioglitazone improves the hepatic regenerative response after partial hepatectomy in obese and diabetic experimental mice by decreasing or preventing an increase in hepatic SOCS-3 messenger RNA. Pioglitazone has been already reported mainly to reduce the overexpression of mRNA for tumour for tumour necrosis factor alpha and both of its receptors in a mouse model of obesity-linked diabetes. After this study, further in vitro studies have also confirmed that PPAR gamma agonists may exert their antidiabetic activity by counteracting the deleterious effects of tumour necrosis factor alpha. By all these signalling activities of these receptors Diabetes can be controlled.

Endocrine function of adipose tissues is mainly modulated by PPAR gamma ligands. To enhance the insulin action and level in muscle and also in liver, the primary target for the drugs like Thiazolidinediones are PPAR gamma ligands. It suggests that regulation of gene expression is also done by these transcription factors. Also, it involved other adipose tissues and signalling is done to those tissues. That's why the adipose tissue has become firmly established as a main target or endocrine organ during the last 10 years. circulating regulators of appetite and also a main source of expenditure of energy is mainly the cells of adipocyte-derived leptin. Other than that, there are more additional adipocytes-secreted proteins that have the major role in insulin-sensitivity are adiponectin and resistin. Other than that, there are more different kinds of polypeptides that are mainly secreted by adipose cells including tumour necrosis factor (TNF-alpha), Plasminogen activator inhibitor 1 (PAI-1) and interleukein-6.

In the setting of lipoatrophy-associated insulin resistance, main sensitizing insulin-function is due to the effect of leptin protein. That is mainly done with leptin administration. These PPAR gamma ligands are mainly used to decrease leptin gene expression. That is the main reason for which the leptin is not that much used in the maintaining insulin sensitivity that is mainly inserted by that of PPAR gamma ligands.

Reduction of the adipocyte gene expression with the usage of TDZs while that of resistin protein, Tumour necrosis factor alpha (TNF-alpha), and interleukien-6, all of these imparted their effect in the process of resistance of insulin. In addition to that, Thiazolidinediones declines the secretion of adipocytes, that is of PAI-1 that is pro-thrombotic. Furthermore, Gene expression of the adiponectin protein and that of insulin-sensitizing adipocyte hormone is mainly done by the usage of PPAR gamma ligands. Therefor the expression of adipocyte hormones is done by PPAR gamma ligands in such a way that prevents insulin resistance and by that diabetes disease can be mitigated.

AIM OF RESEARCH:

The aim of research for the present study was to perform Pharmacophore modelling, virtual screening and molecular docking and generate pharmacophore models by using PPAR gamma as a target molecule for the diabetes mellitus disease.

OBJECTIVES OF RESEARCH:

For achievements of aim of the research, here are list of certain objectives that are given below.

1) To carry out extensive literature search that includes various in-silico approaches for treating the disease Diabetes mellitus for the identification of PPAR gamma agonist.

2) To generate ligand-based pharmacophore model using PPAR gamma agonist as a target.

3) To select best model and perform Virtual screening process for that model amongst all pharmacophore models.

5. PHARMACOPHORE MODELLING:

Paul Enrich is a person who has given and introduce a word pharmacophore and as per him pharmacophore is a molecular framework that carries essential features which are responsible for a drug's biological activity.

For the generation of pharmacophore model, two different methods are mainly used. First one is Structure based Pharmacophore modelling and another one is ligand-gated pharmacophore modelling method. Definition of pharmacophore as per the "International union of Pure and applied Chemistry" (IUPSC)is that a pharmacophore model is " an ensemble of stearic and electronic features that is necessary to ensure the optimal interactions that is of optimal supra-molecular interactions with a specific biological target and to increase or inhibit its biological response." Utilization of this pharmacophore modelling can be done in mainly virtual screening process, in the process of molecular docking and by that it is used in the lead discovery and also in lead improvement process. Pharmacophore approach is mainly known as ana abstract of features that is generating by trial and error continuously and by that generating different features. There are many difficulties related to this pharmacophore modelling techniques specially in the achievement of its normal potential, especially with an interest for decreasing the present high cost related with the disclosure what is more that of improvement of another medication. By removing the normal substance highlights from that of 3D structures for the arrangements of known ligands, this process is typically done. By this, illustrative of basic association is completed between the ligands and a particular molecular target. Features of these pharmacophore should be selected based on SAR studies and that of training set.

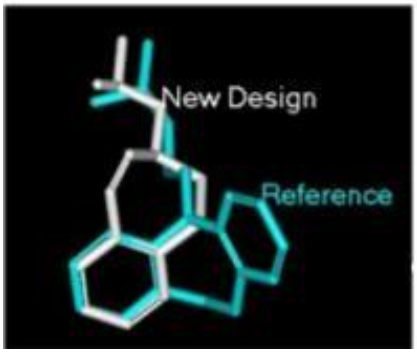
Pharmacophore is basically a set of features common to all known ligands of a particular target. Another definition of Pharmacophore is the spatial orientation of various functional groups or features in 3D necessary to show biological activity. Different pharmacophore features are as below.

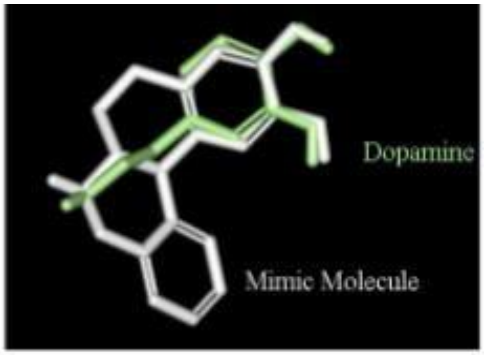
1. Hydrogen bond donor and hydrogen bond acceptor
2. Feature of hydrophobicity
3. Aliphatic Hydrophobic
4. Aromatic hydrophobic
5. Positive ionizable/charge
6. Negative ionizable/charge
7. Aromatic ring system

Each of the above feature that mainly consists of 4 different parts.

- Chemical function
- Weight
- Tolerance in location
- Location and orientation in 3D space.

By examines the features of inactive small molecules and examine the feature of active small molecules, Pharmacophore-based drug designed can be done successfully. By this examining of both the molecules active and inactive a hypothesis is generated and it shows that what chemical groups on the ligands are necessary for showing of biological function, what chemical groups supresses the biological function. By this hypothesis new ligands are generated which have the same necessary chemical groups in the same 3D locations (That mimics the active groups).





In Pharmacophore generation, five steps are there on that bases a pharmacophore can be generated.

1.) Selection of training set
2.) Selection of proper features
3.) Generation of conformation
4.) Common feature alignments
5.) Validation

The training set of molecules that are selected, those having diversity in their structures. It is said mainly based on the assumptions. Also, series of these molecules have maximum information about the structures and having most potent effect. Selection of feature are done based on the structure activity relationship studies of the training set that are taken.

Based on a set of low energy conformation, each training set of molecules should be represented and by this process, generation of conformation is done. Broad coverage of conformation space is ensured by this technique. Active conformations of training set molecules are aligned in order to obtain the best covering of the corresponding features. It is judge by statistical parameter and also by visual inspection of that model.

Without a macromolecular target structure, for encouragement of medication disclosure, the key computational procedure which is used is known as ligand-based pharmacophore modelling method. By removing the normal substance highlights, from that of 3D structure of an arrangement of known ligand targets, this process can be done and it shows their basic association between the ligands and that of particular macromolecular target.

Two basic principle steps are mainly used for the generation of pharmacophore by using the given training set of molecules.

By making the conformation space for each and every possible ligand in the training set to prepared the conformational adaptability of that ligand and by adjusting the various ligands in those preparation sets for the purpose of deciding the basic normal components for the generation of pharmacophore model. Two main key strategies are involved, taking care of that conformation adaptability and by that leading atomic arrangements of the ligands. With fewer considerable advantages, there are also some difficulties with this ligand-targeted pharmacophore modelling process. For that, two useful methodologies were developed in order to overcome this problem. Pre-counting technique is major issue, in that for every particle various adaption are pre-computed and ready to spread in database. Fly strategy is the second one, which includes adaption examination process for the generation of pharmacophore model.

In the ligand-based pharmacophore modelling, second major issue is that of difficulty with having atomic arrangements. This arrangements technique that are mainly separated additionally as atoms, and chemical features of that molecule. Pairs of different atoms, chemical features of the molecule are mainly aligned by using least square methods and fittings in the process of point-based algorithm. By the use of this pharmacophore modelling, there are many kinds of features that can be generated and from that features one can identify the main structure of that particular molecule regarding with its biologic activity. With some variation of closeness, advantages of these arrangements are completed and in the measurements of mainly an intermolecular cover is used for that as a goal work, intermolecular cover of guassians are used in the generation of pharmacophore model.

Making an appropriate choice for the mixture of different training set is one of the major testing issues that can create the problem. This, very basic and also non-specialized issue creates a big problem. The last time produced pharmacophore model that is mainly influenced by different things like the span of the database, different kind of ligand atoms and by its synthetic assorted qualities. The use of different programs in the preparation of diverse training sets, creates extraordinary pharmacophore-based models and that of ligands that are interfacing with the macromolecular target. It is also created by certain calculations too.

By combining the various and different structural moieties together for the generation of novel best molecule, the best computational or in-silico process which is used in the present situation is known as the method of ligand-based or ligand-targeted pharmacophore modelling process. By this process abstract of features can also be generated. In current scenario, for the generation of pharmacophore, different software is used which are shown in the table. From that different software, Genetic algorithm similarity program (GASP) module is currently used to generate the pharmacophore with the use of SYBYL software.

SOFTWARE	PHARMACOPHORE IDENTIFICATION AND RESOURCES
GALAHAD	Genetic algorithm with linear assignment for hyper molecular alignment of database (GALAHALD), Tripos, Inc. http://www.tripos.com/data/SYBYL/GALAHALD_9-7-05.pdf
DISCO & DISCOTECH	Distance comparison for multiple pharmacophores generation. Tripos, Inc. www.tripos.com/data/SYBYL/DISCOtech_072505.pdf
GASP	Genetic algorithm similarity program. A flexible genetic algorithm. Tripos, Inc. www.tripos.com/data/SYBYL/GASP_072505.pdf
Catalyst	An integrated environment for database Management querying task for drug discovery. Accelrys, Inc.
HipHop	HipHop matches the chemical features of compounds without considering activity. The resulting hypothesis can be used to search of the chemical database. Accelrys, Inc. http://www.accelrys.com/products/catalysts/catalystproducts/cathypo.htm
HYPOGEN	By using ligand activity values, hypothesis generator incorporates them and by that scoring function is identified. Accelrys, Inc, www.aacelrys.com/products/catalyst/catalystproducts/Cathypo.html
PHASE	Schrodinger Inc. hhtp://www.schrodiner.com/
FlexS	Flexible shape-based screening of ligands. http://www.bisolveit.de/FlexS/

MPHIL	Mapping pharmacophores in ligands which is different kind of approach known as relaxed MCH approach.
MOE/ pharmacophore modelling	Molecular operating environment. MOE's pharmacophore modelling module is to generate and use 3D genetic information to search for novel active compounds, particularly if there is no availability of receptor geometry. http://chemcomp.com/software.htm
RAPID	Randomized Pharmacophore identification.
SCAMPI	For identification of Pharmacophore, statistical classification is done for activity of the particles. Handle large heterogenous data sets. A random conformational search is combined. Glaxo Welcome, Inc. www.gsk.com/index.htm

Table 3: Available software programs for Pharmacophore identification and Ligand-based design.

5.1 DERIVATION OF ACTIVE PHARMACOPHORE MODELS:

For a ligand-based design approach, generating reliable pharmacophore hypothesis is prerequisite for the 3D pharmacophore database search. Several approaches are available for generating pharmacophore models, including programs to derive the active pharmacophore models or "Virtual receptors" (like 3D QSAR/CoMFA) and also AAA (active analogue approach) and different program to generate hypothetical pharmacophore queries like DISCO & GALLAHARD for database searches of the new chemical scaffolds that match with the target pharmacophore. An example is given below.

In mapping out 3D QSAR, CoMFA program is superior with the comparison of classical QSAR. The CoMFA program provides tools to postulate a biological active conformation and to establish a set of alignment rules to superimpose a molecule under consideration. It will generate a lattice as a box of points around that of molecule and also calculate their electrostatic and stearic fields that each molecule exerts on a probe atom that positioned at each grid or lattice point. Using the method of partial least square statistics, statistical analysis of data is made to derive a linear correlation between the calculated values and that of input biological data. Subsequently it

validates the predictability of the derived model by using the cross-validation. Finally, active ligand CoMFA models are established that can be used to guide novel ligand design and to predict that of the biological activities of that molecule.

This type of analysis has been used to mainly study the pharmacophoric requirements of cannabinoid antagonist, to study the effect of shape on binding on steroids to that of carrier proteins. That is done to reduce the active site of geometries of angiotensin converting enzyme (ACE) and also done to analyse a set of muscarinic receptors.

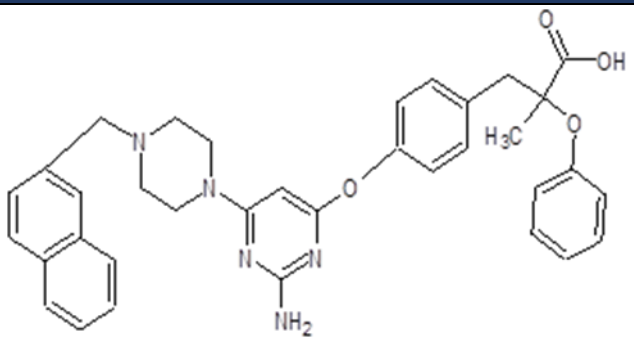
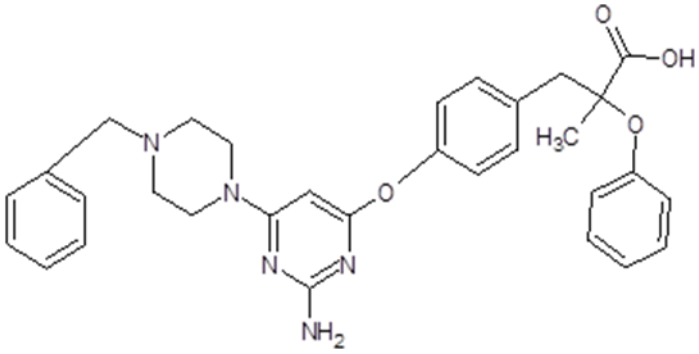
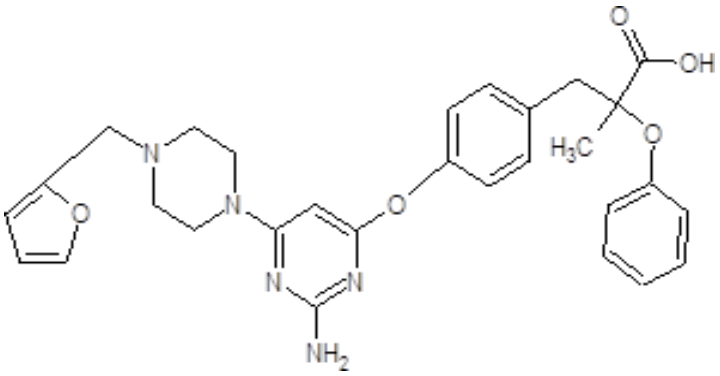
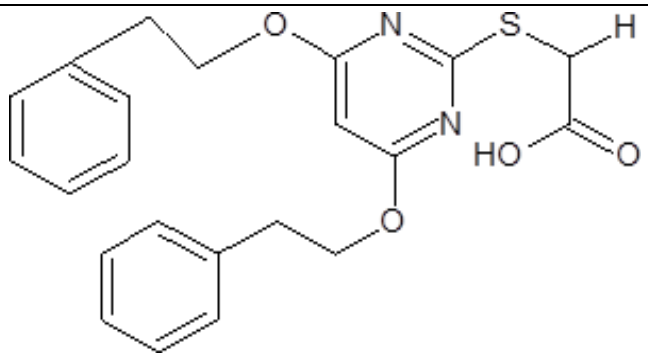
The DISCO (Distance comparison) program often is used to generate multiple pharmacophore models by identifying common pharmacophoric features for that of a given set of active compounds, producing optimally aligned structures and extracting the key features of that of the pharmacophores. Then, these pharmacophore hypotheses can be mainly compared with each other, refined properly and based on that it is used to guide lead generation and lead optimization and in performing the 3D searches of that databases for that of new leads. DISCO has been used in the pharmacophore generation during studies of muscarinic M3 receptors antagonists, muscarinic receptors agonists, and melatonin analogues.

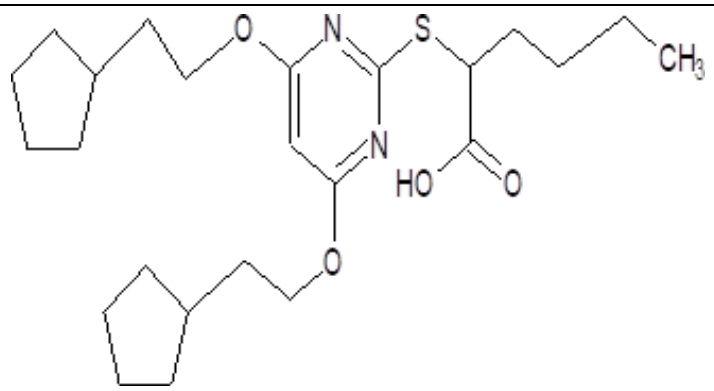
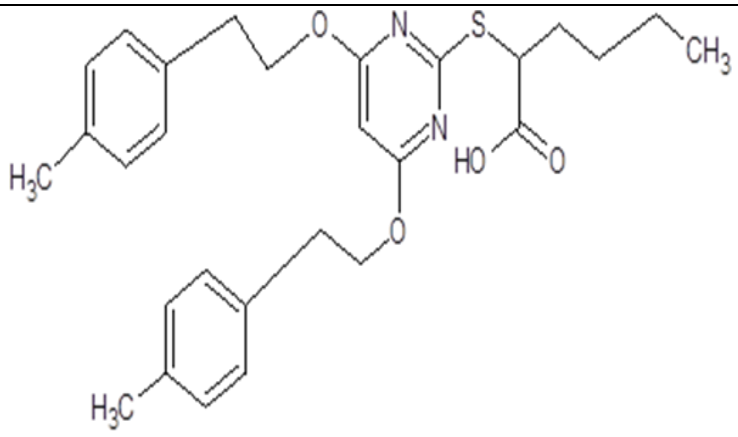
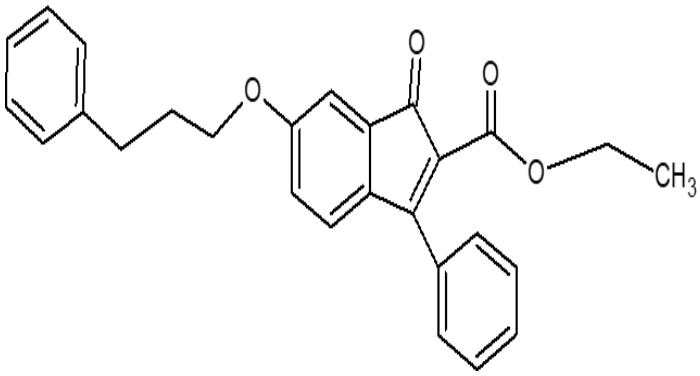
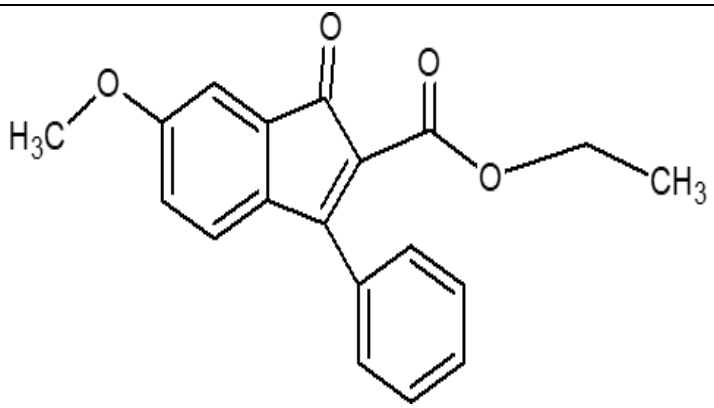
Other Pharmacophore identification approaches that include various types of software through which identification can be easily done. The names of the different software's are Genetic Algorithm similarity program (GASP), Hypotheses generator (HypoGen), HipHop, PHASE, Mapping pharmacophores (MPHIL), Randomised pharmacophore identification (RAPID), Molecular operating environment (MOE) and last one is (SCAMPI) as well as Active analogue approach (AAA) and ensemble distance geometry. The generated various pharmacophore models will be used as hypothetic queries in the database for the identification of lead compound.

5.2 EXPERIMENTAL WORK OF PHARMACOPHORE:

By using DISCOTECH module for the generation of pharmacophore in the SYBYL software, 10 structures are taken having highest, lowest and moderate EC50 activity. After generation of different features, refining of molecules are done with GASP module of SYBYL software. There are basically 5 features that are generated with the usage of PPAR gamma agonist after refining. There are generation of 2 DS- donor site features, 2 AA-acceptor atoms and 1 HY- hydrophobic site is obtained.

From these models, Model 1 shows the highest Demean value and having highest size. So that, taking these features as a consideration, further analysis can be done.

CHEMICAL CLASS	STRUCTURE	EC50 value (μM)
3-{4-[2-Amino-6-(napthalen-1-ylmethyl)-piperazin-1-yl)-pyrimidin-4-yloxyl]-phenyl}-2-methyl-2-phenoxy-propionic acid		3.50
3-{4-[2-Amino-6-(napthalen-1-ylmethyl)-pyrimidin-4-yloxyl]—phenyl}-2-methyl-2-phenoxy-propionic acid		6.62
3-{4-[2-Amino-6-(4-furan-2-ylmethyl-piperazin-1-yl) pyrimidin-4-yloxyl]-2-methyl-2phenoxy-propionic acid		5.01
2-(4-6, -Bis(phenethoxy) pyrimidine-2-ylthio)-ethanoic acid		3.7

2-(4-6, Bis (Penta ethoxy) pyrimidine-2-ylyhio)-hexanoic acid		12.1
2-(4-6, Bis(4-(methyl) phenethoxy) pyrimidine-2-ylthio)-hexanoic acid		7.5
1-Oxo-3 phenyl-6-(3-phenyl-propoxy)-1H-indene-2-carboxylic acid Ethyl ester		0.05
6-Methoxy-1-Oxo-3 phenyl-1H-indene-2-carboxylic acid Ethyl ester		0.30

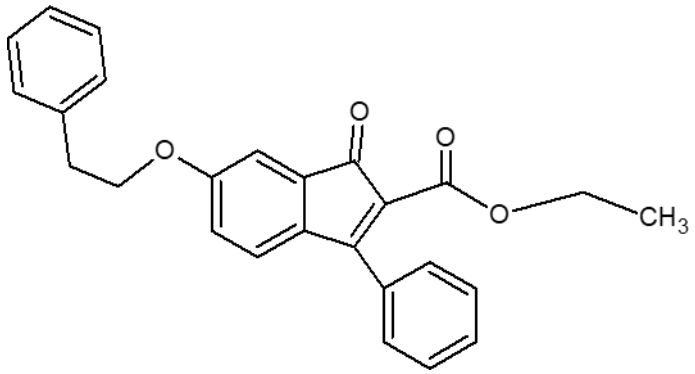
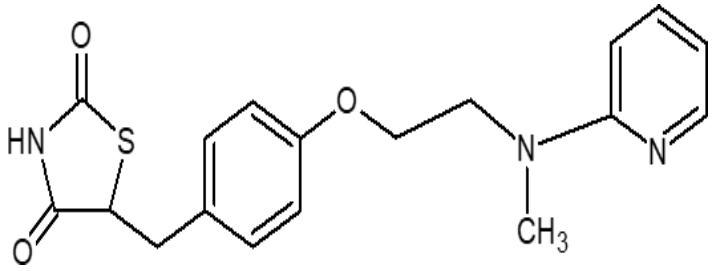
1-Oxo-3 phenyl-6-(3-phenyl-ethoxy)-1H-indene-2-carboxylic acid Ethyl ester		0.21
Rosiglitazone (clinical products)		0.10

Table-4: List of different molecules that are used in the generation of Pharmacophore model

GASP GENERATED PHARMACOPHORE MODEL:

MODEL NUMBER	FITNESS	SIZE	HITS	DMEAN
1	2291.7500	5	10	4.4225
2	1944.1700	0	0	ND
3	1992.2000	0	0	ND
4	1825.0400	5	10	4.3314

Table-5: Details of models that are generated by GASP

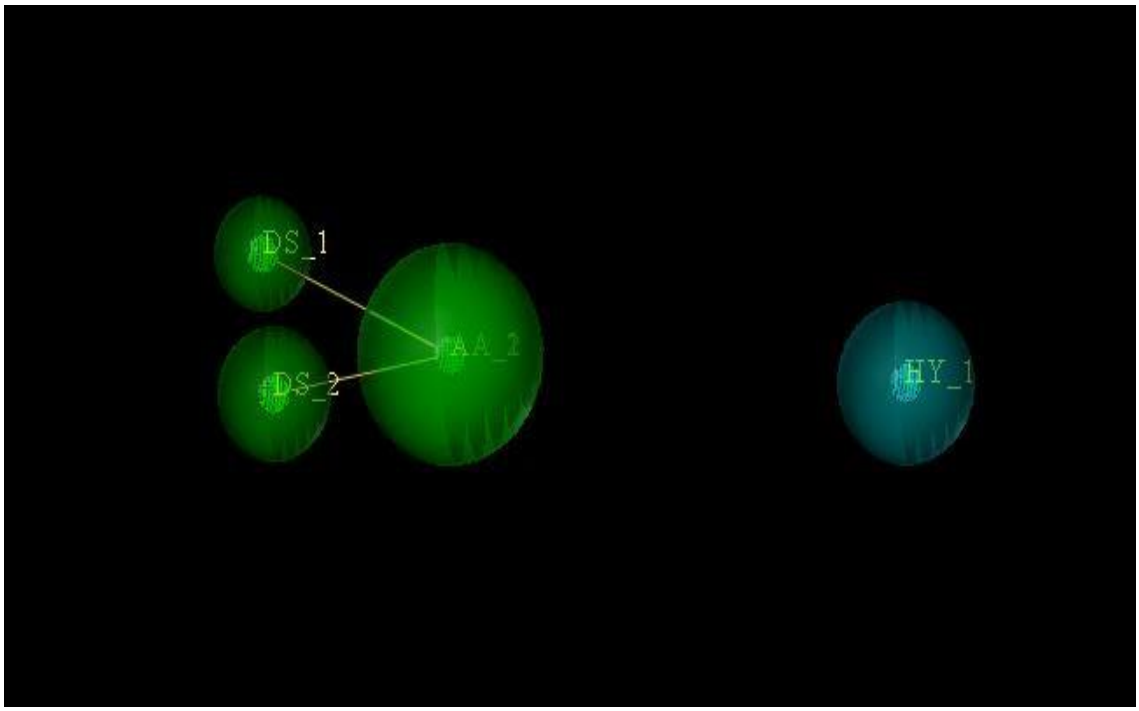


Figure 5: 3D pharmacophore model: AA_1 = acceptor atom, AA_2 = acceptor atom, DS_1 = Donor site, DS_2 = donor site, HY_1 = Hydrophobic site 1

6. VIRTUAL SCREENING:

Virtual screening is basically a method in which biological activity can be identified by using in silico analogue method. A set of structures are taken and they are given scoring, ranking and also filtered by using different computational procedures. After generation of Pharmacophore model using both ligand-targeted and structure targeted methods, it can be used for questioning the three-dimensional substance database that is mainly scanned for the ligands that are potential. This process is known as Pharmacophore based Virtual screening method. In this type of approach mainly, as a concept of format, Pharmacophore consideration is used. Main idea or belief which is considered for approaching this is to find the discovery of leads having mixture highlights as seen in layouts. Some kinds of leads that are like known dynamic mixes and those that are completely new in the platform. When this type of scanning procedures mixes with different types of framework with sharing a natural movement is known as "PLATFORM JUMPING".

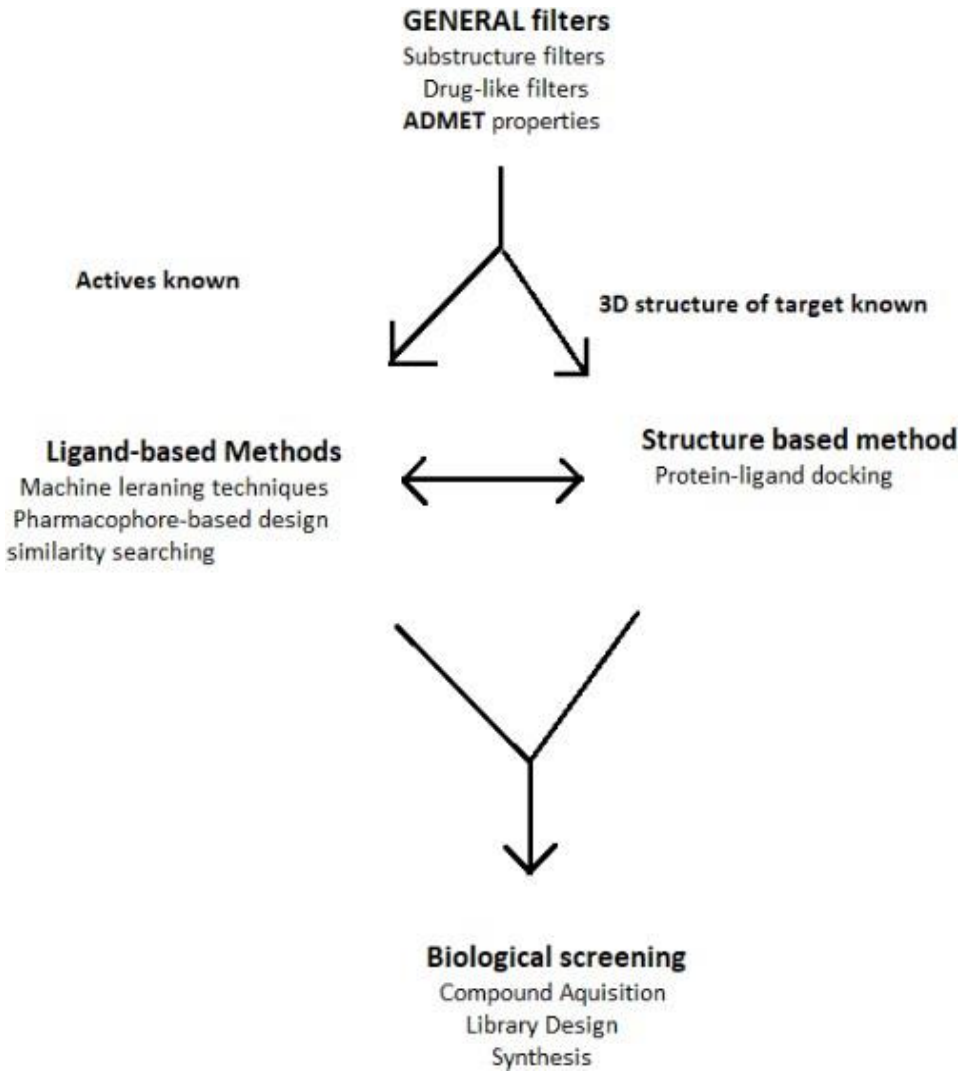


Figure 6: Process of Virtual Screening

The screening process and procedure that includes two possibilities of trouble and also have two different approaches.

Virtual screening can be used,

1) To help in deciding that which compound has to screened.

2) Out of different libraries, it helps in the selection of libraries which will be going to synthesize.

3) It Gives detail about compounds those are purchased from outside and which are not.

4) It is mainly used in identification of the result of this experiment.

5) For different chemical scaffolds, this technique helps to identify novel drug candidates by using different database that are commercial or public or private 3-D database.

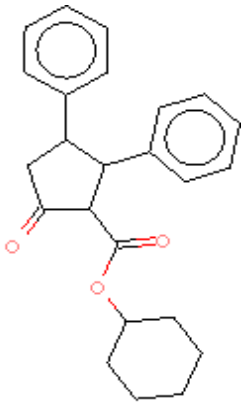
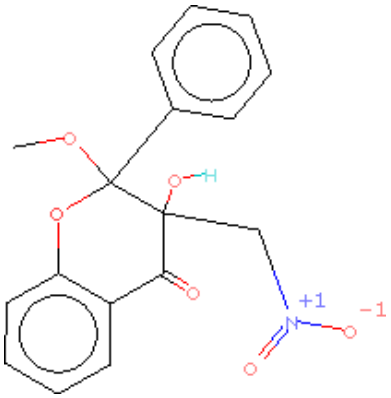
6) Chemical space is mainly reduced by this to give more and accurate promising candidates that are used in the process of lead discovery and optimization.

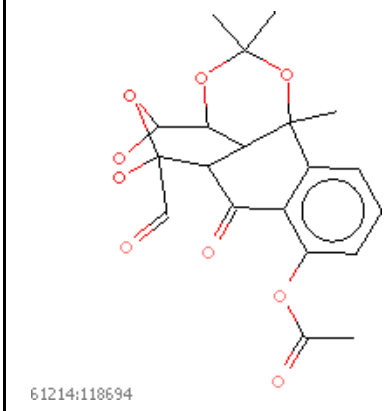
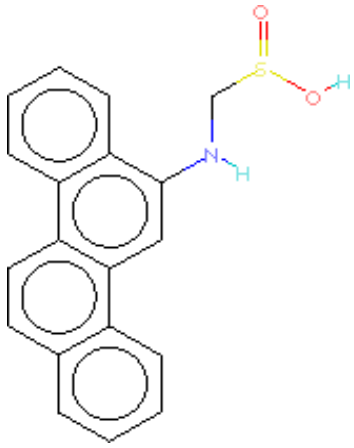
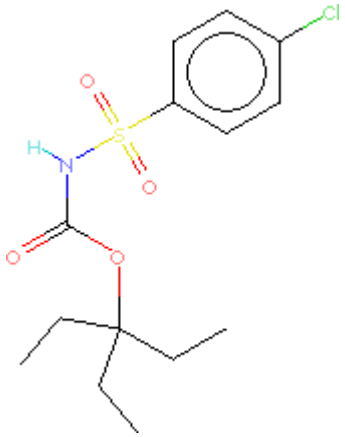
The objective of this process is to differentiate between the molecules having desired effects and properties with those molecules having undesired properties (Poor Pharmacokinetics property). In different term, computational studies help to decrease time effectively and decreased the resources that are required for the synthesis related with biological activity.

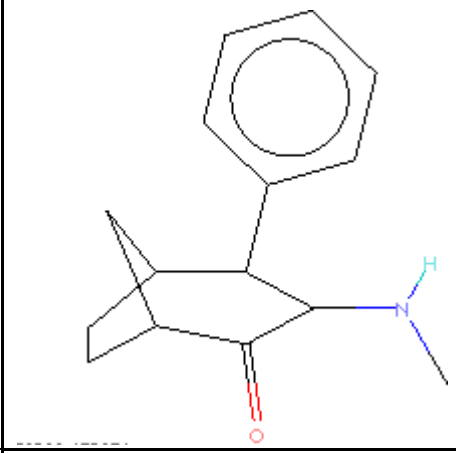
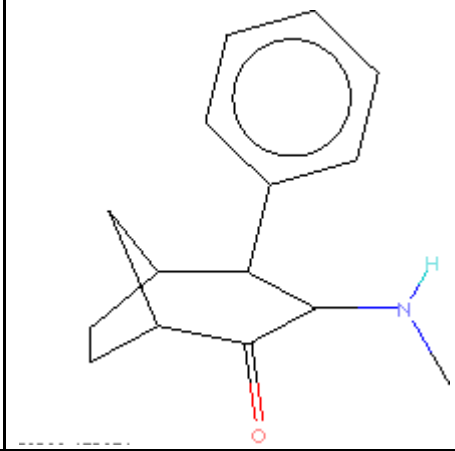
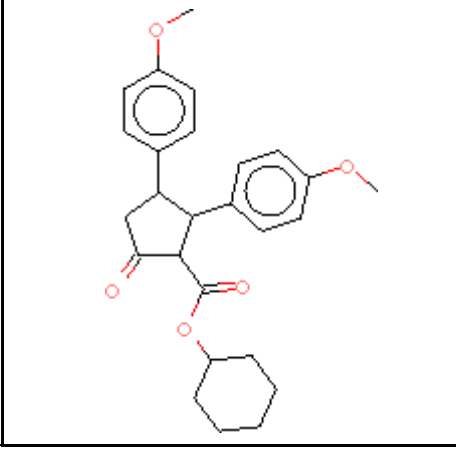
By using this high-throughput virtual screening, advance computational technics or cheminformatics are used to deal with massive amount of compound for their biological behaviour at lower resolution. There are many possible attempts are done for the better improvement of the principles of this Virtual screening techniques. For instance, the scoring functions are basically based on the simplified empirical force fields, and this parameter can be improved by increasing the quantum mechanical calculations and by that overall accuracy will be improved. In short, this virtual screening techniques mainly attempts to incorporate as much of the molecular modelling methods that are discussed above and get the study completed within an acceptable amount of time.
Molecular dynamics also called Monte Carlo schemes are basically designed to explore as much of the relevant regions of conformation space as possible. This technique is mainly used in the field of pharmacy as in the form of many possible things like lead generation, lead selection. In pharmaceutical industries, this technique is used for the optimization of lead compounds that are mainly used in the process of drug discovery.

6.1 EXPERIMENTAL WORK OF VIRTUAL SCREENING:

After generation of Pharmacophore model, features are taken further for virtual screening and some corresponding features are noted as HITS. From that 26012 different molecules are obtained from that 10 molecules having highest QFIT values are taken further for docking and lead generation.

SR NO.	STRUCTURE	QFIT	NUMHITS
1		99.5600	89
2		99.5400	74

3		99.5400	68
4		99.5400	100
5		99.4300	41

6		99.4100	51
7		99.4000	12
8		99.4000	12

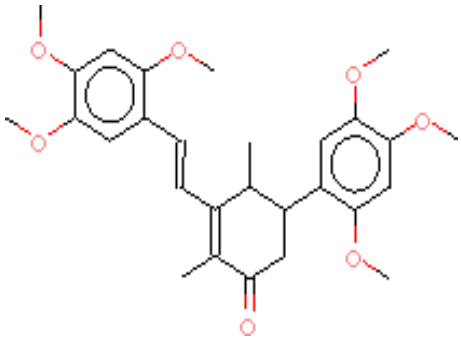
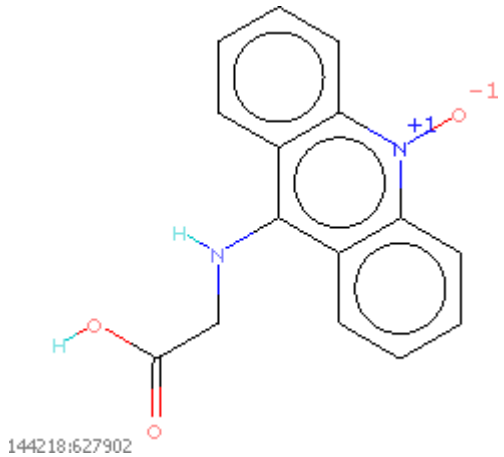
9		99.3500	122
10		99.3400	42

Table 6: Top 10 compounds having best QFIT value after virtual screening.

7. CONCLUSION:

Study on Rational drug approach for identification of potent drug target PPAR gamma can is done based on in-silico approaches. Ligand based pharmacophore modelling by DISCOTECH and GASP module in SYBYL software is done for above target and virtual screening is performed to identify the best structure having highest QFIT value. After generation of pharmacophore models, features are then refined and from that best features are obtained. From that Pharmacophore model, best one is selected and sent for performing virtual screening and lead generation. Here 5 features are generated from pharmacophore model. From which molecules having high score are taken from virtual screening. These molecules are searched against Database and from that 10 different hits are selected based on highest QFIT value and further utilize for the molecular docking.

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