DESIGN, SYNTHESIS AND ANTI- CANCER ACTIVITY OF SOME NOVEL THIAZOLIDINEDIONE MANNICH BASES

Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

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NOVEMBER 2019

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This is to certify that the investigation described in this dissertation entitled **"DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF SOME NOVEL THIAZOLIDINEDIONE MANNICH BASES"** submitted by **Reg. No: 261715353(MUTHUMARI.C)** for the award of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY**, degree comprises of the bonafide work done by her in the was carried out in the Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of pharmacy , Anand nagar, Krishnankoil-626126. Her work was supervised by **Dr. N. Venkateshan., M.Pharm., Ph.D.,** department of pharmaceutical Chemistry, Arulmigu Kalasalingam College of pharmacy, Anand nagar, Krishnan koil.

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Examiners: 1.

2.

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DECLARATION

I, MUTHUMARI.C (Registration no. 261715353), hereby declare that the dissertation work entitled **"DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF SOME NOVEL THIAZOLIDINEDIONE MANNICH BASES"** submitted by me, in partial fulfillment of the requirement for the degree of **MASTER OF PHARMACY** IN **PHARMACEUTICAL CHEMISTRY** to The Tamilnadu Dr. M. G. R. Medical University, Chennai is the result of my original and independent research work carried out under the guidance and supervision of **Dr. N. Venkateshan., M.Pharm., Ph.D.,** during the academic year 2018-2019 and this has not formed the basis for the award of any Degree/ Diploma/ Fellowship or similar title to any candidate of any University.

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ACKNOWLEDGEMENT

I pray our profound gratitude to the almighty God for this invisible help and blessing for the fulfilment of this work.

I take this privilege and pleasure to acknowledgement the contribution of many individuals who has been inspirational and supportive throughout my work undertaken and endowed me most precious knowledge to see the success in my endeavor. My work bears the imprint of this people.

I am grateful to **"Kalvivallal" Thiru T. Kalasalingam., B.Com.,** for providing me the required facilities for extending a rich and also, I convey my sincere thanks to "Ilaiya vallal" Dr. K. Sridharan, Ph.D., vice president of Academic, **Dr. S. Shashi Anand, M.S., Ph.D.,** Dynamic director **Er. S. Arjun Kalasalingam, M.S.,** and the management of our institution for providing me necessary infrastructure.

I am particularly fateful to the person! Without whom this thesis would not be accomplished: my revered mentor **Dr. N. VENKATESHAN, M.Pharm., Ph.D.,** Professor & Principal of Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, to whom I am extremely indebted. He convinced and helped the area of research for this project. Throughout my research studies, he provided me with guidance, supervision and perpetual support. His open-door policy and invaluable advice into the most difficult time of the research made it much easier for me. No written word could do you justice, Thank you Sir.

I express my special thanks to **Dr. R. Rajapandi, M.Pharm., Ph.D.,** Professor, AKCP for providing me necessary facilities and constant source of inspiration and has always encouraged scientific thinking to carry out this dissertation work for providing much of stimuli in the form of suggestions and guidance of enormous support for me during my entire research work. Thank you Sir.

I convey my deep sense of gratitude to **Dr. J. Amutha Iswarya Devi, M.Pharm., Ph.D.,** Associate Professor, AKCP for their suggestions, constant encouragement, inspiration and help throughout my research work.

Besides my advisor, I would like to express my sincere gratitude to my advisor Associate Professor **Mr. V. Rajamanickam, M.Pharm.,(Ph.D)** for his continuous support, motivation and immense knowledge. His guidance helped me in all the time of research.

My sincere thanks also goes to all the lab assistants:**Mrs.V. Shenbagavalli, Mrs. M. Muthulakshmi, Mr. Sivagurusamy, Mr. Ganesan, Mr.Senthil kumar,** of our institution who gave access to the laboratory and research facilities. Also my heartful thanks goes to the librarians **Mr. Abdulkader** and **Mr. Laksmana gurusamy** for their support and co-operation. Without their precious support it would not be possible to conduct this research.

My heartfelt regard goes to my parents **Mr.C.Chelladurai.,** and **Mrs.C.Chellam.,** for their love and moral support. I consider myself to be the luckiest in the world to have such a lovely and caring family, standing beside me with their love and unconditional support.

I thank Almighty for giving me the strength and patience to work through all these years so that I can stand proud with my head held high.

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ABBREVIATION

TZD	-Thiazolidinedione
FTZD	- Furfuryl thiazolidinedione
IAR	- International Agency for Research on Cancer
DNA	- Deoxyribonucleic Acid
CIGLI	- Clglitazone
PIO	- Pioglitazone
TRO	- Troglitazone
ROSI	- Rosiglitazone
PPARγ	- Peroxisome proliferated activator receptor-γ
NFK B	-Nuclear Factor Kappa B
EGF Receptor	- Epidermal growth factor receptor
PAI-1	- Plasminogen activator inhibitor-1
MMPs	- Matrix Metallo proteinases
BaX	- BCL 2- Associated X protein
E-cad	-Electronic computer aided design
mTOR	- Mammalian Target of Rapamycin
AMPK	- Adenosine monophosphate activated protein kinase
PGF 2	- Prostaglandin E_2
COX 2	- Cyclooxygenase-2
PTEN	-Phosphatase and and tensin homolog
P_{13} K	- Phosphoinositide-3-Kinase
MEK	- Methyl Ethyl Ketone

ERK	- Extracellular Signal- regulated kinase
MCF	- Macrophage chemotactic factor
CSD	- Cambridge structural Databases
ACD	- Available chemical Directory
NCI	- National cancer institute Databases

CHAPTER 1

INTRODUCTION

The World Health Organization's cancer agency warns that there will be 22 million new cases of cancer every year within the next two decades. Report from the International Agency for Research on Cancer (IARC) estimated in 2012 that there were 14 million new cases but predicted that the figure would jump significantly due to global ageing and the spread of cancers to developing countries. Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells and it is a fatal disease standing next to the cardiovascular disease in terms of morbidity and mortality. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future. Currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs[1].

What is Cancer?

Our body is composed of many millions of tiny cells, each a self-contained living unit. Normally, each cell coordinates with the others that compose tissues and organs of your body. One way that this coordination occurs is reflected in how your cells reproduce themselves. Normal cells in the body grow and divide for a period of time and then stop growing and dividing. Thereafter, they only reproduce themselves as necessary to replace defective or dying cells.

Cancer occurs when this cellular reproduction process goes out of control. In other words, cancer is a disease characterized by uncontrolled, uncoordinated and undesirable cell division. Unlike normal cells, cancer cells continue to grow and divide for their whole lives, replicating into more and more harmful cells. The abnormal growth and division observed in cancer cells is caused by damage in these cells' DNA (genetic material inside cells that determines cellular characteristics and functioning).

There are a variety of ways that cellular DNA can become damaged and defective. For example, environmental factors (such as exposure to tobacco smoke) can initiate a chain of events that results in cellular DNA defects that lead to cancer. Alternatively, defective

DNA can be inherited from yourparents. As cancer cells divide and replicate themselves, they often form into a clump of cancer cells known as a tumor. Tumors cause many of the symptoms of cancer by pressuring, crushing and destroying surrounding non-cancerous cells and tissues. Tumors come in two forms; benign and malignant. Benign tumors are not cancerous, thus they do not grow and spread to the extent of cancerous tumors. Benign tumors are usually not life threatening. Malignant tumors, on the other hand, grow and spread to other areas of the body[2-4]. The process whereby cancer cells travel from the initial tumor site to other parts of the body is known as metastasis.

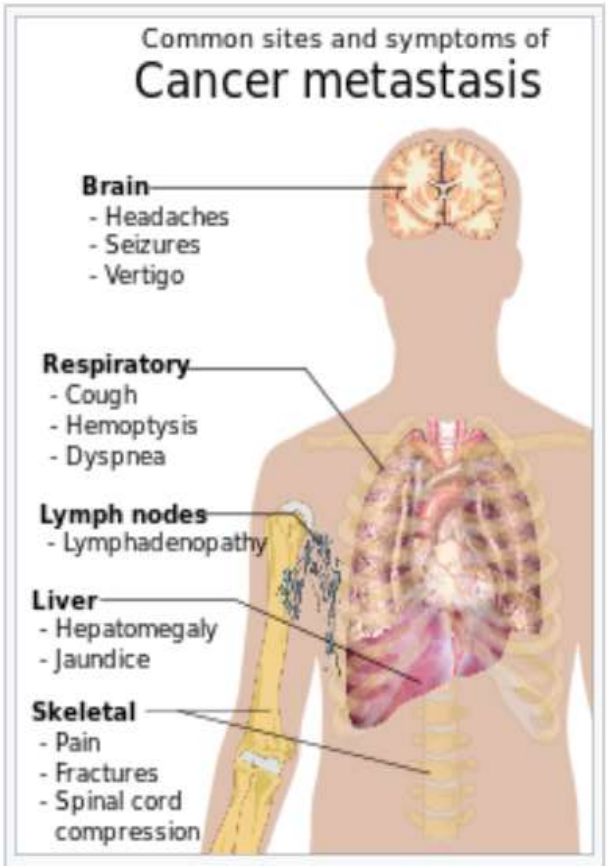


Fig. 1Cancer cells mutations and symptoms of cancer metastasis

Different types of cancer

There are over 200 types of cancer; far too numerous to include in this introductory article.

The general names of some cancers:

Carcinoma: Cancer that begins in the skin or in tissues that line or cover internal organs "skin, lung, colon, pancreatic, ovarian cancers," epithelial, squamous and basal cell carcinomas, melanomas, papilloma's, and adenomas.

Sarcoma: Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue "bone, soft tissue cancers," osteosarcoma, synovial sarcoma, liposarcoma, angiosarcoma, rhabdosarcoma, and brosarcoma

Leukemia: Cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood "leukemia," lymphoblastic leukemias (ALL and CLL), myelogenous leukemias (AML and CML), T-cell leukemia, and hairy-cell leukemia.

Lymphoma and myeloma: Cancers that begin in the cells of the immune system "lymphoma," T-cell lymphomas, B-cell lymphomas, Hodgkin lymphomas, non-Hodgkin lymphoma, and lymphoproliferative lymphomas.

Central nervous system cancers: Cancers that begin in the tissues of the brain and spinal cord "brain and spinal cord tumors," gliomas, meningiomas, pituitary adenomas, vestibular schwannomas, primary CNS lymphomas, and primitiveneuroectodermal tumors.

Multiple Myeloma: Multiple myeloma is cancer that begins in plasma cells, another type of immune cell. The abnormal plasma cells, called myeloma cells, build up in the bone marrow and form tumors in bones all through the body. Multiple myeloma is also called plasma cell myeloma and Kahler disease.

Melanoma: Melanoma is cancer that begins in cells that become melanocytes, which are specialized cells that make melanin (the pigment that gives skin its color). Most melanomas form on the skin, but melanomas can also form in other pigmented tissues, such as the eye.

LYMPHOMA CANCER

Lymphoma is a cancer of the lymphatic system. It affects a type of white blood cells known as lymphocytes. These helps fight disease in the body. They play an important role in the immune system. This type of cancer starts in the white blood cells, or lymphocytes. As it is present in the bloodstream, it can spread, or metastasize, to different parts of the body. Lymphoma can occur at any age, but it is one of the most common causes of cancer in children and young adults aged 15 to 24 years. It is often treatable[5]. In the United States, the lifetime risk of getting Non-Hodgkin lymphoma is 2.1 percent. The risk of getting Hodgkin lymphoma is around 0.2 percent.

Causes

Cancer happens when there is uncontrolled growth of abnormal cells that thrive and spread

instead of dying as they would in the life cycle of a normal cell. Lymphatic tissue is connected throughout the body. If cancer cells develop in the lymphatic system, they can spread easily from their original location to other tissues and organs, including those outside the system[6].Lymphoma most often spreads to the liver, bone marrow, or lungs.

In Hodgkin lymphoma, the cancer usually affects one lymph node after another in order.

In non-Hodgkin lymphoma, tumors may arise in disparate lymph nodes, skipping some nodes.

Exactly what causes lymphoma is unclear, but there are some risk factors.

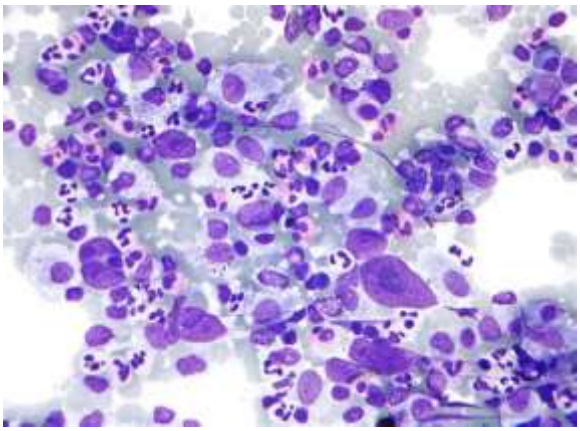


Fig. 2Hodgkin lymphoma

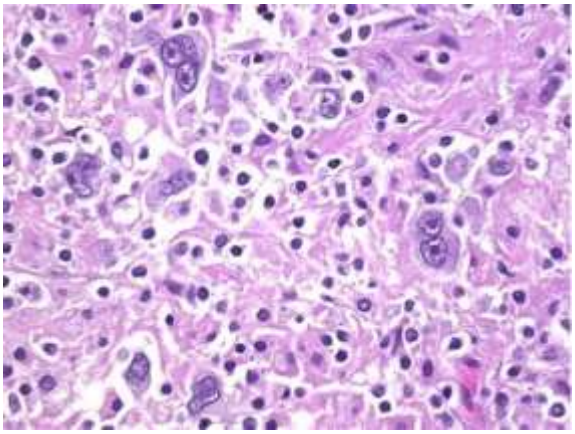


Fig. 3 Non Hodgkin lymphoma

Symptoms

The signs and symptoms of lymphoma are similar to those of illnesses such as viral diseases

and the common cold, but they continue for longer than would normally be expected. Some people will have no symptoms, but some may notice a swelling of the lymph nodes. These are located all around the body, often in the neck, groin, abdomen, or armpits. The swellings are normally painless, but pain may occur if the enlarged glands press on organs, bones, and other structures. This can be confused with back pain[7-9].

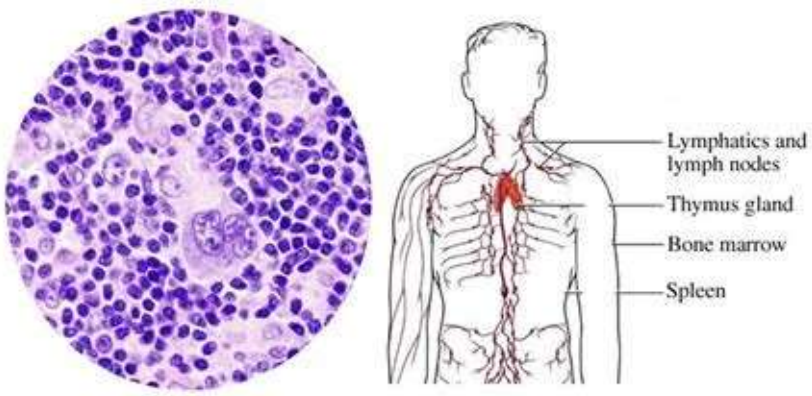


Fig. 4 Lymphoma cancer

Lymph nodes can swell during common infections, such as a cold, but in lymphoma the swelling does not go away. Pain is also more likely to accompany the swelling if it is due to aninfection. The overlap of symptoms can lead to misdiagnosis. Anyone who has ongoing swelling of the glands should see their doctor. Other symptoms of both types of lymphoma may include:

- ongoing fever without infection
- Night sweats, fever, and chills.
- weight loss and loss of appetite
- unusual itching
- persistent fatigue, unusual tiredness, or lack of energy
- pain in lymph nodes after drinking alcohol

Additional symptoms that can indicate Non-Hodgkin lymphoma include:

- persistent coughing
- shortness of breath
- pain or swelling of the abdomen

Pain, weakness, paralysis, or otherwise altered sensation can occur if an enlarged lymph node presses against spinal nerves or the spinal cord. Lymphoma can spread rapidly from the lymph nodes to other parts of the body through the lymphatic system. As cancerous lymphocytes spread into other tissues, the body's ability to fight infection weakens.

Treatment

Treatment depends on the type of lymphoma and the stage it has reached. Indolent, or Slow-growing lymphoma may need only watchful waiting and no treatment[5-6].If treatment is necessary, it can involve:

Biologic therapy: This is a drug treatment that stimulates the immune system to attack the cancer cells by inserting living microorganisms into the body.

Antibody therapy: Synthetic antibodies are inserted into the bloodstream to combat the cancer's antigens.

Chemotherapy: Aggressive drug treatment is used to kills cancer cells.

Radioimmunotherapy: This delivers high-powered radioactive doses directly into the cancerous B-cells and T-cells to destroy them.

Radiation therapy: This is used to focus on small areas of cancer.

Stem-cell transplantation: This can restore damaged bone marrow following high-dose chemotherapy or radiation therapy.

Steroids: These may be injected to treat lymphoma.

Surgery: This can be used to remove the spleen or other organs after the lymphoma hasspread.Surgery is used more often for obtaining a biopsy.

Introduction about Thiazolidinediones

Glitazones, also called thiazolidinediones (TZDs), are remembered carbon ring molecules containing two heteroatoms (nitrogen and sulfur). One carbonyl group in thethiazole at position 4 and another at position 2 make theheterocyclic compound a thiazolidine-2,4-dione. TZDsare ligands of the Peroxisome Proliferator Activated Receptor gamma (PPAR γ)a nuclear receptor inducing upregulation ofspecific genes that decrease insulin resistance, inflammation, VEGF-induced angiogenesis, proliferation, and leptin levels, inducing differentiation of adipocytes, and increasing adiponectin levels. Tis spectrum of actions led to the approval of TZDs for treatment of diabetes mellitus type II.TZDs differ according to the substitution at C5.

Ciglitazone (CIGLI) is the prototype of all TZDs but has never been approved for medication of diabetes mellitusbecause its clinical activity was too weak. Troglitazone (TRO)was the first TZD approved for treatment of diabetes mellitusin 1997. The compound showed beneficial e□ects on glucose levels, insulin sensitivity, and free fatty acid concentration but was withdrawn from the market in 2000due to severe hepatotoxicity. The second TZD, rosiglitazone(ROSI), has been banned in Europe and restricted in the USA because of increased cardiovascular morbidity. Also, the use of pioglitazone (PIO) as the third TZD with anti-diabetic action is restricted due to concerns about a potential facilitation of bladder cancer development[7-9]. The fourth substance with anti-diabetic profile, rivoglitazone, is still under investigation

Mechanism of Antitumor Action by TZDs

Although all TZDs are PPARγ ligands, the observed antitumor effects can only be explained in part by genomic PPARγ activation. Genomic activation is defined as the binding of a nuclear receptor to a response element, which activates the transcription of certain genes. The process is also termed transactivation. Another DNA-mediated effect transrepressor, which describes the binding of receptors to transcription factors (e.g., nuclear factor kappa B (NFkB) or activator protein 1 (AP-1)).PPARγ ligands trigger a conformational change of the PPARγ receptor that attracts transcriptional coactivators of the steroid receptor coactivator family. Once activated by ligand binding, the PPARγ receptor forms heterodimers with the retinoid X-receptor and transcription is initiated. Transcriptional activation may result in decreased proliferation, migration and inflammation and increased differentiation and apoptosis . Inflammatory effects are usually mediated by trans repression.

It illustrates the variety of pathways influencedby genomic activation of PPARγ by TZDs, resulting in downregulation of migration, proliferation, inflammation, and invasion and in upregulation of apoptosis. Common mechanisms involve influence on EGF signaling, cyclins, Ki- 67, c-myc, cyclin-dependent kinases, p53 and PTEN expression, adhesion proteins, metalloproteinases, and cytokines. Hormone-dependent cancers react through different mechanisms to TZDs depending on the hormone receptor status. In androgen-dependent prostate carcinoma, for instance, CIGLI downregulated aromatase activity, while in androgen-independent tumors proliferation was reduced. Different TZDs may act by different mechanisms; while CIGLI downregulated cyclin D1 and upregulated p21 byPPARγ signaling to induce these effects in androgen-independent prostate carcinoma cells. The description of all mechanisms of TZDs is beyond the scope of this review but one important signaling pathway for tumor cells and for surrounding tissue (tumor microenvironment) each illustrates the variety of PPARγ effects.

Tumor biology is not only determined by tumor cells but to a high extent by properties of stromal cells in the tumor microenvironment. Among the diverse cells in the tumor stroma (endothelial cells, cancer-associated fibroblasts, leukocytes, myofibroblasts, and mesenchymal stem cells), tumor-associated macrophages play the most decisive role in tumor progression. For tumor cells, signaling by Epidermal Growth Factor receptor (EGF-receptor, Figure 2) is highly relevant. The signaling cascade of the EGF-receptor involves the ERK cascade, consisting of

Ras-Raf-MEK1/MEK2-ERK1/ERK2 and is seen in several cancer types. ERK may phosphorylate PPARγ and reduce its genomic activity[10].

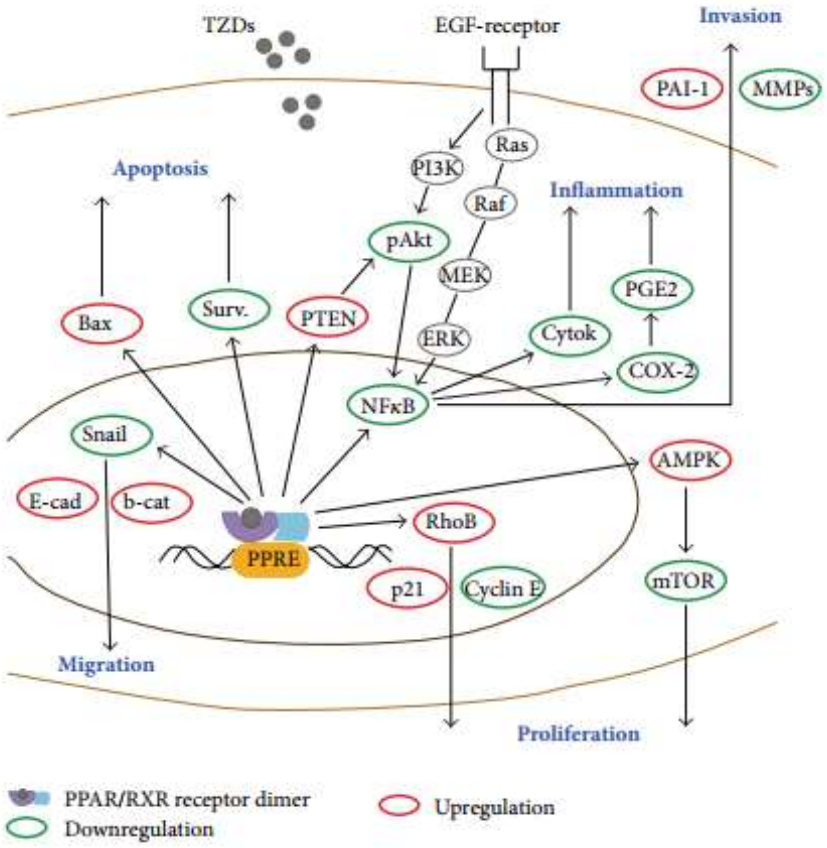
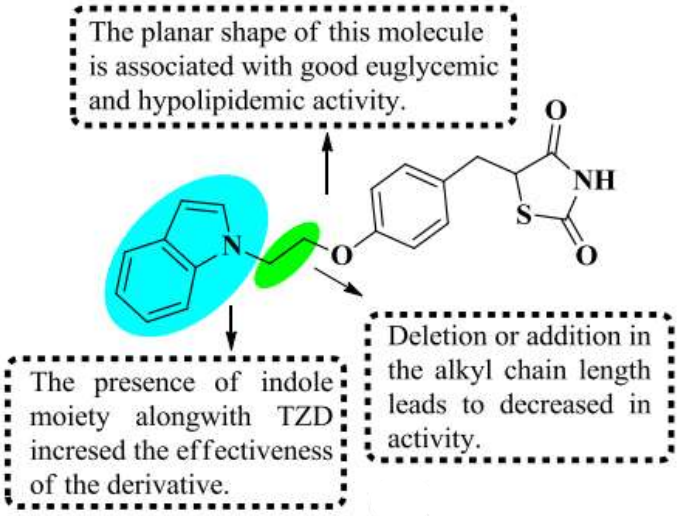


Fig. 5 Effects of TZDs on apoptosis, migration, invasion, and proliferation of cancer cells.

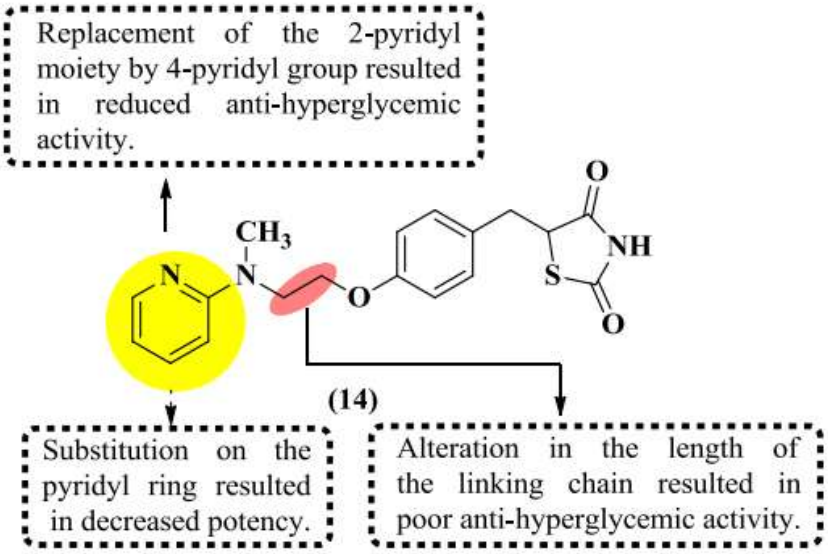
This effect occurs in cancer cell lines and a variety of normal cells alike. TRO, for example, was reported to bind to the EGF receptor and trigger its internalization in EGF-receptor transfected endothelial cells. This action is an example of nongenomic effects of TZDs since no ligand binding to response element occurred. Normal macrophages can transform into tumor associated macrophages under stimulation of PPARγ ligands. ROSI decreased activation of macrophages and thereby reduced inflammation in nondiabetic patients with symptomatic carotid artery stenosis. In murine macrophages, these effects are mediated by interaction of PPARγ with N In these effects, trans repression appears to be the main mechanism. Finally, MEK1 action by ROSI may lead to nuclear export and cytoplasmic retention of PPARγ (wild-type and mutant) co-transfected HEK-293 cells. In this effect nongenomic action of TZDs was involved.

SAR of Thiazolidinediones

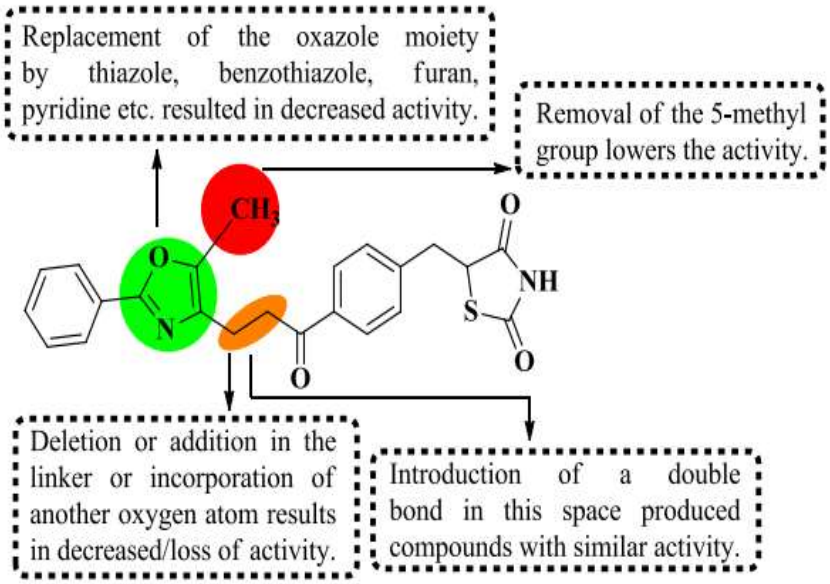
Lohray*et al.*, 1998 synthesized a series of [[(heterocyclyl)ethoxy]benzyl]-2,4- thiazolidinediones by condensation of corresponding aldehydes &2,4-thiazolidinedione followed by hydrogenation[11-12].



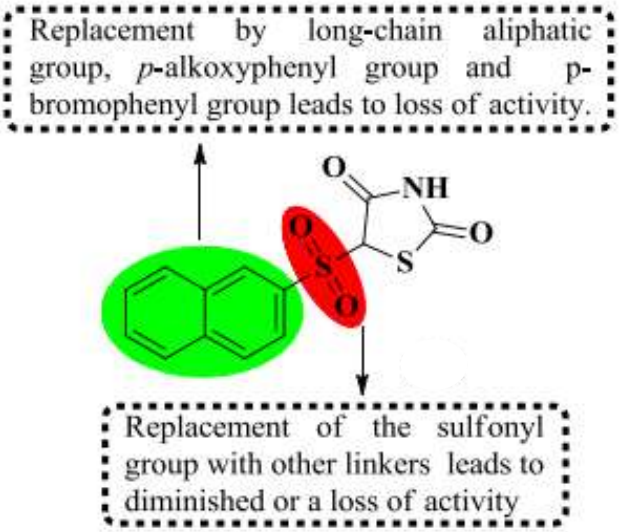
Cantello*et al.*, 1994synthesized a series of [(ureidoethoxy) benzyll- 2,4- thiazolidinediones and [[(heterocyclylamino) alkoxyl benzyl]-2,4-thiazolidinedione derivatives.



Hulin*et al.*,1992 prepared a new series of thiazolidine-2,4-diones by replacing the ether group of Englitazone with ketone, alcohol, or olefin moiety which reduces glucose level in blood in genetically obese and insulin-resistant ob/ob mouse.

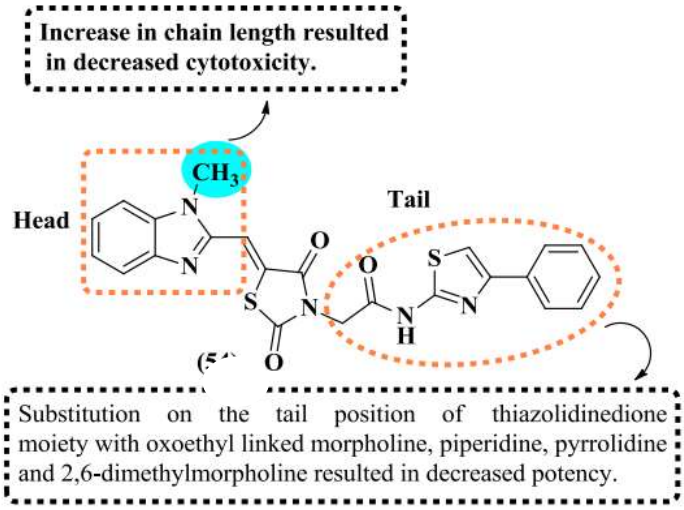


Zask*et al.*,1990 prepared a series of 5-(naphthalenylsulfony1)-2,4-thiazolidinedione derivatives and assessed them for antihyperglycemic activity in an insulin-resistant, genetically diabetic db/db mouse model of non-insulin-dependent diabetes mellitus (NIDDM).

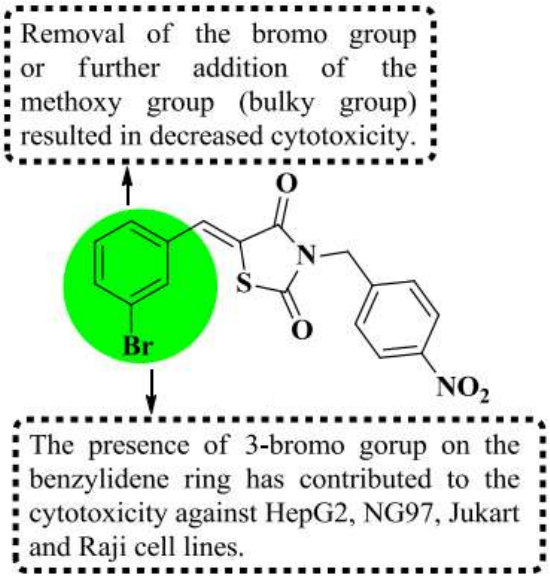


Sharma *et al.*, reported benzimidazole thiazolidinedione hybrids and assessed them against PC-3, DU-145, MDA-MB-231, A549 cancer cell lines for their cytotoxic potential emerged as the

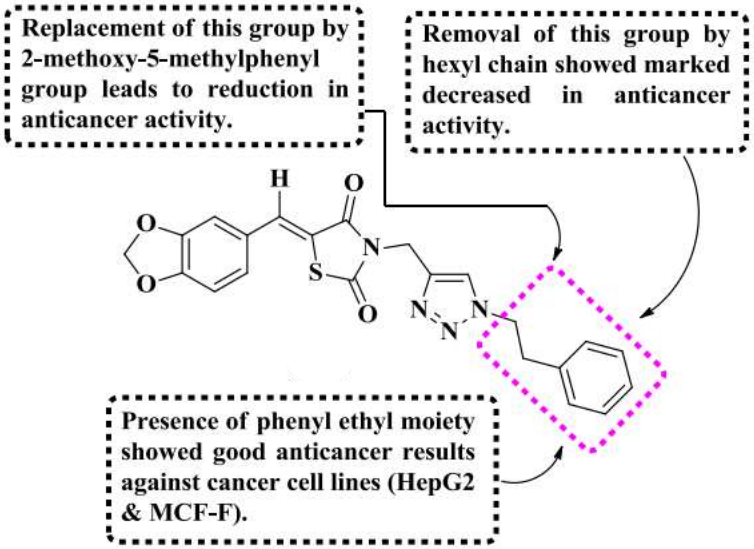
most promising candidate with IC50 value of 11.46 ± 1.46 mM on A549 lung cancer cell without any significant toxicity to MCF10A cells using 5-fluorouracil as the reference drug.



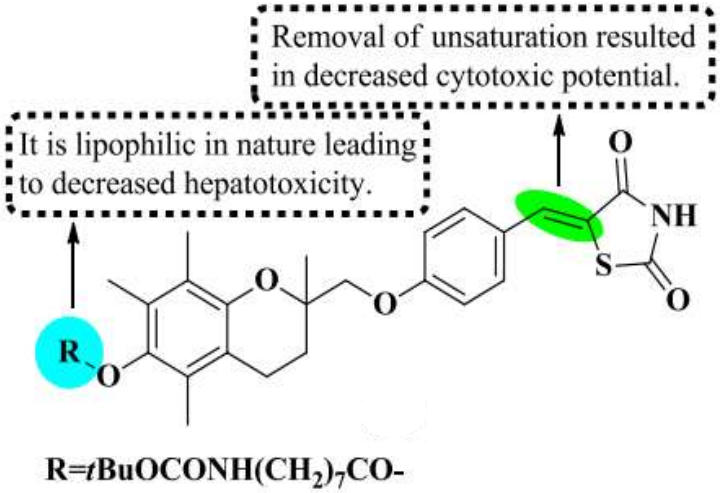
Rego*et al.*, synthesized disubstituted thiazolidinedione derivatives and evaluated their cytotoxic potential on tumor cell lines. It was found to be most potent against T47D, MiaPaca, HepG2, NG97, Raji, Jukart& PBMC tumor cell lines with IC50 > 100 mM, >100 mM, 58.58 mM, 73.73 mM, 58.8 mM, 62.54 mM and >100 mM using Amsacrine as a positive control[41].



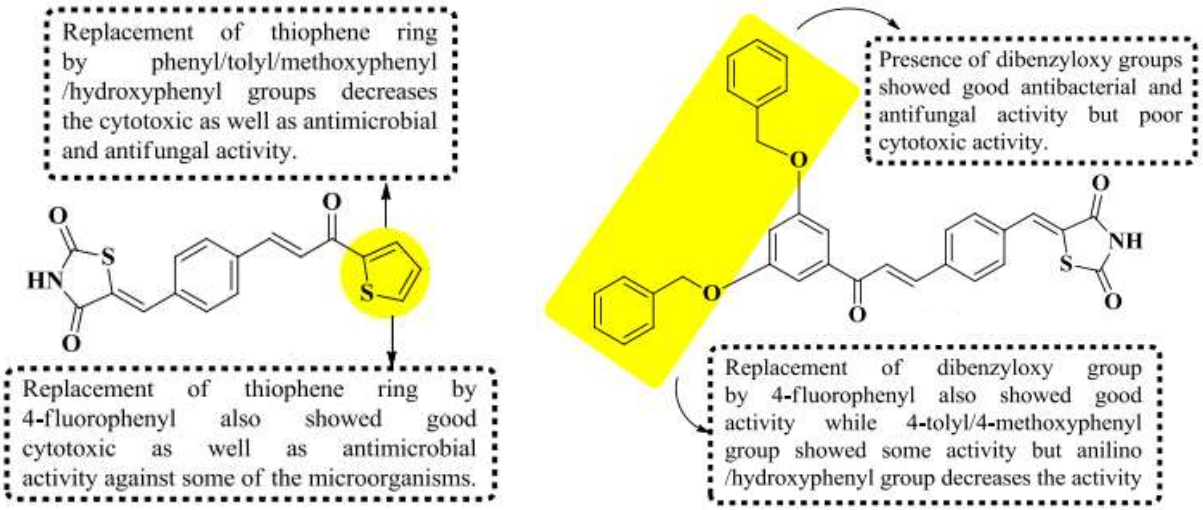
Chinthala*et al.*, reported new thiazolidinedione derivatives and evaluated for good anticancer activity with IC50 values of 31 mg/ml & 30 mg/mL against HepG2 and MCF-7 cancer cell lines using Doxorubicin as the standard drug.



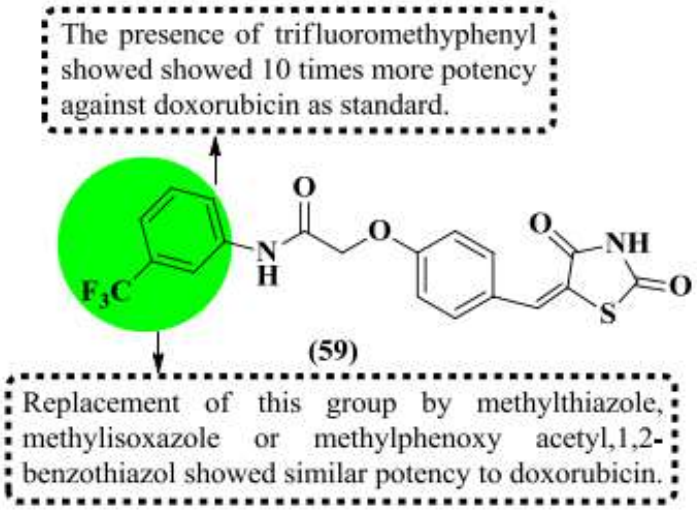
Salamone*et al.*, reported new derivatives of troglitazone and assayed for anticancer activity showed significant results against hormone dependent (MCF-7) and hormone independent (MDA-MDB-231) breast cancer cell lines with IC_{50} of 13.0 ± 1.5 & 3.2 ± 0.3 with 79% hepatocyte viability using troglitazone as reference drug.



Avupati*etal.*, and evaluated for cytotoxicity and antimicrobial activities was found as the most potent cytotoxic agent with an ED50 value of 4 mg/when compared with the standard drug Podophyllotoxin having anED50 value of 3.61 mg/mL.



Patil*et al.*, synthesized 5-benzylidene-2,4-thiazolidinedione derivatives and evaluated their antiproliferative activity against 7 cancer cell lines HOP62, PC3, MCF7, HEPG2, K562, GURAV, and KB at10-fold dilutions of 4 different concentrations (104 to 107 M).



DOCKING:

Molecular docking is an attractive scaffold to understand drug-biomolecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) in to the preferred binding site of the target specific region of the DNA/ Protein (receptor), mainly in a non-covalent fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy. The net predicted binding free energy bond (ΔG_{bind}) is revealed in terms of various parameters. Hydrogen bond ($\Delta G_{\text{ h bond}}$), Electrostatic (ΔG_{elec}) Torsional free energy (ΔG_{tor}), Dispersion and Repulsion (ΔG_{vdw}), Desolvation (ΔG_{desoly}) total internal energy (ΔG_{total}) and unbound systems energy (ΔG_{unb}). Therefore, good understanding of the general ethics that govern predicted binding free energy (ΔG_{bind}) provides additional clues about the nature of various kinds of interactions leading to the molecular docking. Molecular docking of small molecules to a target includes a pre-defined sampling of possible conformation of ligand in the particular groove of target in an order to establish the optimized conformation of the complex. This can be made possible using scoring function of software. Since the infrared spectroscopy, X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy are the techniques for the investigation and establishment of three dimensional structures of any organic molecule/ biomolecular targets. Hence homology modeling makes it possible to determine the tentative structure of proteins of unknown structure with high sequence homology to known structure. There are various databases available, which offer information on small ligand molecules such as CSD (Cambridge Structural Database), ACD (Available Chemical Directory), MDDR (MDL Drug Data Report) and NCI (National Cancer Institute Database). While performing docking, different interacted conformers are generated and compared with each other. The docked conformers according to their experimental binding affinities and binding free energies seem to be more difficult than their binding orientation[13]. To overcome this problem, different scoring functions are employed such as consensus scoring; appliance of number of score functions to the same docked pose in order to eliminate false positives. A huge number of attempts has been made for the development

of efficient docking protocols. No doubt, significant progress has been made in the computational prediction of docking modes.

Approaches of Molecular Docking:

For performing molecular docking, primarily two types of approaches are used.

Simulation approach:

Here the ligand and target is being separated by physical distance and then ligand is allowed to bind into groove of target after "definite times of moves" in its conformational space (Figure 1). The moves involve variations to the structure of ligand either internally (torsional angle rotations) or externally (rotations and translations). The ligand in every move in the conformational limit releases energy, as "Total Energy". This approach is more advantageous in the sense that it is more compatible to accept ligand flexibility[14].

Shape complementarity approach:

This approach employs ligand and target as surface structural feature that provides their molecular interaction (Figures 2 and 3). Here the surface of target is shown with respect to its solvent-accessible surface area and ligand's molecular surface is showed in terms of matching surface illustration. The complementarity between two surfaces based on shape matching illustration helps in searching the complementary groove for ligand on target surface. For example, in protein target molecules, hydrophobicity is estimated by employing number of turns in the main-chain atoms. This approach is rather quick and involves the rapid scanning of numerous thousands of ligands in a few seconds to find out the possible binding properties of ligand on target molecular surface.

Types of Docking:

Comprehensively utilized docking tools employ search algorithms such as genetic algorithm, fragment-based algorithms, Monte Carlo algorithms and molecular dynamics algorithms. Besides this, there are some tools such as DOCK, GOLD, Flex X and ICM which are mainly used for high throughput docking simulations. There are various kinds of molecular docking procedures involving either ligand/target flexible or rigid based upon the objectives of docking simulations like flexible ligand docking (target as rigid molecule), rigid body docking (both the target and ligand as rigid molecules) and flexible docking (both interacting molecules as flexible).

Applications of Molecular Docking:

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing[58]. Some of the major applications of molecular docking are described below;

Lead optimization:

Molecular docking can predict an *optimized orientation* of ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. This can be used to develop more potent, selective and efficient drug candidates.

Hit identifications:

Docking in combination with scoring function can be used to *evaluate large databases* for finding out potent drug candidate *in-silico*, which can target the molecule of interest.

Drug-DNA interaction:

Molecular docking plays a prominent role in the initial prediction of drug's binding properties to nucleic acid. This information establishes the correlation between drug's molecular structure and its cytotoxicity. Keeping this in view, medicinal chemists are constantly putting their efforts to elucidate the underlying anticancer mechanism of drugs at molecular level by investigating the interaction mode between nucleic acid and drugs in presence of copper. Medicinal chemists are doing *in silico* observations where their main finding is to predict whether the compound/drug is interacting with the protein/DNA. If the docking program is predicting the said interaction, then the experimental procedures are made available to find out the real binding mode of the complex. This leads to the development of new anticancer drug. Furthermore, this knowledge would be instrumental in the detection of those structural modifications in a drug that could result in sequence/structure specific binding to their target.

Basic Challenges in Molecular Docking:

Certain basic challenges in docking and scoring are discussed under the following headings.

Ligand chemistry:

The ligand preparation has prominent effect on the docking results because the ligand recognition by any biomolecule depends on 3-dimensional orientation and electrostatic interaction. This confirms that the conformation of both the ligand as well as ligand preparation is important. Earlier, keeping approximate pka values, the structure being most likely optimized by removing or adding hydrogen but the tautomeric and protomeric states of the molecules which are to be docked, still remained a major discrepancy. Since almost all databases keep molecules in their neutral forms but under physiological conditions they are actually ionized. Hence it is compulsory to ionize them prior to docking. But in different programs, the standard ionization is easy to achieve. Regarding the issue of tautomers, the problem still remains there, which tautomer one should use or should one use all possible tautomers.

Receptor flexibility:

This is a major challenge in docking i.e., handling of flexible protein. A biomolecule/protein adopts different conformations depending upon the ligand to which it binds. This confirms that docking done with a rigid receptor will give a single conformation of receptor. However, when the docking is done with flexible receptor, the ligands may require many receptor conformations to bind. In molecular docking studies, usually the most neglected aspect is *different conformational* states of proteins. Since the protein flexibility is important as it accounts for better affinity to be achieved between a given a drug and target. Another aspect of target flexibility is active site water molecules. Water molecules must be rectified to avoid using anti-fact waters in the docking process.

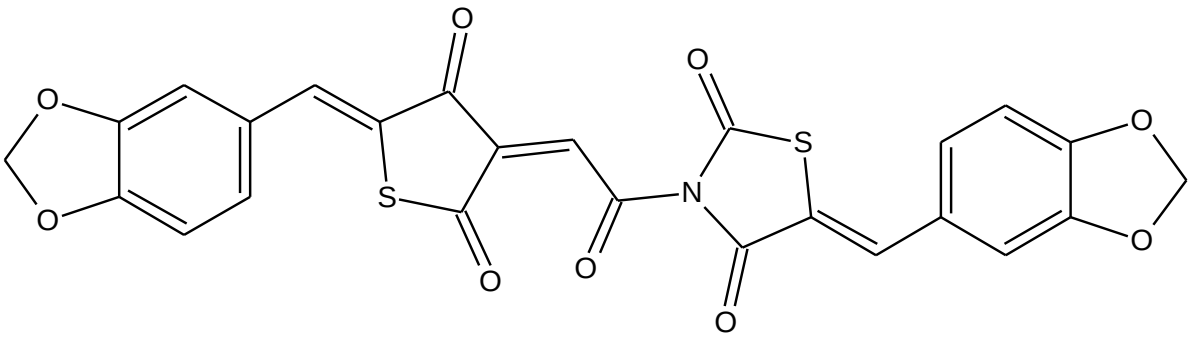
Scoring function:

Another challenge in docking is imperfection in scoring function. Just like search algorithm is having potential to give optimum conformation, scoring function should also be able to differentiate true binding modes from all the other parallel modes. A potential scoring function would be computationally much economical, unfavorable for analyzing several binding modes. When there is accuracy, scoring functions make number of suggestions to evaluate ligand affinity. The physical phenomenon i.e., entropy and electrostatic interactions are disregarded in scoring schemes. Hence the lack of suitable scoring function, both in terms of accuracy and speed, is the main congestion in molecular docking programming.

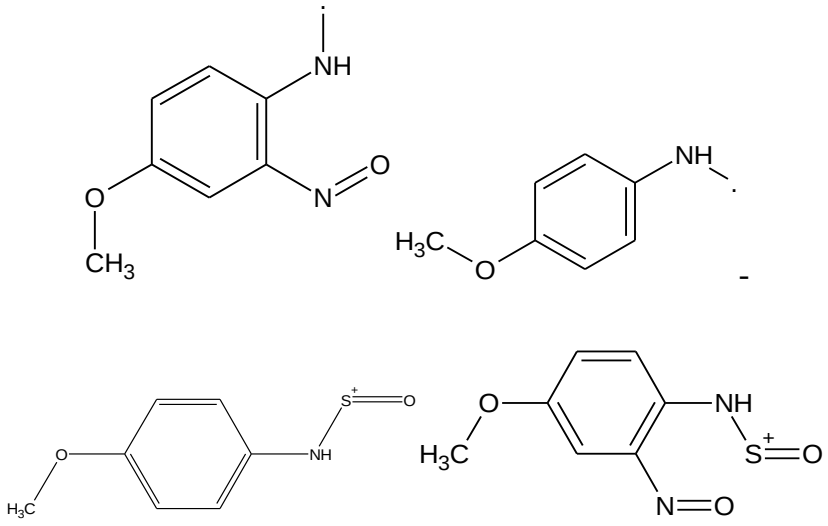
CHAPTER 2

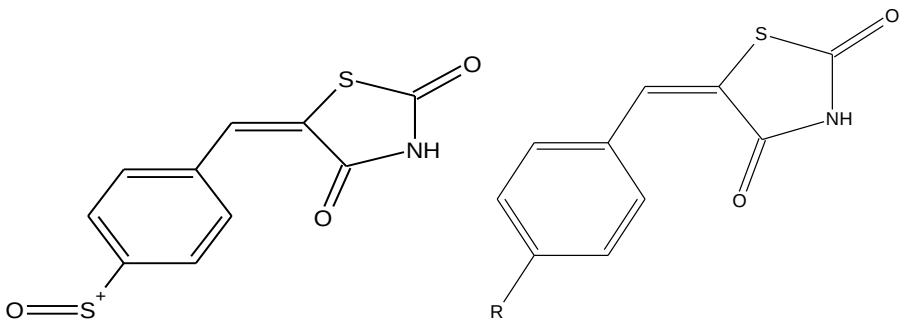
LITERATURE REVIEW

Ahmed *et al.*,**2010**[15]Synthesized the 5-(Benzo[d][1,3] dioxol-5-ylmethylene) thiazolidine -2,4-dione and evaluated for anti-diabetic and anti-hyperlipidemic activity (in vivo) using alloxan animal model.(5*Z*)-5-[(2*H*-1,3-benzodioxol-5-yl)methylidene]-3-[(2*Z*)-2-{(5*Z*)-5-[(2*H*-1,3-benzodioxol-5-yl)methylidene]-2,4-dioxothiolan-3-ylidene}acetyl]-1,3-thiazolidine-2,4-dione

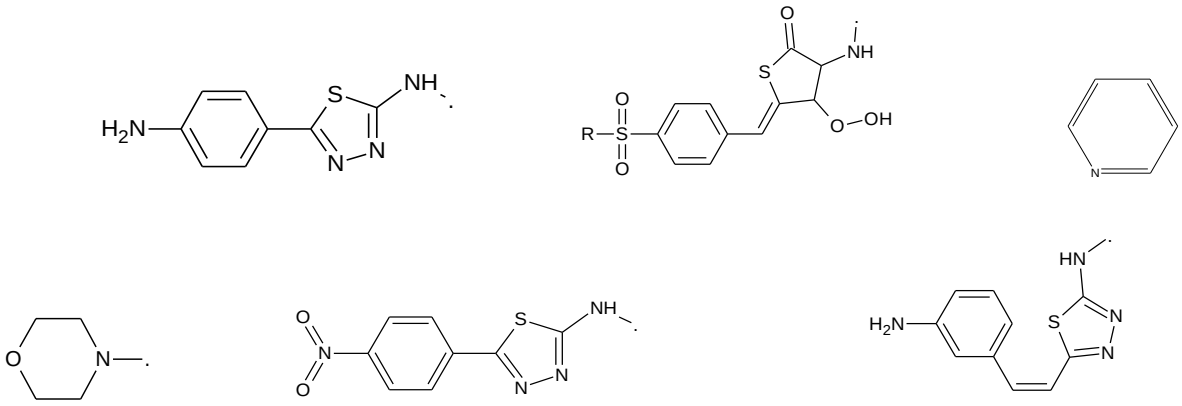


Roy *et al.*,**2011**[16] Synthesized and Evaluate the Some Novel 5-[4-(substituted) benzylidene] 2.4 thiazolidinediones as Oral Antihyperglycemic Agents.

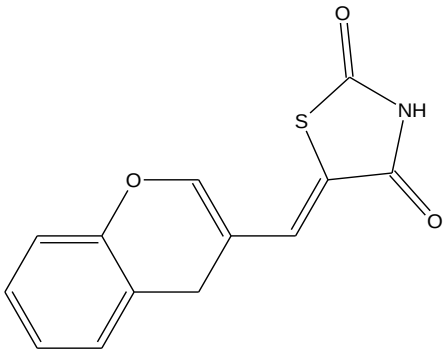




Shashikant *et al.*,(**2009)**[17] Synthesized the Novel 2,4-Thiazolidinedione Derivatives with Antidiabetic Activity.

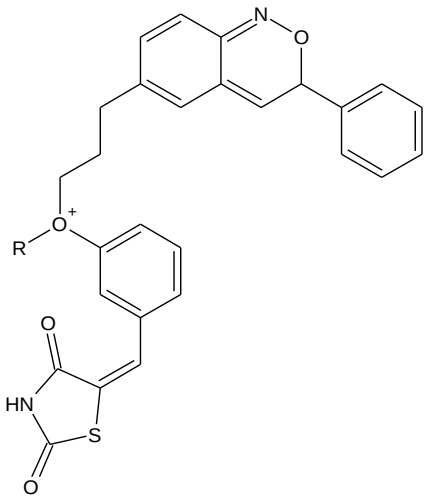


Ashish *et al.*,**2010 [18]**have done the Synthesis and study of (5*Z*)-5 -[(4-oxo-4*H*-chromen-3-yl) methylidene]-1, 3- thiazolidine-2, 4-dione derivative.



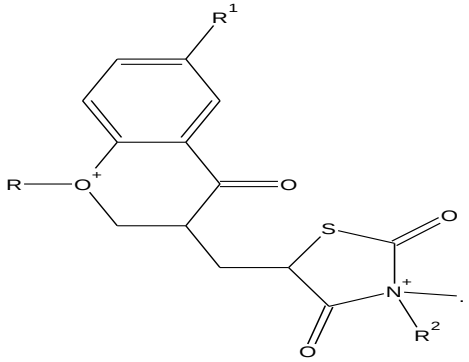
R=H,Hcl,F, NO_2, CH_3

Shriram *et al.*,**2014**[19]synthesized Benzisoxazole containing Thiazolidinediones as Peroxisome Proliferator Activated Receptor-γ Agonists and assess their antidiabetic activity and performed molecular docking.



R=methyl, ethyl, n-propyl

Partha Neogi *et al.*,**2014** [20] have synthesized and studied number of 2,4-thiazolidinedione derivatives of -phenyl substituted cinnamic acid for their PPAR agonist activity.

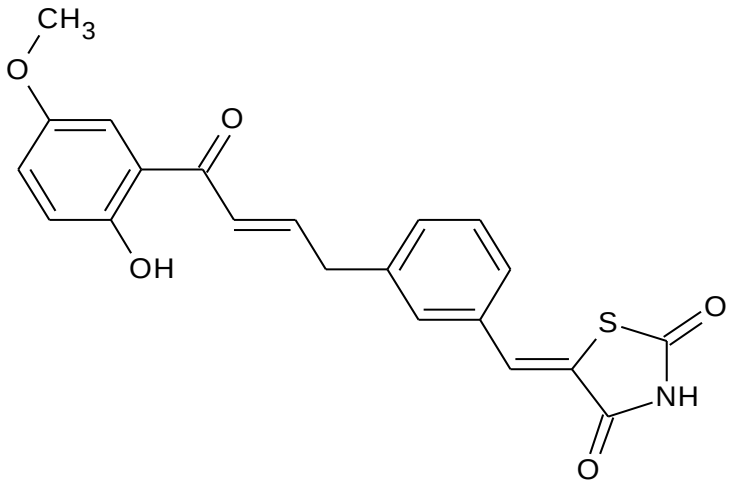


R= cl, F, Br

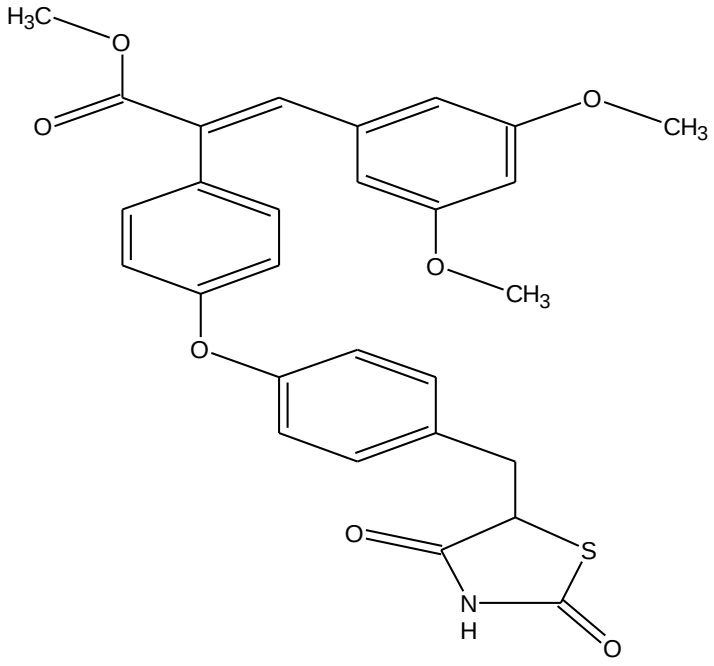
R^1= OCH_3, NO_2

R^2=OH, NO_2, NH_2

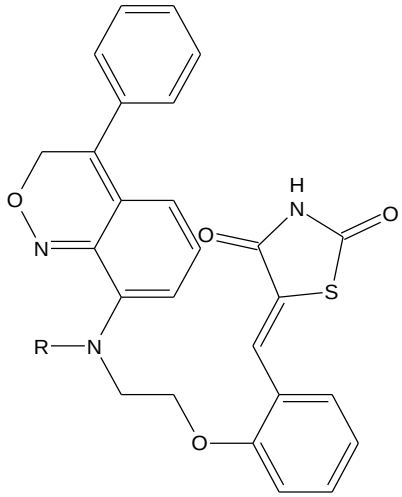
DebarshiKarMahapatra *et al.*,**2015**[21]have done the study of Chalcones and their therapeutic targets for the management of diabetes: Structural and pharmacological Perspective.



Mohdimran *et al.*,**2006**[22]have done the review of recent thiazolidinediones as antidiabetics.

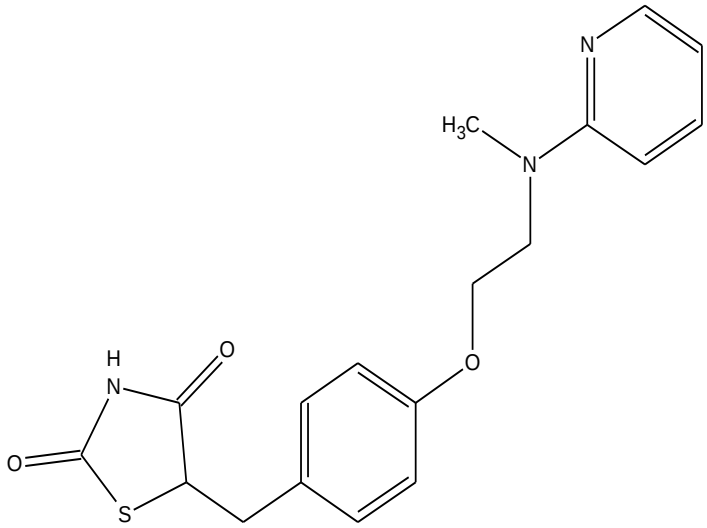


Shriram *et al.*,**2012**[23]predicted the possibility of novel 5-substituted benzisoxazole containing thiazolidine-2, 4- dione derivatives as potent ppar-γ agonists.

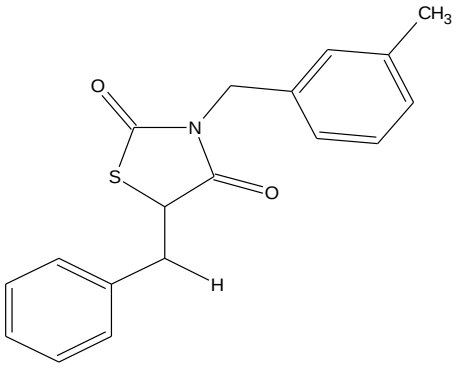


R=methyl, ethyl, n-propyl

Hong Woo Lee *et al.*,**2014**[24] reported the synthesis and antidiabetic activity of novel substituted pyrimidines having thiazolidinedione moiety and were evaluated for their glucose and lipid lowering activity in mice. From the results, novel compounds exhibited considerably more potent biological activity than that of the reference compounds, pioglitazone and Rosiglitazone.

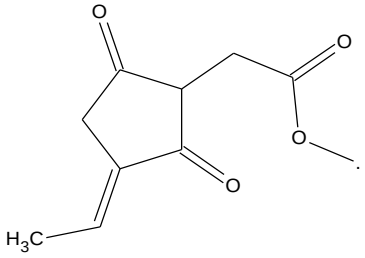


Pitta *et al.,***2005** [25]**s**ynthesized a novel set of acridinylidenethiazolidinediones and benzylidene thiazolidinediones by nucleophilic addition of cyanoacrylates. Some of these compounds were evaluated for their glucose lowering capability and their effects on the triglyceride level in alloxan diabetic mice.



OCH_3, NO_2, Cl, F

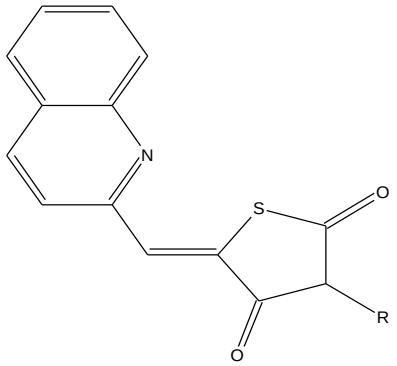
Devi Prasad Sahu *et al.,* **2004**[26]synthesized number of thiazolidine-2,4-diones derivatives having carboxylic ester appendage at N-3 and their antihyperglycemic activity was evaluated. Many of these derivatives as well as their corresponding carboxylic acid showed significant improvement on post-prandial hyperglycemia in normal rats, in contrast to their poor agonist activity at PPAR-γ.



X=COOH, Cl, NH_2

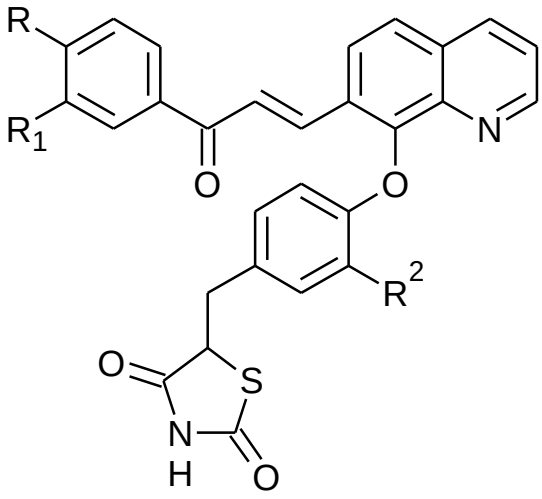
R=NO_2, C_2H_5,CH_3

Riyaz *et al.*,**2004**[27]have done the PEG-600 mediated one pot synthesis of quinolinyllidinethiazolidine 2,4-diones and evaluate as potential anti- hyperglycemic agents.



R=CH_3, C_2H_5, C_4H_{10}, CH_2ph, SO_2ph

Srikanth *et al.*,**2010**[28] synthesized and evaluated the newer quinoline derivatives of thiazolidinediones for their antidiabetic activity.

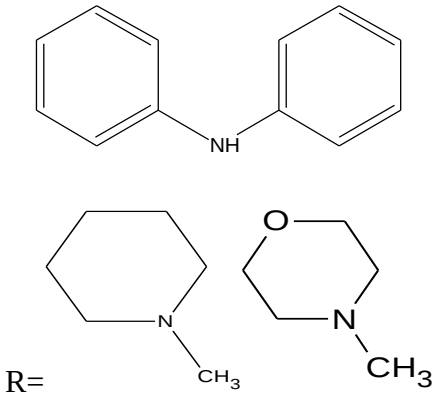


R=H, NO_2, OH, OCH_3, CH_3

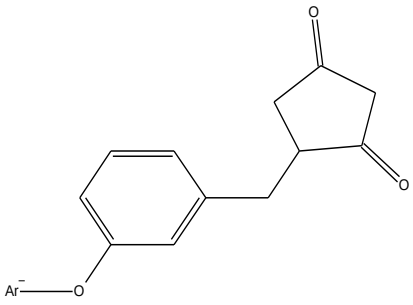
R_1=H, C_2H_5, NO_2, NH_2

R^2=H

Alam *et al.*,**2011**[29]synthesized and characterized the thiazolidinedione Derivatives as oral hypoglycemic agent.

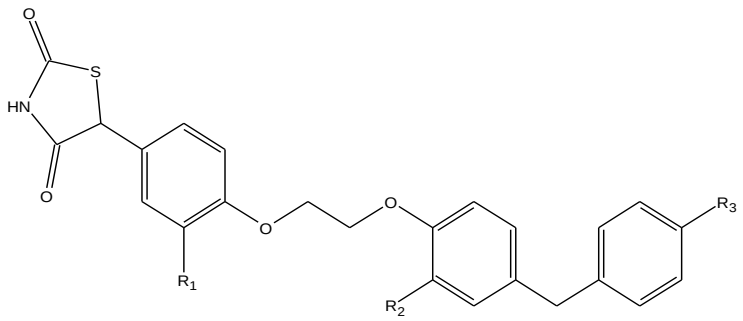


Changyou hou *et al.*,**2012**[30]performed Systematic structure–activity relationship (SAR) studies of a screening lead led to the discovery of a series of thiazolidinediones (TZDs) as potent GPR40 agonists.



Ar=C_6H_5-CL, C_6H_5-Br,C_6H_5-NO_2

Hiroo Koyama *et al.*,**2013**[31]designed, synthesized, and evaluated a series of 5-aryl thiazolidine-2,4-diones containing 4-phenoxyphenyl side chains for PPAR agonist activities. One such compound exhibited comparable levels of glucose correction to rosiglitazone in the type 2 diabetes animal model.

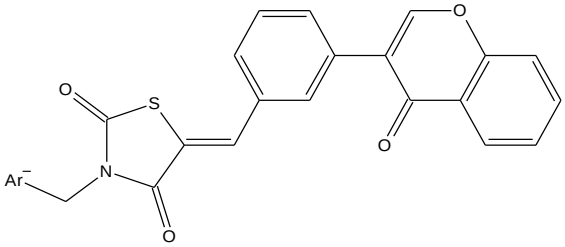


R_1=NO_2, OH

R_2=Cl, Br, F

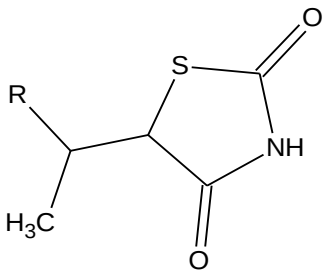
R_3=OMe, C_2H_5

RahmiyeErtan *et al.*, **2012**[32]synthesized series of 3-benzyl (p-substituted benzyl)-5-[4-oxo-1-benzopyran-2-yl)-benzylidene]-2,4- thiazolidinediones. Products were prepared by Knoevenagel reaction and *In vitro* insulinotropic activity was determined.



Ar= C_6H_5-Cl, C_6H_5-Br, C_6H_5-NO_2

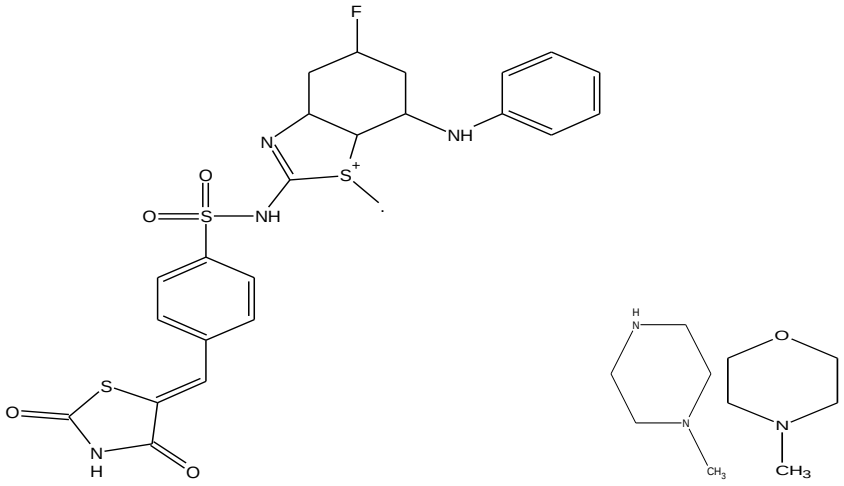
Sachin *et al.*,**2012**[33]have done the review of thiazolidinediones as a plethora of biological load.



R=2-napthyl 4-bromo phenyl 4-fluro phenyl, n-octyl

X=O,CH_2, CH_2S,CH_2SO_2, SO,SO_2

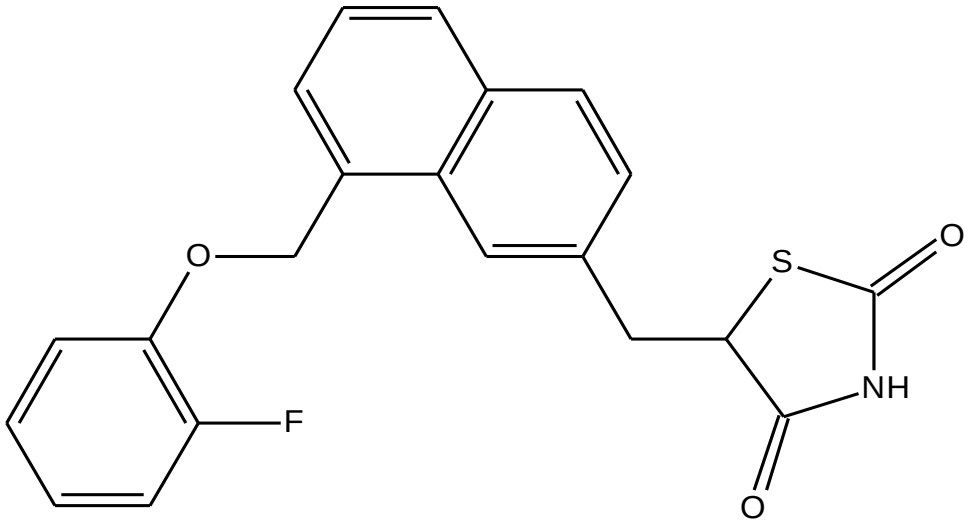
Pattan *et al.*,**2013**[34]synthesized and evaluate the anti-diabetic activity of 2-amino (5-(4-sulphonyl benzylidene) 2,4-thiazolidinedione)-7 -chloro-6- fluorobenzothiazole].



H, m-NO_2, P-COOH

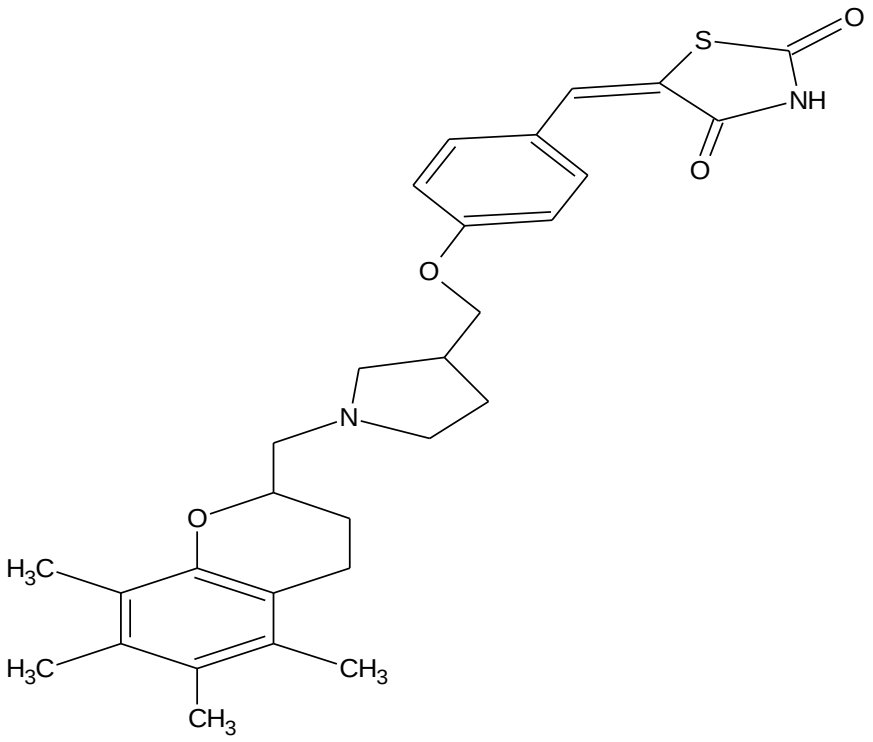
Nanjan *et al.*,**2012**[35]designed some novel glitazones based on the structure activity relationships as possible PPAR-agonists. The manually designed glitazones were synthesized by using the appropriate synthetic schemes and screened for their *in vitro* antihyperglycemic activity by estimating glucose uptake by rat hemi-diaphragm, both in the absence and in the presence of external insulin. Some of the glitazones exhibited good antihyperglycemic activity in presence of insulin.

Fana *et al.*,**2013** [36]evaluate toxicity and toxicokinetic of MCC-555, a treatment candidate for type 2 diabetes, a novel thiazolidinedione which has comparatively high anti-diabetic efficacy in beagle dogs. During the treatment and recovery periods, the effects of the test agent on mortality, body weight, food consumption, hematology, serum biochemistry, urinalysis, electrocardiogram (ECG), organ weights, bone marrow and histopathology were examined. Metabolites and the metabolic style of MCC-555 are to be approved.

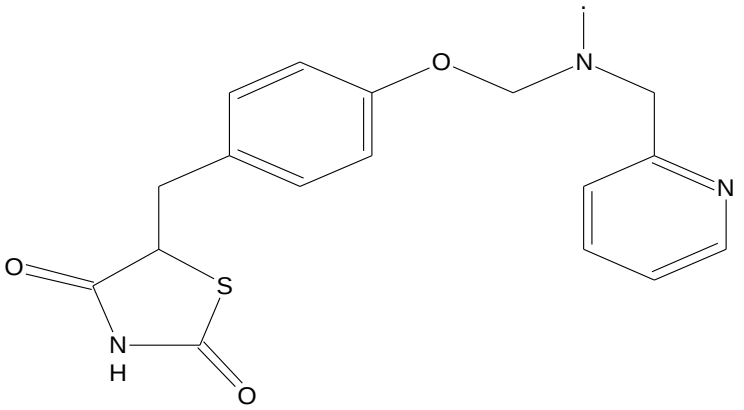


MCC-555

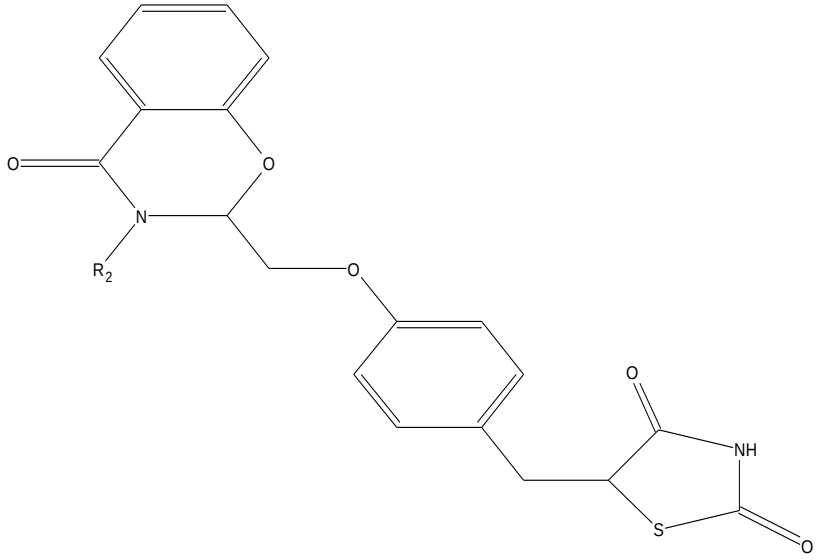
Vaibhav *et al.*,**2013**[37]have done the SAR and computer-aided drug design approaches in the discovery of peroxisome proliferator-activated receptor.



Haruya *et al.*,**2011**[38]reported that PPAR agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein.

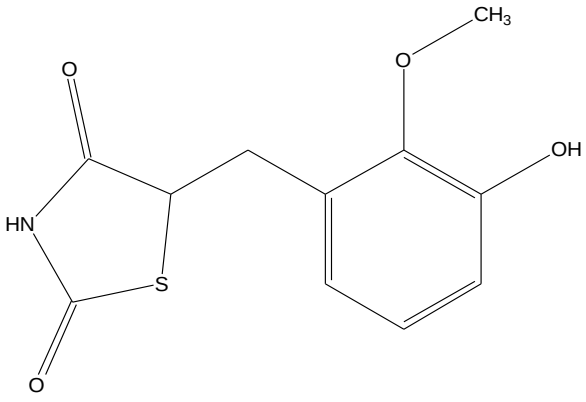


Gurram *et al.*,**2009**[39]synthesized and evaluated 2,4-Thiazolidinedione derivatives of 1,3-benzoxazinone for their PPAR-α and $_{\gamma}$ dual activation. DRF-2519, a compound obtained through SAR of TZD derivatives of benzoxazinone, has shown potent dual PPAR activation. In ob/ob mice, it showed better efficacy than the comparator molecules.

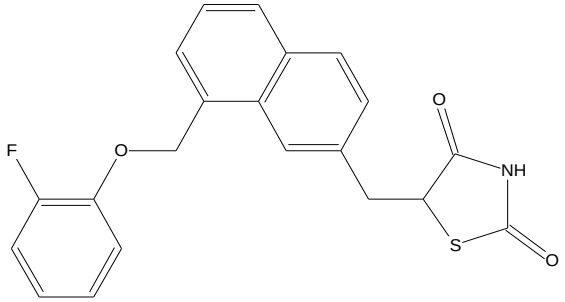


R_2=NH_2, OH, Cl, F, Br

Chaudhary *et al.*,**2010**[40]characterized the pharmacological profiles of NS-1 chemically known as (5Z)-5-[4- hydroxy-3-methoxy-phenyl) methylene] thiazolidine-2, 4-dione), as a selective partial activator of PPAR- γ. Studies suggest that novel compound improves insulin resistance in such animal models through activation of PPAR-γ mediated transcriptional activity and that it would be a new therapeutic candidate with potential for the treatment of type 2 diabetic patients.

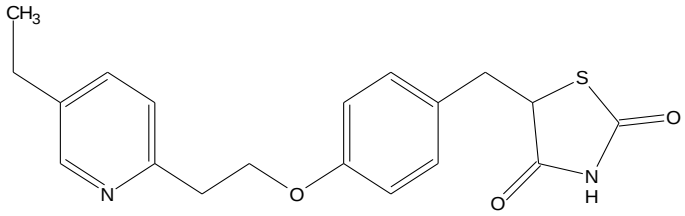


Li Sen Liu *et al.*,**2008**[41]suggested that MCC-555 effect was paralleled by a significant dephosphorylation of IRS-1 on Ser/Thr. In conclusion, MCC-555 rapidly sensitizes insulin stimulated cardiac glucose uptake by enhancing insulin signaling resulting from increased intrinsic activity of PI 3-kinase. Acute activation of protein expression leading to a modulation of the Ser/Thr phosphorylation state of signaling proteins such as IRS-1 (Insulin receptor substrate 1) may be underlying this process. It is may provide a causal therapy of insulin resistance by targeted action on the defective site in the insulin signaling cascade.

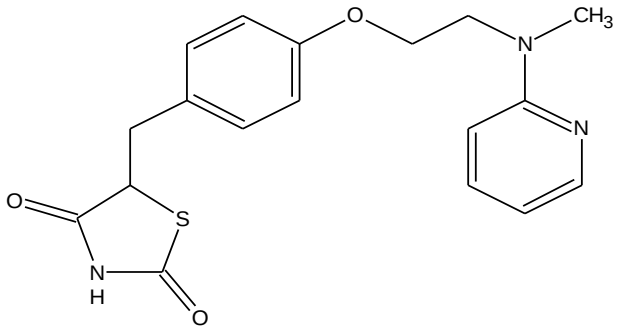


MCC=555

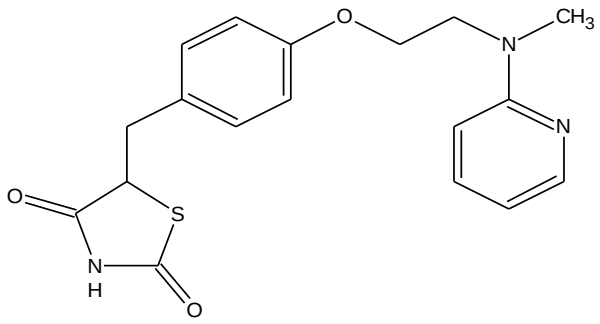
Atanas *et al.*,**2011**[42]reported that Honokiol is a non-adipogenicPPARγ agonist from nature.



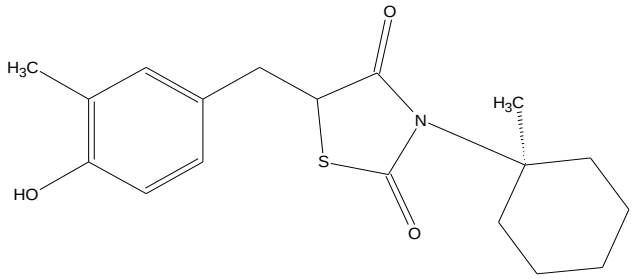
Julieta *et al.*,**2012[43]**reported the Hypoglycemic Action of Thiazolidinediones /Peroxisome Proliferator Activated Receptor- γ by Inhibition of the c-Jun NH2-Terminal Kinase Pathway.



Rai *et al.*,**2011**[44]prescribed fenofibrate and rosiglitazone to treat hypertriglyceridemia and diabetes, respectively. Since fenofibrate improves lipid profile in diabetic patients and improves insulin resistance in animal models, examined the mechanism of antidiabetic effects of fenofibrate in KKAy mouse, an animal model of diabetes and dyslipidemia. Results shown that, amelioration of antidiabetic and hyperlipidemic state by fenofibrate in mice occurred via down regulation of DGAT2, PEPCK and 11_-HSD1 while the undesirable lipogenic effects of T090317 could be dampened by fenofibrate.

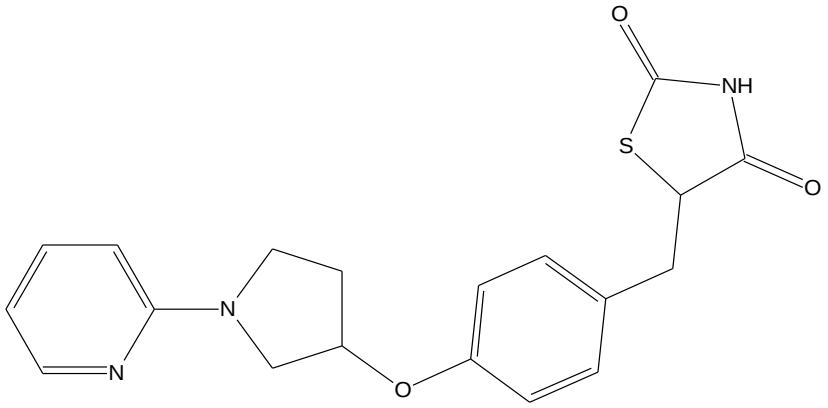


Ching-Shih Chen *et al.*,**2011**[45]used 2CG, a PPAR-$_{\gamma}$ inactive analogue of ciglitazone, to conduct lead optimization able to mediate PPAR-$_{\gamma}$ independent transcriptional repression of androgen receptor (AR) in a tumor cell-specific manner, and to develop a novel class of AR-ablative agents. Structure–activity analysis indicates a high degree of flexibility in realigning 2CG's structural moieties without compromising potency in AR repression, as evidenced by the higher AR-ablative activity of the permuted isomer whose modification which completely inhibited AR expression at low micromolar concentrations.

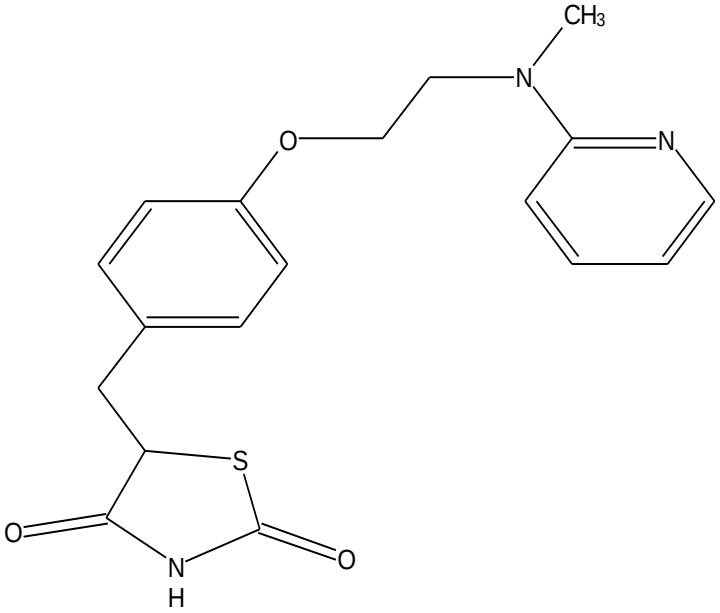


Matthew J. Ellis *et al.*,**2014** [46]developed a novel molecular dynamics (MD) analysis algorithm, DASH, to utilize the sequential nature of MD simulation data. By adjusting a set of parameters, the sensitivity of DASH can be controlled, allowing molecular motions of varying magnitudes to be detected or ignored as desired, with no knowledge of the number of conformations required

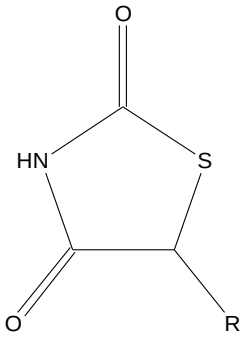
being prerequisite. MD simulations of three synthetic ligands of the orphan nuclear receptor PPAR- γ were generated in vacuo using Tripos's SYBYL and used as the training set for DASH. Two X-ray crystal structures of PPAR-γ complexed with Rosiglitazone were compared to gain knowledge of the pharmacophoric conformation; this showed that the conformation of the ligand is significantly different between the two structures, indicating that there is no distinct conformation in which rosiglitazone binds to PPAR- γ but multiple binding modes. The results show that DASH analysis is as good as Ward analysis in some areas.



Divya *et al.*,**2010**[47]reported that PPAR-γ agonist is an effective strategy for cancer treatment.

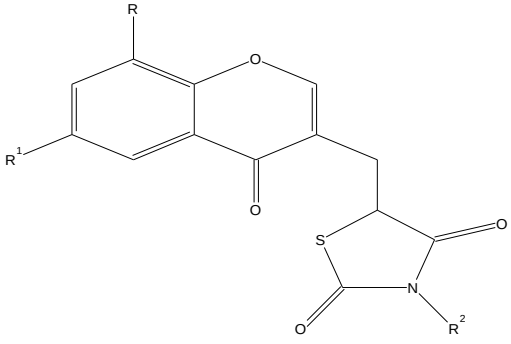


Muchtaridi *et al.*,**2009**[48]have done the in silico evaluation of potent for ppar-$_{\gamma}$ agonist of lignan derivatives from myristicafragranshoutt seeds.



R=H, NO_2, CH_3, OH, Cl

OyaBozdag-Dundar*et al.*,**2010**[49]prepared series of chromonyl-2,4- thiazolidinediones by Knoevenagel reaction with substituted 3-formylchromones and unsubstituted or substituted 2,4-thiazolidinedione. The synthesized compounds were tested for their ability to inhibit rat kidney AR by an *in vitro* spectrophotometric assay. Compound IIIe showed the highest inhibitory activity.

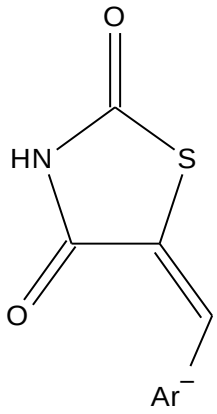


R=Cl, F, Br

R^1=NO_2, OCH_3

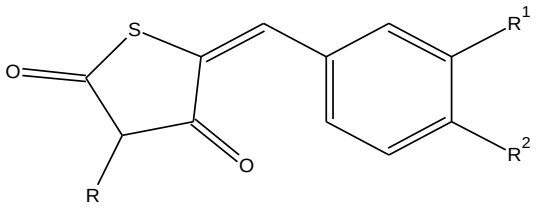
R^2=OH, NO_2, NH_2

Kumar Soni *et al.*,**2008**[50]performed QSAR study on a series of 5-arylidene-2,4-thiazolidinediones using the FujitaBan and the classical Hansch approach and molecular modeling studies employing AM1 calculations to gain structural insight into the binding mode of these molecules to the aldose reductase enzyme.



Ar=C_6H_5-Cl, C_6H_5-Br, C_6H_5-F

Maccari *et al.*,**2011**[51]synthesized and evaluated number of 5-arylidene- 2,4-thiazolidinediones containing a hydroxy or a carboxymethyl group in their 5-benzylidene moiety as *in vitro* aldose reductase (ALR2) inhibitors. Most of them exhibited strong inhibitory activity, with IC50 values in the range between 0.20 and 0.70 lM. Molecular docking simulations into the ALR2 active site highlighted that the phenolic or carboxylic substituents of the 5-benzylidene moiety can favorably interact, in alternative poses, either with amino acid residues lining the lipophilic pocket of the enzyme, such as Leu300, or with the positively charged recognition region of the ALR2 active site.

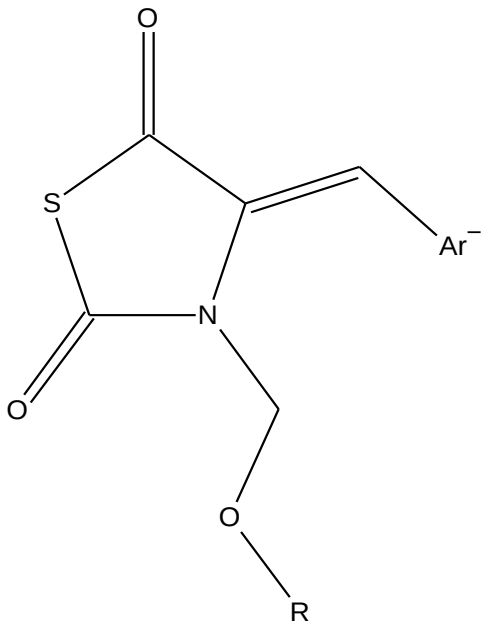


R=H,CH_3, C_2H_5

R^1=Cl, F, Br

R^2=OH, NO_2, OMe

Maccari *et al.*,**2011**[52]reported a series of non-carboxylic acid containing 2,4-thiazolidinedione derivatives, analogues of synthesized carboxylic acids which was very active *in vitro* aldose reductase (ALR2) inhibitors. Although the replacement of the carboxylic group with the carboxamide or N-hydroxy carboxamide one decreased the *in vitro* ALR2 inhibitory effect which led to the identification of mainly non-ionized derivatives with micromolar ALR2 affinity. The 5-arylidene moiety deeply influenced the activity of these 2,4- thiazolidinediones. Induced-fit docking studies suggested that 5-(4-hydroxybenzylidene)-substituted derivatives may bind the polar recognition region of the ALR2 active site by means of the deprotonated phenol group, while their acetic chain and carbonyl group at position 2 of the thiazolidinedione ring form a tight net of hydrogen bonds with amino acid residues of the lipophilic specificity pocket of the enzyme.

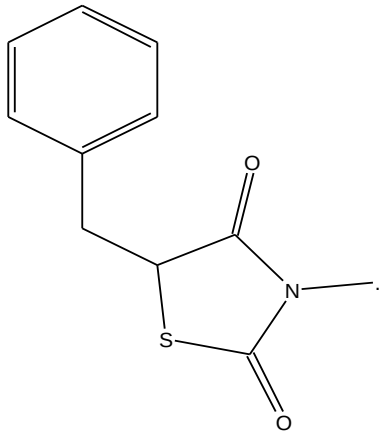


R=OH, NH_2, Na, H

Ar=C_6H_5-Cl, C_6H_5-Br

Soni *et al.*,**2012**[53]reported quantitative structure–activity relationship (QSAR) analysis performed by 3D-QSAR analysis, Hansch analysis, and Fujita-Ban analysis on a series of 5-arylidene-2,4-thiazolidinediones as aldose reductase inhibitors. The 2D & 3D-QSAR models were generated using 18 compounds and Fujita-Ban analysis models were obtained using 23

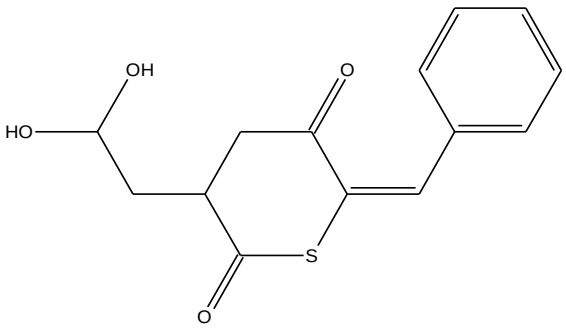
compounds. The predictive ability of the resulting 2D and 3D models was evaluated against a test set of 5compounds. Analyses of results from the present QSAR study inferred that 3rd position of the phenyl ring and acetic acid substitution at N-position of thiazolidinediones play a key role in the aldose reductase inhibitory activity.



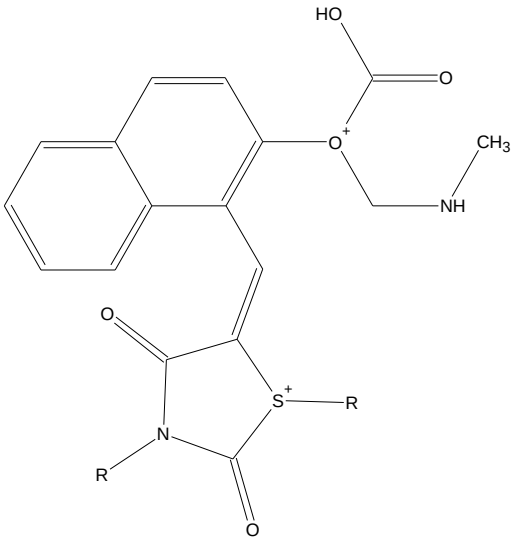
X=Cl, CH_3, C_2H_5

NH_2, NO_2, OH

Maccari *et al.*,**2011**[54]synthesized several (Z)-5-arylidene-2,4- thiazolidinediones and tested as aldose reductase inhibitors (ARIs). The most active of the N-unsubstituted derivatives exerted the same inhibitory activity of Sorbinil. The introduction of an acetic side chain on N-3of the thiazolidinedione moiety led to a marked increase in lending inhibitory activity, conducting to the discovery of a very potent ARI (4c), whose activity level (IC50=0.13 mM) was in the same range of Tolrestat. The substitution pattern on the 5-benzylidene moiety markedly influenced the activity of N-unsubstituted 2,4-thiazolidinediones. Compounds with substituent at the Meta position being generally more effective than the para-substituted one.

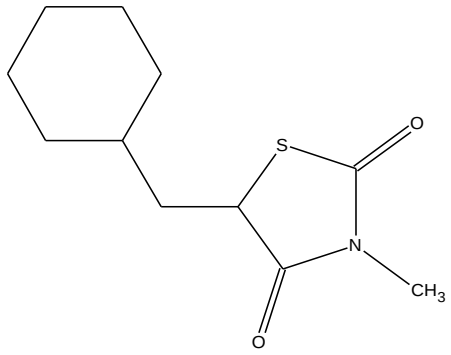


Murata *et al.*,**2010**[55]reported a series of 2-(1-((4-oxo-2-thioxothiazolidin-5- ylidene) methyl) naphthalen-2-yloxy) acetic acid were synthesized and evaluated as aldose reductase inhibitors.



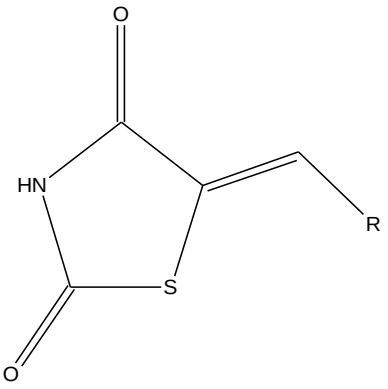
R=CH_3, C_2H_5

Urzhumtsev *et al.*,**2009**[56]suggest the active site of AR to bind tightly to different inhibitors; this happens both upon binding to the inhibitor's hydrophilic heads, and at the hydrophobic and specificity pockets of AR, which can change their shape through different conformational changes of the same residues. This flexibility could explain the large variety of possible substrates of AR.



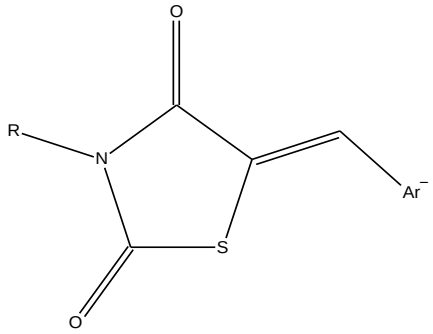
X=CH_3, C_2H_5, OCH_3

Rosaria Ottana *et al.*,**2011**[57]explored more effective 5-arylidene-4-thiazolidinones as aldose reductase inhibitors. Acetic acids **5** proved to be interesting inhibitors of the enzyme as well as excellent antioxidant agents that are potentially able to counteract the oxidative stress associated with both diabetic complications as well as other pathologies.



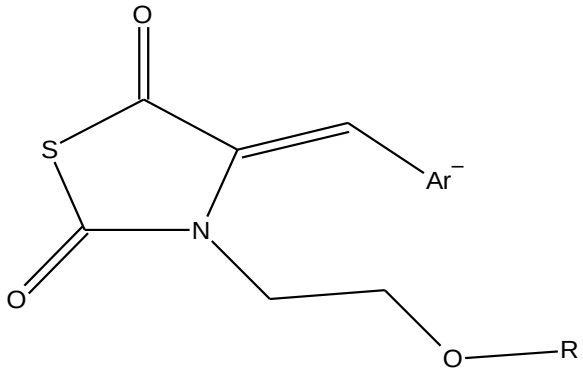
R=OH, NH_2, COOH, OCH_3, NO_2

Maccari *et al.*,**2013**[58]evaluated 2-Thioxo-4-thiazolidinone derivatives were as aldose reductase inhibitors (ARIs) and most of them exhibited good or excellent *in vitro* efficacy. Out of the tested compounds, most N-unsubstituted analogues were found to possess inhibitory effects at low micromolar doses and two of them exhibited higher potency than sorbinil, used as a reference drug.



R=CH_3, C_2H_5, OCH_3

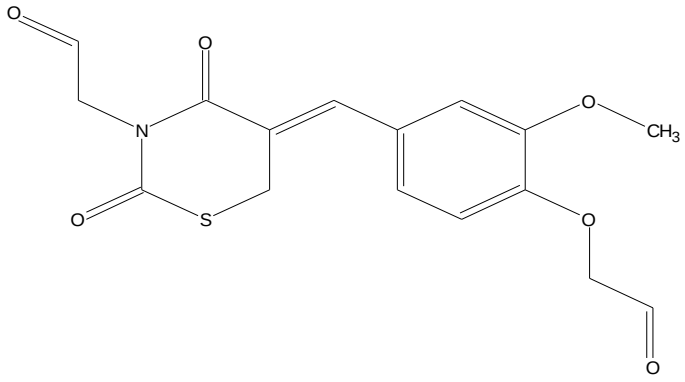
Rosaria Ottana *et al.,* **2013** [59]explored a new set of suitably substituted compounds for more effective 5-arylidene-4- thiazolidinones as aldose reductase inhibitors, Acetic acids Substitution proved to be interesting inhibitors of the enzyme as well as excellent antioxidant agents that are potentially able to counteract the oxidative stress associated with both diabetic complications as well as other pathologies. Molecular docking experiments supported SAR studies.



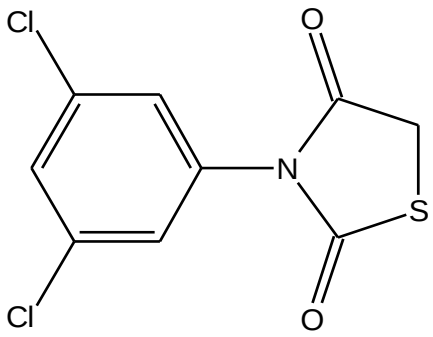
Ar=C_6H_5-cl, C_6H_5-Br,

R=OH, NH_2, Na, H

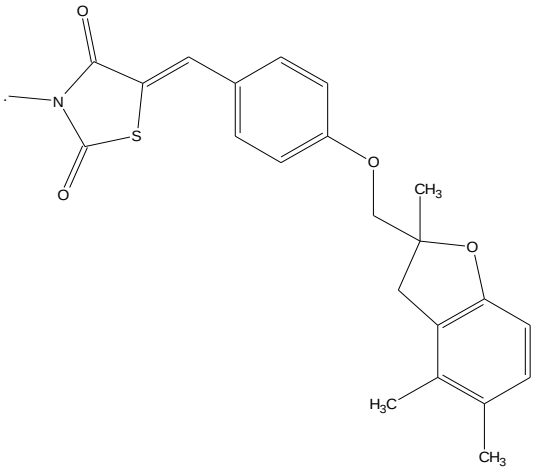
Ossama El-Kabbani *et al.,***2014**[60]determined the structure of aldehyde reductase (ALR1) in ternary complex with the coenzyme NADPH and [5-(3- carboxymethyl- 4-methoxy benzylidene)-2,4-dioxothiazolidin-3 yl] acetic acid (CMD), a potent inhibitor of aldose reductase (ALR2), at 1.99 Å resolution. Molecular modelling calculations and inhibitory activity measurements of CMD and [5-(3- hydroxy-4- methoxybenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid (HMD) indicated that pi stacking interactions with several conserved active site tryptophan residues and hydrogen-bonding interactions with the non-conserved C-terminal residue Leu300 in ALR2 (Pro301 in ALR1) contributed to inhibitor selectivity.



Harvison *et al.*,**2008**[61]suggest that Cytochrome P450 (CYP)-mediated metabolism in the thiazolidinedione (TZD) ring may contribute to the hepatotoxicity of the insulin-sensitizing agents such as troglitazone. Then administered hepatotoxic doses of DCPT (0.6 or 1.0 mmol/kg, i.p.) to male Fischer 344 rats after pretreatment with vehicle, 1- amino benzotriazole (ABT, non-selective CYP inhibitor) and troleandomycin (TAO, CYP3A inhibitor). Both hepatotoxic doses of DCPT induced elevations in serum alanine aminotransferase (ALT) levels that were attenuated by ABT or TAO pretreatment. Enzyme activity and Western blotting experiments with rat liver microsomes confirmed the effects of the various pretreatments. Results suggest that hepatic CYP3A isozymes may be involved in DCPT-induced liver damage in male rats.

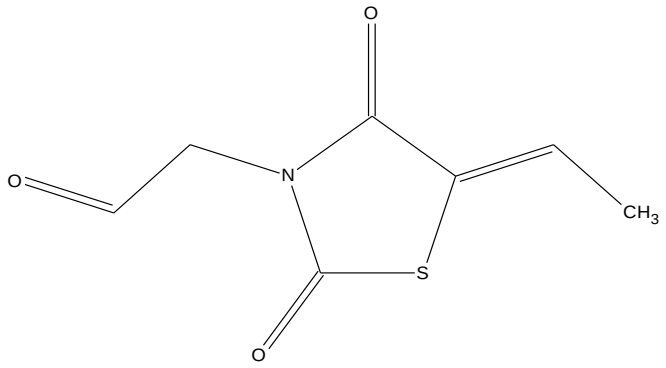


DCPT



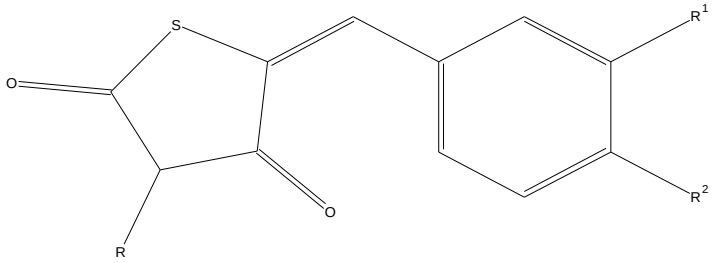
TROGLITAZONE

Dundar *et al.*,**2007**[62]prepared a series of flavonyl-2,4-thiazolidinediones by Knoevenagel reaction. The synthesized compounds were tested for their ability to inhibit rat kidney aldose reductase (AR) and for their insulinotropic activities in INS-1 cells.



FLAVONE

Maccari *et al.*,**2010**[63]synthesized and evaluated number of 5-arylidene -2,4-thiazolidinediones containing a hydroxy or a carboxymethoxy group in their 5-benzylidene moiety as *in vitro* aldose reductase (ALR2) inhibitors. Most of them exhibited strong inhibitory activity, with IC50 values in the range between 0.20 and 0.70 lM. Molecular docking simulations into the ALR2 active site highlighted that the phenolic or carboxylic substituents of the 5-benzylidene moiety can favorably interact, in alternative poses, either with amino acid residues lining the lipophilic pocket of the enzyme, such as Leu300, or with the positively charged recognition region of the ALR2 active site.

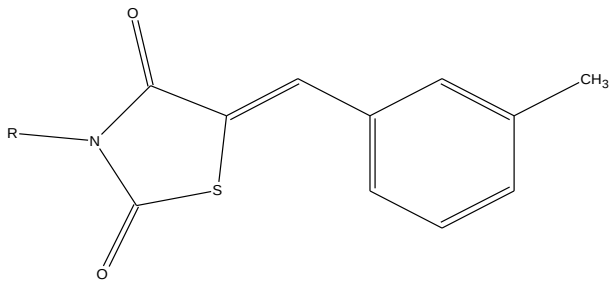


R=H, CH_3, C_2H_5

R^1=Cl, F, Br

R^2=OH, NO_2, OMe

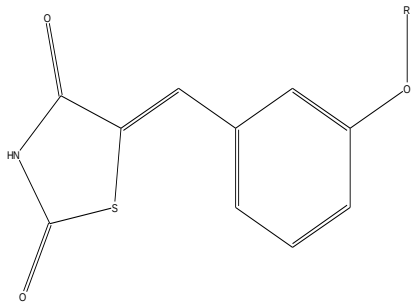
Maccari *et al.*,**2009**[64]synthesized and tested several 5-benzyl-2,4- thiazolidinediones (5–7) as *in vitro* aldose reductase (ALR2) inhibitors. Most of them, particularly N-unsubstituted 5-benzyl -2,4-thiazolidinediones 5 and (5-benzyl-2,4- dioxothiazolidin-3-yl)acetic acids 7, displayed moderate to high inhibitory activity levels. In detail, the insertion of an acetic chain on N-3 significantly enhanced ALR2 inhibitory potency, leading to acids 7 which proved to be the most effective among the tested compounds.



NH_2, NO_2, COOH, Cl, F, Br

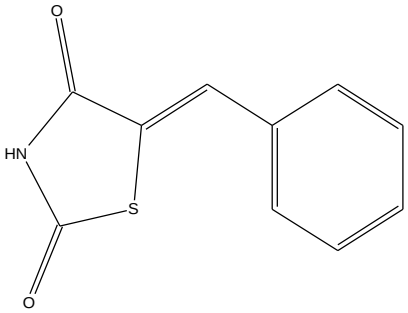
R=CH_3, C_2H_5, OCH_3, OC_2H_5

Hsin-Hsiung Tai *et al.*,**2011**[65] **s**ynthesized a series of benzylidene thiazolidinediones with varied ring structure and methylene bridge to phenyl ring through ether linkage and assayed for inhibitory activity. It was found that compound CT-8 (5-[4-(cyclohexyl ethoxy)benzylidene]-2,4-thiazolidinedione) was the most potent inhibitor effective at nanomolar range.



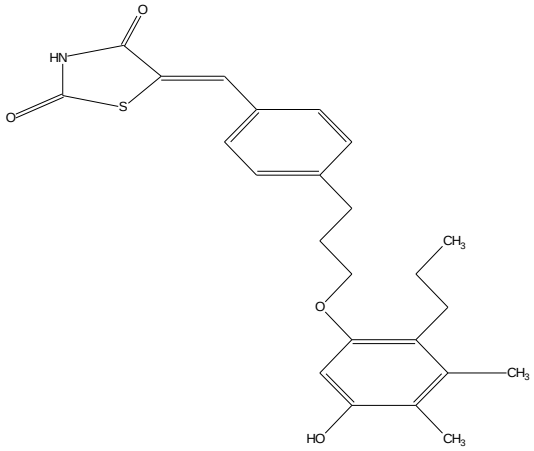
R=CH_3, C_2H_5

Maccari *et al.*,**2010** [66]synthesized and tested several (Z)-5-arylidene-2,4- thiazolidinediones as aldose reductase inhibitors (ARIs). The most active of the N-substituted derivatives exerted the same inhibitory activity of Sorbinil. The introduction of an acetic side chain on N-3 of the thiazolidinedione moiety led to a marked increase in lending inhibitory activity, conducting to the discovery of a very potent ARI (4c), whose activity level (IC50=0.13 mM) was in the same range of Tolrestat.

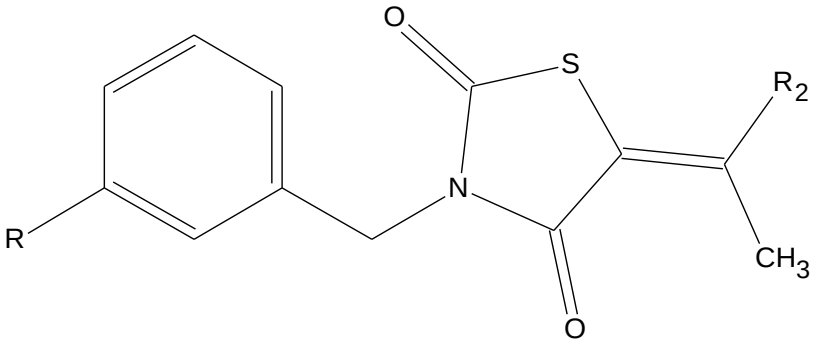


NH_2, NO_2, COOH, Cl, F, Br

Cossy *et al.*,**2012** [67]obtained Troglitazone in 5 steps from 4-bromo-l, ldimethoxy-3-methyibut-2-ene with an overall yield of 7.5%. The formation of the chromone ring was achieved by condensing an unsaturated acetal with trimethylhydroquinone in the presence of bis (trifluoromethyl sulfonyl) imide.



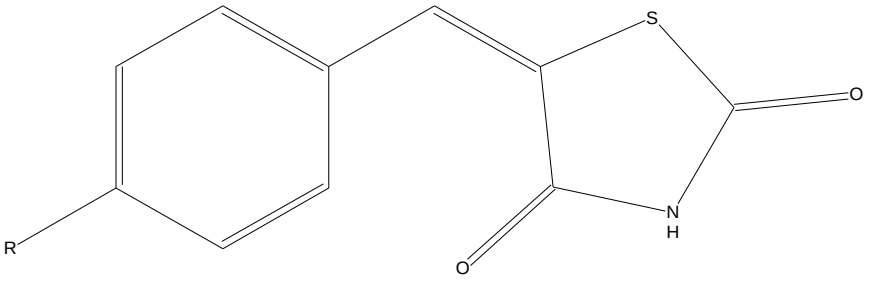
Pitta *et al.*,**2005**[68]synthesized and assayed eight new 5-arylidene -3-benzyl-thiazolidine-2,4-diones with halide groups on their benzyl rings in vivo to investigate their anti-inflammatory activities. These compounds showed considerable biological efficacy when compared to rosiglitazone, a potent and well-known agonist of PPAR-$_\gamma$ which was used as a reference drug. This suggests that the substituted 5-arylidene and 3-benzylidene groups play important roles in the anti-inflammatory properties of this class of compounds.



R_1=NH_2, OCH_3

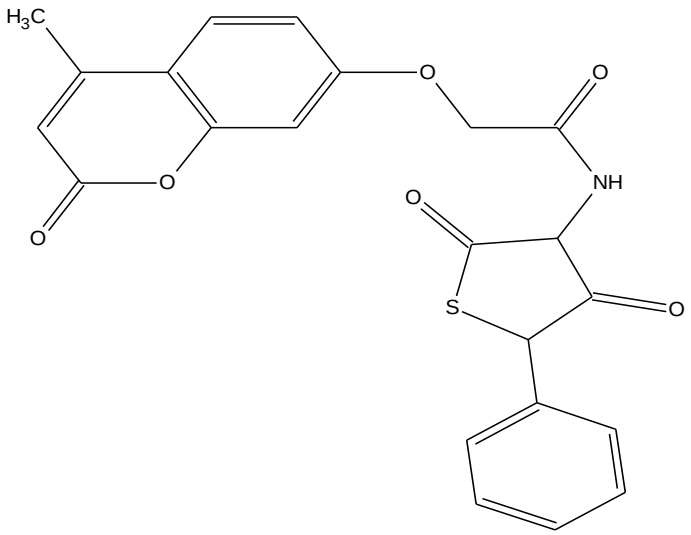
R_2=OH, Cl, NO_2

Rekha *et al.*,**2011**[69]performed the synthesis and evaluation of novel thiazolidinediones for anti-inflammatory activity.



R=OH, Cl, Br, OCH_3, NH_2, NO_2

Cong *et al.*,**2014**[70]synthesized and biologically evaluate the Antibacterial Activity of Analogs of 5-Arylidene-3-(4-methylcoumarin-7-yloxyacetylamino)-1,3-thiazolidin -2,4- dione.



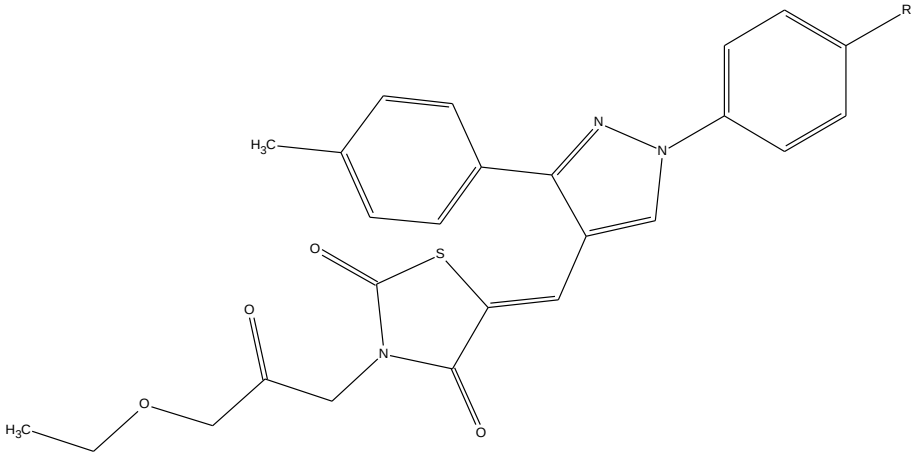
X=4-$N(CH_3)_2$ =2-$N0_2$

=4-Br =4-H

=4-OH =4-$N0_2$

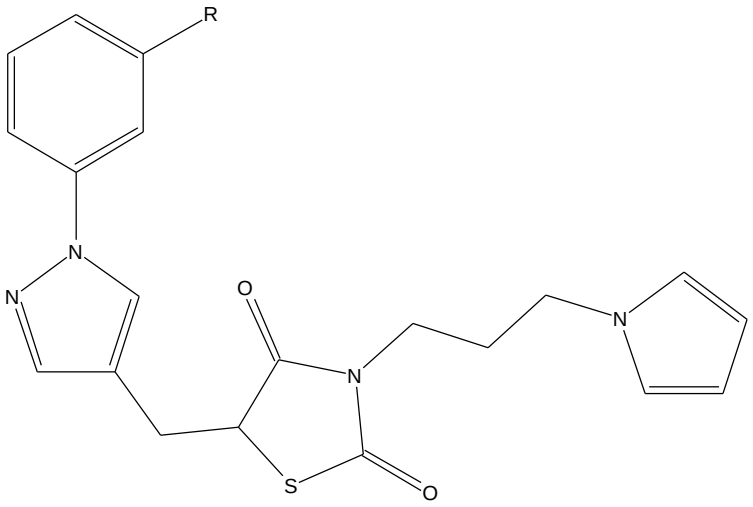
=4-Cl =4-OCH_3

Deepak *et al.*,**2011**[71]Synthesized the pyrazolyl-2, 4-thiazolidinedione and evaluated the antibacterial and antifungal agents.



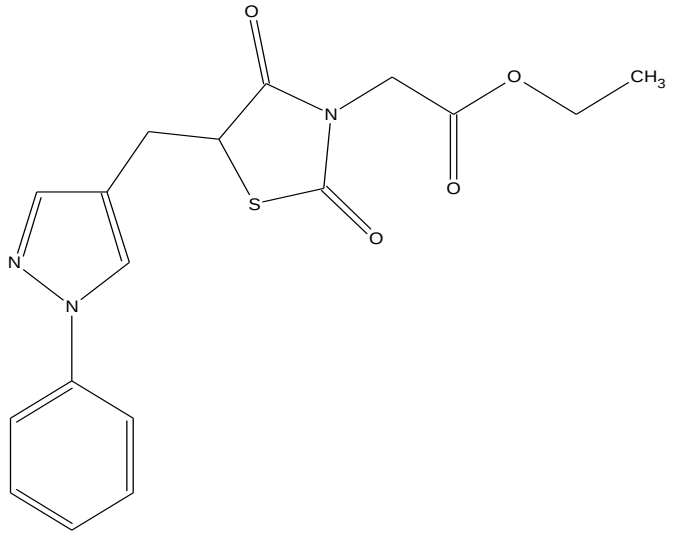
R=H, CH_3, Ethyl n-prop, Cl, F, OH, NO_2

Nisheet *et al.*,**2014**[72]Synthesized and evaluate the *N*-Substituted Thiazolidine-2,4-dione Containing Pyrazole as Potent Antimicrobial Agents.

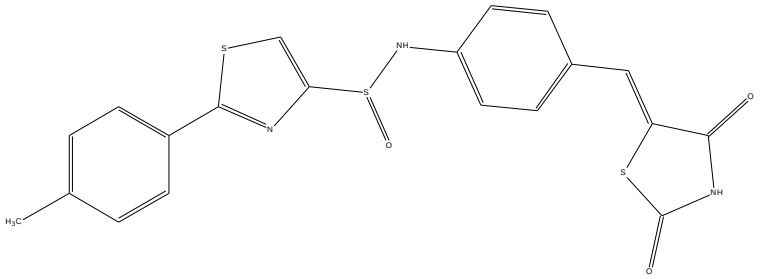


R=OH, NH_2, NO_2, OCH_3

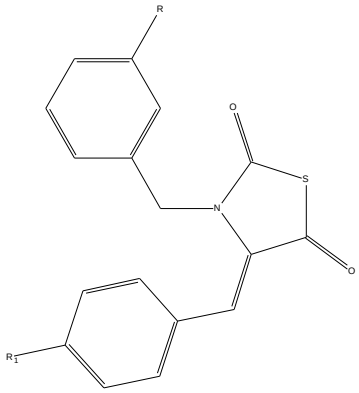
Aneja *et al.*,**2011**[73]discovered synthesis and biological activity of 5-((3-(4- chlorophenyl)-1-phenyl-1Hpyrazol-4-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetate derivatives as antibacterial and antifungal agent.



Nikhil *et al.*,**2012**[74]have done the Microbial studies of *N*-chloro aryl acetamide substituted thaizole and 2,4- thiazolidinedione derivatives.



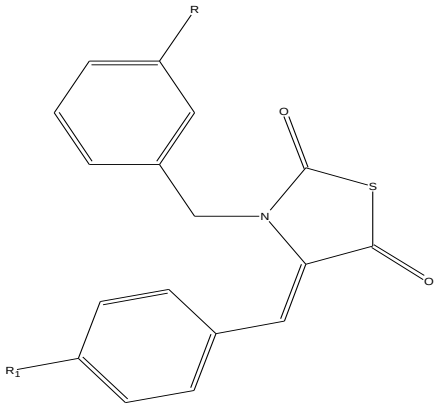
Shriram *et al.*,**2012**[75]have done the synthesis & antimicrobial activity of a new series of 3, 5-disubstituted thiazolidine-2, 4-diones].



R=H, OCH_3, CH_3, C_2H_5

R_1=NO_2, NH_2, CHO

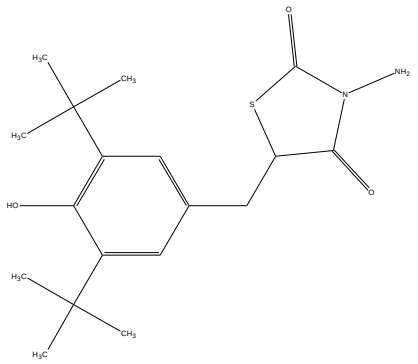
Neeru *et al.*,**2011**[76]performed the synthesis and antimicrobial evaluation of n-substituted-5-Benzylidene-2,4- thiazolidinedione derivatives.



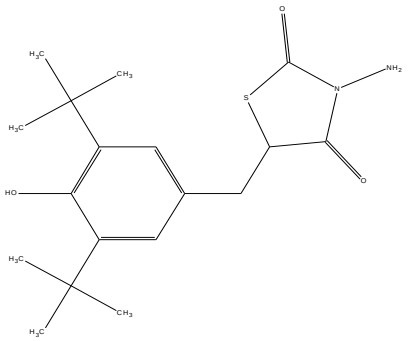
R=H, NO_2, NH_2, CHO

R_1=Cl, F, Br

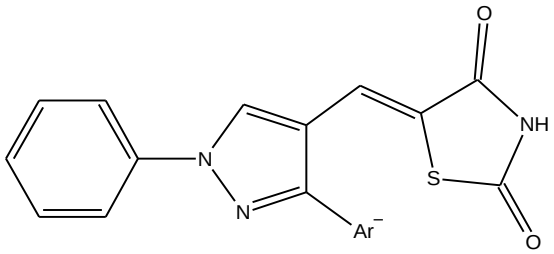
J. Geldenhuys *et al.*,**2010**[77]have done the Structure-based design of a thiazolidinedione which targets the mitochondrial protein mito NEET.



Richard T. Carroll *et al.*,**2010**[78]identified a novel protein, mitoNEET, which was later shown to regulate the oxidative capacity of the mitochondria. This identified an alternative target for the glitazones suggesting a possible new drug target for the treatment of neurodegenerative diseases. Molecular docking studies employing the reported crystal structure revealed five possible binding pockets on mito NEET.

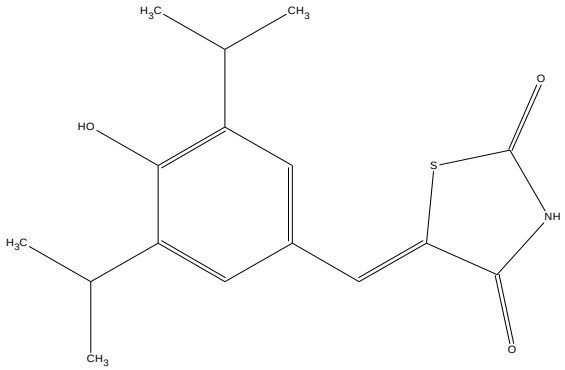


Sudheer Kumar *et al.*,**2011**[79]synthesized the some novel 2, 4-thiazolidinedione incorporated pyrazolederivativesas anticancer agents.

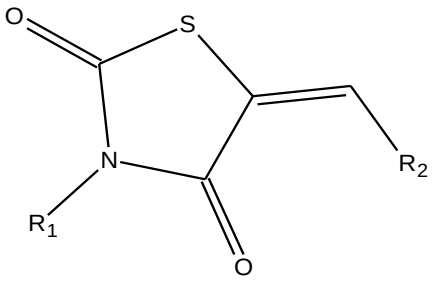


Ar=C_6H_5, 4-cl -C_6H_4, 4-CH_3-C_6H_4, 4-OCH_3-(C_6H_4)2, 2-OCH_3-C_6H_4,4-OH-C_6H_4

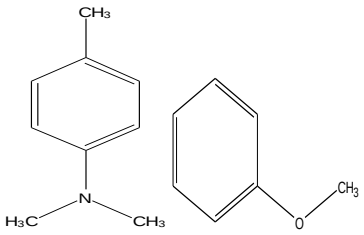
Richard T Carroll *et al.,***2010**[80]have studied SAR and docking studies of thiazolidinedione-type (TZD) compounds with MAO-B inhibitory activity.



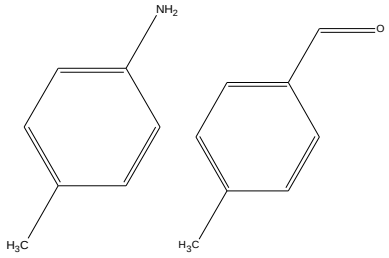
Naresh *et al.,***2013**[81]have done the synthesis, characterization and anti-tubercular activity of some new 3,5- disubstituted-2,4-thiazolidinediones .



R_1



R_2



CHAPTER 3

RESEARCH OBJECTIVES

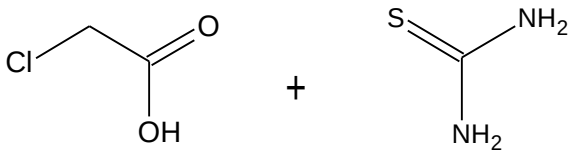
Among the various synthetic products, the first choice of selection of nucleus in our current research work is Heterocyclic compounds i.e. Thiazolidinedione. Thiazolidinedione have played a very important role in the development of theory in heterocyclic chemistry and also extensively in organic synthesis. Thiazolidinedione nucleus is an important pharmacophore and privileged structure in medicinal chemistry. Thiazolidinedione have wide range of biological activities such as anti-microbial, anthelmintic activity, antiulcer activity, antiemetic ant anti-hypertensive. The work was planned to perform that Thiazolidinedione was substituted with Furfuryl thiazolidinedione. Furfuryl thiazolidinedione was substituted by various secondary amines to produce Furfuryl thiazolidinedione Mannich base, exhibit interesting pharmacological activities. The present study is aimed to carry out the synthesis of Furfuryl thiazolidinedione Mannich base derivatives. Then, structures will be assigned by FT-IR and ^{1}H NMR and Mass spectral analysis. Further, the compounds are evaluated for biological activities such as anti-cancer activity *in-vitro.*

The plan of this research work are,

1. To carry out Docking studies of manually designed thiazolidinedione by MolDock Virtual docker.
2. To select the best MolDock Score compound based on the result of MVD.
3. To synthesis the best Scored compound of Furfuryl thiazolidinedione Mannich baseswith specific reaction condition.
4. To characterize the above synthesized compounds by means of their FT-IR & 1H NMR and Mass.

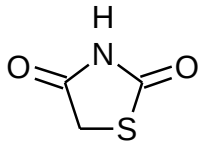
5. To evaluate the synthesized compounds for their *in-vitro* anti-cancer activity used MTT assay method.
6. To evaluate the possible binding interaction against Anaplastic Lymphoma kinase enzyme with amino acid residues with the help of MVD results.

SYNTHETIC SCHEME



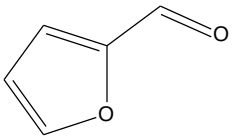
chloroacetic acid thiourea

Reflux for 9 hours with HCL. 100˚C

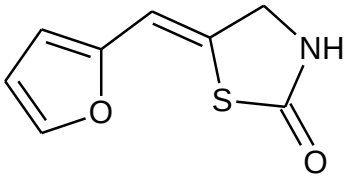


1,3-thiazolidine-2,4-dione

100˚C Reflux for 1 hours. Ethanol, Piperidine

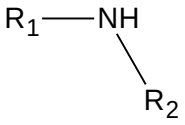


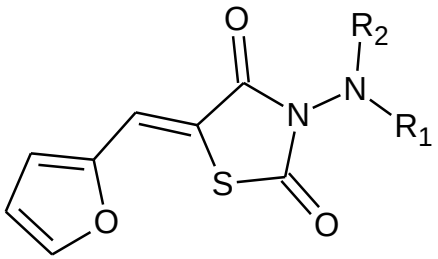
furan-2-carbaldehyde



(5*Z*)-5-[(furan-2-yl)methylidene]-
1,3-thiazolidin-2-one

Reflux for 1 hours 100˚C, HCHO





Furfuryl thiazolidinedione Mannich bases

Scheme: 1 Synthesis of the titled compounds

CHAPTER 4

METHODS AND MATERIALS

CHEMICALS

TABLE 2: Chemicals and Company name of the chemicals used in the synthesis

S.NO	NAME OF THE CHEMICAL	MANUFACTURE'S DETAILS
1	Dimethylamine	Central Drug House (P) Ltd'
2	Diethylamine	Central Drug House (P) Ltd'
3	Morpholin	Central Drug House (P) Ltd'
4	Piperazine	Central Drug House (P) Ltd'
5	Methyl piperazine	Central Drug House (P) Ltd'
6	Diphenylamine	Central Drug House (P) Ltd'
7	Ammonia	Central Drug House (P) Ltd'
8	Benzylamine	Central Drug House (P) Ltd'
9	Ethanol	Changshu Hongsheng Fine Chemical Co.Ltd
10	Thiourea	Central Drug House (P) Ltd'
11	P-chloroacetic acid	Central Drug House (P) Ltd'
12	Formaldehyde	Central Drug House (P) Ltd'

All the chemicals used were analytical grade and were purchased from UNIVERSAL SCIENTIFIC APPLIANCES in Madurai.

INSTRUMENTS

INFRA-RED SPECTROPHOTOMETER

IR spectra was measured using SHIMADZU IR TRACER 100 IR spectrophotometer at IRC, Kalasalingam Acadamy of Research and Education, Srivilliputtur, Virudhunagar(DT).

NUCLEAR MAGNETIC RESONANCE SPECTROPHOTOMETER

^{1}H NMR spectra was measured using BRUCKNER AMX 400 MHz at IARC, Gandhigram Rural Institute, Gandhigram, Dindigul.

Table3: List of instruments used for the characterization of the synthesized compounds

S.NO	**INSTRUMENT**	**MODEL**
1	Digital balance	Shimadzu ELB 300
2	Magnetic stirrer	Remi equipment
3	Melting point apparatus	Sigma melting point apparatus
4	FT-IR spectrophotometer	Shimadzu IR tracer 100
5	^{1}H NMR spectrophotometer	BRUCKNER AMX 400 MHz
6	Heating mantle	Sigma industries
7	Mass spectrophotometer	Shimadzu lab solutions

Hardware

Molegro Virtual Docker 5.0 2010 and [Sybil2] were used for molecular modeling with dual core processors, windows 10, 2GB RAM, 2GB Graphics card.

Reagents for *in vitro* studies

- *U937 cell line*
- Penicillin (100 u/ML)
- streptomycin (100µg/ml)
- 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide
- DMSO
- UV- Vis spectrophotometer

Molecular Docking Studies:

Molegro Virtual Docker, (MVD) its integrated platform for computation drug design. One application of molecular docking is to design pharmaceuticals*in silico* by optimizing lead candidates targeted against proteins. The lead candidates can be found using a docking algorithm that tries to identify the optimal binding mode of a small molecule (ligand) to the active site of a macromolecular target. Thus, the purpose of drug discovery is to derive drugs that more strongly bind to a given protein target than the natural substrate. By doing so, the biochemical reaction that the target molecule catalyzes can be altered or prevented.

Hardware

Molegro Virtual Docker 5.0 2010 and [Sybil2] were used for molecular modeling with dual core processors. Windows 10, 2GB RAM, 2GB Graphics card.

Software Methodology:

In the present molecular docking study, software Molegro Virtual Docker v 6.0 (www.molegro.com) along with Graphical User Interface (GUI) tools available for Windows, Linux, and Mac OS X. was utilized to generate grid, calculate dock score and evaluate conformers. Molegro Virtual Docker offers high-quality protein-ligand docking based on novel optimization techniques combined with a user interface experience focusing on us ability and productivity. The scoring function used by MolDock is derived from the Piecewise Linear Potential (PLP) scoring functions. The active binding site region was defined as a spherical region which encompasses all protein within 15.0 A^o of bound crystallographic ligand atom with selected co-ordinates of X, Y and Z axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 A^o and for each of the 10 independent runs; a maximum number of 2000 of iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, i.e. all non-ring torsions were allowed.

Ligand Preparation:

The structures of Furfuryl thiazolidinedione Mannich base were converted into suitable chemical information using Chemdraw ultra v 10.0 (Cambridge software), copied to Chem3D ultra v 10.0 to create a 3D model and, finally subjected to energy minimization using molecular

mechanics (MM2). The minimization was executed until the root mean square gradient value reached a value smaller than 0.001kcal/mol. Such energy minimized structures are considered for docking and corresponding pdb files were prepared using Chem3D ultra v 10.0 integral option (save as /Protein Data Bank (pdb)).

Protein Preparation:

All EGFR X-ray crystal structures were obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Subsequent to screening for the above specific standards the resultant protein target (**PDB Code: 2XB7**) was selected and prepared for molecular docking simulation in such a way that all heteroatoms (i.e., non-receptor atoms such as water, ions, etc.) were removed and Kollmann charges were assigned.

Software Method Validation:

Software method validation was performed in MVD using Protein Data Bank (PDB) protein 2XB7. The x-ray crystal structure of 2XB7 complex with co-crystallized ligand was recovered from PDB. The bio active co-crystallized bound ligand was docked with in the active site region of 2XB7. The RMSD of all atoms between the two conformations is 2.5Åindicating that the parameters for docking simulation are good in reproducing X-ray crystal structure. On the basis of pilot docking studies, the MolDock-rerank scores were selected for ranking the inhibitor poses, and for all the anaplastic lymphoma kinase docking performed here, the poses selected as the best were taken as those with the highest MolDockre-rank score. Anaplastic lymphoma kinase crystal structure was directly downloaded to the workspace of MVD from the PDB accessed at the URL: (http://www.rscb.org/pdb). The structure of furfuryl thiazolidinedione derivatives have been drawn on ChemBioDrawUltra12.0 software and imported to the MVD workspace in 'sdf'format.

In order to make accurate predictions, it is important that the imported structures have been properly prepared, that is, the atom connectivity and bond orders are correct and partial atomic charges are assigned. PDB files often have poor or missing assignment of explicit hydrogens, and the PDB file format cannot accommodate bond order information. All necessary valency

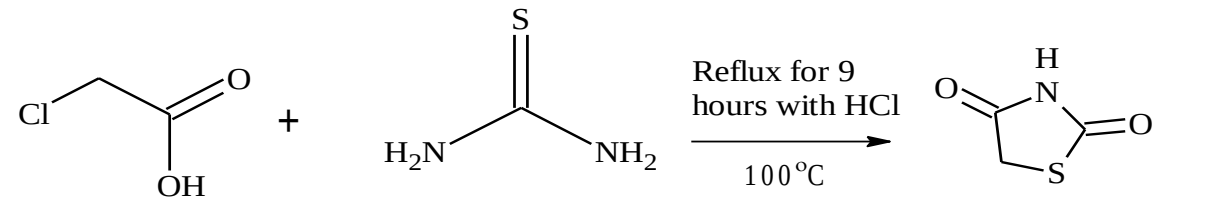
checks and H atom addition were thus performed using the utilities provided in MVD. The binding site specifies the region of interest where the docking procedure will look for promising poses (ligand conformations).

Molecular docking is an optimization problem, where the objective is to find the ligand binding mode with lowest potential energy. The process of docking involves sampling the coordinate space of the target binding site and scoring each possible ligand pose within that site, the highest scoring pose then taken as the predicted binding mode for that compound. There are many different docking programs now available and they differ in the nature of the sampling algorithms they employ, in their manner of handling ligand and protein flexibility, in the scoring functions they use, and in the CPU time they required. In the studies reported here, MVD was used, because it showed higher docking accuracy when benchmarked against other available docking programs (MD: 87%, Glide: 82%, Surflex: 75%, FlexX:58%) and has been shown to be successful in several recent studies, but also for reasons of cost and user friendliness (Thomsen *et al.,* 2006).

SYNTHETIC METHODOLOGY

Step 1

In first step, 9.4 gm of chloroacetic acid is taken with 7.6 gm of thiourea and placed in magnetic stirrer for 15 minutes. To that add 10 ml of Hcl drop by drop. It will form a white precipitate. After it is placed in reflux for 9 hours at 100-110°C to form a product (thiazolidinedione). Then it undergoes filtration and recrystallize with ethanol.

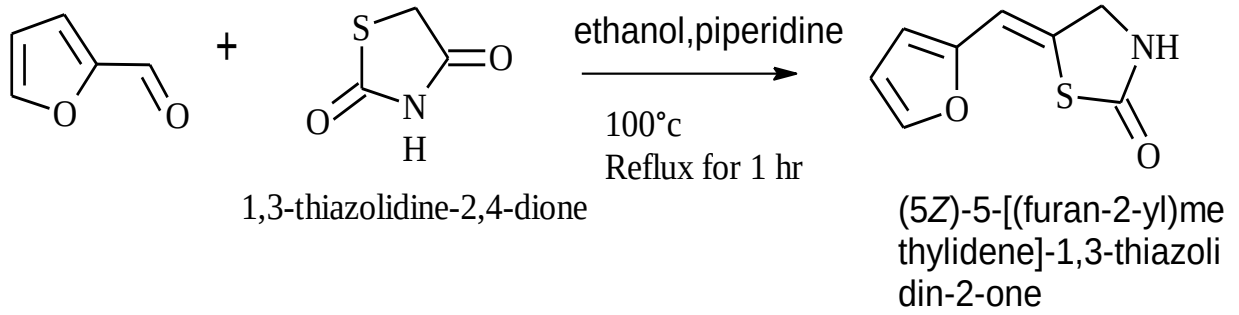


chloroacetic acid thiourea 1,3-thiazolidine-2,4-dione

Scheme: 2 Synthesis of the compound TZD-1

Step 2

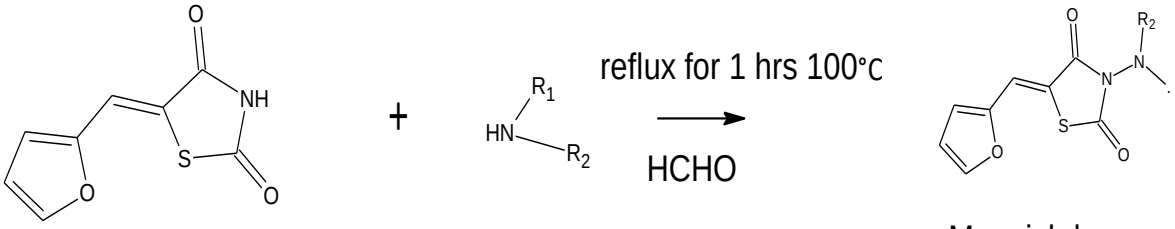
In second step, 4 gm of benzaldehyde, 4.24 gm of thiazolidinedione (obtained from step 1) and piperidine 0.4 ml was taken. After it is placed in reflux for 1 hours at 100-110°C to form a product (furfuryl thiazolidinedione). Then it undergoes filtration and recrystallize with ethanol.



Scheme: 3 Synthesis of the compound TZD-3

Step 3

The second step of Furfuryl thiazolidinedione and added in secondary amines (derivatives) and added in Formaldehyde undergoes reflux for 2 hours at 70-75°C for 5 mins.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Mannich base

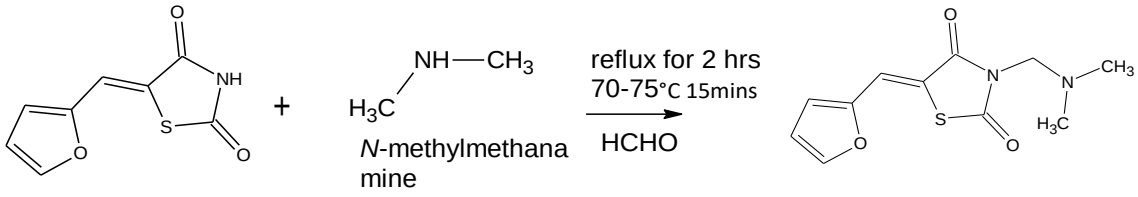
Scheme: 4 General schemes for the synthesis of the compounds TZD 31-TZD 38

Derivates from step 3 product

After second step, 8 types of secondary amines such as dimethylamine, diethylamine, morpholin, methyl piperazine, piperazine, diphenylamine, ammonia and benzylamine. These secondary amines are react with furfuryl thiazolidinedione to form 8 different derivate products.

1) **(5*E*)-3-[(dimethylamino)methyl]-5-(furan-2-ylmethylidene)-1,3-thiazolidine-2,4-dione**

   In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 2.45 gm of dimethylamine undergoes reflux at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

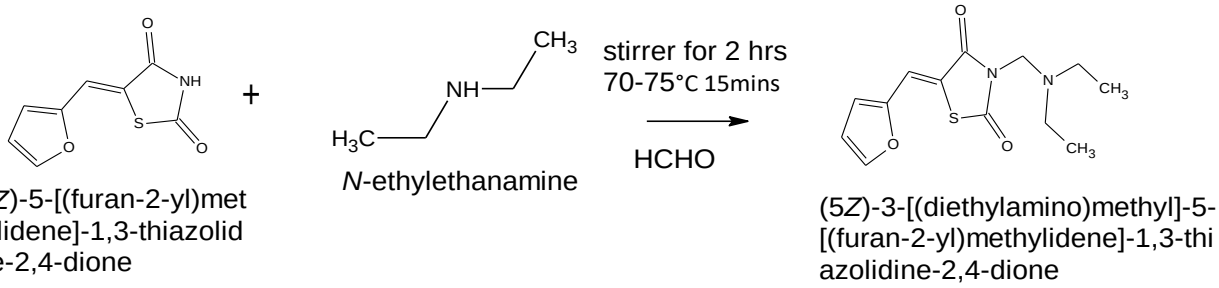
N-methylmethanamine

(5*Z*)-3-[(dimethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Scheme: 5 Synthesis of the compound TZD-31

2) **(5*E*)-3-[(diethylamino)methyl]-5-(furan-2-ylmethylidene)-1,3-thiazolidine-2,4-dione**

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 0.73 gm of diethylamine placed in magnetic stirrer at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

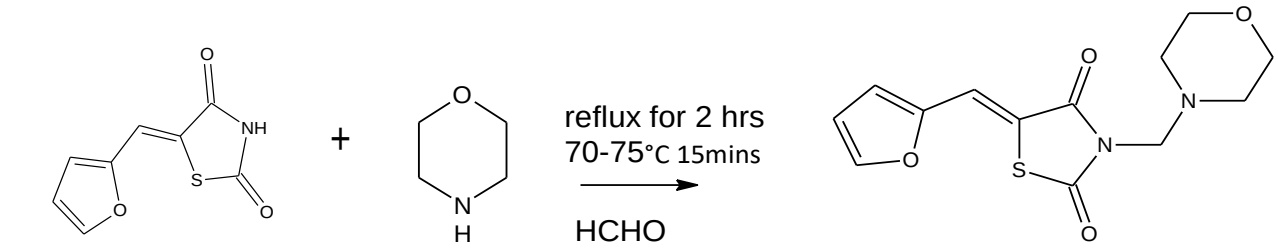
N-ethylethanamine

(5*Z*)-3-[(diethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Scheme: 6 Synthesis of the compound TZD-32

3) **(5E)-5-(furan-2-ylmethylidene)-3-(morpholin-4-ylmethyl)-1,3-thiazolidine-2,4-dione**

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 0.87 gm of morpholin undergoes reflux at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

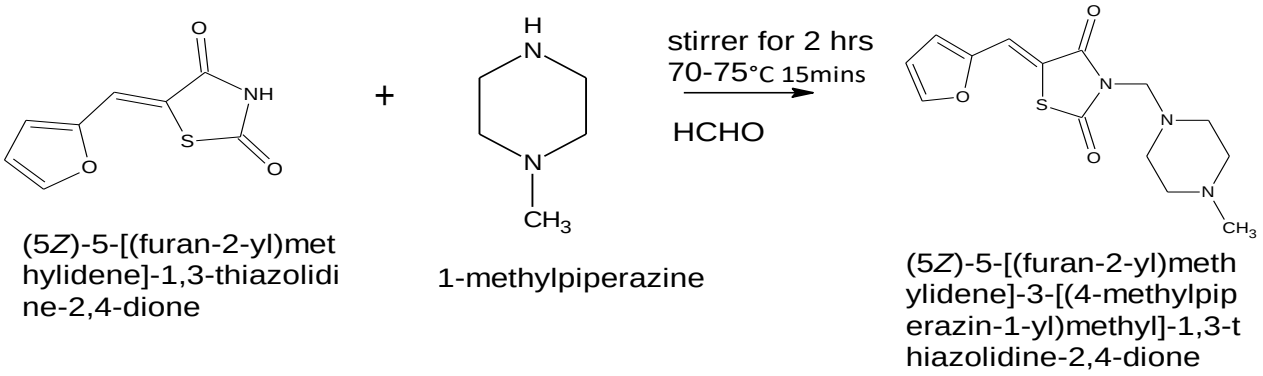
morpholine

(5*Z*)-5-[(furan-2-yl)methylidene]-3-[(morpholin-4-yl)methyl]-1,3-thiazolidine-2,4-dione

Scheme: 7 Synthesis of the compound TZD-33

4) **(5E)-5-(furan-2-ylmethylidene)-3-[(4-methylpiperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione**

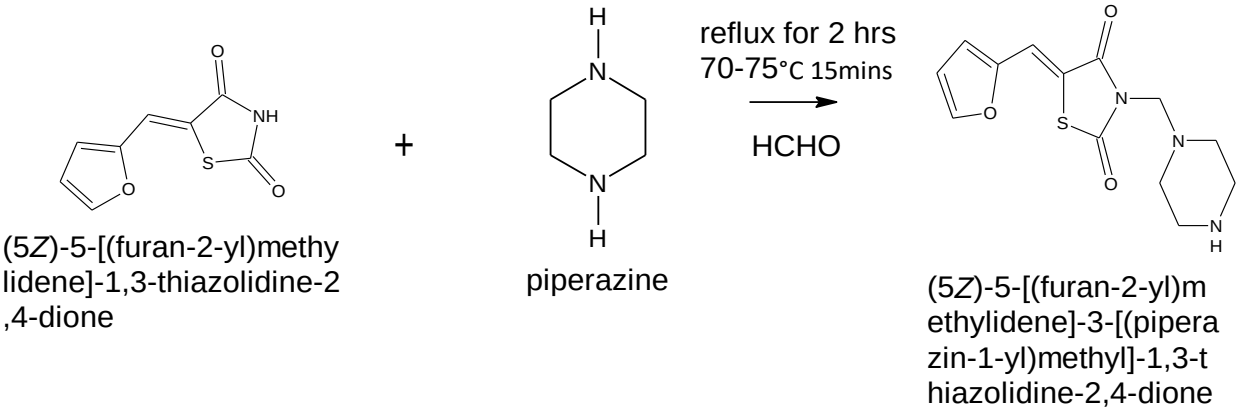
In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 1.00 gm of methyl piperazine placed in magnetic stirrer at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



Scheme: 8 Synthesis of the compound TZD -34

5) **(5E)-5-(furan-2-ylmethylidene)-3-(piperazin-1-ylmethyl)-1,3-thiazolidine-2,4-dione**

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 1.94 gm of piperazine undergoes reflux at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

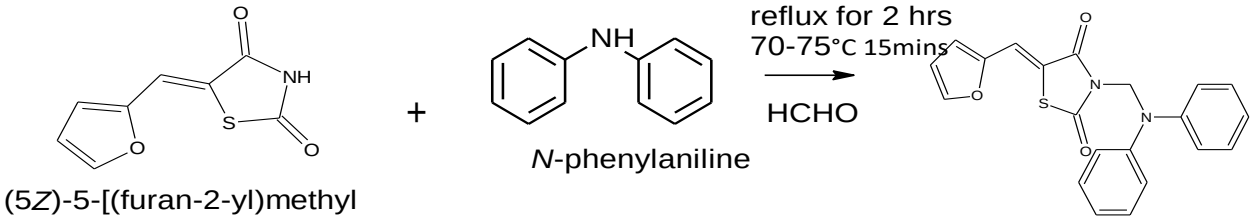
piperazine

(5*Z*)-5-[(furan-2-yl)methylidene]-3-[(piperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione

Scheme: 9 Synthesis of the compound TZD-35

6) (5E)-3-[(diphenylamino)methyl]-5-(furan-2-ylmethylidene)-1,3-thiazolidine-2,4-dione

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 1.69 gm of diphenylamine undergoes reflux at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

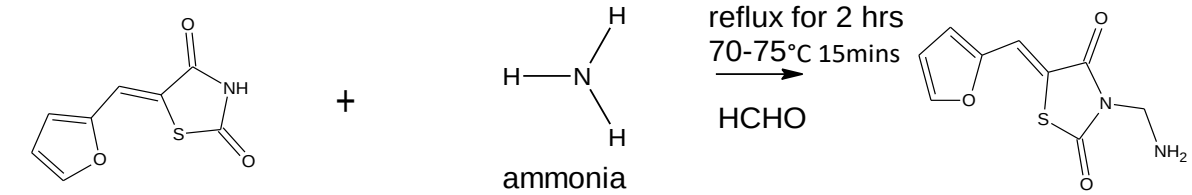
N-phenylaniline

(5*Z*)-3-[(diphenylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Scheme: 10 Synthesis of the compound TZD-36

7) (5E)-3-(aminomethyl)-5-(furan-2-ylmethylidene)-1,3-thiazolidine-2,4-dione

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 0.17 gm of ammonia undergoes reflux at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



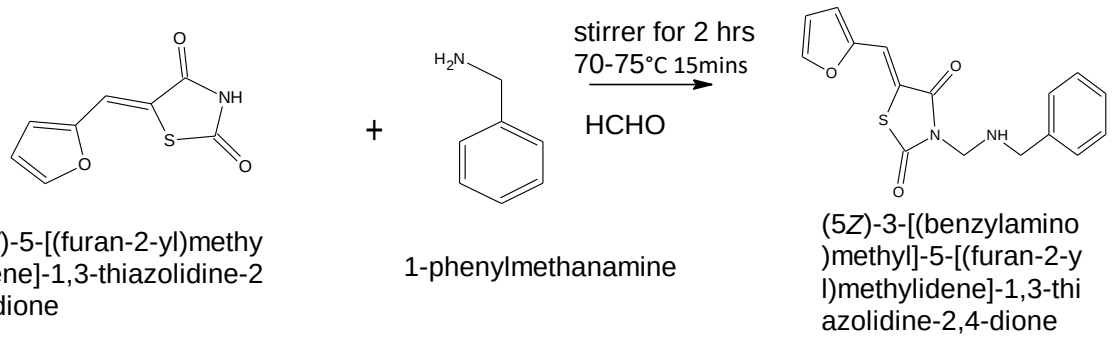
(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

(5*Z*)-3-(aminomethyl)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Scheme: 11 Synthesis of the compound TZD-37

8) (5E)-3-[(benzylamino)methyl]-5-(furan-2-ylmethylidene)-1,3-thiazolidine-2,4-dione

In this method, 0.01M mol of 1.95 gm of furfuryl thiazolidinedione is taken with 1.07 gm of benzylamine placed in magnetic stirrer at 70-75°C for 15 minutes. It will add 0.3ml of formaldehyde drop by drop. Then it will be placed in reflux for 2 hours and placed overnight.



(5*Z*)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

1-phenylmethanamine

(5*Z*)-3-[(benzylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

Scheme: 12 Synthesis of the compound TZD-38

IN VITRO ANTI CANCER EVALUATION

CELL LINE and CULTURE

U937 cell line was obtained from NCCS, Pune.The cells were maintained in Minimal Essential Medium supplemented with 10% FBS.Penicillin (100 u/ML).and streptomycin (100µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37˚C.

PROCEDURE

MTT ASSAY(MOSMANN.1983)

Cells (1×10^5/ were plated in 24-well plates and incubated in 37˚C with 5% CO_2 condition. After the cell reaches the confluence. The various concentrations of the samples were added and incubated for 24 hours.100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation. 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell Viability = A570 of treated cells /A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is incubated in each assay to compare the full cell viability assessments.

CHAPTER 5

CHARACTERIZATION

SOLUBILITY STUDIES

Solubility analysis has been carried out all the synthesized compound using different solvents and important are in table.

Table: 4 Solubility data of the synthesized compounds

Compound code	Solvent					
	Benzene	**$CHCl_3$**	**Ethyl acetate**	**Methanol**	**Distilled water**	**DMSO**
TZD-1	Insoluble	Insoluble	Sparingly Soluble	Soluble	Soluble	Soluble
TZD-2	Insoluble	Insoluble	Soluble	Soluble	Sparingly Soluble	Very Soluble
TZD-31	Insoluble	Insoluble	Insoluble	Soluble	Insoluble	Very Soluble
TZD-32	Soluble	Soluble	Sparingly Soluble	Soluble	Sparingly Soluble	Soluble
TZD-33	Insoluble	Insoluble	Soluble	Soluble	Sparingly insoluble	Soluble
TZD-34	Sparingly Soluble	Soluble	Soluble	Soluble	Insoluble	Very Soluble
TZD-35	Soluble	Soluble	Soluble	Soluble	Soluble	Very Soluble
TZD-36	Insoluble	Sparingly soluble	Sparingly Soluble	Soluble	Insoluble	Soluble
TZD-37	Sparingly Soluble	Insoluble	Sparingly Soluble	Soluble	Sparingly Soluble	Very Soluble
TZD-38	Insoluble	Insoluble	Soluble	Soluble	Insoluble	Soluble

TLC ANALYSIS

The purity of the compound was ascertained by TLC

Adsorbent used : Silica gel-G

Detecting agent : Iodine vapor

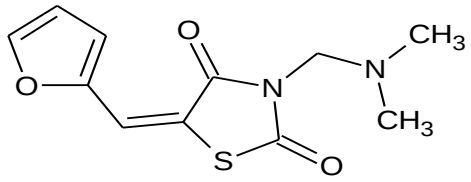
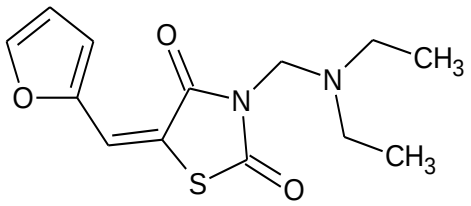
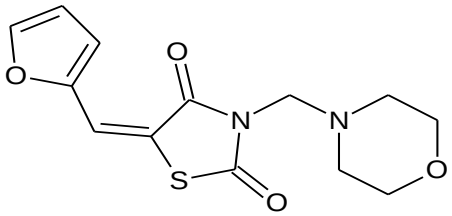
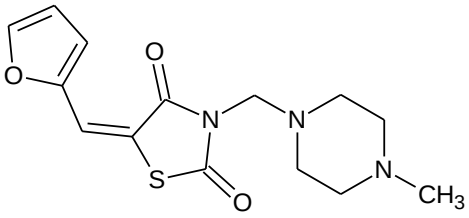
R_f values of the synthesized compounds were calculated by the following formula

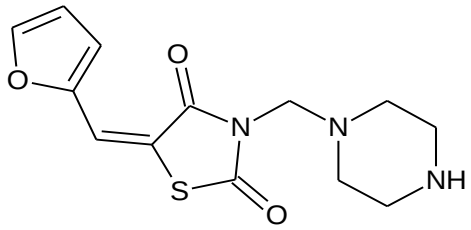
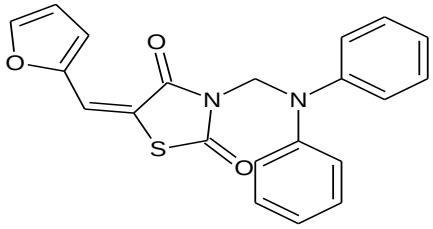
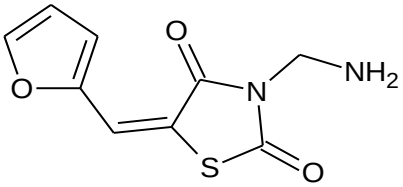
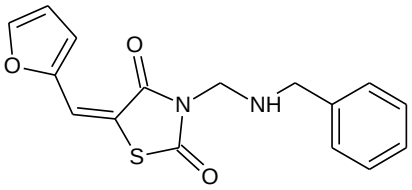
$$R_f \text{value} = \frac{\text{Distance travelled by solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

Table: 5 R_f values of the synthesized compounds

S.No	Compound code	Solvent system ratio	R_f value
		Acetonitrile : Water	
1.	TZD-1	90:10	0.830
2.	TZD-2	90:10	0.711
3.	TZD-31	90:10	0.846
4.	TZD-32	90:10	0.876
5.	TZD-33	90:10	0.938
6.	TZD-34	90:10	0.830
7.	TZD-35	90:10	0.784
8.	TZD-36	90:10	0.753
9.	TZD-37	90:10	0.815
10.	TZD-38	90:10	0.830

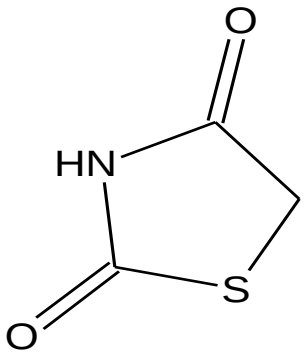
Table: 1 Structure of the titled compounds with IUPAC names

COMPOUD CODE	CHEMICAL NAME	CHEMICAL STRUCTURE
TZD-31	(5*E*)-3-[(dimethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione	
TZD-32	(5*E*)-3-[(diethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione	
TZD-33	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(morpholin-4-yl)methyl]-1,3-thiazolidine-2,4-dione	
TZD-34	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(4-methylpiperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione	

TZD-35	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(piperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione	
TZD-36	(5*E*)-3-[(diphenylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione	
TZD-37	(5*E*)-3-(aminomethyl)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione	
TZD-38	(5*E*)-3-[(benzylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione	

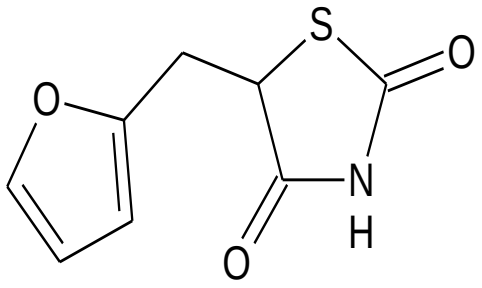
Characterization of the synthesized compounds

1)



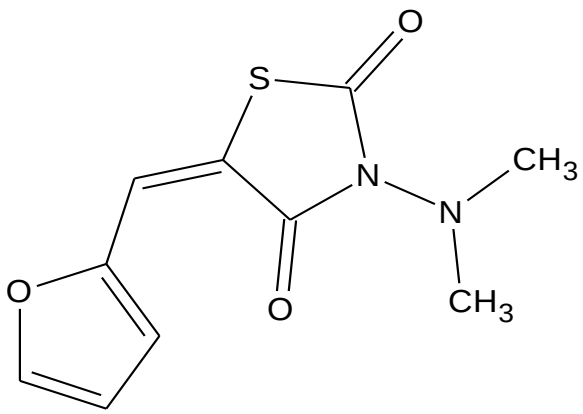
Compound code	:	TZD1
IUPAC name	:	1, 3-thiazolidine-2, 4-dione
Molecular formula	:	$C_3H_3NO_2S$
Molecular weight	:	117.12
Color	:	White color
Melting point	:	118°C
IR Spectrum (cm^{-1})	:	1163, 1228, 1741, 2864, 3007,3367, 3477
^{1}HNMR Spectrum (ppm)	:	4.331 1H, Singlet (NH), 2.675 2H,Singlet (CH_2).
ESI-MS$[M+H]^+$	:	117.540

2)



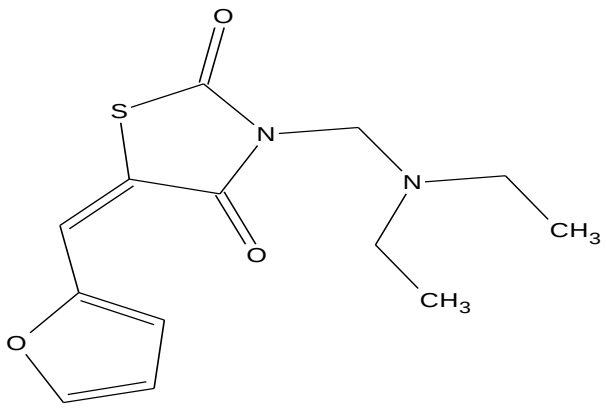
Compound code	:	TZD2
IUPAC name	:	5-[(furan-2-yl)methyl]-2,4-dimethylidine-1,3-thiazolidine
Molecular formula	:	$C_8H_7NO_3S$
Molecular weight	:	197.21
Color	:	White color
Melting point	:	240°C
IR Spectrum(cm^{-1})	:	1020,1147,1166,1217,1612,1678,1691,1732,1776,2802,3360
^{1}H NMR Spectrum (ppm)	:	3.370 1H,Singlet(NH), 3.016 1H, Singlet (CH-Allylic), 6.7-8.0 3H, Multiplet (H- Aromatic).
ESI-MS$[M+H]^+$	:	196.023

3)



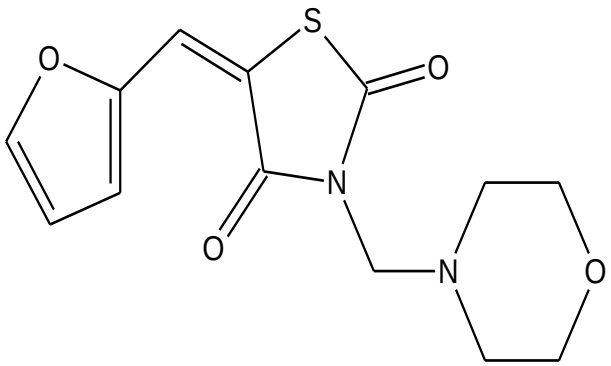
Compound code	:	TZD31
IUPAC name	:	(5*E*)-3-(dimethylamino)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine- 2, 4-dione
Molecular formula	:	$C_{11}H_{12}N_2O_3S$
Molecular weight	:	280.34
Color	:	Brownish color
Melting point	:	124°C
IR Spectrum (cm^{-1})	:	1020, 1060, 1151, 1219, 1614, 1683, 1735, 3122
^{1}H NMR Spectrum(ppm)	:	1.240 3H,Multiplet (CH_3), 2.711 2H,Singlet (CH_2), 2.677 1 H,Singlet (CH-Allylic), 6.780 3H,Multplet (H-Aromatic).
ESI-MS$[M+H]^+$	:	279.05

4)



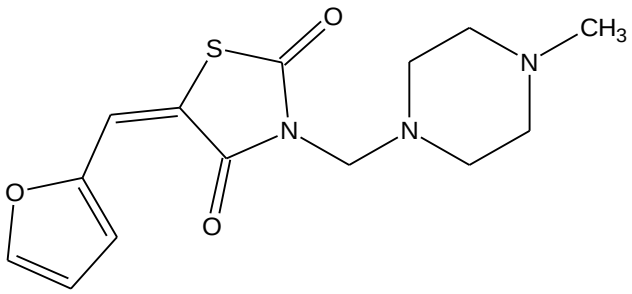
Compound code	:	TZD32
IUPAC name	:	(5*E*)-3-[(diethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione
Molecular formula	:	$C_{13}H_{16}N_2O_3S$
Molecular weight	:	294.32
Color	:	Black color
Melting point	:	234°C
IR Spectrum (cm^{-1})	:	1020,1149,1219,1614,1683,1735,3122
^{1}H NMR Spectrum (ppm)	:	6.7-8.0 3H,Multiplet (H-Aromatic), 2.676 1H,Singlet (CH-Allylic), 2.932, 2H,Singlet (CH_2), 1.238 5H,Singlet (CH_2CH_3).
ESI-MS$[M+H]^+$	:	293.43

5)



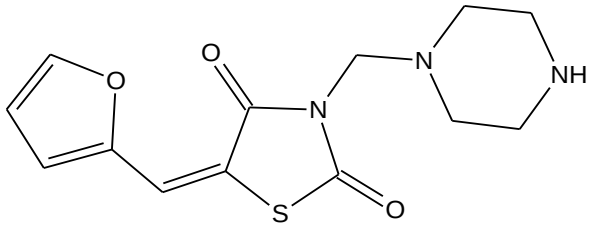
Compound code	:	TZD33
IUPAC name	:	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(morpholin-4-yl)methyl]- 1,3- thiazolidine-2,4-dione
Molecular formula	:	$C_{13}H_{14}N_2O_4S$
Molecular weight	:	294.32
Colour	:	Black color
Melting point	:	102°C
IR Spectrum (cm^{-1})	:	1112,1159,1222,1610,1670,3041,3126
^{1}H NMR Spectrum (ppm)	:	6.7-8.0 11H,Multiplet(H-Aromatic), 2.678 1H,Singlet (CH-Allylic), 3.0 ,2H,Singlet (CH_2).
ESI-MS$[M+H]^+$	:	300.32

6)



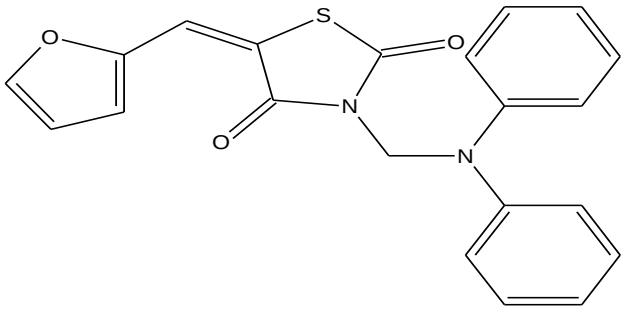
Compound code	:	TZD34
IUPAC name	:	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(4-methylpiperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione
Molecular formula	:	$C_{14}H_{17}N_3O_3S$
Molecular weight	:	307.36
Color	:	Brownish black color
Melting point	:	198°C
IR Spectrum (cm^{-1})	:	1020,1612,1683,1735,2802,3035
^{1}H NMR Spectrum (ppm)	:	6.7-8.0 11H,Multiplet (H-Aromatic), 2.656 1H,Singlet(H-Allylic), 3.016 , 2H,Singlet (CH_2), 1.644 3H,Singlet (CH_3).
ESI-MS$[M+H]^+$	:	307.01

7)



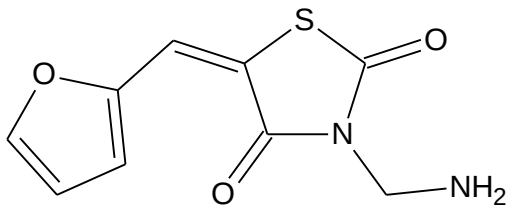
Compound code	:	TZD35
IUPAC name	:	(5*E*)-5-[(furan-2-yl)methylidene]-3-[(piperazin-1-yl)methyl]-1,3-thiazolidine-2,4-dione
Molecular formula	:	$C_{13}H_{15}N_3O_3S$
Molecular weight	:	293.34
Color	:	Brownish black color
Melting point	:	206°C
IR Spectrum (cm^{-1})	:	1058,1093,1165,1193,1614,1676,1734,2829
^{1}H NMR Spectrum (ppm)	:	6.7-8.0 11H,Multiplet (H-Aromatic), 2.656 1H,Singlet (H-Allylic), 3.016 , 2H,Singlet (CH_2), 1.644 3H,Singlet CH_3).
ESI-MS$[M+H]^+$	:	294.24

8)



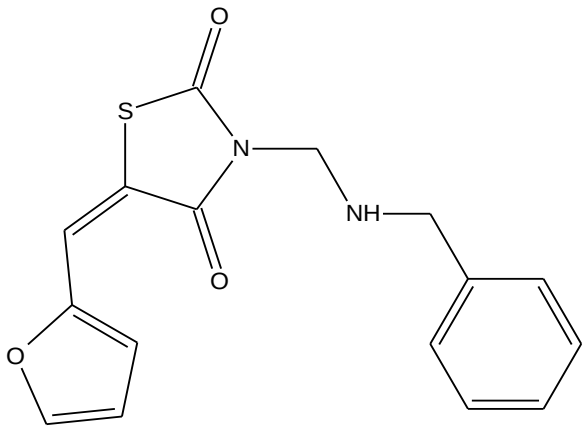
Compound code	:	TZD36
IUPAC name	:	(5*E*)-3-[(diphenylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione
Molecular formula	:	$C_{21}H_{16}N_2O_3S$
Molecular weight	:	376.42
Color	:	Black color
Melting point	:	142°C
IR Spectrum (cm^{-1})	:	1085,1107,1163,1230,1458,1490,1620,1681,1735
^{1}H NMR Spectrum(ppm)	:	6.7-8.0 13H,Multiplet (H-Aromatic), 2.675 1H,Singlet(H-Allylic), 3.021, 2H, Singlet (CH_2).
ESI-MS$[M+H]^+$	:	377.40

9)



Compound code	:	TZD37
IUPAC name	:	(5*E*)-3-(aminomethyl)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine- 2,4-dione
Molecular formula	:	$C_9H_8N_2O_3S$
Molecular weight	:	224.23
Color	:	Black Color
Melting point	:	280°C
IR Spectrum (cm^{-1})	:	1020,1151,1614,1683,2802,3032,3143
^{1}H NMR Spectrum (ppm)	:	6.7-7.9 3H,Multiplet (H-Aromatic), 2.672 1H,Singlet (H-Allylic), 4.568 2H,Singlet (NH_2), 3.010 2H,Singlet (CH_2).
ESI-MS$[M+H]^+$	:	225.20

10)



Compound code	:	TZD38
IUPAC name	:	(5*E*)-3-[(benzylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3- Thiazolidine-2,4-dione
Molecular formula	:	$C_{16}H_{14}N_2O_3S$
Molecular weight	:	314.35
Color	:	Brownish black color
Melting point	:	140°C
IR Spectrum (cm^{-1})	:	1020, 1165, 1217, 1467, 1612, 1683, 1734, 2804, 3034
^{1}H NMR Spectrum (ppm)	:	6.7-7.8 8H, Multiplet (H-Aromatic), 2.677 1H,Singlet (H-Allylic), 3. 360, 2H,Singlet (CH_2), 4.0-4.9 1H,Singlet (NH).
ESI-MS$[M+H]^+$	:	314.32

CHAPTER 6

RESULTS AND DISCUSSION

The titled compounds were designed manually by performing the molecular docking studies using *Molegro Virtual Docker* 5.0 2010. Ligand preparation was done using Chemdraw ultra V 10.0 and all the ligands were energy minimized using the molecular mechanics. X-ray crystal structure of the protein Anaplastic Lymphoma Kinase (PDB code: **2XB7**) was retrieved from Brookhaven Protein Data bank. All the ligands were imported inside the protein cavity with highest volume and measured for their affinity, MolDock score, re-rank score and H-bond score. The docking scores of the ligand molecules with Anaplastic Lymphoma Kinase has been posted on the below table.

S.No	Compound Code	Mol Dock Score	RerankScore	H-Bonding
1	**TZD31**	**-95.1189**	**-67.4579**	**-3.2296**
2	**TZD32**	**-109.725**	**-87.1519**	**-2.5**
3	**TZD33**	**-99.7353**	**-84.2026**	**-1.37424**
4	**TZD34**	**-104.01**	**-87.2115**	**-2.60561**
5	**TZD35**	**-105.88**	**-80.4063**	**-1.1418**
6	**TZD36**	**-132.797**	**-93.4662**	**-1.27137**
7	**TZD37**	**-77.883**	**-65.9582**	**-0.538305**
8	**TZD38**	**-109.845**	**-90.8004**	**-2.56044**
9	TZD39	-108.271	-91.8565	-3.97003
10	TZD 40	-108.221	-90.9616	-2.29516
11	TZD 41	-111.707	-94.492	-2.5
12	TZD 42	-108.358	-87.8184	-1.42707

13	TZD 43	-100.174	-84.8847	-2.5
14	TZD 44	-104.725	-89.4572	-7.3671
15	TZD 45	-97.6618	-81.4772	-2.5
16	TZD 46	-100.092	-85.0631	-2.49937
17	TZD 47	-108.356	-87.4787	-2.53422
18	TZD 48	-120.127	-98.4684	-2.41028
19	TZD 49	-102.55	-82.7096	-2.68517
20	TZD 50	-95.3975	-62.8836	-4.795
21	TZD 51	-85.5074	-75.3551	-2.5
22	TZD 52	-121.153	-96.3112	-3.01003
23	TZD 53	-104.493	-87.1936	-7.07187
24	TZD 54	-111.378	-87.8866	-3.12418
25	TZD 55	-122.253	-69.837	-3.11672
26	TZD 56	-97.6593	-73.6646	0
27	TZD 57	-105.183	-83.9685	-2.9283
28	TZD 58	-111.304	-89.8838	-2.97896
29	TZD 59	-108.665	-83.4734	-1.06886
30	TZD 60	-112.856	-92.3747	-5.29658
31	TZD 61	-108.11	-88.0923	-1.88894
32	TZD 62	-120.742	-97.1966	-2.5

Based on the above table, the compounds with best MolDock scores have been selected for the synthesis. Among the titled compounds, **TZD 32, TZD 34, TZD 35, TZD 36 and TZD 38** bearing Diethylamine, Methyl piperazine, Piperazine, Diphenylamine and Benzylamine moieties showed highest MolDock scores of about -109.725, -104.01, -105.88, -132.797 and -109.845 respectively as well as the re-rank scores of about -87.1519, -87.2115, -80.4063, -93.4662 and -90.8004 respectively. These compounds with better MolDock scores have been selected for the synthesis.

Table 6: MolDock scores of the compounds selected for the synthesis.

S.No	Compound Code	Mol Dock Score	Rerank Score	H-Bonding
1	TZD31	-95.1189	-67.4579	-3.2296
2	TZD32	-109.725	-87.1519	-2.5
3	TZD33	-99.7353	-84.2026	-1.37424
4	TZD34	-104.01	-87.2115	-2.60561
5	TZD35	-105.88	-80.4063	-1.1418
6	TZD36	-132.797	-93.4662	-1.27137
7	TZD37	-77.883	-65.9582	-0.538305
8	TZD38	-109.845	-90.8004	-2.56044

The above compounds with best MolDock scores were used for the synthesis of Furfuryl thiazolidinedione Mannich bases. The first step involved the synthesis of the basic nucleus *1,3-thiazolidine-2,4-dione*. It was synthesized by the cyclization reaction between thiourea and chloroacetic acid. The reaction mixture was stirred in the presence of concentrated hydrochloric acid and refluxed for 8-9 hours at 100º-110ºC. the resultant product *1,3-thiazolidine-2,4-dione*was stable, crystalline and white in color. The melting point of the synthesized compound *1,3-thiazolidine-2,4-dione*was 118ºC. The purity of the synthesized compound was ascertained by TLC and R_f value was found to be 0.830. The synthesized compound *1,3-thiazolidine-2,4-dione*was soluble in DMSO, ethanol, methanol and ethyl acetate.

The chiral Centre at the position 5 of *1,3-thiazolidine-2,4-dione*ring was configurationally unstable under physiological conditions. Hence the second step involves the protection of chiral carbon at 5th position with protecting agent, furan-2-carbaldehyde (furfuraldehyde). In the second step, furan-2-carbaldehyde was incorporated into the synthesized *1,3-thiazolidine-2,4-dione*by heating under reflux for one hour in the presence of the catalyst piperidine and ethanol. The product obtained *(5Z)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione*was stable, amorphous and dark brown in color. The melting point of the synthesized compound *(5Z)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione* was 240ºC, and R_f value was found to be 0.711. The synthesized compound *(5Z)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione*was soluble in DMSO, ethanol, methanol and ethyl acetate.

The third step involved the formation of Mannich bases. The compound *(5Z)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione*was allowed to react with formaldehyde and appropriate amines to get the final product of furfuryl thiazolidinedione Mannich bases. The mechanism of the Mannich base formation followed the nucleophilic addition. The nucleophilic addition of the amines to the carbonyl group of formaldehyde results in the formation of the intermediate iminium ion (Schiff base). The intermediate iminium compound act as an electrophile which undergoes electrophilic addition with the compound containing acidic proton (*(5Z)-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione*) to form the desired Mannich bases. The resultant Mannich bases were stable and all the compounds were freely soluble in ethanol, methanol, ethyl acetate and DMSO. The melting points for all the compounds were determined uncorrected by the open ended capillary tube. The purity of all the synthesized were checked by TLC using silica gel G as the stationary phase. The FT-IR, ^{1}H NMR and Mass spectral data for a
Figure: FT-IR spectrum of the compound TZD 32 (5*E*)-3-[(diethylamino)methyl]-5-[(furan-2-yl)methylidene]-1,3-thiazolidine-2,4-dione

The FT-IR spectra of the synthesized compounds showed the peak at1159.22 cm^{-1} for the confirmation of C=N (azomethine) and all the compounds shows peak at 1610.35 cm^{-1} for the confirmation of C=C and C-H Stretching, The peaks at 1735.93 cm^{-1} for the confirmation of C=O and peaks at 670.35 cm^{-1} for the confirmation of C-O and at 1222.87 cm^{-1} for the

confirmation of C-Sand two absorption peaks at 3126.61 for the confirmation of Mannich base formation N-CH_2-N.

The ^{1}H NMR Spectrum of synthesized compounds of thiazolidinedione Mannich bases showed singlet 2.676-H ppm which assigned for Allylic CH groups of Diethyl amine, CH_2 groups were assigned at 2.932 ppm-2H singlet, CH_2CH_3 group were assigned at 1.238-singlet-SH, Aromatic-H groups were assigned at 6.7-8.0 ppm 3H, multiplet in Diethylamine.The Mass spectra of the synthesized compound were correlated with the expected structures.

The above synthesized compounds with best MolDock scores has been selected for the evaluation of *in vitro* Anti-cancer activity.The compounds TZD 32, TZD 34, TZD 35, TZD 36 and TZD 38 showed with cell viability 44.60%, 32.66%, 29.70%, 23.89% and 31.28% and IC_{50} value of 500 µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml and 125µg/ml respectively.Although all the five compounds showed good anticancer activity against to the targeted protein the compound TZD 36 showed better activity with a cell viability of 23.89% and IC_{50} value of 62.5 µg/ml .

Further the compound with best MolDock score **TZD 36** was subjected to find its major hydrogen bond interaction and stearic interaction with the target protein Anaplastic Lymphoma Kinase. It was found out that the amino acid residues leu 1190, Ser 1189 were the major sources of the hydrogen bond interaction. The amino acid residues Gln 1188, Leu 1190, Ser 1186, Pro 1191, Arg 1113, Ser 1189 Anaplastic Lymphoma Kinase were found to have the stearic interactions with the protein.

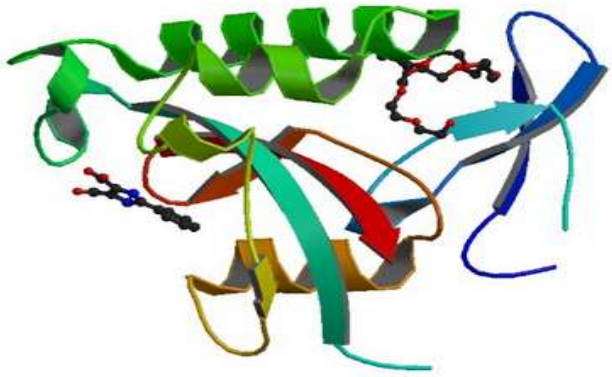


Fig6:structure of the protein anaplastic lymphoma kinase

(PDB CODE: 2XB7).

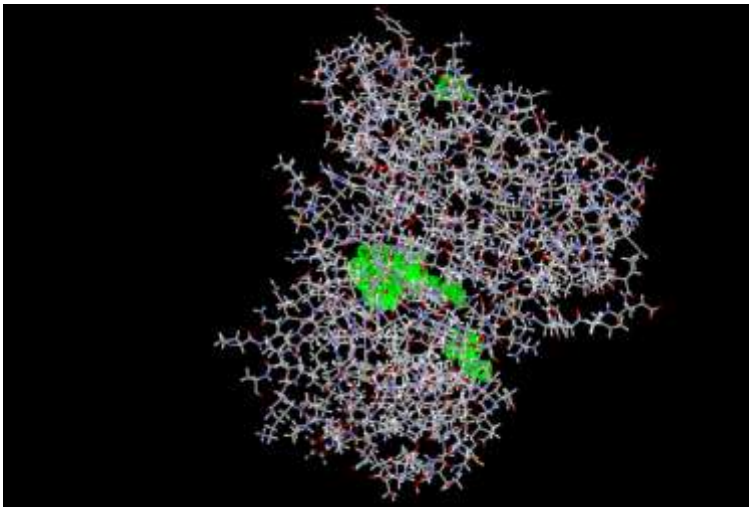


Fig7:structure of the protein with active cavities

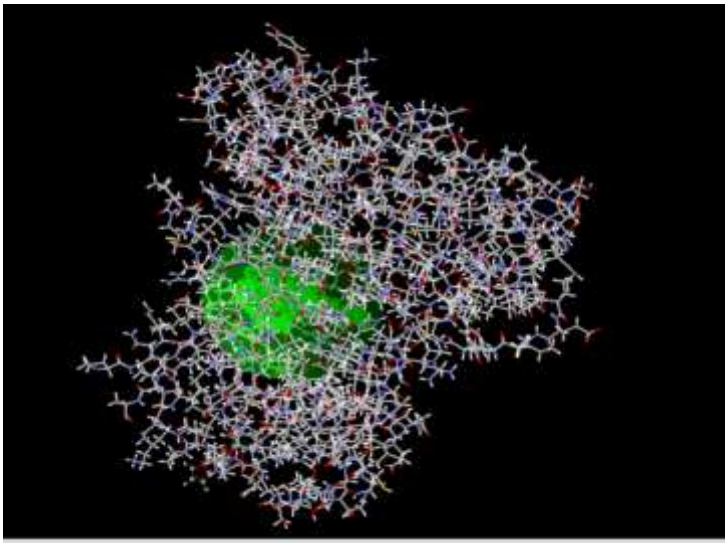


Fig8:docking view of the ligand tzd36 at cavity 1 of the protein (2XB7)

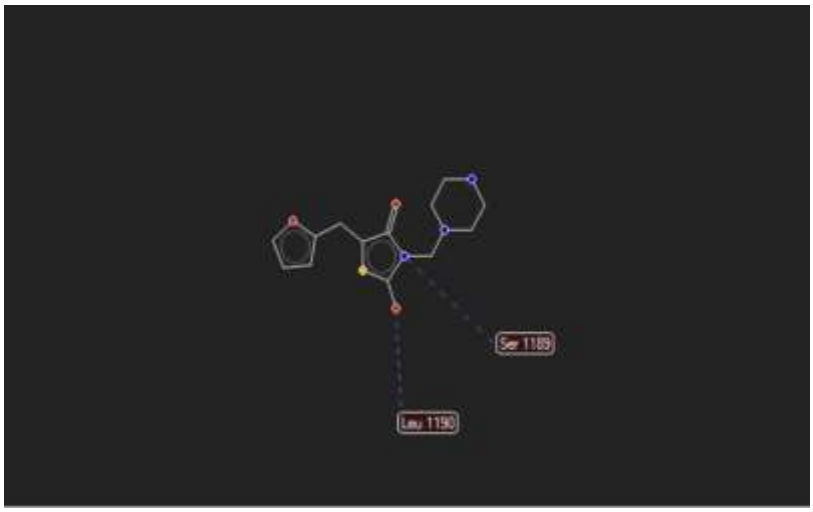


Fig:9 hydrogen bonding interaction of the ligand tzd36 with cavityof the protein(2XB7)

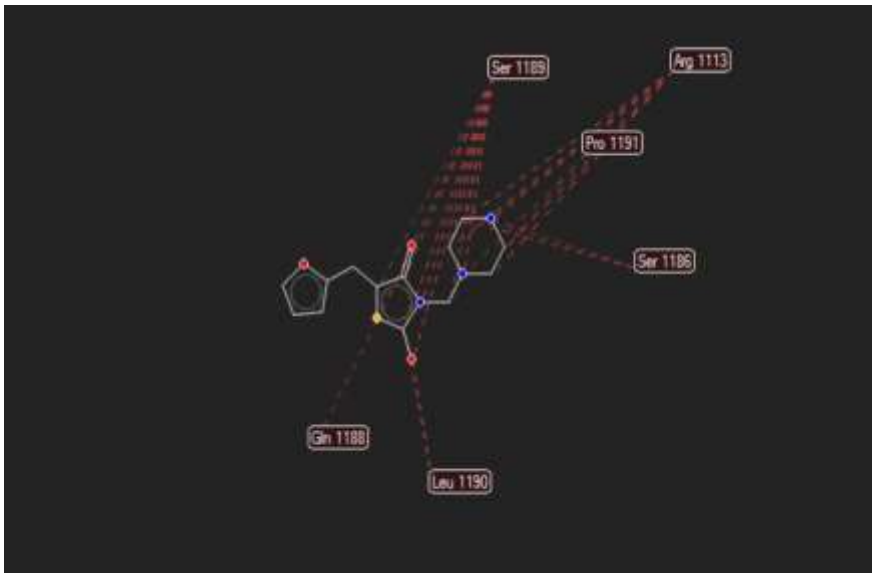


Fig:10 stearic interaction of the ligand tzd36 with cavity 1 of the protein(2XB7)

INVITRO ANTICANCER ACTIVITY

Anticancer effect of sample TZD-32 on cell lineU937

Table7: Anticancer effect of sample TZD-32 U937 on cell line

S.NO	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.422	44.60
2	500	1:1	0.488	51.58
3	250	1:2	0.553	58.45
4	125	1:4	0.618	65.32
5	62.5	1:8	0.685	72.41
6	31.2	1:16	0.754	79.70
7	15.6	1:32	0.816	86.25
8	7.8	1:64	0.881	93.12
9	Cell control	-	0.946	100

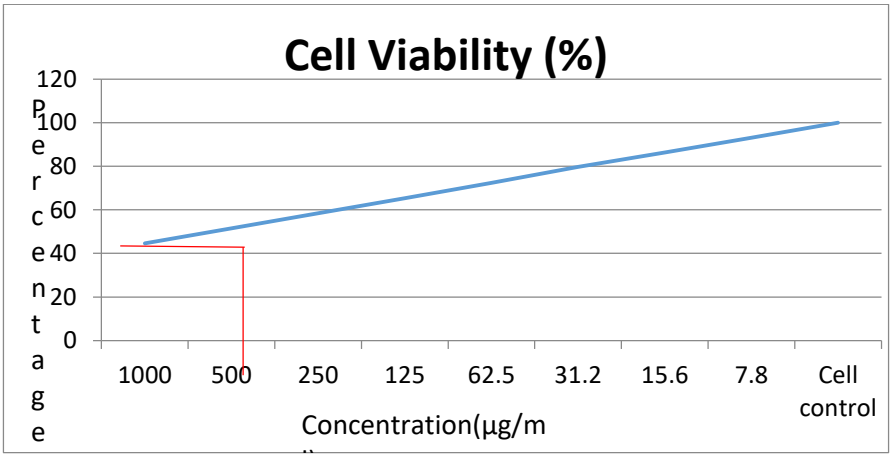
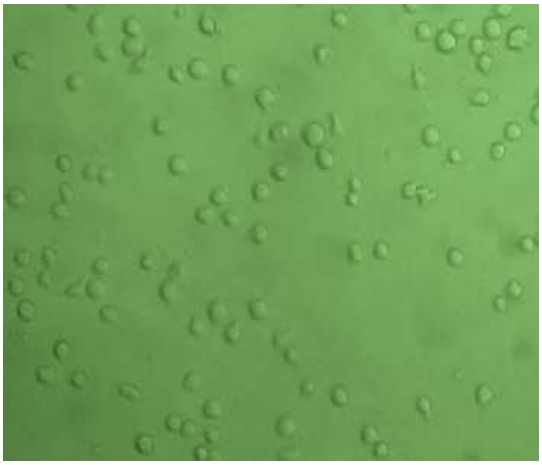


Fig11: Anticancer effect of Sample TZD-32 on U937 cell line

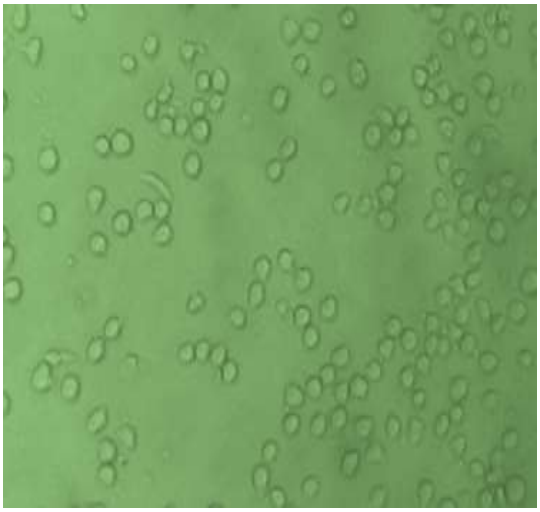
Anti-cancer effect of the Sample TZD-32 on U937 cell line

control

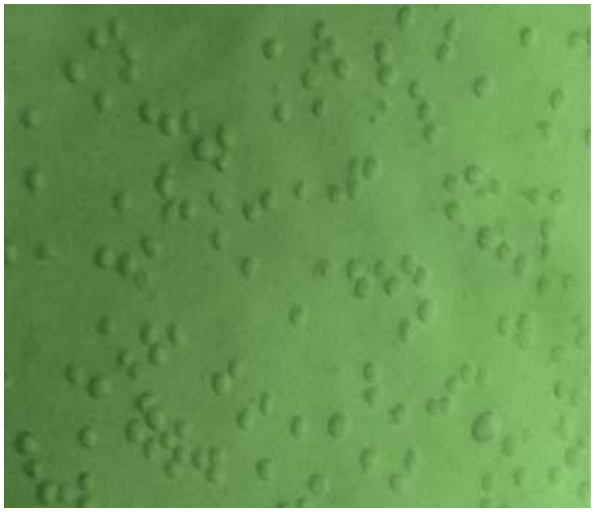
1000µg/ml



500µg/ml



7.8µg/ml



Anticancer effect of sample TZD-34 on cell lineU937

S:NO	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.309	32.66
2	500	1:1	0.398	42.07
3	250	1:2	0.475	50.21
4	125	1:4	0.561	59.30
5	62.5	1:8	0.652	68.92
6	31.2	1:16	0.733	77.48
7	15.6	1:32	0.817	86.36
8	7.8	1:64	0.904	95.56
9	Cell control	-	0.946	100

Table: 8 Anticancer effect of sample TZD-34 U937 on cell line

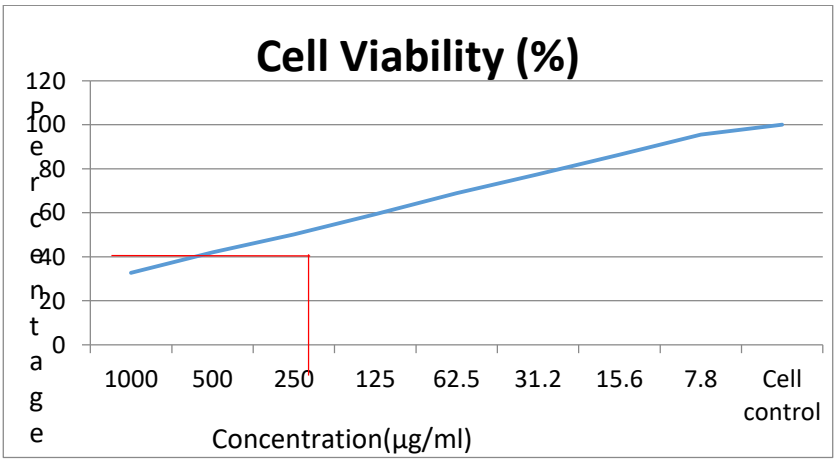
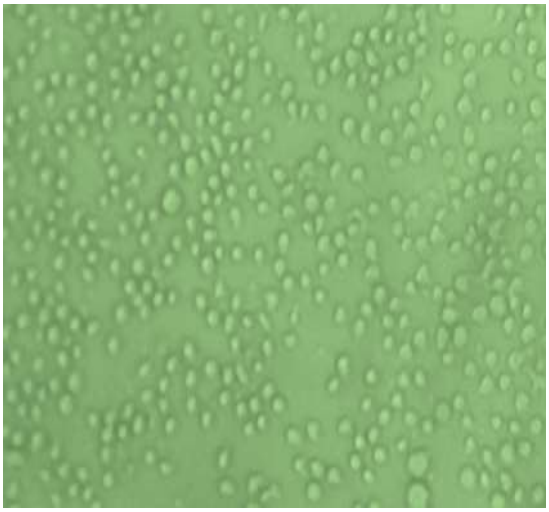


Fig12:Anticancer effectof Sample TZD-34on U937 cell line

Anti-cancer effect of the Sample TZD-34on cell lineU937

control



250µg/ml

1000µg/ml

7.8 µg/ml

Anticancer effect of sample TZD-35 on cell lineU937

S.NO	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.281	29.70
2	500	1:1	0.347	36.68
3	250	1:2	0.407	43.02
4	125	1:4	0.464	49.04
5	62.5	1:8	0.532	56.23
6	31.2	1:16	0.604	63.84
7	15.6	1:32	0.679	71.77
8	7.8	1:64	0.745	78.75
9	Cell control	-	0.946	100

Table: 9 Anticancer effect of sample TZD-35 U937 on cell line

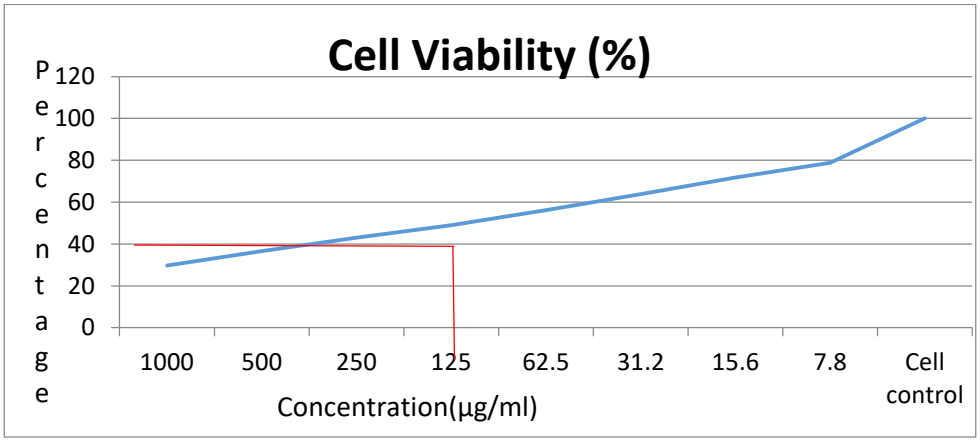


Fig13:Anticancer effect of Sample TZD-35 on U937 cell line

Anti-cancer effect of the Sample TZD-35 on cell lineU937

Control

125µg/ml

1000 µg/ml

7.8µg/ml

Anticancer effect of sample TZD-36 on cell lineU937

S.NO	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.226	23.89
2	500	1:1	0.319	33.72
3	250	1:2	0.406	42.91
4	125	1:4	0.491	51.90
5	62.5	1:8	0.586	61.94
6	31.2	1:16	0.670	70.82
7	15.6	1:32	0.749	79.17
8	7.8	1:64	0.840	88.79
9	Cell control	-	0.946	100

Table: 10 Anticancer effect of sample TZD-36 U937 on cell line

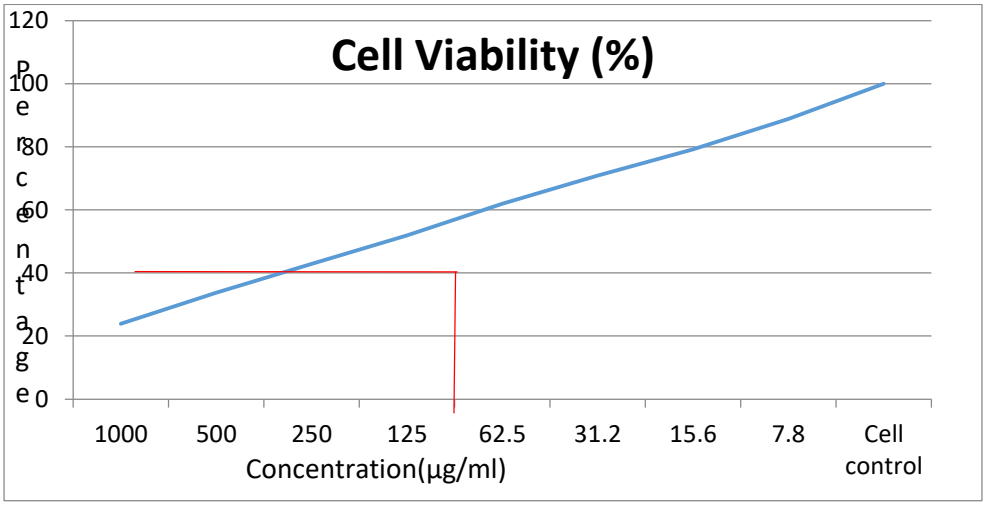


Fig14: Anticancer effectof Sample TZD-36 on U937 cell line

Anti-cancer effect of the Sample TZD-36 oncell lineU937

Control

125µg/ml

1000µg/ml

7.8µg/ml

Anticancer effect of sample TZD-38 onU937 cell line

S.NO	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.296	31.28
2	500	1:1	0.352	37.20
3	250	1:2	0.418	44.18
4	125	1:4	0.487	51.47
5	62.5	1:8	0.562	59.40
6	31.2	1:16	0.628	66.38
7	15.6	1:32	0.691	73.04
8	7.8	1:64	0.762	80.54
9	Cell control	-	0.946	100

Table: 11 Anti-cancer effect of sample TZD-38 U937 on cell line

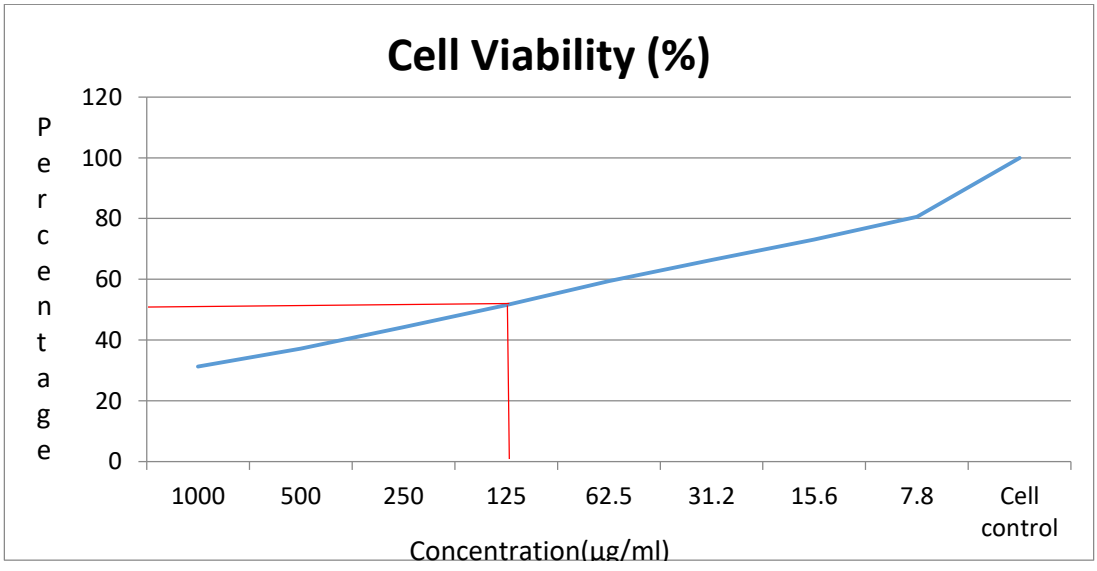
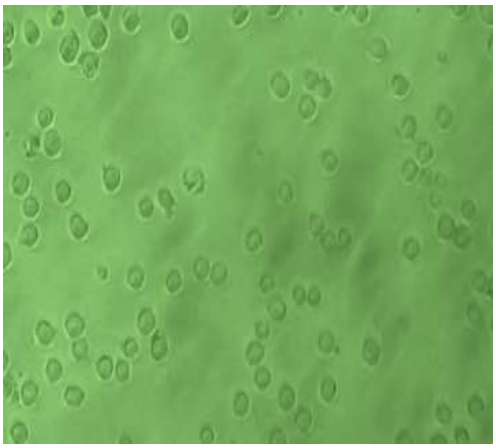


Fig15: Anti-cancer effect of Sample TZD-38 on U937 cell line

Anti-cancer effect of the Sample TZD-38 on cell lineU937

control

1000µg/ml



125µg/ml

7.8µg/ml

CHAPTER 7

SUMMARY AND CONCLUSION

The novel furfuryl thiazolidinedione Mannich bases were designed manually using Molgro Virtual Docker. The designed compounds were analyzed in order to predict the best score compounds . Based on the *in silico* docking studies, the best scored compounds were selected for the synthesis using scheme 1. The synthesized compounds were characterized by their FT-IR, ^{1}H NMR and their Mass spectra. The confirmed compounds were subjected to *in vitro* anti-cancer activity with U937 using MTT method. All the compounds showed good ani-cancer activity. Among them, the compound TZD 36 [(5*E*)-3-[(diphenylamino)methyl]-5-[(furan-2-yl)methylidene]- 1,3-thiazolidine-2,4-dione] showed better anti-cancer activity with a cell viability of 23.89% and IC_{50} value of 62.5 µg/ml. The anti-cancer activity compound TZD 36 was subjected to *in silico* docking studies in order to predict the probable binding site against anaplastic lymphoma kinase (PDB code: 2XB7). From the *in silico* studies, compound 36 showed amino acids are linked via hydrogen bonding wit the ligand and amino acids like were shoed stearic interaction with the ligand. From the above results, we conclude that these interactions maybe the reason for the better activity than the other synthesized compounds. Moreover, we planned to analyze further all the compounds for other ailments.

CHAPTER 8

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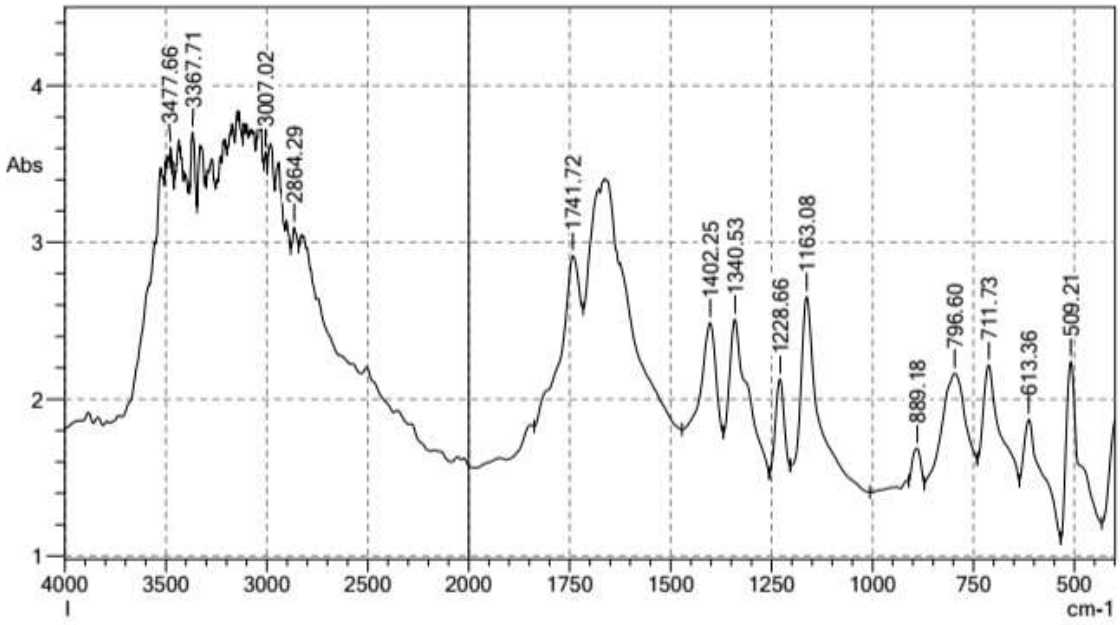

Fig16: FT-IR spectrum of the compound TZD-1

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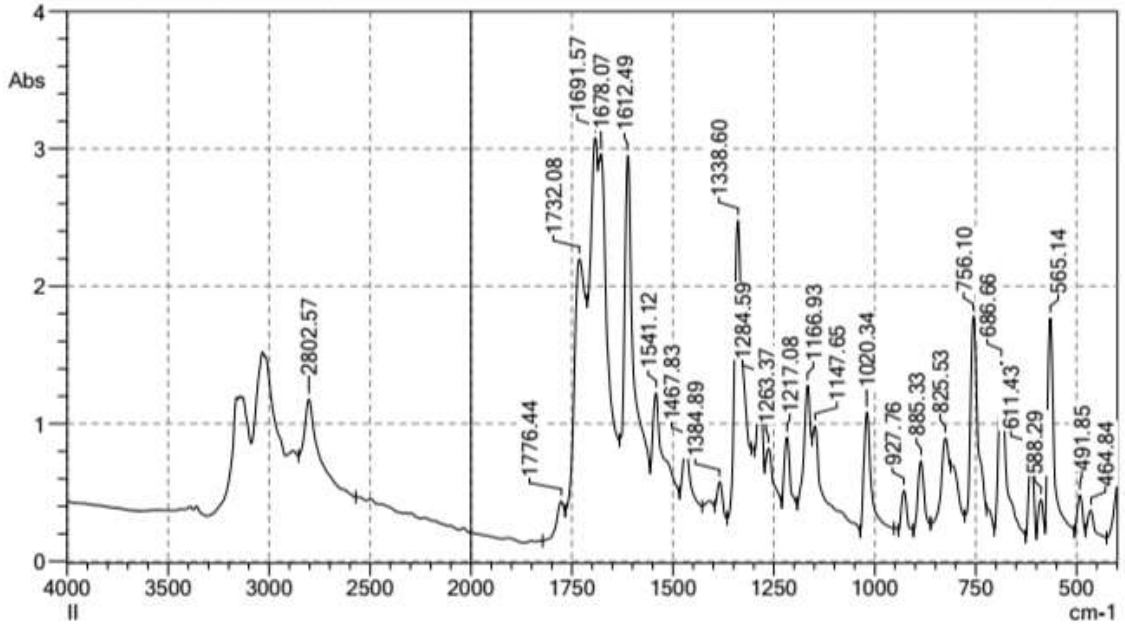

Fig17: FT-IR spectrum of the compound TZD-2

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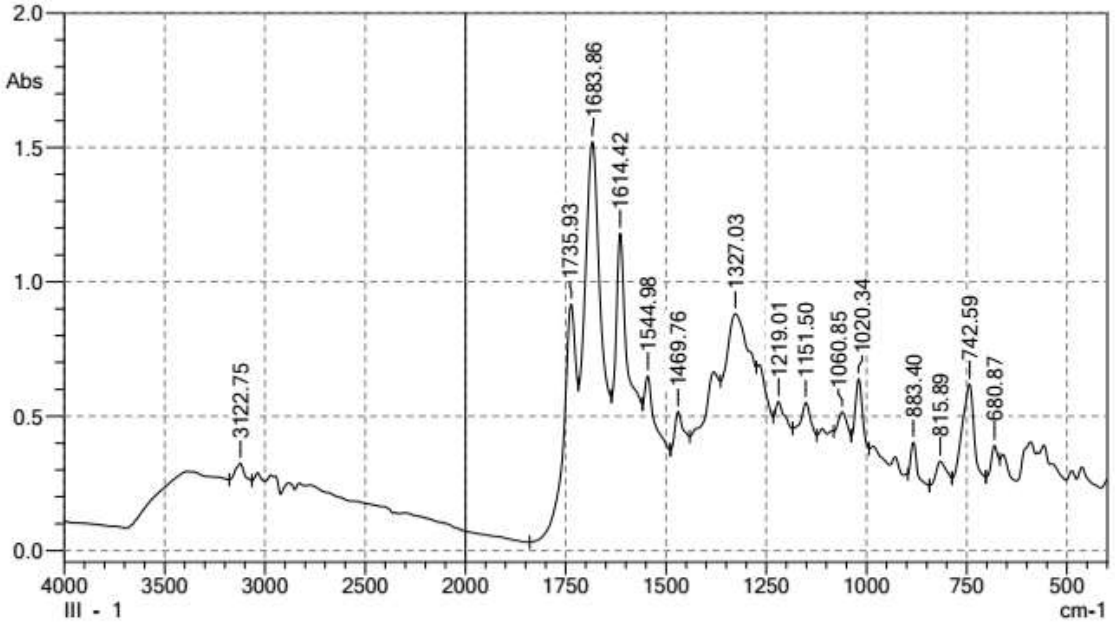

Fig18: FT-IR spectrum of the compound TZD-31

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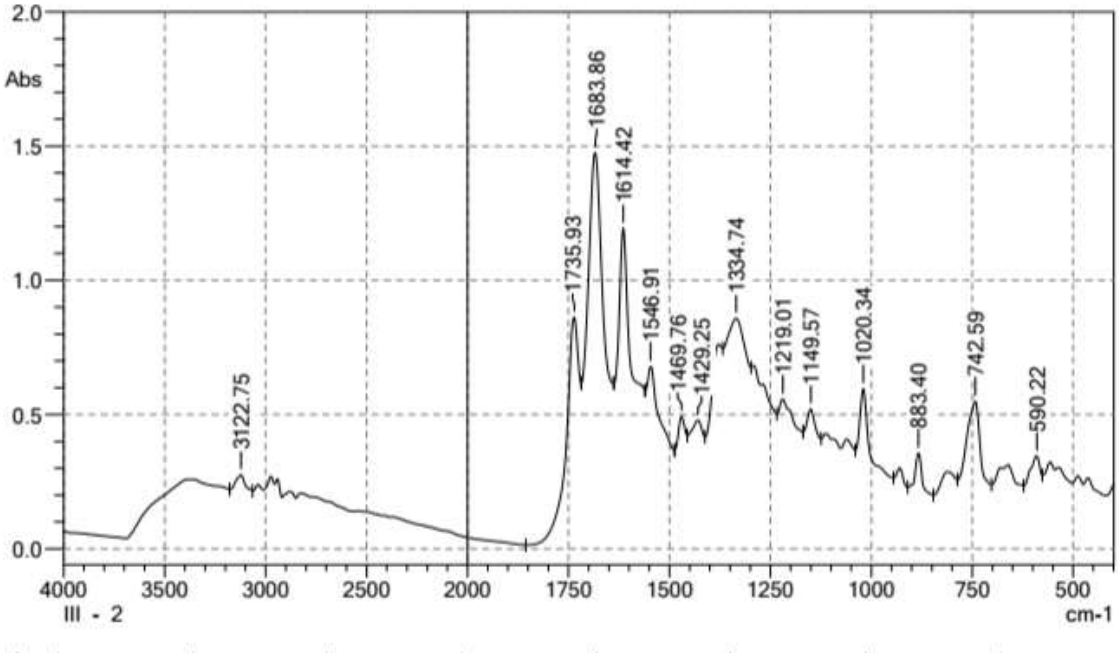

Fig19: FT-IR spectrum of the compound TZD-32

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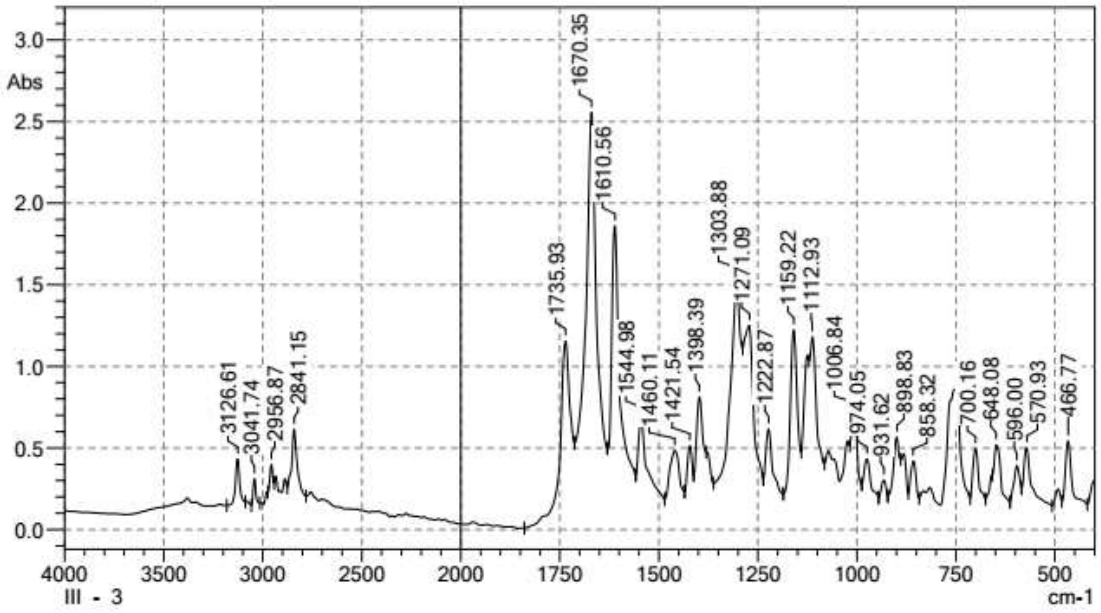

Fig20: FT-IR spectrum of the compound TZD-33

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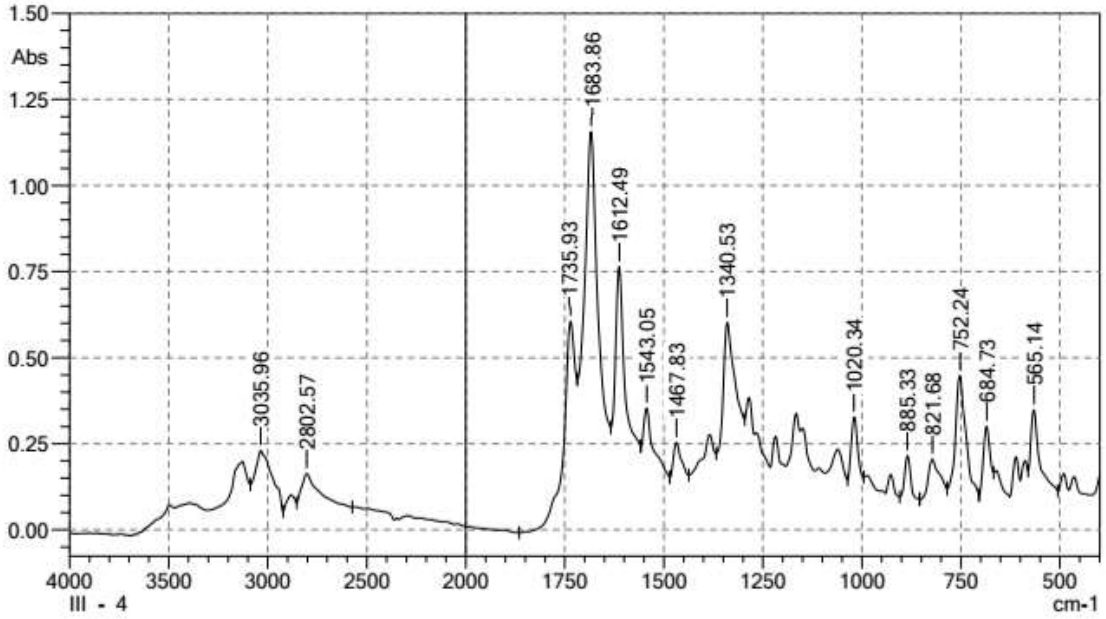

Fig21: FT-IR spectrum of the compound TZD-34

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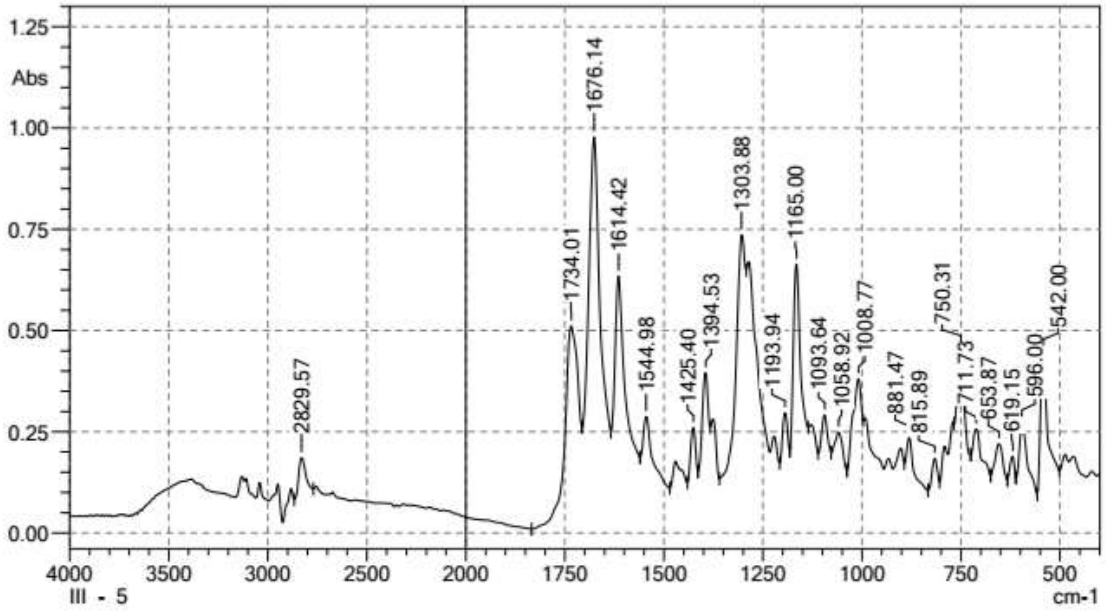

Fig22: FT-IR spectrum of the compound TZD-35

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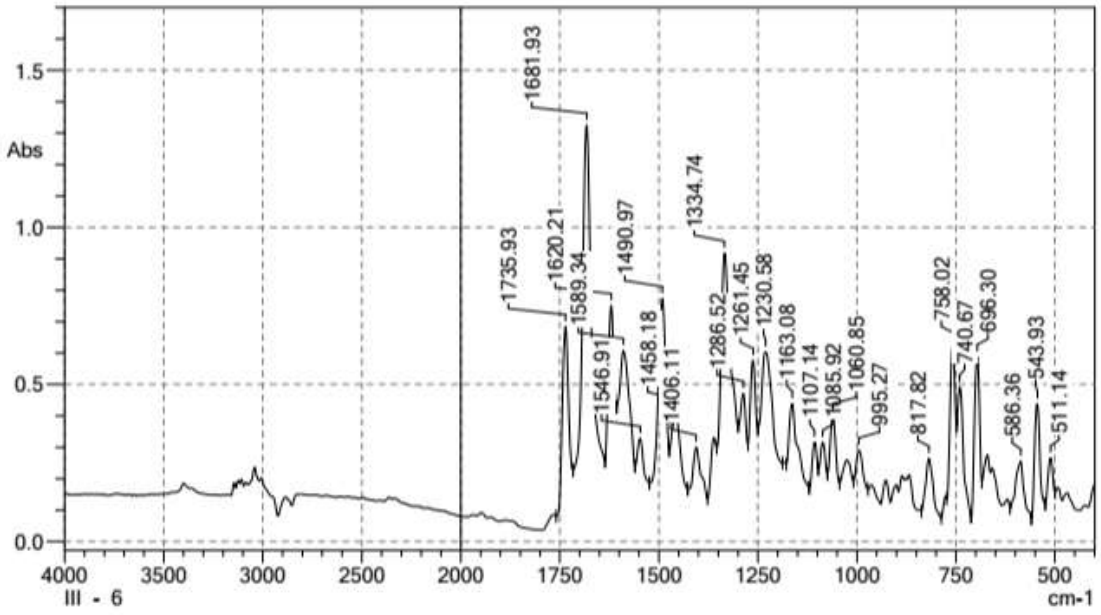

Fig23: FT-IR spectrum of the compound TZD-36

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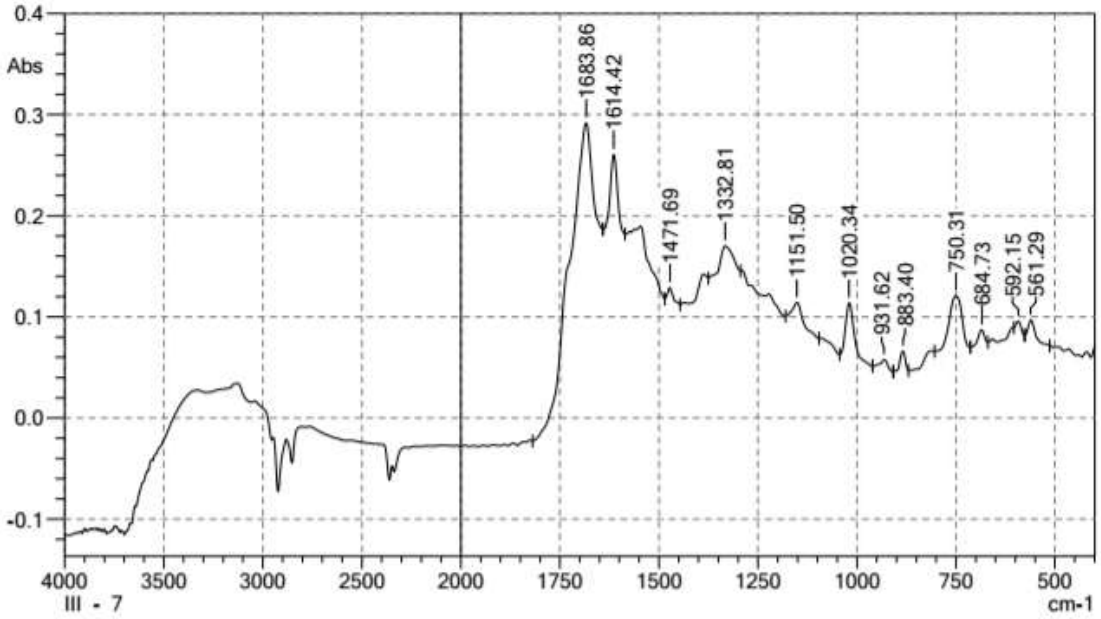

Fig24: FT-IR spectrum of the compound TZD-37

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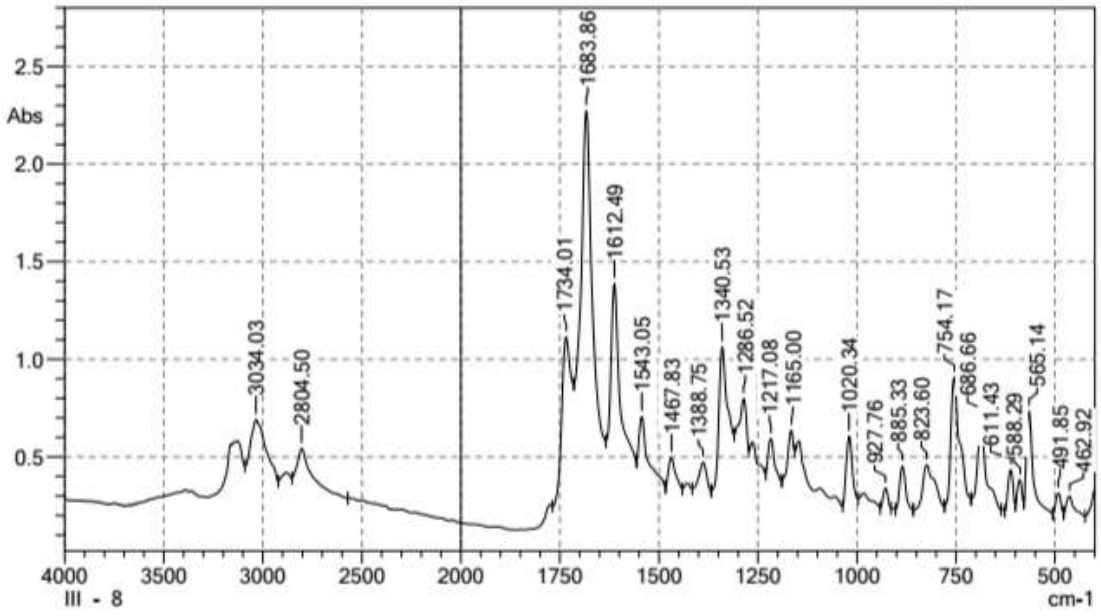

Fig 25: FT-IR spectrum of the compound TZD-38

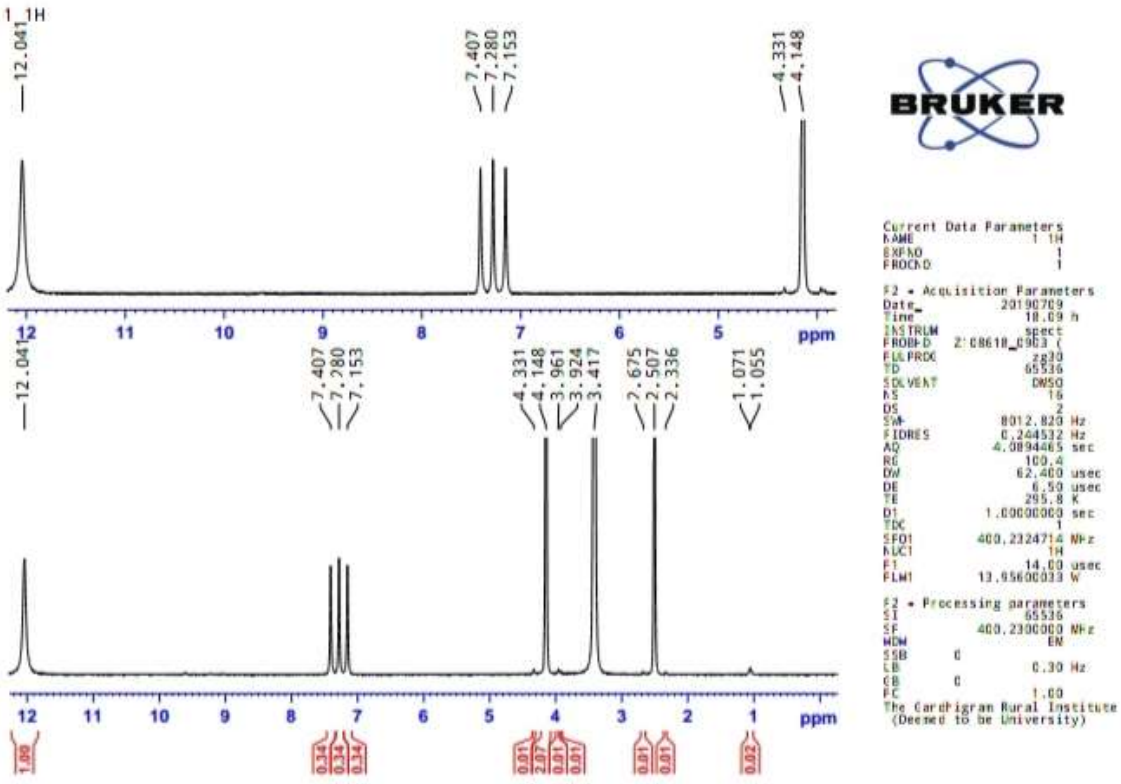

Fig 26: ^{1}H NMR spectrum of the compound TZD-1

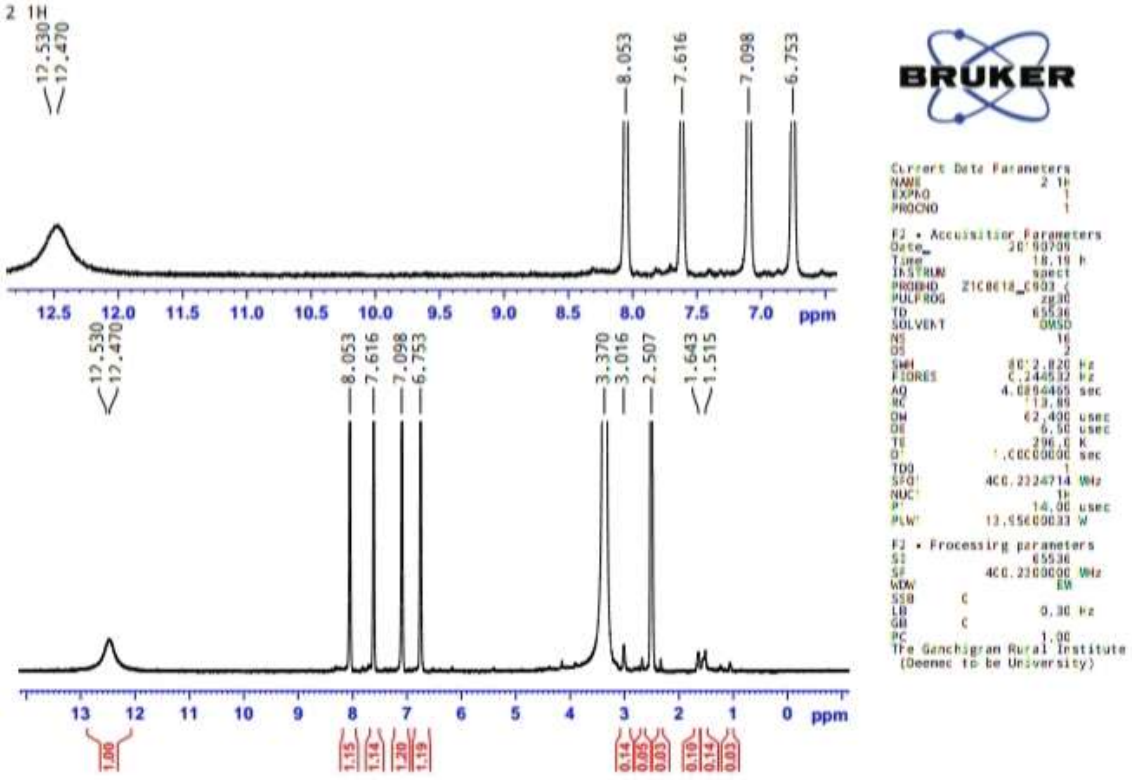

Fig27: ^{1}H NMR spectrum of the compound TZD-2

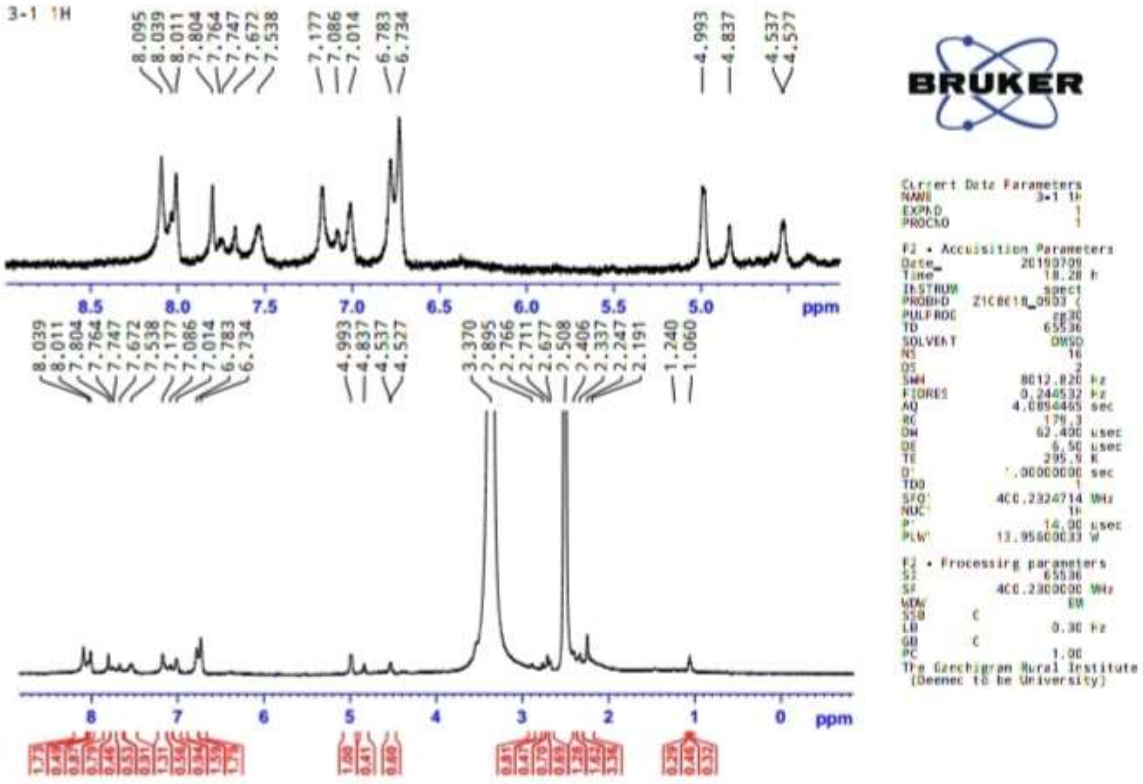

Fig 28: ^{1}H NMR spectrum of the compound TZD-31

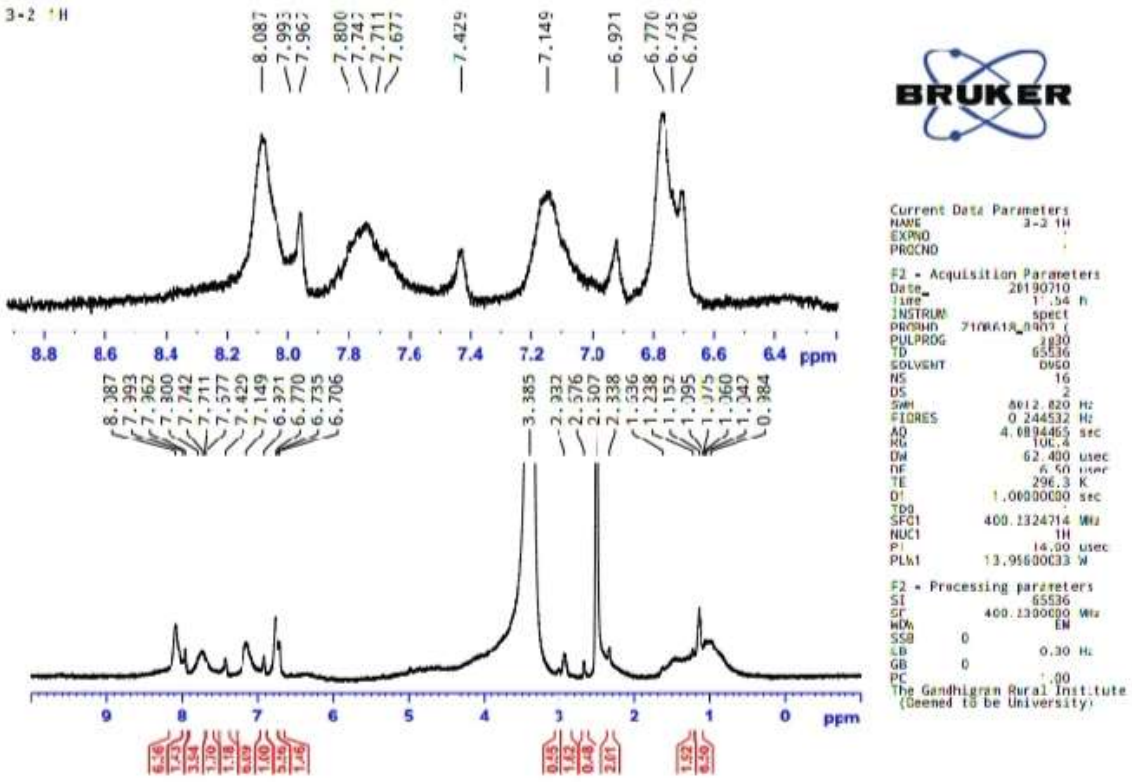

Fig 29: ^{1}H NMR spectrum of the compound TZD-32

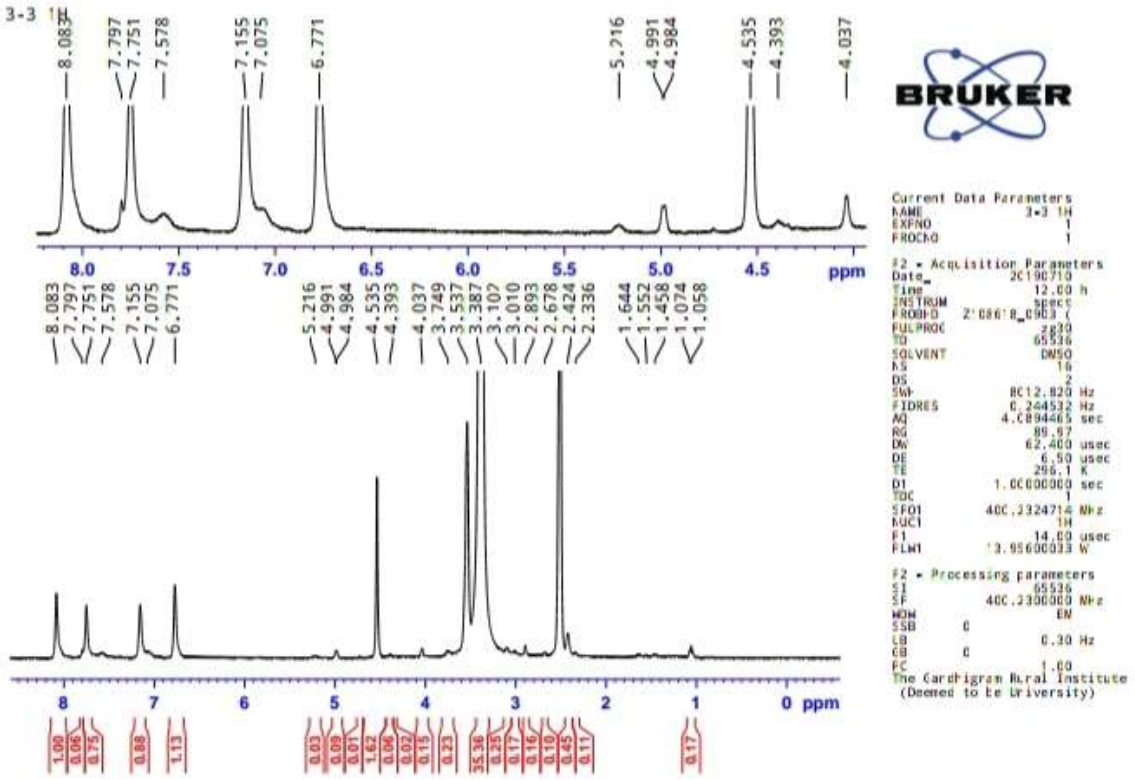

Fig 30: ^{1}H NMR spectrum of the compound TZD-33

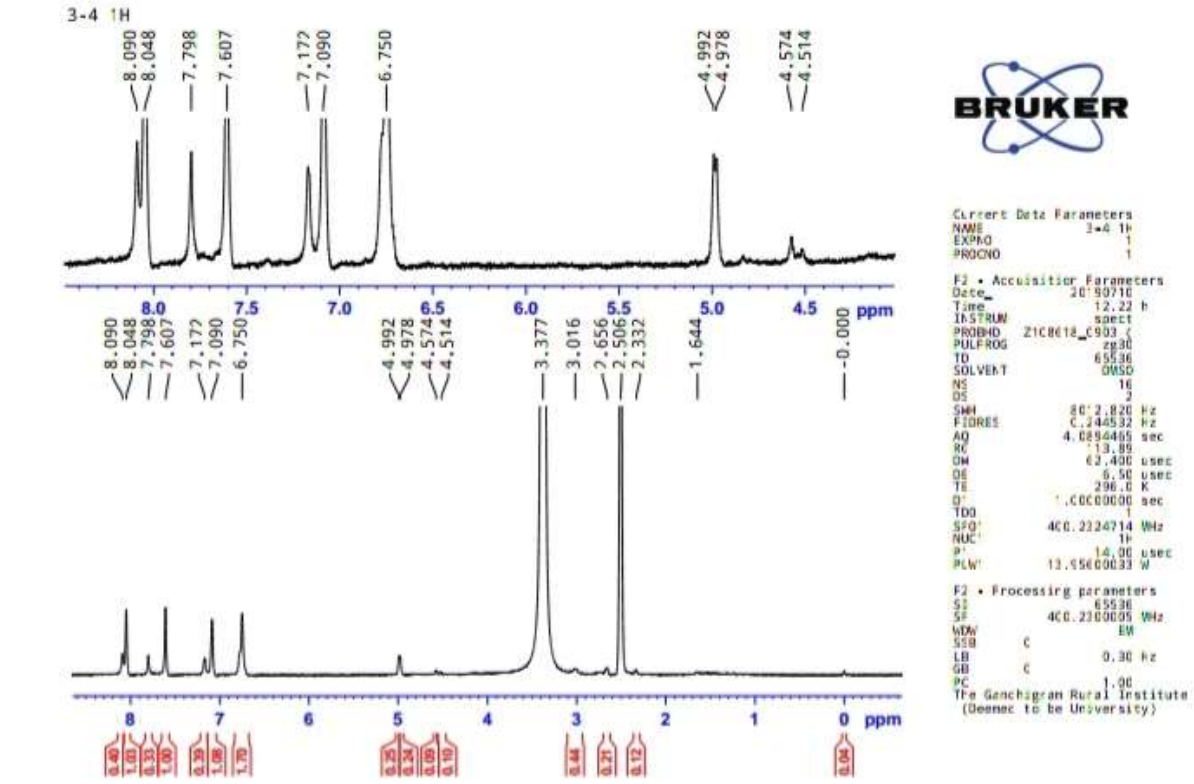

Fig31: ^{1}H NMR spectrum of the compound TZD-34

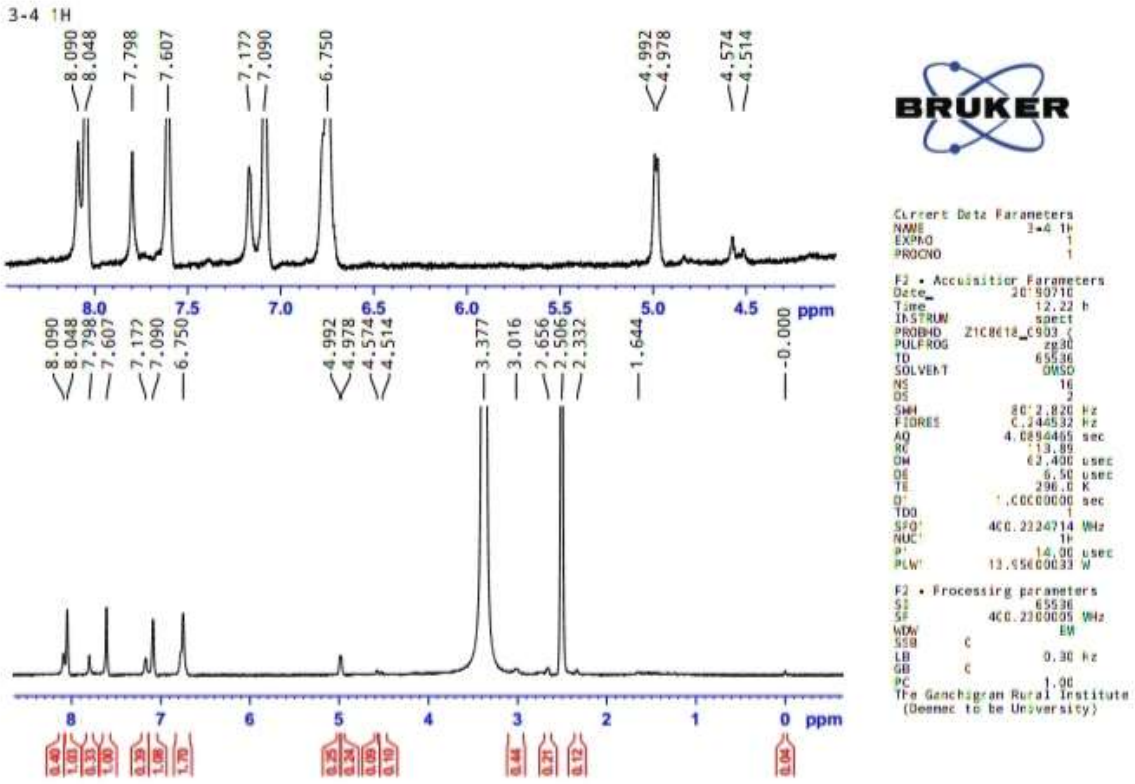

Fig 32: ^{1}H NMR spectrum of the compound TZD-35

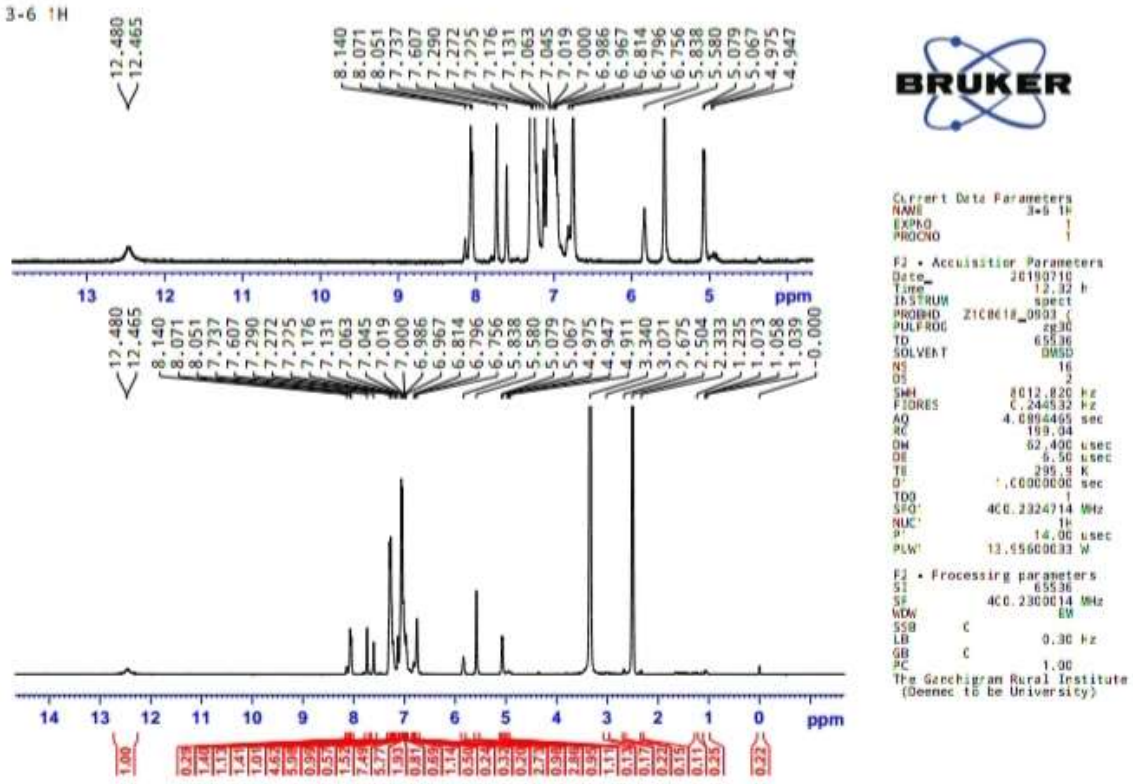

Fig33: ^{1}H NMR spectrum of the compound TZD-36

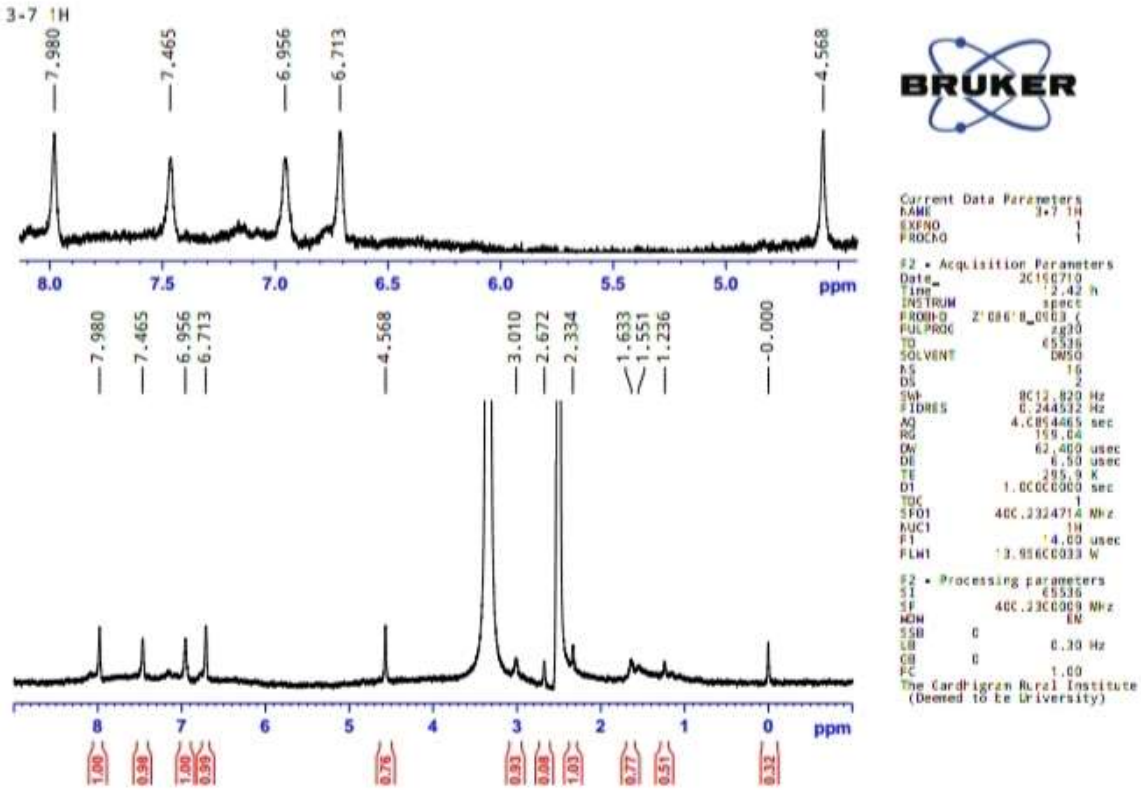

Fig 34: ^{1}H NMR spectrum of the compound TZD-37

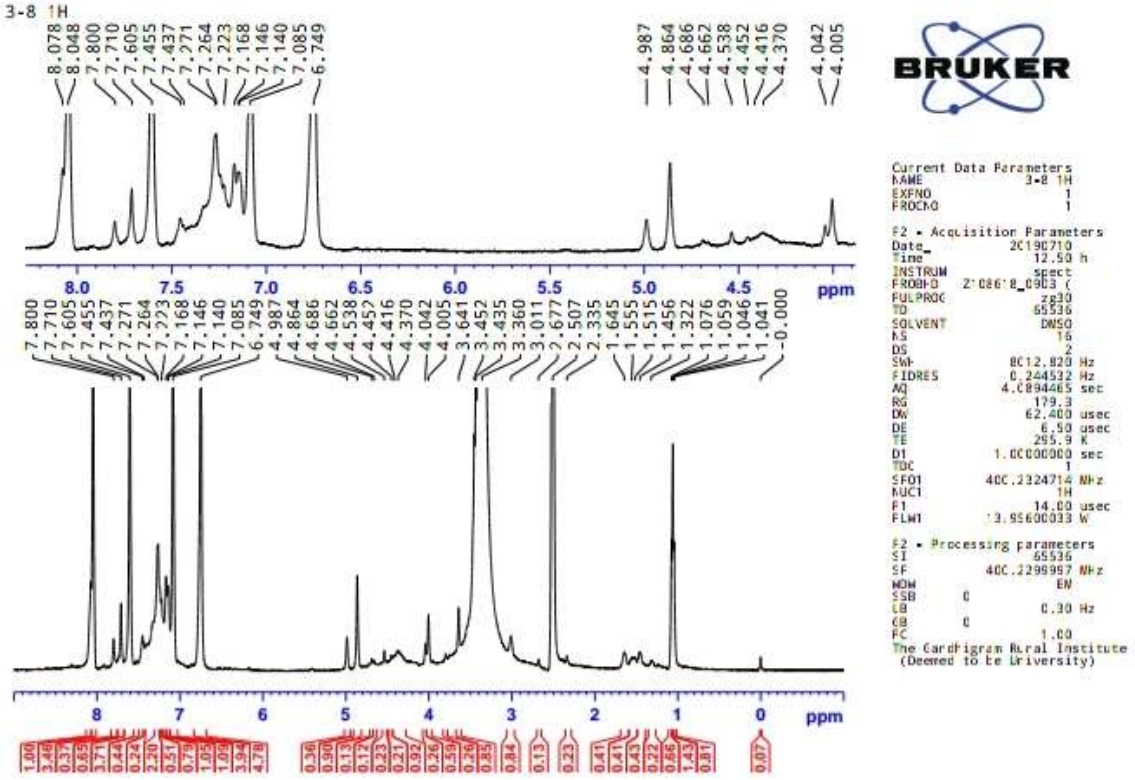

Fig 35:[1]H NMR spectrum of the compound TZD-38

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Analysis Report

<Sample Information>

Sample Name	: DPA/007/19-20		
Sample ID	: Kalasalingam_Synthetic_Samples_09_09_19		
Data Filename	: Kalasalingam_Synthetic_Samples_09_09_19.lcd		
Method Filename	: Scan method.lcm		
Vial #	: 1-31	Sample Type	: Solid (Samples)
Injection Volume	: 10 uL		
Date Acquired	: 09-09-2019 13:40:16	Acquired by	: System Administrator
Date Processed	: 09-09-2019 15:05:21	Processed by	: System Administrator

<TZD 32>

Negative

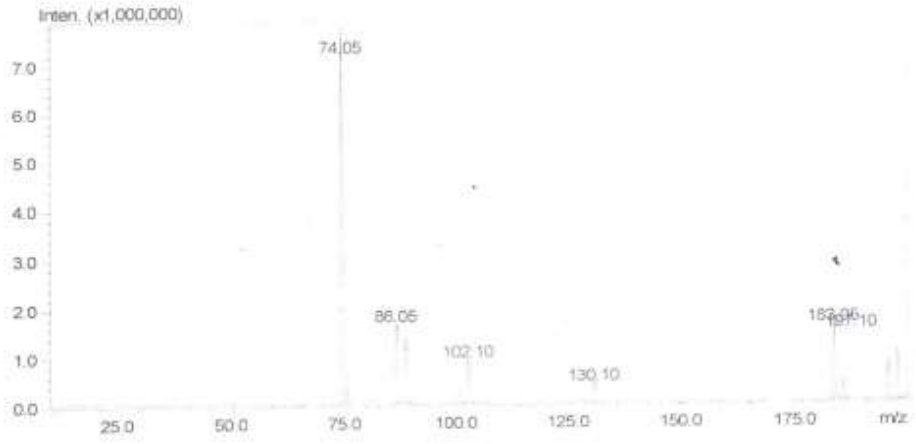

Fig36: Mass spectrum of the compound TZD-32

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Analysis Report

<Sample Information>

Sample Name	: DPA/007/19-20		
Sample ID	: Kalasalingam_Synthetic_Samples_09_09_19		
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Method Filename	: Scan method.lcm		
Vial #	: 1-31	Sample Type	: Solid (Samples)
Injection Volume	: 10 uL		
Date Acquired	: 09-09-2019 13:40:16	Acquired by	: System Administrator
Date Processed	: 09-09-2019 15:05:21	Processed by	: System Administrator

<TZD 34>

Positive

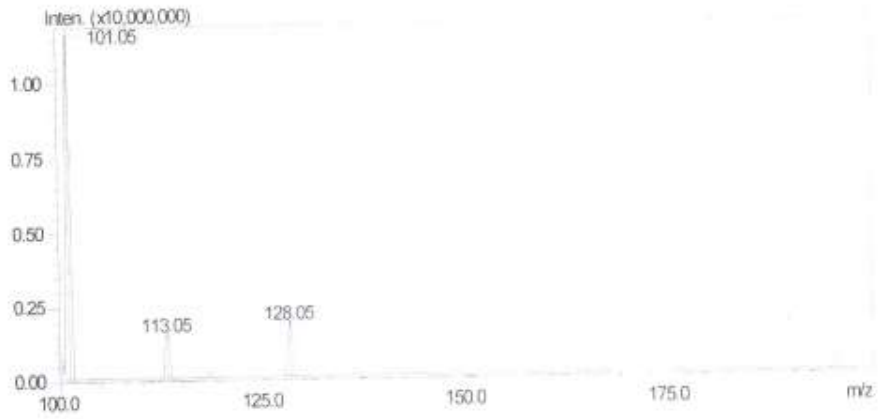

Fig37: Mass spectrum of the compound TZD-34

SHIMADZU
LabSolutions

Analysis Report

<Sample Information>

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Sample ID	: Kalasalingam_Synthetic_Samples_09_09_19		
Data Filename	: Kalasalingam_Synthetic_Samples_09_09_19.lcd		
Method Filename	: Scan method.lcm		
Vial #	: 1-31	Sample Type	: Solid (Samples)
Injection Volume	: 10 uL		
Date Acquired	: 09-09-2019 13:40:16	Acquired by	: System Administrator
Date Processed	: 09-09-2019 15:05:21	Processed by	: System Administrator

<TZD 35>

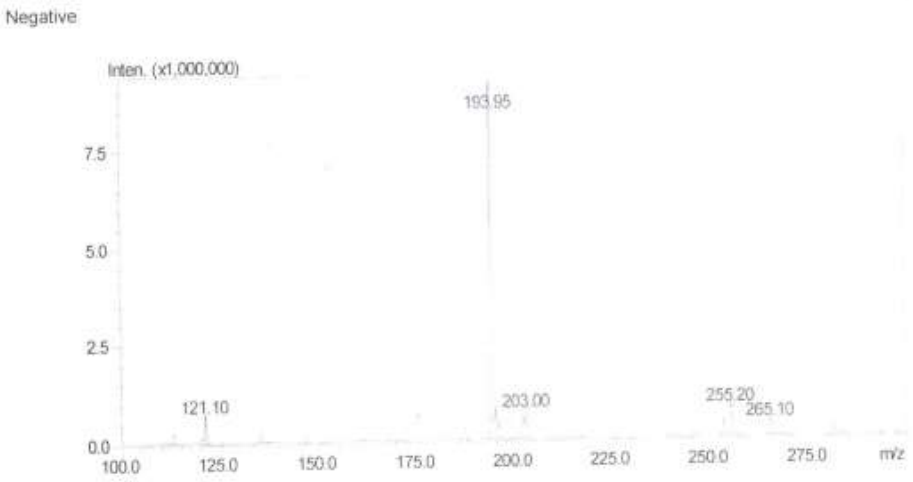

Fig 38: Mass spectrum of the compound TZD-35

SHIMADZU LabSolutions

Analysis Report

<Sample Information>

Sample Name	: DPA/007/19-20		
Sample ID	: Kalasalingam_Synthetic_Samples_09_09_19		
Data Filename	: Kalasalingam_Synthetic_Samples_09_09_19.lcd		
Method Filename	: Scan method.lcm		
Vial #	: 1-31	Sample Type	: Solid (Samples)
Injection Volume	: 10 uL		
Date Acquired	: 09-09-2019 13:40:16	Acquired by	: System Administrator
Date Processed	: 09-09-2019 15:05:21	Processed by	: System Administrator

<TZD 36>

Negative

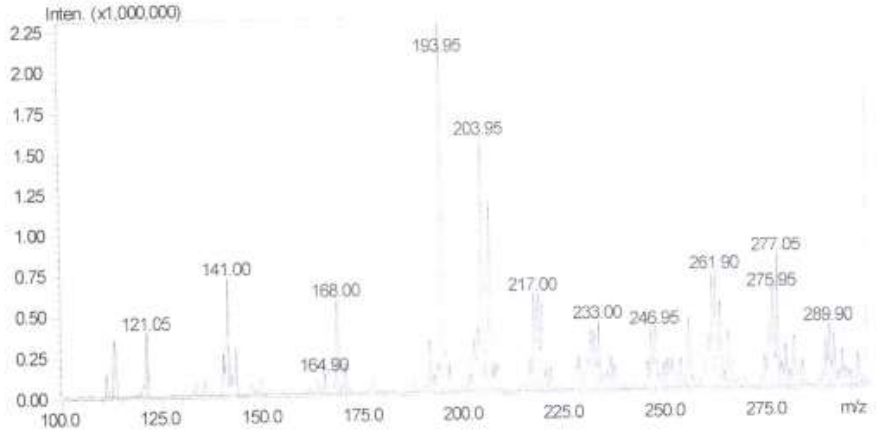

Fig39: Mass spectrum of the compound TZD-36

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Analysis Report

<Sample Information>

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Sample ID	: Kalasalingam_Synthetic_Samples_09_09_19		
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Method Filename	: Scan method.lcm		
Vial #	: 1-31	Sample Type	: Solid (Samples)
Injection Volume	: 10 uL		
Date Acquired	: 09-09-2019 13:40:16	Acquired by	: System Administrator
Date Processed	: 09-09-2019 15:05:21	Processed by	: System Administrator

<TZD 38>

Positive

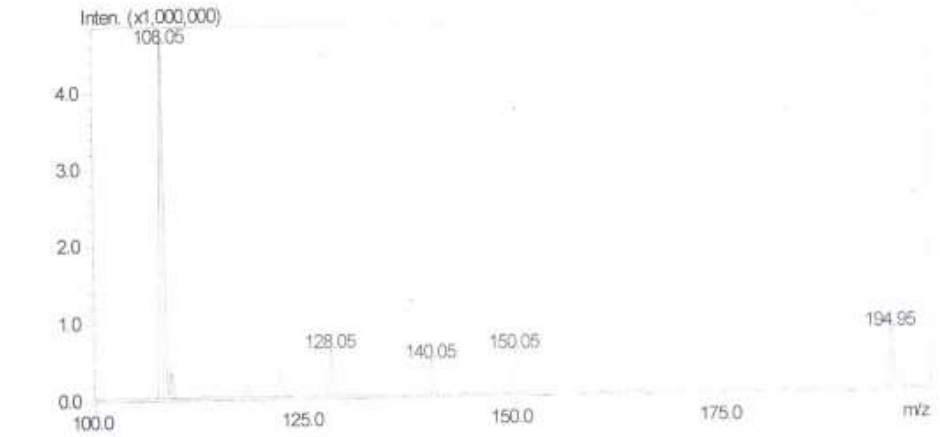

Fig: 40 Mass spectrum of the compound TZD-38