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DRUG DESIGN AND *IN SILICO* BINDING ANALYSIS OF PYRIMIDINE APPENDED THIAZOLIDINEDIONE ANALOGUES AS PPAR-γ AGONISTS

[1]*Arunlal V. B. and [2]G. Babu

[1]Research Scholar, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.
[2]Professor and Principal, Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala.

***Corresponding Author: Arunlal V. B.**
Research Scholar, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.

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ABSTRACT

Drug design is the creative process of finding new remedies based on the knowledge of a biological target. This work gave the various approaches of drug design, lead discovery, lead modification & various types of drug discovery. Drug design may have a significant role in the alteration of pharmakinetics of a lead molecule. The process of drug discovery by laboratory experiments is time consuming and very expensive as compared to computational methods. Thiazolidin-2,4-diones (TZDs) are primarily used as antidiabetic agents but also exhibit diverse therapeutic activities, such as antimicrobial, antiviral, anticancer, and antioxidant activities etc. The TZD nucleus plays a central role in the biological functioning of several essential molecules and possesses significant medicinal potential. The discovery of new TZD analogues is not easily attainable. Thiazolidinedione nucleus upon the substitution of various functional groups is provides a wide spectrum of biological activity by the use of different mechanism on different target sites. The present work attempted to explore the anticancer potential of TZDs on PPAR-γ (peroxisome proliferator-activated receptor gamma) in breast cancer. Thiazolidinediones are synthetic PPAR-γ agonists hence incorporation with pyrimidine derivatives expected the synergetic activity. Thiazolidinedione moiety has more specific binding interactions with the receptor sites but N-substituted Thiazolidine-2,4-dione derivatives (pyrimidine appended analogous) have been shown moderate interactions with receptors when compared with standard anticancer drug methotrexate. The results avail to understand the type of interactions that occur between pyrimidine linked thiazolidinediones analogues with PPAR-γ binding site region and explain the importance of various computational tools in the designing of newer molecule against breast cancer.

KEYWORDS: Thiazolidinedione, Pyrimidine, PPAR-γ, Molinspiration, ADMET, molecular docking.

INTRODUCTION

Now a day, Pharmaceutical chemists are at the front most of discovery, Structural active relationship, genomics structural study, computational chemistry, Computer aided drug design & pharmacology to innovate & develop new drugs & at the cellular level investigate their interaction. For development of novel drugs from synthetic origin many efforts are being made in the design.

Rational drug discovery

In contrast to traditional methods of drug discovery, which rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design begins with a hypothesis that modulation of a specific biological target may have therapeutic value. In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will have therapeutic value. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease states. The second is that the target is "drugable". This means that it is capable of binding to a small molecule and that its activity can be modulated by the small molecule.

Computer-aided drug design (CADD)

CADD uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when the small molecule binds to it.

Molecular Modelling

Molecular modelling describes the generation, representation and/or manipulation of 3D structure of chemical and biological molecules, along with determination of physicochemical properties that can help to interpret structural activity relationship (SAR) of the biological molecules. Many computational (*in-silico*) methods are developed and widely applied in drug design, development, and therapeutic applications. These methods have frequently been used in the discovery and optimization of novel molecules with affinity to a target. Molecular modelling is an accumulation of methods used in the fields of computational chemistry, molecular mechanics, and dynamics, drug design and docking etc. Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. Hit identification using virtual screening(structure or ligand-based design)
2. Hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)
3. Lead optimization optimization of other pharmaceutical properties while maintaining affinity

Types of Drug Designing

There are two major types of drug design.

- **Ligand-based drug design**
- **Structure-based drug design**

Ligand-based Drug Design (LBDD)

Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target. Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogous.

Structure-based Drug Design (SBDD)

Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Drug targets are most often proteins and enzymes in these pathways. SBDD uses the known 3D geometrical shape or structure of proteins to assist in the development of new drug compounds. Alternatively various automated computational procedures may be used to suggest new drug candidates.

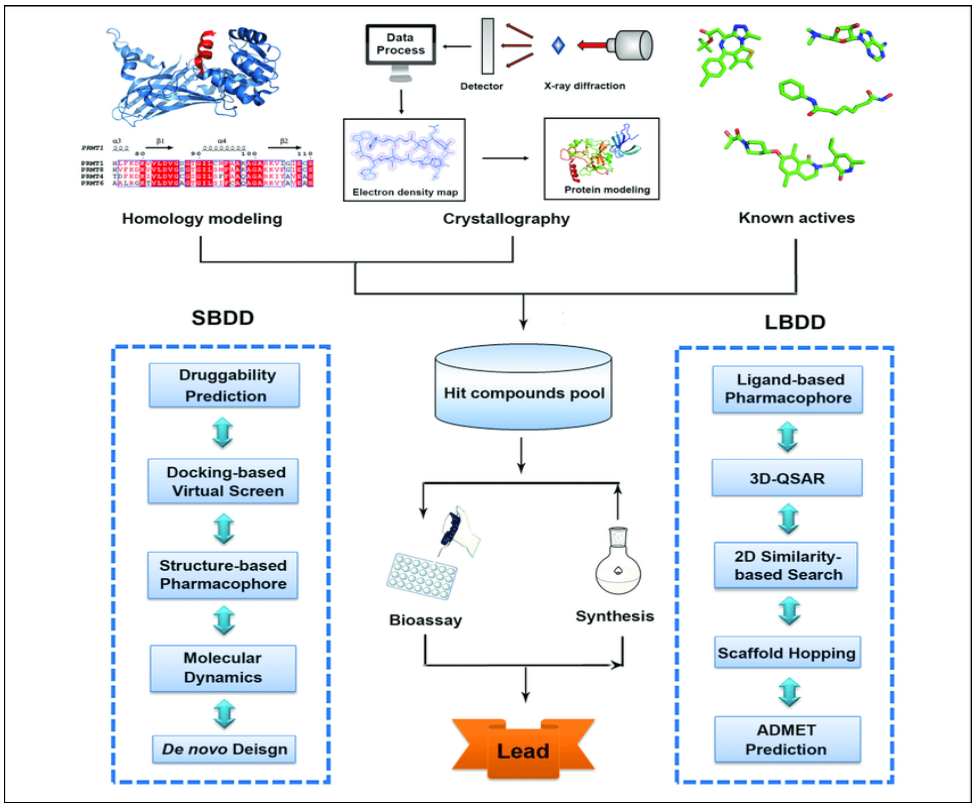


Fig. 1: Workflow of structure-based drug design and ligand-based drug design.

Docking

Molecular docking is a key tool in structural biology and computer assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use scoring function that currently ranks candidate dockings. Docking can be used to perform Virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligand inhibits the target, which is invaluable in lead optimization. Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligand which would form a complex with overall minimum energy. The small molecule, known as ligand usually fits within protein's cavity which is predicted by the search algorithm. These protein cavities become active when come in contact with any external compounds and are thus called as active sites.

The results are analyzed by a statistical scoring function which converts interacting energy into numerical values called as the docking score; and also the interacting energy is calculated. The 3D pose of the bound ligand can be visualized using different visualizing tools like Pymol, Rasmol etc. which could help in inference of the best fit of ligand.

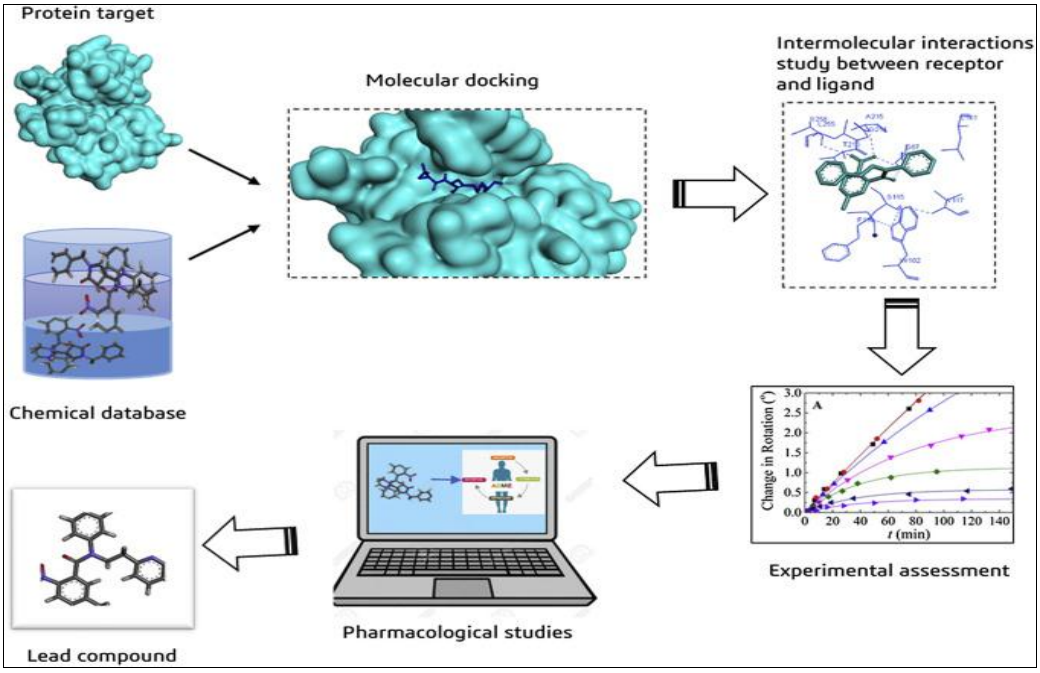
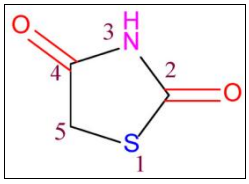


Fig. 2: Docking-Based Drug Design.

Thiazolidine-2,4-dione a versatile lead molecule



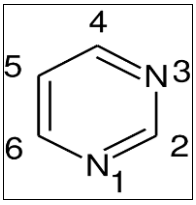
Thiazolidin-2,4-dione, also called glitazone, pentacyclic heterocyclic moiety that consists of a five-membered saturated thiazolidine ring with sulfur at 1 and nitrogen at 3, along with two carbonyl functional groups at the 2 and 4 positions. Substitutions of various moieties are possible only at the third and fifth positions of the Thiazolidin-2,4-dione (TZD) scaffold. The analogues of TZD offer a wide range of structural varieties and also possess a proven range of diversified therapeutic potentials, such as antidiabetic, analgesic, anti-inflammatory, wound healing, antiproliferative, antimalarial, antitubercular, hypolipidemic, antiviral, antimicrobial, antifungal, antioxidant properties, anticonvulsant, antiviral, anticancer, anti-inflammatory, antioxidant, anti-HIV (human immunodeficiency virus) and antituberculosis etc.

Many of the approved drugs having thiazolidinedione moiety are available in commercial market as anti-hyperglycemic active drugs, some of them are ciglitazone, troglitazone, pioglitazone, englitazone, darglitazone, rosiglitazone etc.

TZDs produce their biological response by stimulating the PPARγ receptor. PPARs are a subfamily of transducer proteins that act as transcriptional factors, belonging to the nuclear superfamily of retinoic acid receptors (RARs)/steroid receptors/thyroid hormone receptors (TRs), which are involved in different processes and also help in the regulation and expression of various genes that are vital for glucose and lipid metabolism. There are three different types of PPARs have been identified, i.e., PPAR-α, PPAR-β, and PPAR-γ, which are encoded by different genes.

TZD ring has been used as a scaffold to develop this novel class of anticancer agents, encouraged by the literature reported that. TZDs are designed for the treatment of human cancers expressing high levels of PPAR-γ because it is assumed that activation of PPAR-γ mediates their anticancer activity. The thiazolidine-2,4-diones derivatives showing anticancer activity are mainly derivatives modified in the position 5 of thiazolidine-2,4-dione ring.

Pyrimidine



Pyrimidine is a heterocyclic aromatic organic compound containing two nitrogen atoms at positions 1 and 3 of the six member ring shows wide range of biological activities. Pyrimidine posses wide spectrum of biological activities like including anti-tubercular, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory**,** Anti-malarial activity, anti-cancer and anti-neoplastic activity, anti-HIV activity etc. Nitrogen containing heterocyclic ring such as pyrimidine is a promising structural moiety for drug design.

In medicinal chemistry pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity. The literature indicated that the compounds having pyrimidine nucleus possess broad range of biological activities. Like 5-fluorouracil as anticancer; idoxuridine and trifluoridine as antiviral; zidovudine and stavudine as anti-HIV, trimethoprim, sulphamethiazine and sulphadiazine as antibacterial; sulphadoxin as antimalarial and antibacterial; minoxidil and prazosin as antihypertensive; barbiturates e.g. phenobarbitone as sedative, hypnotics and anticonvulsant; propylthiouracil as antihyroid; thionzylamine as H1-antihistamine; and toxoflavin and fervennuline as antibiotics.

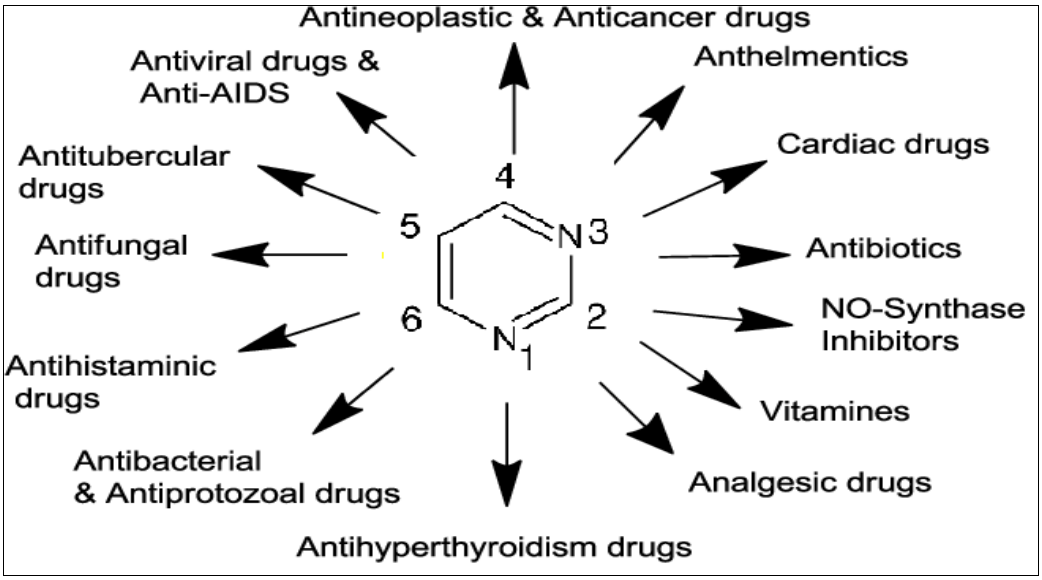


Fig. 3: Medicinal significances of Pyrimidine.

Breast Cancer

Breast cancer happens when cells in your breast grow and divide in an uncontrolled way, creating a mass of tissue called a tumor. Signs of breast cancer can include feeling a lump in your breast, experiencing a change in the size of your breast and seeing changes to the skin on your breasts. Breast cancer is one of the most frequent malignancies in females, with one woman in eight or nine developing breast cancer in her life.

Breast cancer cells often express prominent levels of PPAR-γ (Peroxisome proliferators-activated receptors). Peroxisome proliferators-activated receptors are a class of ligand-dependent nuclear receptor transcription factors discovered in 1990 that can regulate the transcription of many genes involved in various cellular physiological processes. The dysregulation of these physiological processes is highly correlated with the occurrence of various diseases, including malignant tumors. Additionally, a large number of reports have indicated that the transcriptional regulation function of peroxisome proliferator-activated receptors and its abnormal expression are related to breast cancer.

A couple of studies have shown that TZDs can inhibit proliferation and induce differentiation-like changes in breast cancer cell lines both in vitro and growing in nude mice. Additional studies have found that the combination of moiety with a PPAR-γ ligand synergistically inhibited the growth and induced apoptosis of a series of breast

cancer cell lines both in vitro and in engrafts growing in nude mice. In vivo experiments have shown that administration of a PPAR-γ ligand inhibited the development of carcinogen-induced breast cancer in rats. The overall role PPAR-γ plays in breast cancer is somewhat murky. In vitro and in vivo data show that PPAR-γ ligands can suppress breast cancer growth; and this can be enhanced by retinoids.

MATERIALS AND METHOD

Computational platform used: All the computational procedures of this work have been carried out with Intel CORE i5 processor on windows 10.0. All the docking studies were carried out on the server LP CORE i5 processor (32GB RAM) and modem used was JioWifi.

Softwares Used: Molinspiration Cheminformatics online tool, PreADMET online tool, ACD Chemsketch 17.0, Chemdraw 17.0, Chem 3D 17.0, Glide Maestro 12.5 Schrödinger 2020-3 software etc.

Designing of proposed analogues

There are different drugs are available in market with thiazolidinedione ring moiety and mainly used as anti-diabetic agent. Examples as follows.

Literature shows that, Thiazolidine2,4-dione ring play an important role in many dysfunctions of body like diabetes, epilepsy, hypertension, retroviral diseases, cancer etc. It was clearly proved that presence of heterocyclic ring at target molecule would increases the potency of the molecule weather it is at binder site or effecter site. So planned to incorporate the heterocyclic rings like pyrimidine in the thiazolidine 2,4-dione by without the lost of active sites. For the above purpose we studied the marketed antidiabetic drugs and their designing of proposed analogues is represented in the flowchart.

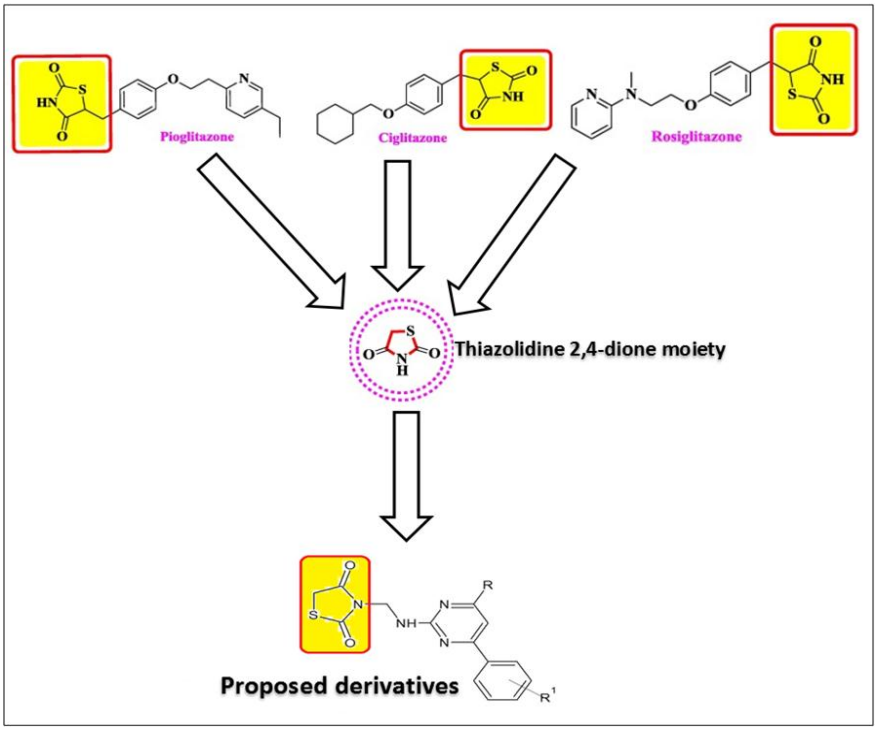


Fig. 4: Reported thiazolidinedione containing drugs along with proposed pyrimidine linked Thiazolidinedione anticancer molecule.

SOFTWARE METHODOLOGY

A) Molinspiration Cheminformatics

Molinspiration supports internet chemistry community by offering free on-line services for calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors). Lipinski's rule of five (RO5) is used to evaluate whether the chemical compound possess the properties of drug likeness (i.e. orally active) in humans.

B) *IN-SILICO* ADMET STUDIES

In silico approaches are being used today in drug discovery to assess the ADME (Absorption, Distribution, Metabolism and Excretion) and toxicity properties of compounds at the early stages of discovery/development. ADME properties are also increasingly urgent because of the implementation of combinatorial chemistry and high-throughput screening, since this can generate vast numbers of potential lead compounds. The Pre-ADMET program was accessed at http://preadmet.bmdrc.org/.

C) CHEMSKETCH

ACD ChemSketch software (Developed by Advanced Chemistry Development, Canada.) is a chemically intelligent molecular structure drawing application, which helps us to, Draw chemical structures, reactions, and scheme, and access a variety of graphical tools and templates; generate names from molecular structures; calculate molecular properties from chemical structure; generate SMILE notation, SMILE notation to structure etc.

D) DOCKING METHODOLOGY

Docking is a term that covers a large class of computer algorithms that attempt to find an optimal placement of a rigid or flexible ligand in the receptor binding site. The ligands is typically a small molecule; peptide-protein and protein-protein docking algorithms are currently under active development. Docking algorithms also generate a score that attempts to distinguish between molecules that bind strongly in their optimal placement from these that bind weakly. In this tutorial, you will learn to use the program Glide, which is developed and sold by Schrodinger, LLC.

The main software for docking is called Glide. 3 levels of precisions are available: HTVS (High Throughput Virtual Screening), SP (Standard Precision), XP (Extra Precision). During the docking (which performs "a systematic search of the conformational, orientational, and positional space of the docked ligand"), a score is calculated which is similar to a binding free energy in kcal/mol. The three precisions differ by the scoring function and also by how they dock the ligands. The first one takes less than 1 second per structure, the second one around 30 seconds per structure and the last one roughly 5 minutes.

For docking, two files are needed: a protein grid and a ligand library. Tools are provided by Schrödinger Inc. for preparing both. One important tool in the Schrödinger suite is Maestro. It can be downloaded from http://www.schrodinger.com/freemaestro/ and is also useful for post-processing. However, for preparing the ligands or the protein other softwares from Schrödinger Inc. are needed, and they are only installed on Odyssey since they are commercial.

Docking of the ligand that was originally present in the crystal structure to the same protein is one of the easiest docking tasks. Many docking programs correctly identify a ligand pose very similar to the experimentally observed pose as one with one with the highest score. The success of such re-docking does not always indicate that the docking of novel compounds will be reliable with the same program. First, comparison of novel compounds involves scoring of these compounds against each other; this step is not needed for docking a single compound. Second, the shape of the binding pocket during docking of novel compounds is usually not allowed to change to accommodate the novel ligand, and thus ligands larger than the original ligand may not fit into the pocket according to the docking program. In reality, proteins are flexible, and it is likely that the active site will change slightly to accommodate slightly larger ligands that otherwise make good contacts with the receptor. You can prove this by trying to dock known HIV reverse transcriptase inhibitors.

The molecular docking simulations were performed to analyze the binding interactions of the proposed structures using the Glide Maestro 12.5 Schrödinger 2020-3 software.

Schrödinger protocol

X-ray crystal structures of proteins were retrieved from Protein Data Bank server (www.pdb.org). In the maestro 12.5 program; the protein preparation was carried out using 'Protein Preparation Wizard'. In the protein preparation stage, water molecules and other structure in the protein were deleted and hydrogen atoms were added. The energy minimization of structure was done by using the force field OPLS3e. The potential binding sites in the receptor was identified by using Site Map and the best binding site identified based on Site Score and D Score. Ligands were prepared with 'LigPrep' using OPLS3e force field. 'Receptor Grid Generation' creation module is run by clicking on any atom of the ligand and default grid box is prepared. Glide XP Docking was performed between ligands and the grid box of the protein. Results were evaluated according to XP GScore.

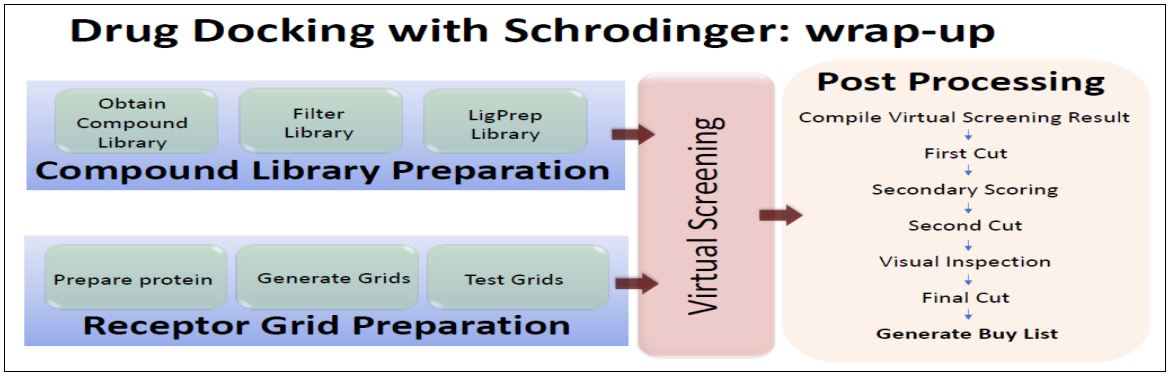
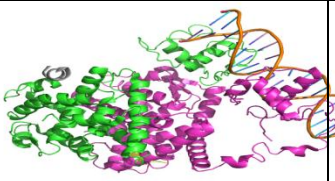


Fig. 5: Steps involved in the ligand docking with Schrodinger.

Target Identification and Retrieval
Crystallographic structures of the targets of interest were obtained from PDB.

Table 1: Protein target and PDB ID.

Target	PDB ID
 Helical structure of PPAR-γ	2PRG

1. Preparation of protein (Protein preparation wizard)
Crystallographic structures of proteins of interest were retrieved from RCSB protein data bank as a PDB file. Typical PDB structure is not suitable for immediate use.

Using Schrödinger's Protein Preparation Wizard, we can convert a raw PDB structure into all-atom, fully prepared protein models in minutes instead of hours or days, while also ensuring the accuracy of all downstream modeling simulations. The Protein Preparation Wizard enables this increased efficiency in structure preparation by including tools.

2. Preparation of ligands (LigPrep)
The preparation of the co-crystallized ligand from the 1FJS structure for used in virtual screening. This is a typical step for cognate ligand docking, as it provides important validation prior to screening a larger ligand data set. The following steps provide an example of how you would prepare a ligand data set using LigPrep. Ligand files can be sourced from numerous places, such as vendors or databases, often in the form of 1D or 2D structures with unstandardized chemistry. Before being used in a virtual screen, ligands must be converted to 3D structures, with their chemistry properly standardized and extrapolated.

3. Visualizing Protein-Ligand Complexes
Presets can be used in a variety of ways, from de-cluttering your structure to creating publication-quality images. We will analyze the protein-ligand complex by looking at the interactions that are made and the surface of the binding pocket. Finally, we will save an image of the complex.

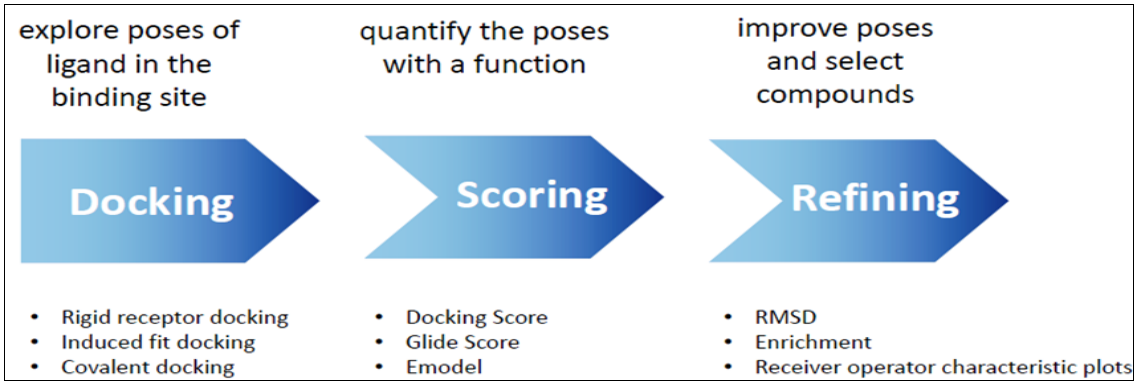


Fig. 6: Steps of structure based virtual screening.

First way to search for best binding pose for the prepared ligand will carried out with the help of Glide. Glide docking grid will help to specify where to dock the grid. Ligand is flexible but receptor shown as rigid. Glide provides accurate and rational workflow of virtual screening from the HTVS (high-throughput virtual screening) mode for efficiently enriching million compound libraries, to the SP (standard precision) mode for reliably docking tens to hundreds of thousands of ligand with high accuracy, to the XP (extra precision) mode where further false positives are eliminated. Glide reliably finds the correct binding modes and exhibits excellent docking accuracy.

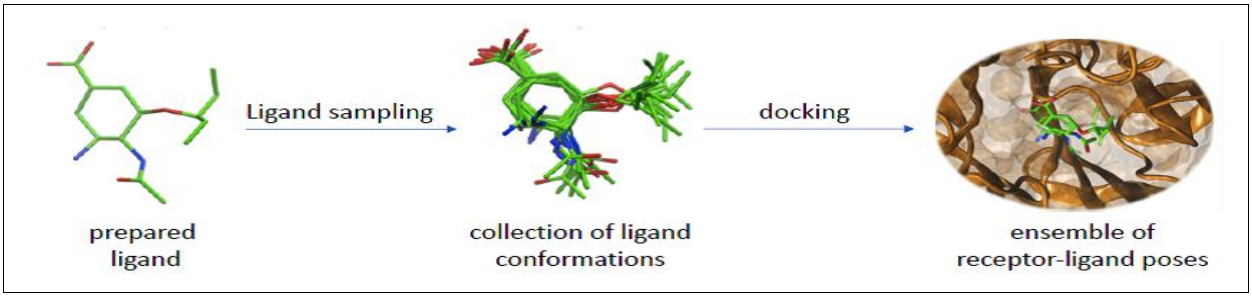


Fig. 7: Schematic representation of Docking poses receptor and ligand.

Scoring

Binding affinity of the compounds was demonstrated in terms of binding energy, calculated in term of negative energy. Binding affinity is more when binding energy is less. Docking scores were shown as numerical value of interaction energy which is statistical evaluation function for displaying the results. Different visualization tools were used to visualize the 3D pose of the ligand interaction with receptor.

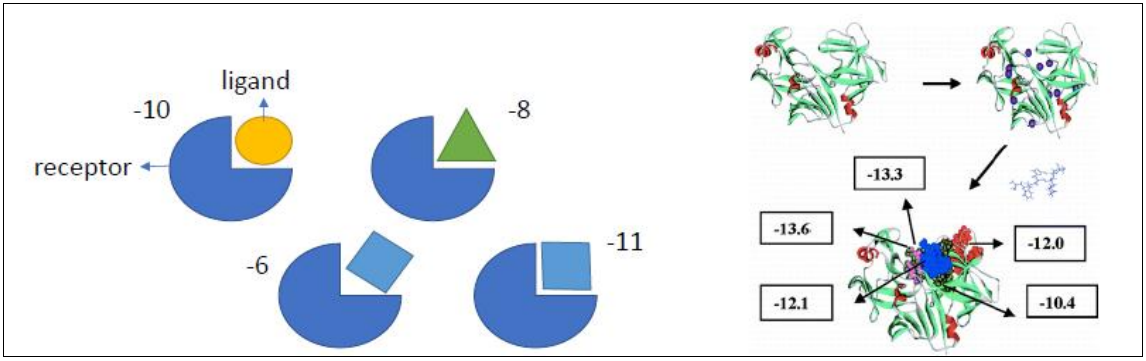


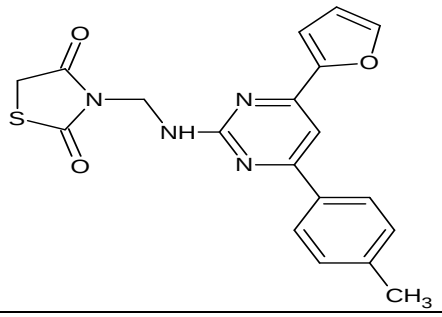
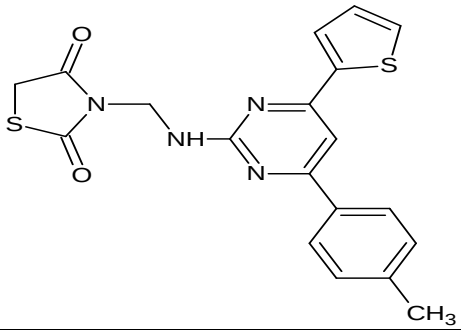
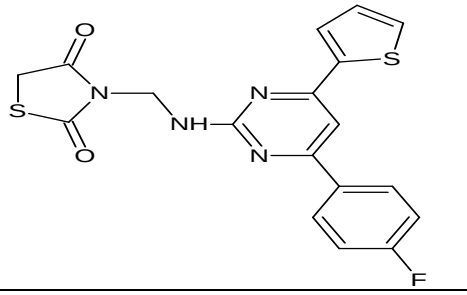
Fig. 8: Binding modes/poses illustration and scoring function.

RESULTS AND DISCUSSION

Result and Discussion of *In-Silico* Molecular Studies

By using different computational tools 52 Pyrimidine linked thiazolidine 2,4–dione derivatives were screened and only 3 compounds selected for further studies.

Table 2: List of selected compound's structures and its name.

SI. NO	Compound code	Structure	Name
01	TZPY 2		3-[(E)-{[4-(furan-2-yl)-6-(4-methylphenyl) pyrimidin-2-yl]imino}methyl]-1,3-thiazolidine-2,4-dione
02	TZPY 28		3-[(*E*)-{[4-(4-methylphenyl)-6-(thiophen-2-yl) pyrimidin-2-yl]imino}methyl]-1,3-thiazolidine-2,4-dione
03	TZPY 45		3-[(*E*)-{[4-(4-fluorophenyl)-6-(thiophen-2-yl) pyrimidin-2-yl]imino}methyl]-1,3-thiazolidine-2,4-dione

A) Molinspiration

By using Molinspiration online tool (https://www.molinspiration.com/cgi-bin/properties) the drug likeness properties of Pyrimidine linked thiazolidine 2,4–dione derivatives were determined.

Table 3: Lipinski rule analysis of selected proposed derivatives by Molinspiration.

Comp. code	Factors of Lipinski rule of 5					
	log P (<5)	MW (<500)	H bond donor (<5)	H bond acceptor (<10)	No. of rotatable bonds (<10)	No. of Violations
TZPY 2	3.35	380.42	1	7	5	0
TZPY 28	4.00	396.48	1	6	5	0
TZPY 45	3.71	400.44	1	6	5	0

B) Pre ADMET

We have predicted the ADMET (Absorption, Distribution, Metabolism, Elimination and Toxicity) properties of the 52 designed compounds to assess the drug-likeness and pharmacokinetic properties. The PreADMET online tool was used for the evaluation of some important absorption, distribution, metabolism, elimination and toxicity (ADMET) parameters and selected compounds and its permissible rangers are listed below.

Table 4: Predicted ADMET parameters of selected compounds by PreADMET software.

Compound code	ADMET Properties				
	Human intestinal absorption	In-vitro PPB	BBB	In-vitro CaCO2 cell permeability	Carcinogenicity
TZPY 2	97.852250	86.290241	0.509897	23.3376	NO Risk
TZPY 28	98.343187	89.083595	0.641335	27.3869	NO Risk
TZPY 45	98.177196	90.214759	0.969443	22.3408	NO Risk

Proposed derivative's physicochemical properties like LogP, molecular weight, hydrogen bond donors, hydrogen bond acceptors, rotating bond etc. were compared and studied. Predicted drug likeness and pharmacokinetic properties is essential molecular descriptors in rational drug design. All the proposed compounds which were not violated the Lipinski rule of five; those were selected for further study. All these pharmacokinetic studies help us to achieve a good pharmacokinetic profile of the newer analogues. Pharmacokinetic studies used to ensure to evaluate the acceptability of the proposed analogues.

C) ACD Chemsketch

ACD ChemSketch helped us to draw chemical structures, and to view them as 2D two dimensional and three dimensional (3D) models. Chemsketch helped to name designed proposed molecules. SMILES helped to describe a chemical structure in simple text characters.

Table 5: SMILES notation of selected compounds by Chemsketch.

COMPOUND CODE	SMILE NOTATION
TZPY 2	O=C1CSC(=O)N1CNc1nc(cc(n1)c1ccco1)c1ccc(C)cc1
TZPY 28	O=C1CSC(=O)N1CNc1nc(cc(n1)c1cccs1)c1ccc(C)cc1
TZPY 45	O=C1CSC(=O)N1CNc1nc(cc(n1)c1cccs1)c1ccc(F)cc1

D) Docking Studies

As observed from the literature review that the thiazolidine 2,4–dione analogues have good anticancer activity in breast cancer. PPAR-γ found in many region of the body especially in breast cells. The activation of PPAR-γ may produce an effective action in breast cancer treatment. Heterocyclic moiety like Pyrimidine was designed to incorporated with thiazolidine 2,4–dione. The designed compounds were carried out the docking studies by using the docking software Schrodinger. Total 52 compounds were predicted for docking and studied the interactions between the receptors and the designed ligands. So, with the help of docking studies, the best interactions given 3 compounds were selected for further studies.

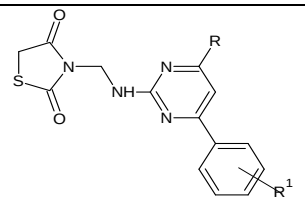
Result and Discussion of Docking Studies

The protein (PPAR-γ 2PRG) was first prepared using the Protein Preparation wizard and the docking studies were performed using the Schrédinger Glide software Maestro with extra-precision (XP) mode. The best fitted conformations of the designed structure of 3D pose were docked with the receptor protein. The interaction pose of 2D and 3D visualization of designed analogues were observed.

Molecular docking study revealed that the designed Pyrimidine incorporated with thiazolidine 2,4–dione derivatives exhibited moderate interaction with crucial amino acids of receptor protein. The best-fitted compounds **TZPY 2, TZPY 28** and **TZPY 45** showed

the docking scores of **-8.13, -8.13** and **-8.21,** respectively in comparison to standard drug Ciglitazone (marketed TZD containing drug, docking score -11.30) and Methotrexate (marketed Anticancer drug, docking score-11.42). Docking score of Pyrimidine linked Thiazolidine-2, 4-dione proposed derivatives (52 derivatives) on PPAR-γ (2PRG) are given below.

Table 6: Docking score of Pyrimidine linked Thiazolidine-2, 4-dione proposed derivatives.



SI. NO.	Comp. code	R (Aldehyde derivatives)	R^1 (Acetophenone derivatives)	Docking score (kcal/mol)
1	TZPY 1	Furfuraldehyde	Acetophenone	-7.15
2	**TZPY 2**	Furfuraldehyde	4-methyl acetophenone	**-8.13**
3	TZPY 3	Furfuraldehyde	3-methyl acetophenone	-6.54
4	TZPY 4	Furfuraldehyde	2-methyl acetophenone	-7.70
5	TZPY 5	Furfuraldehyde	2-Bromo 4-methyl acetophenone	-7.67
6	TZPY 6	Furfuraldehyde	4-nitro acetophenone	-6.62
7	TZPY 7	Furfuraldehyde	3-nitro acetophenone	-7.22
8	TZPY 8	Furfuraldehyde	2-nitro acetophenone	-6.72
9	TZPY 9	Furfuraldehyde	4-amino acetophenone	-4.99
10	TZPY 10	Furfuraldehyde	3-amino acetophenone	-6.59
11	TZPY 11	Furfuraldehyde	2-amino acetophenone	-6.88
12	TZPY 12	Furfuraldehyde	4-amino 3,5-dichloro acetophenone	-7.01
13	TZPY 13	Furfuraldehyde	4-methoxy acetophenone	-7.65
14	TZPY 14	Furfuraldehyde	4-hydroxy acetophenone	-7.33
15	TZPY 15	Furfuraldehyde	3-hydroxy acetophenone	7.38
16	TZPY 16	Furfuraldehyde	2-hydroxy acetophenone	-6.84
17	TZPY 17	Furfuraldehyde	3-chloro 4-hydroxy acetophenone	-6.94
18	TZPY 18	Furfuraldehyde	4-hydroxy 3-nitro acetophenone	-7.25
19	TZPY 19	Furfuraldehyde	4-fluoro acetophenone	-7.78
20	TZPY 20	Furfuraldehyde	4-bromo acetophenone	-7.53
21	TZPY 21	Furfuraldehyde	4-chloro acetophenone	-8.11
22	TZPY 22	Furfuraldehyde	4-iodo acetophenone	-7.77
23	TZPY 23	Furfuraldehyde	4-acetoxy acetophenone	-7.23
24	TZPY 24	Furfuraldehyde	4-phenyl acetophenone	-7.28
25	TZPY 25	Furfuraldehyde	4-methyl thioacetophenone	-7.46
26	TZPY 26	Furfuraldehyde	4-ethyl acetophenone	-7.87
27	TZPY 27	Thiophene-2- carbaldehyde	Acetophenone	-7.43
28	**TZPY 28**	Thiophene-2- carbaldehyde	4-methyl acetophenone	**-8.13**
29	TZPY 29	Thiophene-2- carbaldehyde	3-methyl acetophenone	-5.05
30	TZPY 30	Thiophene-2- carbaldehyde	2-methyl acetophenone	-5.29
31	TZPY 31	Thiophene-2- carbaldehyde	2-Bromo 4-methyl acetophenone	-7.88
32	TZPY 32	Thiophene-2- carbaldehyde	4-nitro acetophenone	-6.99
33	TZPY 33	Thiophene-2- carbaldehyde	3-nitro acetophenone	-7.77
34	TZPY 34	Thiophene-2- carbaldehyde	2-nitro acetophenone	-6.97
35	TZPY 35	Thiophene-2- carbaldehyde	4-amino acetophenone	-7.09
36	TZPY 36	Thiophene-2- carbaldehyde	3-amino acetophenone	-6.95
37	TZPY 37	Thiophene-2- carbaldehyde	2-amino acetophenone	-7.36
38	TZPY 38	Thiophene-2- carbaldehyde	4-amino 3,5-dichloro acetophenone	-7.72
39	TZPY 39	Thiophene-2- carbaldehyde	4-methoxy acetophenone	-6.03
40	TZPY 40	Thiophene-2- carbaldehyde	4-hydroxy acetophenone	-7.77
41	TZPY 41	Thiophene-2- carbaldehyde	3-hydroxy acetophenone	-6.18
42	TZPY 42	Thiophene-2- carbaldehyde	2-hydroxy acetophenone	-7.59
43	TZPY 43	Thiophene-2- carbaldehyde	3-chloro 4-hydroxy acetophenone	-7.51

44	TZPY 44	Thiophene-2- carbaldehyde	4-hydroxy 3-nitro acetophenone	-6.94
45	**TZPY 45**	Thiophene-2- carbaldehyde	4-fluoro acetophenone	**-8.21**
46	TZPY 46	Thiophene-2- carbaldehyde	4-bromo acetophenone	-6.36
47	TZPY 47	Thiophene-2- carbaldehyde	4-chloro acetophenone	-7.50
48	TZPY 48	Thiophene-2- carbaldehyde	4-iodo acetophenone	-6.86
49	TZPY 49	Thiophene-2- carbaldehyde	4-acetoxy acetophenone	-7.36
50	TZPY 50	Thiophene-2- carbaldehyde	4-phenyl acetophenone	-6.98
51	TZPY 51	Thiophene-2- carbaldehyde	4-methyl thioacetophenone	-6.26
52	TZPY 52	Thiophene-2- carbaldehyde	4-ethyl Acetophenone	-7.48

Mainly there types of interactions were shown in the docking studies, they are H bonding interactions, hydrophobic interactions and polar interactions. All these interactions are essential factors for the proper binding of ligands to its active pockets.

❖ **H Bonding interactions**

2D diagram of each thiazolidine-2,4–dione analogues were obtained and identified the interacting amino acid residues. All the amino acid residues which were interacted with proposed derivatives were enlisted below with its code, ALA: Alanine, ARG: Arginine, GLN: Glutamine, GLU: Glutamic acid, GLY: Glycine, ILE: Isoleucine, LEU:Leucine, MET: Methionine, PHE: Phenylalanine, SER: Serine, THR: Threonine, TRP: Tryptophan, TYR: Tyrosine, VAL: Valine, CYS: Cysteine and HIE: Histamine in protonated form.

Table 7: Docking score and interacting amino acid residues of proposed derivatives against PPAR-γ.

Compound code	Docking score (kcal/mol)	Interactingamino acid residues in PPAR-γ (2PRG)
TZPY 2	-8.13	LEU330, LEU333, MET329, TYR327, ILE341, HIE323, ALA292, SER289, ARG288, LEU469, HIE449, GLY286, LEU469, CYS285, PHE282, ILE281, PHE326, MET348, VAL 339, ILE341 **(22 amino acids)**
TZPY 28	-8.13	LEU330, LEU333, MET329, TYR327, ILE341, HIE323, ALA292, SER289, ARG288, GLY286, CYS285, TYR475, LEU469, LEU449, PHE282,PHE363, MET364, MET348, VAL339, ILE341, LEU353 **(22 amino acids)**
TZPY 45	-8.21	IEU353, VAL339, MET348, ILE341, LEU330, LEU333, MET329, TYR327, ILE326, HIE323, ALA292, SER289, ARG288, GLN286, CYS285, PHE282, TYR475, LEU449 , LEU453, HIE449, PHE363, MET364 **(22 amino acids)**
Ciglitazone (Marketed TZD containing drug)	-11.30	MET334, LEU333, MET348, LEU353, LEU330, TYR327, ILE469, TYR473, HIE323, SER289, ARG288, HIE449, LEU453, GLN286, CYS285, PHE282, PHE363, MET364, ILE281, VAL339, LEU340, ILE341, SER342 **(24 amino acids)**
Methotrexate (Marketed anticancer drug)	-11.42	ARG280,ILE281,PHE282,GLY284, TYR473, CYS285, GLN286, ARG288, SER289, LEU453, LEU465, LEU469, HIE449, HIE323, LYS367, ILE326, TYR327, MET364, PHE363, LEU330, LEU333, MET348, LEU353, VAL339, LEU340, ILE341, SER342 **(27 amino acids)**

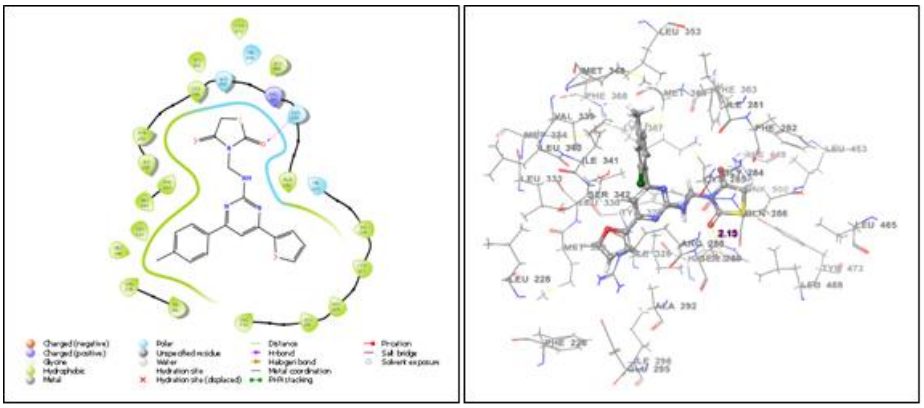


Fig. 9: Best affinity mode and H bond interactions of Compound PZPY2.

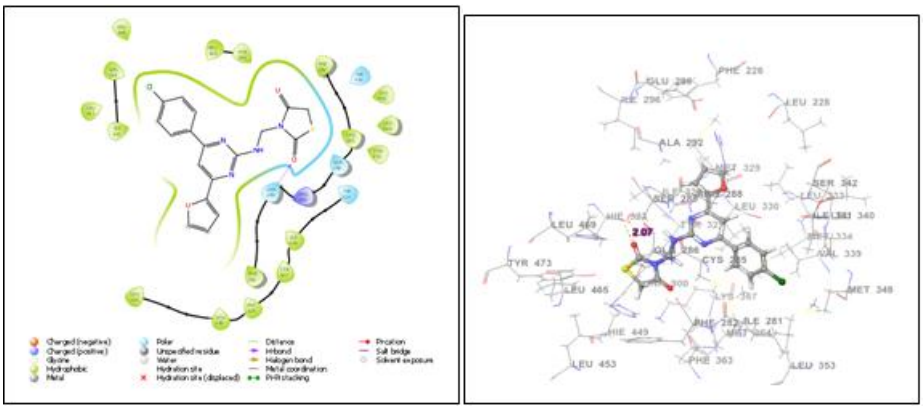


Fig. 10: Best affinity mode and H bond interactions of Compound PZPY28.

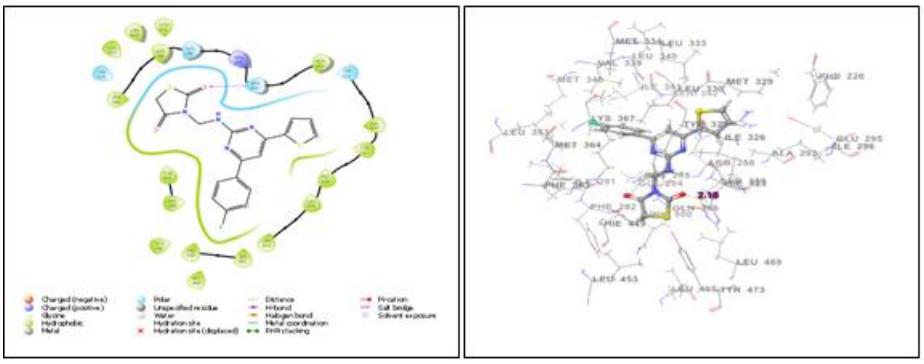


Fig. 11: Best affinity mode and H bond interactions of Compound PZPY45.

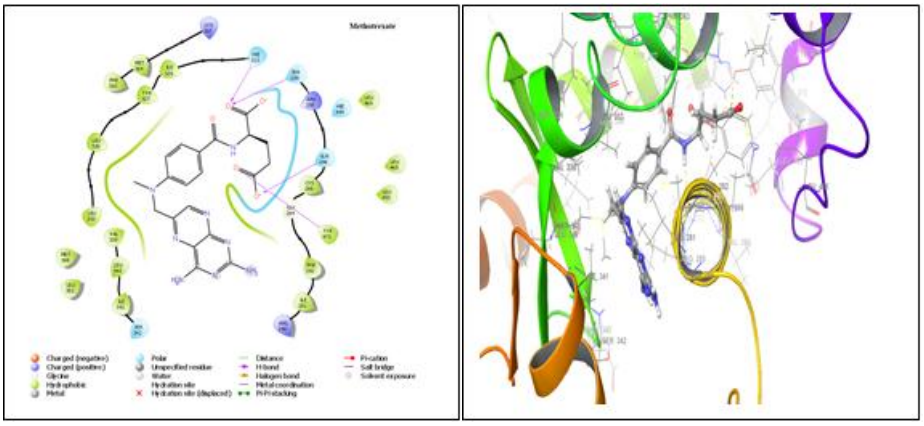


Fig. 12: Best affinity mode and H bond interactions of Methotrexate

Table 8: Docking score, interacting amino acid residues and its H-bond length of selected proposed derivatives and standard drugs against PPAR-γ.

Comp. Code	Docking Score (kcal/mol)	H bonding interacted functional groups in ligand	H bonding interacted amino acid residues in receptor	H Bond length (A^0)
Ciglitazone (marketed TZD containing drug)	-11.30	C=O	GLN 286	2.01
		N--H	TYR 473	1.98
		C=O	HIE 323	1.75
		C=0	SER 289	1.80
Methotrexate (marketed anticancer drug)	-11.42	C=O	GLN 286	1.65
		N--H	TYR 473	1.77
		C=O	HIE 323	2.10
		C=0	SER 289	1.82

TZPY 2	-8.13	C=0	SER 289	2.15
TZPY 28	-8.13	C=0	SER 289	2.07
TZPY 45	-8.21	C=0	SER 289	2.18

Hydrophobic interactions

2D and 3D Model of docking images of each thiazolidine-2, 4–dione analogues has shown its hydrophobic interactions with the amino acid residues. If higher the positive valve, then the molecule will be more hydrophobic. It is used to predict the membrane proteins.

2D and 3D images of thiazolidine-2, 4–dione analogues has been shown that H bonding interactions and Hydrophobic interactions play a major role the active docking score. It was observed thiazolidine-2, 4-dione nucleus have a major role in the interactions of ligand with the receptor.

There are three thiazolidine-2,4–dione derivatives (TZPY 2, TZPY 28 and TZPY 45) shown moderate H bonding interactions with amino acid residues SER289. N-substituted pyrimidine ring gave low docking score. Thiazolidine 2, 4-dione moiety is more easily make a bond with different amino acids of the targeted receptor (PPARγ). And docking study also reveals presence of thiazolidinedione ring imparts good cytotoxic activity.

CONCLUSIONS

In-silico molecular modeling helped to calculate the pharmacokinetic properties and to design the proposed pyrimidine linked thiazolidine-2,4-dione derivatives. The drug likeness and ADMET properties was checked by using the online tools like Molinspiration cheminformatics and PreADMET software. The values of drug likeness and ADMET properties indicate that the proposed derivatives have potential to be new drug candidates. All the proposed derivatives were docked with protein target PPAR-γ (PDB ID:2PRG) for anticancer activity by using the software Schrödinger. All the designed 52 compounds were docked with receptor PPAR-γ (2PRG) which was downloaded from protein data bank. Data received all are compared with each other. 2D and 3D images by the binding interactions between the ligand and receptors sites were saved. Different types of interactions were studied, (H bonding, Hydrophobic interactions and polar interactions) and H bond interaction varies with each derivatives. H-bond length between the Thiazolidine-2,4-dione moiety and the amino acids was measured and noted. Out of 52 derivatives for the best posed 3 compounds were short out. Pyrimidine-thiazolidine-2,4-dione series gave moderate docking score compared to standard anticancer drug methotrexate. Thiazolidinedione moiety has more specific binding interactions with the receptor sites but N-substituted thiazolidine-2,4-dione derivatives have been shown least interactions with receptors. This work will provide information regarding the developments of drug design. Drug design is the creative process of finding new remedies based on the knowledge of a biological target.

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CONFLICT OF INTREST

There is no conflict of interest in the work presented in this manuscript.

REFERENCES

1. Hongyu Z et al., Design, synthesis, cytoselective toxicity, structure–activity relationships, and pharmacophore of thiazolidinone derivatives targeting drug-resistant lung cancer cells. J Med Chem, 2008; 51: 1242–125.
2. Vagdevi HM, Vaidya VP, Latha KP, Padmashali B, Synthesis and pharmacological examination of some Thiazolidinone derivatives of Naphtofuran. Indian J Pharm Sci., 2006; 68(6): 19-725.
3. A Garg, P. Chawla, D. Panjabi., Synthesis of Some Novel Thiazolidinediones as bioactive agents. International Journal of Pharmaceutical Sciences and Nano technology, 2014; 4: 254-258.
4. Anna Pratima G Nikalje, Diwali Deshpande and Hemant D. One, Facile synthesis and in vivo hypoglycemic activity of novel 2,4-Thiazolidinedione derivatives, European Journal of Experimental Biology, ISSN-22489215, 2012; 2(2): 343-353.
5. A. Rekha and Shantharam U., Synthesis and evaluation of novel thiazolidinediones for anti-diabetic activity, International Journal of Pharmacy and Pharmaceutical Sciences ISSN-0975-1491, 2011; 3(5): 113-117.
6. Aaron Mathew Thomas Molecular docking based virtual design of substituted 2, 4-thiazolidinediones as HMG COA reductase inhibitor ISSN-2278–4357, 2014; 3(6): 1298-1304.
7. Deepthy chandran, Leena. K. Pappachen, Manju Prathap, Jinsha.M.J, Jilsha.G. *Insilico* drug design and molecular docking studies of some novel benzothiazole derivatives as anti-cancer and anti-inflammatory agents, International Journal of Pharmacy and Pharmaceutical Sciences, ISSN-0975-1491, 2014; 6(2): 203-208.
8. Fajeelath Fathima, Abitha Haridas and Baskar Lakshmanan, Docking studies Of 3,5-disubstituted thiazolidinedione chalcones as PPAR-γ agonist, Journal of Pharmaceutical Chemistry, 2016; 3(3): 1-3.
9. Metta Madhuri, Cheepurupalli Prasad, Vasudeva Rao Avupati, *In Silico* Protein-Ligand Docking Studies on Thiazolidinediones as Potential

Anticancer Agents, International Journal of Computer Applications, ISSN-09758887, 2014; 9(5): 13-16.
10. Sachin Malik, Prabhat Kumar Upadhyaya and Sandeep Miglani, Thiazolidinediones: A Plethro of Biological Load, International Journal of Pharm Tech Research, ISSN-0974-4304, 2011; 3(1): 62-75.
11. Santosh L. Gaonkar, Namratha B, Nitinkumar S. Shetty and Hiroki Shimizu Microwave-assisted synthesis and evaluation of N-substituted thiazolidine-2,4-dione derivatives as antimicrobial agents, Interactive Medicinal Chemistry, ISSN-2053-7107, 2014; 2(2): 7-9.
12. Swastika Ganguly Molecular docking studies of novel thiazolidinedione analogs as HIV-1-RT inhibitors, Medicinal chemistry research, 2012; 1(2): 4-16.
13. Wei Wei and Yihong Wan Thiazolidinediones on PPARγ: The Roles in Bone Remodeling, PPAR Research, ISSN-867180, 2011; 1(2): 1-9.
14. Shasikant Pattan, Manisha Kedar and Jayashri. Synthesis and Evaluation of Some Novel 2, 4-Thiazolidinediones for Antibacterial, Antidiabetic activity. Indian Journal of Chemistry, 2012; 1513: 1421-1425.
15. Santosh L. Gaonkar, Namratha B1, Nitinkumar S. Shetty and Hiroki Shimizu, Microwave-assisted synthesis and evaluation of N-substituted thiazolidine-2,4-dione derivatives as antimicrobial agents, Interactive Medicinal Chemistry, ISSN-2053-7107, 2014; 2(2): 107-112.
16. Mohammed Shahnaz and Pattel Kannu Bhai. Synthesis and Characterisation of 2, 4-Thiazolidinedione Derivatives and Evaluation of Their Antioxidant activity. Journal of Drug Delivery and Therapeutics, 2013; 3(6): 96-101.
17. Panigrahy D and Shen LQ. Therapeutic Potential of Thiazolidinediones as Anticancer Agents.US National Library of Medicine, 2003; 12(2): 1925-1937.
18. S. Kushibiki, K. Hodate, H. Shingu et al., "Insulin resistance induced in dairy steers by tumor necrosis factor alpha is partially reversed by 2,4-thiazolidinedione, Domestic Animal Endocrinology, 2001; 21(1): 25–37.