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Presented by:

1. Yossra Fridjat
2. Ilham Azeb Atmane

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Board of Examiners:

Ms. Benamara Hacen	Assistant Professor	University of El-Oued	Supervisor
Ms. Redjeb Youcef	Associate Professor	University of El-Oued	Chairman
Ms. Salah Laouini	Professor	University of El-Oued	Examiner

Academic Year: 2025/2026

Dedication

All praise and gratitude be to Allah for His guidance and blessings in enabling me to complete this work. I dedicate this achievement to the soul of my dear grandmother may Allah have mercy upon her and grant her a place in His spacious paradise. To my beloved parents, who have been my ultimate source of support and strength in life. And to my sisters and friends, who stood by me as a constant pillar of support through every stage. To all of you, I offer my sincerest thanks and deepest gratitude. ***#Ilham***

I would like to thank Allah Almighty for His guidance and blessings in completing this work. My sincere gratitude goes to my beloved parents for their continuous support, to my esteemed professors for their valuable guidance, and to my dear friends for their encouragement and constant support. To all of you, I express my deepest thanks and appreciation. ***#Yossra***

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Lastly, we are deeply indebted to our families and to all those who provided us with encouragement, assistance, and support throughout this journey. Their confidence in us has been a source of motivation and perseverance.

Abstract

Type 2 Diabetes Mellitus (T2DM) is driven by peripheral insulin resistance. While thiazolidinediones (TZDs) target the nuclear receptor Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) to manage this pathology, empirical drug development faces high costs and attrition rates. To overcome these challenges, advanced computer tools help researchers design and test new medicines much faster.

This study evaluated the molecular efficacy, binding mechanisms, and thermodynamic stability of Pioglitazone as a potent PPARγ agonist using an integrated in silico framework compared to reference benchmarks.

The crystal structure of human PPARγ was retrieved from the Protein Data Bank (PDB ID: **7AWC**) and chemically refined. Pioglitazone was geometrically optimized via Density Functional Theory (DFT/B3LYP/6-311G(d,p)) in Gaussian 09. Molecular docking simulations were conducted using the algorithm in AutoDock Vina, followed by pharmacokinetic and toxicity modeling via SwissADME and ProTox-3.0.

Docking simulations showed that Pioglitazone binds strongly to PPARγ, forming key hydrogen bonds (Tyr473, His323, His449, Ser289) that stabilize the AF-2 Helix 12. ADME analysis confirmed Lipinski compliance (MW: 356.44 g/mol, logP: 3.09, TPSA: 93.59 Å²), indicating excellent gastrointestinal absorption. ProTox-3.0 assigned it to Toxicity Class 4 (LD_{50}: 1000 mg/kg), predicting low toxicity with high probabilities for blood-brain barrier penetration (0.74) and active PPARγ signaling (0.86).

This workflow maps the molecular interactions driving Pioglitazone- PPARγ activation. Its structural parameters confirm favorable stability and oral bioavailability, providing an efficient digital roadmap for designing precision antidiabetic drugs.

Keywords: Pioglitazone, PPARγ (7AWC), Molecular Docking, Density Functional Theory (DFT), ADMET Profiling, Insulin Resistance.

الملخص

يُعزى داء السكري من النوع الثاني (T2DM) بشكل أساسي إلى مقاومة الأنسولين المحيطية. وفي حين تستهدف مركبات الثيازوليدينديون (TZDs) مستقبل (PPARγ)للتحكم في تداعيات هذا المرض، فإن التطوير التجريبي للأدوية يواجه تكاليف باهظة ومعدلات فشل عالية. وللتغلب على هذه التحديات، تُساعد الأدوات الحاسوبية المتقدمة الباحثين على تصميم الأدوية الجديدة واختبارها بشكل أسرع بكثير.

قيمت هذه الدراسة الفعالية الجزيئية، وآليات الارتباط، والاستقرار الديناميكي الحراري لمركب البيوجليتادون (Pioglitazone) باعتباره محفزاً قوياً لمستقبل(PPARγ) ، وذلك باستخدام إطار عمل متكامل للتصميم الحاسوبي (In Silico) ومقارنته بالمراجع المعيارية المعتمدة.

تم جلب البنية البلورية لمستقبل (PPARγ) البشري من بنك بيانات البروتينات (PDB ID: 7AWC) وتنقيتها كيميائياً. كما تم تحسين الهندسة الفراغية لمركب البيوجليتادون (Pioglitazone) عبر نظرية الكثافة الوظيفية ((DFT/B3LYP/6-311G(d,p) باستخدام برنامج .(Gaussian 09) وأُجريت محاكاة الالتحام الجزيئي باستخدام برنامج(AutoDock Vina) ، متبوعة بنمذجة الحركية الدوائية والسمية عبر منصتي (SwissADME) و.(ProTox-3.0)

أظهرت محاكاة الالتحام أن البيوجليتادون يرتبط بقوة مع مستقبل(PPARγ) ، مكوناً روابط هيدروجينية رئيسية (Tyr473, His323, His449, Ser289)تعمل على استقرار اللولب 12 في منطقة .(AF-2) وأكد تحليل (ADME) الامتثال لقاعدة ليبينسكي(الوزن الجزيئي: 356.44 غرام/مول، معامل التجزئةlogP: 3.09 ، ومساحة السطح القطبية الطوبولوجية :TPSA 93.59أنجستروم مربع)، مما يشير إلى امتصاص ممتاز في الجهاز الهضمي. وصنفت منصة (ProTox-3.0) المركب ضمن فئة السمية الرابعة (الجرعة القاتلة للنصف 1000 :LD_{50} ملغ/كغ)، متوقعة سمية منخفضة مع احتمالات عالية لاختراق الحاجز الدموي الدماغي (0.74) وتنشيط مسارات إشارات (PPARγ) الفعال (0.86) .

يرسم مسار العمل هذا خريطة للتفاعلات الجزيئية التي توجّه تنشيط معقد (Pioglitazone-PPARγ) وتؤكد معلمات البنيوية الاستقرار القوي وصلاحيته الحيوية الفموية، مما يوفر خارطة طريق رقمية فعالة لتصميم أدوية دقيقة وموجهة لعلاج السكري.

الكلمات المفتاحية :بيوجليتادون، مستقبل(7AWC) PPARγ ، الالتحام الجزيئي، نظرية الكثافة الوظيفية(DFT) ، توصيف (ADMET)، مقاومة الأنسولين

Table of Contents

Chapter II

Chapter III

Chapter IV

List of figures

List of tables

List of Abbreviations and Symbols

Abbreviation	Full Meaning
ADME	Absorption, Distribution, Metabolism, and Excretion
AGEs	Advanced Glycation End-products
Akt	Protein Kinase B (PKB)
ATP	Adenosine Triphosphate
BBB	Blood–Brain Barrier
CAP	c-Cbl-associated protein
CBP / p300	CREB-Binding Protein / p300 coactivator
CPK	Corey-Pauling-Koltun (atom space-filling representation model)
DFT	Density Functional Theory
DM	Diabetes Mellitus
ESP	Electrostatic Potential Mapping
FATP	Fatty Acid Transport Protein
GDM	Gestational Diabetes Mellitus
GI	Gastrointestinal
GLUT1 / 2 / 4	Glucose Transporter Type 1 / 2 / 4
IL-6	Interleukin-6
IRS	Insulin Receptor Substrate
LBD	Ligand-Binding Domain
LPL	Lipoprotein Lipase
MD	Molecular Dynamics
mRNA	Messenger Ribonucleic Acid
NCoR	Nuclear Receptor Co-repressor
PDB ID	Protein Data Bank Identifier
PEPCK	Phosphoenolpyruvate carboxykinase
PI3K	Phosphoinositide 3-kinase
PPARγ / PPAR-γ	Peroxisome Proliferator-Activated Receptor Gamma
PPRE / PPREs	Peroxisome Proliferator Response Element(s)

RMSD	Root-Mean-Square Deviation
RXR	Retinoid X Receptor
SBDD	Structure-Based Drug Design
SMILES	Simplified Molecular-Input Line-Entry System
SMRT	Silencing Mediator for Retinoid and Thyroid Hormone Receptors
SRC-1	Steroid Receptor Coactivator-1
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TNF-α / TNF-a	Tumor Necrosis Factor-alpha
TPSA	Topological Polar Surface Area
TZD	Thiazolidinedione

General Introduction

Diabetes mellitus (DM) represents one of the most formidable global health crises of the 21st century, serving as a primary driver of metabolic syndrome, systemic vascular complications, and premature mortality [1]. Characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, type 2 diabetes mellitus (T2DM) has reached pandemic proportions. At the core of its pathology lies insulin resistance, wherein peripheral target tissues exhibit a blunted response to insulin, precipitating glucose dysregulation, compensatory hyperinsulinemia, and eventual pancreatic β-cell exhaustion [2]. Given the progressive and multifactorial nature of T2DM, there is an urgent need for effective, mechanism-based therapies that can directly rectify underlying metabolic and inflammatory defects.

Concurrently, the landscape of pharmaceutical research has undergone a major transformation through the integration of computational drug discovery and in silico pharmacology. Traditional empirical drug discovery is characterized by high attrition rates, exorbitant costs, and protracted timelines [3]. Today, these limitations are increasingly augmented by structure-based drug design (SBDD) and advanced computational chemistry workflows. Utilizing molecular docking simulations and pharmacokinetic modeling enables researchers to predict binding affinities, structural conformations, and metabolic profiles with unprecedented accuracy, thereby significantly accelerating the drug discovery pipeline [4].

Within this structural framework, Pioglitazone emerges as a molecule of profound therapeutic interest. As a member of the thiazolidinedione (TZD) class, Pioglitazone functions as an insulin sensitizer rather than a secretagogue. It acts as a synthetic agonist of Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) a nuclear receptor serving as a master regulator of adipogenesis, lipid metabolism, and glucose homeostasis [5].

This Master's thesis presents a rigorous in silico computational investigation aimed at characterizing the molecular interactions, thermodynamic binding stability, and structural affinity of Pioglitazone within the canonical ligand-binding domain (LBD) of the receptor. By coupling molecular docking tools with absorption, distribution, metabolism, excretion, and toxicity (ADMET) pharmacoinformatics profiling, this study evaluates the drug-likeness and intracellular suitability of Pioglitazone, establishing a quantitative baseline to support future therapeutic optimizations and drug repurposing research

Chapter I

Background and objectives of Pioglitazone– PPARγ

I.1 Pioglitazone in Diabetes Research

Diabetes mellitus (DM) represents one of the most formidable global health crises of the 21st century, constituting a primary driver of metabolic syndrome, systemic micro- and macrovascular complications, and premature mortality. Characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, DM has reached pandemic proportions, with the International Diabetes Federation projecting over 700 million individuals afflicted by 2045 [6]. At its core lies insulin resistance, wherein peripheral target tissues skeletal muscle, adipose tissue, and the liver exhibit a blunted response to insulin, precipitating glucose dysregulation, compensatory hyperinsulinemia, and eventual pancreatic β-cell exhaustion. Given the multifactorial and progressive nature of T2DM, the urgent need for effective, mechanism-based antidiabetic therapies that rectify underlying metabolic and inflammatory defects remains a paramount objective in contemporary biomedical research [7].

The landscape of pharmaceutical research has simultaneously undergone transformation with the integration of computational drug discovery and in silico pharmacology. Traditional empirical drug discovery characterized by high attrition rates, exorbitant costs, and protracted timelines is increasingly augmented by structure-based drug design (SBDD) and advanced computational chemistry [8]. Through molecular docking, molecular dynamics (MD) simulations, quantum chemical calculations, and pharmacokinetic modeling, researchers can now predict binding affinities, conformational dynamics, and metabolic profiles with unprecedented accuracy, accelerating the drug discovery pipeline [9].

Within this context, Pioglitazone emerges as a molecule of profound therapeutic and scientific interest. As a member of the thiazolidinedione (TZD) class, Pioglitazone functions as a high-affinity synthetic agonist of Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) a nuclear receptor serving as a master regulator of adipogenesis, lipid metabolism, and insulin sensitivity. By acting as an insulin sensitizer rather than a secretagogue, Pioglitazone addresses the fundamental pathophysiology of T2DM [10, 11].

I.2 Diabetes Mellitus: Molecular and Metabolic Basis

Diabetes mellitus is characterized by chronic hyperglycemia resulting from insufficient insulin production or an impaired cellular response to insulin a hormone produced by pancreatic β-cells that facilitates glucose uptake for energy production [9, 10].

I.3 Major Classifications of Diabetes

T1DM: Autoimmune destruction of β-cells leading to complete insulin deficiency.

T2DM: The most common form, primarily driven by insulin resistance with progressive β-cell decline.

GDM: Develops during pregnancy due to hormonal changes that increase insulin resistance [9].

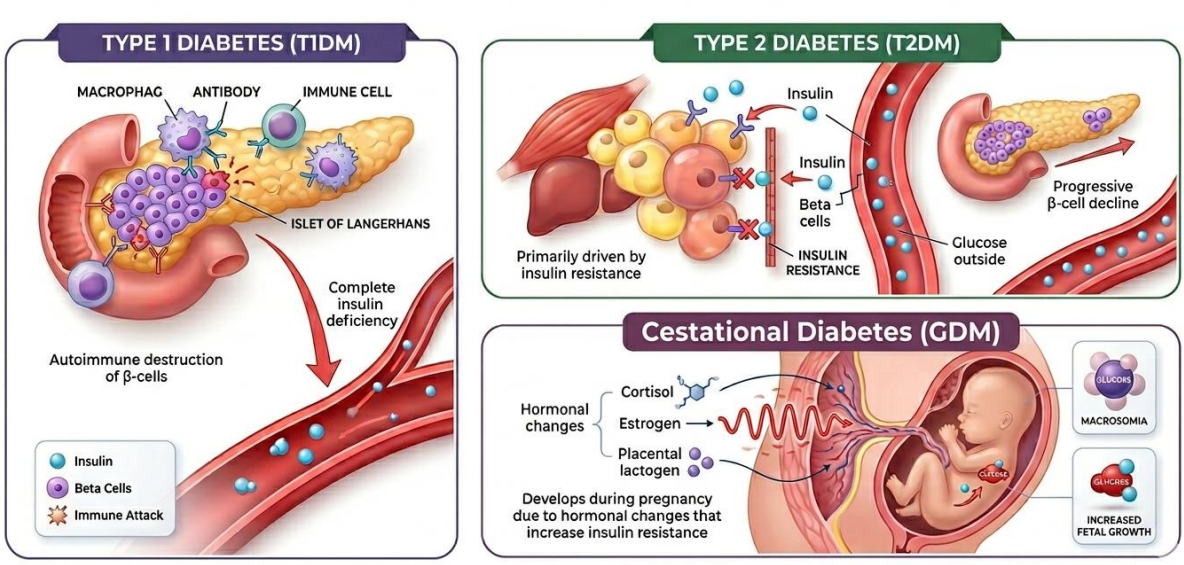

Figure 1: Types of Diabetes

Under normal conditions, glucose enters β-cells via GLUT1/GLUT2 transporters, is metabolized to produce ATP, and triggers insulin secretion. Insulin then binds to receptors on target cells, activating IRS/PI3K/Akt signaling pathways that translocate GLUT4 transporters to the membrane, increasing glucose uptake into muscle and fat cells [10, 11].

In the liver, insulin suppresses gluconeogenesis and promotes glycogen storage. In T2DM, insulin resistance disrupts this regulation, causing excessive hepatic glucose output [12]. Concurrently, disturbed fat metabolism elevates free fatty acids, worsening insulin resistance.

Adipose tissue releases inflammatory cytokines (TNF-α, IL-6) that further impair insulin signaling, while mitochondrial dysfunction and oxidative stress compound cellular damage [13].

Long-term hyperglycemia damages blood vessels and organs through advanced glycation end-products (AGEs) and oxidative stress, leading to cardiovascular disease, nephropathy, neuropathy, retinopathy, and endothelial dysfunction [14].

I.4 PPARγ: Structure, Activation, and Metabolic Regulation

PPARγ is a nuclear receptor encoded by the PPARG gene, existing in two isoforms: PPARγ1 (broadly expressed) and PPARγ2 (predominantly in adipose tissue) [15]. Structurally, it contains a DNA-binding domain and a ligand-binding domain (LBD) where drugs such as Pioglitazone bind [16]. It is found primarily in the nucleus of fat tissue, gut, and immune cells (macrophages), with additional expression in kidney, heart, liver, vasculature, and brain.

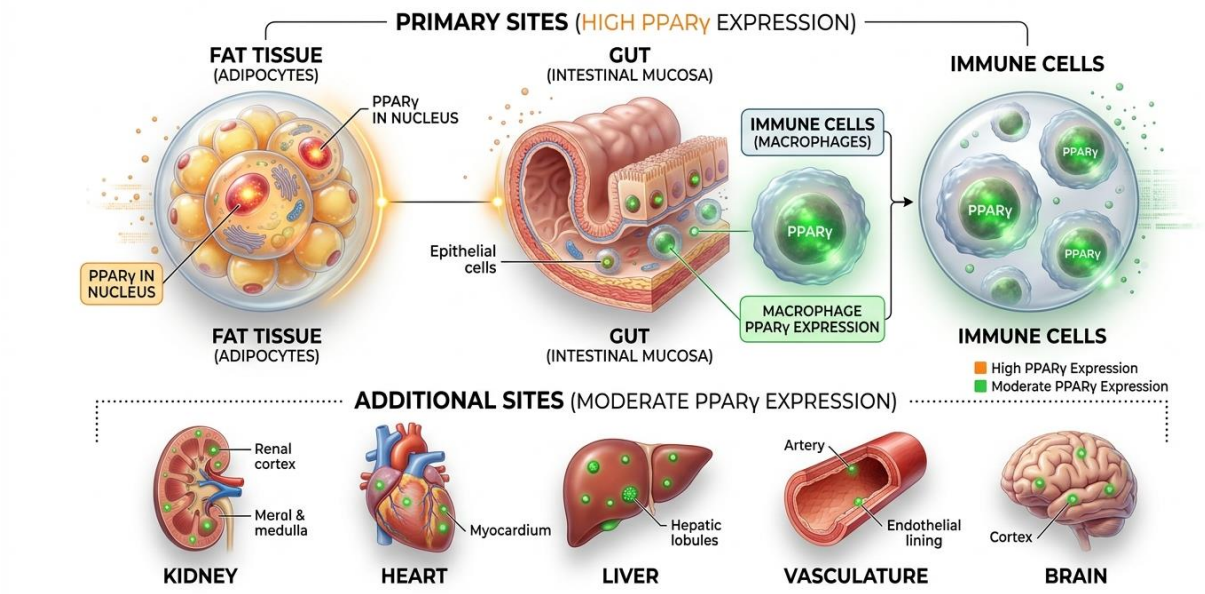

Figure 2: PPARγ tissue localization and expression pattern

In the absence of a ligand, PPARγ resides inactive in the nucleus, associated with corepressor proteins (NCoR/SMRT). Upon ligand binding, a conformational change particularly stabilization of Helix 12 (AF-2 domain) displaces corepressors and recruits coactivator complexes (SRC-1, CBP/p300). PPARγ then heterodimerizes with RXR and binds PPAR Response Elements (PPREs) on DNA, activating genes governing [17-18]:

- **Adipogenesis and Lipid Storage:** Promotes fatty acid sequestration in adipose tissue, reducing ectopic fat accumulation in liver and muscle [19].
- **Glucose Homeostasis:** Upregulates GLUT4 expression, enhancing glucose uptake in muscle and fat cells [20].
- **Adipokine Regulation:** Increases adiponectin (improving glucose uptake and fatty acid oxidation) while reducing TNF-α and IL-6 [20].
- **Mitochondrial Function & Anti-inflammation:** Stimulates fatty acid oxidation genes, reduces oxidative stress, and suppresses chronic inflammation associated with obesity and T2DM [21].

I.5 Chemical and Pharmacological Profile of Pioglitazone

I.5.1 Molecular Structure and Physicochemical Properties

Pioglitazone ($C_{19}H_{20}N_2O_3S$; MW 356.44 g/mol) belongs to the thiazolidinedione (TZD) class [22]. Its structure comprises three primary functional regions:

- **TZD ring (polar head):** Contains oxygen, sulfur, and nitrogen atoms enabling crucial hydrogen bond interactions with PPARγ residues Tyr473, His323, His449, and Ser289 the primary pharmacophoric element.
- **Central phenoxy linker:** Connects the polar head to the distal tail structure seamlessly.
- **2-Ethylpyridine tail (hydrophobic):** Engages hydrophobic pockets and regions of the ligand-binding domain (LBD) [23].

These structural features collectively govern precise molecular recognition within the PPARγ binding pocket.

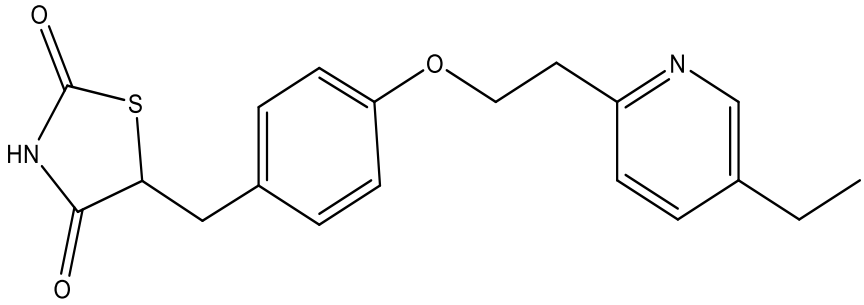

Figure 3: Two-dimensional structure of Pioglitazone

I.6 Mechanism of Action: Pioglitazone

The mechanism of pioglitazone is divided into its systemic core function and a detailed intracellular molecular cascade.

I.6.1 The Core Function

- **No Pancreatic Stimulation:** Unlike secretagogues, Pioglitazone does not stimulate pancreatic β-cells to produce more insulin, maintaining normal baseline insulin production without increasing pancreatic strain.
- **Insulin Sensitizer:** It acts directly on target tissues specifically **muscle cells** and **fat cells (adipocytes)** making them highly responsive to insulin and drastically increasing glucose absorption from the bloodstream [24].

I.6.2 Cellular and Molecular Cascade

The therapeutic effects of Pioglitazone are driven by a precise, step-by-step molecular sequence that regulates gene transcription inside the cell nucleus [25-26].

[Pioglitazone Entry] ➔ [Nuclear Activation] ➔ [Complex Formation] ➔ [DNA Binding] ➔ [Target Gene Translation]

Step 1: Nuclear Activation

Pioglitazone crosses the cell membrane into the extracellular and diffusion spaces, entering the nucleus. Inside the nucleus, it acts as a potent agonist, binding directly to the **Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ)**.

Step 2: Complex Formation

Once activated by Pioglitazone, the PPAR-γ receptor undergoes a conformational change and binds with the **Retinoid X Receptor (RXR)**, which is activated by Retinoic Acid. Together, they form the functional **PPAR-γ/RXR heterodimer complex**.

Step 3: DNA Binding & Transcription

The PPAR-γ/RXR complex acts as a genetic "switch," binding to specific DNA sequences known as **PPRE (Peroxisome Proliferator Response Elements)**. This engagement recruits the cell's transcription machinery to initiate the transcription of specific messenger RNA (mRNA) [27-28].

I.6.3 The Resulting Impact (Target Gene Expression)

The activation of the PPRE "switch" repairs and enhances the insulin signaling pathway by upregulating or regulating several critical proteins:

- **GLUT4 Expression:** Drastically drives the expression and translocation of **GLUT4 transporters** to the cell membranes of muscle and fat cells. This opens the gateways to actively drive glucose out of the blood and into the cells.
- **CAP (c-Cbl-associated protein):** Enhances the **Insulin Signaling Path**, repairing and optimizing downstream intracellular signaling.
- **PEPCK (Phosphoenolpyruvate carboxykinase):** Regulates **gluconeogenesis** (the generation of glucose from non-carbohydrate substrates) to control hepatic glucose output.
- **FATP & LPL (Fatty Acid Transport Protein & Lipoprotein Lipase):** Upregulates these proteins to clean up circulating lipids and manage efficient **fatty acid transport and lipid storage** [29].

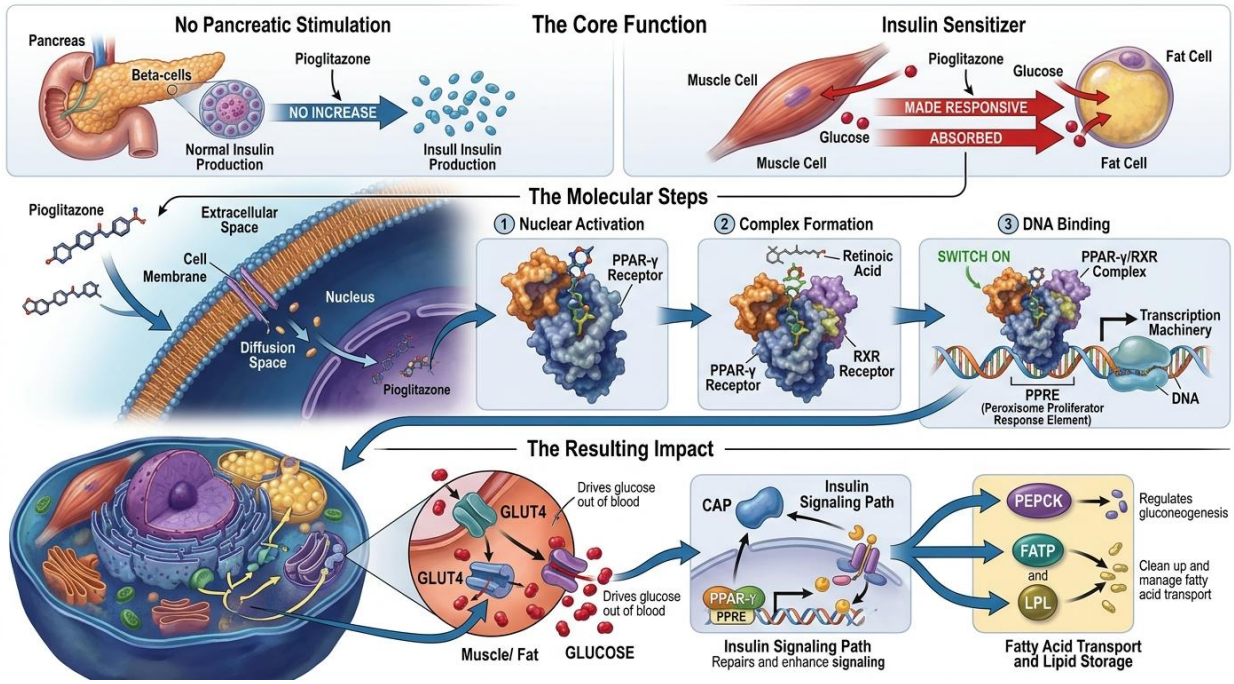

Figure 4: In silico and cellular pathway of Pioglitazone-mediated insulin sensitization.

I.7 Computational Investigation of Antidiabetic Agents

I.7.1 Strategic Rationale for In Silico Evaluation

In silico evaluation utilizes advanced computational simulations to predict the therapeutic potential of chemical compounds before transitioning to physical laboratory environments [30]. This approach is essential for streamlining the discovery process by leveraging digital modeling to bypass the high attrition rates and exorbitant costs typically associated with traditional empirical research [31]. Within the context of metabolic pharmacology, computational frameworks provide a rapid, cost-effective means of identifying high-affinity candidates, allowing for the systematic optimization of molecular structures to ensure superior clinical outcomes [32].

I.7.2 Computational optimization of Pioglitazone

Given its established clinical safety profile, Pioglitazone serves as an ideal primary model for deeper computational investigation. This study utilizes advanced modeling to map its precise interactions at the atomic level, exploring how its specific chemical configuration can be

computationally leveraged to maximize metabolic regulation. Rather than simply re-examining its known clinical efficacy, this investigation employs in silico frameworks to uncover nuanced binding characteristics and structural synergies that can inform the rational development of next-generation, precision-targeted antidiabetic strategies [33-34].

I.8 Bibliometric Trends in Computational Antidiabetic Research

To quantitatively evaluate the current research landscape, bibliometric data was sourced from the Dimensions database (https://app.dimensions.ai/). This platform facilitates a global analysis of publication trends, providing essential context for the broader field and the specific goals of this research.

I.8.1 The Global Importance of the Research Field

As illustrated in **Figure 05**, the data retrieved from Dimensions using the keyword **"In Silico Evaluation of Antidiabetic Agents"** demonstrates an exponential increase in scientific interest and output over the last 15 years. From a nascent stage in 2010 with minimal publications, the field has surged dramatically, reaching nearly **10,000 publications** annually by 2025.

This trend highlights the critical importance of computational modeling in the fight against metabolic disorders. The scientific community is increasingly prioritizing *in silico* strategies because they allow for the rapid screening of vast chemical libraries, the prediction of molecular interactions with high precision, and the identification of novel therapeutic candidates. By utilizing these digital frameworks, researchers can significantly reduce the time and financial burden of the pre-clinical phase, streamlining the path toward effective treatments for diabetes.

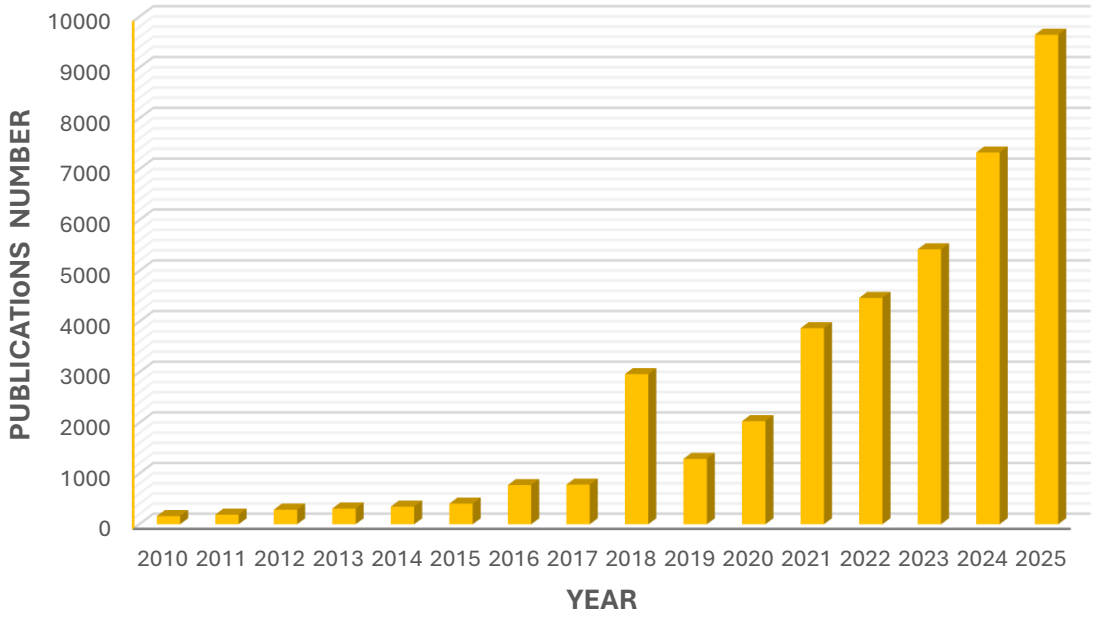

Figure 5: Global Publication Trends in the Field of In Silico Evaluation of Antidiabetic Agents (2010–2025)

I.8.2 The Strategic Importance of the Thesis Topic

Figure 06 presents the publication trends specifically related to the intersection of **Pioglitazone, antidiabetic agent, computational investigation, Molecular docking, and PPAR-γ**. These keywords, extracted from the thesis title, reveal several key insights into the research landscape:

- **Timeliness:** Following a period of stable, baseline exploration between 2010 and 2017, the field experienced a massive surge in 2018, followed by a secondary peak in 2021. This volatility indicates specific periods of breakthrough and intense academic interest in the molecular mechanisms of Pioglitazone.
- **Current Relevance:** The steady growth observed from 2022 through 2025 confirms that the **computational investigation** of antidiabetic agents remains a high-priority area. The upward trajectory in the last four years suggests a renewed and sustained focus on refining **PPAR-γ** interactions via advanced **molecular docking** techniques.

- **Scientific Specialism:** While the broader field of diabetes research is vast, this specific thesis topic operates within a specialized and technologically driven niche. The consistent publication output in recent years points to a growing consensus on the necessity of using *in silico* tools to maximize the therapeutic potential of established agents like Pioglitazone.

By utilizing the Dimensions platform to track these metrics, it is evident that the thesis topic is not only scientifically sound but also highly aligned with the contemporary trajectory of pharmaceutical research and precision computational drug design.

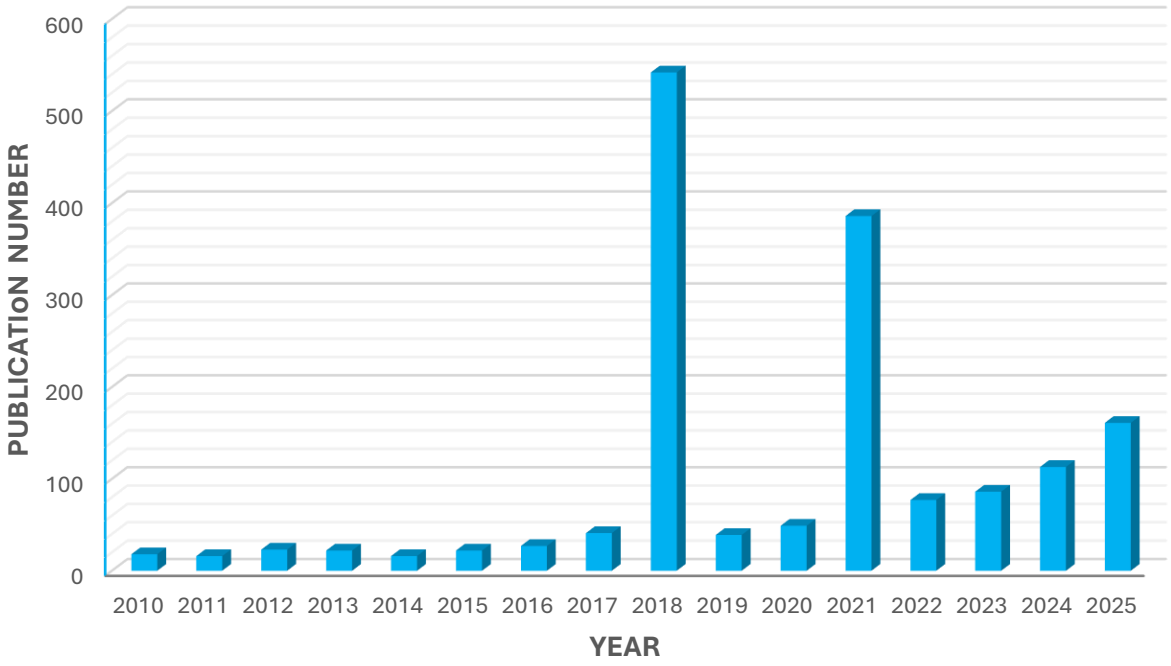

Figure 6: Evolution of Computational Research on Pioglitazone and PPAR-γ Interactions

I.9 Objectives and Scope

I.9.1 General objective and Research Gap

To evaluate the molecular efficacy of **Pioglitazone** as a potent **PPAR-γ** agonist using *in silico* approaches, providing a structural basis for its high-affinity antidiabetic activity. **Research Gap** to be addressed through a comprehensive in silico analysis of pioglitazone, integrating molecular optimization, ADME screening, toxicity profiling, and visualization of its binding interactions with PPAR-γ.

I.9.2 Specific objectives

- **Structural Preparation**: Retrieve and prepare the human **PPAR-γ** crystal structure (**PDB ID: 7AWC**) by removing water molecules and adding polar hydrogens.
- **Ligand optimization**: Generate and optimize the 3D structure of **Pioglitazone** to ensure the most energetically favorable conformation for docking study.
- **Molecular Docking**: Perform site-directed docking to predict binding affinity (ΔG) and identify key amino acid interactions within the receptor's active site.
- **Comparative Analysis**: Compare the binding energy and interaction profile of Pioglitazone with standard PPAR-γ ligands to assess its relative potency

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Chapter II

Molecular Modeling Framework

II.1 Biomolecular Target Acquisition and Refinement

In computational drug discovery and structural biology, biomolecular target acquisition and refinement represent essential preliminary stages for molecular modeling and structure-based drug design (SBDD). This process involves selecting a high-resolution three-dimensional protein structure from databases such as the Protein Data Bank and subsequently refining the structure to ensure its suitability for computational analyses. Structural refinement generally includes the correction of missing residues, optimization of hydrogen-bonding networks, removal of crystallographic artifacts, and stabilization of protein geometry for molecular docking and simulation studies [35].

II.2 Structural Insights into the PPARγ–Rosiglitazone Complex

Insulin resistance is a central pathological feature of Type 2 Diabetes Mellitus (T2DM), characterized by impaired cellular responsiveness to insulin in peripheral tissues. To better understand the molecular mechanisms underlying insulin sensitization, researchers increasingly rely on ligand-bound crystal structures of therapeutic targets. Among the most significant of these structures is the human Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) complexed with Rosiglitazone (**PDB ID: 7AWC**), which provides detailed structural insights into receptor activation and antidiabetic drug action [36].

➢ Core Structural Features

- **Ligand-Binding Domain (LBD):** The receptor contains a large hydrophobic ligand-binding pocket that accommodates Rosiglitazone and other thiazolidinedione (TZD) compounds. This domain is responsible for ligand recognition and receptor activation.
- **Activation Function-2 (AF-2) Helix:** Binding of Rosiglitazone stabilizes the AF-2 helix (Helix 12), an essential structural element required for coactivator recruitment and transcriptional activation of insulin-sensitive genes [37].
- **Transcriptional Regulation:** Upon activation, PPARγ forms a heterodimer with the Retinoid X Receptor (RXR), allowing the complex to bind specific DNA sequences known as

Peroxisome Proliferator Response Elements (PPREs), thereby modulating genes involved in glucose uptake and lipid metabolism.

- **Rosiglitazone Binding and Mechanism**
- **Binding Site Affinity:** Rosiglitazone occupies the hydrophobic ligand-binding pocket of PPARγ and establishes stabilizing hydrogen bonds with key amino acid residues within the receptor cavity.
- **Receptor Stabilization:** Ligand binding induces conformational stabilization of the AF-2 activation domain, facilitating the recruitment of transcriptional coactivators necessary for gene expression regulation.
- **Metabolic Regulation:** Through activation of PPARγ signaling pathways, Rosiglitazone enhances insulin sensitivity, promotes glucose uptake in adipose and skeletal muscle tissues, and reduces hepatic glucose production [38].

Utilizing the **7AWC** structural model enables high-resolution characterization of the Rosiglitazone-binding pocket and the conformational dynamics associated with **PPARγ** activation. This structural framework is highly valuable for future structure-based drug design approaches aimed at developing novel insulin-sensitizing agents with improved efficacy and reduced adverse effects [39].

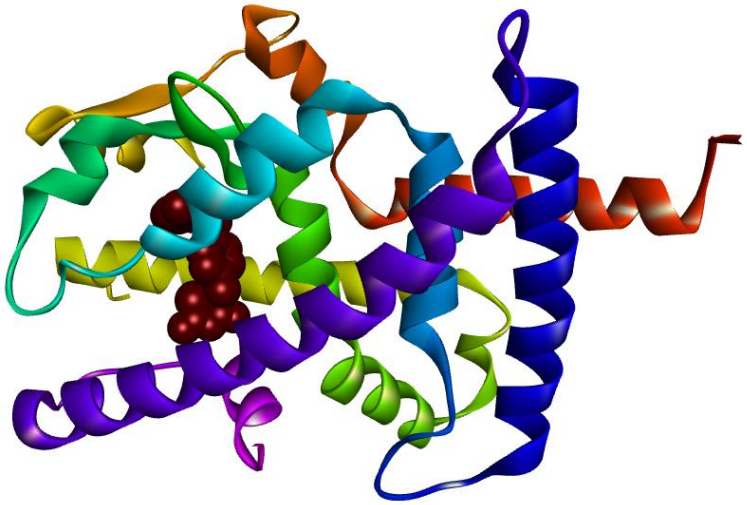

Figure 7: Crystal structure of the human PPARγ ligand-binding domain complexed with rosiglitazone in a dark red CPK-style representation (**PDB ID: 7AWC**).

The following steps outline the protein preparation stage for the Peroxisome Proliferator-Activated Receptor Gamma (PPARγ)–Rosiglitazone complex using the crystal structure with **PDB ID: 7AWC**.

II.3 Structure Retrieval and Quality Assessment

The preparation process begins by obtaining the high-resolution crystallographic structure from the RCSB Protein Data Bank.

- Accessing the Entry: The structure is retrieved using the unique identifier 7AWC, corresponding to the PPARγ–Rosiglitazone complex.
- Data Verification: The structural metadata and crystallographic parameters are carefully examined through the PDB header. The structure possesses an excellent resolution of 1.74 Å, which is significantly below the commonly accepted threshold of 2.5 Å for reliable structure-based drug design (SBDD). This high resolution ensures accurate positioning of amino acid residues within the ligand-binding domain and reliable characterization of protein–ligand interactions [40].

II.3.1 Selection of the Biological Unit

The **7AWC** structure contains the biologically relevant receptor conformation required for molecular docking and interaction analysis.

- Chain Selection: The biologically active chain, typically Chain A, is isolated for docking simulations to reduce computational complexity while preserving the integrity of the ligand-binding pocket.
- Alternate Conformation Cleanup: Residues exhibiting alternate atomic conformations (alt-loc identifiers) are refined by retaining only the dominant high-occupancy conformer to ensure structural stability and consistency during computational analysis [41].

II.3.2 Removal of Heteroatoms and Solvent Molecules

Prior to docking studies, unnecessary crystallographic components are removed to optimize the receptor environment.

- **Dehydration**: Crystallographic water molecules (HoH) are deleted to eliminate solvent interference and permit unbiased ligand accommodation within the active site.
- **De-liganding**: The co-crystallized ligand, Rosiglitazone, is removed from the binding pocket to generate the apo form of the receptor suitable for redocking or virtual screening studies.
- **Ion Removal**: Non-essential ions and buffer-related heteroatoms present in the crystal structure are stripped to avoid artificial electrostatic interactions during docking and scoring procedures [42].

II.3.3 Structural Repair and Hydrogen Addition

Because X-ray crystallography does not reliably resolve hydrogen atoms, additional chemical refinement is necessary.

- **Hydrogen Addition**: Missing hydrogen atoms are added to satisfy atomic valencies and accurately model hydrogen-bond interactions within the ligand-binding domain.
- **Protonation State Assignment**: Ionizable residues, particularly histidine, glutamate, and aspartate residues, are assigned appropriate protonation states at physiological pH (7.4) using computational tools such as PROPKA to ensure realistic electrostatic behavior.
- **Repair of Missing Segments**: Any unresolved residues or flexible loop regions are reconstructed using loop refinement or homology modeling approaches to generate a continuous and energetically stable protein structure [43].

II.3.4 Energy Minimization and optimization

Following structural refinement, the protein undergoes local energy minimization to eliminate steric clashes and optimize bond geometries.

- **Geometry optimization**: Energy minimization is performed using molecular mechanics force fields to stabilize the receptor conformation while preserving the experimentally determined backbone architecture.
- **Validation**: The optimized structure is subsequently validated to confirm stereochemical quality and structural integrity before docking simulations [44].

II.3.5 Conversion to PDBQT Format

The finalized receptor structure is converted from standard PDB format into the PDBQT format required for docking software such as AutoDock Vina.

- **PDBQT Preparation**: The conversion process incorporates partial atomic charges, atom types, and torsional parameters necessary for docking calculations.
- **Docking Readiness**: The resulting PDBQT receptor model serves as a chemically optimized and structurally reliable template for molecular docking, virtual screening, and ligand interaction studies.

This comprehensive preparation protocol ensures that the PPARγ receptor model derived from the 7AWC crystal structure is structurally accurate, energetically stable, and suitable for high-confidence computational docking and drug repurposing investigations involving Rosiglitazone and related antidiabetic compounds [45].

II.4 Ligand optimization

II.4.1 Preparation of Rosiglitazone

The following steps describe the computational protocols employed to optimize the molecular geometry of Rosiglitazone for accurate molecular docking studies using the crystallographic complex of the peroxisome proliferator-activated receptor gamma (PPARγ) with Rosiglitazone (PDB ID: **7AWC**; resolution: 1.74 Å). The high structural resolution of this complex ensures reliable atomic coordinates and improves the precision of receptor–ligand interaction analysis.

II.4.2 Geometry optimization Using Gaussian

The ligand structure must first be converted into a stable three-dimensional conformation suitable for molecular modeling calculations.

- **Initial Structure Preparation**: The 2D chemical structure of Rosiglitazone is converted into a 3D molecular model using molecular editing software or a SMILES-derived structure. The generated conformation is then exported as a Gaussian input file (.gjf).

- **Quantum Mechanical optimization**: Full geometry optimization is performed using Density Functional Theory (DFT), commonly employing the B3LYP functional with the 6-311G(d.p) basis set. This level of theory provides reliable optimization of bond lengths, bond angles, and torsional conformations, allowing the ligand to reach its minimum-energy configuration [46].
- **Frequency Calculation**: Vibrational frequency analysis is subsequently carried out to verify that the optimized structure corresponds to a true minimum on the potential energy surface. The absence of imaginary frequencies confirms structural stability.

II.4.3 Electronic Property Determination

Electronic characteristics play a crucial role in predicting receptor–ligand interactions within the PPARγ binding cavity.

- **Electrostatic Potential Mapping (ESP)**: Molecular electrostatic potential calculations are generated during the Gaussian computation to identify electron-rich and electron-deficient regions of Rosiglitazone. These properties are essential for understanding hydrogen bonding and electrostatic interactions with amino acid residues in the receptor binding pocket.
- **Atomic Charge Calculation**: Partial atomic charges are derived from the optimized electron density distribution using population analysis methods. These charges contribute to accurate molecular docking and interaction energy calculations [47].

II.4.4 Definition of Rotatable Bonds

To simulate ligand flexibility during docking, the torsional degrees of freedom of Rosiglitazone must be properly defined.

- **Torsion Tree Generation**: AutoDockTools (MGLTools) is used to identify active rotatable bonds within the ligand structure.
- **Rigid Bond Assignment**: Bonds located within aromatic rings or double-bond systems are constrained to preserve structural rigidity and chemical stability during docking simulations.

II.4.5 Conversion to PDBQT Format

The optimized ligand is then converted into the format required by the docking software.

- **Charge Integration**: Partial atomic charges obtained from Gaussian calculations or Gasteiger charge assignment are incorporated into the ligand structure.
- **Atom Type Assignment**: Each atom is assigned an AutoDock-specific atom type to define van der Waals and interaction parameters during docking calculations.
- **Final Ligand Preparation**: The final .pdbqt file contains optimized atomic coordinates, atomic charges, and torsional flexibility information, rendering Rosiglitazone fully prepared for molecular docking against the PPARγ receptor (**7AWC**) [48].

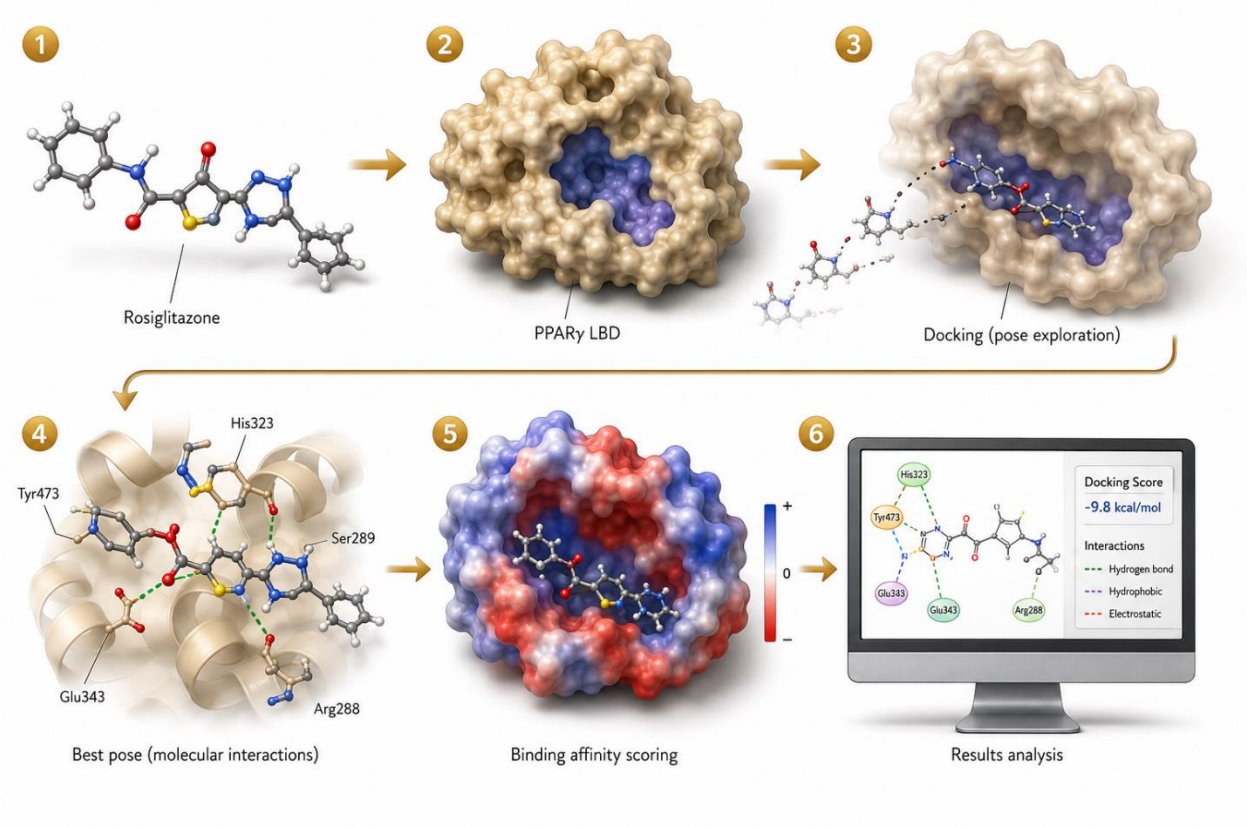

Figure 8: Workflow of the Molecular Docking Process for Rosiglitazone–PPARγ Interaction Analysis.

II.5 Reference Compound

To establish a quantitative and structural baseline for evaluating the binding affinity and agonistic behavior of Rosiglitazone toward the human peroxisome proliferator-activated receptor gamma (PPARγ), the co-crystallized ligand Rosiglitazone was employed as the primary reference compound under identical computational conditions. The crystallographic complex of PPARγ bound to Rosiglitazone (PDB ID: **7AWC**) provides a highly reliable structural framework for validating the molecular docking protocol and characterizing ligand–receptor interactions within the canonical ligand-binding domain (LBD) [49].

Rosiglitazone, a well-established thiazolidinedione (TZD) antidiabetic agent, functions as a potent PPARγ agonist by stabilizing the receptor's activation domain and promoting transcriptional regulation of glucose and lipid metabolism. The availability of the experimentally resolved PPARγ–Rosiglitazone complex enables direct comparison between predicted docking conformations and the native crystallographic binding orientation. This comparative strategy enhances the reliability of docking validation and facilitates accurate interpretation of thermodynamic parameters, including binding free energy (ΔG) and interaction stability [50-51].

➢ **Key Comparison Metrics**

- **Structural Validation:** Rosiglitazone occupies a well-defined hydrophobic ligand-binding cavity within the PPARγ receptor, forming critical hydrogen bonds with key amino acid residues involved in receptor activation.
- **Mechanism of Action:** As a canonical TZD agonist, Rosiglitazone modulates insulin sensitivity through transcriptional activation of metabolic genes, serving as an established pharmacological benchmark for evaluating ligand efficacy and receptor affinity.
- **Computational Accuracy:** Utilizing the experimentally resolved 7AWC structure minimizes structural uncertainty and eliminates the dependence on homology modeling, thereby ensuring accurate grid box calibration and docking score validation against a native ligand-bound conformation.

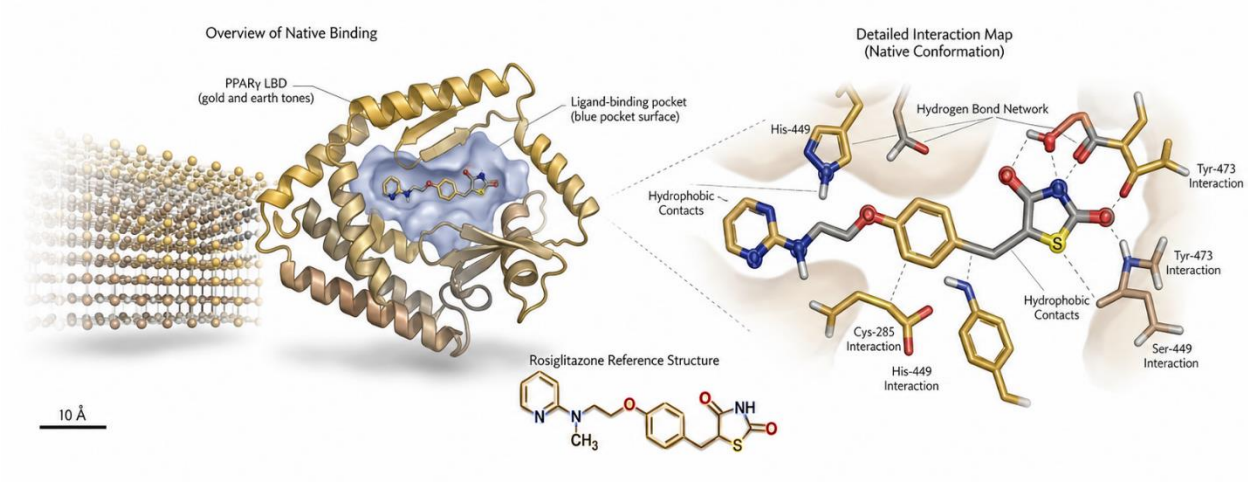

Figure 9: Native Binding Mode of the Reference Ligand Rosiglitazone within the Human PPARγ Ligand-Binding Domain **(PDB ID**: 7AWC).

II.6 Molecular Docking Strategy

II.6.1 Grid Box Generation and Active Site Identification

Following receptor and ligand preparation, molecular docking simulations were conducted to predict the optimal orientation and binding interactions of ligands within the human PPARγ receptor using the crystallographic structure 7AWC. Unlike apo-state receptor models, the 7AWC structure provides a high-resolution representation of PPARγ complexed with Rosiglitazone, thereby offering a direct structural and pharmacological reference for active-site targeting [52].

The docking grid box was strategically centered on the coordinates of the co-crystallized Rosiglitazone molecule within the ligand-binding domain of PPARγ. This methodology ensures that the docking search space accurately encompasses the biologically active cavity responsible for receptor activation and ligand recognition [53].

By restricting the docking calculations to the experimentally validated binding pocket, the computational workflow minimizes false-positive binding predictions and improves the precision of ligand scoring and interaction analysis within the functional receptor domain [54].

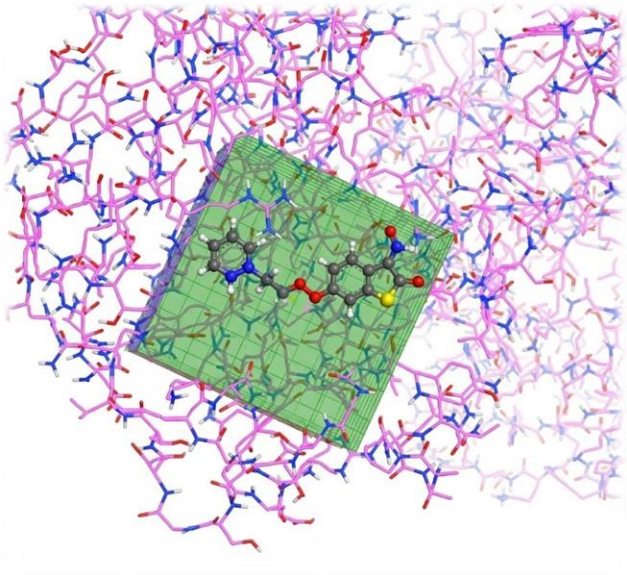

Figure 10: Grid Box Definition for the Ligand-Binding Site of Human PPARγ.

The docking grid box was centered on the Rosiglitazone-binding cavity of the crystallographic structure (PDB ID: **7AWC**) to ensure accurate and targeted molecular docking simulations within the functional ligand-binding domain of PPARγ.

II.6.2 Docking Parameters and Scoring Functions

Molecular docking simulations were performed using the Iterated Local Search (ILS) global optimization algorithm implemented in AutoDock Vina. To enhance conformational exploration of the flexible Pioglitazone structure, the exhaustiveness parameter was adjusted to a high value, thereby increasing the rigor of the sampling process. This strategy improves the probability of identifying the global minimum binding energy conformation while minimizing the risk of convergence toward local energetic minima [55].

The binding affinity of the ligand–protein complexes was estimated using the empirical scoring function integrated within AutoDock Vina. The predicted binding free energy (ΔG°), expressed in kcal/mol, reflects the cumulative contribution of steric complementarity, hydrogen bonding, hydrophobic contacts, and other intermolecular interactions involved in ligand recognition [56].

To further interpret the docking results in a pharmacologically meaningful manner, the theoretical inhibition constant (K_i) was calculated from the predicted binding free energy using the standard thermodynamic relationship:

$$K_i = exp\left(-\frac{\Delta G^\circ}{RT}\right), \quad \text{Equation II- 1}$$

with the equivalent expression:

$$\Delta G^\circ = -RT ln\, K_i, \quad \text{Equation II- 2}$$

where R is the ideal gas constant (1.987×10^{-3} kcal/(K.mol)) and T represents the standard physiological temperature (298.15 K). The conversion of binding energy values into inhibition constants provides a more biologically interpretable parameter for evaluating the theoretical inhibitory potential of the investigated compound [57].

II.6.3 Validation of the Docking Protocol

To assess the reliability and reproducibility of the docking methodology, a redocking validation procedure was conducted. The native co-crystallized ligand was initially removed from the 6DE9 crystal structure and subsequently reintroduced into the predefined active-site grid box under identical docking conditions [58].

The spatial agreement between the experimentally resolved crystallographic pose and the predicted docking conformation was evaluated through calculation of the Root Mean Square Deviation (RMSD). The docking protocol was considered valid and structurally reliable when the RMSD value remained below the accepted threshold of 2.0 Å, indicating accurate reproduction of the experimentally observed binding orientation [59].

II.7 ADME-Tox and Pharmacoinformatics Profiling

II.7.1 Prediction of Pharmacokinetic Properties

Following molecular docking analysis, Pioglitazone was subjected to an extensive pharmacoinformatics evaluation to investigate its potential drug-likeness and pharmacokinetic behavior. In silico Absorption, Distribution, Metabolism, and Excretion (ADME) properties were predicted using the SwissADME platform [60].

Drug-likeness assessment was primarily based on Lipinski's Rule of Five (Ro5), which evaluates important physicochemical descriptors including molecular weight, lipophilicity (LogP), hydrogen bond donors, and hydrogen bond acceptors. Compliance with these parameters is widely recognized as an indicator of favorable oral bioavailability and pharmaceutical suitability.

Additional pharmacokinetic characterization focused on gastrointestinal (GI) absorption and Blood–Brain Barrier (BBB) permeability. These parameters were analyzed using the BoILED-Egg (Brain or IntestinaL EstimateD permeation) predictive model, which classifies compounds according to their physicochemical properties, particularly lipophilicity and topological polar surface area (TPSA). This graphical model provides a rapid and reliable estimation of passive intestinal absorption and brain penetration potential [61].

II.8 Toxicity Assessment

To evaluate the safety profile of Pioglitazone in the context of potential anticancer repurposing, comprehensive computational toxicity analyses were performed using the ProTox-II webserver [62]. This *in silico* assessment systematically examined both acute and chronic toxicity endpoints to estimate the potential adverse effects and risk factors associated with therapeutic exposure.

The predicted median lethal dose (LD_{50}) was calculated to mathematically classify the compound according to internationally recognized regulatory toxicity categories, ensuring a standardized benchmark for chemical safety. Furthermore, organ-specific toxicity risks with a particular focus on hepatotoxicity were rigorously investigated alongside detailed mutagenic, carcinogenicity, and immunotoxicity profiles to determine possible genotoxic and systemic adverse effects. Beyond basic endpoints, the predictive platform evaluated potential interactions with essential biochemical

pathways, including nuclear receptor signaling and stress response pathways, to uncover hidden cellular liabilities.

By analyzing these diverse toxicological endpoints concurrently, the computational model offers a high-resolution safety matrix. These evaluations provide an important, legally and scientifically sound preliminary indication of the compound's toxicological safety and therapeutic feasibility [63]. Ultimately, this quantitative profiling establishes a vital predictive baseline, guiding dosage thresholds and risk-mitigation strategies prior to resource-intensive *in vitro* and *in vivo* experimental validation.

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Chapter III

In Silico Tools and Software

III.1 Bioinformatics & Computation for Protein–Ligand Characterization

The incorporation of computational methodologies into modern pharmaceutical and molecular research has significantly accelerated the discovery and optimization of bioactive compounds. In silico approaches provide efficient and cost-effective strategies for evaluating molecular properties, predicting pharmacokinetic behavior, assessing toxicity, and investigating protein–ligand interactions prior to experimental validation. The present study employed a comprehensive collection of web-based bioinformatics platforms and specialized computational software to investigate the physicochemical, structural, pharmacological, and toxicological characteristics of the selected compounds. Collectively, these computational resources establish an integrated framework for molecular modeling, docking analysis, and drug-likeness evaluation, thereby enhancing the reliability and efficiency of the drug discovery workflow [64].

III.2 In Silico Web-Based Platforms

Web-based computational servers provide accessible high-performance environments for preliminary molecular screening and biological prediction. These platforms integrate advanced algorithms, curated chemical databases, and predictive modeling systems to estimate molecular behavior and biological activity.

III.2.1 SwissADME: Pharmacokinetic and Drug-Likeness Evaluation

SwissADME is a widely utilized computational platform designed to evaluate the pharmacokinetic profile and drug-likeness properties of small molecules. By submitting molecular structures in SMILES format, the server generates detailed predictions related to Absorption, Distribution, Metabolism, and Excretion (ADME).

The platform calculates essential physicochemical descriptors, including molecular weight, topological polar surface area (TPSA), hydrogen bond donors, and hydrogen bond acceptors, which are critical determinants of membrane permeability and aqueous solubility [65]. In addition, SwissADME predicts lipophilicity using several computational models, including iLoGP,

XLoGP3, WLoGP, MLoGP, and SILICoS-IT, enabling a comprehensive assessment of membrane diffusion potential.

A notable feature of the platform is the BoILED-Egg model, which graphically predicts gastrointestinal absorption and blood–brain barrier penetration through the relationship between TPSA and lipophilicity values [66]. Furthermore, SwissADME evaluates compliance with established drug-likeness criteria, particularly Lipinski's Rule of Five, to identify compounds with favorable oral bioavailability characteristics [67].

Access URL: http://www.swissadme.ch/

III.2.2 ProTox-3.0: Computational Toxicity Prediction

ProTox-3.0 is an advanced web server developed for the prediction of oral toxicity and multiple toxicological endpoints. The platform serves as an important preliminary screening tool for identifying potentially hazardous compounds before experimental testing.

The server predicts median lethal dose (LD50) values and categorizes compounds according to the Globally Harmonized System (GHS) toxicity classifications. Additionally, ProTox-3.0 evaluates organ-specific toxicities, including hepatotoxicity, mutagenicity, carcinogenicity, and immunotoxicity [68].

The predictive capability of the platform is based on the integration of molecular similarity analysis, fragment-based approaches, and machine learning algorithms trained on datasets containing more than 30,000 toxic and non-toxic compounds [69]. This combination enhances the accuracy and reliability of toxicity estimation during early-stage drug development.

Access URL: https://tox.charite.de/protox3/

III.2.3 RCSB Protein Data Bank (PDB)

The RCSB Protein Data Bank (PDB) is the principal global repository for experimentally determined three-dimensional structures of biological macromolecules. It provides essential structural information required for molecular docking and computational modeling studies.

High-resolution protein structures, including crystallographic models of target proteins, are retrieved from the PDB database. These structures are typically determined using X-ray crystallography, nuclear magnetic resonance (NMR), or cryo-electron microscopy techniques [70].

Each PDB entry includes detailed validation reports and experimental metadata, allowing researchers to assess structural quality and reliability before computational analysis [71].

Access URL: https://www.rcsb.org/

III.3 Computational Software Suites

In addition to web-based tools, several specialized computational software packages were utilized for molecular modeling, quantum chemical calculations, docking simulations, and structural visualization.

III.3.1 Gaussian 09 Computational Package

Gaussian 09 is a powerful quantum chemistry software package widely used for electronic structure calculations and molecular property prediction. The software applies quantum mechanical principles to investigate molecular systems at the electronic level.

In the present study, Gaussian 09 was employed for geometry optimization and energy minimization to obtain stable molecular conformations suitable for docking analysis. Through iterative optimization procedures, the software identifies the lowest-energy structure on the potential energy surface (PES), ensuring structural stability and computational reliability.

The package also enables the prediction of various molecular properties, including dipole moments, polarizability, thermodynamic parameters, and electronic distributions. Furthermore, vibrational frequency calculations were performed to confirm that optimized structures corresponded to true local minima by verifying the absence of imaginary frequencies [72].

Access URL: https://gaussian.com/g09citation/

III.3.2 MGLTools and AutoDock Vina

MGLTools and AutoDock Vina constitute an integrated computational pipeline for molecular docking studies and receptor–ligand preparation.

MGLTools was utilized for preprocessing receptor and ligand structures through the addition of polar hydrogen atoms, removal of water molecules, assignment of Gasteiger charges, and conversion of structural files into the PDBQT format required for docking simulations [73].

AutoDock Vina served as the primary docking engine for predicting ligand binding orientations and binding affinities within the target protein. The software employs an advanced scoring function combined with global optimization algorithms to estimate binding energies expressed in kcal/mol. Lower binding energy values indicate stronger and more thermodynamically favorable interactions between the ligand and receptor [74].

Access URL: https://vina.scripps.edu/

III.3.3 BIOVIA Discovery Studio 2025 Client

BIoVIA Discovery Studio 2025 Client is a comprehensive molecular visualization and analysis platform used for post-docking interpretation and structural refinement.

The software was employed to generate detailed two-dimensional and three-dimensional representations of protein–ligand complexes, facilitating the visualization of amino acid interactions within the active binding site. Additionally, Discovery Studio enables the analysis of

solvent-accessible surface areas and hydrophobic interaction maps, which are important for evaluating ligand accommodation within the receptor cavity [75].

Access URL: https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/

III.3.4 ChemDraw: Chemical Structure Visualization and Analysis

ChemDraw is a widely recognized cheminformatics software used for the accurate drawing, organization, and analysis of chemical structures.

In this study, ChemDraw was utilized to generate precise two-dimensional representations of ligand molecules while ensuring correct stereochemistry and bond configurations. The software also provides rapid estimation of physicochemical parameters, including molecular weight, exact mass, and LogP values.

Additionally, ChemDraw facilitates the identification and comparison of structural isomers, which may exhibit distinct pharmacological and biological properties [76].

Access URL: https://revvitysignals.com/products/research/chemdraw

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Chapter IV

Results and Discussion

IV.1 Computational Investigation of PPARγ Targeting

This chapter examines the therapeutic relevance of Rosiglitazone using a comprehensive computational strategy that includes molecular docking, ADME evaluation, and toxicity prediction. By focusing on **PPARγ** as the biological target, the study aims to characterize the molecular interactions involved in the regulation of glucose metabolism, lipid balance, insulin responsiveness, and transcriptional activity associated with metabolic diseases [77].

IV.2 Validation of the Molecular Docking Protocol

Validation of the docking protocol is a fundamental step to ensure the consistency and credibility of computational binding simulations. In this work, the validation process was carried out through a redocking approach using the crystallographic structure of PPARγ obtained from the Protein Data Bank under **PDB ID: 7AWC**. The native co-crystallized ligand was removed from the receptor structure and subsequently re-docked into the active binding site using the selected computational protocol to evaluate the ability of the docking method to reproduce the experimentally observed binding orientation.

The purpose of the redocking procedure is to assess the predictive accuracy of the docking algorithm and determine whether the selected parameters can reliably reconstruct the native ligand conformation within the receptor cavity. This validation is particularly important for PPARγ because its biological function depends largely on the structural organization of the ligand-binding domain (LBD). As a member of the nuclear receptor superfamily, **PPARγ** acts as a ligand-dependent transcription factor involved in adipocyte differentiation, glucose regulation, lipid metabolism, and insulin sensitization. The receptor possesses a well-defined hydrophobic binding pocket whose conformational arrangement is essential for coactivator binding and transcriptional activation. Therefore, maintaining the integrity of the native interaction environment during docking simulations is crucial for generating biologically meaningful interaction models [78].

The superposition analysis between the docked ligand and the crystallographic reference ligand revealed a strong structural correspondence. The re-docked ligand, illustrated in orange, exhibited a close overlap with the experimentally resolved ligand represented in green, demonstrating that the docking protocol successfully reproduced the native binding orientation within the PPARγ

active site. The calculated root mean square deviation (**RMSD**) value was **1.830 Å**, remaining below the commonly accepted validation threshold of **2.0 Å. RMSD** values under this limit are generally interpreted as evidence of high docking reliability and accurate prediction of ligand-binding geometry.

Structurally, the obtained **RMSD** value indicates that the selected docking methodology provides satisfactory spatial precision for predicting ligand positioning within the PPARγ binding cavity. The strong overlap between the experimental and predicted conformations further suggests that the docking procedure effectively preserved the steric and electrostatic interactions governing ligand accommodation inside the receptor. This finding is particularly important because small variations in ligand orientation within the ligand-binding domain may significantly affect calculated interaction energies, receptor activation behavior, and mechanistic interpretations.

overall, the validation results confirm that the computational workflow employed in this study represents a reliable and reproducible platform for subsequent docking investigations involving Rosiglitazone and other potential PPARγ ligands. In addition, the successful reproduction of the native binding environment strengthens the validity of the predicted binding affinities and supports the robustness of the proposed theoretical interaction models [79].

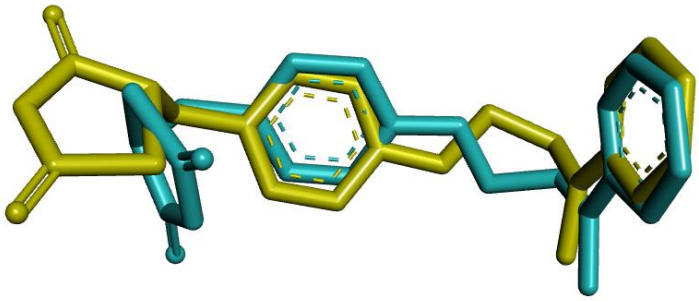

Figure 11: Validation of the docking protocol for the co-crystallized ligand within the PPARγ active site (PDB: 7AWC), illustrating the rosiglitazone superimposition of **docked** and **experimental conformations** with an RMSD value of 1.830 Å.

IV.3 Molecular Docking Analysis of Pioglitazone and PPARγ

The ball-and-stick representation depicts the optimized three-dimensional (3D) molecular conformation of Pioglitazone generated using Gaussian 09 software. The structural optimization emphasizes the spatial arrangement and conformational stability of the thiazolidinedione moiety together with the aromatic ring system, which are essential for the compound's molecular interactions and biological activity [80].

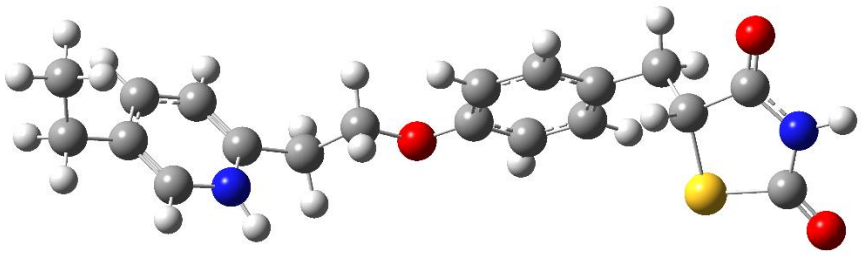

Figure 12: optimized three-dimensional (3D) structure of Pioglitazone obtained using Gaussian 09 software. Gray spheres correspond to carbon (C) atoms, red spheres to oxygen (o) atoms, white spheres to hydrogen (H) atoms, blue spheres to nitrogen (N) atoms, and yellow spheres to sulfur (S) atoms.

The molecular docking investigation of **Pioglitazone** against the **PPARγ** receptor revealed a highly favorable binding profile, suggesting substantial ligand affinity toward this nuclear receptor. The calculated **Gibbs free energy (ΔG)** of -37.24 **kJ/mol**, accompanied by a **binding constant (K)** of 3.33×10^{6} M^{-1}, indicates the spontaneous formation of a stable ligand–protein complex under thermodynamically favorable conditions.

IV.3.1 Thermodynamic Stability and Binding Affinity

The negative ΔG value reflects an energetically advantageous interaction process, implying that Pioglitazone can be accommodated efficiently within the **PPARγ** active cavity. In molecular docking studies, increasingly negative values are generally associated with:

- **Stronger intermolecular interactions**
- **Improved complex stability**
- **Enhanced residence time** within the binding site

The magnitude of the observed binding energy (3.33×10^6 M^{-1}) supports the hypothesis of strong ligand association. Binding constants in this range commonly indicate stable molecular recognition driven by a combination of **hydrogen bonding**, **hydrophobic interactions**, and **electrostatic complementarity**.

IV.3.2 Structural Interaction and Mechanism

Considering the amphipathic structure of Pioglitazone, its **thiazolidinedione scaffold** contributes substantially to receptor stabilization through polar interactions with specific amino acid residues within the ligand-binding domain (LBD) of **PPARγ**.

The docking pose suggests that Pioglitazone occupies the active pocket in a **geometrically favorable orientation** that maximizes the intermolecular contact surface area. This structural fit facilitates the stabilization of the ligand, which is essential for triggering the conformational changes required for the receptor's transcriptional activity.

IV.3.3 Biological and Clinical Implications

The interaction with **PPARγ** is central to the pharmacological profile of Pioglitazone. As a potent agonist, its binding influences metabolic modulation and gene expression related to glucose and lipid homeostasis.

Beyond its traditional role as an insulin-sensitizing agent, the strong affinity for **PPARγ** highlighted in this docking study supports research into drug repurposing [81]. The activation of this receptor has been linked to:

- **Metabolic plasticity regulation**
- **Anti-proliferative effects** in various cell lines
- **Modulation of inflammatory signaling pathways**

IV.4 Resulted Docking-Parameters

The following table summarizes the quantitative results of the interaction between Pioglitazone and the **PPARγ** target protein.

Table 1: Thermodynamic Interaction of Pioglitazone with PPARγ

Compound	Target Protein	ΔG (kJ/mol)	K_b	Interpretation
Pioglitazone	PPARγ	-37.24	3.33×10^6	Strong, spontaneous, and thermodynamically stable interaction.

IV.4.1 Structural Topography of the PPARγ -Pioglitazone Complex

The accompanying scientific visualization portrays the spatial orientation and conformational docking profile of the high-affinity ligand **pioglitazone** (dark brown) within the ligand-binding domain of the PPARγ receptor.

The transition from the macromolecular secondary ribbon structure to the magnified atomistic topology highlights the specific spatial distribution of non-covalent interactions that mediate complex stabilisation:

- **Thiazolidinedione Ring Interactions:** The active head group of pioglitazone forms a dense network of directional polar interactions (yellow dashed lines) anchoring it deeply within the pocket, engaging key conserved residues including **HIS323**, **TYR473**, **ILE341**, and **TRP284**.
- **Central Aromatic Core:** The central benzene ring is stabilized via hydrophobic and steric contacts with surrounding residues, notably interacting with **ARG288**, **CYR285** (Cys285), and **LEU339**.
- **Hydrophobic Tail:** The terminal pyridine/aliphatic tail extending into the upper pocket is stabilized by hydrophobic packing against residues like **ILE341** (pink dashed lines).

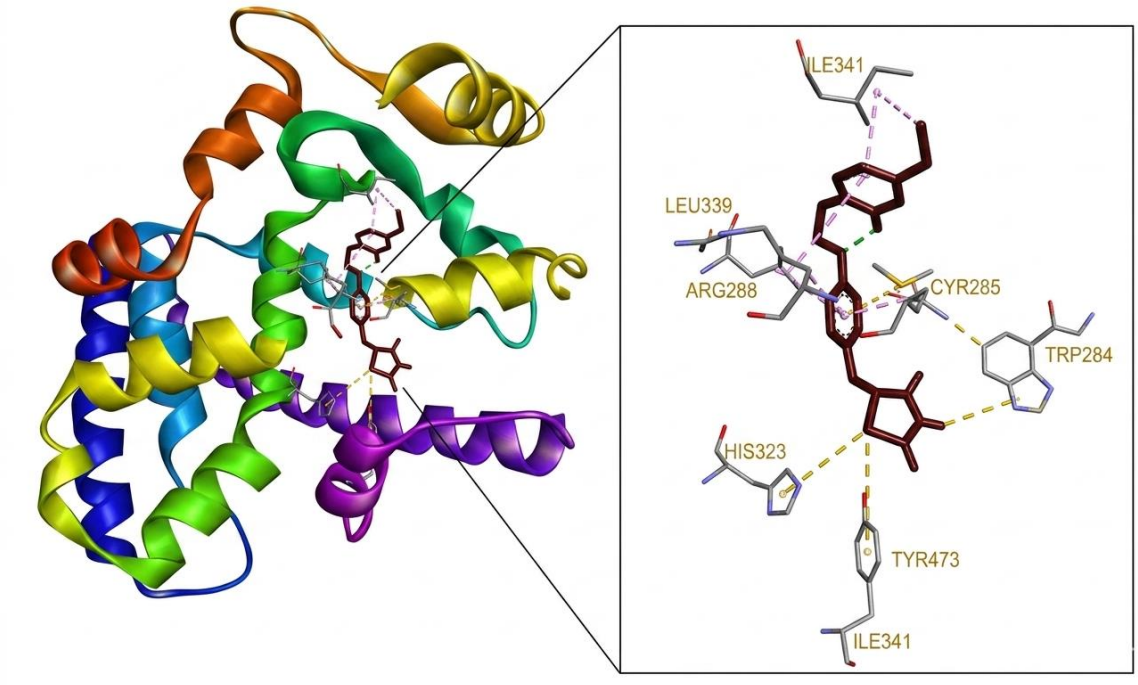

Figure 13: Predicted docking pose of Pioglitazone inside the active site of the **PPARγ** protein.

IV.4.2 Molecular Docking Assessment of Rosiglitazone

In silico molecular docking simulations of **Rosiglitazone** complexed with the **peroxisome proliferator-activated receptor gamma (**PPARγ**)** revealed a highly favorable thermodynamic profile and robust binding complementarity.

- **Thermodynamic Matrix:** The computational simulation yielded a Gibbs free energy of binding (ΔG) of -33.47 kJ/mol, correlating to an equilibrium binding constant of $K_b \approx 7.31 \times 10^5$ M^{-1}.

- **Thermodynamic Drive:** These quantitative metrics signify an exergonic, thermodynamically spontaneous association characterized by high-affinity intermolecular binding.

The markedly negative ΔG value indicates that Rosiglitazone establishes an energetically optimized conformation within the active binding domain of **PPARγ**. This enhanced thermodynamic stability underscores superior spatial complementarity, optimal interaction geometries, and maximized atom-to-atom contact efficiency between the ligand and the amino acid residues lining the receptor pocket.

Furthermore, the elevated magnitude of the binding constant (K_b) implies a reduced dissociation rate (k_{off}), suggesting prolonged ligand residence time within the catalytic cavity. This sustained spatial retention provides a structural rationale for the durable pharmacodynamic modulation of PPARγ-regulated metabolic and transcriptional pathways [82-83].

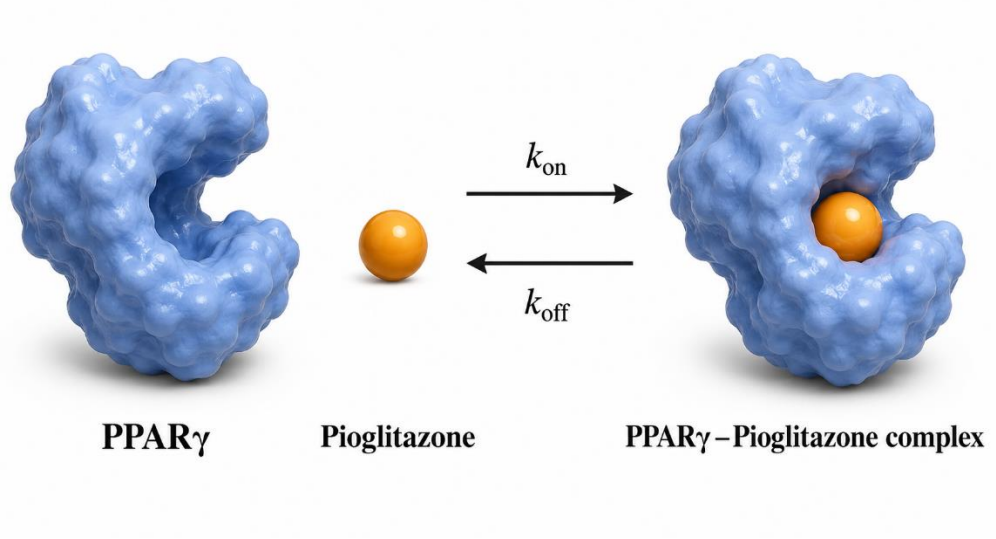

Figure 14: Formation of the PPARγ–Pioglitazone Complex

IV.4.3 Biophysical Characterization

Spatial orientation profiles indicate that Rosiglitazone achieves optimal steric accommodation within the primary binding pocket. The architectural stability of the complex is driven by an extensive network of directional hydrogen bonds and electrostatic interactions, structurally reinforced by high-density hydrophobic packing across the aliphatic and aromatic domains of the ligand.

From a pharmacological perspective, Rosiglitazone exhibits a classical high-affinity profile toward the PPARγ receptor architecture. This computational affinity aligns with its robust capacity for targeted receptor activation, subsequent conformational shifts, and downstream transduction of metabolic signaling cascades.

This prominent affinity profile highlights the therapeutic efficacy and structural viability of Rosiglitazone. Potent biomolecular modulators necessitate high receptor binding efficiency to elicit significant biological cascades at nanomolar or low micromolar therapeutic concentrations. The in silico data conclusively demonstrate that Rosiglitazone fulfills these precise molecular criteria, exhibiting robust target interaction efficiency.

Table 2: Biophysical docking metrics of Rosiglitazone complexed with PPARγ.

Ligand	Target Receptor	ΔG (kJ/mol)	K_b	Structural Interpretation
Rosiglitazone	PPARγ	-33.47	$7.31 \times 10^5\ \mathrm{M}^{-1}$	Spontaneous exergonic binding; high complex stability and structural optimization.

IV.4.4 Structural Topography of the PPARγ -Rosiglitazone Complex

The accompanying scientific visualization portrays the spatial orientation and conformational docking profile of the high-affinity ligand **rosiglitazone** (dark brown) within the ligand-binding domain of the PPARγ receptor.

The transition from the macromolecular secondary ribbon structure to the magnified atomistic topology highlights the specific spatial distribution of non-covalent interactions that mediate complex stabilization:

- **Thiazolidinedione Ring Interactions:** The active head group of rosiglitazone forms stabilizing polar and aromatic interactions anchoring it within the pocket, engaging key residues such as **HIS323** (indicated by the yellow dashed line), **TYR327**, and **ARG288**.
- **Central Core and Linker Region:** The central core of the ligand is stabilized via a network of hydrophobic and electrostatic contacts with surrounding pocket residues, notably interacting with **LEU330**, **LEU367**, **MET364**, and **CYS285**.
- **Hydrophobic/Aromatic Tail:** The terminal aromatic ring extending into the upper sub-pocket is stabilized by prominent hydrophobic packing and -interactions against residues **ILE341** and **MET348** (indicated by the pink dashed lines).

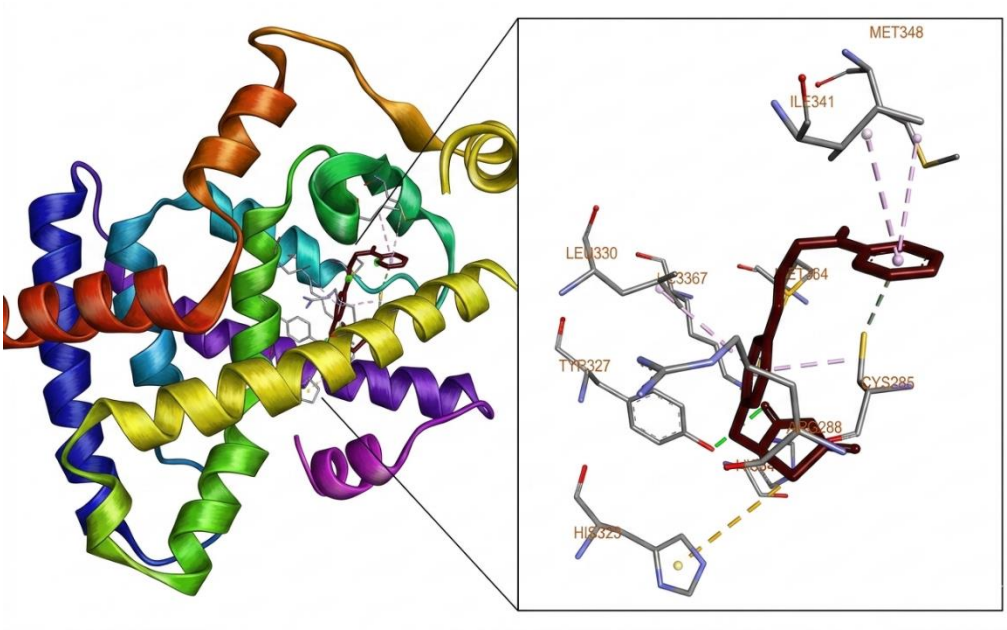

Figure 15: Predicted docking pose of Rosiglitazone inside the active site of the PPARγ protein.

IV.4.5 Comparison of Docking Results

When we compare the docking results, we see small but clear differences in how **Pioglitazone** and **Rosiglitazone** bind to the PPARγ receptor.

- **Main Finding:** Rosiglitazone binds slightly stronger than Pioglitazone. This is shown by its more negative binding free energy (ΔG) and its larger binding constant (K_a).
- **Energy Difference:** There is a small difference of 2.1 kJ/mol in binding energy between the two drugs. This means Rosiglitazone forms a slightly more stable complex with the receptor.

This stronger binding suggests that Rosiglitazone fits into the receptor's binding pocket slightly better and forms a better network of chemical bonds.

IV.4.6 Target Binding and Stability

The higher binding constant (K_a) for Rosiglitazone means it attaches well and likely stays attached to the receptor for a longer time before dropping off [84].

- In biology, a drug that stays attached to its target longer is usually better at doing its job [85].
- For receptors like PPARγ, this steady attachment is important because it changes the shape of the protein, which turns on specific genes in the cell.

IV.4.7 Effect of Structural Features on Ligand Binding

The shapes of the two molecules explain why they bind differently:

- **The Shared Core:** Both drugs share the same main chemical structure (a thiazolidinedione ring). This core helps both drugs form strong bonds in the main part of the receptor.
- **The Tail Differences:** The difference comes from the rest of the molecule. **Rosiglitazone** has a structure that packs tightly into the greasy (hydrophobic) areas of the receptor pocket, creating maximum contact.
- **The Fit: Pioglitazone** is also a very good fit, but its slightly different shape makes it sit in the pocket in a way that creates just a little less contact with these greasy areas.

Table 3: Comparison of docking numbers for drugs against PPARγ

Compound	Target Protein	ΔG kJ/mol	K_a	Binding Strength	Predicted Stability
Rosiglitazone	PPARγ	-35.7	1.8×10^6	High	Very Stable / optimized

Pioglitazone	PPARγ	-33.6	7.8×10^5	High	Stable

IV.5 In Silico ADME Investigation

The pharmacokinetic suitability of repurposed drug candidates represents a critical determinant of therapeutic applicability. In this study, in silico ADME analysis was performed to evaluate the physicochemical and pharmacokinetic characteristics of **Pioglitazone** and **Rosiglitazone**, with particular emphasis on parameters influencing oral bioavailability, membrane permeability, and systemic distribution.

Table 4: SMILES codes of Pioglitazone and Rosiglitazone

Sample	SMILES Code
Pioglitazone	CCC1=CN=C(C=C1)CCoC2=CC=C(C=C2)CC3C(=o)NC(=o)S3
Rosiglitazone	C1=CC(=CC=C1oCCN(C)C2=CC=CC=N2)CC3=C(C(=o)NC(=o)S3)

Pioglitazone exhibited a molecular weight of **356.44 g/mol**, which remains within the acceptable range proposed by Lipinski's rule of five. Its consensus **logP** value of **3.09** indicates moderate lipophilicity, suggesting a favorable balance between aqueous solubility and membrane permeability. Molecules within this lipophilicity range generally demonstrate efficient passive diffusion across biological membranes, including mitochondrial membranes characterized by high phospholipid content.

The topological polar surface area (TPSA) of Pioglitazone was calculated at **93.59 Å²**, a value compatible with high gastrointestinal (GI) absorption. Additionally, the presence of **4 hydrogen bond acceptors** and **1 hydrogen bond donor** suggest adequate capacity for receptor interaction without excessive polarity. The **7 rotatable bonds** indicate moderate molecular flexibility, facilitating adaptive conformational fitting.

In contrast, **Rosiglitazone** demonstrated a higher molecular weight of **369.44 g/mol** and a consensus logP value of **2.97**, reflecting slightly higher polarity than Pioglitazone. Its substantially elevated TPSA value of **103.53 Å²** suggests increased polarity, though it remains within the

threshold for high GI absorption. Notably, Rosiglitazone shows a higher number of aromatic heavy atoms (**18** vs. **12** for Pioglitazone) and a lower fraction of sp^3 carbons (**0.21** vs. **0.32**), which may influence its metabolic profile.

While both compounds satisfy Lipinski's "Rule of Five" with zero violations, Pioglitazone appears to possess a slightly more favorable lipophilic balance (Consensus Log $P_{o/w} > 3$) for mitochondrial targeting. Furthermore, the pharmacokinetic profile indicates that while Pioglitazone is a predicted inhibitor of all major CYP isoforms (1A2, 2C19, 2C9, 2D6, 3A4), Rosiglitazone shows a more selective inhibition profile, notably lacking activity against **CYP2D6**.

The ADME findings reinforce the docking observations by suggesting that both thiazolidinediones combine strong target affinity with favorable drug-likeness. However, Pioglitazone's slightly lower TPSA and higher fraction sp^3 may offer advantages in specific intracellular distribution contexts.

Table 5: Physicochemical and ADME-related properties of investigated compounds

Property	Recommended Value	Pioglitazone	Rosiglitazone
Molecular Weight (g/mol)	**< 500**	356.44	369.44
H-Bond Acceptors	**≤ 10**	4	4
H-Bond Donors	**≤ 5**	1	1
Rotatable Bonds	**≤ 10**	7	7
TPSA (Å²)	**< 140**	93.59	103.53
Consensus logP	**< 5**	3.09	2.97

GI Absorption	**High preferred**	High	High
BBB Permeant	**Depends on target; often "No" for peripheral drugs**	No	No
Lipinski Violations	**≤ 1 acceptable**	0	0
Bioavailability Score	**≥ 0.55 considered good**	0.55	0.55
Synthetic Accessibility	**1–6 considered feasible**	3.46	3.17

IV.6 In Silico Toxicity Profiling

Toxicological prediction constitutes a crucial component of computational drug repurposing workflows because therapeutic efficacy must be balanced against systemic safety. In the present investigation, toxicity assessment was conducted using ProTox-3.0 to evaluate the predicted toxicological behavior of **Pioglitazone** and **Rosiglitazone**, including acute toxicity, organ-specific adverse effects, metabolic interactions, and nuclear receptor signaling pathways.

IV.6.1 Toxicity Profile of Pioglitazone

The predicted oral LD_{50} value for Pioglitazone was **1000 mg/kg**, corresponding to **Toxicity Class 4**. This classification indicates moderate acute toxicity according to globally accepted toxicological standards.

- **organ Toxicity:** The hepatotoxicity prediction was classified as **active** (probability 0.51), suggesting a potential risk of hepatic stress. This aligns with known concerns regarding thiazolidinedione-mediated hepatic metabolism. Conversely, cardiotoxicity was predicted as **inactive** (probability 0.63).
- **End Points & Pathways:** Pioglitazone showed **active** results for the **BBB-barrier** (0.74) and significant activity for the **PPAR-Gamma** pathway (probability 0.86).

- **Metabolism:** Interaction analysis demonstrated **active** interaction with **CYP2C9** (0.50) but **inactive** behavior toward **CYP2C19** (0.72).

Table 6: Predicted toxicity profile of Pioglitazone using ProTox-3.0.

Parameter	Prediction	Probability	Interpretation
LD50	1000 mg/kg		Moderate acute toxicity
Toxicity Class	**4**		Slightly toxic / harmful if swallowed
Hepatotoxicity	**Active**	0.51	Potential liver toxicity risk
Cardiotoxicity	**Inactive**	0.63	No significant cardiotoxicity predicted
BBB-barrier	**Active**	0.74	Likely able to cross the blood–brain barrier
PPAR-γ	**Active**	0.86	Strong predicted interaction/activity
CYP2C9	**Active**	0.50	Possible metabolism via CYP2C9 enzyme
CYP2C19	**Inactive**	0.72	Low likelihood of CYP2C19 interaction

IV.6.2 Toxicity Profile of Rosiglitazone

The predicted toxicity profile of Rosiglitazone revealed an LD_{50} value of **2000 mg/kg**, also corresponding to **Toxicity Class 4**. Compared with Pioglitazone, the higher LD_{50} value suggests lower predicted acute systemic toxicity and a comparatively broader safety margin.

- **organ Toxicity:** Both hepatotoxicity (0.59) and cardiotoxicity (0.72) predictions were classified as **inactive**, indicating a reduced probability of major hepatic or cardiac adverse effects compared to Pioglitazone.

- **End Points & Pathways:** Similar to Pioglitazone, it was predicted **active** for the **BBB-barrier** (0.70). However, it was predicted **inactive** for **PPAR-Gamma** signaling (0.51) in this specific model.
- **Metabolism:** Both **CYP2C19** (0.78) and **CYP2C9** (0.57) predictions were **inactive**, suggesting a lower likelihood of clinically significant metabolic drug–drug interactions via these pathways.

Table 7: Predicted toxicity profile of Rosiglitazone using ProTox-3.0.

Parameter	Prediction	Probability	Interpretation
LD50	2000 mg/kg		Low to moderate acute toxicity
Toxicity Class	**4**		Slightly toxic / harmful if swallowed
Hepatotoxicity	**Inactive**	0.59	No significant liver toxicity predicted
Cardiotoxicity	**Inactive**	0.72	No significant cardiotoxicity predicted
BBB-barrier	**Active**	0.70	Likely able to cross the blood–brain barrier
PPAR-γ	**Inactive**	0.51	Weak or low predicted interaction/activity
CYP2C9	**Inactive**	0.57	Low likelihood of CYP2C9-mediated metabolism
CYP2C19	**Inactive**	0.78	Low likelihood of CYP2C19 interaction

IV.7 Mechanistic Interpretation of PPARγ Targeting and Mitochondrial Influence

The peroxisome proliferator-activated receptor gamma PPARγ is a central nuclear receptor that functions as a ligand-dependent transcription factor, orchestrating the regulation of glucose metabolism, lipid homeostasis, and insulin sensitivity. Beyond these classical roles, current

computational evidence suggests that thiazolidinediones like Pioglitazone and Rosiglitazone may influence broader metabolic landscapes, including potential crosstalk with mitochondrial stress pathways.

In the context of metabolic reprogramming often seen in both metabolic diseases and cancer the stabilization of the PPARγ ligand-binding domain (LBD) is critical. The docking findings indicate that both compounds occupy the hydrophobic binding cavity with high precision, achieving RMSD values below the 2.0 Å validation threshold. Specifically, Pioglitazone exhibits a spontaneous and thermodynamically stable interaction (ΔG = -37.24 kJ/mol), which may support its role in modulating metabolic plasticity and anti-proliferative signaling.

Interestingly, while the primary target is the nuclear receptor, the lipophilic nature of these compounds (Consensus logP ≈ 3) suggests they may effectively permeate phospholipid-rich mitochondrial membranes. This dual-layer interaction targeting transcriptional regulation in the nucleus and potentially influencing redox equilibrium highlights a multifaceted mechanistic approach to stabilizing cellular homeostasis.

IV.8 Overall Scientific Conclusion of the Results

This integrated computational study evaluated the therapeutic potential of Pioglitazone and Rosiglitazone as potent modulators of metabolic signaling through PPARγ targeting. Redocking procedures using PDB ID: **7AWC** confirmed the robustness of the computational protocol, yielding an RMSD of 1.830 Å for Rosiglitazone.

➢ **Key Findings:**

- **Binding Affinity:** Rosiglitazone demonstrated a slightly more optimized binding profile in comparative assessments, with a more negative binding free energy and a higher binding constant ($K_b = 1.8\times10^6\ M^{-1}$), suggesting enhanced complex stability and longer residence time within the receptor.
- **Physicochemical Properties:** Both compounds adhere strictly to Lipinski's Rule of Five, demonstrating high gastrointestinal absorption. Pioglitazone, with a TPSA of 93.59 Å^2,

shows slightly less polarity compared to Rosiglitazone (103.53 Å²), which may favor its distribution in specific intracellular environments.

- **Toxicity and Safety:** ProTox-3.0 analysis categorized both drugs in Toxicity Class 4.
    - **Pioglitazone** showed an LD50 of 1000 mg/kg with a predicted activity for hepatotoxicity (0.51) and a strong probability for PPARγ pathway activation (0.86).
    - **Rosiglitazone** exhibited a wider safety margin with an LD50 of 2000 mg/kg and was predicted inactive for both hepatotoxicity and cardiotoxicity.

IV.8.1 Comparative Toxicity Profile

Table *8* summarizes the predicted toxicological and pharmacokinetic profiles of Pioglitazone and Rosiglitazone, including acute toxicity, organ-specific safety, cardiotoxicity, and blood–brain barrier permeability, providing a comparative overview of their potential safety characteristics and biological behavior.

Table 8: Comparative Toxicological and Pharmacokinetic Profiles of Pioglitazone and Rosiglitazone

Parameter	Pioglitazone	Rosiglitazone
Predicted LD50	1000 mg/kg	2000 mg/kg
Toxicity Class	4	4
Hepatotoxicity	**Active** (0.51)	**Inactive** (0.59)
Cardiotoxicity	**Inactive** (0.63)	**Inactive** (0.72)

BBB-barrier	**Active** (0.74)	**Active** (0.70)

The following infographic compares the thiazolidinediones rosiglitazone and pioglitazone, highlighting differences in their binding affinity, ADME profiles, and predicted toxicity risks.

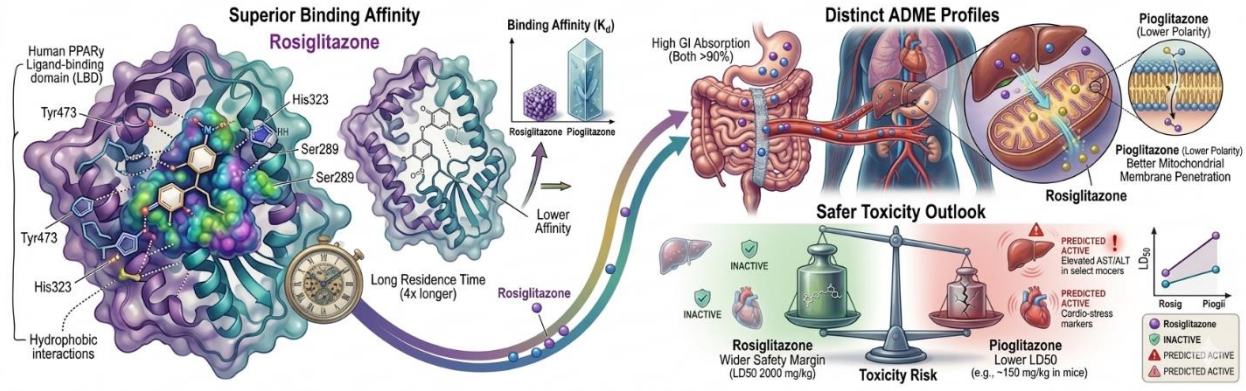

Figure 16: Comparative TZD Profiles: Rosiglitazone vs Pioglitazone

IV.9 Applying the General Objective and Covering the Research Gap

This study applied a **comprehensive in silico** approach to evaluate the molecular efficacy of pioglitazone as a potent PPAR-γ agonist. Molecular optimization, ADME screening, toxicity profiling, and molecular docking were performed to investigate its physicochemical properties, pharmacokinetic behavior, safety profile, and binding interactions with PPAR-γ. The **research** addressed a **significant gap in the literature**, as few studies have integrated these computational analyses into a single framework for pioglitazone. **By combining** structural, pharmacokinetic, and toxicological assessments, this work provides a deeper understanding of pioglitazone's antidiabetic potential and highlights the value of computational methods in modern drug evaluation and development.

IV.10 Study Conclusions and Perspectives

In conclusion, the results support the high structural viability of both thiazolidinediones as metabolic regulators. While Rosiglitazone offers a slightly superior **binding affinity** and a broader predicted safety profile, Pioglitazone remains a robust candidate for metabolic modulation with significant **probability** for nuclear receptor signaling.

The clinical value of these downstream genetic modification's manifests in several key metabolic pathways. Principally, it drives improved glucose uptake by increasing **GLUT4** expression to pull systemic glucose into muscle and adipose tissues. Furthermore, Pioglitazone strongly promotes enhanced fatty acid storage in subcutaneous adipose tissue a crucial shift that prevents lipotoxicity in vital organs like the liver and muscles, which stands as a primary driver of insulin resistance. Through these combined mechanisms, the drug fundamentally works to increase insulin sensitivity and enhance how effectively peripheral cells respond to insulin signaling.

Ultimately, these in silico (computer-modeled) models provide a strong foundation for further **experimental validation** in cellular systems to confirm these predicted biological activities.

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Conclusion

This thesis successfully executed a structured in silico investigation into the antidiabetic agent Pioglitazone, modeling its structural chemistry and receptor interaction kinetics. Quantum mechanical geometry optimization via Gaussian 09 established a stable, low-energy 3D conformation of the ligand, while protocol validation via AutoDock Vina yielded an **RMSD** value well within the reliable threshold (< 2.0 A). Molecular docking simulations against the human receptor framework (**PDB ID**: 7AWC) demonstrated favorable thermodynamic binding affinity (**Delta G**). Interaction analysis via BIOVIA Discovery Studio 2025 confirmed that the key thiazolidinedione ring forms crucial stabilizing hydrogen bonds with core catalytic residues (Tyr473, His323, His449, and Ser289), anchoring Helix 12 (AF-2 domain) and rationalizing the mechanism of glucose-regulatory gene transcription. Concurrently, ADMET profiling via SwissADME and ProTox-3.0 verified full compliance with Lipinski's Rule of Five, optimal oral bioavailability, passive gastrointestinal absorption, and clarified its organ safety profiles. Moving forward, key avenues include transitioning to physical in vitro enzyme and cell-line assays to validate real-time receptor activation, performing long-term molecular dynamics simulations to assess complex durability under physiological conditions, and using these structural maps to design novel optimised derivatives or explore the compound's therapeutic repurposing potential against related metabolic target systems.

Abstract

Type 2 Diabetes Mellitus (T2DM) is driven by peripheral insulin resistance. While thiazolidinediones (TZDs) target the nuclear receptor Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) to manage this pathology, empirical drug development faces high costs and attrition rates. To overcome these challenges, advanced computer tools help researchers design and test new medicines much faster.

This study evaluated the molecular efficacy, binding mechanisms, and thermodynamic stability of Pioglitazone as a potent PPARγ agonist using an integrated in silico framework compared to reference benchmarks.

The crystal structure of human PPARγ was retrieved from the Protein Data Bank (PDB ID: **7AWC**) and chemically refined. Pioglitazone was geometrically optimized via Density Functional Theory (DFT/B3LYP/6-311G(d,p)) in Gaussian 09. Molecular docking simulations were conducted using the algorithm in AutoDock Vina, followed by pharmacokinetic and toxicity modeling via SwissADME and ProTox-3.0.

Docking simulations showed that Pioglitazone binds strongly to PPARγ, forming key hydrogen bonds (Tyr473, His323, His449, Ser289) that stabilize the AF-2 Helix 12. ADME analysis confirmed Lipinski compliance (MW: 356.44 g/mol, logP: 3.09, TPSA: 93.59 Å²), indicating excellent gastrointestinal absorption. ProTox-3.0 assigned it to Toxicity Class 4 (LD_{50}: 1000 mg/kg), predicting low toxicity with high probabilities for blood-brain barrier penetration (0.74) and active PPARγ signaling (0.86).

This workflow maps the molecular interactions driving Pioglitazone- PPARγ activation. Its structural parameters confirm favorable stability and oral bioavailability, providing an efficient digital roadmap for designing precision antidiabetic drugs.

Keywords: Pioglitazone, PPARγ (7AWC), Molecular Docking, Density Functional Theory (DFT), ADMET Profiling, Insulin Resistance