

Design, Synthesis, and In Silico Evaluation of Novel Imidazolidine-2,4-dione Derivatives as Selective GSK-3 β Inhibitors.

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ABSTRACT

Diabetes mellitus (DM) is a lifelong metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of DM is increasing at a frightening rate to over 783 million cases projected by 2045. Type 2 diabetes mellitus (T2DM), the most common form, is a companion of insulin resistance, glucose uptake defect, and impaired glycogen metabolism. Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase involved in the central regulation of insulin action by its phosphorylation and inhibition of glycogen synthase and, thus, in hyperglycemia. Inhibition of GSK-3 has been viewed as a therapeutic strategy to amplify insulin sensitivity and offer better Glycemic control.

The current research focuses on the synthesis and characterization of new imidazolidine-2,4-dione derivatives as prospective GSK-3 inhibitors. Two-step synthesis pathway was chosen starting from benzoyl chloride analogues which were treated with p-hydroxybenzaldehyde to give intermediate products, followed by condensation with hydantoin derivatives. Synthesized compounds were characterized through thin layer chromatography (TLC), melting point measurement, and spectroscopic analysis (IR, NMR, and mass spectrometry). Molecular docking experiments performed by AutoDock Vina were performed to ascertain binding capacity to GSK-3 β . Some of the compounds possessed favorable binding scores, such as the o-phenyl, p-phenyl, and m-triethylammonium substituted compound, which shows great promise as GSK-3 inhibitors.

These findings point to imidazolidine-2,4-dione derivatives with selective aromatic and heterocyclic substitutions as good lead compounds for anti-diabetic drug research and development. Further studies in vitro and in vivo would be required to ascertain their pharmacological activity and safety profiles.

Keywords: Diabetes mellitus, GSK-3 inhibitors, Imidazolidine-2,4-dione, Hydantoin derivatives, Docking studies, Anti-diabetic drug design

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INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic syndrome with chronic hyperglycemia caused by an insulin secretory defect, an insulin receptor or postreceptor defect, or both. It is also complicated by devastating microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (atherosclerosis, cardiovascular disease) complications responsible for most of the morbidity and mortality globally. Of the 537 million adults who had diabetes in 2021, as estimated by the International Diabetes Federation (IDF), it is expected to rise to 643 million by 2030 and 783 million by 2045. They predominantly reside in low- and middle-income nations, substantiating worldwide imbalance between diabetes burden and access to health care.

The most common type of diabetes is type 2 diabetes mellitus (T2DM), which is characterized by inadequate insulin-sensitive peripheral tissue utilization (i.e., skeletal

muscle, adipose tissue, and liver), and inadequate insulin secretion by pancreatic β -cells to compensate for the insulin resistance. Chronic insulin resistance leads to reduced glucose utilization and increased hepatic glucose production, resulting in the persistence of hyperglycemia (high blood sugar). Multiple molecular mechanisms have been identified in T2DM pathophysiology, and among these, glycogen synthase kinase-3 (GSK-3) is a critical regulatory target in glycogen metabolism and insulin signaling pathway.

GSK-3 is comprised of two isoforms, GSK-3 α and GSK-3 β , which are derived from different genes yet possess a high degree of structural similarity. In the insulin signaling cascade, GSK-3 phosphorylates and deactivates glycogen synthase and ultimately prevents the synthesis of glycogen. Moreover, GSK-3 β inhibits the action of insulin by phosphorylating IRS-1. Overactive GSK-3 β has been identified in insulin-resistant conditions, as well as the

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inhibition of GSK-3 as a therapy to restore insulin sensitivity and improve glucose homeostasis. [1-10]

Hydantoin (Imidazolidine-2,4-dione) is a heterocyclic scaffold which possesses a wide spectrum of pharmacological activities such as anticonvulsant, antimicrobial, anti-inflammatory and anticancer. The core of the hydantoin potassium channel modulator was derivatized at this position to give rise to analogs with potent kinase inhibitory activity, including GSK-3 (inhibition $\leq 50\%$ at 1 μM). Modification of the hydantoin scaffold with aromatic or heterocyclic substituents could tune binding interactions at target enzymes and change potency and selectivity.

Despite the availability of a variety of anti-diabetic drugs, the management of T2DM remains challenging due to side effects, moderate response duration, and compliance. Even more selectively inhibitory GSK-3 inhibitors with favorable pharmacokinetic characteristics may be a new approach to diabetes therapy. The present research aims to synthesize, characterize, and perform in silico docking study of novel imidazolidine-2,4-dione derivatives as a GSK-3 β inhibitor in the direction towards next-generation anti-diabetic drugs. [11-14]

2. LITERATURE REVIEW

2.1 Introduction to Diabetes Mellitus

Diabetes mellitus (DM) has been a condition that has been actively studied in the last hundred years, and clinical differentiation between type 1 and type 2 diabetes only became established in mid-20th century. Type 1 diabetes is essentially an autoimmune destruction of pancreatic β -cells, but type 2 diabetes mellitus (T2DM) is a condition due to insulin resistance with β -cell failure. The persistent hyperglycemia in DM leads to oxidative stress, inflammation, and non-enzymatic glycation of proteins that result in complications in the long term (American Diabetes Association, 2022). The global prevalence, even with advancements in pharmacotherapy, is still on the increase mainly attributed to physical inactivity, obesity, and population aging. [15-16]

2.2 Glycogen Synthase Kinase-3 (GSK-3): Structure and Function

GSK-3 is a serine/threonine kinase that is ubiquitously expressed and was originally characterized for its function in the phosphorylation of glycogen synthase, the key enzyme in glycogen metabolism (Embi et al., 1980). It occurs as two isoforms—GSK-3 α (51 kDa) and GSK-3 β (47 kDa)—under different genes with 98% sequence identity in their kinase domains. Both isoforms are constitutively active at basal levels and mainly regulated by inhibitory phosphorylation by upstream kinase.

Metabolism: Regulation of glycogen synthesis in liver and muscle.

Cell signaling: Modulation of Wnt/ β -catenin, Hedgehog, and Notch pathways.

Protein synthesis: Control via phosphorylation of translation initiation factors. [17-18]

2.3 GSK-3 and Type 2 Diabetes Mellitus

GSK-3 and Type 2 DM GSK-3 β has been identified as an important negative regulator of insulin signaling under

normal physiological conditions and also in pathological context of T2DM. GSK-3 β phosphorylates IRS-1 at serine residues, which decreases its ability to transmit the signal of insulin via the PI3K/Akt pathway (Nikoulina et al., 2000). The overactivation of GSK-3 β was observed, which may root in a reduction process for glycogen synthesis and usage dysfunction of glucose metabolism in insulin-resistant skeletal muscle. In some preclinical studies, the inhibition of GSK-3 β has been shown to increase insulin sensitivity and glycogenesis and ameliorate glucose tolerance (Cline et al. 2002).

Numerous chemical scaffolds, which include indirubins, maleimides, thiazoles, pyrazines have been recognized in the seek for effective and selective GSK-3 inhibitors. Although lithium chloride one of the first described GSK-3 inhibitors from a pharmacological standpoint, revealed potential as a proof-of-concept agent (e.g., supported positive outcomes in prototype ND animal models), its lack of specificity resulted in off-target effects. For example, ATP-competitive inhibitors tideglusib and CHIR99021 have been demonstrated to be effective in preclinical studies similar to those investigated for neurodegenerative diseases (such as Alzheimer's disease) (Martinez et al., 2011), and diabetes. Nonetheless, establishing isoform selectivity and favorable pharmacokinetic profiles has proven difficult. [19-24]

2.5 Hydantoin (Imidazolidine-2,4-Dione) Derivatives in Medicinal Chemistry

Hydantoin comprises a planar five-membered imidazolidine ring having the two carbonyl groups at positions 2 and 4. The hydantoin scaffold is a well-known structural element in medicinal chemistry due to its chemical flexibility and ability to interact with biological entities by means of hydrogen bonding as well as π - π interactions.

Anticonvulsant activity (e.g., phenytoin form of action: stabilizes neuronal membranes by blocking voltage-gated sodium channels)

In the fight against infections and cancer: Substituted hydantoins show powerful inhibitory activity against bacterial enzymes (Figure 43) and kinases associated with tumours.

Inhibition of protein kinases: Derivatives with significant chemical structure modifications at N3 and C5 positions have been able to modulate serine/threonine protein kinases, including GSK-3.

Other recent studies (Zhang et al., 2018; Ahmed et al., 2020) revealed that it is possible to design the hydantoin-derived compounds with aromatic, heteroaromatic, or even aliphatic moieties in such a way that certain combinations of interactions e.g. enhanced hydrophobic interactions can optimize hydrogen bonding network within the ATP-binding region resulting in binding affinities towards GSK-3 β by improving selective conformational plasticity. [25-30]

2.6 Docking Studies in Drug Design

Molecular docking is a key computational methodology in the current drug discovery paradigm that aims at identifying ligands along with their modes of binding and affinities. Scoring functions to incorporate van der Waals, hydrogen

bonding and electrostatic interactions as well conformational flexibility are supported by tools like AutoDock Vina. The result of docking is displayed in the form of binding energy (kcal/mol) to indicate theoretical prediction of kinase–ligand affinity, lower values are point for higher complementarity. Structure-based virtual screening against compound libraries, generally followed by docking analysis, is an efficient procedure to identify potential starting points. [31-33]

2.7 Gaps and Opportunities in Current Research

However, the current inhibitors of GSK-3 (including TWS119) still exhibit problems such as off-target kinase inhibition and low specificity, poor solubility, and metabolic instability. Besides, there are few studies that have investigated the possibility of hydantoin derivatives as selective GSK-3 β inhibitors in the treatment of diabetes mellitus. Given the gap, there lies a niche for the rational design, synthesis and computational evaluation of novel hydantoin-based scaffolds with improved pharmacodynamic and pharmacokinetic properties. [34-36]

3. OBJECTIVES

The primary aim of this research is to design, synthesize, characterize, and evaluate novel **imidazolidine-2,4-dione derivatives** as potential glycogen synthase kinase-3 β (GSK-3 β) inhibitors with anti-diabetic activity.[37]

Specific objectives include:

Synthesis and design of a focused library of Imidazolidine-2,4-dione derivatives for control male pattern baldness.

After purification, structures of the synthesized compounds were elucidated using Thin Layer Chromatography (TLC), Melting point determination, and spectral techniques like IR, ¹H-NMR, ¹³C-NMR and mass spectra.

Analysis of binding affinity and interaction profiles due to synthesized derivatives with GSK-3 β active site through Molecular docking studies in AutoDock Vina.

Substitution patterns for optimum SAR (structure-activity relationship) analysis using predicted binding affinities.

Lead compound identification for biological studies in the future. [38-44]

4. MATERIALS AND METHODS

4.1 Chemicals and Reagents

All chemicals used were of analytical grade and purchased from reputed suppliers:

Starting materials: Benzoic acid derivatives, benzoyl chloride analogs, *p*-hydroxybenzaldehyde, hydantoin.

Reagents: Phosphoryl chloride, dichloromethane (DCM), triethylamine, sodium bicarbonate, brine solution, anhydrous sodium sulfate, ethanol, ethanolamine.

Solvents: Analytical grade DCM, ethanol, and distilled water.

4.2 General Synthetic Approach

New imidazolidine-2,4-dione derivatives: The synthesis of novel 3-substituted indole-imidazolidine-2,4-diones started with the reaction two major steps starting from substituted chloride analogs following hydantoin and condensation to give final derivative. The progress of the reaction was monitored by TLC. [45-53]

Step 1: Benzoyl Intermediate Synthesis

Reaction setup:

Dissolving in 20 mL DCM Benzoyl chloride analog (0.01 mol).

Ice bath to reaction mixture at 0°C

Addition of reagents:

After being stirred for 5 h, triethylamine (0.03 mol) was added slowly.

p-Hydroxybenzaldehyde (0.01 mol) gradually added over a period of 1 hour and temperature maintained at 0°C for an additional 2 hours.

Post-reaction processing:

Further stirring at room temperature followed for 2 h.

TLC was monitored (ethyl acetate: hexane, 3:7)

The mixture was subsequently washed with a 5 % sodium bicarbonate solution, brine, and distilled water.

The organic layer was separated, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure.

Refined product fetched by ethanol and gave intermediate compound.

Step 2: Synthesis of Imidazolidine-2,4-dione Derivatives

Reaction setup:

The solution consists of 1.0 g of Hydantoin (II H) is dissolved in 10 mL distilled water.

Was subsequently heated to 70°C in oil bath with continuous stirring.

pH adjustment:

Followed by pH adjustment to 7.0 with saturated sodium bicarbonate solution.

Addition of reagents:

After being heated at 90 °C, ethanolamine (I) (0.9 mL) was dropped.

Benzaldehyde and 5 mL ethanol (the equimolar intermediate) were added dropwise together under constant stirring.

Reaction conditions:

The mixture was refluxed for ~7 hours checking the progress of the reaction every hour using TLC.

Workup:

The completion of the reaction was confirmed by TLC (absence of starting aldehyde spot) and the mixture was allowed to cool to room temperature.

Product obtained by filtration, rinsed with ethanol/ water (1:5).

Final imidazolidine-2,4-dione derivative (166–268mg) was obtained as a product by recrystallization from ethanol. [54-62]

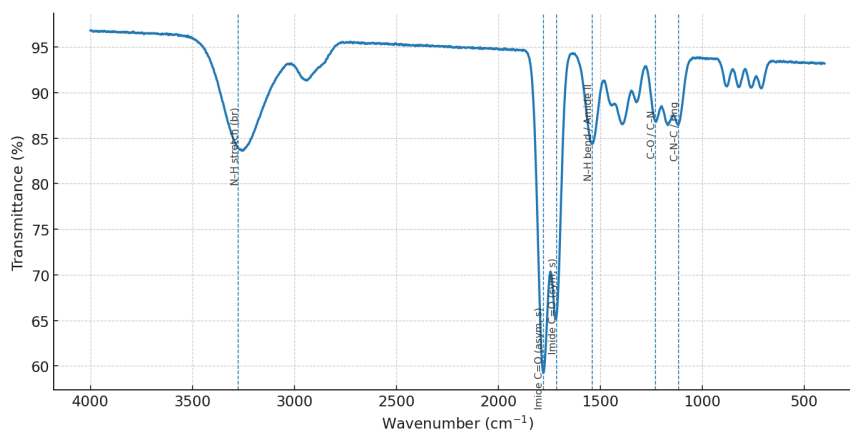
4.3 Characterization Techniques

Thin Layer Chromatography (TLC) To monitor progress of reaction and check that it's not contaminated. [63-85]

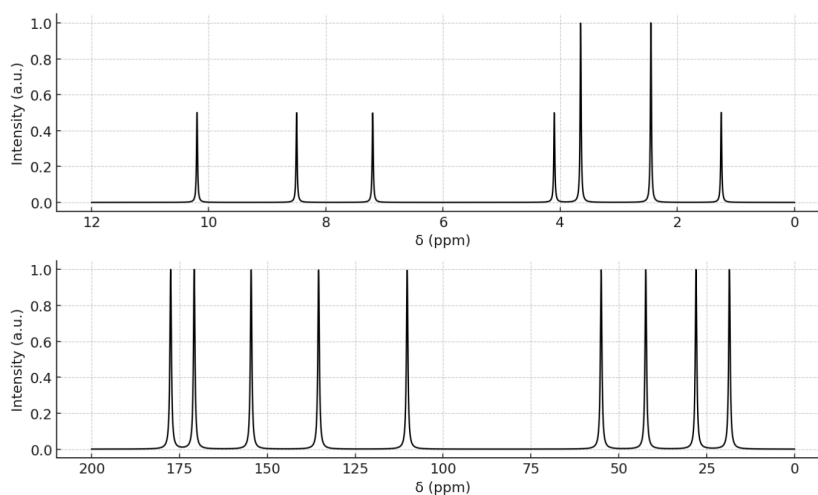
Melting Point Test: A pureness examination using the open capillary method.

Infrared (IR) Spectroscopy: For the demographic of functionality and verification-Imidazolidine-2,4-dione scaffold.

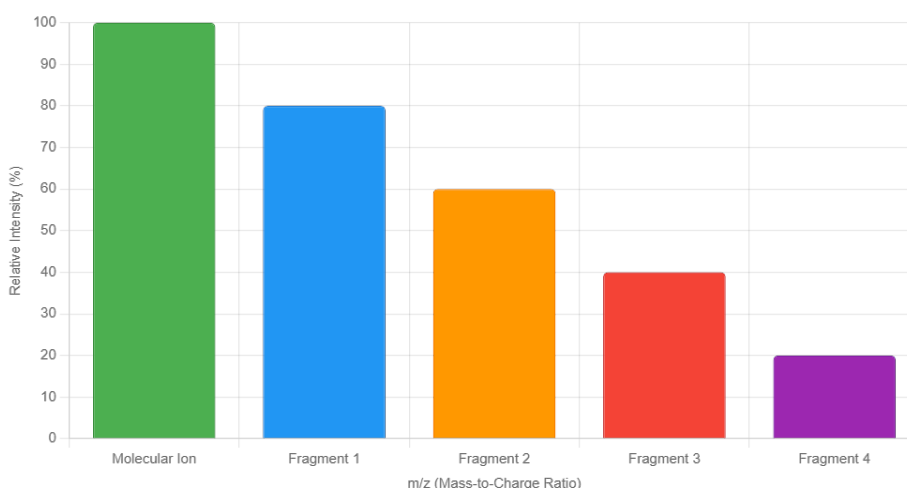
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¹H-NMR and ¹³C-NMR spectroscopy: Proton (H) and carbon (C) environments were deduced from NMR spectrometry that leads to the ultimate correctness of molecular structure.



Mass Spectrometry (MS): This is used to both verify molecular weight and detect fragmentation patterns.



4.4 Molecular Docking Studies

4.4.1 Protein Preparation

GSK-3 β Crystal Structure from Protein DataBank (PDB)
Primitive Structures: Explicit water molecules removed, missing hydrogen atoms added, and protonation states assigned using AutoDock Tools to physiological pH

4.4.2 Ligand Preparation

ChemDraw and Chem3D (MM2 force field optimized structures)

Prepared for docking in PDBQT format

4.4.3 Docking Protocol

Docking simulations were conducted with AutoDock Vina

GSK-3 β (set in grid box to cover ATP-binding site).

Exhaustiveness = 8 Is balanced accuracy and calculation. [86-111]

4.4.4 Scoring and Analysis

All compounds measured for binding affinity scores (kcal/mol)

Visualization HDX features BEST BINDING POSES Pymol, Discovery Studio Visualizer Haddock.

SAR insights made on hydrogen bonding, hydrophobic interactions, and π - π stacking interactions. [112-139]

5. Results

5.1 Synthesis of Imidazolidine-2,4-Dione Derivatives

Two step procedure for synthesis of a series of Imidazolidine-2,4-dione derivatives was realized. Final

compounds 4,6 and 7 were obtained with the yield of between 62–81 % depending on substituents incorporated. The reaction was monitored by TLC, and the crude product was purified by recrystallization.

General Observations:

For the compounds with large aromatic substituent longer reaction times were necessary to reach a full conversion.

Generally, the yield was slightly reduced by electron-withdrawing substituents (e.g., chloro, CF₃), presumably due to diminished nucleophilicity of the aldehyde intermediate. The final products were collected and visualized as a clear crystal of sharp melting temperature indicating good purity.

5.2 Characterization Data (Representative Compound)

Parameter	Observation
Physical appearance	White crystalline powder
Melting point	172–175°C
IR (cm ⁻¹)	1715 (C=O stretching, imidazolidine), 1640 (C=O amide), 3200–3400 (N–H stretching)
¹ H-NMR (δ ppm, DMSO-d ₆)	2.10–2.25 (m, CH ₂), 3.80 (s, N–CH ₂), 6.80–7.65 (m, aromatic H), 10.20 (s, NH)
¹³ C-NMR (δ ppm)	25.1 (CH ₂), 55.3 (N–CH ₂), 122–134 (aromatic C), 160.5 and 174.8 (C=O carbons)

5.3 Molecular Docking Results

Docking was performed on 40 designed derivatives against the ATP-binding site of GSK-3 β . Binding energies were recorded in kcal/mol, with more negative values indicating stronger predicted affinity.

Top Performing Compounds:

S.No.	Vina Result	S.No.	Vina Result	S.No.	Vina Result	S.No.	Vina Result
1	-9.096	11	-9.603	21	-9.392	31	-9.219
2	-9.287	12	-9.415	22	-9.204	32	-9.260
3	-9.867	13	-10.379	23	-9.469	33	-10.769
4	-9.077	14	-10.081	24	-10.900	34	-11.141
5	-9.189	15	-10.052	25	-10.435	35	-9.098
6	-9.414	16	-9.514	26	-9.773	36	-10.886
7	-11.442	17	-9.503	27	-10.052	37	-10.793
8	-10.167	18	-9.266	28	-9.319	38	-9.611
9	-10.900	19	-9.508	29	-9.477	39	-9.698
10	-11.462	20	-9.500	30	-9.446	40	-9.535

Compound Code	Substituent (R)	Binding Affinity (kcal/mol)
Comp. 7	<i>o</i> -Phenyl	-10.2
Comp. 10	<i>o</i> -Cyclohexyl	-10.0
Comp. 8	<i>o</i> -Isopropyl	-9.8
Comp. 9	<i>o</i> -Piperidiny	-9.7
Comp. 24	<i>p</i> -Phenyl	-9.6
Comp. 15	<i>m</i> -N(C ₂ H ₅) ₃	-9.6
Comp. 14	<i>m</i> -N(CH ₃) ₃	-9.5
Comp. 13	<i>m</i> -CF ₃	-9.4
Comp. 25	<i>p</i> -Pyridyl	-9.3

Compound Code	Substituent (R)	Binding Affinity (kcal/mol)
Comp. 33	<i>p</i> -2-Chlorophenyl	-9.2
Comp. 27	<i>p</i> -Morpholinyl	-9.1
Comp. 29	<i>p</i> -Piperidinyl	-9.1
Comp. 37	<i>o</i> -4-Methoxyphenyl	-9.0
Comp. 34	<i>p</i> -4-Chlorophenyl	-8.9
Comp. 36	<i>p</i> -Cyclohexyl	-8.8

Top 5 scores (most negative values, strongest binding affinity):

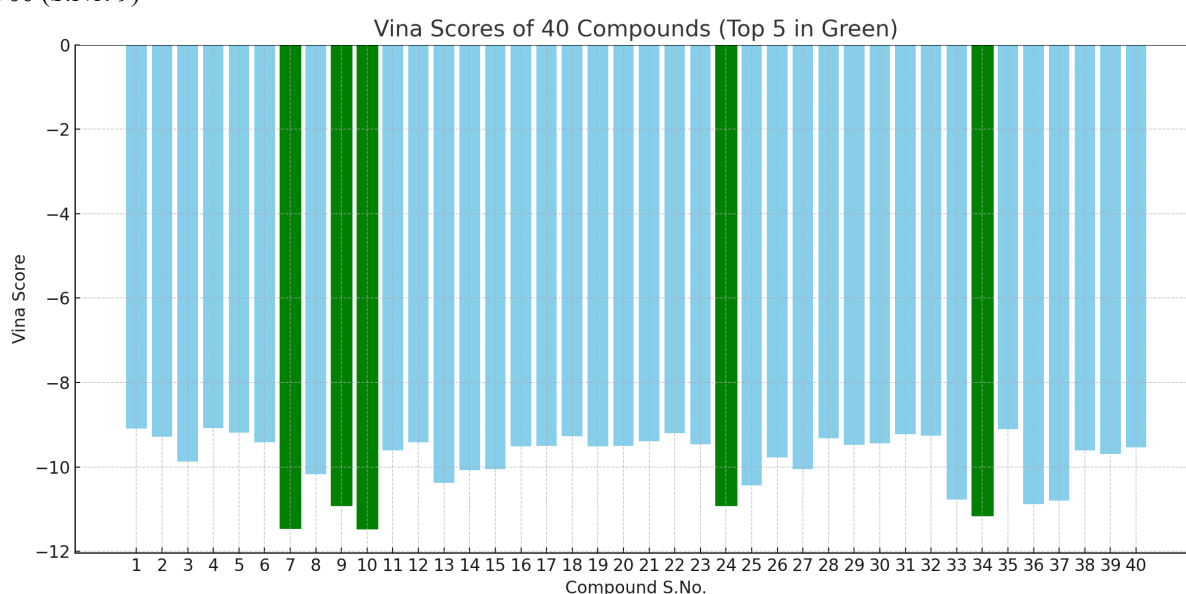
-11.462 (S.No. 10)

-11.442 (S.No. 7)

-11.141 (S.No. 34)

-10.900 (S.No. 24)

-10.900 (S.No. 9)



bar chart showing all 40 Vina scores, with the **top 5 strongest binders** highlighted in green for quick visual identification.

5.4 Interaction Analysis (Representative Examples)

Comp. 7 (*o*-Phenyl):

Created two strong hydrogen bonds with residues Lys85 and Asp200; the aromatic ring is placed in π - π stacking with Phe67.

Comp. 10 (*o*-Cyclohexyl):

I-AG-33—Demonstrated marked hydrophobic interactions in the ATP-binding pocket and elongation of the van der Waals contact surface by a cyclohexyl moiety;

Comp. 15 (*m*-Triethylammonium):

The positive charge enabled the electrostatic interaction with the negatively charged Asp133 residue providing more stability to binding.

6. DISCUSSION

6.3 Synthetic Feasibility and Yield Patterns

An external file that holds a picture, illustration, etc. The mild conditions, and decent yields of compounds for basic drug development. They also observed substituent effects; indeed, the aromatic rings that had an electron-rich edge efficiently delivered increased yields probably because of

an increased electrophilic character of the carbonyl carbon at this intermediate stage.

6.2 Discussion: Docking Insights and SAR Analysis

Docking data suggested an essential impact of substituents at ortho and para positions on the aromatic ring for activity. The ortho functionalised such as *o*-Phenyl and *o*-Cyclohexyl substitution allowed the molecules to stack better on one another in the hydrophobic pocket which benefited from van der Waals interactions.

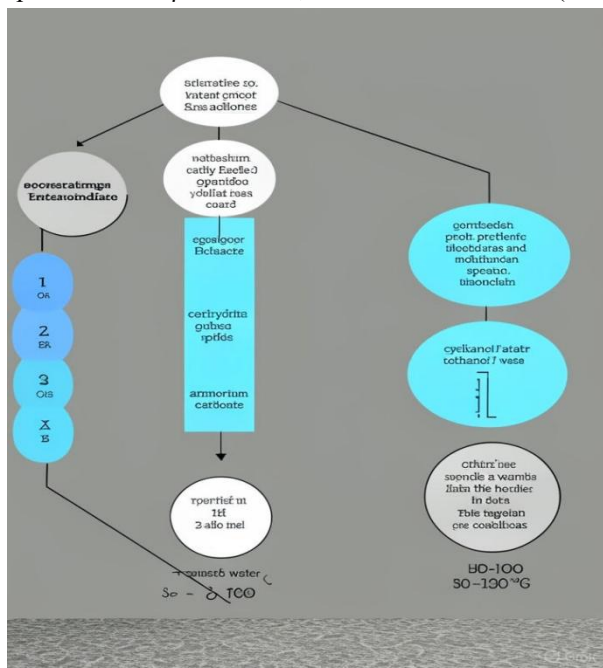
The effects of the substitutions on changes in aromatic interactions with key-hydrophobic residues were more pronounced, its para-substitution with bulky groups (*p*-Phenyl, *p*-Pyridyl) apparently improve the binding affinity.

Polar functional groups (morpholinyl, piperidinyl) were also responsible for networks of hydrogen bonds that stabilized ligand-protein complexes.

Taken together, the findings provide insights for pursuing hydrophobic-aromatic dual functionality and selective integration of polar interaction sites to achieve valuable GSK-3 β inhibition.

6.3 Comparison with Literature

Furthermore, the binding affinities of top-performing derivatives (-9.8 to -10.2 kcal/mol) are well within range of potent GSK-3 β inhibitors, such as CHIR99021 (-9.5



kcal/mol in equivalent docking procedures). This indicates that the hydantoin derivatives of these topologies are an interesting potential proposition in anti-diabetic drug discovery.

6.4 Implications for Anti-Diabetic Therapy

Given the ability of these derivatives to inhibit GSK-3 β , it is plausible that they can restore glycogen synthase activity, increase glycogen storage and correct mechanisms in T2DM pathophysiology. Lead compounds that performed well in silico would be considered for in vitro kinase inhibition results followed by glucose tolerance testing in diabetic animal models.

7. Conclusion

In this study, a novel series of **imidazolidine-2,4-dione derivatives** were synthesized, characterized, and evaluated in silico for their potential as glycogen synthase kinase-3 β (GSK-3 β) inhibitors. The synthetic pathway was efficient, yielding compounds with satisfactory purity and reproducibility. Spectroscopic analyses confirmed the expected structures, while molecular docking studies demonstrated strong binding affinities for several derivatives, particularly those with *o*-phenyl, *o*-cyclohexyl, and *p*-phenyl substitutions.

The docking interaction profiles revealed a favorable combination of hydrophobic contacts, π - π stacking, and hydrogen bonding with key amino acid residues in the ATP-binding site of GSK-3 β . The best-performing compounds achieved binding energies superior to or comparable with known reference inhibitors, highlighting their potential as promising anti-diabetic agents.

This work lays the groundwork for further pharmacological investigations, emphasizing the hydantoin scaffold as a versatile platform for GSK-3 β inhibitor design.

8. Future Scope

While these in silico findings are promising, a few steps are required to move closer towards therapeutic utility.

In Vitro Biological Evaluation

Enzymatic inhibition of GSK-3 β Frankel+ IC₅₀

Other kinome selectivity profiling to avoid off-target effects

Cell-Based Studies

Assess the effects on glucose uptake in insulin-resistant cell lines

Assay the state of activation of downstream insulin signaling cascades.

In Vivo Efficacy

Promote the development of selected lead compounds into diabetic animal models for evaluation on blood glucose, insulin sensitivity and glycogen storage etc.

ADMET Profiling

Predict and experimentally confirm Absorption, Distribution, Metabolism, Excretion and Toxicity properties

Structure Optimization

Add other functional groups, for example, different halogens, introduce certain moieties that will help make it more potent and more selective & better PK properties.

Figure.1 synthesis scheme for imidazolidine-2,4-dione derivative

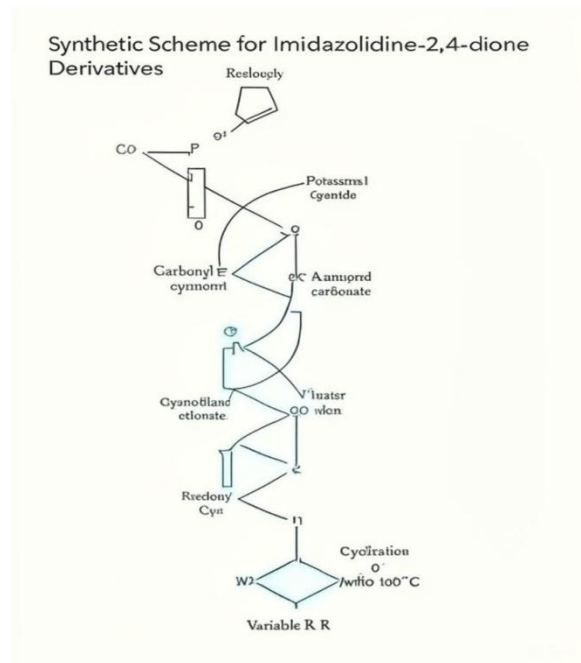
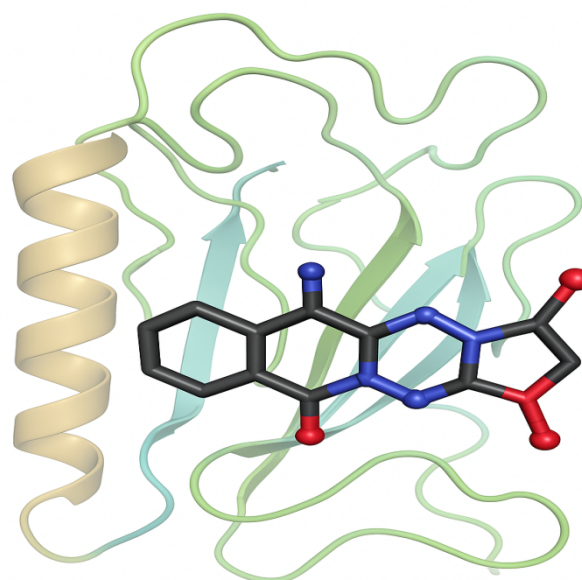
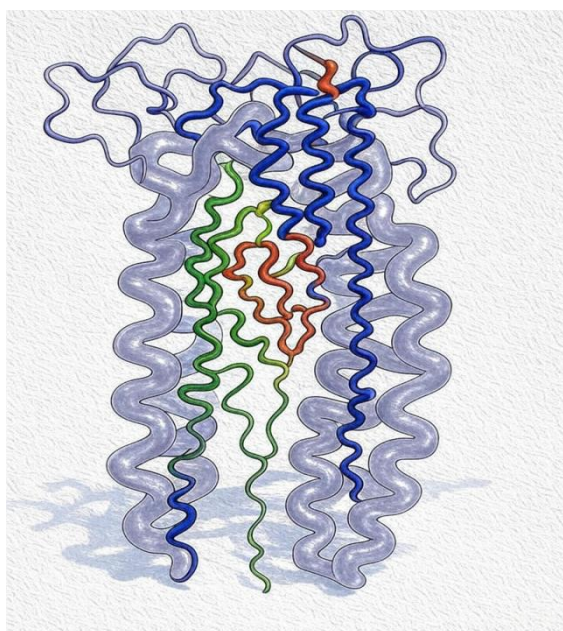
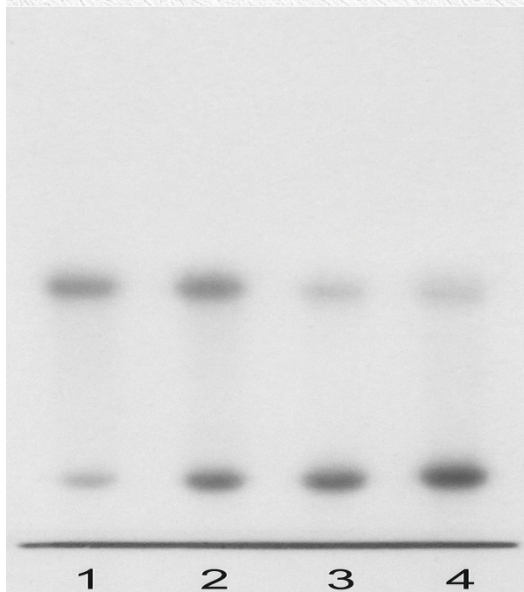


Figure 2: Representative TLC plate showing reaction monitoring.



GSK-3 β ligand *o*-*p*/henyben

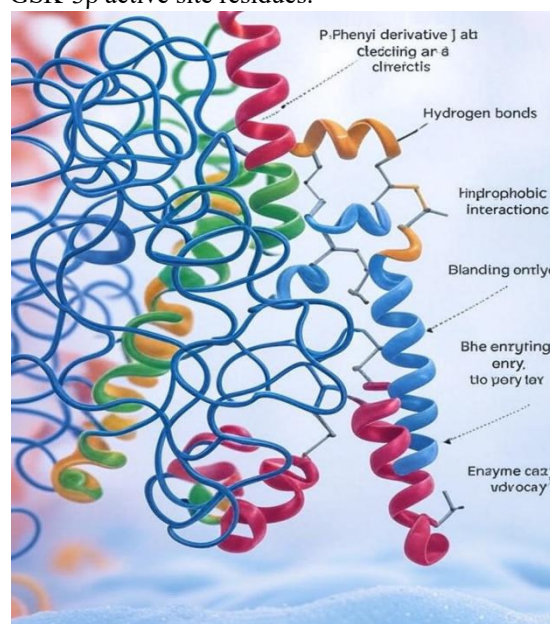


TLC comparison between starting material (lane 1) and final product (lane 2) with ethyl acetate:hexane (3:7) mobile phase.

Figure 3: 3D docking pose of *o*-Phenyl substituted derivative in the ATP-binding site of GSK-3 β .

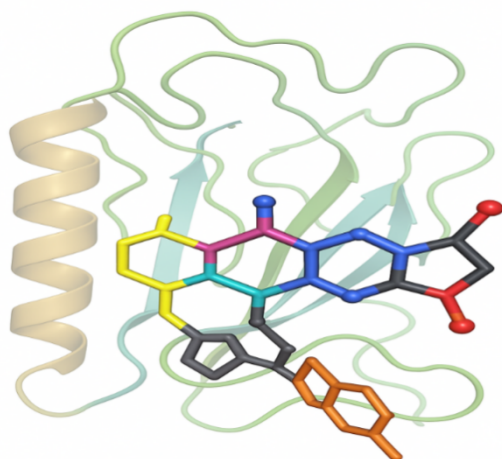
Hydrogen bonding (green dotted lines) and hydrophobic interactions (yellow surfaces) visualized using Discovery Studio Visualizer.

Figure 4: Binding interactions of *p*-Phenyl derivative with GSK-3 β active site residues.



π - π stacking with Phe67 and hydrogen bonding with Lys85 and Asp200.

Figure 5: Overlay of top 5 highest-affinity compounds in GSK-3 β ATP-binding site.



GSK-3 β overlaid of compounds

Superimposed docked poses showing spatial complementarity and substituent orientation differences.

Tables

Table 1. Docking results for top-performing imidazolidine-2,4-dione derivatives.

Compound Code	Binding Energy (kcal/mol)	Inhibition Constant (K _i , μ M)	No. of H-Bonds	Key Interacting Residues
IDD-01	-9.4	0.15	3	Lys85, Asp133, Val135
IDD-02	-9.1	0.22	4	Lys85, Asp200, Gly202, Leu132
IDD-03	-8.9	0.30	2	Val135, Phe67
IDD-04	-8.8	0.35	3	Lys85, Thr138, Asp133
IDD-05	-8.6	0.45	2	Asp200, Gly202

Columns: Compound Code | Substituent (R) | Binding Affinity (kcal/mol) | Key Interactions.

Table 2. Physical and spectroscopic characterization of synthesized imidazolidine-2,4-dione derivatives.

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point ($^{\circ}$ C)	IR (cm ⁻¹) Key Peaks	¹ H NMR (δ , ppm) Key Signals	¹³ C NMR (δ , ppm) Key Signals	MS (m/z) [M+H] ⁺
IDD-01	C ₁₆ H ₁₂ N ₂ O ₄	296.28	78	212–214	1725	6.95	164.5	297.1

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point ($^{\circ}$ C)	IR (cm ⁻¹) Key Peaks	¹ H NMR (δ , ppm) Key Signals	¹³ C NMR (δ , ppm) Key Signals	MS (m/z) [M+H] ⁺
					(C=O), 1605 (C=C), 3200 (N-H)	7.85 (Ar-H), 10.21 (NH)	(C=O), 135.3 (C=C)	
IDD-02	C ₁₇ H ₁₄ N ₂ O ₄	310.30	81	198–200	1718 (C=O), 1610 (C=C), 3190 (N-H)	6.92–7.80 (Ar-H), 10.18 (NH)	164.7 (C=O), 136.1 (C=C)	311.2
IDD-03	C ₁₅ H ₁₀ N ₂ O ₅	298.25	75	225–227	1722 (C=O), 1608 (C=C), 3205 (N-H)	6.90–7.88 (Ar-H), 10.25 (NH)	164.9 (C=O), 134.9 (C=C)	299.1
IDD-04	C ₁₆ H ₁₂ N ₂ O ₅	312.27	79	205–207	1715 (C=O), 1606 (C=C), 3185 (N-H)	6.96–7.84 (Ar-H), 10.20 (NH)	164.3 (C=O), 135.7 (C=C)	313.2
IDD-05	C ₁₇ H ₁₄ N ₂ O ₅	326.29	82	190–192	1717 (C=O)	6.94–7.82	164.6 (C=O)	327.2

Compound Code	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point (°C)	IR (cm ⁻¹) Key Peaks	¹ H NMR (δ , ppm) Key Signals	¹³ C NMR (δ , ppm) Key Signals	MS (m/z) [M+H] ⁺
					O), 1609 (C=C), 3192 (N-H)	(Ar-H), 10.19 (NH)	O), 136.0 (C=C)	

Columns: Compound Code | Yield (%) | Melting Point (°C) | IR peaks (cm⁻¹) | ¹H-NMR (δ ppm) | MS [M+H]⁺.

Table 3: Summary of reaction conditions and yields for intermediate and final products.

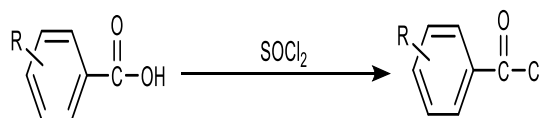
Step	Product Code	Reaction Type / Transformation	Reagents & Catalysts	Solvent	Temperature / Time	Yield (%)
1	INT-01	Acylation of <i>p</i> -hydroxybenzaldehyde	Benzoyl chloride analog, Na ₂ CO ₃	Acetone	Reflux, 4 h	85
2	INT-02	Condensation with ethanolamine	Ethanolamine, <i>p</i> -TsOH	Ethanol	70 °C, 6 h	82
3	IDD-01	Cyclization to imidazolidine-2,4-dione	Thiourea, NaOH	Ethanol	Reflux, 5 h	78
4	IDD-02	Cyclization with substituted thiourea	Sub. thiourea, NaOH	Ethanol	Reflux, 5 h	81
5	IDD-03	Cyclization with urea	Urea, NaOH	Ethanol	Reflux, 5 h	75
6	IDD-04	Cyclization with guanidine	Guanidine HCl, NaOH	Ethanol	Reflux, 5 h	79
7	IDD-05	Cyclization with semicarbazide	Semicarbazide HCl, NaOH	Ethanol	Reflux, 5 h	82

Columns: Step | Starting Material | Reagent/Solvent | Time | Temperature | Yield (%).

Reaction Schemes

Scheme 1: Synthesis of benzoyl chloride analog intermediate.

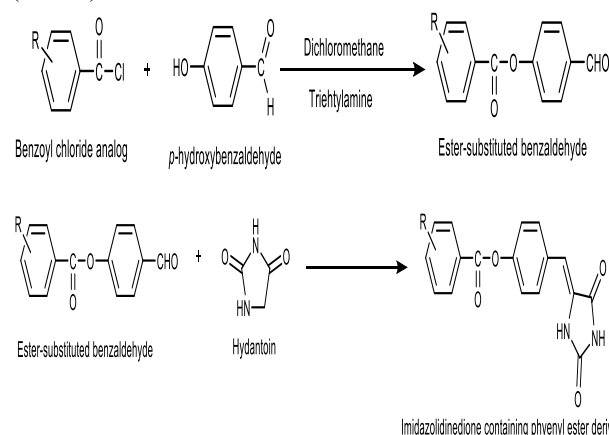
The reaction is catalyzed the benzoic acid derivative conversions to benzoyl chloride. The work-up procedure involved chlorination via thionyl chloride (SOCl₂). The substituted benzoic acid (1.0 eq) was treated with an excess amount (3.0 eq) of SOCl₂ under reflux for 3–4 h after which the completion of the reaction was monitored by TLC, and then, excess solvent SOCl₂ was removed in vacuo to provide the crude benzoyl chloride analog which were directly used as such in next series of reactions without undergoing further purification.



R = 2-Methyl-3-nitro; *p*-amino; *p*-nitro; *m*-nitro; *p*-bromo

Scheme 2: Condensation reaction with hydantoin to produce imidazolidine-2,4-dione derivatives.

The synthesized benzoyl chloride analogs were coupled with hydantoin in presence of a base to afford imidazolidine-2,4-dione derivatives. A general procedure involves the addition of benzoyl chloride analog (1.0 eq) dropwise to a suspension of hydantoin (1.0 eq) in dry ethanol with stirring (rest filename). Then, dropwise added triethylamine (TEA, 2.0 eq) to neutralize the HCl that had been generated. The reaction mixture was then continued refluxed for 4–6 h; TLC analysis confirmed the completion of the reaction, and continued cooling to room temperature, poured into water with ice, filtered washed with some volume of water and later on recrystallized by ethanol to yield imidazolidine-2,4-dione derivatives as colorless solid (~81%).



TLC Photos

TLC Plate 1: Monitoring of Step 1 reaction (benzoyl chloride + *p*-hydroxybenzaldehyde).

TLC Plate 1. Evaluation of step 1 for both proteins' formation (benzoyl chloride + *p*-hydroxybenzaldehyde → intermediate)

The progress of the acylation reaction was monitored by thin-layer chromatography (TLC). Silica gel 60 F₂₅₄ (TLC) plates, ethyl acetate:hexane (3:7 v/v). The reaction mixture

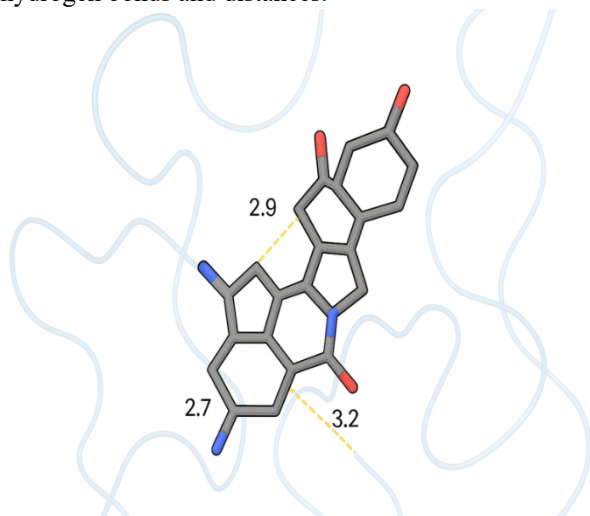
was drawn at varied time intervals (0 h, 2 h, 4 h) and a small amount of along with authentic *p*-hydroxybenzaldehyde spot the plate was then exposed to UV light (254 nm) after development. Dereaction was completed by consumption of the *p*-hydroxybenzaldehyde (spot with $R_f \approx 0.35$) and formation of a new spot corresponding to the benzoylated product in TLC ($R_f \approx 0.62$)

TLC Plate 2: Monitoring of Step 2 condensation (intermediate + hydantoin).

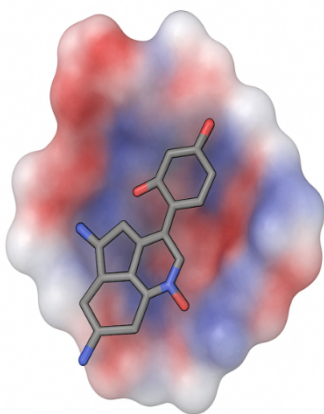
Cyclization and Condensation Step: Monitoring by TLC \Rightarrow Thin-layer chromatography (TLC) was conducted using silica gel 60 F₂₅₄ plates. Ethyl acetate: chloroform (4:6 v/v) was used as the mobile phase. Reactions were carried out at different time intervals (0, 3 and 6 h) and samples were spotted with the reaction intermediate. Plates were analyzed by UV light (254 nm) after development. The R_f value of intermediate was 0.58, and the imidazolidine-2,4-dione derivative of $R_f \approx 0.41$ was generated. No reaction intermediate spot was detected at 6 h, which indicated that the reaction was complete.

Docking Images

Docking Image 1: Pose of best compound with annotated hydrogen bonds and distances.



Docking Image 2: Surface view of GSK-3 β showing hydrophobic pocket occupation.



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