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CADD based designing and biological evaluation of novel triazole based thiazolidinedione coumarin hybrids as antidiabetic agent

Anchal Sharma¹✉, Anmol Narang², Nitish Kumar^{1,4}, Rupali Rana¹, Megha³, Pooja¹, Muskan Dhir¹, Harmandeep Kaur Gulati^{1,5}, Jyoti¹, Aanchal Khanna¹, Jatinder Vir Singh¹, Sukhraj Kaur² & Preet Mohinder Singh Bedi¹✉

A series of 5-(substituted benzylidene) thiazolidine-2,4-dione and coumarin hybrids (I-1 to I-16) were designed and synthesized to explore key structural requirements for effective α -glucosidase inhibitors. Molecular docking studies were conducted to investigate their interactions with various targets, including DPP-4, α -glucosidase, α -amylase, and PPAR- γ . The docking scores and binding energies indicated that Compound I-1 emerged as the optimal scaffold for drug design, excluding α -amylase. Compound I-1 was synthesized based on the insights gained from molecular docking and simulations, which helped predict interactions and identify critical structural features. Pharmacokinetic properties were evaluated through drug-likeness and ADMET studies. Additionally, density functional theory (DFT) analyses were performed to assess the stability and reactivity of potential diabetes mellitus drug candidates. Dynamic simulation studies further elucidated the stability and interaction dynamics of the top-ranked compound I-1. In vitro evaluation against the α -glucosidase enzyme yielded an IC_{50} value of 1.49 μ g/ml. In vivo studies demonstrated that Compound I-1 significantly reduced blood glucose levels, with values of 94.15 mg/dL and 74.60 mg/dL at doses of 10 mg/kg and 20 mg/kg, respectively. Furthermore, Compound I-1, like Acarbose, resulted in significant reductions in ALT, AST, ALP, urea, LDH, and creatinine levels, suggesting improved liver and kidney function.

Keywords Molecular docking, Molecular dynamics simulation, DFT investigation, α -glucosidase, α -amylase, DPP-4, PPAR- γ

Diabetes Mellitus (DM) is one of the main health issues in the world. Patients with DM have been increasing rapidly, and by 2030, there could be 552 millions cases worldwide. The hallmark of this long-term illness is hyperglycaemia, which is brought on by insufficient insulin synthesis or the body's partial resistance to insulin¹. It is well known that DM is linked to serious health issues such as retinopathy, nephropathy, and cardiovascular disorders. Consequently, such aligned complications can be avoided or postponed with proper control of high blood glucose levels². Antidiabetic drugs include various classes of drugs that target distinct biological pathways to regulate blood sugar levels. These include sulfonylureas and meglitinides, which stimulate insulin secretion from the pancreas; biguanides, primarily metformin, which decrease liver glucose production and enhance insulin sensitivity; thiazolidinediones (glitazones), which improve insulin action in tissues; dipeptidyl peptidase-4 (DPP-4) inhibitors and incretin mimetics, which augment the actions of incretin hormones to increase insulin secretion and suppress glucagon release; sodium-glucose co-transporter 2 (SGLT2) inhibitors, which increase glucose excretion in the urine; and α -glucosidase inhibitors, which slow down carbohydrate absorption in the intestines³. α -Glucosidase inhibitors have been a popular treatment option for treating

¹Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab 143005, India.

²Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab 143005, India. ³Department of Chemistry, Guru Nanak Dev University, Amritsar, Punjab 143005, India. ⁴Sri Sai College of Pharmacy, Badhani, Pathankot, Punjab 145001, India. ⁵Dashmesh College of Pharmacy, Faridkot, Punjab 151203, India. ✉email: anchalsharma4619@gmail.com; preet.pharma@gndu.ac.in

postprandial hyperglycaemia, which plays a significant role in the onset, progression, and consequences of diabetes Mellitus⁴. By blocking α -glucosidase, which catalyses the breakdown of 1,4- α glycosidic linkages in complex dietary carbohydrates, these inhibitors slow down the intestinal absorption of glucose⁵. Examples of α -glucosidase inhibitors (Fig. 1) that are clinically given for the treatment of type 2 diabetes include voglibose, Miglitol and acarbose^{3,5}. The widespread clinical application of these drugs has been hindered by the complexity of their industrial production and the occurrence of adverse reactions. These adverse reactions include flatulence, diarrhoea, and gastrointestinal disturbances⁶. Thus, there has been a lot of attention lately focused on finding novel small compounds with strong α -glucosidase inhibitory action and little adverse effects.

Thiazolidinedione is a widely used scaffold in drug development due to its remarkable pharmacological properties, which include antibacterial⁷, anticancer⁸, antihistaminic, anti-inflammatory⁹, anticonvulsant,

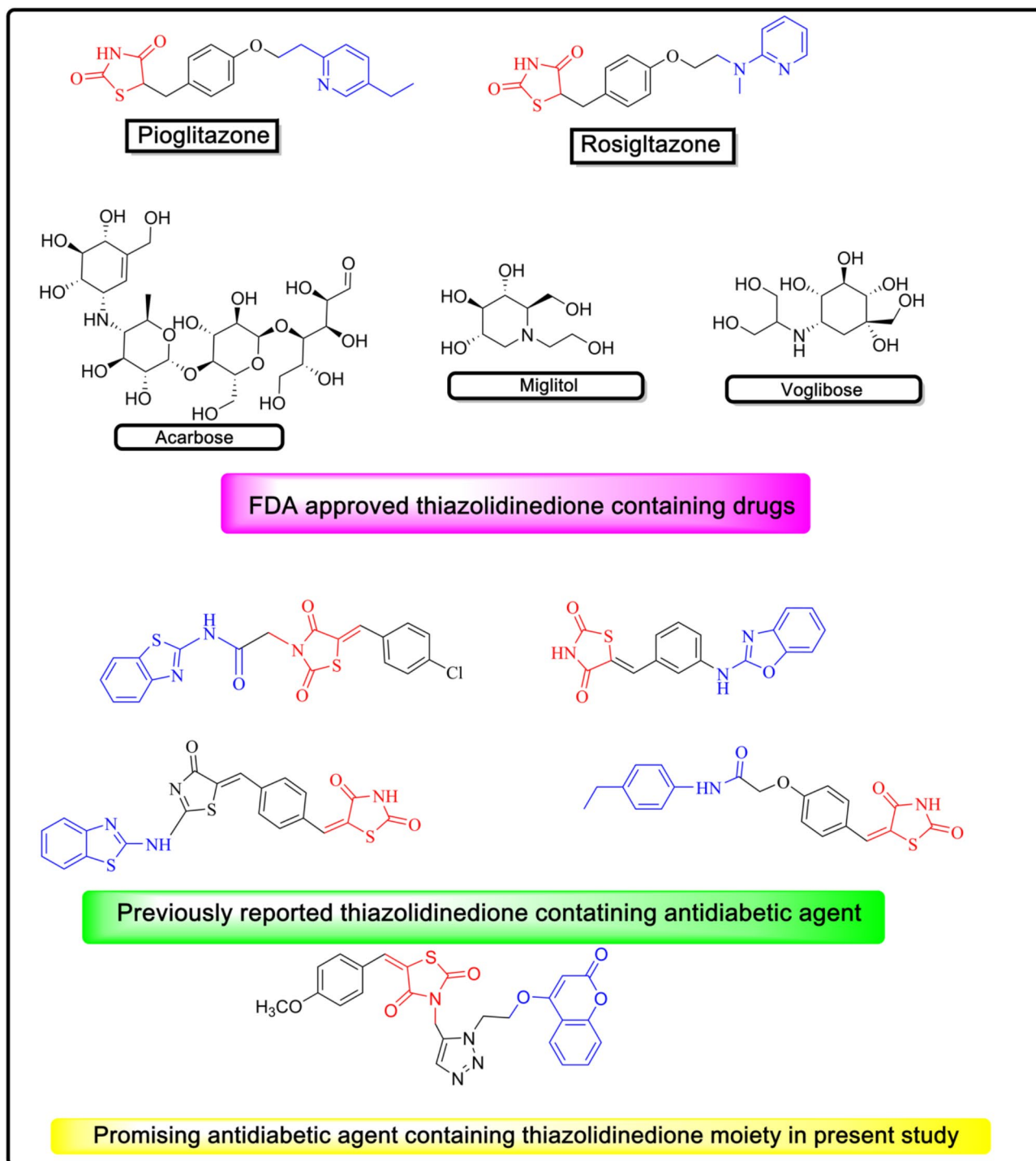


Fig. 1. Thiazolidinedione derivatives: FDA-approved drugs, literature precedents, and a novel compound.

antitubercular, and antiviral properties¹⁰. Its biological potential has been attributed to the keto group located at position four and the N-C-S bond in thiazolidinediones^{11,12}. In addition to this, the thiazolidinedione heterocycle's ability to treat diabetes has been extensively studied, as shown by the efficacious usage of several antidiabetic drugs in clinical settings, including rosiglitazone, pioglitazone, lobiglitazone and troglitazone^{13,14}. The hypoglycaemic action of these drugs is exerted through the peroxisome proliferator activity receptor (PPAR- γ). There have also been published findings on significant hypoglycaemic effects caused by thiazolidinediones acting on additional Molecular targets such aldose reductase (ALR2), protein tyrosine phosphatase 1B (PTP1B), α -glucosidase, and α -amylase^{15,16}.

Coumarin is oxygen containing bicyclic heterocycle that abundantly applied in the design of new potent therapeutic hybrid Molecules^{17,18}. Numerous hybrid structures of coumarin derivatives and various potentially bioactive pharmacophores with inhibitory activities against cholinesterase, Monoamines oxidase, aldose reductase, alkaline phosphatase, urease, carbonic anhydrase, histone deacetylase, lipoxygenase, topoisomerase, tyrosinase, and cyclooxygenase and α -glucosidase have been introduced^{19,20}. They act as antidiabetic drugs by repairing pancreatic β -cells damage and by reducing oxidative stress. They help in improving abnormal insulin signalling and activating AMPK²¹. One of the coumarin derivatives which can be used as a pharmacophoric unit in the design of new α -glucosidase inhibitors is 4-hydroxycoumarin since simple derivatives of this structure exhibited strong inhibitory activity against α -glucosidase^{22,23}. For example, biscoumarin-thiourea hybrids exhibited excellent α -glucosidase inhibitory activity^{19,22}.

Another one of interest building blocks in the design bioactive hybrid structures is 1,2,3-triazole ring²⁴. Hybrid molecules containing 1,2,3-triazole possess a wide variety of pharmacological properties including antibacterial, anticancer, anti-Alzheimer and antidiabetic's activities^{8,25,26}. Tris-heterocyclic compounds have emerged as promising candidates in the realm of diabetes mellitus drug discovery due to their unique structural features and potential biological activities. These compounds, characterized by the presence of three heterocyclic rings within their molecular structure, offer several advantages such as enzyme inhibition, insulin sensitization, antioxidant properties and structural versatility²⁷. The presence of multiple heterocyclic rings within the tris-heterocyclic framework provides a vast array of structural possibilities. This diversity allows for the design and synthesis of compounds with tailored properties and improved potency. Also, The incorporation of various functional groups into the heterocyclic rings can further modulate the compounds' biological activities. This approach enables fine-tuning of properties like solubility, metabolic stability, and target affinity²⁸.

Hence, prompted by above observations and in continuation to our attempt in development of α -glucosidase inhibitors, herein we designed and synthesized for the first time a series of 1,2,3-triazole based thiazolidinedione-coumarin hybrids²⁹. These compounds were tested for their in-vitro inhibitory activity against yeast α -glucosidase. Furthermore, and in-silico studies were performed to gain an insight toward the interactions of title compounds with α -glucosidase. It was observed from the literature that, thiazolidinedione and coumarin fragments are present in the number of reported α -glucosidase and α -amylase inhibitors^{12,30,31}. In this study, α -glucosidase inhibitory potential of thiazolidinedione-coumarin hybrids are reported. Therefore, considering the emerging importance of thiazolidinedione and coumarin fragments, especially with regard to their α -glucosidase inhibitory properties, and as continuation of our efforts for the development of new potent α -glucosidase inhibitors, We have devised a strategy to integrate these pharmacophoric units into a single molecular framework using the molecular hybridization approach, with the goal of achieving their synergistic effects.

Results and discussion

Designing

Considering the emerging importance of thiazolidinedione, coumarin and triazole fragments, especially with regard to their α -glucosidase inhibitory properties, and as continuation of our efforts for the development of new potent α -glucosidase inhibitors (Fig. 2).

Molecular docking studies (LeadIT software)

In order to rationalize antidiabetic activity of compounds Molecular docking studies was performed. In order to validate docking parameters, the co-crystallized ligand in RCSB with PDB ID: 7KBJ (α -glucosidase), and IX70 was redocked into the active site of enzymes. The outcome of redocking was relatively identical, and the RMSD value (0.02 Å) confirmed the docking Method reliability. In order to have a real insight into structural binding mode of all the target total sixteen compounds were designed, and docking process was performed using LeadIT software. The result analysis showed that the Docking score of target compounds for α -glucosidase, DPP-4, PPAR- γ and alpha amylase were (-13.362 to -22.546, -19.565 to -30.987, -13.841 to -27.465, -15.364 to -28.882 kcal/mol and compared to Acarbose, Sitagliptin, rosiglitazone and acarbose were (-6.582, -38.966, -22.335 and to -15.084 kcal/mol respectively) (Table S1 in supplementary file). The docking scores for compound I-1 were almost 3-fold More than standard drug acarbose, and they also had one conventional hydrogen bonding (with Arg624) and one II- sulphur bonding (with Met565). In this investigation, we have selected compounds I-1 for further biological studies. The 3D binding Mode of the ligand I-1 with α -glucosidase and α -amylase, DPP-4 and PPAR- γ of docking of all synthesized compounds at the receptor active site revealed that the potent compound (score > 20 kcal/mol) with the dibromoethane as a substitute on 2,4-thiazolidinedione core had More favourable interactions with α -glucosidase at a hydrophobic pocket. A Map of 2D and 3D representation of the compound I-1 in the active site of α -glucosidase, DPP-4, PPAR- γ and α -amylase (PDB code: KBJ, 1 \times 70, 2PRG and 2QV4 respectively) is shown in Figs. 3 and 4.

ADMET studies (SwissADME online software)

The hypothesis was supported by the moieties' structural and physicochemical properties. The series of all the designed compound molecules (I-1 to I-16) along with their physicochemical characteristics (Molecular

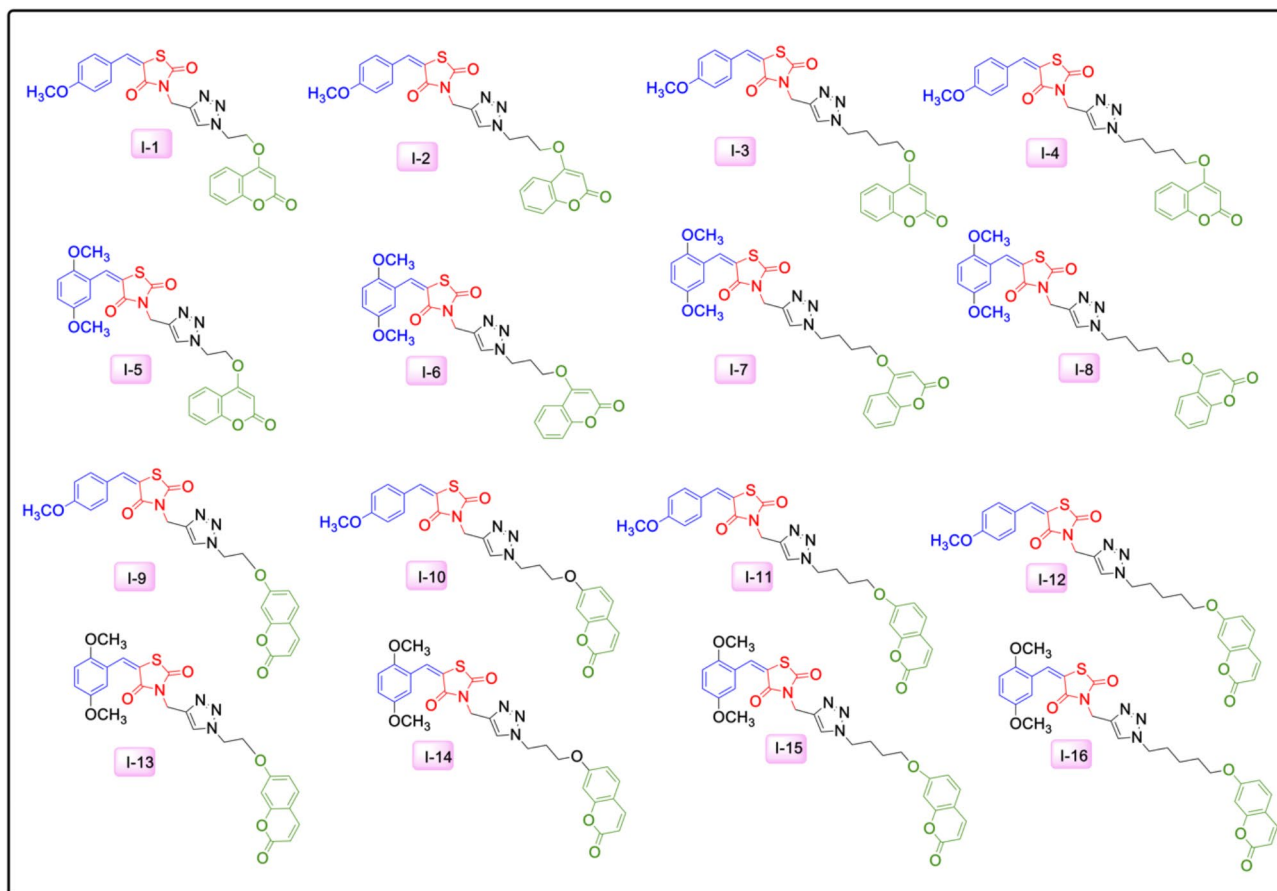


Fig. 2. Chemical structures of designed compounds I-1 to I-16, highlighting the incorporation of diverse pharmacophoric units into a single molecular architecture.

weight, heavy atoms, aromatic heavy atoms, H-bond acceptors and donors, molar refractivity, lipophilicity, and water solubility), Drug likeliness, medicinal chemistry, lipophilicity and water solubility were discussed in the Tables (Tables S2, S3 and S4 refer to supplementary file).

Evaluation of physicochemical characteristics (comparative data of I-1 and acarbose)

Both the compounds I-1 and Acarbose, not comply with Lipinski's rule. This suggests that their molecular makeup is similar to that of oral medicines. Acarbose is not within the established ranges of ≤ 500 , ≤ 5 , $\leq 140 \text{ \AA}^2$, and ≤ 10 , respectively, for molecular weight (MW), the number of hydrogen bond donors (nHBD), topological polar surface area (TPSA), logP, and the number of hydrogen bond acceptors (nHBAs) (Fig. 5).

On the other hand I-1 is close to these recommended values of every parameters as compared to standard drug Table 1. Compound I-1 feature 8 rotatable bond which lies within the range but, in case of acarbose no. of rotatable bond (13) exceeds the limit. The molar refractivity was discovered to be the same as 136.71 and 137.92 for I-1 and Acarbose respectively. Total polar surface area of standard drug was found to be 329.01 \AA^2 while 142.06 \AA^2 for I-1.

MW: molecular weight; nHA: no. of heavy atom; nAHA: no. of arom. heavy atom; F.Csp³: no. of sp³ hybridized carbon out of total carbon count; nRB: no. of rotatable bonds; nHBA: no. of H-bond acceptors; nHBD: no. of H-bond donors, MR: molar refractivity; TPSA: topological polar surface area.

Lipophilicity and water solubility

The partition coefficient I-1 and acarbose preferred into the water compartment, is shown by their log Po/w values, which ranged from 2.91 to -6.24. Both the compounds were predicted as moderately soluble to highly soluble. For the I-1 and acarbose, Log S is aqueous solubility and has the value of -4.73 and 2.57, which is less than the specified range of -4 to 0.5 log mol/L, as indicated Table 2.

Characteristics of pharmacokinetics

Pharmacokinetics is crucial to obtain the intended pharmacological outcome of a drug. This suggests that every compound's pharmacokinetic characteristic has the potential to impact a drug's pharmacological profile. Low GI absorption was found for both substances I-1 and acarbose according to the SwissADME database. Figure 6 indicate the boiled-egg graphs for I-1 and Acarbose, respectively. Compound I-1 showed borderline

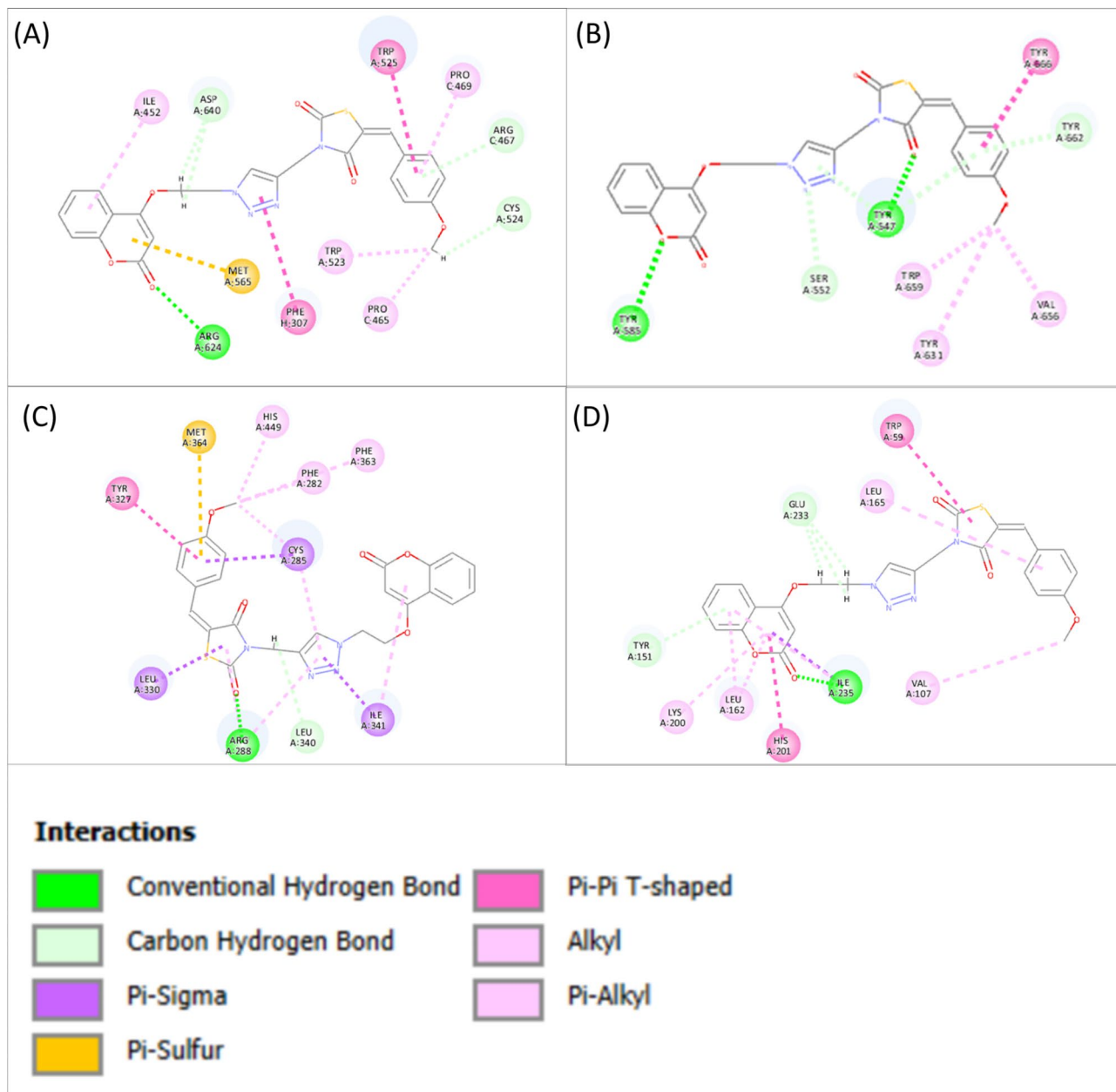


Fig. 3. 2D-interaction diagram of representation of docking interaction by ball and stick model (a) compound I-1 with α -glucosidase enzyme (PDB ID: 7kbj) (b) compound I-1 with DPP-4 (PDB ID: IX70) (c) compound I-1 with PPAR- γ (PDB ID: 2PRG) (d) compound I-1 with α -amylase (PDB ID: 2QV4).

GI absorption, indicating that compound may be suitable for intestinal absorption. Both the I-1 and Acarbose cannot pass the blood-brain barrier. In the case of drug metabolism of I-1 it was found to be CYP2C19, CYP2C9 and CYP3A4 inhibitors. However, acarbose found to be unaffected by CYP2C19, CYP2C9, CYP1A2, CYP2D6 and CYP3A4. The bioavailability score for I-1 and Acarbose was 0.55 and 0.17 respectively (Table 3).

Drug-likeness

According to Table 4, the five drug-likeness approaches (Lipinski, Muegge, Ghose, Veber, and Egan) are violated by the compounds I-1 and standard drug acarbose.

Medicinal chemistry

Thiazolidinedione-coumarin hybrid (I-1) have no PAINS alert, without α -screen artifacts and frequent hitters, and with reasonable reactivity. Brenk-structural-alert indicated a couple of reactive groups in these selected Compound with Michael acceptor and thioester and coumarin.

For the qualitative identification of a Molecule that turns out to be an oral Medicine with admirable bioavailability, drug likeness parameters prediction can be useful. Five rules, including Lipinski, Ghose, Veber,

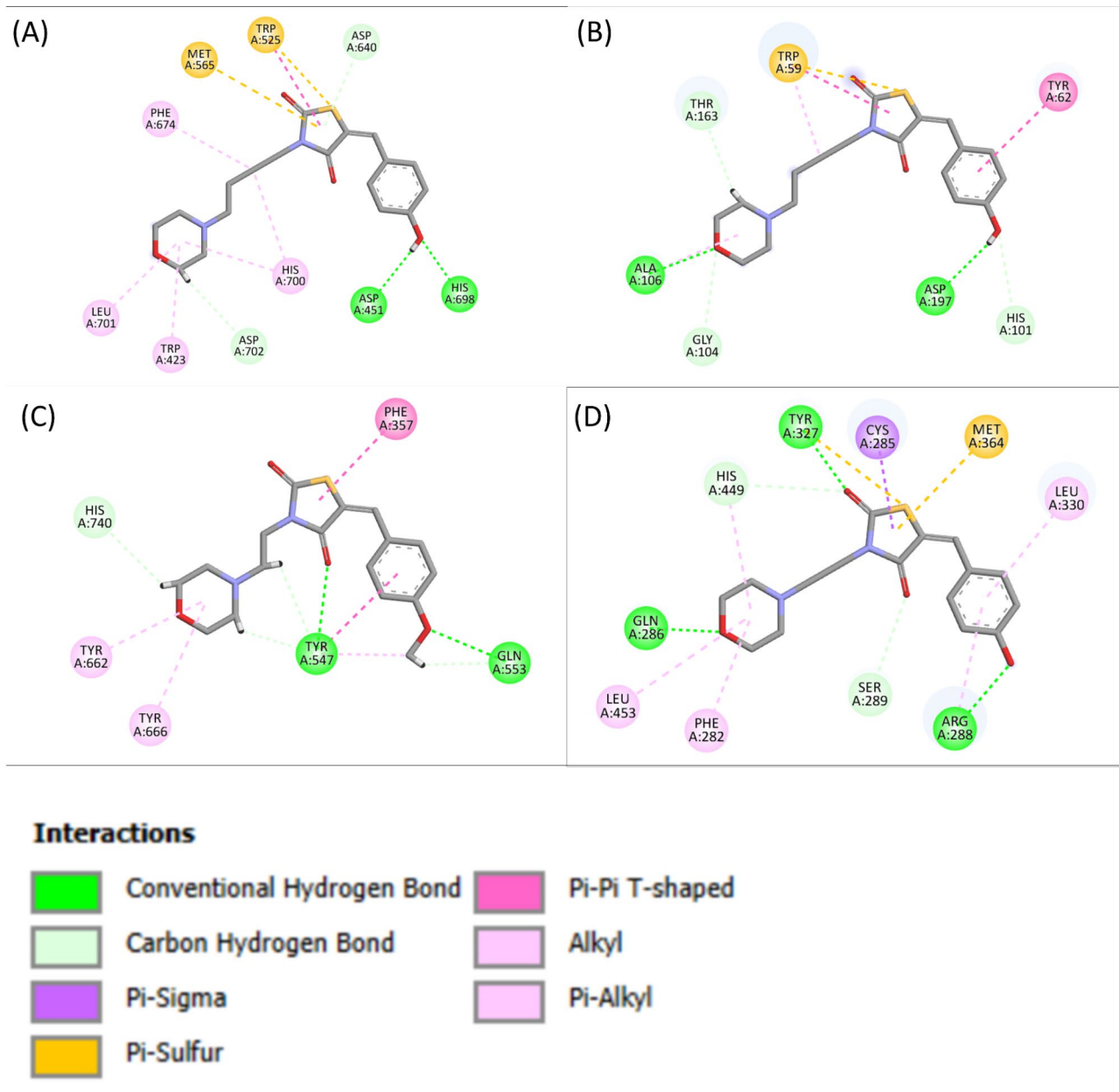


Fig. 4. 3D- Representation of docking interaction by ball and stick model (A) compound I-1 with α -glucosidase enzyme (PDB ID: 7kbj) (B) compound I-1 with α -amylase (PDB ID: 2QV4). (C) compound I-1 with DPP-4(PDB ID: IX70) (D) compound I-1 with PPAR- γ (PDB ID:2PRG).

Egan and Muegge, have been examined in the current work in order to evaluate the oral bioavailability and drug likeness of the developed compounds. Compared to Acarbose, these metrics demonstrated a good bioavailability score and were in good agreement with the exercised criteria for the compounds I-1.

DFT studies

Interaction stability based on FMO analysis

The stability of both intermolecular and intramolecular interactions between rosiglitazone and the thiazolidinedione-based compound I-1 was investigated using frontier molecular orbital (FMO) analysis at the B3LYP/6-31G** level, as implemented in the Gaussian 09 software package. The frontier molecular orbitals, specifically the HOMO and LUMO, which represent electron donation and acceptance, are critical in delineating the interaction pathways between the compounds and the reference drug (Fig. 7). The energy gap between these FMOs was analysed to evaluate the chemical reactivity of the molecules. An increase in HOMO energy indicates a greater capacity for electron donation and a heightened susceptibility to oxidation, while a decrease in LUMO energy reflects the opposite. In compound I-1, the HOMO is predominantly localized over the triazole moiety, whereas the LUMO is concentrated on the thiazolidinedione and benzaldehyde ring. The negative energy

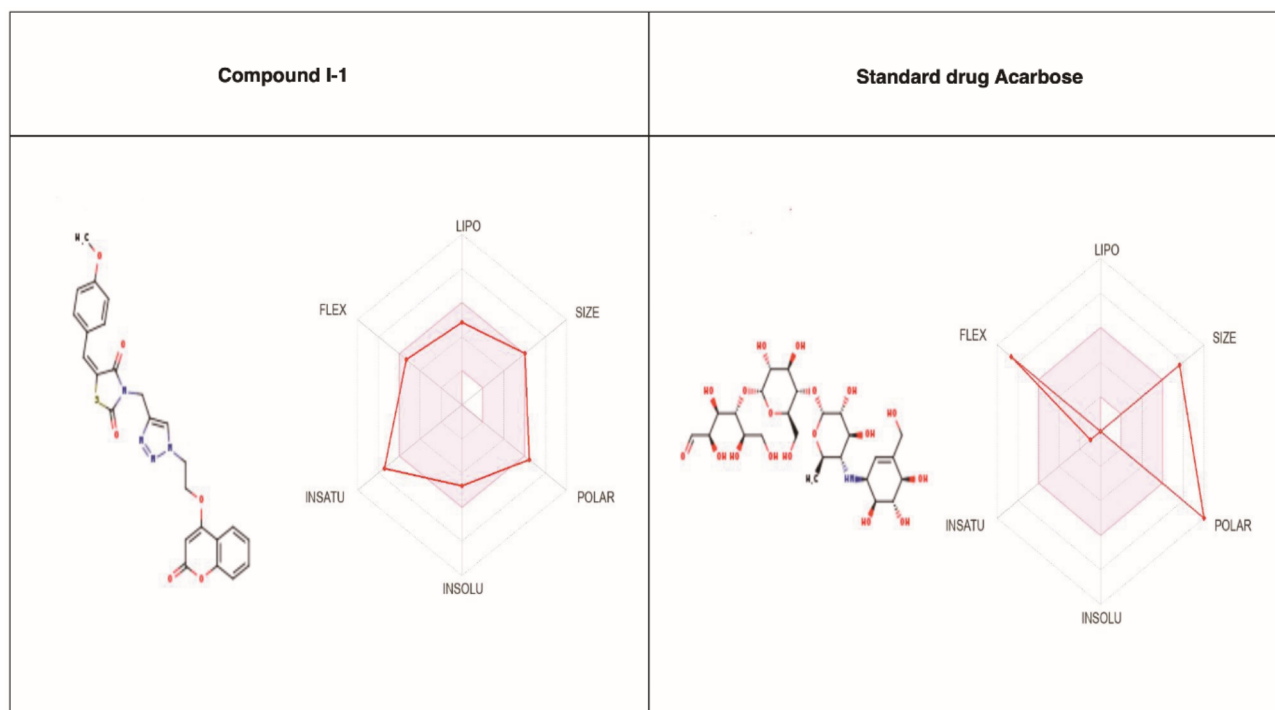


Fig. 5. The radar charts of compounds I-1 and Acarbose depending on the physicochemical properties.

Ligand	MW (g/mol)	nHA	nAHA	F.Csp ³	nRB	nHBA	nHBD	MR	TPSA (Å ²)
I-1	504.51	36	21	0.16	8	8	0	136.71	142.06
Acarbose	645.60	44	0	0.88	13	19	14	137.92	329.01
Recommended value	< 500	15–50	-	-	< 9	0–10	0–5	40–140	20–140

Table 1. The physicochemical property of (I-1 and Acarbose) derivatives calculated with the SwissADME database.

Ligand	Lipophilicity	Water solubility					
	Consensus Log P _{o/w}	Log S (ESOL)	Solubility Class	Log S (Ali)	Solubility class	Log S (SILICOS-IT)	Solubility class
I-1	2.91	4.73	Moderately soluble	-5.60	Moderately soluble	-7.27	Poorly soluble
Acarbose	-6.24	2.57	Highly soluble	2.69	Highly soluble	6.23	Soluble
Recommended value	-2 to -10	- 4 to -0.5	-	-	-	-	-

Table 2. The characteristics of lipophilicity and water solubility of thiazolidinedione coumarin derivatives (I-1 and Acarbose).

values of both HOMO and LUMO suggest an intramolecular electron transfer from thiazolidine-2,4-dione to benzaldehyde. The energy gap (ΔE) is crucial for assessing the stability of the biomolecule within the receptor. The findings indicate that compound I-1 exhibits a ΔE of 2.89 eV, which is significantly lower than that of the standard drug at 4.47 eV, implying a more favourable interaction between the FMO and compound I-1.

Establishment of SAR (structure activity relationship)

The following study represents that the hybrid of coumarin and thiazolidinedione Moiety effective against Type II diabetes. Presence of -NH- group in the thiazolidinedione ring plays an important role in the enhancement of activity against α -glucosidase and α -amylase enzymes. Presence of aldehyde and hydroxy group in the coumarin ring increases the activity. On the other hands, electron donating group (-OCH₃-) present at Meta and para-position of benzaldehyde increases the activity against α -amylase enzyme (Fig. 8). By increasing the length of carbon chain, the activity of the compound increases against α -glucosidase enzyme. Electron-donating and electron-withdrawing groups were well tolerated, and the substitution at the C-5 aldehyde group influenced the compounds' activity profile^{32–34}. The introduction of methoxy groups at 3 positions of the benzene ring

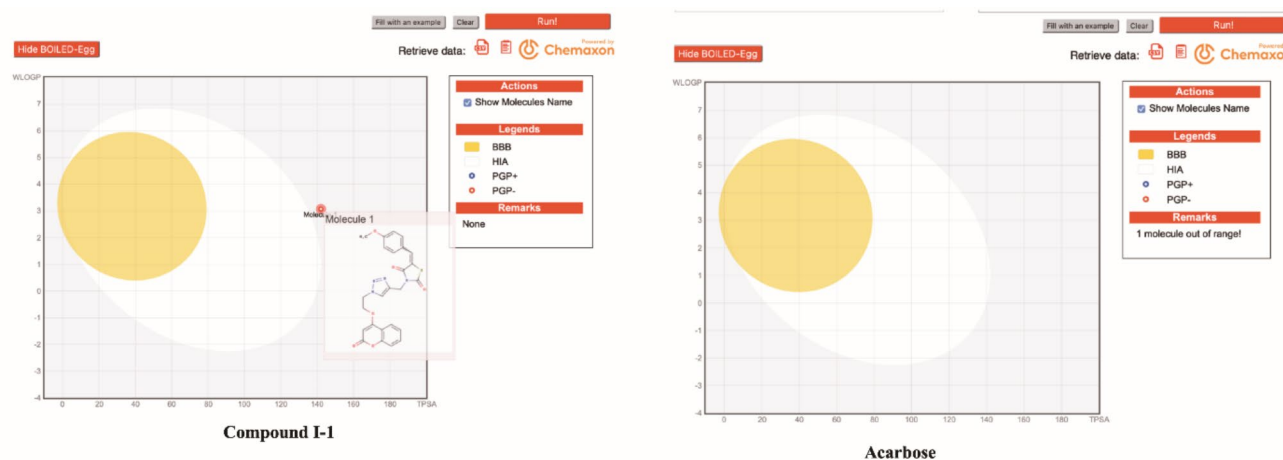


Fig. 6. Boiled egg representation of I-1 and Acarbose.

Ligand	GI abs	Bioavailability score	BBB	P-gp substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
I-1	Low	0.55	No	No	No	Yes	Yes	No	Yes
Acarbose	Low	0.17	No	Yes	No	No	No	No	No

Table 3. The bioavailability score and pharmacokinetics of derivatives calculated with the SWISS ADME database.

Ligand	Drug-likeness rules					Medicinal chemistry		
	Lipinski	Ghose	Veber	Egan	Muegge	PAINS	Brenk	Lead likeness
I-1	Yes 1 violation; MW > 500	No 2 Violations; MW > 480, MR > 130	No 1 Violation, TPSA > 140	No 1 Violation, TPSA > 131.6	Yes	0 alert	2 alerts Michael_acceptor; Thioester; coumarine	No; 2 violation: MW > 350; Rotors > 7
Acarbose	No 3 violation; MW > 500 NorO > 10, NHorOH > 5	No 4 Violations; MW > 480, MR > 130 Atoms > 70 WLOGP < -0.4	No 2 Violation, TPSA > 140 Rotors > 10	No 1 Violation, TPSA > 131.6	No 5 Violations; MW > 600, WLOGP3 < -2 TPSA > 150 H-acc > 10 H-don > 5	0 alert	2 alerts Aldehyde, isolated_alkene	No; 2 violation: MW > 350; Rotors > 7

Table 4. Lists the characteristics of compounds I-1 and Acarbose that relate to their drug-likeness. Some studied chemical complies well with the bioavailability score, which is 0.55. There were no pan assay interference compounds (PAINS) alarms, indicating an excellent pharmacokinetic profile for the lead Molecules.

in compounds I-1 increases the activity whereas, at 4 and 5 position lowered the activity of molecules. I-1 was active against α -glucosidase while displaying weak inhibitory activity against α -amylase^{35–37}. Further, compound I-1 also showed potent antioxidant activity and was also found to be non-toxic. Replacing the 7- hydroxy coumarin with the 4-hydroxy coumarin gave one the most potent inhibitor of α -glucosidase endowed with good antioxidant properties and no cytotoxic activity³⁸.

Correlation of DFT, SAR, docking studies and biological activity

A series of novel anti-diabetic compounds were designed and synthesized based on the thiazolidinedione scaffold. Computational studies indicated efficient electron transfer within the molecular structure and strong electrostatic potential, suggesting favourable interactions with target proteins. Molecular docking simulations revealed high binding affinities for both α -glucosidase and α -amylase enzymes, indicating potential dual inhibitory activity. Experimental validation confirmed the superior α -glucosidase inhibitory activity of the lead compound compared to the standard drug acarbose. The low energy gap (ΔG) between molecular orbitals further supports strong binding interactions with the target proteins³⁹. The molecule with low ΔG has high polarizability, softness, chemical reactivity, and nucleophilicity (thereby easily offering electrons to receptor) and vice versa (Tables S5 and S6 refer to supplementary file) showed the low ΔG values (-2.89 eV.) for the (I-1)

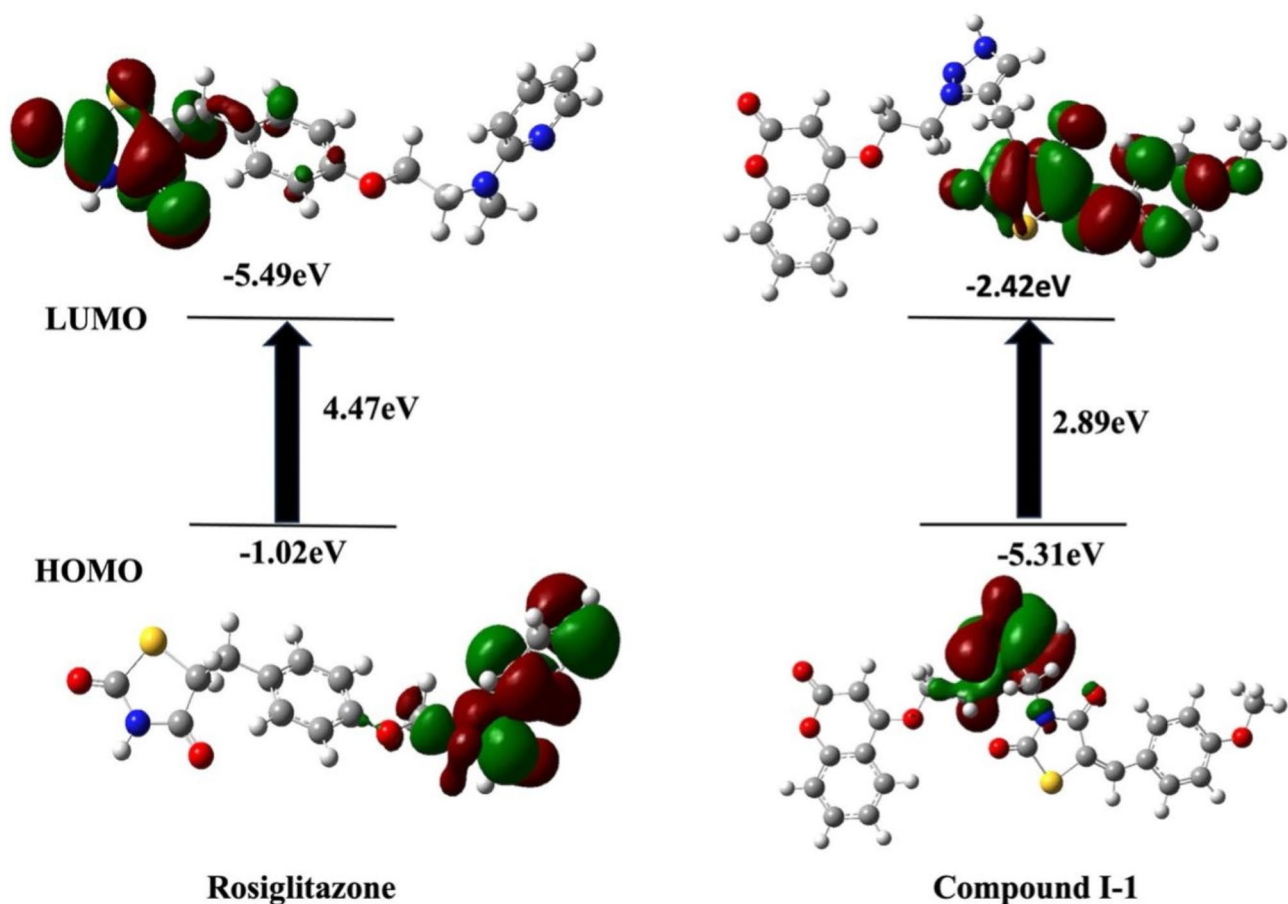


Fig. 7. Representations of comparative energies of frontier orbitals of standard drug and compound I-1 complex.

compound, and (-4.47 eV) for standard drug that may join to form a high degree of reactivity to the biological environment³⁹.

Correlating FMO analysis with docking studies (enhanced reactivity in I-1)

The lower ΔE (-2.89 eV) in compound I-1 compared to rosiglitazone (-4.47 eV) suggests that I-1 is more chemically reactive. This higher reactivity can translate to Stronger intermolecular interactions I-1 might exhibit more favourable interactions with the receptor's binding site due to its increased electron donor/acceptor capabilities. This could lead to Higher binding affinity Potentially resulting in more potent drug activity and More stable protein-ligand complexes. Stronger interactions can contribute to increased residence time of I-1 at the receptor site.

Intramolecular charge transfer

The FMO analysis indicates intramolecular electron transfer from thiazolidine-2,4-dione to benzaldehyde in I-1. This internal charge redistribution can influence ligand conformation The charge transfer might induce conformational changes in I-1, potentially influencing its binding orientation within the receptor. It also Modulate ligand-receptor interactions that resulted in charge distribution could create more favourable electrostatic interactions with specific amino acid residues in the receptor binding site. The FMO data can be used to refine the scoring functions used in docking simulations. For example, electrostatic interactions more heavily due to the potential for strong electrostatic interactions between I-1 and the receptor. The FMO analysis can provide insights into potential binding orientations and key interaction points within the receptor binding site. The lower ΔE and observed intramolecular charge transfer in I-1, as revealed by FMO analysis, suggest that this compound may exhibit enhanced reactivity and potentially stronger interactions with the receptor compared to rosiglitazone. These findings can be effectively utilized to guide and refine docking studies, ultimately leading to a better understanding of the binding mechanism and the design of more potent and selective thiazolidinedione-based compounds.

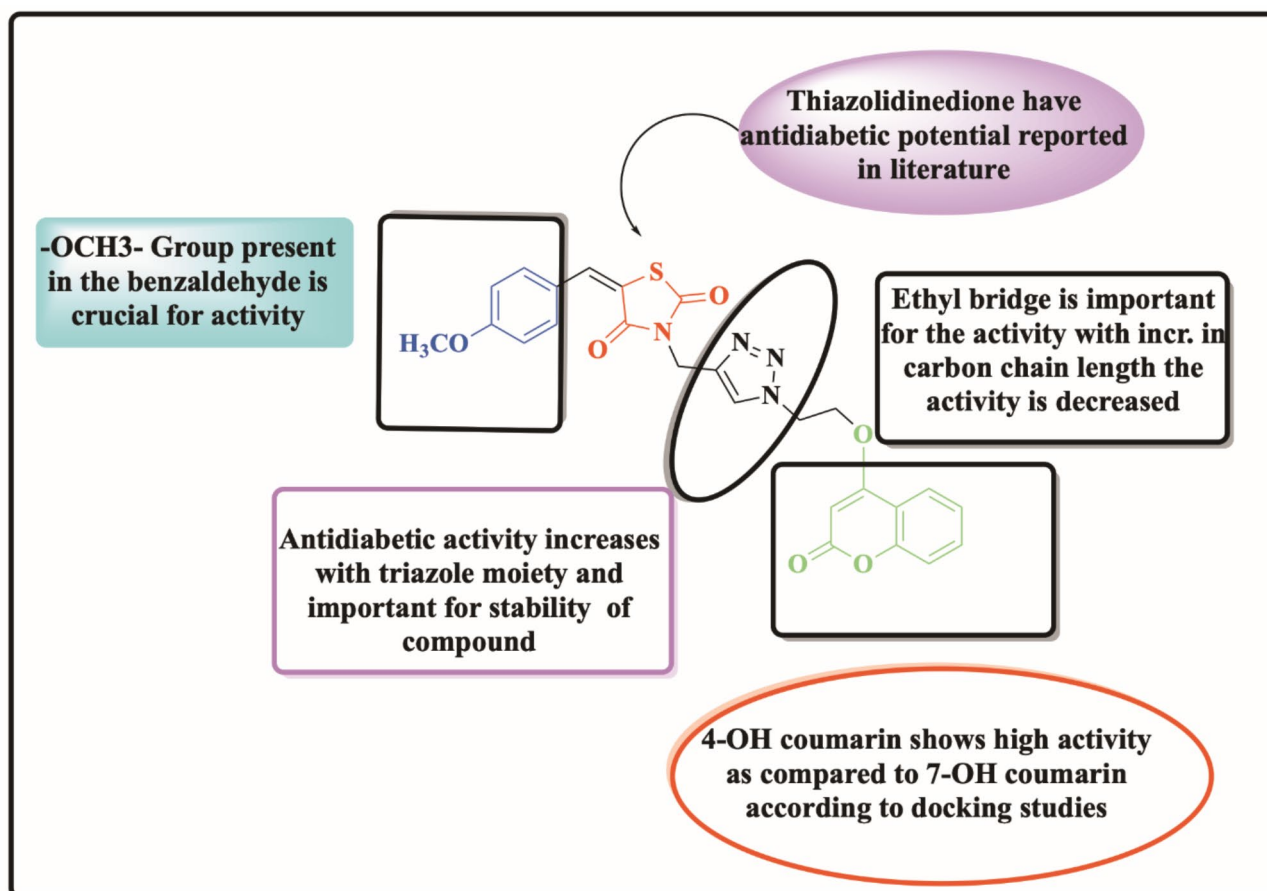


Fig. 8. Establishment of structure-activity relationship of compound I-1 against α -glucosidase enzyme on the basis of molecular docking studies.

Molecular simulation studies

Root mean square deviation analysis (RMSD) against PPAR- γ and DPP-4

The analysis of root mean square deviation (RMSD) serves as a crucial indicator of the system's stability during molecular dynamics (MD) simulations³¹. In this study, the RMSD of the backbone atoms of the protein was examined in both its unbound state and in complex with compound I-1 and the co-crystallized ligand (PPAR- γ , DPP-4). As illustrated in Figs. 9 and 10, equilibrium was achieved at approximately 100 ns for both the free and bound systems. While in the case of PPAR- γ , DPP-4 the protein RMSD was 2.1 Å (Fig. 9), 1.8 Å (Fig. 10) and RMSF was observed similar for both ligands i.e., 0.5–2.5 Å respectively.

This finding highlights the inherent stability of the protein, even amid dynamic interactions with the studied compounds throughout the simulation duration. In the case of the co-crystallized ligand both the protein and ligand RMSD trajectories demonstrated alignment, although the average RMSD was slightly higher at 2.6 Å. Collectively, these results suggest that the introduction of compound I-1 and the co-crystallized ligand does not significantly compromise the overall stability of the protein, as evidenced by the relatively consistent RMSD values and aligned protein-ligand trajectories throughout the simulation. Further analysis of the MD results, particularly the alignment of ligand RMSD with respect to the protein's backbone RMSD, revealed that ligand variations remained below 3 Å. The ligand RMSD of I-1 against α -glucosidase displayed minimal deviation compared to those of DPP-4 and PPAR- γ . Although a slight increase was noted, it remained within the 3 Å margin, indicating that the protein-ligand complex was stable for 15ns without any major orientational changes. Additionally, local conformational changes within the protein residues were assessed by calculating the root mean square fluctuations (RMSF), with vertical green lines in the graph indicating interactions between ligand atoms and protein amino acid residues (Fig. 9b). The results showed that the protein backbone exhibited minimal fluctuations concerning the compound, with RMSF values ranging between 0.5 Å and 2.5 Å, particularly in residues interacting with I-1. The RMSF of I-1 exhibited a similar fluctuation pattern, suggesting that there were no significant fluctuations in the protein backbone relative to the ligands. Subsequently, the interaction patterns of these ligands over time were analyzed (Fig. 9d), revealing that interactions predominantly occurred with residues TRP-423, PHE-571, ASN-572, VAT-576, ARG-624, ASP-640, HIS-647, ILE-650, PHE-673, LYS-674 and ASP-960 throughout the simulation. At any given time, a maximum of fifteen residues were observed in contact with the ligand. Most of the interactions for I-1 were through hydrogen bonding and water bridges, with fewer hydrophobic and ionic interactions. Water bridges were notably more prominent than other interaction

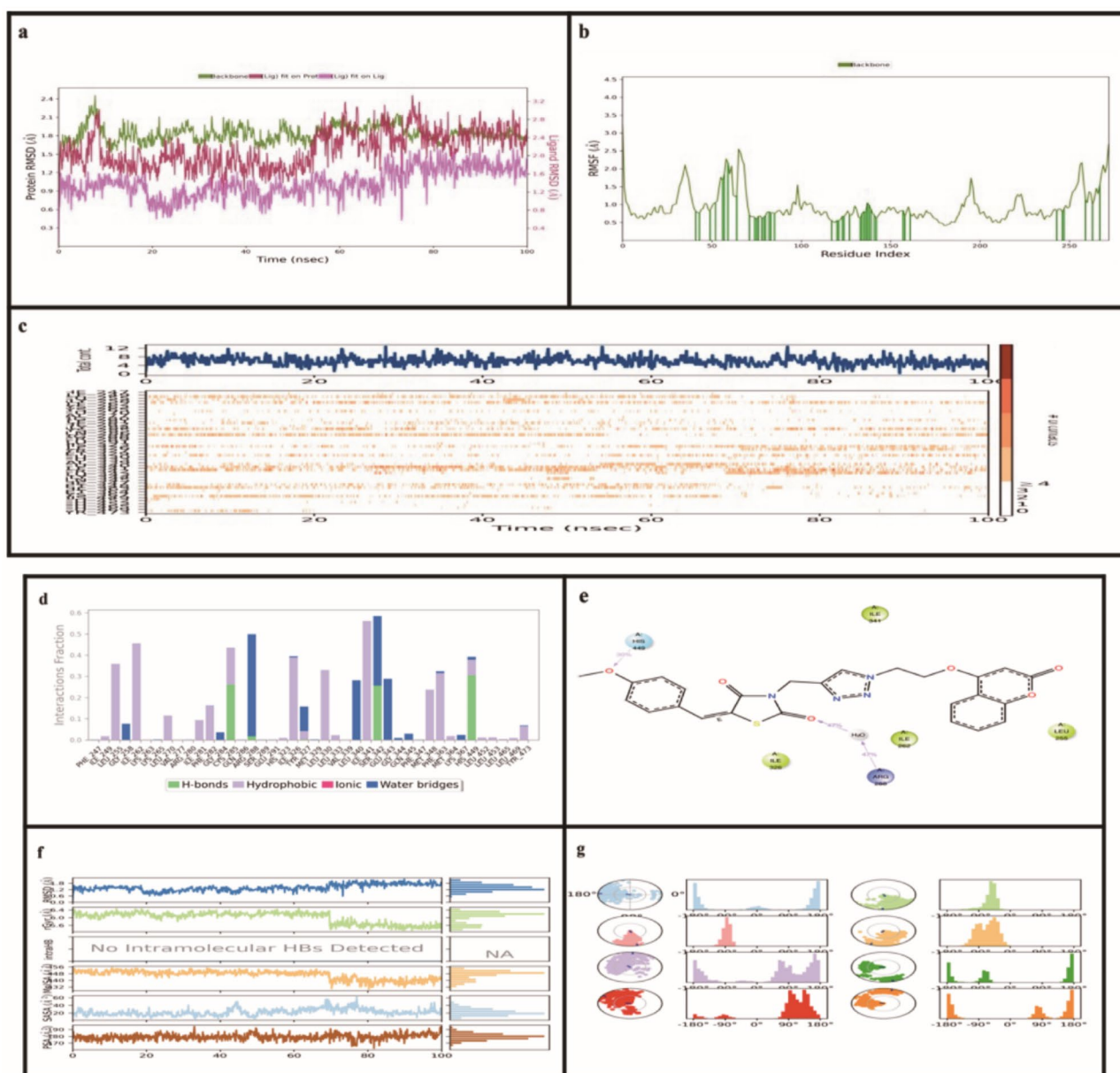


Fig. 9. Representing results for 100 ns MD simulation of **I-1** compound (a) Aligned RMSD of **I-1** and PPAR- γ complex, (b) Fluctuation of protein residues during the simulation, (c) Representing protein ligand contact points via amino acids residues, (d) Histogram shows the type of protein-ligand interaction, (e) 2D interaction diagram of **I-1** with protein residues, (f) Represents plots for Rgyr, intra HB, MolSA, SASA and PSA values of **I-1** and (g) The ligand torsions plot summarizes the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory.

types, indicating that **I-1** has a greater propensity to interact directly with the residues of proteins. In addition to these observations, analyses of solvent-accessible surface area, radius of gyration (Rgyr), molecular surface area (MolSA), and polar surface area (PSA) further supported the stability of the protein-ligand complex (Fig. 9f). However, significant fluctuations were observed in intramolecular hydrogen bonding, which may be attributed to the flexibility of the system.

Chemistry

The triazole based thiazolidinedione-coumarin hybrids (**I-1** to **I-16**) were designed and top most active compounds **I-1** was synthesized by six steps synthetic pathway as outlined in Fig. 11. Initially, the condensation reaction of thiazolidinedione and substituted aldehyde was carried out in the presence of Piperidine (basic condition) to yield benzylidene-thiazolidinedione intermediates. Further, these intermediates were propargylated with propargyl bromide in DMF containing K_2CO_3 as a base to get the propargylated intermediates. On the other side, alkylation of coumarins was carried out with alkylating agents 1,2-dibromoethane and followed by the

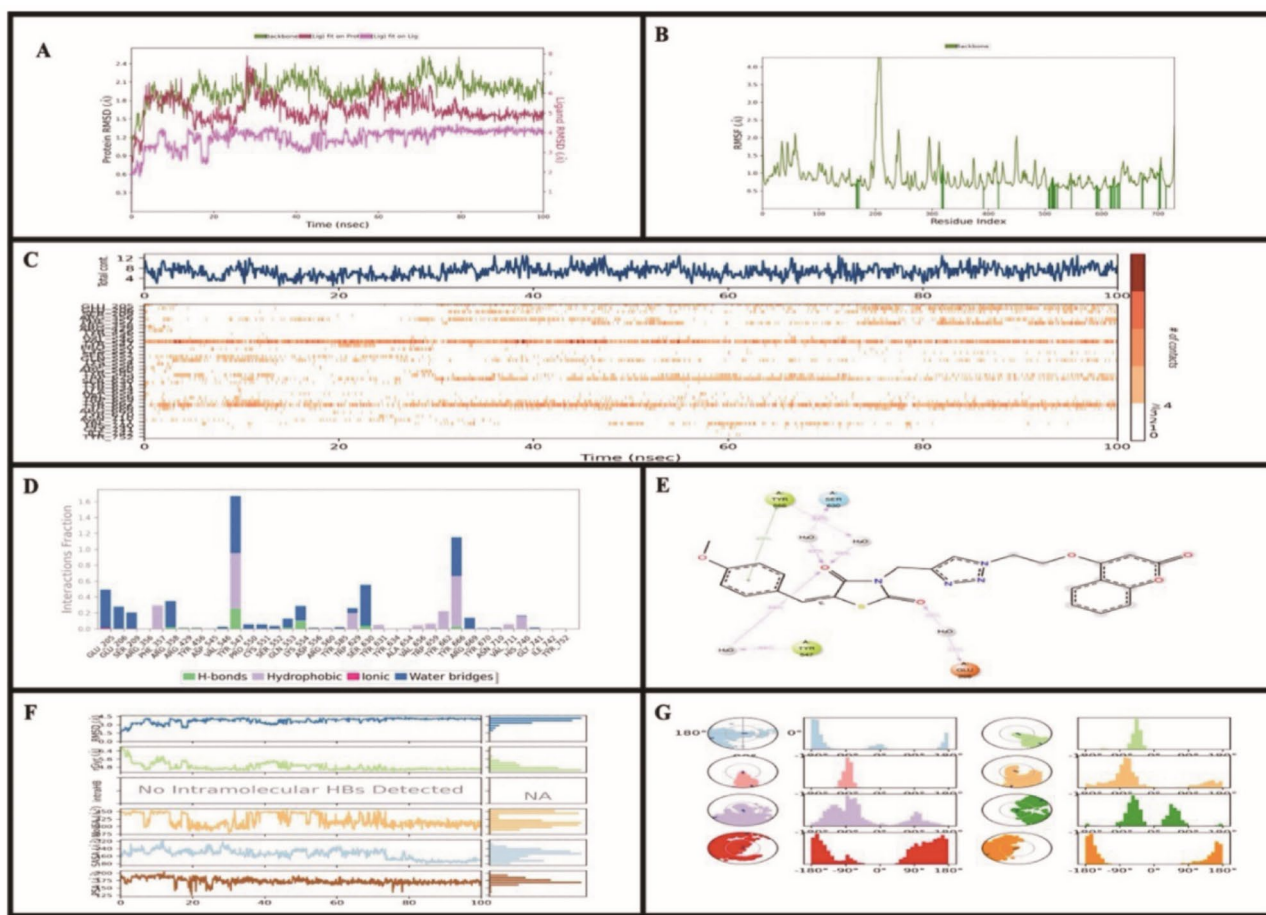


Fig. 10. Representing results for 100 ns MD simulation of I-1 compound (A) Aligned RMSD of I-1 and DPP-4 complex, (B) Fluctuation of protein residues during the simulation, (C) Representing protein ligand contact points via amino acids residues, (D) Histogram shows the type of protein-ligand interaction, (E) 2D interaction diagram of M with protein residues, (F) Represents plots for rgyr, intra HB, Molsa, SASA and PSA values of I-1 and (G) The ligand torsions plot summarizes the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory.

azidation of alkylated coumarin with sodium azide. In the final step, hybrid was synthesized by propargylated compound (1.0 mmol) and azidated coumarins were kept aside at room temperature with pinch of CuSO_4 and sodium ascorbate (1.0 mmol) in DMF that resulted in the formation of triazole based thiazolidinedione-coumarin hybrids. The structures of the compounds were fully characterized by ^1H NMR, ^{13}C NMR spectroscopic analysis.

Synthesis scheme

Spectral data of compound I-1 and their intermediated

- (1) Thiazolidine-2,4-dione: Yield: 78%, mp: 125–127 °C; Colour: White. ^1H NMR (DMSO- d_6 , 500 MHz, δ , TMS = 0) δ 11.98 (s), 4.15 (s). ^{13}C NMR (DMSO- d_6 , 125 MHz, δ , TMS = 0) δ 174.34, 173.58, 36.27. MS calcd for $\text{C}_3\text{H}_3\text{N}_2\text{O}_2\text{S}$ [M] : 117.12, found: 117.
- (2) (E)-5-(4-Methoxybenzylidene)thiazolidine-2,4-dione: Yield: 80%, mp: 85–95 °C; Colour: Yellow. ^{13}C NMR (DMSO- d_6 , 125 MHz, δ , TMS = 0) δ 168.55, 168.13, 161.45, 132.54, 132.19, 126.01, 120.96, 115.39, 115.39, 55.96. MS calcd for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_3\text{S}$ [M] : 235.03 found: 235.05.
- (3) (E)-5-(4-Methoxybenzylidene)-3-(prop-2-yn-1-yl)thiazolidine-2,4-dione: Yield: 65%, mp: 110 °C; Colour: Brown. ^1H NMR (DMSO- d_6 , 500 MHz, δ , TMS = 0) δ 7.94 (s, 1 H), 7.61 (d, J = 8.2 Hz, 2 H), 7.13 (d, J = 8.3 Hz, 2 H), 4.44 (s, 2 H), 3.85 (s, 3 H), 3.32 (d, J = 2.5 Hz, 1 H). ^{13}C NMR (DMSO- d_6 , 125 MHz, δ , TMS = 0) δ 166.97, 165.11, 161.85, 134.33, 132.89, 132.89, 125.75, 117.87, 115.51, 115.51, 77.74, 74.94, 56.03, 39.49. MS calcd for $\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_3\text{S}$ [M] : 273.05 found: 273.05.
- (4) 4-(2-azidoethoxy)-2 H-chromen-2-one: Yield 76%, mp: 92.3 °C, White powder. ^1H NMR (500 MHz, CDCl_3) δ 7.89–7.88 (d, J = 5 Hz, 1 H), 7.60–7.57 (m, 1 H), 7.35–7.30 (m, 2 H), 5.68 (s, 1 H), 4.47 (t, J = 5 Hz,

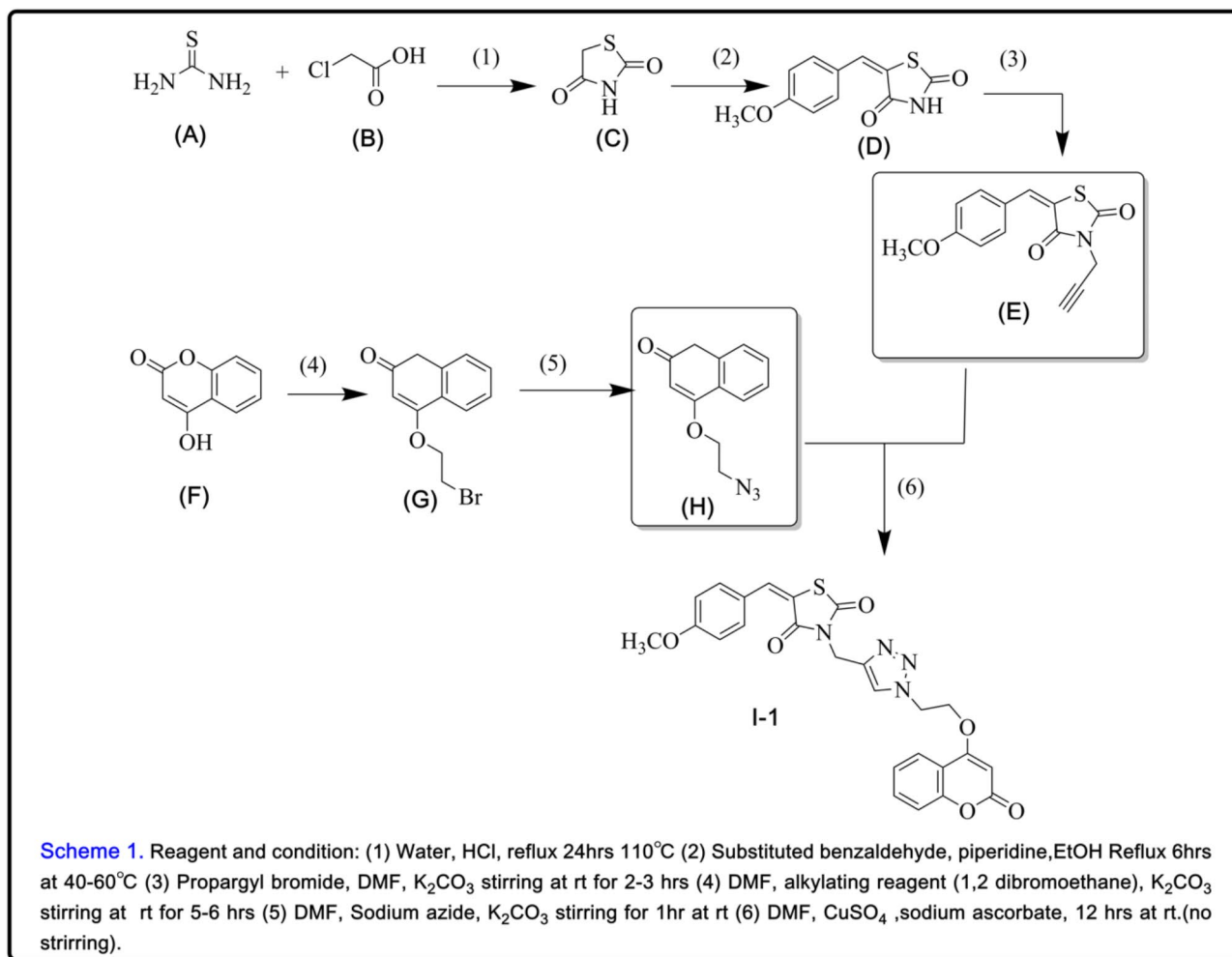


Fig. 11. Step wise synthetic scheme for the intermediates and compound I-1.

2 H), 3.79–3.76 (m, 2 H). ¹³C NMR (125 MHz, CDCl₃) δ 164.92, 162.58, 153.37, 132.68, 124.08, 123.11, 116.81, 90.90, 68.49, 30.91, 27.57. MS calcd for C₉H₅N₃O₃ [M]⁺: 203.03 found: 203.

- (5) (E)-5-(4-Methoxybenzylidene)-3-((1-(2-((2-oxo-2 H-chromen-4-yl)oxy)ethyl)-1 H-1,2,3-triazol-4-yl)Methyl)thiazolidine-2,4-dione: Yield: 50%, mp: 150 °C; Colour: Brown. ¹H NMR (DMSO-*d*₆, 500 MHz, δ, TMS = 0) δ 8.29 (s, 1 H), 7.87 (s, 1 H), 7.67 (d, J = 8.0 Hz, 1 H), 7.60 (dd, J = 12.5, 7.7 Hz, 3 H), 7.39–7.30 (m, 2 H), 7.12 (d, J = 8.4 Hz, 2 H), 5.93 (s, 1 H), 4.90 (d, J = 6.9 Hz, 4 H), 4.61 (t, J = 5.1 Hz, 2 H), 3.84 (s, 3 H). ¹³C NMR (DMSO-*d*₆, 125 MHz, δ, TMS = 0) δ 167.43, 165.68, 164.68, 161.92, 161.75, 153.17, 133.87, 133.27, 132.78, 124.97, 124.67, 123.22, 118.20, 116.85, 115.50, 115.37, 91.54, 68.17, 56.01, 48.87, 37.02. MS calcd for C₂₅H₂₀N₄O₆S [M]⁺: 504.51 found: 504.

Biological evaluation

In-vitro α-glucosidase assay

Among all the designed compounds (I-1 to I-16) only I-1 found to be most active against α-glucosidase and showed variable degree of percentage inhibition against α-glucosidase values ranging from 27 ± 0.23 to 100 ± 0.22 μg/ml (Table S7 in supplementary file). Compound I-1 (IC₅₀ = 1.49 μg/ml) was observed and found to be the most potent than standard drug acarbose (Fig. 12).

In-vitro antioxidant activity

The antioxidant compounds can protect β-cells from reactive oxygen species (ROS), thereby preventing diabetes induced by ROS. ROS causes oxidative stress, leading to lipid peroxidation, protein glycation/oxidation and nitration, enzyme inactivation, and DNA damage. These processes contribute to various pathological conditions, including diabetes mellitus (DM) and neurodegenerative diseases. Fortunately, these effects can be mitigated by endogenous or exogenous antioxidant systems. The antioxidant activity of the hybrid compounds (I-1) was assessed using the stable DPPH assay (Table S8 in supplementary file). The results, shown in Fig. 13, present IC₅₀ values, which indicate the concentration required to achieve 50% of the reducing power for hydrogen-donating or radical scavenging. The scavenging effect increased in the following trend: I-1 > standard drug (ascorbic acid).

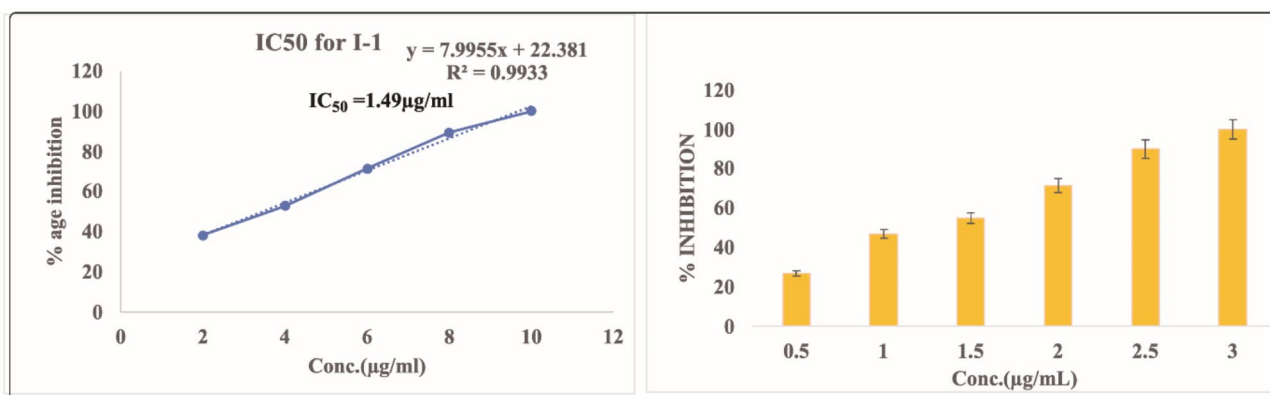


Fig. 12. Graphical representation of percentage inhibition against different concentration of compound I-1 for α -glucosidase enzyme with the IC_{50} value.

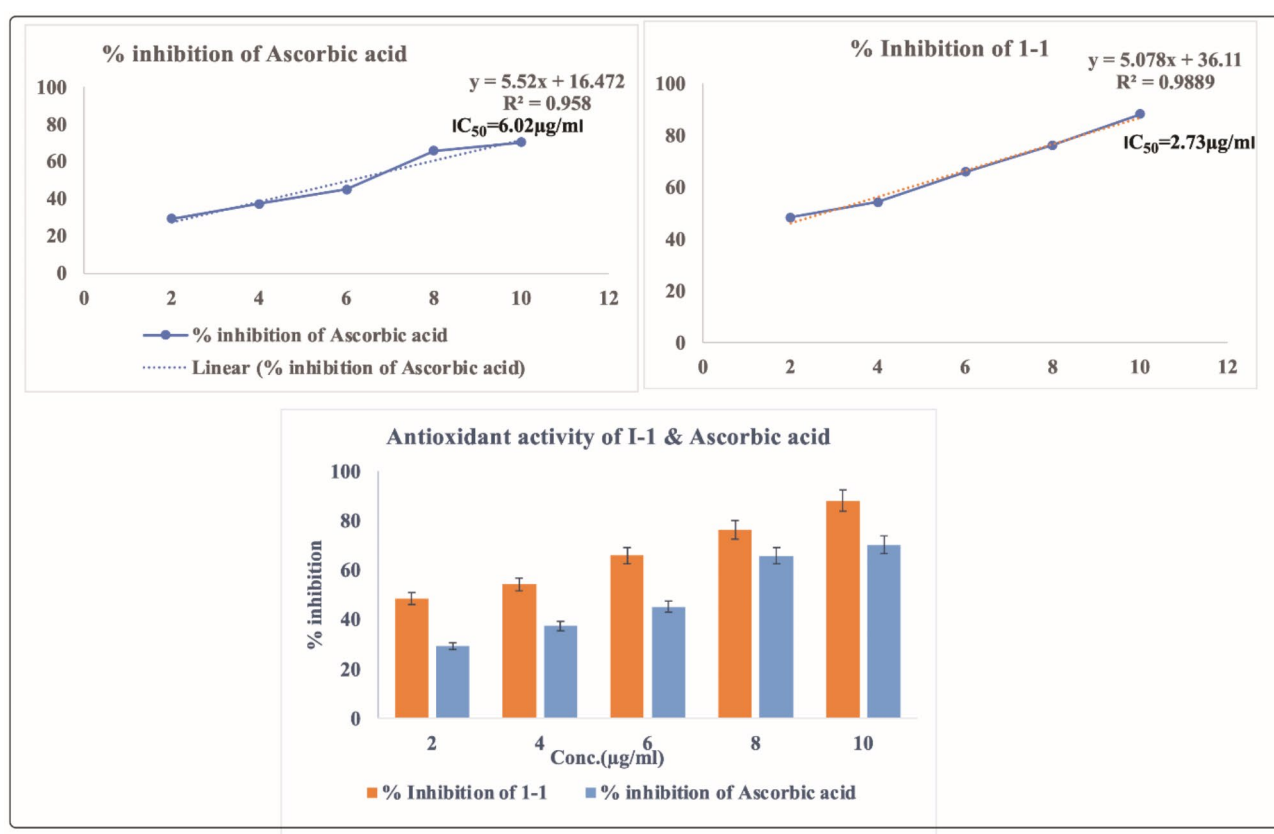


Fig. 13. Graphical representation of percentage inhibition against different concentration of compound I-1 and ascorbic acid in DPPH radical scavenging assay.

Specifically, compounds (I-1) demonstrated higher antioxidant activity than the standard, with IC_{50} value of 2.73 μ g/ml for I-1 and 6.02 μ g/ml for ascorbic acid, as illustrated in Fig. 13.

In-vivo studies

Acute oral toxicity study

For the acute toxicity study of the compound I-1, a limit test dose of 2000 mg/kg body weight was selected for experimental rats. During the LD_{50} evaluation, it was observed that the animals remained safe and unharmed at this Maximum dose, showing no abnormal behavioural changes or Mortality. According to OECD guidelines, the LD_{50} for compound I-1 falls within class IV, with no signs or symptoms of acute toxicity noted at the 2000 mg/kg dose. Pharmacological evaluations were then conducted at doses of 10, 20 & 30 mg/kg body weight.

In-vivo antidiabetic activity

Compound I-1 was assessed for its antidiabetic efficacy in an alloxan-induced diabetic rat Model. Doses of 10, 20, and 30 mg/kg were administered, with Acarbose as a standard drug. The study evaluated fasting blood glucose levels, body weight, lipid profile, serum insulin, and hepatic and renal biomarkers. Diabetic rats exhibited hyperglycaemia compared to healthy controls. Treatment with compound I-1 significantly attenuated hyperglycaemia in a dose-dependent Manner. The most pronounced antidiabetic effect was observed after four weeks of treatment (Fig. 14). At doses of 10 and 20 mg/kg, Compound I-1 reduced blood glucose levels to 94.15 and 74.60 mg/dL, respectively. The Acarbose-treated group achieved a blood glucose level of 80.44 mg/dL.

Effect of tested compound on lipid profile

The study evaluated the effects of compound I-1 and the standard drug acarbose on serum lipid profiles in diabetic rats. Both treatments significantly reduced total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels compared to the diabetic control group (Fig. 15). Additionally, levels of high-density lipoprotein (HDL) were elevated in both the I-1 and acarbose-treated groups compared to the diabetic control, indicating a positive impact on lipid metabolism.

Effect of compound I-1 on body weight and insulin level

Diabetic rats showed a significant decrease in body weight compared to healthy controls. However, treatment with I-1 and acarbose resulted in weight gain relative to the diabetic control group Table 5. Furthermore, serum insulin levels were inversely correlated with blood glucose levels. On day 28, diabetic rats exhibited a significant reduction in serum insulin. In contrast, administration of I-1 and acarbose led to a maximal increase in serum insulin levels by day 28, suggesting an improvement in metabolic function.

Effect of tested compound I-1 on liver and renal serum biomarkers

Diabetic rats displayed elevated levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea, lactate dehydrogenase (LDH), and creatinine, indicating impaired liver and kidney function. These biomarkers were significantly higher in diabetic rats compared to healthy controls. However, treatment with I-1 and acarbose led to a significant reduction in these parameters, suggesting an improvement in liver and kidney function in the treated groups as shown in Fig. 16.

Discussion

The primary goal of every research project is to discover novel and more potent compounds without compromising the safety issue. The present study evaluated the pharmacokinetic studies via SwissADME and stability through DFT studies and molecular simulation of the newly synthesized compound followed by antidiabetic and antioxidant potential through in vitro assays and in-vivo studies.

Thiazolidinedione and coumarin are heterocyclic molecules have been evaluated for their ability to lower blood glucose level. The derivatives consists of a six-membered ring combined with a five-membered thiazolidinedione ring with triazole moiety. According to the traditional literature, thiazolidinedione has been

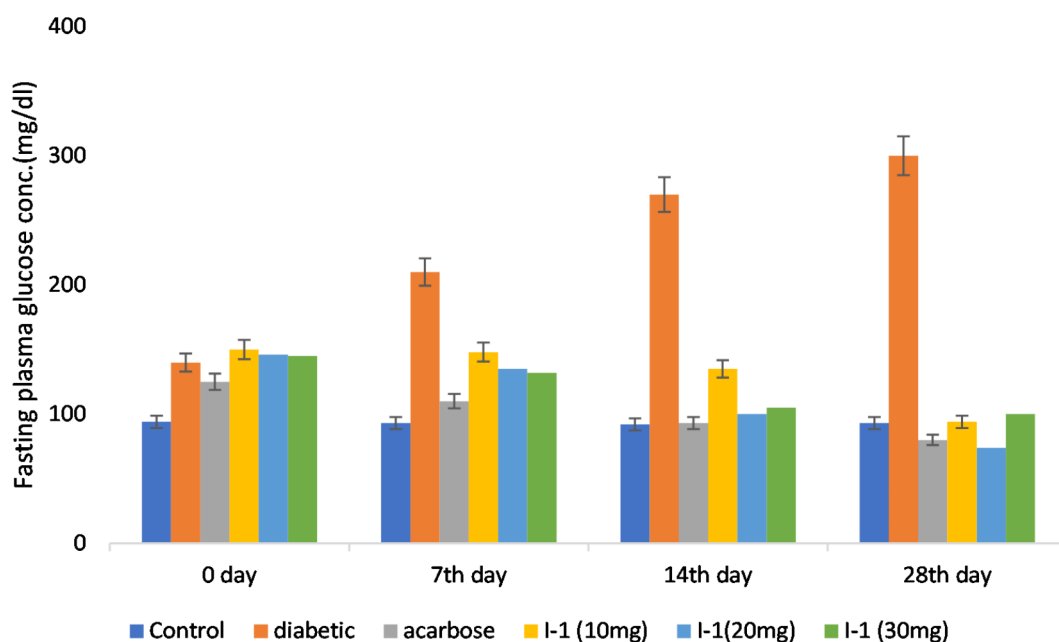


Fig. 14. Effect of tested compound I-1 on fasting plasma glucose level. Values were presented as Mean \pm SEM (number of animals, $n = 6$) and were different significantly $^*p < 0.05$ in comparison to normal control group, $^*p < 0.05$ when compared with diabetic control group.

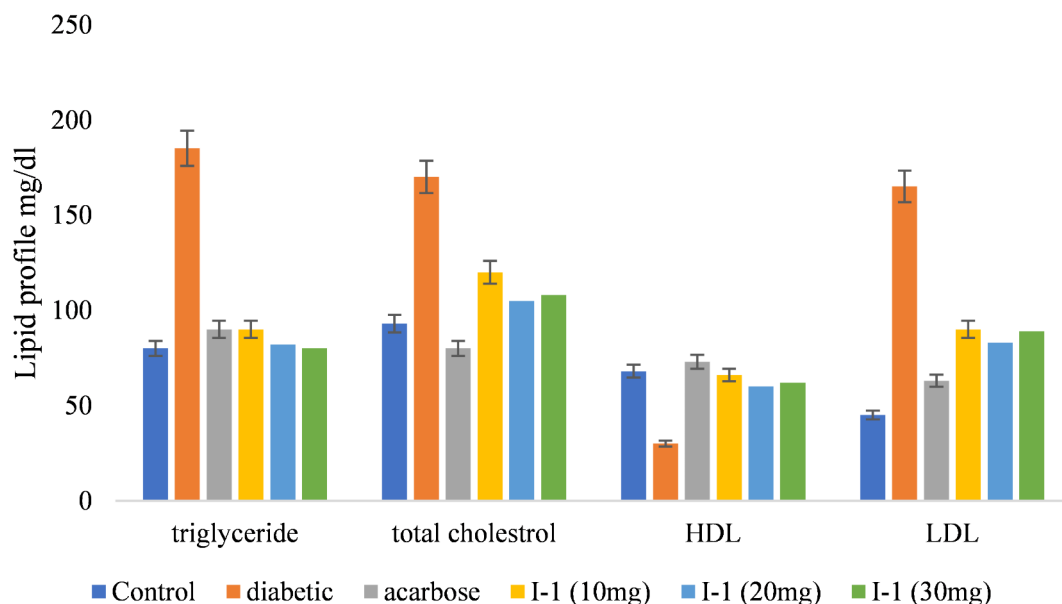


Fig. 15. Effect of tested compound I-1 on lipid profile. Values were presented as Mean \pm SEM (number of animals, $n = 6$) and were significantly different at $p < 0.05$ in comparison to normal control group, $p < 0.05$ when compared with diabetic control group.

Group	Changes in body weight (gm)			Insulin Level (Mean \pm SEM) in mg/dl	
	Before induction	After induction	After treatment	Initial reading	Final reading
Control	193.23 \pm 1.24	196.35 \pm 1.45	189.35 \pm 1.56	0.73 \pm 0.12	0.79 \pm 0.87
Diabetic	188.86 \pm 2.12 ^{ns}	178.80 \pm 1.63 ^{###}	148.65 \pm 2.54 ^{###}	0.84 \pm 0.26 ^{ns}	0.55 \pm 0.13 ^{ns}
Acarbose	179.98 \pm 1.67 ^{ns}	162.74 \pm 2.24 ^{**}	178.90 \pm 4.24 ^{***}	0.82 \pm 0.54 ^{ns}	0.80 \pm 0.18 ^{ns}
I-1 (10 Mg)	191.23 \pm 1.50 ^{ns}	156.28 \pm 3.50 ^{***}	182.43 \pm 4.70 ^{***}	0.80 \pm 0.28 ^{ns}	0.67 \pm 0.25 ^{ns}
I-1 (20 Mg)	200.23 \pm 1.76 ^{ns}	147.34 \pm 1.50 ^{ns}	188.38 \pm 5.67 ^{***}	0.76 \pm 0.48 ^{ns}	0.75 \pm 0.18 ^{ns}
I-1 (30 Mg)	204.66 \pm 3.60 ^{***}	171.60 \pm 1.90 ^{ns}	198.19 \pm 4.66 ^{***}	0.73 \pm 0.38 ^{ns}	0.70 \pm 0.12 ^{ns}

Table 5. Effect of compound I-1 on body weight and insulin level. Values are presented as Mean \pm SEM (no. Of animals, $n = 6$) and were significantly different at ^{###} $p < 0.001$ in comparison to normal control group, while ^{***} $p < 0.001$, ^{**} $p < 0.01$, ^{ns} $p > 0.05$ when compared with diabetic control group.

used to treat inflammatory conditions, cancer, antimicrobial, antidiabetic, antioxidant anti-HIV and infections. Industry and academics have been attracted by its varied pharmacological profile to synthesize novel synthetic thiazolidinedione derivatives with a range of biological functions. The study aimed to synthesize triazole based thiazolidinedione-coumarin hybrids referred to as I-1, by refluxing substituted aromatic aldehyde with thiazolidinedione es in ethanol with the addition of a catalytic amount of piperidine followed by the propargylation. Coumarin were azidated with sodium azide in DMF followed by the molecular hybridization of propargylated and azidated coumarin with the addition of CuSO₄ and sodium ascorbate to yield the compound I-1. The structures of I-1 and their intermediates were characterized and confirmed by using ¹H NMR, ¹³C NMR, and mass spectroscopy. Lipinski's rule of five is required for practical drug creation, according to the results of the pharmacokinetic investigation of the thiazolidine-coumarin hybrid I-1 and Acarbose were compared. All the compounds were tested computationally against α -glucosidase, DPP-4, PPAR- γ . Among all the compounds only I-1 was found to be potent and comparable to the standard drug acarbose. Molecular docking studies results revealed that compound I-1 was found to be potent for all the enzymes except α -amylase. Also, compound I-1 were found to be most potent according to their binding energy and dock score value. Further, from these results only compounds (I-1) were selected for the synthesis to save the chemicals and time as only compound I-1 were found to be active. After synthesizing, the final compound and their intermediated were characterized by NMR spectroscopy. The compounds were further biologically evaluated (In-vitro) for alpha- glucosidase as well as antioxidant activity (DPPH assay). The percentage inhibition was determined and results revealed that compound I-1 found to be most active against alpha glucosidase with 100% inhibition. Further Compound I-1 then evaluated for In-vivo studies and results revealed that treatment with compound I-1 and Acarbose resulted in a significant reduction in these parameters, which suggesting the improved liver and kidney function.

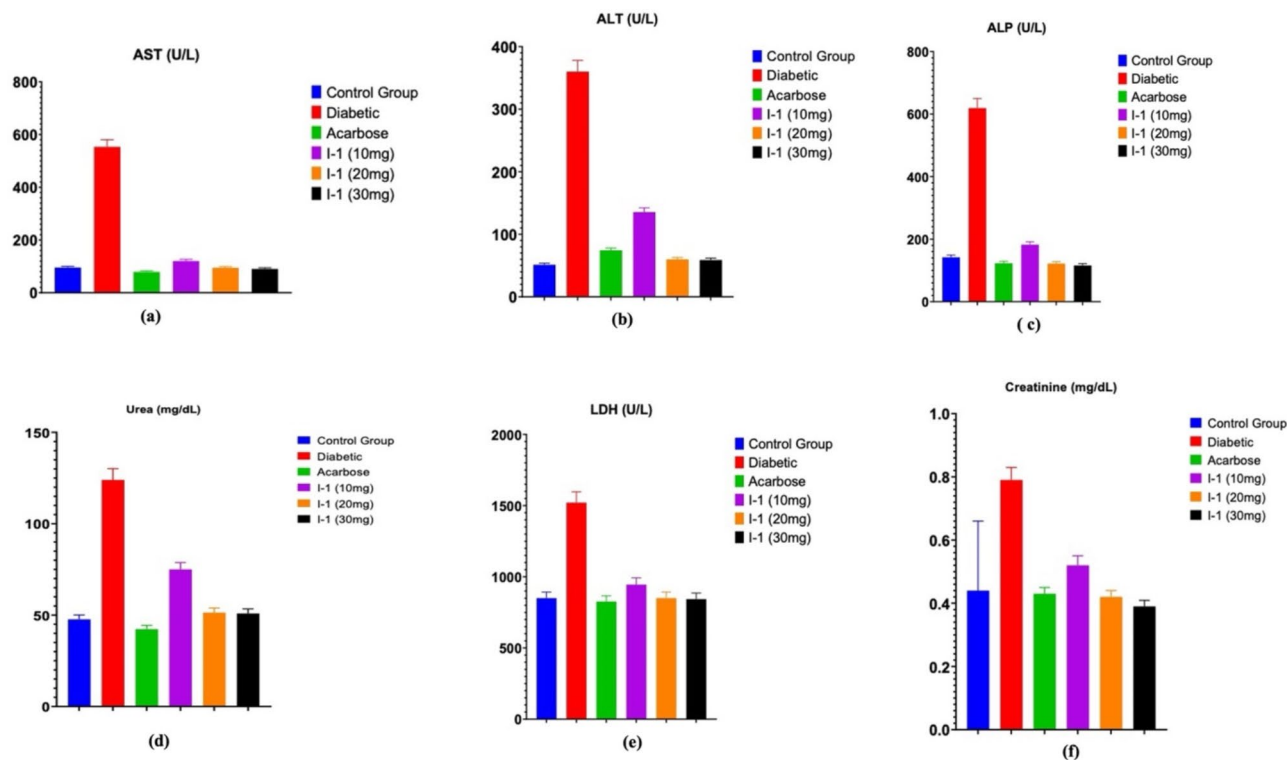


Fig. 16. Graphs represents the (a) AST (b) ALT (c) ALP (d) Urea (e) LDH (f) Creatinine levels and values were presented as Mean \pm SEM (number of animals, $n = 6$) and were significantly different at $p < 0.001$ in comparison to normal control group, while $p < 0.001$ when compared with diabetic control group, ns (not significant); $p > 0.05$.

Conclusion

Bioactive Moieties like thiazolidinedione and coumarin are active for antidiabetic activity and derivatization of these Molecules helps to form potent compounds. Computational studies such as Molecular docking, In the process of finding and developing new drugs, compounds with high bioactivity and low toxicity are probably desired. Designing possible therapeutic candidates is made easier with the help of molecular structure-based in silico ADMET (absorption, distribution, Metabolism, excretion, and toxicity) property screening. During the drug discovery process, the pharmacokinetic failure rate in clinical phases is greatly decreased when ADME features are predicted early on. Results revealed that the compound I-1 were found to be potent in vitro and in In-vivo studies.

Experimental

Molecular docking (LeadIT software)

Preparation of library

Structure of designed compound drawn and were prepared by using standard parameters of “prepare ligands” Module of DS Biovia’s Discovery studio software which removes duplicate entries, enumerates isomers and tautomer, and generates 3D conformations and Minimizes those conformations. Then the Prepared ligands were screened using standard protocols of “Filter by Lipinski and Veber Rules” Module of DS Biovia’s Discovery studio.

Preparation of proteins

3D structure was downloaded from RCSB Protein Data Bank (PDB code: 7KBJ, 2QV4, IX70 and 2PRG). The PDB file was prepared using standard parameters of prepare proteins|| tool of DS Biovia’s Discovery studio software which cleans the protein, optimizes side chain conformation for residues with inserted atoms, removes water molecules Models Missing loop regions based on SEQRES information. Later, the binding sphere was generated by selecting the position of co-crystallized compound within the active site to define the binding sites.

High throughput screening of ligands

The prepared protein was loaded into the libdockscore Module of DSBDS and binding site sphere was select from the available options. Later, the prepared and screened ligand library was loaded and rigid docking was performed using standard protocols. This process generated various conformations and poses of the ligands w.r.t. α -glucosidase, α -amylase, DPP-4 and PPAR- γ . After the successful completion of the docking process, the

resultant file displays libdockscore, which was then utilized to screen and prioritize the appropriate poses of docked ligands.

Setup ligand protein docking calculation

Select a protein and a ligand from your library. Modify advanced parameters during the simulation, such as number of runs, number of evaluations etc.

Evaluation of results

Choose an image from the image gallery or render in Molecular Docking Server and analyse the secondary interactions between the protein and ligand. Scoring of ligand poses and calculation of Binding Energies: Scoring of ligand poses played a crucial role in the screening and evaluation of the best binding pose and also helped in rapidly estimating the binding affinity of a ligand. These usually utilize empirical fitting approach and knowledge-based statistical approach and. The protein and ligands to be scored, were loaded into the protocol of Score ligand poses dialog-box and scoring was performed. Later, binding energy was calculated to estimate the binding energy between the enzyme and the ligand (Table S1 in supplementary file). These were calculated by the following Eq.

$$\text{Energy}_{\text{Binding}} = \text{Energy}_{\text{Complex}} - (\text{Energy}_{\text{Ligand}} + \text{Energy}_{\text{Receptor}}) \quad (1)$$

ADME studies

ADMET study was done using Swiss ADME, which is available at <http://www.swissadme.ch>, the ADME parameters of the proposed compounds were examined.

DFT calculation

Theoretical calculations for the ground state energy optimizations of compound **I-1** were conducted using Gaussian 09 suite of programmes employing the B3LYP/6-31G(d, p) basis set³⁷. The energy optimised structures and frontier molecular orbital pictures were visualized on using Gauss-View 5.0.9 software. Also, Electrostatic potential map (ESP) was generated for all the synthesized molecules, quantum chemical parameters such as the energy gap (ΔE_{GAP}), dipole moment (μ), hardness (η), local softness (σ) can be calculated using the (Frontiers molecular orbitals) highest occupied molecular orbital (E_{HOMO}), lowest unoccupied molecular orbital (E_{LUMO}).

Molecular simulation studies

To replicate a biological system, a protein-ligand complex was constructed using the “system builder” module of D. E. Shaw’s Desmond software. An SPC solvent model was chosen with an orthorhombic box of dimensions $10 \times 10 \times 10$ dimensions. Counter ions (Na^+ or Cl^-) were added for neutralization, with a concentration set to 0.15 M, and the OPLS3e force field was applied. A brief molecular dynamics (MD) simulation lasting 100 ns was conducted DPP-4 (1 \times 70), PPAR- γ (2PRG) and the top ligand (**I-1**) using the “Molecular Dynamics” module of Desmond. Prior to the final MD simulation, the model underwent a five-stage minimization process: (a) Stage 1: Brownian Dynamics NVT at 10 K, with small timesteps and restraints on solute heavy atoms for 100 ps. (b) Stage 2: NVT at 10 K, with small timesteps and restraints on solute heavy atoms for 12 ps. (c) Stage 3: NPT at 10 K, with restraints on solute heavy atoms for 12 ps. (d) Stage 4: NPT with restraints on solute heavy atoms for 12 ps. (e) Stage 5: NPT with no restraints for 24 ps. (f) Stage 6: The final simulation lasted 100 ns, encompassing approximately 200,000 steps⁴⁰.

Synthesis

Materials and methods

The used starting Materials and solvents were purchased from the commercial sources and purified using standard procedures whenever required. Yields refer to isolated yields of products after purification. The completion of the reactions and the homogeneity of the products were Monitored by thin layer chromatography (TLC) using pre-coated TLC-sheets (ALUGRAM[®] Xtra SIL G / UV254). The plates were examined under UV cabinet at 254 nm. The Melting points were determined on Stuart melting point apparatus and were uncorrected. The structures of the synthesized compound and their intermediated were confirmed by ¹H NMR, ¹³C NMR spectroscopy. ¹H and ¹³C NMR spectra were recorded on Bruker (500 mhz) spectrometer in deuterated dimethyl sulfoxide (DMSO- d6). The chemical shifts were reported in parts per Million (ppm) using tetramethylsilane as internal standard. The spin Multiplicities were indicated by the symbols: bs (broad singlet), s (singlet), d (doublet), dd (doublet of doublet), t (triplet) and M (Multiplet).

General procedure for the synthesis of thiazolidinedione

It includes the reaction of thiourea with chloroacetic acid in dry RBF, thiourea(1mmol) was added in of chloroacetic acid (1mmol) with 20 ml of water. HCL was added to the reaction Mixture drop wise with continuous stirring till all the white precipitates were dissolved. When the clear Mixture was observed, the reaction was shifted to reflux for 10–12 h at 100–110 °C. The reaction was Monitored by TLC. After the completion of the reaction crushed ice was added into mixture and kept inside for sometimes to obtain the precipitates. Then it was filtered and air dried to obtain the precipitates of product i.e. Thiazolidinedione. The characterization of the compound was done using NMR¹⁴.

General procedure for the synthesis of benzylidene 2,4-thiazolidinedione

It includes reaction of thiazolidinedione (1mmol) with aromatic aldehydes (1mmol) in double necked RBF (Round Bottom Flask), ethanol (20–30 ml) was added. Piperidine (1mmol) was added as catalyst and reaction Mixture stirred for 16–24 h. at 110 °C. After the completion of the reaction (evident by TLC), reaction mixture was poured on crushed ice and kept aside for some time. Then it was filtered and air dried to obtained Benzylidene 2,4- thiazolidinedione as product. The characterization of the compound was done using NMR.

General procedure for preparation of propargylated benzylidene 2,4-thiazolidinedione

It includes propargylation of Benzylidene 2,4-thiazolidinedione in a dry conical flask, thiazolidinedione (1mmol) was added along with DMF (10–15 ml) as solvent and potassium carbonate (1.5 mmol) as base. The reaction was stirred for 5 min, propargyl bromide was added after sometime, then the reaction mixture was stirred for 1 h. The reaction was monitored by TLC. After the completion, reaction mixture was poured on crushed ice and kept inside till precipitates was observed. Then it was filtered and air dried to obtained Propargylated Benzylidene 2,4- thiazolidinedione as product. The characterization of the compound was done using NMR.

General procedure for preparation of alkylation of coumarin with alkylated agents

It including alkylation of coumarin (1 mmol) with dibromoethane (4 mmol), (alkylated agents) at room temperature with stirring for 5–6 h. DMF used as solvent and potassium carbonate as base to form alkylated coumarin. After completion of reaction (evident by TLC), reaction mixture was poured on crushed ice and kept aside for some time. Then it was filtered and air dried to obtained alkylated product.

General procedure for preparation of azidated coumarin with sodium azide

It includes azidation of alkylated coumarin (1mmol) with sodium azide (1mmol) at room temperature with stirring for 5–6 h. DMF was used as a solvent and potassium carbonate as base to form azidated coumarin. After completion of reaction (evident by TLC), reaction mixture was poured on crushed ice. Then it was filtered and dried to obtain crude azidated products, which was further purified by column chromatography (Hexane/EtOAc).

General procedure for preparation of propargylated benzylidene 2,4-thiazolidinedione and alkylated coumarin hybrids

It includes hybridization of propargylated Benzylidene 2,4-thiazolidinedione (1 mmol) and azidated coumarin (1 mmol) at room temperature with addition of sodium ascorbate and cupric oxide and DMF as solvent, the reaction mixture will keep for 24 h. The reaction will monitor by TLC. After completion of reaction the workup was done by pouring reaction mixture on the crushed ice by passing from silica bed. The characterization of compound was done using NMR spectroscopy.

Biological evaluation

In vitro α -glucosidase inhibition assay

The α -glucosidase inhibitory activities of compound I-1 was tested as per earlier described procedures^{22,43}. In a 96- well plate, 60 μ l of sample solution, prepared by dissolving test compound in 5% DMSO and phosphate buffer [pH 6.8]), and 50 μ l of 0.1 M phosphate buffer (pH 6.8) containing α -glucosidase solution (*Saccharomyces cerevisiae*) (0.2 μ /ml) was incubated at 37 °C for 10 min. To this solution, 50 μ l of 5 mm p-nitrophenyl- α -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added and incubated at 37 °C for another 20 Min. To stop the reaction, 40 μ l of 0.8 M sodium carbonate was added to each well and the absorbance was recorded at 405 nm by microplate reader⁴¹. For control, 60 μ l of 5% DMSO was taken in place of the sample solution and acarbose was used as standard. The tests were performed in triplicate and percentage inhibition was calculated as follows:

$$\text{Inhibition percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The concentration of inhibitors corresponding to 50% of the α -glucosidase inhibition under the assay conditions was defined as the IC_{50} value.

In-vitro antioxidant assay (DPPH assay)

2, 4, 6, 8 & 10 μ g/ml concentrations of ligands and ascorbic acid were prepared. From this stock solution 1 ml has been pipette out and 5 ml methanol solution of DPPH was added, shaken well and the mixture was incubated at 37 °C for 30 min absorbance of all samples were measured against blank at 517 nm. The absorbance of DPPH reagent alone was taken as control. The Percentage radical scavenging activity can be calculated following formula:

$$\text{Inhibition percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Animal studies (in-vivo studies)

Approval of animal studies

Animals were approved by Institutional Animal Ethics Committee (IAEC), GNDU, Amritsar in its meeting held on 31/05/2022 and total 72 rats has been sanctioned under the registration no. 226/CPCSEA/2022/06.

Procurement of animals

72 Sprague-Dawley Female rats were procured from Animal house facility Punjab university, Chandigarh and In vivo studies were performed on female Sprague-Dawley rats and were kept in the animal house at Guru Nanak Dev University Amritsar until experiments were performed. All the animals were acclimatized for 1 week under standard conditions with a temperature of 22 ± 1 °C and humidity of $50 \pm 5\%$ with a 12 h light/dark cycle and had free access to commercial food pellets and fresh tap water. The experimental protocol was approved by the Animals were approved by Institutional Animal Ethics Committee (IAEC), GNDU, Amritsar.

Animal ethics statement

And all the experiment were carried out under the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were in compliant with ARRIVE guidelines.

Toxicological screening

Sprague-Dawley rats weighing 150–230 gm were utilized for our studies. In the antidiabetic studies, rats weighing 150–230 gm were used, whereas for the toxicity studies, rats aged 8–12 weeks were used. The animals were acclimatized for a period of seven days to the laboratory environment before all the experimental procedures and were given sufficient food and water. For the acute toxicity study, a limit test on the laboratory animals at 2000 mg/kg body weight was selected and the up and down procedure (UDP) was followed as per the guidelines of the Organization for Economic Cooperation and Development (OECD). The purpose was to evaluate the dose necessary to perform various pharmacological activities.

Selection of animals

Healthy young adult female rats of about 8 to 12 weeks old were selected for dosing.

Preparation of animals

The rats were randomly selected, Marked for an individual identification, and retained in their cages for at least 5 days before dosing to acclimatize to the laboratory conditions. Prior to dosing, the animals were fasted and they were refrained from food, not water, for 16–18 h⁴².

Calculation and administration of dose

After the fasting period, the weight of each animal was determined and according to the body weight of the animals, the dose at 2000 mg/kg body weight was calculated for each animal. A unique dose of the studied compound I-1 was administered to the animals for the UDP study by the oral route using an intragastric cannula, and it was planned to dose the five animals on the same day. After the 1st 30 Min of dosing, the animals were observed at least once; after the first 24 h, the animals were observed periodically with special care given during the first four hours and thereafter observed daily for 14 days. The onset time, toxic reactions, and length of the recovery period determined the duration of the observation and when considered necessary, may be extended. The important duration is the time at which toxicity signs appear and disappear. Individual observations were recorded and Maintained systematically for each animal. Observations such as changes in the Mucous Membranes and eyes, skin, and fur, the circulatory, respiratory, central, and autonomic nervous systems, the motor activity, and the behaviour pattern were included. Observations of the salivation, tremors, convulsions, lethargy, diarrhoea, sleep, and coma were also considered³¹.

Antidiabetic activity

The antidiabetic activity of the synthesized compound I-1 was evaluated using a Alloxan-induced diabetic rats Model.

Animals selection, care, and handling

Female Sprague-Dawley rats weighing 150 to 230 gm were used for the study which were housed in polyacrylic cages (no More than six animals per cage) with dimensions of $38 \times 23 \times 10$ cm and Maintained under standard laboratory conditions with a dark/light cycle of 14 h of dark and 10 h of light at a temperature of 25 °C. The animals were provided with a standard diet (dry pellet) and water. Before the start of the experimental procedures, the rats were acclimatized to laboratory conditions for 10 days. All the experiments were approved and performed as per the guidelines of the Institutional Animal Ethics Committee (IAEC), GNDU, Amritsar under letter No. 226/CPCSEA/2022/06.

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

Non-insulin-dependent diabetes Mellitus was induced in fasted rats weighing 150–230 gm using a single dose of Alloxan at a dose of 40 mg/kg intraperitoneally. After the induction of diabetes, the increase in the plasma glucose level was measured at 72 h and then Measured on the 7th day to confirm hyperglycaemia. For the diagnosis of diabetes, the threshold value of the fasting plasma glucose level was considered to be more than 126 mg/dl. For the study, only the animals with permanent NIIDM were utilized⁴³.

Experimental design

The animals were classified into 5 groups with 6 animals in each group ($n = 6$). Group I comprised of normal control rats which were daily administered drinking water only; group II served as diabetic control rats (disease group); group III served as the positive control and were administered 0.5 mg/kg of the standard drug acarbose; and group IV, V and VI served as the test group rats and were administered the test compound I-1 at 10, 20 and 30 mg/kg, respectively, for twenty-eight days. The fasting glucose concentration was Measured on the 0, 7th,

14th, and 28th day of the administration of the compound. During the experimental tenure, the weights of the rats were determined daily and the Mean change was recorded.

Insulin level estimation

After the 28th day of treatment with the test compound I-1, the blood samples were taken for the determination of the insulin levels.

Determination of biochemical parameters

The animals were euthanized (sacrificed) via cervical dislocation on the 28th day to observe the biochemical parameters. The parameters studied were triglyceride (TGL), cholesterol, low-density lipoprotein, and high-density lipoprotein (LDL and HDL). The serum samples from the rats were also checked for alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), serum alkaline phosphatase (ALP), and creatinine and urea levels utilizing commercially available kits of Crest Biosystems. The statistical analysis was also performed as per the previous procedure.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Received: 7 October 2024; Accepted: 31 January 2025

Published online: 04 February 2025

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Acknowledgements

Acknowledgement The authors are thankful to, Dassault Systemes BIOVIA, DE Shaw Desmond and Guru Nanak Dev University, Amritsar for providing various basic facilities to complete the work. Authors are also thankful to, Dr. Sumanpreet Kaur and her student Garima for providing central instrumentation facility, Lovely Professional university in the Mass spectra analysis.

Author contributions

A.S.: Conceptualisation, Methodology, Resources, Validation, Writing-original draft, writing review & editing. A.N.: In-vitro studies. N.K.: Molecular simulation, editing and review. R.R.: Formatting, editing. P.: Methodology, In-vivo studies. M.D.: Synthesis and data curation. M.: DFT studies and result validation. H.K.G.: writing review and data curation. J.: Data curation. A.K.: Data curation. J.V.S.: Data curation & review. S.K.: Resources and validation. P.M.S.B.: Resources, validation & supervision.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-88944-y>.

Correspondence and requests for materials should be addressed to A.S. or P.M.S.B.

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