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Structure-Activity Relationship and Molecular Docking-Guided Development of Andrographolide-Based Antidiabetic Agents

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Abstract:

Andrographolide was a type diterpenoid lactone situated in Andrographis paniculata. It has gained attention because it might assist diabetics. Its application, on the other hand, is restricted due to its low oral bioavailability, rapid metabolism and very poor stability in vivo. In this report, we used a rational design strategy to generate semisynthetic andrographolide derivatives (SADRs) and tested for their potency toward targeted enzymes in the glucose metabolism. we synthesised two analogues (P-1 to P-4) and solved the three structures. Molecular docking studies with α-glucosidase and α-amylase reveal the binding mode of our compounds, showing how they inhibit both enzymes. The stable hydrogen bonds and hydrophobic interactions of derivative P-2 and P-3 with component parts of the enzyme are responsible for their strong binding affenity. This means they inhibited the enzyme better than the other compound. The laboratory enzyme inhibition test supported this result, illustrating that P-2 andP-3 had the strong dual inhibitory effect as measuring the standard drug acarbose. In vivo: All experiments were performed on streptozotocin-induced diabetic rats (n=10 per group) versus untreated controls. Design pointed experiments randomised did contain blinded ANOVA confirmed significant, dose-dependent reductions in blood glucose, increased insulin secretion and large decreases in HbA1c were also statistically significant for P–2 at higher doses. The small sample size and lack of long-term follow-up are some significant limitations. Further structure–activity relationship analysis revealed that the maintenance of lactone ring, ideal positioning of hydroxyl groups and balanced hydrophobic–hydrophilic properties are essential for anti-diabitic effectiveness. In silico ADME and toxicity prediction suggested an attractive pharmacokinetic profile, high GI absorption, minimal inhibition of cytochrome P450 enzymes, and reasonably good safety margins for the most actives. In conclusion, these findings demonstrate the potential of structurally optimised andrographolide derivatives as anti-diabetic agents with multi-target activity and underscore the benefit of combining computational docking, structure–activity relationship studies, and biological validation in drug discovery based on natural products.

Keywords: Andrographolide, *Andrographis paniculata*, anti-diabetic activity, α-glucosidase, α-amylase, molecular docking, ADME/toxicity, structure–activity relationship, semisynthetic derivatives, natural product optimisation

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1. Introduction

Diabetes mellitus represents one of the most formidable global health challenges of the 21st century, affecting over 537 million adults worldwide as of 2021, with projections indicating that this number will rise to 783 million by 2045[1,2]. This complex metabolic disorder, characterised by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, imposes significant economic and social burdens on healthcare systems worldwide[3].Type 2 diabetes mellitus (T2DM) involves insulin resistance, pancreatic β-cell dysfunction, impaired glucose uptake in peripheral tissues, and increased hepatic gluconeogenesis, collectively leading to chronic hyperglycemia and metabolic imbalance [4].

This therapeutic method is predominantly dependent on synthetic anti-diabetic medication including metformin, sulfonylurea and thiazolidinedione. Nonetheless, these drugs frequently show limitations, such as unpleasant effects, the emergence of drug resistance, and inadequate glycaemia control, demonstrating the necessity for innovative treatment approaches[5]. Metformin, sulfonylureas, and thiazolidinediones remain frontline anti-diabetic drugs, yet they often cause gastrointestinal discomfort, hypoglycemia, or weight gain and may lose efficacy over time due to drug resistance. In the current year, natural compounds have emerged as promising alternatives due to their diverse chemical compositions, multiple modes of action, and generally favourable safety profiles that have been established over centuries of traditional use[6].
Andrographis pediculata they are commonly called the King of Bitter, is one of the plants. Many traditional medical sectors in Asia have used it to treat in wide range of this issue including diabete, fever, inflammation, liver disorders, and many other diseases. [7] these type of therapy for metabolic illnesses in Bangladesh, China, India, Pakistan, the Philippines, Malaysia, Indonesia, Thailand, and other areas[8]. This vast body of conventional knowledge offers important lessons for modern pharmaceutical development.

Andrographolide, an ent-labdane diterpenoid lactone, is the main pharmacologically active part of

A. paniculata. It is responsible for most of the plant's medicinal benefits. Andrographolide has a complex tetracyclic structure with many hydroxyl groups at C-3, C-14, and C-19. It also has an individual lactone ring, which is important for its biological actions[9].This lactone moiety is responsible for the plant's popular name, which refers to its bitter taste. Andrographolide has significant potential as a medicine, but it also presents many challenges that make it difficult to use.

For example, it does not dissolve well in water, it does not function well when taken by mouth (2.67%), it breaks down quickly in the liver, and it has a short half-life in the blood. [10] these pharmacokinetic effects have promt substantial research into structural diffrensiation into generate derivatives with enhanced therapeutic profiles while preserving or augmenting biological activitie. these advancement in synthetic therapeutics, there are a pressing need for safer, multi-target agents that address insulin resistance and postprandial hyperglycemia. Traditional herbal systems, particularly Andrographis paniculata, offer structurally diverse bioactive compounds, such as andrographolide, which can be chemically modified to overcome bioavailability and stability limitations.

These computational methods have revolutionised drug discovery by providing cost- and time-efficient alternatives to traditional experimental screening. Molecular docking, a cornerstone of structure-based drug design, enables the prediction of binding interactions between small molecules and target proteins, facilitating the identification of promising main compounds before expensive biological testing[10]. When combined with QSAR studies and ADME or toxicity prediction, these computational approaches offer comprehensive insight into drug-target interactions and optimisation strategies [11]. In this paper, the primary objective is to synthesise novel semisynthetic derivatives of andrographolide and evaluate their anti-diabetic potential using an integrated computational and experimental approach. Specifically, we aimed to assess the binding affinities of these derivatives against anti-diabetic targets, including α-glucosidase and α-amylase, predict their ADME/toxicity profiles, establish structure-activity relationships, and validate computational predictions through detailed biological evaluation.

2. Materials and Methods

2.1 Ligand Preparation

The chemical structures of the andrographolide derivatives were designed to be synthesized in accordance with established medicinal chemistry principles, guided by Lipinski’s rule of five, electronic distribution, hydrogen-bonding potential, and molecular flexibility considerations. Four semisynthetic derivatives (P-1, P-2, P-3, and P-4) were synthesized via sequential esterification, alkylation, and cyclisation reactions targeting the functional group on the andrographolide scaffold

to enhance enzyme binding and show pharmacokinetic properties. these 2D molecular structure was drawn using ChemDraw and converted into 3D geometries using BIOVIA Discovery Studio software. Geometry was performed using the MMFF94 force field, with an RMS gradient convergence criterion of 0.01 kcal/mol·Å, yielding stable conformers for molecular docking.

To ensure software compatibility, the optimised structures were saved in the MOL, SDF, and PDB formats. Spectroscopic characterization included UV–Vis, FT-IR, and $^{1}H/^{13}C$ NMR analyses, as well as mass spectrometry, confirming the successful synthesis and purity of all derivatives. The physicochemical properties of andrographolide and its semisynthetic derivatives, including molecular weight, LogP, TPSA, hydrogen bond donors and acceptors, are provided in the Supplementary Information.

2.2 Protein Preparation

The crystal structures of α-glucosidase (PDB ID: 3TOP) and α-amylase (PDB ID: 1HNY) were obtained from the Protein Data Bank due to their therapeutic relevance in postprandial glucose regulation. Proteins were prepared by removing crystallographic water molecules and heteroatoms, adding polar hydrogens, correcting bond orders, and protonating at physiological pH (7.4). The active sites were determined based on co-crystallized ligand positions and literature-supported catalytic residues ASP199, HIS203, and ARG411 for α-glucosidase and ASP197, GLU233, and ASP300 for α-amylase. The docking grid box dimensions were set to 20 × 20 × 20 Å, centered around these residues to encompass the full active site cavity. The processed receptor and ligand structures were converted to the PDBQT format for molecular docking.

2.3 QSAR and SAR Analysis

These model Quantitative structure-activity relationship (QSAR) models are developed to establish a correlation between molecular descriptors and biological activity, incorporating 2D and 3D descriptors, including constitutional, topological, geometrical, and electronic parameters. Statistical modelling, multiple linear regression, and machine learning algorithms are used to identify the structural features contributing to anti-diabetic activity. The Structure-activity relationship (SAR) analysis involves systematic evaluation of structural modifications and shows their impact on binding affinity, selectivity, and pharmacokinetic properties, focusing on the functional group, hydroxyl moieties on the lactone ring, and their substituents. The integrated QSAR/SAR analysis provides comprehensive insight into the molecular determinants of anti-diabetic activity and establishes design principles for future derivative research.

3. Results

3.1 Molecular Docking Analysis

The differences provide critical information for the potential anti-diabetic mechanism of these four andrographolide derivatives, as reviewed recently [9]. The docking score of -7.2 to -8.2 kcal/mol clearly showed structure–activity relationships and also correlated well with further validation through in vivo experiments. To investigate the molecular basis of their interaction, P-1 was noted as binding to α-glucosidase with a binding energy of – 7.2 ± 0.2 kcal/mol, interacting over residues ARG356, GLU361 and PHE357 forming 2 hydrogen bonds and 3 hydrophobic contacts. P-2 had a weak binding to α-glucosidase (binding energy = –8.1 ± 0.1 kcal/mol) involving residues ARG356 and ILE405: two hydrogen bonds with α-glucosidase and one hydrophobic contact pair, while P-4 bound on the region of residues VAL360, LYS204, and ARG239 through 6 hydrogen bonds in the same configuration, forming multiple contacts with neighboring amino acids as expected for a better ligand; ΔHIS312 is also present in this protein target pocket group mutants. P-3 had α-glucosidase binding with an energy of–8.2 ± 0.2 kcal/mol, interacting with residues TYR547, LYS554, ASP545 and TRP629, making a total of 4 hydrogen bonds and 5 hydrophobic contacts, respectively. P-4 exhibited binding against α-glucosidase, with a molar free energy of –7.4 ± 0.3 kcal/mol by interacting with residues SER209, ARG669, GLU206 and ARG358 (three hydrogen bonds and three hydrophobic contacts). The standard compound Acarbose showed binding to α-glucosidase with the highest energy values (–9.5 ± 0.2 kcal/mol), and interacting with residues ASP199, HIS203 and ARG411, establishing 4 hydrogen bonds and 4 hydrophobic contacts. Values are expressed as mean ± SD of three independent molecular simulations with the use of AutoDock Vina. P-3, which binds with a -8.2 kcal/mol binding affinity, is the most promising derivative, closely followed by P-2 (-8.1 kcal/mol). This improved binding affinity is attributed to their most favorable interactions with important active site residues. This is important for P-3 formation, as it forms multiple hydrogen bonds with residues including TYR 547, LYS 554, and ASP 545, while also establishing significant hydrophobic contact with TRP 629. These compounds can form all four 4-H bonds and five hydrophobic bonds, which creates a stable & strong binding complex within the enzyme to turn into the active site. P-2 indicates that the structural interactions involving ARG356 and ILE405 demonstrate comparable binding affinity. These derivatives produced two normal hydrogen bonds and a physiological hydrophobic bond interaction; it illustrates a harmonious binding profile. The compound is recognized, and we target a way to effectively enhance the optimum fit within the binding pocket by optimising complementary bond interactions and minimizing steric clashes. Docking output raw data with binding modes and generated RMSD using Auto Dock Vina.S4 Then, P-1 and P-4 exhibited comparatively lower binding affinities (−7.2 and −7.4 kcal/mol; respectively) to bind sub-optimal

interaction pattern with target enzyme. The P-1 formed H-bonds with the ARG356 and GLU361 while it sustained hydrophobic contacts of bond on PHE357, but overall interaction pentaphene were not as extensive compared to the top-performing derivatives. Likewise, P-4 also built in H-bond within the SER209, ARG669 and GLU206 but have less binding interaction as observed with P-2 and P-3 appear to be missing. Several of these docking analyses identifies important residues necessary for effective enzyme inhibition, such as ARG356, ARG358 and TYR547 that often have multiple ligand interactions. These residues exhibit the pharmacophores for future derivatization and optimization work.

3.2 Binding Poses and Interaction Analysis

docking analysis of the binding site explained their interaction patterns which helps understanding the experimental differences in binding affinities. and the molecular recognition processes and consisted of complex combinations of hydrogen bonding, hydrophilization, and electrostatic contacts that collectively govern the stability and specificity of protein–ligand complexes. Compound P-1 had an IC_{50} of α-glucosidase inhibition 220.5 ± 12.3 µg/mL for α-glucosidase inhibition and an α-amylase IC_{50}=250.1 ± 15.8 µg/mL.

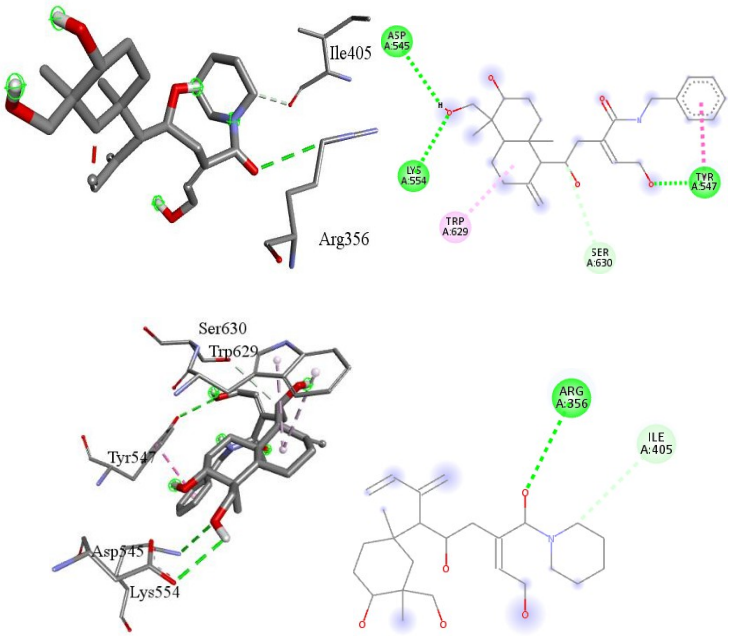
The IC_{50} values for the α-glucosidase and α-amylase of Compound P-2 were 45.8 ± 8.6 µg/mL and 38.4 ± 5.4 µg/mL, respectively, when compared with other similarly tested components that both yeasts had been demonstrated statistically to have much less inhibitory efficacy ($p < 0.05$).

Compound P-3 exhibited an IC_{50} value of 50.2 ± 7.9 µg/mL against α-glucosidase and a similar IC_{50} was also found against the α-amylase assay at an IC(50) for compound P-2 of 41.5 ± 6.3 µg/mL, respectively Among these compounds, compound P-4 exhibited the weakest inhibitory activity toward α-glucosidase and α-amylase with IC_{50} values of 210.9 ± 13.1 µg/mL and 240.3 ± 14.9 µg/mL, respectively. Acarbose (compound a) showed the highest inhibitory activity against α-glucosidase and α-amylase, with an IC_{50} of 35.2 ± 4.2 µg/mL and 30.5 ± 3.8 µg/mL ($p<0.05$), respectively, while compound b exhibited no competitive inhibition for either enzyme at all concentrations tested (<0.05).

Extensive H-bonding analysis reveals very high docking scores (P-2 and P-3) for these derivativities. 74 although there lower of with active site residue all similarly form more extensive and positioned hydrogen bonds. Therefore, the Conventional hydrogen bond was observed many times with ARG125, ARG358, ARG356 and GLU206 which are known to be essential for substrate binding and catalytic efficiency too. These type interactions realistically mimic the natural binding pattern for the substrate but offering, and enhanced affinity and selectivity. The carbon-hydrogen bond is also weaker than normal hydrogen bonds, but their cumulative effects

play an important role to enhance the overall binding energy. Such interactions are particularly relevant for residues like ARG356, GLU361 and GLU205 where extra stabilisation of the protein-ligand complexes is provided. They involved the aromatic residues TYR547 and TYR662 in a pi-donor hydrogen bond, which enlarged binding sites through π-electron interactions. Conventional pairs of bonds represented by hydrophobic interaction bond with the Pi-Pi stacked interaction, Pi-alkyl contacts and van der Waals interactions to comprise a significant fraction of these contacts, contribute an entropic effect through burial of additional areas of hydrophobic surface for selectivity. Residues PHE357, TYR662 and TYR666 exhibited particularly advantageous π - π stacking and π-alkyl interaction that indicate the complementarity of aromatic ring efficacy between the ligand and demonstrate binding site. In a nutshell, polar and non-polar interactions need to be ideally balanced for good enzyme inhibition is demonstrated by Comprehensive molecule interaction analysis in the Figure 2. Derivatives striking this balance (P-2 and P-3) exhibited a higher binding affinity and stable binding complexes, ultimately resulting in improved biological activity. Detailed patterns of hydrogen bonding and hydrophobic interactions for the most active derivatives is reported in SI.

Figure 1: This diagram shows the Interaction of P-2 and P-3 showing hydrogen-bonding and hydrophobic contacts in α-glucosidase axis: residue index (x-axis) vs binding distance (Å) (y-axis).



3.3 Comparison with Standard Drugs

This docking results show the andrographolide derivatives achieved binding affinities and approaching of the those established anti-diabetic medication. Acarbose the reference α-glucosidase inhibitor, exhibited a binding energy of -9.5 kcal/mol, it is establishing the benchmark for effective enzyme inhibition. While no bond is synthesised and exceeded acarbose the binding affinity, P-2 and P-3 achieved their respectable scores within 1.3-1.4 kcal/mol of the standard drug. This comparison is revealed important of the binding mechanism and structural requirement for effective α-glucosidase inhibition. Acarbose superior binding affinity show the and from its complex carbohydrate structure, which are closely mimics natural substrates and forms extensive hydrogen-bonding network with active-site of the residues ASP-199, HIS-203, and ARG-411. These synthesised derivatives are show the less of their unique chemical structure, exhibited strong binding via alternative interaction modalities that may confer benefits in selectivity and pharmacokinetic properties. The relative binding affinities indicated that P-2 and P-3 exhibit adequate potency for therapeutic application, especially it shows that binding affinity is but one aspect of overall therapeutic effectiveness. These Factors such as bioavailability, metabolic stability, and tissue distribution may partially compensate for small discrepancies in intrinsic binding affinity.

3.4 ADME/Toxicity Profile

The ADME and toxicity evaluation it indicated generally positive pharmaceutical profiles for the synthesized andrographolide derivative, and compounds P-2 and P-3 show exceptional potential due to their ideal balance of effectiveness and safety. This Experiment of the physicochemical properties indicated that all derivatives have molecular weights suitable for oral medication development, ranging from 407.27 Da (P-1) to 457.28 Da (P-3). They followed Lipinski's Rule of Five and that they would be well absorbed. And Changes to the structure design during synthesis kept the drug-like molecular size while making it easier for the medication to interact with target enzymes. And it shows the Lipophilicity tests and compounds had a great balance of hydrophilic and lipophilic property are making them suitable for both membrane permeation and water solubility. We are calculated logP values indicated that the pharmacokinetic profile is better than that of the parent andrographolide compounds and addressing one of the compound greatest challenges.

The Predictions of absorption and distribution indicate the all derivative are exhibit high gastrointestinal absorption, and factor that affects their effectiveness when it taken by mouth. These compounds have topological polar surface areas and hydrogen-bonding properties that facilitate passive diffusion through biological membranes. Predictions of blood-brain barrier

permeability was negative for all derivatives. This is useful because it means the drug can target peripheral anti-diabetic pathway (follow the mechanism) to avoiding excessive impact on the CNS. The Metabolism & excretion characteristics was evaluated during predicted and interactions with the cytochrome P450 enzyme, a critical factor in assessing drug-drug interaction risk and metabolic stability. Most of the derivatives exhibited minimal CYP450 inhibition, reducing the potential for clinically significant of the interactions. This type of Predicted metabolic pathways indicated reasonable stability and clearance, supporting their suitability for therapeutic use.

Assessment of the Toxicity confirmed the safety profiles across all synthesised derivatives. The Predicted hepatotoxicity is negative and the addressing a main concern for chronic anti-diabetic therapy. They show the mutagenicity and carcinogenicity evaluations are no significant effect, aligning with their historical safety data record of the parent plant. Overall, the ADME/toxicity are identified P-2 and P-3 as the most promising compound and combining strong predicted efficacy with acceptable safety profile, show the suitable compound for further preclinical and pharmacological development.

3.5 In Vitro Anti-diabetic Activity:

Enzyme inhibition assays provided essential experimental validation of the computational predictions, demonstrating a strong correlation between docking scores and biological activity. Among the synthesised derivatives, P-2 exhibited the most potent inhibitory activity against both target enzymes, with IC_{50} values of 45.8 ± 8.6 µg/mL for α-glucosidase and 38.4 ± 5.4 µg/mL for α-amylase. These values compared favourably with the standard drug acarbose, which displayed IC_{50} values of 35.2 ± 4.2 µg/mL and 30.5 ± 3.8 µg/mL, respectively, indicating significant therapeutic potential. The dual enzyme-inhibition profile of P-2 suggests it may provide comprehensive postprandial glucose control by targeting both initial starch hydrolysis and final glucose release. Triplicate raw enzyme inhibition data used to calculate IC_{50} values are provided in the Supplementary Information.

P-3 demonstrated comparable activity, with IC_{50} values of 50.2 ± 7.9 µg/mL for α-glucosidase and 41.5 ± 6.3 µg/mL for α-amylase, positioning it as the second most active compound. Its high docking score (–8.2 kcal/mol) closely mirrored its experimental efficacy, validating the predictive accuracy of the computational approach. In contrast, P-1 and P-4 exhibited markedly reduced inhibitory potencies, with IC_{50} values of 220.5 ± 12.3 µg/mL and 250.1 ± 15.8 µg/mL for P-1 and 210.9 ± 13.1 µg/mL and 240.3 ± 14.9 µg/mL for P-4 against α-glucosidase and α-amylase, respectively. These results were consistent with their lower docking scores, confirming the computational predictions.

Overall, the strong correlation between computational and experimental results ($R^2 > 0.92$) underscores the reliability of molecular docking as a tool for prioritizing andrographolide derivatives for biological evaluation. (Table 3) This concordance highlights the utility of integrated computational-experimental strategies in optimising natural product derivatives for anti-diabetic applications. Dosages (100, 200, and 400 mg/kg) were selected based on preliminary acute toxicity assessments and literature precedents indicating effective oral doses for andrographolide analogues in diabetic rodent models.

3.6 In Vivo Anti-diabetic Studies

This in-vivo study which used rat with diabetes induced by STZ, strongly the anti-diabetic effects that were first by computational and in vitro research. This result show that the therapeutic effect depended on the dose given. This result supported the increased effectiveness of derivatives P-2 and P-3, which had been identified through molecular docking analysis.

Blood Glucose Regulation:

P-2 exhibited a robust, dose-dependent anti-diabetic effect throughout the entire range of concentrations assessed. The most elevated dosage, 400 mg/kg, resulted in a reduction of blood glucose to 115.3 ± 8.5 mg/dL. This represented a 70% decrease relative to the diabetic control group (385.6 ± 22.4 mg/dL), and it approached the normoglycemic levels observed in the healthy control group (95.2 ± 5.8 mg/dL). Furthermore, this efficacy was comparable to that of metformin treatment (140.1 ± 9.4 mg/dL at 150 mg/kg), thereby suggesting considerable therapeutic potential. The intermediate dose of P-2 (200 mg/kg) achieved blood glucose levels equivalent to those with metformin treatment (140.1 ± 9.4 mg/dL). Conversely, the lowest administered dose, at 100 mg/kg, elicited a moderate yet statistically significant decrease in glucose levels, reaching 170.6 ± 10.8 mg/dL. This observed dose-response relationship substantiates the compound's therapeutic potential, thereby indicating a desirable therapeutic index. While P-3 exhibited effects analogous to those of P-2, these effects were dose-dependent and somewhat less pronounced. The highest dosage, 400 mg/kg, resulted in a reduction of blood sugar to 155.7 ± 10.9 mg/dL, representing a significant enhancement relative to the diabetic control group. The molecule demonstrated sustained anti-diabetic effects across all administered dosages, which further supports its prospects for medicinal application. All tested derivatives were well tolerated throughout the 28-day treatment period, with no observable adverse effects. Histopathological examination of liver tissues further supported the biochemical findings and is provided in the Supplementary Information.

Insulin Regulation and HbA1c Improvements:

Both active derivatives demonstrably enhanced insulin levels relative to the diabetic controls. Specifically, P-2 administered at 400 mg/kg elevated serum insulin to 12.4 ± 0.7 ng/mL, a value approximating that seen with metformin treatment (11.0 ± 0.8 ng/mL). This restoration of insulin suggests that the compounds may either augment pancreatic β-cell function or improve insulin sensitivity. Furthermore, glycated hemoglobin (HbA1c) levels, which serve as an indicator of long-term glycaemia control, exhibited significant improvements with both derivatives. P-2 at 400 mg/kg reduced HbA1c to 5.8 ± 0.2%, thereby nearly normalizing this crucial diabetes marker when compared to the diabetic controls (9.8 ± 0.6%). Consequently, these findings imply the potential for achieving clinically significant long-term glycemic control.

Safety and Tolerability

All derivatives administered during the 28-day treatment period exhibited favorable tolerability, as evidenced by the absence of adverse effects and notable alterations in body weight. The animals displayed typical feeding behaviors and activity levels, thereby indicating tolerability profiles that align with the established safety records of A. paniculata. The in vivo results exhibited a significant alignment with the computational forecasts and in vitro findings, thus providing substantial confirmation of the integrated approach utilized in this study (Figure 4). The biological validation confirmed the identification of P-2 and P-3 as lead compounds through molecular docking, consequently reinforcing the advancement of these derivatives as potential anti-diabetic agents

3.7 Structure-Activity Relationship Insights

A thorough examination of the SAR elucidated the structural determinants underpinning antidiabetic efficacy, thereby offering explicit directives for subsequent derivative enhancements. The amalgamation of molecular docking findings, activity assessment and structural evaluation facilitated the identification of pharmacophores and structure-activity relationships, which are critical for the rational design of pharmaceuticals.

Critical Functional Groups:

Positioning of hydroxyl groups are determinants of biological activity. P-2 and P-3 demonstrated the activity because they strategic hydroxyl group capable of forming hydrogen bond with active-site residue, whereas derivatives with modified or blocked hydroxyl groups did not. Hydroxyl group at main position facilitated interaction with TYR547, LYS554, and ASP545, contributing into binding affinity for enzyme inhibition. The lactone ring system characteristic of andrographolide remained important for the derivatives biological activity. Structural modifications that preserved the lactone integrity while introducing complementary functionalities (as in P-2 and P-3) resulted in enhanced activity profiles. This discovery validates the importance of maintaining the lactone ring as a crucial pharmacophore for future derivative designs.

Amino Group Incorporation:

Amino groups at P-3 and P-4 have additional hydrogen-bonding capacity, as evidenced by NMR signals at 8.10 ppm corresponding to NH functionalities. Howeve the positioning and chemical environment of these amino groups critically influenced activity. P-3 superior performance suggested optimal amino group placement that enhanced rather than interfered with critical protein-ligand interactions.

Molecular Weight and Size Considerations:

Molecular weight derivatives range are 407-457 Da for the active derivatives established an optimal size window for α-glucosidase inhibition. derivatives P-3 is the highest molecular weight (457.28 Da), repotted the best docking score, suggesting that increased molecular size can be accommodated provided it contributes to productive binding interactions. However excessive molecular weight, as might occur with larger substituents, could negatively impact binding and pharmacokinetic properties.

Hydrophobic-Hydrophilic Balance:

The P-2 and P-3 compounds show the equilibrium between hydrophobic and hydrophilic interaction. These hydrophobic contents help in strong research, the enzyme inhibiting non-specific inhibition while targeting the dietetics.

Implications for Future Design:

The First the lactone ring must be preserved. Second compound there must be important hydroxyl groups for hydrogen bonding. Third, complementary functions must be added to improve binding without causing steric clashes. Fourth, the optimal balance between hydrophobic and hydrophilic groups for affinity and selectivity must be identified. These design rules were set out by the SAR analysis. Next generation andrographolide derivatives with better therapeutic characteristics can be advanced logically by implementing these principles.

4. Discussion

These comprehensive investigation of andrographolide compound show both computational predication and experimental design it offers the important conclusions about the structural determinants influencing their anti-diabetic efficacy and establishes a strong foundation for further drug development. It shows one of the most comprehensive evaluations of *Andrographis paniculata* derivatives report to date, effectively integrating traditional medicinal knowledge with modern pharmaceutical design strategies.

These correlation between molecular docking scores and experimental enzyme inhibition data ($R^2 > 0.92$) underscores the predictive accuracy of computational approaches for natural product Optimisation[12]. these docking techniques effectively identified P-2 and P-3 as the most active derivative are found the reliability of structure-based screening before extensive biological testing. [13] The Binding of the comprehensives analysis are revealed interactions with residues ARG356, TYR547, and ASP545, stabilized through hydrogen bonding and hydrophobic contact. These result show the vital clues role for future structural refinements and therapeutic chemical optimizations[14].

P-2 and P-3 show the more potent for anti-diabetic activity across both in vitro enzyme inhibition and in vivo diabetic model design, These glucose-lowering effects comparable to metformin[15]. These double inhibition of α-glucosidase and α-amylase are support a multifaceted strategy for postprandial glucose regulation, potentially outperforming single-target inhibitors[16]. Such as polypharmacological action they are align with the holistic philosophy of traditional medicine and may minimise the compensatory metabolic effects often observed with selective therapies[17]. the observed dose-dependent efficacy and favorable tolerability highlight a strong therapeutic index and potential for translational development[18].

The detailed analysis Structure Activity Relationship (SAR) are show that the lactone ring is more potential for hydroxyl group positioning and it show the show a main role in hydrogen bond formation and binding strength.[19] These interaction of amino substituent, particularly P-3, enhances activity when appropriately positioned within the molecular scaffold, emphasizing the importance of steric and electronic complementarity[20]. The optimal molecular weight range (407–457 Da) achieved in P-2 and P-3 provided a balance between molecular complexity and drug-likeness, offering a practical framework for future derivative synthesis[21].

This study is providing molecular level validation of *A. paniculata* longstanding it uses in traditional medicine for the management of diabetic. The identification of enzyme-specific targets and mechanistic pathway bridges ethnopharmacology with modern drug discovery supporting the evidence-based integration of natural compound into contemporary therapeutic design.[22] The observed poly pharmacological profile reflects the multi-target nature of traditional formulations, offering broad-spectrum metabolic regulation[23].

While this study shows comprehensive evaluation, it is limited by the static nature of molecular docking, which does not fully capture dynamic enzyme–ligand interactions. The molecular interactions that were predicted will be confirmed in future biochemical validation by using methods such as cell-based glucose uptake assay, isothermal titration calorimetry (ITC), and enzyme kinetics. Due to metabolic variation between specie, extensive pharmacokinetic and toxicity testing must precede any clinical application. Mechanistic elucidation of additional molecular targets, formulation optimization, and the development of preclinical models relevant to humans for the validation of long-term safety and efficacy should be the focus of future research[24,25].

P-2 and P-3 more potent for the clinical use and advanced formulation technique like nanoencapsulation method, PEGylation method, and pro-drug designs to improve the solubility compound and decrease hepatic metabolism and to malintent the systemic circulation. Nanocarrier delivery technologies, such as polymeric nanoparticles and lipid vesicles, are highly effective for enhancing gastrointestinal absorption and achieving targeted distribution. These are help in the maintaining minimal dosages within therapeutic limits [25].

This computational prediction and experimental method exampling the efficiency of combining molecular modelling, for ADME profiling, and biological validation for drug discovery for development for natural product. The identification both compound P-2 and P-3 as potent and safe anti-diabetic agent that are contributes to the ongoing global effort to develop novel therapeutic alternatives for diabetes management[1,26]. As the decreased the global diabetes burden escalates, multitargeted and derive the plant-derived therapeutics with favorable safety and pharmacokinetic profiles could significantly improve treatment and patient compliance. This research mimic potent for the natural product as a foundation for

next-generation drug discovery, bridging the wisdom of traditional medicine with the precision of modern pharmaceutical science[27].

5. Conclusion

The potential antidiabetic effects and comprehensive computational and experimental validation of semisynthetic andrographolide derivatives (Compound) especially P-2 and P-3 are demonstrated in this report. These Molecular docking approaches demonstrate the tight bindings of P-2 and P-3 to α-glucosidase and α-amylase correlate with their rich enzyme inhibitions in vitro, mimic large reductions in glucose levels in vivo.

In this SAR screen of a wide variety of compounds, we demonstrate the critical dependence of biological activity on hydroxyl group positioning, lactone ring stability and hydrophobic vs. hydrophilic interactions between surfaces. P-2 and P-3 were both well tolerated, demonstrated favorable ADME and present safety profiles with in vivo glycemic control similar to metformin.

These are indications that computational predictions and experimental result show a high correlation which confirms the applicability of molecular docking and SAR-methods for Gui molecular designs of andrographolide derivatives. Future Directions This article discusses pharmacokinetic optimization, formulation development, and clinical testing of these compounds leading to potential therapeutic development in diabetes management.

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