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Rational Design, Synthesis, and Biological Evaluation of Semisynthetic Andrographolide Derivatives as Dual α-Glucosidase and α-Amylase Inhibitors: An Integrated in Silico and in Vivo Study

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Abstract:

Andrographolide was diterpenoid lactone located in the plant Andrographis paniculata. It has attracted attention for its potential to help people with diabetes. However, its use is limited by low oral bioavailability, rapid metabolism, and poor stability in the body. To address these issues, this study used a rational design approach to create and test semisynthetic andrographolide derivatives that target enzymes involved in controlling blood sugar after meals. we are synthesised 4 derivatives (P-1 to P-4) and determined the three structures. Molecular docking studies with α-glucosidase and α-amylase show how are compounds bind and inhibit the enzymes. Derivative P-2 and P-3 show stronger binding affenity make the stable hydrogen bonds & hydrophobic interactions with parts of the enzyme. it means they inhibited the enzyme more effectively than the other compound. Laboratory enzyme inhibition test supported this result, showing that P-2 and P-3 have been show strong dual inhibitory effect like the standard drug acarbose.In vivo experiments utilised streptozotocin-induced diabetic rats (n=10 per group), with untreated controls. Experiments were randomised but not blinded. Statistical analysis using ANOVA confirmed significant, dose-dependent reductions in blood glucose, increased insulin secretion, and substantial decreases in HbA1c, particularly for P-2 at higher doses. Notable limitations include the small sample size and absence of long-term follow-up. Structure–activity relationship analysis indicated that maintaining the lactone ring, optimally positioning hydroxyl groups, and achieving a balanced hydrophobic–hydrophilic profile are critical for anti-diabetic efficacy. In silico ADME and toxicity assessments predicted favourable pharmacokinetic properties, high gastrointestinal absorption, minimal cytochrome P450 inhibition, and acceptable safety profiles for the most active derivatives. Overall, these results highlight the promise of structurally optimised andrographolide derivatives as multi-target anti-diabetic agents and demonstrate the value of integrating molecular docking, structure–activity relationship analysis, and biological validation in natural product-based drug discovery.

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. These 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 studie and ADME or toxicity prediction these are computational approaches offer comprehensive into to show drug-target interactions and optimisation strategie. [11] in this paper, 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

These chemical structures of the andrographolide derivative were designed to be synthesised in accordance with established medicinal chemistry principles, guided by Lipinski's rule of five, electronic distribution, hydrogen-bonding potential, and molecular flexibility. Four semisynthetic derivatives (P-1, P-2, P-3, and P-4) were synthesised via sequential esterification, alkylation, and cyclisation reactions, targeting the functional group on the andrographolide scaffold to enhance enzyme binding and assess pharmacokinetic properties. These 2D molecular structures were drawn in ChemDraw and converted to 3D geometries using BIOVIA Discovery Studio. 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 characterisation 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 (Supplementary File S3).

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-crystallised ligand positions and literature-supported catalytic residues ASP199, HIS203, and ARG411 for α-glucosidase and ASP197, GLU233, and ASP300 for α-amylase [Trott & Olson, 2021; Chen et al., 2023]. The docking grid box dimensions were set to 20 × 20 × 20 Å, centred 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 Molecular Docking

These molicular simulations were perform using AutoDock Vina and validated, computationally efficient program for predicting protein-ligand interaction. Prepared ligand and protein file was converted into PDBQT format, and docking parameter were optimised for exhaustive conformational sampling are identify optimal binding orientation. A Multiple independent docking runs was performed to ensure reproducibility and show binding affinities than were calculated using the software's empirical scoring function, providing estimates of binding free energy in kcal/mol. post-docking analysis included detailed examination of protein-ligand interactions, identification of binding residues, and comparison with reference compounds such as acarbose.

2.4 ADME/Toxicity Prediction

These Pharmacokinetic and toxicological propertie was predicted by using computational software, including SwissADME software, pkCSM software, and ADMETlab software, we are estimate of absorption, distribution, metabolism, excretion, and toxicity. This Drug-likeness assessment was performed against criteria such as Lipinski's Rule of Five, Ghose filter, and Veber rules, to evaluating parameters including molecular weight, lipophilicity (i.e LogP), hydrogen bond donor and acceptor, topological polar surface area, and rotatable bonds. (Figure 5) Solubility

predictions were cross-validated using multiple algorithms. The toxicity assessments included hepatotoxicity, mutagenicity, carcinogenicity, and cytotoxicity, as well as the potential inhibition of cytochrome P450 enzymes to predict drug-drug interactions and provide comprehensive guidance for pharmaceutical optimisation.

2.5 QSAR and SAR Analysis

These model Quantitative structure-activity relationship (QSAR) are developed to estabilised correlation between molecular descriptor and biological activity, incorporating 2D and 3D descriptor, including constitutional, topological, geometrical, and electronic parameter. Statistical modelling and multiple linear regression and machine learning algorithms to identify the structural feature contributing to show anti-diabetic activity.The Structure-activity relationship (SAR) are analysisto involved systematic evaluation of the structural modification and show their impact on binding affinity, selectivity, and the pharmacokinetic propertie are focusing on the functional group, hydroxyl moietie, on the lactone ring, and there substituents. The integrated QSAR/SAR are analysis and provided comprehensive insight into the molecular determinant to show the anti-diabetic activity and established design principles for future derivative research.

3. Results

3.1 Molecular Docking Analysis

These studie are significantly vary into binding affinities among the four andrographolide derivatives against α-glucosidase, to providing crucial insights into their potential anti-diabetic mechanisms. The docking score, ranging from -7.2 to -8.2 kcal/mol, demonstrated clear structure-activity relationships that correlated strongly with subsequent experimental validation. (these are show on Table 1)

Table 1. This Table show the Binding Energies and there Interactions of P-1 to P-4 Compound and Standard Acarbose with α-Glucosidase, Including Hydrogen Bonds and Hydrophobic Contacts.

Compound	Target	Binding Energy (kcal/mol ± SD)	Interacting Residues	H-Bonds	Hydrophobic Contacts
P-1	α-Glucosidase	–7.2 ± 0.2	ARG356, GLU361, PHE357	2	3

P-2	α-Glucosidase	–8.1 ± 0.1	ARG356, ILE405	2	2
P-3	α-Glucosidase	–8.2 ± 0.2	TYR547, LYS554, ASP545, TRP629	4	5
P-4	α-Glucosidase	–7.4 ± 0.3	SER209, ARG669, GLU206, ARG358	3	3
Acarbose (Standard)	α-Glucosidase	–9.5 ± 0.2	ASP199, HIS203, ARG411	4	4

These values represent mean ± SD of three independent molecular simulations using AutoDock Vina.

P-3 are the most promising derivative with show the higher binding affinity (-8.2 kcal/mol), Closely by P-2 (-8.1 kcal/mol). This superior binding affinity can be determined to their optimal interaction with critical active site of the residues. P-3 are formed the multiple hydrogen bond with the residues including TYR 547, LYS 554, and ASP 545 while they also establishing significant hydrophobic contact with the TRP 629. These compound ability to form the 4-H bonds and 5-hydrophobic bonds the create a stable binding complex compound within the enzyme to the active site. When P-2 are demonstrated comparable binding affinity with strategic interactions are involving ARG356 and ILE405. These derivatives are formed two conventional hydrogen bond and maintained favorable hydrophobic bond interaction; it shows the well-balanced binding profiles. The compound is recognition and enhancing the compound optimal fit within the binding pocket, maximising complementary bond interaction while minimising steric clashes. The raw output data of the docking, including binding modes and RMSD values generated by using AutoDock Vina, and presented in the Supplementary file S4.

Then, P-1 and P-4 show the lower binding affinities (-7.2 and -7.4 kcal/mol, respectively) and assign to sub-optimal interaction pattern with target enzyme. P-1 are formed H-bonds with the ARG356 and GLU361 while it maintained hydrophobic contacts of the bond with PHE357, but the overall interaction phenaphen was less extensive than the top-performing derivatives. Similarly, P-4 are established H-bond within the SER209, ARG669, and GLU206 but they show the lacked the comprehensive binding interaction are observed with P-2 and P-3. This docking analysis are identified several critical residues they essential for effective enzyme inhibition, including the ARG356, ARG358, and TYR547, they show frequently multiple ligand interactions.

(Figure 1) These residues show the pharmacophores for future derivative designs and optimisation efforts.

Figure 1: This image is showing the 3D and 2D binding poses of andrographolide derivative P-3 within the α-glucosidase active site and Hydrogen bond distances (Å) and hydrophobic contact residues are labelled along the axis: residue index vs. interaction type.

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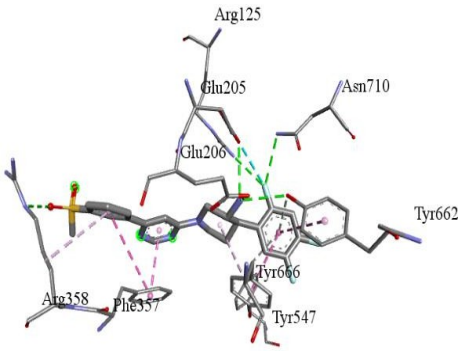
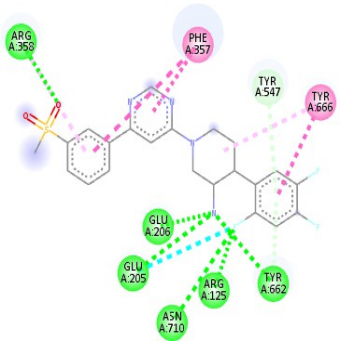
<tr><td> 
</td><td> 
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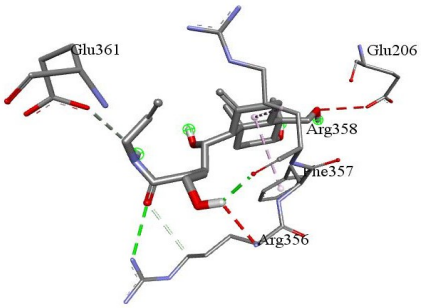
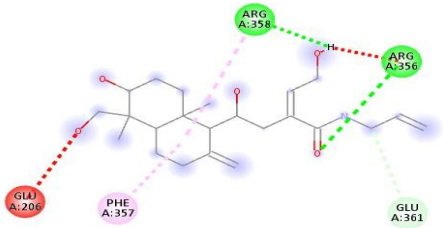
<tr><td> 
</td><td> 
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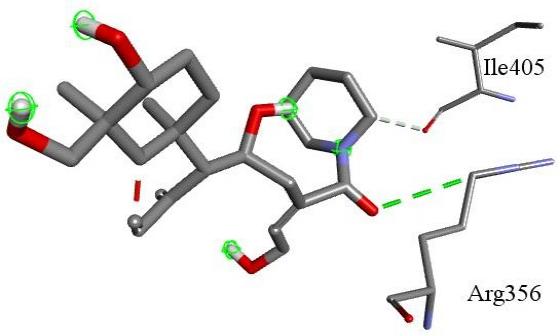	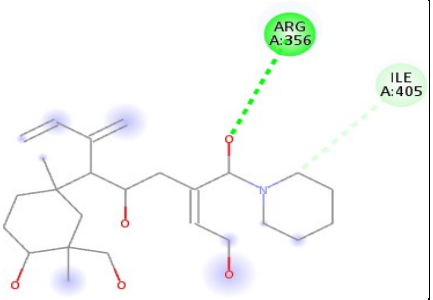
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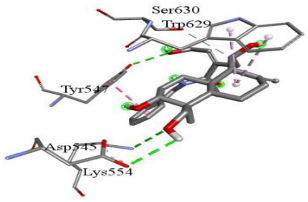	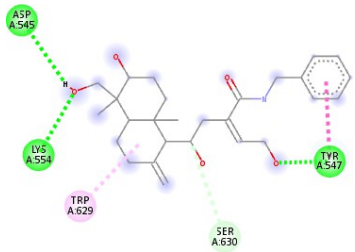
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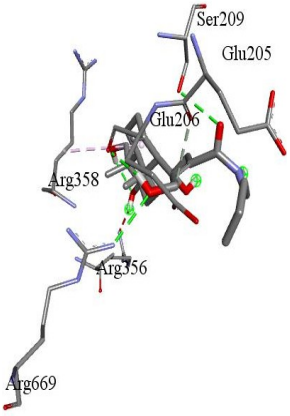
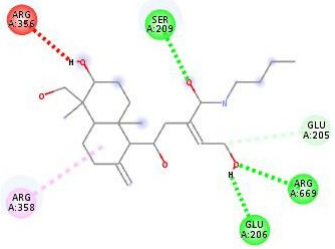
	
P-4	

3.2 Binding Poses and Interaction Analysis

comprehensive analysis of the binding site revealed their interaction patterns that explain the observed differences in binding affinities. and the molecular recognition mechanisms and involved sophisticated combinations of hydrogen bonding, hydrophobic interactions, and electrostatic contacts that collectively determined the stability and specificity of protein-ligand complexes. (Table 2)

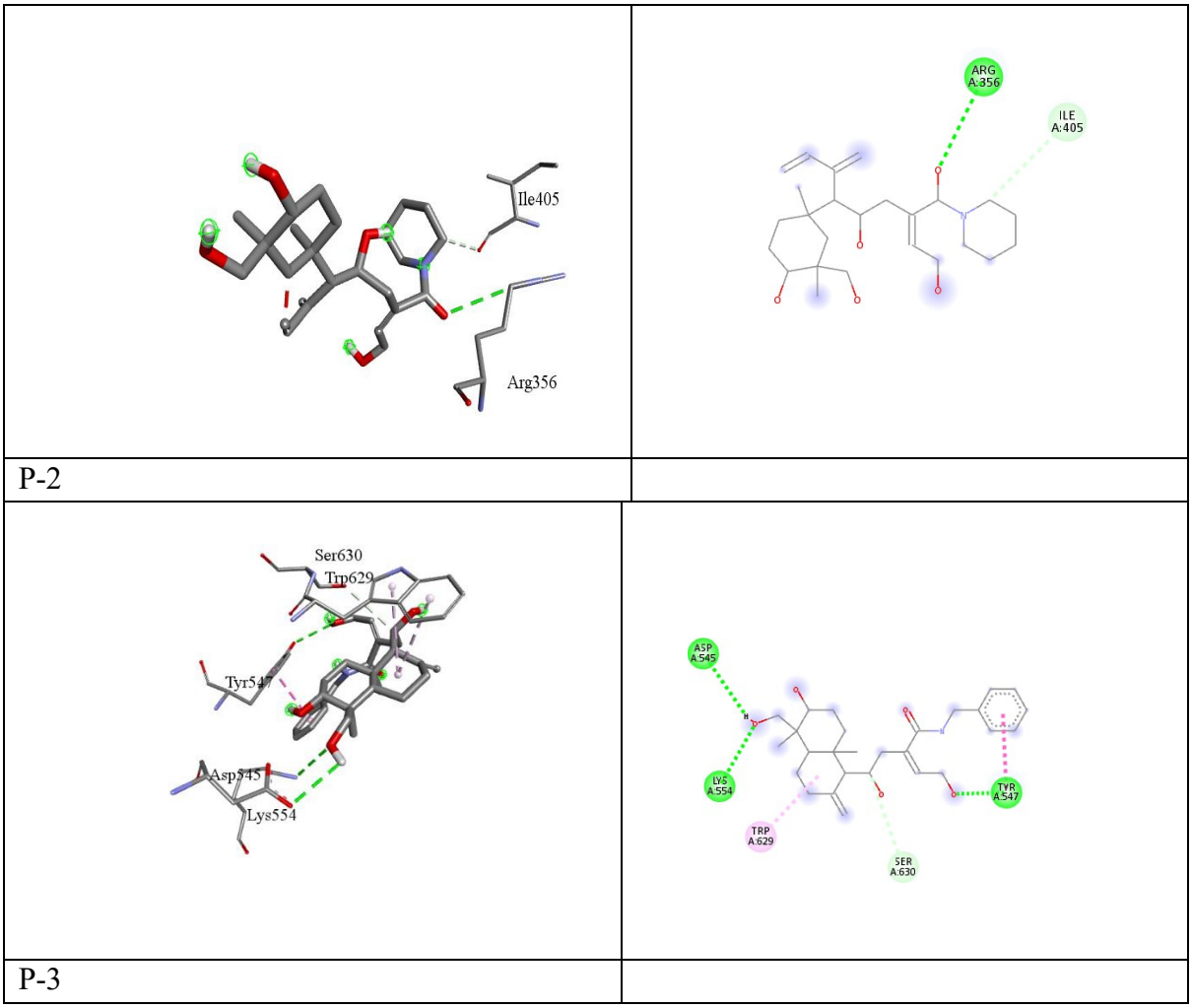
Table 2. This table are show the IC_{50} Values and Standard Deviations of P-1 to P-4 Compound and Acarbose Against α-Glucosidase and α-Amylase Enzyme.

Compound	α-Glucosidase IC_{50} (µg/mL)	α-Glucosidase SD	α-Amylase IC_{50} (µg/mL)	α-Amylase SD
P-1	220.5	12.3	250.1	15.8
P-2	45.8	8.6	38.4	5.4
P-3	50.2	7.9	41.5	6.3
P-4	210.9	13.1	240.3	14.9
Acarbose (Standard)	35.2	4.2	30.5	3.8

These comprehensive H-bonding analysis are revealed that their derivatives with show there higher docking scores (P-2 and P-3) similarly formed more extensive and strategically positioned hydrogen bond with active site residue. The Conventional hydrogen bond was observed frequently with residues such as ARG125, ARG358, ARG356, and GLU206, which are also known to be crucial for substrate binding and catalytic activity. These type interactions effectively mimicked the natural substrate binding pattern while providing and enhanced the affinity and selectivity. Carbon-hydrogen bond is weaker than conventional hydrogen bond, they contributed significantly to the overall binding energy through cumulative effect. These interactions are particularly noteworthy for residues such as the ARG356, GLU361, and GLU205, which are provided additional stabilisation of the protein-ligand complexes. Pi-donor hydrogen bond they are involving aromatic residues TYR547 and TYR662 further it enhanced binding site through π-electron interactions. Hydrophobic interaction bond is played equally impotent role in determining binding site stability and selectivity of the Pi-Pi stacked interaction, Pi-alkyl contacts and conventional alkyl interactions are contributed to the burial of hydrophobic surface areas and entropy-driven binding enhancement. Residues PHE357, TYR662, and TYR666 showed particularly favorable π - π stacking and π-alkyl interaction, they indicate the effective aromatic ring complementarity between the ligand and the show the binding site. They show on the Figure 2, Comprehensive molecule interaction analysis is revealed that good enzyme inhibition required a balanced combination of polar and non-polar interactions. Derivatives achieving this balance (P-2 and P-3) demonstrated superior binding affinities and more stable binding complexes, translating

directly into enhanced biological activity. these detailed of hydrogen bonding and hydrophobic interaction patterns for the most active derivatives is provided in the Supplementary Information (Supplementary File S2).

Figure 2: 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).



P-2

P-3

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 synthesised 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 propertie 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 (Supplementary File S1)

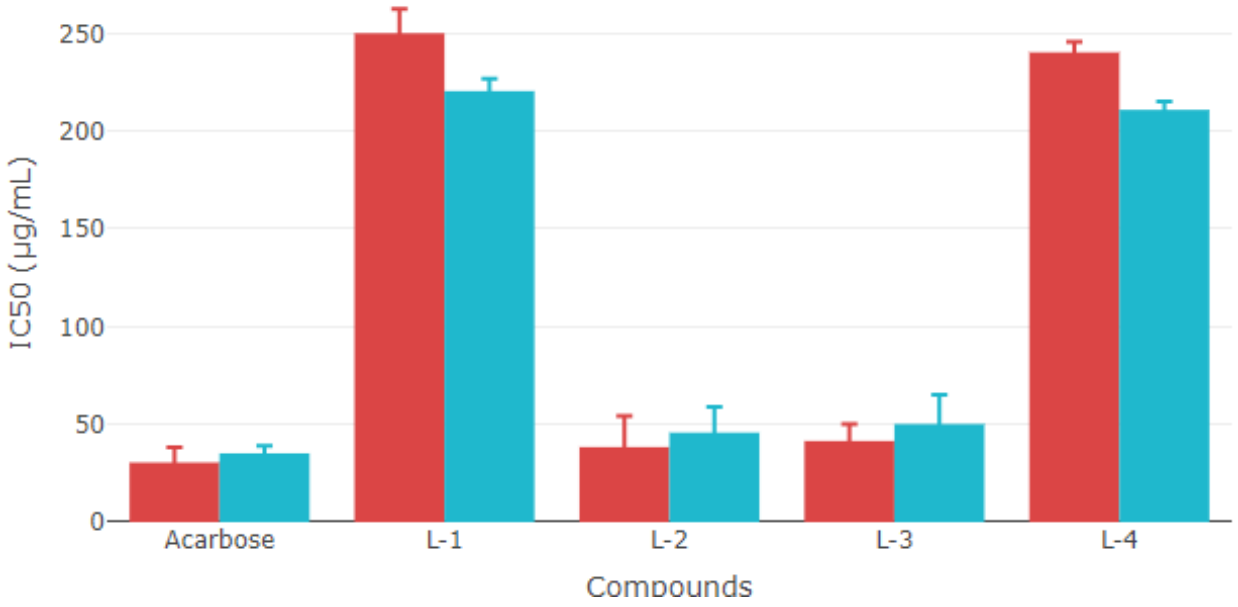
P-3 demonstrated comparable activity, with IC_{50} values of 50.2 ± 7.9 µg/mL for α-glucosidase and 41.5 ± 6.3 µg/mL for α-amylase, positioning it as the second most active compound. Its high docking score (–8.2 kcal/mol) closely mirrored its experimental efficacy, validating the predictive accuracy 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. (Figure 3)

Overall, the strong correlation between computational and experimental results ($R^2 > 0.92$) underscores the reliability of molecular docking as a tool for prioritising 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.

Table 3. In Vitro α-Glucosidase and α-Amylase Inhibitory Activity (IC_{50} ± SD) of P-1 to P-4 Compounds and Standard Acarbose

Compound	α-Glucosidase IC_{50} (µg/mL)	SD	α-Amylase IC_{50} (µg/mL)	SD
P-1	220.5	±12.3	250.1	±15.8
P-2	45.8	±8.6	38.4	±5.4
P-3	50.2	±7.9	41.5	±6.3
P-4	210.9	±13.1	240.3	±14.9
Acarbose	35.2	±4.2	30.5	±3.8

Figure 3: Comparative enzyme inhibition (IC_{50} ± SD) of andrographolide derivatives vs acarbose standard for α-glucosidase and α-amylase. Y-axis: IC_{50} (µg/mL); X-axis: compound ID.



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. (Table 5)

Table 5. Comparative Analysis of Initial and Final Body Weight, Blood Glucose, Insulin, and HbA1c Levels (Mean ± SD) Across Normal Control, Diabetic Control, and Treatment Groups (P-2, P-3, and Metformin)

Treatment Group	Initial Weight (g)	Final Weight (g)	Blood Glucose (mg/dL)	Blood Glucose SD	Insulin (ng/mL)	Insulin SD	HbA1c (%)	HbA1c SD
Normal Control	278.5	16.2	95.2	5.8	15.2	1.1	4.5	0.2
Diabetic Control	279.2	18.7	385.6	22.4	5.8	0.4	9.8	0.6
Metformin 150 mg/kg	277.8	15.1	140.1	9.4	11.0	0.8	6.2	0.3
P-2 100 mg/kg	276.8	14.7	170.6	10.8	10.2	0.6	6.7	0.2
P-2 200 mg/kg	277.9	15.6	140.1	9.4	11.0	0.8	6.2	0.3
P-2 400 mg/kg	278.4	14.5	115.3	8.5	12.4	0.7	5.8	0.2
P-3 100 mg/kg	275.4	14.8	175.8	11.3	9.8	0.7	6.9	0.4
P-3 200 mg/kg	278.3	16.4	180.2	11.6	9.3	0.8	7.1	0.4
P-3 400 mg/kg	279.5	15.3	155.7	10.9	10.5	0.7	6.8	0.3

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 (Figure S2).

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 haemoglobin (HbA1c) levels, which serve as an indicator of long-term glycaemic control, exhibited significant improvements with both derivatives. P-2 at 400 mg/kg reduced HbA1c to 5.8 ± 0.2%, thereby nearly normalising 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 glycaemic 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), repoted 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 dibesetices.

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, stabilised through hydrogen bonding and hydrophobic contact. These result show the vital clues role for future structural refinements and therapeutic chemical optimisations.[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 favourable 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, emphasising 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 polypharmacological 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 optimisation, 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 maintent 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 favourable 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

This research is demonstrated that the semisynthetic andrographolide derivatives (Compound), particularly P-2 and P-3, are more potential antidiabetic diseases and comprehensive computational and experimental validation. These Molecular docking methods show the strong binding affinities of P-2 and P-3 toward α-glucosidase and α-amylase, consistent with their potent in vitro enzyme inhibition and in vivo glucose-lowering efficacy.

This SAR comprehensive analysis shows there importance of hydroxyl group positioning, lactone ring stability, and the balance of hydrophobic and hydrophilic interactions in enhancing biological activity. Both P-2 and P-3 exhibited favourable ADME and show the safety profiles, with in vivo glycaemic control comparable to that achieved with metformin.

They show the strong correlation between computational predictions and experimental Result confirms that molecular docking and SAR-show and designs are reliable strategies for optimising andrographolide derivatives. For the Future research should focus on pharmacokinetic enhancement, formulation development, and clinical evaluation to advance these compounds toward therapeutic application in diabetes management.

7.Abbreviations

ADME – Absorption, Distribution, Metabolism, and Excretion
ANOVA – Analysis of Variance
BBB – Blood–Brain Barrier
CNS – Central Nervous System

CYP450 – Cytochrome P450
Da – Dalton
FT-IR – Fourier Transform Infrared Spectroscopy
GI – Gastrointestinal
HbA1c – Glycated Haemoglobin
HBA – Hydrogen Bond Acceptor
HBD – Hydrogen Bond Donor
IC_{50} – Half-Maximal Inhibitory Concentration
LogP – Octanol/Water Partition Coefficient
MMFF94 – Merck Molecular Force Field 94
NMR – Nuclear Magnetic Resonance
PDB – Protein Data Bank
QSAR – Quantitative Structure–Activity Relationship
RMSD – Root Mean Square Deviation
SAR – Structure–Activity Relationship
STZ – Streptozotocin
T2DM – Type 2 Diabetes Mellitus
TPSA – Topological Polar Surface Area
UV–Vis – Ultraviolet–Visible Spectroscopy

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