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RESEARCH PAPER

In silico screening of azo acetohydrazide derivatives as potential antidiabetic agents through alpha glucosidase inhibition

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Abstract: Type 2 diabetes mellitus is a progressive metabolic disorder marked by persistent hyperglycemia resulting from insulin resistance, insufficient insulin secretion, or a combination of both conditions. A potential therapeutic target for treating this illness is the suppression of the alpha-glucosidase enzyme, which is a key regulator of postprandial glucose absorption and carbohydrate metabolism. This work conducted a thorough in silico analysis to evaluate the efficacy of three synthesised azo-acetohydrazide derivatives (designated compounds **C**, **D**, and **E**) as alpha-glucosidase inhibitors. Pharmacokinetic properties and drug-likeness characteristics were evaluated using the SwissADME platform. At the same time, molecular docking simulations were conducted using AutoDockTools against the alpha-glucosidase enzyme structure (PDB ID: 3W37), with acarbose serving as a reference inhibitor. All three compounds adhered to essential drug-likeness criteria, including those established by Lipinski, Veber, and Ghose, and exhibited advantageous physicochemical characteristics, such as appropriate molecular weight, lipophilicity, topological polar surface area, and aqueous solubility. The compounds were anticipated to have significant gastrointestinal absorption and were unable to penetrate the blood-brain barrier, suggesting a minimal risk of central nervous system damage. Compound **E** demonstrated the highest binding affinity among the compounds, with a docking score of –7.80 kcal/mol, and formed multiple stabilizingstabilizing interactions within the enzyme's active site, including hydrogen bonds, hydrophobic contacts, and electrostatic interactions. The novelty of this study lies in the computational discovery of azo-acetohydrazide scaffolds, which are inadequately investigated chemical entities with potential antidiabetic properties. These findings establish a theoretical basis for continued development and substantiate future endeavours in the synthesis and experimental validation of these molecules. This study validates the efficacy of structure-based drug design in discovering novel alpha-glucosidase inhibitors. It identifies compound E as a promising candidate for further in vitro and in vivo research in the treatment of type 2 diabetes.

Keywords: Type 2 Diabetes Mellitus, Alpha Glucosidase, Azo-Acetohydrazide, in Silico Study.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder characterised by elevated blood glucose levels resulting from reduced insulin sensitivity, insufficient insulin production, or a combination of these mechanisms [1]. This disease is one of the leading causes of global mortality, with the number of affected individuals increasing steadily each year. According to the latest data from the IDF Diabetes Atlas 2025, China has the highest diabetes-related mortality rate worldwide, with approximately 760,000 deaths, followed by the United States (360,000), India (330,000), Pakistan (230,000), Indonesia (130,000), and Mexico (120,000) [2]. Based on these statistics, Indonesia is one of the top six nations with the highest rates of diabetes mortality, highlighting the urgent need for safer, more effective, and more affordable treatment approaches.

One of the commonly applied therapeutic strategies to control blood glucose levels in type 2 diabetes mellitus (T2DM) is the inhibition of the alpha-glucosidase enzyme, which plays a crucial role in the final stage of carbohydrate hydrolysis into glucose within the small intestine [3]. Alpha-glucosidase inhibitors, including acarbose, miglitol, and voglibose, are widely used in clinical practice; however, their administration is often associated with gastrointestinal side effects such as diarrhea, flatulence, and abdominal discomfort, which can reduce patient compliance [4,5]. Therefore, the discovery of alternative compounds that can selectively inhibit alpha-glucosidase with minimal side effects is urgently required. As a validated target, alpha-glucosidase directly modulates glucose absorption from complex carbohydrates. Inhibition of this enzyme has been shown to delay glucose absorption and assist in glycemic control [6]. One promising approach to discovering new alpha-glucosidase inhibitors is the use of molecular design methods, particularly in silico molecular docking simulations.

In recent years, compounds based on azo (-N=N-) and acetohydrazide ($-COCH_2NHNH_2$) functional groups have attracted considerable interest owing to their diverse biological activities, including antimicrobial, anticancer, and antidiabetic properties [7–15]. Azo groups can interact strongly with aromatic residues in enzyme active sites via π–π stacking interactions, while acetohydrazide groups offer conformational flexibility and strong hydrogen bonding capacity [16,17]. Combining these two moieties is believed to yield hybrid compounds with high binding affinity toward biological targets such as alpha-glucosidase. Additionally, introducing substituents such as hydroxyl or methoxy groups on aromatic rings is expected to enhance molecular interactions and improve solubility in biological environments.

As illustrated in Figure 1, three azo-acetohydrazide derivatives (**C–E**) were designed with varied aromatic substituents to evaluate the impact of structural modification on binding affinity and pharmacokinetic profile. The three derivatives consist of a para-hydroxyl substituted compound, a meta-methoxy substituted compound, and an unsubstituted parent structure. The hydroxyl group was placed in the para position rather than the ortho position to minimise steric hindrance and facilitate favourable hydrogen bonding interactions. These structural variations are expected to provide insight into the contribution of functional groups to molecular interactions with alpha-glucosidase.

This study investigates the inhibitory potential of three designed compounds against alpha-glucosidase through an in silico approach. The investigation was conducted using AutoDock Tools for molecular docking against the alpha-glucosidase enzyme (PDB ID: 3W37), while pharmacokinetic profiles and drug-likeness parameters were assessed via the SwissADME platform. The findings are intended to serve as a starting point for the development of novel oral antidiabetic options with higher efficacy and fewer adverse effects.

MATERIALS AND METHODS

Research Tools

This study was conducted on a personal computer (ASUS VivoBook X403FA_K403FA) running Windows 11 Home Single Language 64-bit, equipped with an Intel® Core™ i5-8265U CPU @ 1.60 GHz (8 CPUs) and 8 GB of RAM. Several software tools were used throughout the research workflow. MarvinSketch version 24.1.3 was used to draw and energy-minimise the ligand structures. AutoDockTools version 1.5.6 was used to prepare both proteins and ligands, as well as to conduct molecular docking simulations. The pharmacokinetic and drug-likeness properties of the ligands were predicted using the SwissADME web platform (https://www.swissadme.ch), developed by the Swiss Institute of Bioinformatics. Molecular interaction analysis and visualization were performed using Biovia Discovery Studio Visualizer v20.1.0.19295 and UCSF Chimera version 1.13.1.

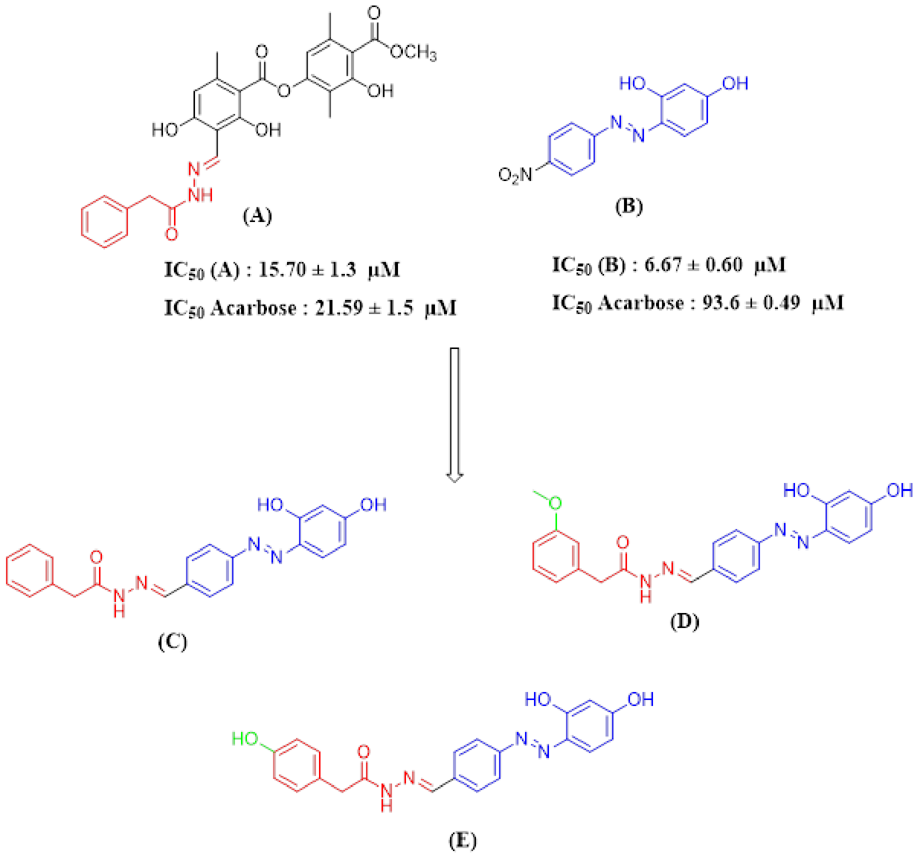


Figure 1. Design of azo-acetohydrazide derivatives (compounds C–E). Compound A (red: azo-acetohydrazide moiety; black: reference scaffold) showed IC$_{50}$ = 15.70 ± 1.3 µM vs. acarbose (21.59 ± 1.5 µM). Compound B (red: azo-acetohydrazide moiety; blue: substituted aromatic ring) exhibited IC$_{50}$ = 6.67 ± 0.60 µM vs. acarbose (93.6 ± 0.49 µM). Compounds C–E incorporate the red azo-acetohydrazide scaffold, blue aromatic pharmacophore, and green additional substituents for optimisationoptimizationoptimization. The different IC$_{50}$ values of acarbose in Figures A and B reflect distinct assay conditions and serve as contextual benchmarks.

In Silico Prediction of Physicochemical, Pharmacokinetic, and Drug-Likeness Properties

The physicochemical characteristics, pharmacokinetic profiles, and drug-likeness of the three designed azo-acetohydrazide derivatives (compounds **C–E**) and the reference drug acarbose were predicted using the SwissADME web platform developed by the Swiss Institute of Bioinformatics. This platform incorporates advanced computational algorithms, including multiple linear regression, binary classification, and support vector machine (SVM), trained on large datasets of drug-like and non-drug-like molecules [18]. Evaluated parameters included water solubility (Log S), lipophilicity (Log P), topological polar surface area (TPSA), number of hydrogen bond donors and acceptors (HBD and HBA), blood–brain barrier (BBB) penetration, gastrointestinal absorption, and potential interactions with cytochrome P450 enzymes. Drug-likeness evaluation was conducted using Lipinski's Rule of Five, along with Ghose and Veber filters, and predictive models such as the BOILED-Egg and Bioavailability Radar. These predictions served as the basis for evaluating the potential of the candidate compounds as safe and effective oral drugs.

Protein Preparation and Ligand Validation

The 3D crystal structure of alpha-glucosidase (PDB ID: 3W37) was obtained from the Protein Data Bank and downloaded as a file in pdb format. Before molecular docking, the protein structure was prepared using AutoDockTools by eliminating crystallographic water molecules, incorporating polar hydrogen atoms, and assigning Kollman partial atomic charges to ensure accurate electrostatic representation. The native co-crystallised ligand, acarbose, was separated from the complex and employed as a reference ligand for the redocking validation step. Subsequently, both the protein and ligand structures were converted into pdbqt format to be compatible with AutoDock. This comprehensive preparation step ensures the reliability and reproducibility of the docking simulation results.

Docking Protocol Validation

To ensure the reliability of the molecular docking procedure, protocol validation was performed through redocking of the native ligand, acarbose, into the active site of the prepared alpha-glucosidase structure. The docking accuracy was assessed by comparing the predicted binding pose with the original crystallographic conformation using root-mean-square deviation (RMSD) analysis.

Docking Simulation of Designed Compounds

Compounds **C**, **D**, and **E** were drawn using MarvinSketch and their geometries were optimised using the Merck Molecular Force Field (MMFF94) to obtain the most stable conformers. The ligands were saved in .mol format, converted to .pdb, and finally to .pdbqt using AutoDockTools. The grid box parameters were defined to encompass the active site region, guided by the binding coordinates of the native ligand. Molecular docking was performed using the Lamarckian Genetic Algorithm (LGA) with 100 genetic algorithm runs. The results were saved in .dlg format for further analysis.

Docking Simulation of Designed Compounds

Molecular docking simulations of the designed azo-acetohydrazide derivatives were conducted to predict their binding affinity and interaction patterns within the active site of alpha-glucosidase. The docking results, including binding energy values and interaction profiles, were analysed using Biovia Discovery Studio Visualizer and UCSF Chimaera. These visualisation platforms enabled a comprehensive examination of ligand orientations and conformational fit within the binding pocket. Critical non-covalent interactions, including hydrogen bonding, hydrophobic contacts, π–π stacking, and electrostatic interactions, were analysed to evaluate the stability and binding specificity of the ligand–receptor complexes. This interaction profiling offered meaningful insights into the molecular mechanisms underlying ligand affinity, contributing to the evaluation of their potential inhibitory activity against alpha-glucosidase.

RESULTS AND DISCUSSION

In Silico Analysis of Physicochemical, Pharmacokinetic, and Drug-Likeness Properties

The physicochemical characteristics, pharmacokinetic parameters, and drug-likeness profiles of the designed azo-acetohydrazide derivatives (**C–E**) were evaluated in silico using the SwissADME platform, with acarbose serving as a reference compound. The primary objective of this analysis was to assess absorption, distribution, metabolism, and excretion (ADME) properties, as well as oral drug-likeness based on parameters such as molecular weight (MW), topological polar surface area (TPSA), number of hydrogen bond acceptors (HBA) and donors (HBD), rotatable bonds, lipophilicity (LogP), aqueous solubility, and compliance with established drug-likeness rules (Lipinski, Ghose, and Veber). The physicochemical data for all compounds are presented in Table 1.

Compounds **C–E** displayed molecular weights ranging from 374 to 404 g/mol, which fall within the optimal range for oral drug candidates (160–480 g/mol), indicating potential for favourable bioavailability and membrane permeability [19]. In contrast, acarbose exhibited a significantly higher molecular weight (645.60 g/mol), which may impair its absorption through the gastrointestinal tract. The TPSA values of compounds **C–E** ranged between 106.84 and 126.87 Å², remaining below the critical threshold of 140 Å², suggesting good potential for transmembrane diffusion [20]. Acarbose, however, had a TPSA of 329.01 Å², indicating extremely low permeability, which is consistent with its predicted low gastrointestinal absorption. The LogP values for compounds **C–E** ranged from 3.13 to 3.54, which is within the permissible range for oral medicines (0-5), indicating favourable lipid membrane permeability [19]. In contrast, acarbose had a significantly negative LogP value (–6.24), indicating its high polarity and hydrophilicity, as well as low membrane penetration. Compounds C-E have a moderate solubility, whereas acarbose is very water-soluble; however, this benefit is countered by its excessively high MW and TPSA.

Table 1. Physicochemical properties of azo-acetohydrazide derivatives (C–E) and acarbose

Compound	MW (g/mol)	TPSA (Å²)	HBA	HBD	Rotatable Bonds	Moriguchi's LogP	Water Solubility
C	374.39	106.84	6	3	7	3.54	Moderately Soluble
D	404.42	115.87	7	3	8	3.48	Moderately Soluble
E	390.39	126.87	7	4	7	3.13	Moderately Soluble
Acarbose	645.60	329.01	19	14	13	-6.24	Highly Soluble

As shown in Table 2, drug-likeness predictions revealed that compounds **C-E** fulfilled all of the requirements set by the Lipinski, Ghose, and Veber guidelines, indicating their potential as orally bioavailable medication candidates. In comparison, acarbose violated more than five Ghose filter parameters. It failed to meet two important Veber rule criteria, indicating that it is unsuitable for oral administration according to established drug-likeness criteria.

Table 2. Pharmacokinetic properties and drug-likeness prediction of azo-acetohydrazide derivatives (C–E) and acarbose

Compound	GI Absorption	BBB Perm.	P-gp Substrate	CYP1A2 Inhib.	CYP2C19 Inhib.	CYP2C9 Inhib.	CYP2D6 Inhib.	CYP3A4 Inhib.	Log Kp (cm/s)	Lipinski	Ghose	Veber	Bioav. Score
C	High	No.	No	No	No	Yes	No	No	-5.88	Yes	Yes	Yes	0.55
D	High	No.	No	No	No	Yes	No	No	-6.07	Yes	Yes	Yes	0.55
E	High	No.	No	No	No	Yes	No	No	-6.21	Yes	Yes	Yes	0.55
Acarbose	Low	No.	Yes	No	No	No	No	No	-16.50	No	No	No	0.17

From a pharmacokinetic standpoint, compounds **C–E** demonstrated high predicted gastrointestinal absorption and an inability to traverse the blood–brain barrier (BBB), suggesting minimal risk of central nervous system toxicity [21]. Acarbose also does not penetrate the BBB due to its high polarity, but was predicted to have low GI absorption. Additionally, none of the designed compounds were identified as P-glycoprotein (P-gp) substrates, implying low potential for intestinal efflux. Regarding cytochrome P450 (CYP) enzyme interactions, only weak inhibitory potential against CYP2C9 was observed for compounds **C–E**, while no inhibition was predicted for major isoforms such as CYP3A4 or CYP2D6 [22], indicating a low likelihood of hepatotoxicity or significant drug–drug interactions.

The three designed azo-acetohydrazide derivatives (**C–E**) exhibited favourable ADME profiles, each with a predicted oral bioavailability score of 0.55. These findings suggest their potential suitability for oral administration. Compared to acarbose, which failed to meet multiple drug-likeness criteria, the designed compounds demonstrated improved compliance with Lipinski's Rule of Five and other drug-likeness filters, reflecting their superior pharmacokinetic properties in silico. Despite these promising results, it is essential to emphasise that experimental validation—encompassing in vitro and in vivo assays—is still required to confirm their pharmacological efficacy and safety profile before further development as therapeutic candidates for type 2 diabetes mellitus.

Molecular Docking Results

To evaluate the binding affinity of the designed azo-acetohydrazide derivatives (**C–E**), molecular docking studies were performed against the alpha-glucosidase enzyme (PDB ID: 3W37) using AutoDockTools version 1.5.6. The 3D structure of the target protein was obtained from the Protein Data Bank in .pdb format and subsequently prepared by deleting crystallographic water molecules, incorporating polar hydrogen atoms, and assigning Kollman partial charges.

For precise ligand positioning and reliable interaction analysis, the molecular docking simulations were performed using a grid box configuration specifically defined to encompass the active site region of alpha-glucosidase. The grid box was centred at coordinates (X = 0.305, Y = –1.705, Z = –23.114), with dimensions of 30 × 50 × 30 grid points in the X, Y, and Z directions, respectively. The grid spacing was set at 0.375 Å, allowing for high-resolution sampling of potential binding conformations. The number of genetic algorithm (GA) runs was set to 100, which provided robust conformational sampling for reliable prediction of binding poses. The electrostatic potential was calculated using a distance-dependent dielectric constant of –0.1465, and a smoothing factor of 0.5 Å was applied to retain minimum energy values within a defined radius, thereby improving the accuracy of the energy map.

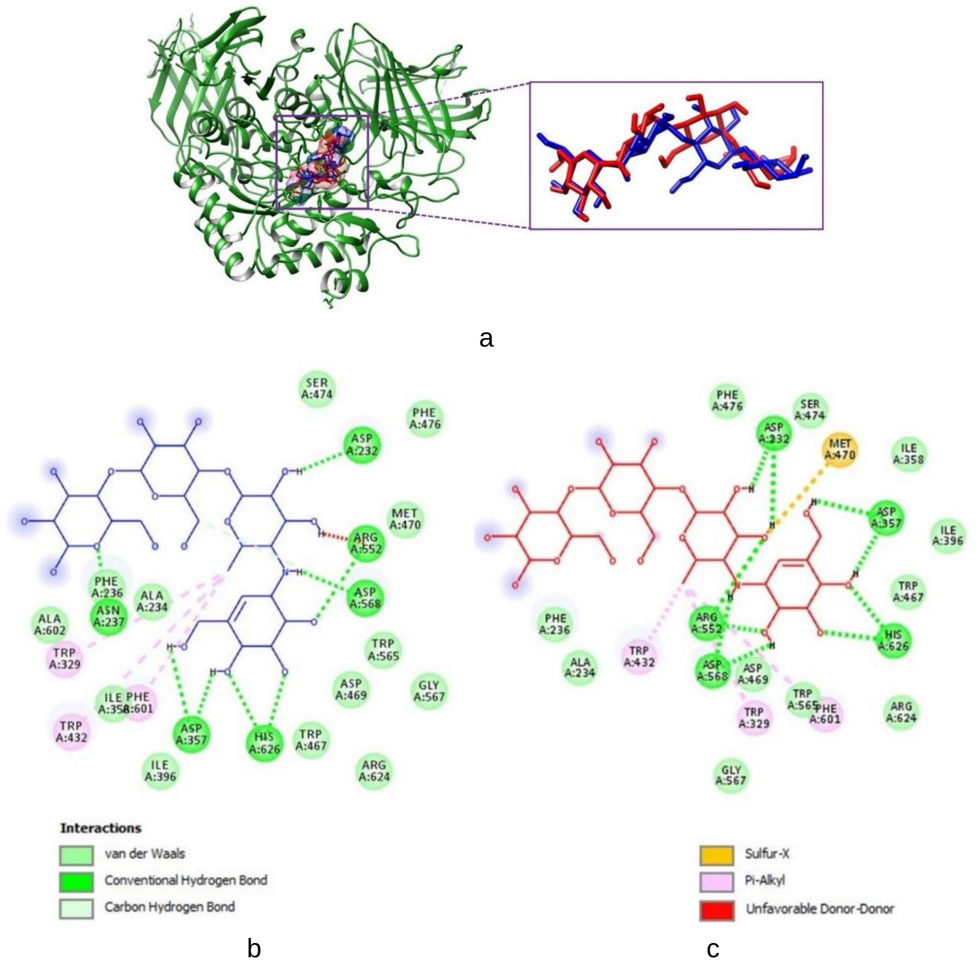
To ensure the reliability of the docking methodology, a redocking procedure was carried out using the native ligand, acarbose, which was originally co-crystallised with the alpha-glucosidase enzyme. The ligand was extracted from the crystal complex and re-docked into the active site under the same docking parameters applied to the test compounds. The resulting root mean square deviation (RMSD) between the redocked pose and the original crystallographic conformation was 1.44 Å, as illustrated in Figure 2, which is within the commonly accepted validation threshold of ≤2.0 Å. The outcome supports the robustness and accuracy of the molecular docking approach applied in this study [23]. Furthermore, the redocked conformation of acarbose retained key molecular interactions with active site residues, closely mirroring those observed in the native crystal structure [24].

The docking results for compounds **C–E** and acarbose are summarised in Table 3 and illustrated in Figure 3. Among the three compounds, compound **E** exhibited the most favourable binding energy (–7.80 kcal/mol), suggesting the strongest binding affinity to the alpha-glucosidase active site. It formed hydrogen bonds with six key residues: Compared with C and D, compound E formed more stabilising hydrogen bonds and complementary hydrophobic contacts, which likely enhanced its binding strength. Electrostatic interactions included attractive charge contacts with ASP A:232 and ASP A:469, as well as an unfavourable positive–positive interaction with LYS A:506. This repulsive electrostatic interaction, which occurs between two positively charged residues, may slightly reduce the stability of the ligand–receptor complex due to increased binding energy [25]. Nonetheless, the overall stability remained favourable, due to several strong hydrogen bonds and hydrophobic contacts, suggesting that additional beneficial interactions offset the destabilising effect. The interaction profile of compound E differs markedly from that of acarbose. Acarbose predominantly interacts with the catalytic residues ASP A:232, ASP A:568, ASP A:357, ARG A:552, and HIS A:626 through hydrogen bond formation, in addition to hydrophobic contacts with TRP A:432, TRP A:329, and PHE A:601. In contrast, compound E establishes supplementary hydrogen bonds with ASN A:237 and LYS A:506 and forms alternative hydrophobic interactions with ILE A:233 and ALA A:234. These additional contacts indicate a broader stabilisation of the active site, which may underlie a stronger inhibitory potency compared with acarbose.

Compound **C** displayed a binding energy of –6.96 kcal/mol and an RMSD of 17.73 Å (Table 3). Compared to compound E, its binding affinity was lower; however, it still established interactions with several key active site residues, suggesting potential as a selective inhibitor. Electrostatic interactions included attractive contacts with ASP A:232 and ASP A:469, along with an unfavourable positive–positive interaction with LYS A:506, which may contribute to reduced binding stability. Overall, these interactions highlight the moderate yet significant inhibitory potential of compound C against α-glucosidase.

In contrast, compound **D** showed the weakest binding affinity, with a binding energy of –5.85 kcal/mol and the highest RMSD value of 23.69 Å (Table 3). Additionally, a π–π-anion electrostatic interaction was observed with ASP A:630. The weaker binding affinity suggests that its binding configuration and interaction profile are less optimal for alpha-glucosidase inhibition.

As a reference, acarbose yielded a binding energy of –6.75 kcal/mol and an RMSD of 1.44 Å (Table 3), and formed five hydrogen bonds with ASP A:232, ASP A:568, ARG A:552, ASP A:357, and HIS A:626. Its hydrophobic contacts included π–alkyl interactions with TRP A:432, TRP A:329, and PHE A:601, as well as one sulfur-X electrostatic interaction with MET A:470. These results align with established interaction patterns reported in previous studies.



a

b c

Figure 2. (a) Superimposition of the native binding conformation of acarbose (blue) and the redocked conformation (red) within the active site of alpha-glucosidase (PDB ID: 3W37). (b) Molecular interactions between native acarbose and active site residues of alpha-glucosidase. **(c)** Molecular interactions formed between redocked acarbose and the corresponding active site residues.

Table 3. Molecular docking result of azo-acetohydrazide derivatives (C–E) and acarbose (redocked) with alpha-glucosidase (PDB ID: 3W37)

Compound	**Binding Energy (Kcal/mol)**	**RMSD (Å)**	**Type of Interaction**		
			Hydrogen bond	**Hydrophobic interaction**	**Electrostatic interaction**
C	–6.96	17.73	LYS A:506, ASP A:568, HIS A:626, ASP A:357	π–π sigma: ILE A:233	Unfavorable positive-positive: LYS A:506 Attractive charge: ASP A:232, ASP A:469
D	–5.85	23.69	ASP A:469, ASP A:568, ARG A:552	π–π T-shaped: TRP A:432, TRP A:329 π–alkyl: MET A:470, ALA A:628, ALA A:602, PHE A:601	π–anion: ASP A:630
E	–7.80	20.51	ASN A:237, LYS A:506, ARG A:552, ASP A:568, HIS A:626, ASP A:357	π–sigma: ILE A:233 π–alkyl: ALA A:234	Attractive charge: ASP A:232, ASP A:469 Unfavourable positive-positive: LYS A:506
Acarbose (redocked)	–6.75	1.44	ASP A:232, ASP A:568, ARG A:552, ASP A:357, HIS A:626	π–alkyl: TRP A:432, TRP A:329, PHE A:601	Sulfur-X: MET A:470

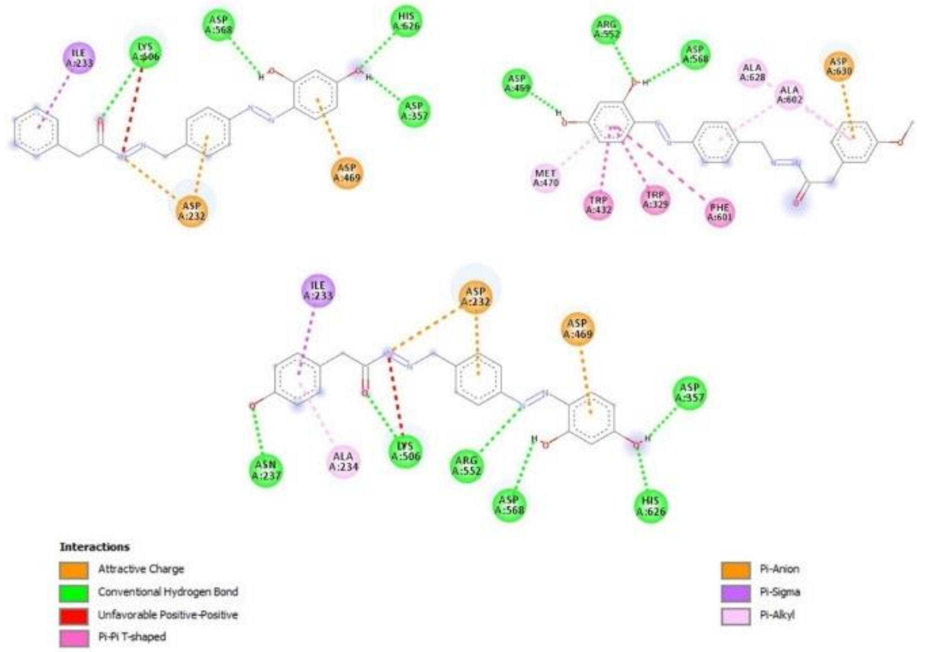


Figure 3. Molecular interactions of (a) compound C, (b) compound D, and (c) compound E within the active site of alpha-glucosidase (PDB ID: 3W37)

Compound **E** exhibited the most advantageous interaction profile regarding binding energy and the quantity of stabilising interactions, establishing it as the most robust contender among the three derivatives. Although compounds **D** and **C** exhibited diminished binding affinities, their energy levels remained within an acceptable range for subsequent experimental analysis. The results indicate that all three azo-acetohydrazide derivatives have potential as alpha-glucosidase inhibitors, with compound **E** identified as the most promising candidate for further development. It is important to recognise that the scoring functions used in AutoDock mainly focus on estimating binding free energy through enthalpic contributions. At the same time, they do not comprehensively consider entropic and solvation effects. Consequently, although the docking results offer significant insights into binding modes and relative affinities, they ought to be considered as predictive indicators rather than definitive measures of inhibitor potency.

CONCLUSION

According to the in silico evaluation of physicochemical characteristics, pharmacokinetics, drug-likeness, and molecular docking, the three azo-acetohydrazide derivatives (**C–E**) demonstrate significant potential as alpha-glucosidase inhibitors for the treatment of type 2 diabetes mellitus. All compounds exhibited advantageous oral pharmacokinetic properties, encompassing suitable molecular weight, TPSA values conducive to membrane permeability, ideal lipophilicity and aqueous solubility, and adherence to Lipinski's, Ghose's, and Veber's criteria. Furthermore, their anticipated oral bioavailability ratings recommend continued development. Docking simulations indicated that compound **E** exhibited the highest binding affinity for alpha-glucosidase (–7.80 kcal/mol), establishing numerous stabilising hydrogen bonds and hydrophobic interactions with critical active-site residues, whereas compounds **C** and **D** demonstrated significant binding patterns but with relatively lower affinity. Compound **E** emerged as the most promising contender, exhibiting robust molecular interactions and good pharmacokinetic properties. This study is limited by its dependence on in silico predictions, the restricted number of developed compounds, and the inherent limitations of docking scoring systems, which inadequately consider entropic and solvation effects. To enhance the translational significance of these findings, subsequent research should include molecular dynamics simulations to evaluate binding stability over time, followed by the synthesis of the most promising derivatives and thorough in vitro enzymatic testing. Ultimately, in vivo pharmacological and toxicological investigations will be necessary to validate their therapeutic efficacy and safety profile.

Acknowledgements

The authors confirm that there are no conflicts of interest associated with this study. All relevant data, analyses, and interpretations have been fully disclosed within this manuscript. No additional information has been withheld, and the findings are transparently presented to support reproducibility and further investigation.

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