



OPEN Substituted piperazine conjugated to quinoline-thiosemicarbazide as potent α -glucosidase inhibitors to target hyperglycemia

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Diabetes mellitus, particularly type 2 diabetes, is a growing global health challenge characterized by chronic hyperglycemia due to insulin resistance. One therapeutic approach to managing this condition is the inhibition of α -glucosidase, an enzyme involved in carbohydrate digestion, to reduce postprandial blood glucose levels. In this study, a series of thiosemicarbazide-linked quinoline-piperazine derivatives were synthesized and evaluated for their α -glucosidase inhibitory activity, to identify new agents for type 2 diabetes management. Structure-activity relationship (SAR) analysis revealed that the nature and position of substituents on the aryl ring significantly impacted the inhibitory potency. Among the synthesized derivatives, the 2,5-dimethoxy phenyl substitution (7j) exhibited the most potent activity with an IC_{50} value of 50.0 μ M, demonstrating a 15-fold improvement compared to the standard drug acarbose. Kinetic studies identified compound 7j as a competitive inhibitor, with a K_i value of 32 μ M. Molecular docking simulations demonstrated key interactions between compound 7j and the active site of α -glucosidase, while molecular dynamics simulations confirmed the stability of the enzyme-ligand complex, reflected in low RMSD and RMSF values.

Keywords α -glucosidase, Diabetes mellitus, Thiosemicarbazide, Quinoline-piperazine

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia) due to disruption in insulin secretion, insulin action, or both. The disease affects millions of people worldwide and is associated with various complications, including cardiovascular diseases, kidney failure, neuropathy, and retinopathy¹. Diabetes is typically classified into two major types: type 1 diabetes, resulting from autoimmune destruction of pancreatic β -cells, and type 2 diabetes, which is associated with insulin resistance and inadequate insulin production². Type 2 diabetes accounts for approximately 90–95% of all diabetes cases and is closely linked to lifestyle factors such as diet and physical activity³.

One of the primary goals of diabetes management is to regulate blood sugar levels and prevent postprandial hyperglycemia, which is a major contributor to the long-term complications of diabetes⁴. Among the therapeutic strategies employed to manage postprandial glucose levels, α -glucosidase inhibitors play a significant role. α -Glucosidase is an enzyme located in the brush border of the small intestine that plays a key role in carbohydrate digestion. It catalyzes the breakdown of complex carbohydrates, such as starch and disaccharides, into simple sugars like glucose⁵. The inhibition of α -glucosidase slows down the digestion and absorption of carbohydrates, thereby reducing the rapid rise in blood glucose levels after a meal⁶.

α -Glucosidase inhibitors are an essential class of drugs for managing type 2 diabetes. By delaying carbohydrate digestion, these inhibitors prevent sudden increases in blood glucose, helping to maintain better glycemic control. Common α -glucosidase inhibitors, such as acarbose, miglitol, and voglibose, are widely used

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in clinical practice⁷. Developing novel α -glucosidase inhibitors with improved efficacy and reduced side effects is an ongoing area of research in medicinal chemistry. Through structure-activity relationship (SAR) studies, researchers aim to discover new inhibitors that offer better therapeutic outcomes and minimal adverse effects, such as gastrointestinal discomfort commonly associated with current inhibitors⁸.

Recent advances in the design of α -glucosidase inhibitors have focused on exploring a variety of heterocyclic scaffolds, including quinoline⁹, isatin¹⁰, and benzimidazole^{11,12} derivatives. Among these, quinoline-based α -glucosidase inhibitors have garnered significant attention due to their promising bioactivity and structural versatility. Numerous series of α -glucosidase inhibitors containing a quinoline ring have been synthesized, with structural modifications aimed at enhancing potency and specificity^{13–15}. For example, quinoline carboxylic acid has demonstrated significant inhibitory potency compared to the standard acarbose ($IC_{50} = 66.5 \pm 1.5 \mu\text{g/mL}$)¹⁶. Additionally, Schiff bases derived from quinoline analogs (Compound A, Fig. 1) have shown IC_{50} values ranging from 6.20 to 48.50 μM . SAR studies revealed that the presence of electron-donating groups in these analogs generally diminished inhibitory activity, suggesting that electron-withdrawing substituents may enhance binding interactions with the enzyme's active site¹⁷. Compound B also exhibited highly potent α -glucosidase inhibitors.

Thiosemicarbazide moieties have also emerged as potent α -glucosidase inhibitors. For instance, thiosemicarbazide-isatin derivatives (C) exhibit impressive inhibitory activity, with IC_{50} values ranging between 1.20 ± 0.10 and $35.60 \pm 0.80 \mu\text{M}$, significantly outperforming acarbose ($IC_{50} = 38.60 \mu\text{M}$)¹⁸. Also in another study, chromones conjugated to thiosemicarbazide (D, $IC_{50} = 0.11 \pm 0.01 \mu\text{M}$), demonstrated superior activity, suggesting that the thiosemicarbazide framework provides a strong basis for further development of α -glucosidase inhibitors¹⁹.

Similarly, aryl-piperazine-linked benzimidazole derivatives have shown noteworthy inhibitory activity against α -glucosidase. The most potent analog, compound E, acts as a competitive inhibitor with a K_i value of 120 μM . Molecular docking studies reveal key interactions with amino acid residues contributing to the inhibitory effect²⁰. The benzimidazole-piperazine derivatives (compound F) exhibited significant inhibition against both α -glucosidase ($IC_{50} = 0.85\text{--}29.72 \mu\text{M}$) and α -amylase ($IC_{50} = 4.75\text{--}40.24 \mu\text{M}$), further highlighting their therapeutic potential²¹. A very recent study applied a fused design strategy by incorporating a quinoline core with a flexible piperazine linker (compound G), yielding promising results²².

In the current study, fusing and fragment-based design strategies were employed by combining distinct pharmacophores—thiosemicarbazide, quinoline, and piperazine—into a single molecule to achieve enhanced therapeutic effects. The newly designed compounds were evaluated for their α -glucosidase inhibitory activity, with their inhibition mechanisms further investigated through kinetic studies, molecular docking, and molecular dynamics simulations. These computational analyses provided a comprehensive understanding of the molecular interactions between the synthesized compounds and the enzyme, highlighting key structural features that contribute to their inhibitory activity. These findings offer a robust foundation for future optimization and the development of more potent α -glucosidase inhibitors.

Results and discussion

Chemistry

In this work, we synthesized a novel series of 2-(4-(3-((2-carbamothioylhydrazineylidene)methyl)quinolin-2-yl)piperazin-1-yl)-N-phenylacetamide derivatives 7a–n by reaction 2-(4-(3-formylquinolin-2-yl)piperazin-1-yl)-N-phenylacetamide 6a–n and thiosemicarbazide. In the previous step, nucleophilic reaction between 2-(piperazin-1-yl)quinoline-3-carbaldehyde 4 and 2-chloro-N-phenylacetamide derivatives 5a–n in the presence of K_2CO_3 leads to synthesis of 2-(4-(3-formylquinolin-2-yl)piperazin-1-yl)-N-phenylacetamide 6a–

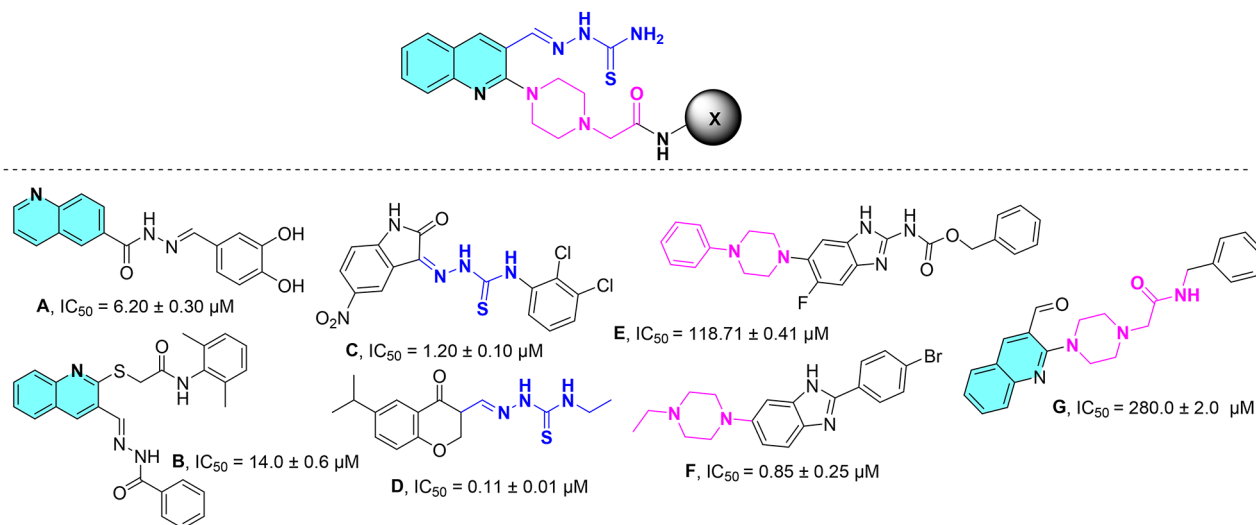


Fig. 1. The rationale for the design of substituted piperazine conjugated to quinoline-thiosemicarbazide as new α -glucosidase inhibitors

n derivatives. At this step, substances **4** and 1 eq K_2CO_3 were initially mixed in DMF for half an hour at room temperature. Then, 2-chloro-N-phenylacetamide was added to the reaction mixture, and temperature was raised to 80 °C. After completing the reaction, water was added to the reaction mixture, resulting in the formation of a yellow precipitate. The precipitate was filtered and washed several times with water. As outlined in Scheme 1, via Vilsmeier–Haack process chloroquinoline-3-carbaldehyde **2** was synthesized then reaction between compound **2** and 1-Boc-piperazine in the presence of KI and K_2CO_3 and deprotection of *tert*-butyl 4-(3-formylquinolin-2-yl)piperazine-1-carboxylate **3** leads to the synthesis of 2-(piperazin-1-yl)quinoline-3-carbaldehyde **4** (Scheme 1).

Structure-activity relationships to target α -glucosidase

The SAR for this series of compounds based on α -glucosidase inhibitory activity reveals important insights into the effect of various substituents on the aryl moieties. The inhibitory potency, as reflected by the IC_{50} values, shows a wide range and specific trends can be identified (Table 1).

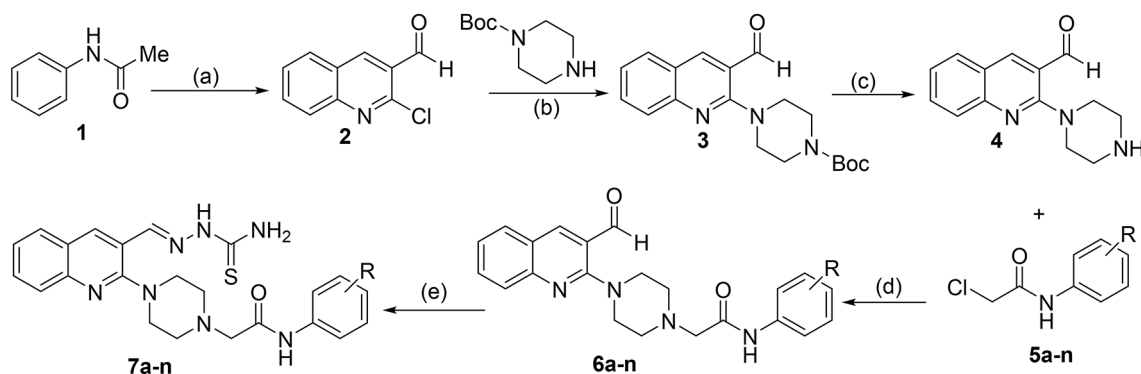
The unsubstituted phenyl ring exhibits moderate inhibitory activity (compound **7a**, IC_{50} = 501.5 μ M), serving as a baseline for comparison with substituted derivatives. Halogen substitution reveals important trends. Fluorine substitution of the phenyl ring, whether alone (compound **7b**, 4-F) or in combination with chlorine (compound **7d**, 3-Cl-4-F), leads to weak inhibitory activity. Fluorine is a small, highly electronegative atom that can influence electron distribution, but its size and low polarizability may reduce strong interactions with the enzyme. On the other hand, chlorine substitution at the 4-position (compound **7c**) significantly enhances inhibitory activity (IC_{50} = 249.2 μ M). Chlorine, being bulkier than fluorine and more polarizable, likely enhances hydrophobic and van der Waals interactions within the enzyme's active site. This suggests that chlorine offers a more favorable balance of steric and electronic effects, improving binding compared to fluorine. The trifluoromethyl substitution on the phenyl ring (compound **7e**) also shows good inhibition (IC_{50} = 389.0 μ M). The electron-withdrawing nature of 4- CF_3 contributes to enzyme interaction but may introduce steric hindrance due to its larger size compared to chlorine, which could explain its reduced potency relative to the 4-Cl derivative.

Nitro substitution demonstrates a position-dependent effect on enzyme inhibition. The nitro group at the 2-position of the phenyl ring (compound **7f**) shows poor inhibitory activity (IC_{50} > 750 μ M), likely due to steric clash within the enzyme active site. However, shifting the nitro group to the 3-position (compound **7g**) significantly improves activity (IC_{50} = 309.4 μ M).

Methyl substitution at the *ortho* position generally leads to diminished activity in compound **7h**. Methyl group is electron-donating, and they do not contribute to hydrogen bonding. The absence of significant electronic effects or steric interactions likely explains the reduced activity in this case. Methoxy substitution shows position effects. Substitution with a methoxy group at the 4-position of the phenyl ring (compound **7i**) results in poor inhibitory activity (IC_{50} > 750 μ M). The methoxy group, being electron-donating, may increase electron density on the phenyl ring, but at the 4-position, it may not favorably interact with the enzyme's active site. However, the di-substitution of methoxy groups at the 2- and 5-positions on the phenyl ring (compound **7j**) significantly enhances inhibitory activity (IC_{50} = 50.0 μ M). This compound showed 15-fold better activity vs. acarbose as the positive control. This dramatic improvement suggests that methoxy groups in these positions increase binding affinity.

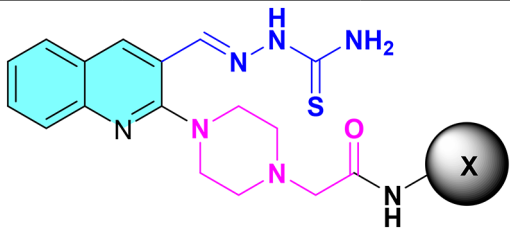
Isoquinoline substitution (compound **7l**) also shows moderate inhibitory potency (IC_{50} = 395.2 μ M), reflecting improved activity compared to some phenyl derivatives. Isoquinoline introduces an additional nitrogen atom into the ring, which may engage in additional hydrogen bonding or electrostatic interactions with the enzyme.

The benzyl substitution (compound **7m**) leads to weak inhibitory activity (IC_{50} > 750 μ M). The 4-methoxy benzyl derivative (compound **7n**) also exhibits poor activity, indicating that neither the larger size nor the electron-donating methoxy group favors binding. So it seems that in this case the length might increase the flexibility and rotation of molecules in the binding site of the enzyme and reduce the potency.



Scheme 1. Synthesis of 2-(4-(3-(1 H-benzo[d]imidazol-2-yl)quinolin-2-yl)piperazin-1-yl)-N-phenylacetamide derivatives **7a–n**: (a) $POCl_3$, DMF, 0 °C, then 80 °C, 12 h; (b) KI (10 mol%), K_2CO_3 (1 eq), 80 °C, 2 h; (c) HCl, MeOH, RT, 3 h; (d) K_2CO_3 (1 eq), DMF, 80 °C, 2 h; (e) thiosemicarbazide (1.5 eq), PTSA (20 mol%), DMF, 80 °C, 3 h.

Compounds	X	IC ₅₀ (μM)
7a		501.5 ± 0.5
7b		> 750
7c		249.2 ± 1.9
7d		457.7 ± 1.4
7e		389.0 ± 0.3
7f		> 750
7g		309.4 ± 0.6
7h		> 750
7i		> 750
7j		50.0 ± 2.0
7k		> 750



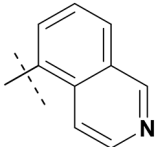
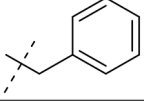
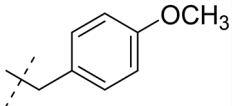
Compounds	X	IC ₅₀ (μM)
7l		395.2 ± 2.4
7m		> 750
7n		> 750
Acarbose ^b		750.0 ± 1.2

Table 1. α-Glucosidase inhibition assay of 7a–n^a. ^aEach value represents the mean ± SD. ^bPositive control.

Enzyme kinetic studies

According to Fig. 2a, the Lineweaver-Burk plot showed that the K_m gradually increased and V_{max} remained unchanged with increasing inhibitor concentration indicating a competitive inhibition. The results show Compound 7j binds to the active site on the enzyme and competes with the substrate for binding to the active site. Furthermore, the plot of the K_m versus different concentrations of the inhibitor gave an estimate of the inhibition constant, K_i of 32 μM (Fig. 2b).

Molecular docking study

Molecular docking is a powerful tool in drug discovery, used to simulate and analyze the interactions between ligand and its target receptor. It helps identify the key factors influencing the drug's effectiveness against disease by visualizing how the ligand binds within the active site of the enzyme. In this study, molecular docking was performed on the homology-modeled α-glucosidase enzyme to gain insights into the binding interactions of the most potent analog. As expected, the most active compound, 7j, demonstrated several important interactions with the enzyme's binding site, confirming its strong affinity (Fig. 3). Specifically, the quinoline ring of the analog formed two key pi-cation interactions with Arg312, which are crucial for stabilizing the ligand in the binding pocket. Additionally, a strong hydrogen bond interaction was observed between His279 and the sulfur atom of the thiosemicarbazide moiety, further enhancing the binding stability. Moreover, two additional hydrogen bond interactions were identified: one between the NH group of the amide linker moiety and Pro309, and the other between the carbonyl group (C=O) of the amide and Asn241. These interactions play a significant role in securing the ligand within the enzyme's active site. The terminal 2,5-methoxyphenyl group of the molecule exhibited a pi-pi stacking interaction with Phe231, which further contributes to the stability of the enzyme-ligand complex. Each part of the molecule, from the quinoline core to the terminal substituted phenyl group, participates in distinct interactions, confirming the high affinity of the compound for the enzyme.

Molecular dynamic simulation

Molecular dynamic (MD) simulations were conducted to study the behavior of the 7j-enzyme complex over 300 ns in a simulated biological environment. This approach allows for the evaluation of the stability and conformational changes in the complex under dynamic conditions. Root Mean Square Deviation (RMSD) analysis was used to monitor structural deviations and stability throughout the simulation. RMSD is an essential metric that reflects how much the structure of the enzyme or complex changes relative to its initial configuration during the simulation. Generally, an RMSD (root mean square deviation) below 3 Å is considered acceptable, indicating that the protein remains close to its average thermal structure. For the α-glucosidase-7j complex, the RMSD remained below this threshold, suggesting that the system was stable and well-equilibrated during the simulation. Moreover, after 180 ns of simulation, the 7j-enzyme complex exhibited an even lower RMSD value of less than 2 Å, further confirming its stability. This low deviation suggests that the 7j forms a stable interaction with the enzyme, maintaining the overall conformation of the complex with minimal structural changes. In contrast, when the enzyme was analyzed without the ligand, a larger conformational shift was observed, with the RMSD reaching approximately 4.5 Å. (Fig. 4)

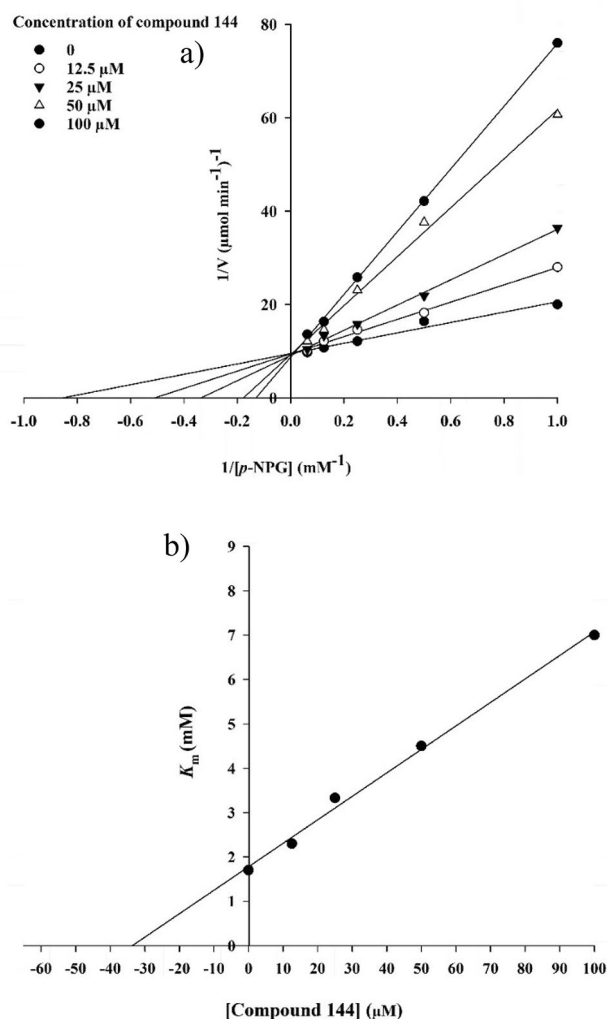


Fig. 2. Kinetics of α -glucosidase inhibition by 7j. (a) The Lineweaver–Burk plot in the absence and presence of different concentrations of 7j; (b) The secondary plot between K_m and various concentrations of 7j.

The root mean square fluctuation (RMSF) of the backbone atoms in the 7j-enzyme complex was calculated to evaluate the flexibility of the complex. RMSF analysis provides insights into the mobility of specific regions of the enzyme, focusing on the fluctuations of α -glucosidase residues in the presence of the ligand. This analysis aims to determine how ligand binding affects the stability of various enzyme regions, particularly the active site and the A domain.

The results revealed that compound 7j significantly interacted with the B domain, active site, and A domain, leading to reduced fluctuations in these key regions (Fig. 5a). This reduction in flexibility suggests that the intermolecular interactions between the enzyme and ligand are robust, effectively restricting movement and stabilizing the enzyme's structure. Furthermore, the decreased flexibility in the active site indicates that the enzyme-ligand complex is highly stable, reinforcing the potential of 7j as a potent α -glucosidase inhibitor.

The stability of the enzyme-ligand complex can be attributed to several key interactions between compound 7j and α -glucosidase (Fig. 5b). The quinoline moiety of 7j plays a central role by forming hydrogen bond with Arg312. The piperazine acetamide group of 7j is also involved in important interactions, forming hydrogen bonds with His239, which helps to maintain the ligand within the enzyme's binding pocket. Furthermore, the amine group attached to 2,5-dimethoxyphenyl group of 7j forms an additional hydrogen bond with Ser308, adding to the overall stability of the enzyme-ligand complex. Lastly, the NH group of the thiosemicarbazide moiety forms two hydrogen bonds with Thr215 and Glu276 further reinforcing the interactions between the ligand and the enzyme. These multiple and diverse interactions—hydrogen bonding, pi-pi stacking, and hydrophobic contacts—create a highly stable complex that restricts the movement of key enzyme residues, as evidenced by the RMSF analysis. This highlights the potential of compound 7j as a strong α -glucosidase inhibitor, effectively stabilizing the enzyme and reducing its activity.

Hydrogen bonding is one of the most critical interactions for stabilizing protein-ligand complexes. The number of hydrogen bond interactions between compound 7j and the protein residues over the simulation

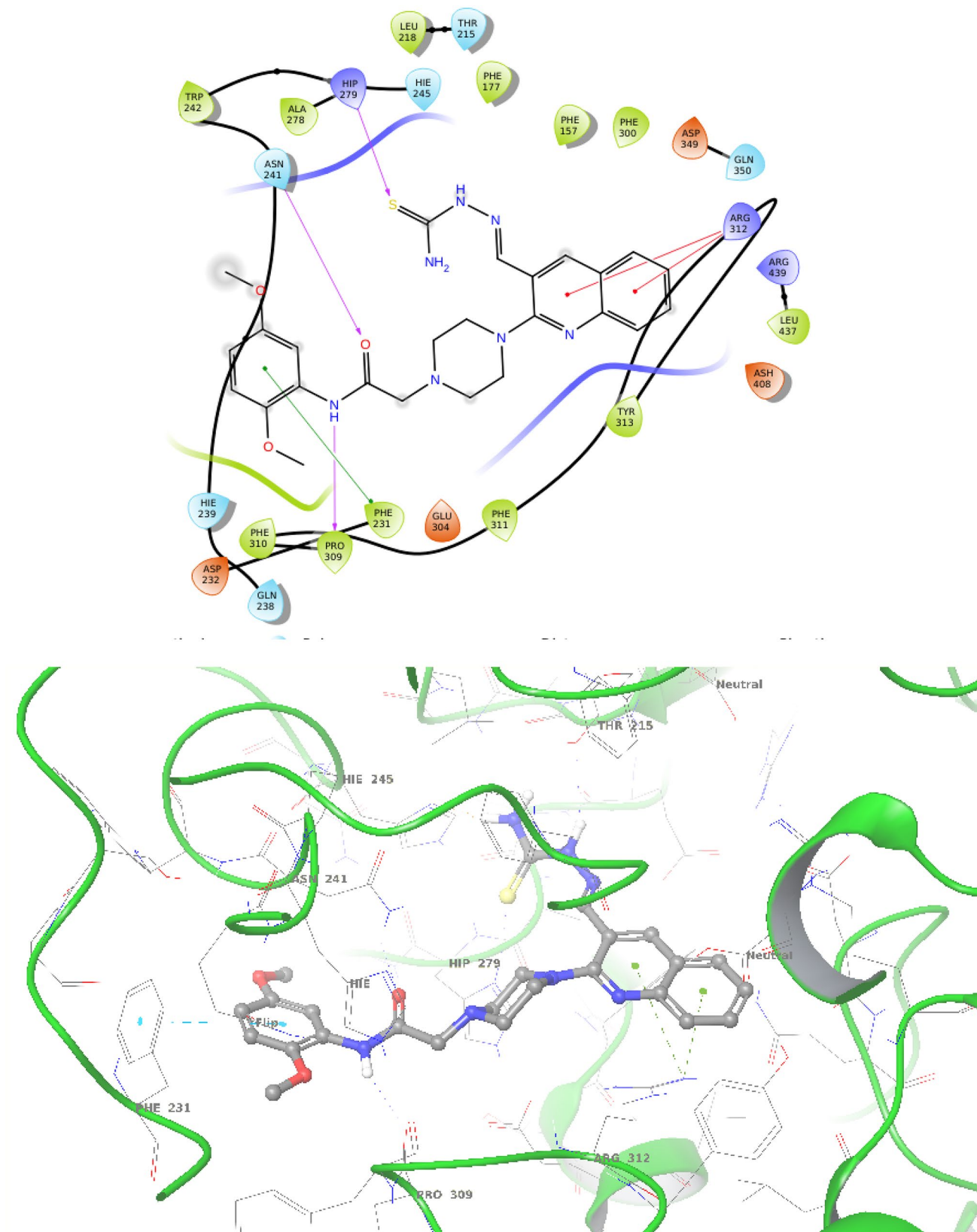


Fig. 3. A docking model of α -glucosidase-7j complex (two- and three-dimensional interactions).

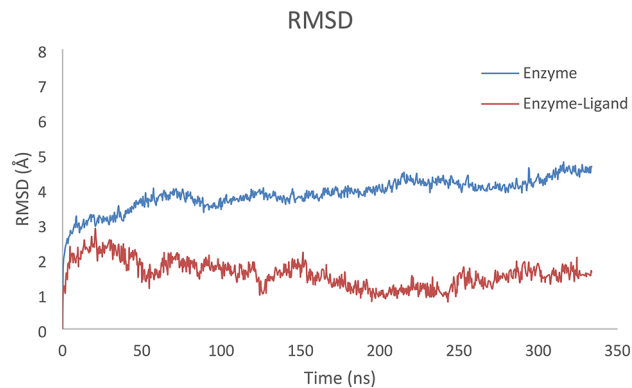


Fig. 4. RMSD plot of α -glucosidase backbone (blue) and compound **7j** (red), throughout the 300 ns of the simulation time.

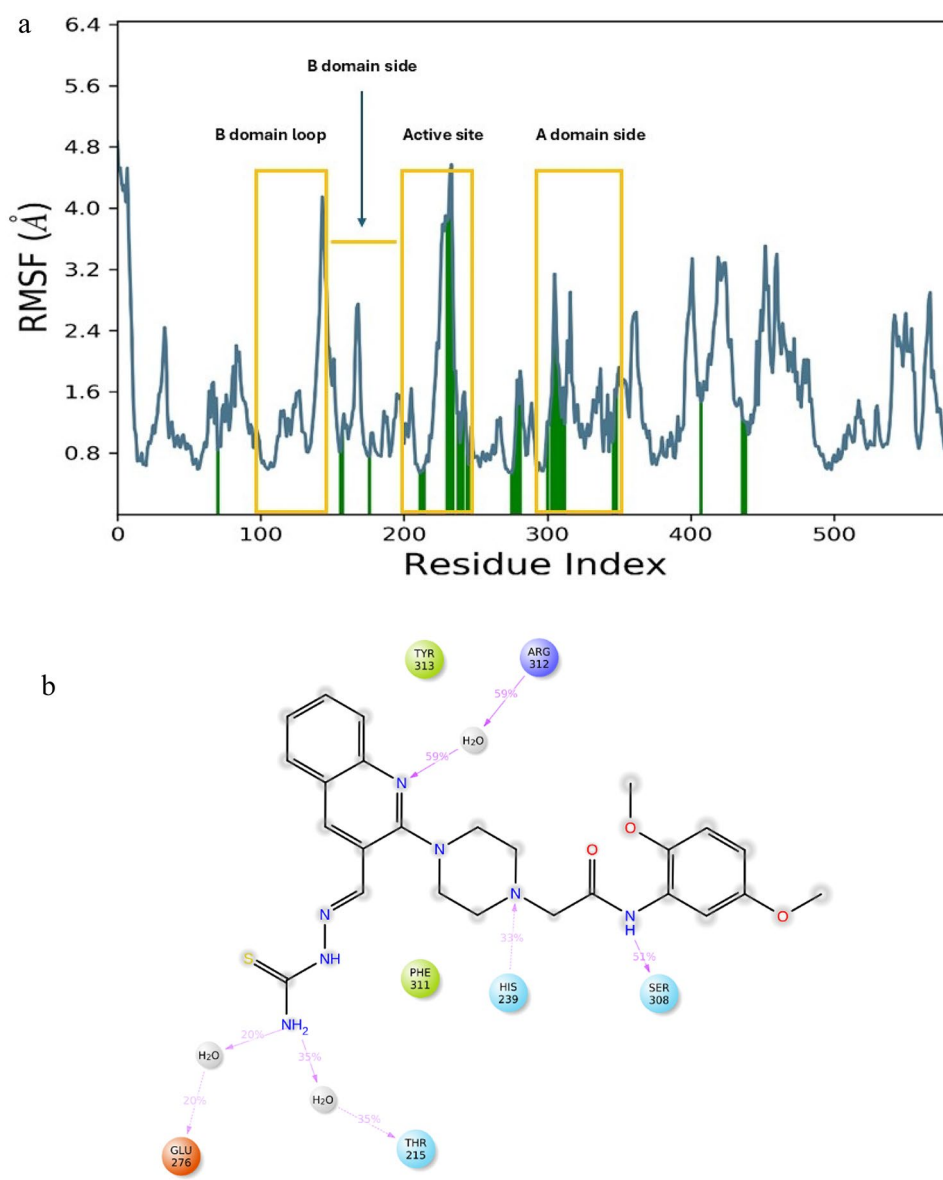


Fig. 5. (a) RMSF graph of the Ca atoms of α -glucosidase in complex with **7j** and enzyme, (b) Interactions that happened for over 25% of the simulation.

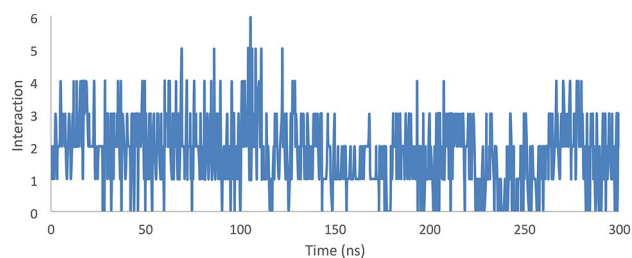


Fig. 6. Number of hydrogen bond interactions formed during MD simulation.

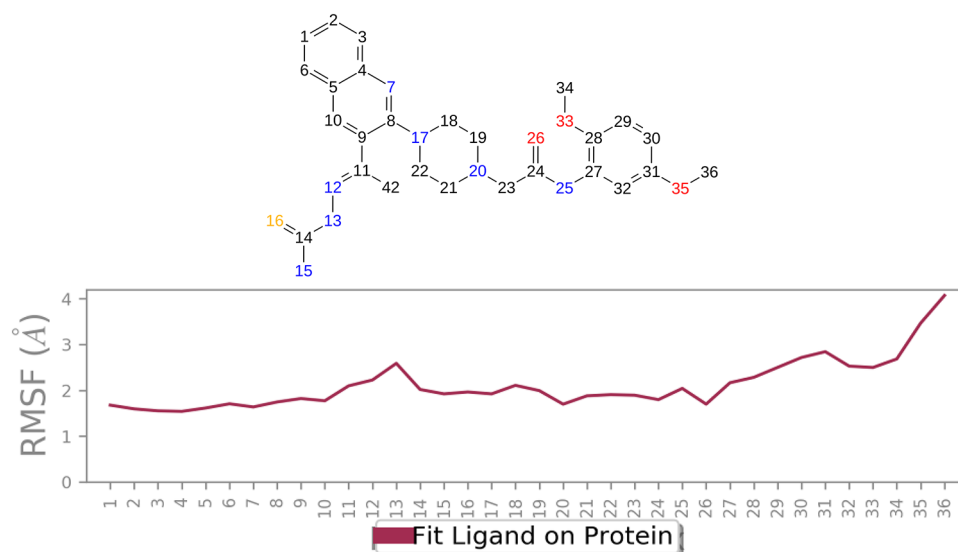


Fig. 7. RMSF of 7j within enzyme active site throughout the 300 ns of the simulation time.

time was also monitored. As shown in Fig. 6, in most frames, 7j successfully participated in hydrogen bond interactions, confirming its high potency.

Additionally, the stability of the enzyme-ligand complex was further confirmed through the evaluation of the RMSF of the ligand (compound 7j) alone within the enzyme's binding site, as shown in (Fig. 7). The RMSF values for all atoms of the ligand remained below 3 Å, indicating minimal fluctuation and confirming the stability of the ligand in the binding pocket. However, slight deviations were observed in atom 36, which exhibited slightly higher fluctuations compared to the rest of the ligand atoms. Despite these localized increases in RMSF, the overall low fluctuation of the majority of 7j confirms its stable binding, which demonstrated strong interactions between compound 7j and the critical residues of α -glucosidase.

Comparison between docking and MD simulations

The comparison between docking and MD simulations also highlights the dynamic and adaptive nature of protein-ligand interactions, emphasizing the importance of MD simulations for capturing realistic binding behaviors. As shown in Table 2, key residues such as Arg312 and His279 consistently participated in strong interactions during both docking and MD simulations, confirming their pivotal role in stabilizing the ligand within the active site. Residues like Thr215, His239, Glu276, and Ser308 formed hydrogen bond or water bridge interactions during MD simulations that were absent in docking results, highlighting the significance of accounting for dynamic water-mediated effects and conformational flexibility. In contrast, residues such as Phe231 and Pro309 exhibited interactions in docking but showed reduced persistence during MD simulations, indicating that these interactions might be weaker or context-dependent under dynamic conditions. Additionally, interactions involving residues like Asn241 and Glu276 demonstrated a combination of hydrophobic and water-mediated contributions, reinforcing their role in ligand stabilization. This analysis demonstrates the complementary strengths of docking and MD simulations in providing a comprehensive understanding of protein-ligand interactions, offering valuable insights for further optimization of the ligand's design for enhanced inhibitory activity and stability.

Conclusion

In this study, we synthesized and evaluated a series of substituted piperazine conjugated to quinoline-thiosemicarbazide for their α -glucosidase inhibitory activity. The SAR analysis revealed that substitutions at

Residue	Interaction type	Docking	MD simulation (persistence)
Thr215	Hydrogen bond and water bridge	No	Yes (moderate frequency)
Phe231	Pi-pi stacking	Yes	Rare
His239	Hydrogen bond and water bridge	No	Yes (moderate frequency)
Asn241	Hydrogen bond and hydrophobic	Yes	Yes (moderate frequency)
Glu276	Hydrogen bond and water bridge	No	Yes (moderate frequency)
His279	Hydrogen bond and hydrophobic	Yes	Yes (high frequency)
Ser308	Hydrogen bond and water bridge	No	Yes (moderate frequency)
Pro309	Hydrogen bond and hydrophobic	Yes	Rare
Arg312	Pi-cation and water bridge	Yes	Yes (high frequency)

Table 2. Comparison of protein-ligand interactions observed in docking and MD simulations.

specific positions on the aryl moieties significantly influenced inhibitory potency. Among the synthesized compounds, compound **7j** (2,5-dimethoxyphenyl derivative) exhibited the most potent inhibitory activity, with an IC_{50} value of 50.0 μ M, demonstrating a 15-fold improvement compared to the standard acarbose. Kinetic studies indicated that compound **7j** acts as a competitive inhibitor, as confirmed by the Lineweaver-Burk plot, with a K_i value of 32 μ M. Molecular docking and MD simulations provided further insight into the binding interactions between the compound and the α -glucosidase enzyme. Key interactions, including hydrogen bonding, pi-pi stacking, and hydrophobic interaction, contributed to the stabilization of the enzyme-**7j** complex. The low RMSD and RMSF values observed in the MD simulations confirmed the stability of the complex, supporting the potential of these compounds as strong inhibitors.

Overall, the findings suggest that thiosemicarbazide-linked quinoline-piperazine derivatives, particularly compound **7j**, are promising candidates for the development of novel α -glucosidase inhibitors. These results provide valuable insights for further optimization and development of potent antidiabetic agents with improved efficacy and reduced side effects.

Experimental Chemistry

Organic chemicals and solvents were sourced from Merck and used without additional purification. Analytical TLC was conducted with pre-coated aluminum sheets and silica gel to check reaction completion and compound purity. Melting points were measured using an Electrothermal 9100 apparatus. 1H NMR and ^{13}C NMR spectra were recorded on Bruker 600 MHz instruments with TMS as the internal standard and $CDCl_3$ as solvents.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-phenylacetamide

Yellow solid; Yield= (91%, 122.8 mg); mp: 135–137 °C. 1H NMR (600 MHz, $CDCl_3$) δ 11.27 (s, 1 H), 9.80 (s, 1 H), 8.97 (s, 1 H), 8.44–8.06 (m, 3 H), 7.86–7.62 (m, 5 H), 7.48–7.24 (m, 3 H), 7.07 (s, 1 H), 3.29–3.12 (m, 5 H), 2.98–2.67 (m, 5 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.4, 168.6, 159.7, 147.0, 139.2, 139.0, 135.8, 130.6, 129.1, 128.5, 127.6, 125.4, 125.2, 123.9, 121.5, 119.9, 62.2, 53.0, 50.9. HRMS (ESI) m/z : Calcd for $C_{23}H_{25}N_7OS$ [M + H]⁺ 448.1920, Found 448.1927. FT-IR δ 3385, 3253, 1641, 1471, 773, 732 cm^{-1} .

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(4-fluorophenyl)acetamide

Yellow solid; Yield= (84%, 117.2 mg); mp: 141–143 °C. 1H NMR (600 MHz, $CDCl_3$) δ 11.72 (s, 1 H), 9.88 (s, 1 H), 8.97 (s, 1 H), 8.42–8.09 (m, 3 H), 7.89–7.58 (m, 5 H), 7.51–7.41 (m, 1 H), 7.24–7.06 (m, 2 H), 3.26 (s, 6 H), 2.91–2.72 (m, 4 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.4, 168.6, 159.7, 159.3, 157.7, 147.0, 139.3, 135.4, 130.6, 128.5, 127.6, 125.4, 125.2, 121.9, 121.8, 121.5, 115.7, 115.6, 62.2, 53.0, 50.8. HRMS (ESI) m/z : Calcd for $C_{23}H_{24}FN_7OS$ [M + H]⁺ 466.1825, Found 466.1819. FT-IR δ 3419, 3275, 1631, 1475, 1155, 771. 720 cm^{-1} .

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(4-chlorophenyl)acetamide

Yellow solid; Yield= (81%, 116.9 mg); mp: 142–144 °C. 1H NMR (600 MHz, $CDCl_3$) δ 11.71 (s, 1 H), 10.04 (s, 1 H), 8.99 (s, 1 H), 8.56–8.02 (m, 3 H), 7.87–7.25 (m, 8 H), 3.15–2.60 (m, 9 H), 1.23 (s, 1 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.5, 158.8, 146.8, 145.8, 139.0, 138.3, 137.4, 135.9, 130.8, 129.2, 128.5, 127.6, 125.9, 125.5, 121.6, 121.3, 52.6, 29.4, 21.2. HRMS (ESI) m/z : Calcd for $C_{23}H_{24}ClN_7OS$ [M + H]⁺ 482.1530, Found 482.1521. FT-IR δ 3330, 3279, 1634, 1449, 830, 734, 720 cm^{-1} .

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(3-chloro-4-fluorophenyl)acetamide

Yellow solid; Yield= (90%, 134.8 mg); mp: 140–142 °C. 1H NMR (600 MHz, $CDCl_3$) δ 11.72 (s, 1 H), 10.06 (s, 1 H), 8.96 (s, 1 H), 8.49–7.19 (m, 10 H), 3.17 (s, 6 H), 2.82 (s, 4 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.5, 168.9, 159.7, 154.5, 152.8, 147.0, 139.3, 136.3, 135.8, 130.6, 128.5, 127.6, 125.4, 125.2, 121.5, 120.4, 120.3, 119.5, 119.4, 117.3, 117.1, 62.0, 53.0, 50.7. HRMS (ESI) m/z : Calcd for $C_{23}H_{23}ClFN_7OS$ [M + H]⁺ 500.1436, Found 500.1426. FT-IR δ 3420, 3270, 1643, 1468, 1159, 835, 771, 725 cm^{-1} .

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(4-(trifluoromethyl)phenyl)acetamide

Yellow solid; Yield= (90%, 139.1 mg); mp: 144–146 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.71 (s, 1 H), 10.24 (s, 1 H), 8.99 (s, 1 H), 8.35 (s, 1 H), 8.26 (s, 1 H), 8.15 (s, 1 H), 7.98–7.67 (m, 7 H), 7.53–7.37 (m, 1 H), 3.26 (s, 5 H), 2.89 (m, 3 H), 2.73 (s, 2 H), 1.31–1.17 (m, 2 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 162.7, 146.9, 142.5, 139.2, 135.8, 130.7, 128.5, 127.6, 126.5, 125.7, 125.4, 125.3, 123.9, 121.5, 119.8, 52.9, 36.2, 31.2. HRMS (ESI)*m/z*: Calcd for C₂₄H₂₄F₃N₇OS [M+H]⁺ 516.1793, Found 516.1799. FT-IR δ 3390, 3267, 1630, 1485, 1156, 793, 741 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(2-nitrophenyl)acetamide

Yellow solid; Yield= (85%, 125.5 mg); mp: 130–132 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.73 (s, 1 H), 11.61 (s, 1 H), 8.99 (s, 1 H), 8.65 (s, 1 H), 8.46–8.06 (m, 4 H), 7.96–7.56 (m, 4 H), 7.53–7.19 (m, 2 H), 3.17 (s, 5 H), 3.07–2.60 (m, 5 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 170.2, 162.7, 159.7, 147.0, 139.3, 137.6, 136.1, 158.3, 148.4, 130.6, 128.5, 127.6, 126.1, 125.4, 124.1, 122.2, 121.5, 62.1, 53.2, 51.0. HRMS (ESI)*m/z*: Calcd for C₂₃H₂₄N₈O₃S [M+H]⁺ 493.1770, Found 493.1778. FT-IR δ 3392, 3296, 1619, 1530, 1479, 1319, 780, 731 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(3-nitrophenyl)acetamide

Yellow solid; Yield= (80%, 118.1 mg); mp: 130–132 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.69 (s, 1 H), 10.43 (s, 1 H), 9.01 (s, 1 H), 8.71 (s, 1 H), 8.44–8.08 (m, 3 H), 8.07–7.89 (m, 2 H), 7.88–7.73 (m, 2 H), 7.66 (s, 2 H), 7.52–7.40 (m, 1 H), 3.26–2.66 (m, 9 H), 2.27 (s, 1 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 162.7, 158.3, 148.4, 146.9, 146.1, 139.9, 139.1, 138.0, 135.9, 130.8, 128.5, 127.6, 126.0, 125.5, 121.4, 118.7, 114.2, 52.8, 36.2, 31.2. HRMS (ESI)*m/z*: Calcd for C₂₃H₂₄N₈O₃S [M+H]⁺ 493.1770, Found 493.1777. FT-IR δ 3383, 3279, 1655, 1529, 1476, 1310, 761, 738 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(o-tolyl)acetamide

Yellow solid; Yield= (89%, 123.1 mg); mp: 135–137 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.74 (s, 1 H), 9.46 (s, 1 H), 8.98 (s, 1 H), 8.56–8.05 (m, 3 H), 7.99–6.89 (m, 8 H), 3.27 (s, 6 H), 2.86 (s, 4 H), 2.26 (s, 3 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.5, 168.1, 159.6, 147.0, 139.4, 136.5, 135.8, 130.7, 130.6, 129.5, 128.5, 127.6, 126.6, 125.4, 125.2, 124.9, 122.7, 121.5, 61.9, 53.2, 51.0, 17.9. HRMS (ESI)*m/z*: Calcd for C₂₄H₂₇N₇OS [M+H]⁺ 462.2076, Found 462.2083. FT-IR δ 3432, 3257, 1660, 1473, 783, 713 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(4-methoxyphenyl)acetamide

Brown solid; Yield= (93%, 133.1 mg); mp: 150–152 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.67 (s, 1 H), 9.94 (s, 1 H), 9.02 (s, 1 H), 8.46–8.07 (m, 3 H), 7.99–7.41 (m, 6 H), 9.92 (d, *J*=8.4 Hz, 2 H), 3.73 (s, 3 H), 3.43 (s, 8 H), 2.89 (s, 1 H), 2.73 (s, 1 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 162.7, 156.0, 146.8, 139.0, 135.9, 131.8, 130.7, 128.5, 127.6, 125.6, 125.5, 125.4, 121.5, 121.4, 114.3, 55.6, 52.7, 36.2, 31.2. HRMS (ESI)*m/z*: Calcd for C₂₄H₂₇N₇O₂S [M+H]⁺ 478.2025, Found 478.2033. FT-IR δ 3420, 3246, 1649, 14772, 1231, 1028, 785, 759 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(2,5-dimethoxyphenyl)acetamide

Brown solid; Yield= (84%, 127.8 mg); mp: 139–141 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.72 (s, 1 H), 9.80 (s, 1 H), 8.99 (s, 1 H), 8.57–8.06 (m, 3 H), 8.05–7.36 (m, 5 H), 6.98 (s, 1 H), 6.62 (s, 1 H), 3.98–3.62 (m, 6 H), 3.28–2.65 (m, 10 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 168.3, 159.6, 153.6, 146.9, 142.7, 140.7, 139.2, 135.8, 130.6, 128.5, 127.6, 125.4, 125.3, 121.5, 112.1, 107.8, 106.1, 61.9, 57.0, 55.8, 53.1, 51.3. HRMS (ESI)*m/z*: Calcd for C₂₅H₂₉N₇O₃S [M+H]⁺ 508.2131, Found 508.2139. FT-IR δ 3410, 3279, 1650, 1489, 1230, 1035, 789, 721 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(3,4-dimethoxyphenyl)acetamide

Brown solid; Yield= (88%, 133.9 mg); mp: 149–151 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.70 (s, 1 H), 9.70 (s, 1 H), 9.00 (s, 1 H), 8.52–8.00 (m, 3 H), 7.93–7.57 (m, 3 H), 7.50–6.84 (m, 4 H), 4.00–3.64 (m, 6 H), 2.88 (s, 8 H), 1.36–0.75 (m, 2 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 149.0, 146.9, 145.5, 139.1, 138.0, 135.8, 132.5, 130.7, 128.6, 128.5, 127.6, 125.9, 125.4, 121.4, 112.4, 111.9, 106.1, 56.1, 55.8, 52.9, 31.4, 22.5. HRMS (ESI)*m/z*: Calcd for C₂₅H₂₉N₇O₃S [M+H]⁺ 508.2131, Found 508.2122. FT-IR δ 3380, 3252, 1636, 1479, 1220, 1033, 791, 766 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(isoquinolin-5-yl)acetamide

Yellow solid; Yield= (87%, 130.0 mg); mp: 141–143 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.73 (s, 1 H), 10.20 (s, 1 H), 9.35 (s, 1 H), 8.99 (s, 1 H), 8.58 (s, 1 H), 8.44–7.30 (m, 11 H), 3.42 (s, 6 H), 3.20–2.76 (m, 4 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 159.6, 153.1, 147.0, 143.3, 139.2, 135.8, 132.8, 130.7, 130.1, 129.1, 128.5, 127.7, 127.6, 125.9, 125.4, 125.3, 125.1, 125.0, 121.5, 115.6, 53.1, 50.7, 21.2. HRMS (ESI)*m/z*: Calcd for C₂₆H₂₆N₈OS [M+H]⁺ 499.2029, Found 499.2022. FT-IR δ 3430, 3210, 1666, 1471, 785, 711 cm⁻¹.

N-benzyl-2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)acetamide

Yellow solid; Yield= (91%, 126.0 mg); mp: 138–140 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.72 (s, 1 H), 8.97 (s, 1 H), 8.70–7.98 (m, 4 H), 7.97–7.04 (m, 9 H), 4.34 (s, 2 H), 3.15 (s, 6 H), 2.76 (s, 4 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.5, 169.6, 159.7, 147.0, 140.1, 139.4, 135.7, 130.6, 128.7, 128.5, 127.6, 127.1, 126.0, 125.4, 125.1, 121.5, 61.7, 53.2, 50.8, 42.4. HRMS (ESI)*m/z*: Calcd for C₂₄H₂₇N₇OS [M+H]⁺ 462.2076, Found 462.2071. FT-IR δ 3393, 3270, 1652, 1491, 789 cm⁻¹.

2-(4-(3-((2-carbamothioylhydrazono)methyl)quinolin-2-yl)piperazin-1-yl)-N-(4-methoxybenzyl)acetamide

Brown solid; Yield= (80%, 117.9 mg); mp: 143–145 °C. ¹H NMR (600 MHz, CDCl₃) δ 11.68 (s, 1 H), 8.99 (s, 1 H), 8.57–8.02 (m, 4 H), 7.93–7.60 (m, 3 H), 7.53–7.38 (m, 1 H), 7.32–7.10 (m, 2 H), 7.00–6.77 (m, 2 H), 4.27 (s, 2 H), 3.73 (s, 3 H), 3.28–2.61 (m, 10 H). ¹³C NMR (151 MHz, CDCl₃) δ 178.4, 169.5, 159.7, 158.6, 147.0, 139.2,

135.2, 132.1, 130.6, 129.0, 128.5, 127.6, 125.4, 125.2, 121.5, 114.1, 61.7, 55.5, 53.2, 50.8, 41.8. **HRMS (ESI)** m/z : Calcd for $C_{25}H_{29}N_7O_2S$ $[M+H]^+$ 492.2182, Found 492.2173. **FT-IR** δ 3386, 3277, 1679, 1480, 1229, 1051, 790, 735 cm^{-1} .

In vitro α -glucosidase inhibition assays

In vitro inhibition assay of the new compounds, **7a–n** was performed according to previously reported work^{11,23}.

Enzyme kinetic studies

The mode of inhibition of the most active compound (**7j**), identified with the lowest IC_{50} , was investigated against α -glucosidase activity with different concentrations of *p*-nitrophenyl α -D-glucopyranoside (1–4 mM) as substrate in the absence and presence of **7j** at different concentrations (0, 12.5, 25, and 50, 100 μ M). A Lineweaver–Burk plot was generated to identify the type of inhibition and the Michaelis–Menten constant (K_m) value was determined from the plot between the reciprocal of the substrate concentration ($1/[S]$) and the reciprocal of enzyme rate ($1/V$) over various inhibitor concentrations. The experimental inhibitor constant (K_i) value was constructed by secondary plots of the inhibitor concentration $[I]$ versus K_m ^{24,25}.

Docking study

Molecular modeling of the selected compound was conducted on modeled α -glucosidase based on our previously reported works²⁶. The induced molecular docking was conducted using the Schrödinger Suites Maestro molecular modeling platform.

Molecular dynamics simulations

The starting model was obtained by imposing the induced fit docking to α -glucosidase. MD simulations were conducted using the Desmond v5.3 of Schrodinger's suit maestro according to previously reported procedures^{23,27,28}.

Data availability

All data generated or analyzed during this study are included in the published article and its supplementary information file.

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References

1. Bandy, M. Z., Sameer, A. S. & Nissar, S. Pathophysiology of diabetes: an overview. *Avicenna J. Med.* **10** (4), 174–188 (2020).
2. Antar, S. A. et al. Diabetes mellitus: Classification, mediators, and complications; a gate to identify potential targets for the development of new effective treatments. *Biomed. Pharmacother.* **168**, 115734 (2023).
3. Eizirik, D. L., Pasquali, L. & Cnop, M. Pancreatic β -cells in type 1 and type 2 diabetes mellitus: different pathways to failure. *Nat. Rev. Endocrinol.* **16** (7), 349–362 (2020).
4. Galicia-Garcia, U. et al. Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci.* **21** (17). (2020).
5. Sohrabi, M. et al. A review on α -glucosidase inhibitory activity of first row transition metal complexes: a futuristic strategy for treatment of type 2 diabetes. *RSC Adv.* **12** (19), 12011–12052 (2022).
6. Hossain, U. et al. An overview on the role of bioactive α -glucosidase inhibitors in ameliorating diabetic complications. *Food Chem. Toxicol.* **145**, 111738 (2020).
7. van de Laar, F. A. et al. α -Glucosidase inhibitors for patients with type 2 diabetes: results from a cochrane systematic review and meta-analysis. *Diabetes Care* **28** (1), 154–163 (2005).
8. Zolotareva, D. et al. Morpholine, piperazine, and piperidine derivatives as antidiabetic agents. *Molecules* **29** (13), 3043 (2024).
9. Safapoor, S. et al. Synthesis, ADMET prediction, and in vitro and in silico α -glucosidase inhibition evaluations of new quinoline–quinazolinone–thioacetamides. *RSC Adv.* **13** (28), 19243–19256 (2023).
10. Abbasi, I. et al. Isatin-hydrazide conjugates as potent α -amylase and α -glucosidase inhibitors: synthesis, structure and invitro evaluations. *Bioorg. Chem.* **116**, 105385 (2021).
11. Moghadam Farid, S. et al. Synthesis and structure–activity relationship studies of benzimidazole-thioquinoline derivatives as α -glucosidase inhibitors. *Sci. Rep.* **13** (1), 4392 (2023).
12. Noori, M. et al. Design, synthesis, and in silico studies of quinoline-based-benzo[d]imidazole bearing different acetamide derivatives as potent α -glucosidase inhibitors. *Sci. Rep.* **12** (1), 14019 (2022).
13. Ghomi, M. K. et al. Synthesis, in vitro potency of inhibition, enzyme kinetics and in silico studies of quinoline-based α -glucosidase inhibitors. *Sci. Rep.* **14** (1), 501 (2024).
14. Avula, S. K. et al. Synthesis of novel substituted quinoline derivatives as diabetics II inhibitors and along with their in-silico studies. *J. Mol. Struct.* **1274**, 134560 (2023).
15. Elebiju, O. F. et al. Recent advances in functionalized quinoline scaffolds and hybrids-exceptional pharmacophore in therapeutic medicine. *Front. Chem.* **10**, 1074331 (2022).
16. Lee, H. W., Yang, J. Y. & Lee, H. S. Quinoline-2-carboxylic acid isolated from ephedra pachyclada and its structural derivatives show inhibitory effects against α -glucosidase and α -amylase. *J. Korean Soc. Appl. Biol. Chem.* **57** (4), 441–444 (2014).
17. Taha, M. et al. Synthesis of quinoline derivatives as diabetic II inhibitors and molecular docking studies. *Bioorg. Med. Chem.* **27** (18), 4081–4088 (2019).
18. Rahim, F. et al. Isatin based thiosemicarbazide derivatives as potential inhibitor of α -glucosidase, synthesis and their molecular docking study. *J. Mol. Struct.* **1222**, 128922 (2020).
19. Basri, R. et al. Synthesis, biological evaluation and molecular modelling of 3-Formyl-6-isopropylchromone derived thiosemicarbazones as α -glucosidase inhibitors. *Bioorg. Chem.* **139**, 106739 (2023).
20. Saeedian Moghadam, E. et al. Synthesis, bioactivity, and molecular docking of benzimidazole-2-carbamate derivatives as potent α -glucosidase inhibitors. *J. Mol. Struct.* **1278**, 134931 (2023).
21. Özil, M. et al. Synthesis of benzimidazoles containing piperazine ring as potential therapeutic agents against diabetes mellitus and antioxidant activities. *J. Mol. Struct.* **1304**, 137714 (2024).
22. Ghasemi, M. et al. Quinoline-piperazine derivatives as potential α -glucosidase inhibitors: synthesis, biological evaluation, and in silico studies. *J. Mol. Struct.* **1323**, 140561 (2025).

23. Iraj, A. et al. Cyanoacetohydrazide linked to 1,2,3-triazole derivatives: a new class of α -glucosidase inhibitors. *Sci. Rep.* **12** (1), 8647 (2022).
24. Hamedifar, H. et al. Aryl-quinoline-4-carbonyl hydrazone bearing different 2-methoxyphenoxyacetamides as potent α -glucosidase inhibitors; molecular dynamics, kinetic and structure–activity relationship studies. *Sci. Rep.* **14** (1), 388 (2024).
25. Nasli Esfahani, A. et al. Design and synthesis of phenoxymethylbenzimidazole incorporating different aryl thiazole-triazole acetamide derivatives as α -glycosidase inhibitors. *Mol. Divers.* **26** (4), 1995–2009 (2022).
26. Pedrood, K. et al. Design, synthesis, and molecular docking studies of diphenylquinoxaline-6-carbohydrazide hybrids as potent α -glucosidase inhibitors. *BMC Chem.* **16** (1), 57 (2022).
27. Azmi, A. et al. Alpha-glucosidase inhibitory and hypoglycemic effects of imidazole-bearing thioquinoline derivatives with different substituents: in silico, in vitro, and in vivo evaluations. *Bioorg. Chem.* **144**, 107106 (2024).
28. Asadi, M. et al. Synthesis, α -glucosidase inhibitory activity, and molecular dynamic simulation of 6-chloro-2-methoxyacridine linked to triazole derivatives. *Sci. Rep.* **14** (1), 17338 (2024).

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Author contributions

Author contribution M. G, M. M, M. D, and M. E synthesized compounds, S. M, and M. A. F performed biological assessments, A.I supervise the biological procedures, A. Al-H supervises the synthetic procedures.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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