

Design, Synthesis, and Computational Analysis of 4-Amino-1,2,4-Triazol-3-one Schiff Base Derivatives as Potent α -Glucosidase Inhibitors

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Abstract

Type 2 diabetes mellitus (T2DM) remains a major global health challenge, driving the search for novel α -glucosidase inhibitors with improved efficacy and safety profiles over existing therapies. Here, we report the design, synthesis, and biological evaluation of a new series of Schiff bases derived from 4-amino-1,2,4-triazol-3(4H)-one (compounds 1–10) as potential antidiabetic agents. The compounds were synthesized and characterized by ^1H -NMR and EI-MS, and their α -glucosidase inhibitory activities were assessed *in vitro* using acarbose as the reference standard. The series exhibited a broad range of potencies, with IC_{50} values ranging from 10.2 ± 0.19 to 39.4 ± 0.15 $\mu\text{g/mL}$. Notably, compounds 8 (10.2 ± 0.19 $\mu\text{g/mL}$) and 10 (11.0 ± 0.68 $\mu\text{g/mL}$) exhibited significantly stronger inhibition than acarbose (23.0 ± 0.13 $\mu\text{g/mL}$), while compounds 4 (17.5 ± 0.56 $\mu\text{g/mL}$) and 5 (18.0 ± 0.41 $\mu\text{g/mL}$) demonstrated comparable or slightly higher activity.

To elucidate structure-activity relationships and potential multitarget antidiabetic profiles, density functional theory (DFT)-optimized geometries were subjected to molecular docking against three validated targets: α -glucosidase (AglA, PDB ID: 10BB), dipeptidyl peptidase-4 (DPP-4, PDB ID: 1NU6), and sodium-glucose cotransporter-2 (SGLT2, PDB ID: 8HDH). Several derivatives, particularly compounds 1 and 7, exhibited binding affinities comparable to or exceeding those of reference ligands acarbose, sitagliptin, and empagliflozin. These ligands formed crucial hydrogen bonds with catalytic residues, including GLU205, ARG125, and TYR631, and engaged in stabilizing π - π stacking interactions, thereby supporting the experimentally observed inhibitory trends.

In silico ADMET and drug-likeness analyses using the ADMETSAR 3.0 indicated that the majority of the derivatives comply with Lipinski's and Veber's criteria, show favorable lipophilicity ($\log P$ 0–3), moderate aqueous solubility ($\log S > -4$), and low predicted toxicity. Within the series, one candidate displayed the most balanced pharmacokinetic and safety profile, combining high intestinal absorption, acceptable metabolic stability, and low predicted hepatotoxicity.

These integrated experimental and computational findings identify 4-amino-1,2,4-triazol-3-one Schiff bases, particularly compounds 8 and 10, as promising lead scaffolds for the development of next-generation oral antidiabetic agents that target α -glucosidase and potentially other clinically relevant enzymes.

Introduction

Diabetes mellitus (DM) continues to be a significant global health challenge, defined by persistent hyperglycemia due to impairments in insulin secretion, insulin action, or both [1]. According to recent World Health Organization (WHO) estimates, the prevalence of diabetes continues to rise sharply, with type 2 diabetes mellitus (T2DM) accounting for more than 90% of diagnosed cases worldwide [2]. The disease is often associated with long-term metabolic complications, including neuropathy, nephropathy, cardiovascular diseases, and retinopathy, all of which contribute significantly to morbidity and mortality [3]. One of the major therapeutic strategies in the management of T2DM involves controlling postprandial hyperglycemia by inhibiting carbohydrate-hydrolyzing enzymes such as α -glucosidase and α -amylase, which break down complex carbohydrates into absorbable monosaccharides [4]. Inhibition of these enzymes delays glucose absorption, thereby attenuating the sharp rise in blood glucose levels after meals [4].

Despite the availability of clinically approved α -glucosidase inhibitors such as acarbose, miglitol, and voglibose, their therapeutic application is often limited by adverse gastrointestinal effects and poor selectivity [4]. These limitations underscore the need to design and develop new, potent, and safer α -glucosidase inhibitors with improved pharmacological profiles. In this regard, heterocyclic compounds bearing nitrogen-rich scaffolds have gained remarkable attention due to their diverse biological activities and favorable drug-like properties [5]. Among these, 1,2,4-triazoles and their derivatives occupy a privileged position in medicinal chemistry owing to their broad pharmacological spectrum, including antidiabetic activities [6]. Incorporating Schiff base functionality into triazole frameworks further amplifies biological potential, as Schiff bases are known to exhibit wide-ranging bioactivities attributed to their azomethine ($-\text{CH}=\text{N}-$) group, which facilitates electron delocalization and interactions with active sites of enzymes and receptors [7].

In recent years, the combination of synthetic chemistry and computational drug design has become indispensable for accelerating the discovery of novel enzyme inhibitors [8]. Molecular docking studies provide insight into the possible binding conformations and interactions of synthesized compounds within the catalytic site of target enzymes, offering predictive validation of their inhibitory potential [9]. Thus, integrating experimental and computational approaches can therefore enhance the rational design of molecules with improved affinity and selectivity for biological targets [10].

This study discusses the synthesis and computational assessment of novel 4-amino-1,2,4-triazol-3-one Schiff base derivatives as possible α -glucosidase inhibitors. This study's findings offer significant insights into the structure-activity relationships (SAR) governing the α -glucosidase inhibitory potential of triazole-based Schiff base derivatives. Furthermore, the mechanism of action of the studied compounds was investigated through molecular docking simulations. Finally, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds were evaluated to predict their pharmacokinetic features and safety characteristics. This may aid the development of novel antidiabetic agents with improved efficacy and reduced side effects.

Materials and Methods

Synthesis of 4-amino-2H-1,2,4-triazol-3(4H)-one (1)

Carbonohydrazide weighing 0.09 g (1 mmole) was dissolved in 40 mL of water, and the mixture was stirred vigorously for about 30 minutes at 60°C, 0.147 mL of triethyl orthoformate (0.148 g) was added to the mixture, and the reaction was refluxed for an additional three hours. To monitor the reaction's progress, Thin-Layer Chromatography (TLC) was used. After one hour of refluxing, the solution turns pinkish, and the color deepens as the reaction proceeds to completion. Thereafter, the crude product was immediately filtered, yielding a deep pink-coloured filtrate and a white powdery solid residue. The filtrate was left standing, which settled to form more of the white residue at the base of the round-bottom flask. The residues were harvested, washed several times in distilled water, dried, and re-crystallized in ethanol, which afforded 4-amino-2H-1,2,4-triazol-3(4H)-one (a) as shown in Scheme-1.

General procedure for the synthesis of 4-amino-2H-1,2,4-triazol-3(4H)-one Schiff base derivatives (1–10)

One mmole of 4-amino-2H-1,2,4-triazol-3(4H)-one (a) was accurately weighed and solubilised in 20 mL methanol using a 100 mL flask, 1 mmole each of the appropriate aldehyde was added to the resulting solution, and a catalytic amount of acetic acid was added. The mixture was stirred for about 4 hours, as shown in Scheme 2. The mixture was poured into crushed ice for precipitation, the precipitate was filtered, dried, weighed, and kept in sample bottles prior to analysis.

α -Glucosidase inhibition assay

The α -glucosidase enzyme was inhibited using varying concentrations of α -glucosidase inhibitors (0.0625, 0.125, 0.3, and 0.4 mM) and varying inhibitor-substrate concentrations (p-nitrophenyl R-D-maltoside (NPM)) at 0.1, 0.2, 0.4, 0.8, and 1.0 mM. Deionised water was used to dissolve the enzyme, yielding a final concentration of 0.2 mg/mL in each well. The pH of the PIPES buffer was 7.0. At 25°C, the reaction mixture was incubated for 30 minutes, with samples collected at 1-minute intervals. The 96-well plate (BioTek XL-800, USA) was used to measure absorbance with an ELISA reader, following the reported method [11].

In Silico Studies

Molecular Docking of the Synthesized Ligands with the Potential Targets.

To evaluate the antidiabetic potential of synthesised compounds, molecular docking was performed against three validated targets: DPP-4 (PDB ID: 1NU6, Resolution: 2.10 Å), α -glucosidase A (AglA, PDB ID: 10BB, Resolution: 1.90 Å), and the SGLT2-MAP17 complex (PDB ID: 8HDH, Resolution: 3.10 Å). We retrieved high-resolution X-ray crystal structures of the target proteins from the Protein Data Bank (<http://www.rcsb.org/pdb/>). These proteins were selected for their roles in enhancing insulin secretion, delaying carbohydrate absorption, and promoting renal glucose excretion, respectively. FDA-approved inhibitors-Sitagliptin, Acarbose, and Empagliflozin-served as reference ligands.

With the aid of AutoDock Vina via PyRx and focusing on the active sites of each receptor, docking simulations were conducted. Resulting complexes were visualised and analysed using ChimeraX v1.9 and Biovia Discovery Studio.

Quantum Chemical Calculation

Before docking, the compound geometries were optimized using density functional theory (DFT) at the B3LYP/6-31G(d,p) level of theory in Gaussian 16 on the Lengau cluster at the Center for High-Performance Computing (CHPC) in Cape Town, South Africa. Additionally, frequency calculations were performed to confirm that the optimized structures corresponded to a true energy minimum and had no imaginary frequencies. The three-dimensional optimized structures of the molecules are shown in Fig. 1.

Pharmacokinetic and Drug-Likeness Evaluation

To evaluate the pharmacokinetic and drug-likeness profiles of the top five compounds identified from molecular docking against diabetes-related targets, we performed an *in silico* ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis. This analysis used the online model predictions available through the ADMETSAR 3.0 server (<https://lmmd.ecust.edu.cn/admet3/>). The key parameters evaluated included lipophilicity (log Po/w), water solubility (log S), bioavailability score, and comprehensive pharmacokinetic and toxicity profiles. This analysis offers valuable insights into the compounds' potential efficacy, safety, and overall behavior in biological systems, which are crucial for early-stage drug discovery and risk assessment in the development of diabetes therapies.

Results and discussion

The reactions proceeded smoothly for each aromatic aldehyde, affording the desired products in uniformly high yields of 78–88%. This good yield range indicates a favourable condensation reaction.

The melting point data show pronounced substituent-dependent trends. Compounds with strong electron-withdrawing groups and multiple hydrogen-bonding sites, such as the nitro- and chloro-nitro derivatives (1 and 3), exhibited the highest melting points (282–

283°C and 251–252°C, respectively). This behavior is consistent with enhanced intermolecular interactions, increased molecular polarity, and improved crystal lattice packing. Similarly, compound 8, containing multiple oxygenated substituents capable of extensive hydrogen bonding, displayed the highest melting point in the series (286°C).

In the ^1H NMR spectrum, diagnostic signals confirm Schiff base formation. The azomethine proton ($\text{N}=\text{CH}$) appeared as a sharp singlet in the δ_{H} 9.16–10.15 ppm range, while the exocyclic NH proton resonated downfield between δ_{H} 11.92–12.24 ppm. These narrow chemical shift ranges indicate that substitution on the aromatic ring. However, subtle trends are evident: derivatives bearing strong electron-withdrawing groups, particularly nitro-substituted compounds (1 and 3), showed slightly more deshielded azomethine and NH signals, consistent with reduced electron density and enhanced hydrogen bonding. In contrast, electron-donating substituents such as dimethylamino (9) and methyl groups (6) resulted in marginal upfield shifts of these protons, reflecting increased electron density across the imine linkage. Phenolic OH protons in compounds 4 and 10 appeared as highly deshielded singlets, in some cases above δ_{H} 12 ppm, suggesting strong intermolecular or intramolecular hydrogen bonding.

The aromatic regions of the ^1H NMR spectra further corroborate the substitution patterns. Para-substituted derivatives (4 and 9) displayed AA'BB' systems, whereas ortho-substituted and polysubstituted compounds 2, 3, 6, and 10 exhibited more complex splitting patterns due to non-equivalent proton environments and increased coupling interactions. Alkyl and alkoxy substituents were unambiguously identified by characteristic singlets or multiplets in the aliphatic region, such as methyl resonances around δ_{H} 2.3–3.0 ppm and ethoxy signals in compound 10.

The EI-MS data across the series provide complementary structural confirmation and reveal consistent fragmentation behavior. Molecular ion peaks (M^+ or M^++H) were observed for all compounds, though their relative intensities varied obviously with substitution. Electron-donating substituents, as in compounds 4, 7, and 9, enhanced molecular ion stability, resulting in comparatively intense M^+ peaks. In contrast, nitro-substituted derivatives, compounds 1 and 3, showed weaker molecular ions, reflecting facile fragmentation under electron impact conditions. Halogenated compounds 2 and 3 exhibited the expected chlorine isotope patterns, providing unequivocal evidence for chloro substitution. Remarkably, a dominant fragment at m/z 84–85 was consistently observed across most derivatives, corresponding to cleavage within the triazolone core, emphasizing its intrinsic stability and serving as a diagnostic fragment for this scaffold. A benzyloxy-containing compound, 7, showed a prominent tropylium ion at m/z 91, while alkyl-substituted derivatives generated stabilized benzylic cations, highlighting the influence of substituents on fragmentation pathways.

Spectral data of synthesized 4-amino-2H-1,2,4-triazol-3(4H)-one and its Schiff base Derivatives (1–10)

4-(3-nitrobenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (1)

White amorphous powder, Yield: 0.8 g (87%), M.p: 282–283 °C; ^1H -NMR: (400 MHz, $\text{DMSO}-d_6$) δ 12.13 (s, 1H, NH), 9.59 (s, 1H, $\text{N}=\text{CH}_{\text{imine}}$), 8.44 (s, 1H, NH-ring), 8.61 (t_{like}, $J=12.0$ Hz, 1H, H-2'), 8.34 (dd, $J=2.5, 8.5$ Hz, 1H, H-6'), 8.23(d, $J=7.5$ Hz, 1H, H-4'), 7.80 (d, $J=7.5$ Hz, 1H, H-5').

EI-MS: m/z (rel. abund. %) 234 (M^+ , 2.3), 233 (9.0), 149 (21.0), 103 (18.1), 89 (10.6), 85 (100), 76 (19.3), 63 (12.8), 41 (21.0).

4-(2-chlorobenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (2)

White amorphous powder, Yield: 0.7 g (85%), M.p: 267–268 °C; ^1H -NMR: (400 MHz, $\text{DMSO}-d_6$) δ 12.15 (s, 1H, NH), 9.96 (s, 1H, $\text{N}=\text{CH}_{\text{imine}}$), 8.39 (s, 1H, NH-ring), 8.01 (dd, $J=1.5, 8.0$ Hz, 1H, H-6'), 7.60 (dd, $J=1.0, 10.0$ Hz, 1H, H-3'), 7.48 (dd, $J=7.5, 8.0$ Hz, 1H, H-5'), 7.54 (dd, $J=7.5, 8.0$ Hz, 1H, H-4').

EI-MS: m/z (rel. abund. %) 224 (M^++2 , 3.7), 222 (M^+ , 10.8), 187 (15.7), 140 (31.5), 139 (26.0), 138 (87.5), 137 (45.2), 111 (24.8), 102 (35.2), 89 (19.1), 85 (100), 75 (18.6).

4-(2-chloro-5-nitrobenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (3)

White amorphous powder, Yield: 0.6 g (87%), M.p: 251–252 °C; ^1H -NMR: (400 MHz, $\text{DMSO}-d_6$) δ 12.24 (s, 1H, NH), 10.00 (s, 1H, $\text{N}=\text{CH}_{\text{imine}}$), 8.72 (d, $J=2.5$ Hz, 1H, H-6'), 8.52 (s, 1H, NH-ring), 8.34 (dd, $J=3.0, 9.0$ Hz, 1H, H-4'), 7.92 (d, $J=3.0$ Hz, 1H, H-3').

EI-MS: m/z (rel. abund. %) 268 (M^++H , 5.4), 267 (M^+ , 22.4), 232 (16.4), 185 (18.2), 183 (63.0), 139 (15.9), 137 (43.4), 136 (10.7), 123 (16.9), 110 (20.7), 101 (10.5), 86 (14.4), 84 (100), 73 (10.2), 41 (49.8).

4-(4-hydroxybenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (4)

White amorphous powder, Yield: 0.7 g (85%), M.p: 189 °C; ^1H -NMR: (400 MHz, $\text{DMSO}-d_6$) δ 11.99 (s, 1H, NH), 10.15 (s, 1H, OH-4'), 9.25 (s, 1H, $\text{N}=\text{CH}_{\text{imine}}$), 8.31 (s, 1H, NH-ring), 7.64 (d, $J=8.5$ Hz, 2H, H-2', H-6'), 6.87 (d, $J=8.5$ Hz, 2H, H-3', H-5').

EI-MS: m/z (rel. abund. %) 205 ($M^+ + H$, 6.2), 204 (M^+ , 51.9), 120 (64.6), 119 (100), 93 (20.4), 86 (22.2), 85 (18.8), 65 (28.2).

4-(benzylideneamino)-2H-1,2,4-triazol-3(4H)-one (5)

Off white amorphous powder, Yield: 0.8 g (88%), M.p: 181–183 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 12.06 (s, 1H, NH), 9.43 (s, 1H, N = CH_{imine}), 8.39 (s, 1H, NH-ring), 7.81 (dd, J = 1.5, 8.5 Hz, 2H, H-2', H-6'), 7.52 (m, 3H, H-3', H-4', H-5').

EI-MS: m/z (rel. abund. %) 189 ($M^+ + H$, 2.9), 188 (M^+ , 19.7), 104 (100), 103 (33.7), 89 (11.1), 85 (94.1), 77 (51.1), 50 (12.5), 41 (17.9).

4-(2,4-dimethylbenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (6)

White amorphous powder, Yield: 0.6 g (87%), M.p: 201–202 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 12.04 (s, 1H, NH), 9.61 (s, 1H, N = CH_{imine}), 8.39 (s, 1H, NH-ring), 7.75 (d, J = 8.6 Hz, 1H, H-3'), 7.13 (d, J = 5.5 Hz, 2H, H-5', H-6'), 2.42 (s, 3H, CH₃-2'), 2.31 (s, 3H, CH₃-4').

EI-MS: m/z (rel. abund. %) 216 (M^+ , 8.6), 132 (27.3), 131 (100), 130 (23.1), 117 (15.0), 116 (41.1), 84 (10.7), 77 (11.7).

4-(4-(benzyloxy)-2-methylbenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (7)

Off white amorphous solid, Yield: 0.7 g (85%), M.p: 268–269 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 12.01 (s, 1H, NH), 9.33 (s, 1H, N = CH_{imine}), 8.33 (s, 1H, NH-ring), 7.76 (d, J = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, J = 7.0 Hz, 2H, H-2'', H-6''), 7.41 (t, J = 7.5 Hz, 2H, H-3'', H-5''), 7.34 (d, J = 5.0 Hz, 1H, H-4''), 7.15 (d, J = 8.5 Hz, 2H, H-3', H-5'), 5.18 (s, 1H, O-CH₂).

EI-MS: m/z (rel. abund. %) 295 ($M^+ + H$, 15.4), 294 (M^+ , 80.5), 92 (57.1), 91 (100), 90 (10.5), 65 (27.3).

4-(3,5-dimethoxyl-4-hydroxybenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (8)

White flaky powder, Yield: 0.6 g (82%), M.p: 286 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 12.01 (s, 1H, NH), 9.33 (s, 1H, N = CH_{imine}), 8.33 (s, 1H, NH-ring), 7.76 (d, J = 9.0 Hz, 2H, H-2', H-6'), 7.47 (d, J = 7.0 Hz, 2H, H-2'', H-6''), 7.41 (t, J = 7.5 Hz, 2H, H-3'', H-5''), 7.34 (d, J = 5.0 Hz, 1H, H-4''), 7.15 (d, J = 8.5 Hz, 2H, H-3', H-5'), 5.18 (s, 1H, O-CH₂).

EI-MS: m/z (rel. abund. %) 295 ($M^+ + H$, 15.4), 294 (M^+ , 80.5), 92 (57.1), 91 (100), 90 (10.5), 65 (27.3).

HR-EIMS: calcd for C₁₁H₁₂N₄O₄: m/z = 264.0866, theoretical m/z = 264.0859.

4-(4-dimethylbenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (9)

White amorphous powder, Yield: 0.7 g (83%), M.p: 220–221 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 11.92 (s, 1H, NH), 9.16 (s, 1H, N = CH_{imine}), 8.28 (s, 1H, NH-ring), 7.61 (d, J = 11.0 Hz, 2H, H-2', H-6'), 6.78 (d, J = 11.5 Hz, 2H, H-3', H-5'), 3.01 (s, 6H, N-CH₃-4').

EI-MS: m/z (rel. abund. %) 233 ($M^+ + 2$, 1.3), 232 ($M^+ + H$, 11.2), 231 (M^+ , 100), 147 (18.6), 146 (81.6), 145 (81.4), 119 (9.2), 118 (8.5), 105 (7.3), 91 (7.0), 77 (8.2), 41 (7.9).

4-(2-hydroxyl-3-ethoxybenzylideneamino)-2H-1,2,4-triazol-3(4H)-one (10)

White amorphous powder, Yield: 0.6 g 78%), M.p: 251–252 °C; ^1H -NMR: (400 MHz, DMSO- d_6) δ 14.11 (s, 1H, NH), 12.50 (s, 1H, OH-2'), 9.39 (s, 1H, N = CH_{imine}), 8.47 (s, 1H, NH-ring), 7.36 (d, J = 7.50 Hz, 1H, H-6'), 7.16 (d, J = 7.5 Hz, 1H, H-4'), 6.89 (d, J = 8.0 Hz, 1H, H-5'), 4.09 (q, J = 6.9 Hz, 2H, OCH₂-3'), 1.35 (s, 3H, OCH₂-CH₃-3').

EI-MS: m/z (rel. abund. %) 248 (M^+ , 1.5), 233 (10.4), 232 (66.1), 231 (23.9), 217 (51.3), 216 (16.8), 215 (100), 203 (13.7), 188 (15.5), 187 (45.2), 175 (32.4), 161 (11.1), 149 (15.9), 148 (10.1), 147 (12.2).

In vitro α -glucosidase inhibitory activities of the synthesized

The α -glucosidase inhibitory activities of the synthesized Schiff bases of 1,2,4-triazol-3(4H)-one derivatives (1–10), as shown in Table 1, were evaluated and compared with those of acarbose, a standard α -glucosidase inhibitor used clinically for the management of postprandial hyperglycemia. The results, expressed as IC₅₀ $\pm$ SEM, revealed clear variations in inhibitory potential across the series, reflecting the influence of different substituents on the triazole Schiff base scaffold. Compounds 8 and 10 exhibited the most potent inhibitory effects, with IC₅₀ values of 10.2 $\pm$ 0.19 μ g/mL and 11.0 $\pm$ 0.68 μ g/mL, respectively, surpassing the standard drug acarbose (23.0 $\pm$ 0.13 μ g/mL) by more than twofold. Their remarkable activity suggests that the electronic or steric nature of the substituents on these derivatives enhances enzyme binding affinity, possibly through additional hydrogen bonding or π - π stacking interactions with active-site residues. Compounds 4 and 5 also demonstrated strong inhibitory effects (17.5 $\pm$ 0.56 and 18.0 $\pm$ 0.41 μ g/mL, respectively), showing comparable or slightly higher potency than acarbose, indicating that moderate electron-withdrawing or donating groups at specific positions on the aromatic moiety may favorably modulate activity.

In contrast, derivatives 1, 2, 3, 6, 7, and 9 displayed moderate to low inhibitory effects, with IC₅₀ values ranging from 22.1 ± 0.39 to 39.4 ± 0.15 µg/mL. Compound 3, in particular, showed the least activity (39.4 ± 0.15 µg/mL), implying that its substituent configuration may sterically hinder proper orientation within the enzyme active site or reduce electronic compatibility with catalytic residues. Compounds 6, 7, and 9, although less active than the most potent analogs, still exhibited comparable inhibitory strength to acarbose, highlighting the overall promise of this triazole-based Schiff base framework as an α-glucosidase inhibitory pharmacophore. The observed trend in activity suggests that substitution patterns significantly influence enzyme–ligand interactions, with an optimal balance of hydrophobicity and hydrogen bonding enhancing inhibitory potency.

Molecular Docking Analysis

The binding affinities and key molecular interactions of the synthesized compounds were evaluated against three validated antidiabetic targets, which are human dipeptidyl peptidase IV (DPP-4; PDB ID: 1NU6), α-glucosidase (AglA; PDB ID: 10BB), and human sodium/glucose cotransporter 2 (SGLT2; PDB ID: 8HDH). These proteins were selected for their established roles in regulating blood glucose levels. The docking results are summarized in Table 2 and Figs. 1–4. Molecular docking remains a widely adopted technique in the pharmaceutical industry for screening novel candidates prior to pre-clinical and clinical trials (Wu et al., 2020). The calculated binding free energies (ΔG°) ranged from – 8.3 to -6.2 kcal/mol for DPP-4, -8.8 to -6.3 kcal/mol for AgIA, and – 10.2 to -3.1 kcal/mol for SGLT2. Among the reference drugs, Sitagliptin showed the highest affinity for DPP-4 (-8.3 kcal/mol), Acarbose for AgIA (-8.8 kcal/mol), and Empagliflozin for SGLT2 (-10.2 kcal/mol).

Of the compounds studied, compound 5 has the weakest binding across all targets, while 1 and 7 demonstrated the most favorable affinities. Specifically, compound 1 exhibited ΔG° values of -7.6 (DPP-4) and – 8.6 kcal/mol (SGLT2), while compound 7 showed – 7.6, -8.2, and – 8.5 kcal/mol values for DPP-4, AgIA, and SGLT2, respectively. These findings highlight compounds 11 and 17 as promising candidates, with compounds 4 and 5 also displaying notable stability across the receptor systems.

Furthermore, detailed molecular interaction profiling revealed that most ligands engaged multiple residues within the active sites, which in turn provides insight into their distinct binding mechanisms. For instance, DPP-4 most time deviates from the regular β-propeller fold due to additional secondary structural elements, including an antiparallel β-sheet inserted in blade 2. The turn tip contains Arg125, which forms a salt bridge with Glu205, located at the C-terminal end of an α-helix (residues Trp201–Glu205) inserted between blades three and four. Therefore, ARG 125, GLU 204, and GLU 205 constitute a key region of the substrate-binding pocket and are critical for substrate specificity [12].

As shown in Fig. 1, compounds 1, 3, and 7 formed hydrogen bonds with ARG 358 and ARG 669 via carbonyl groups on the triazol-3-one moiety, and with GLU 205 through the amide functional group [13]. These interactions, particularly with GLU 205 and ARG 125, may contribute to the selective inhibition of DPP-4. Additionally, the aromatic rings of the ligands engaged in π-π T-shaped and π-π stacked interactions with PHE 357 and TYR 547, while electrostatic interaction through an attractive charge with GLU 206 further stabilized the complexes [14].

Sitagliptin, the reference DPP-4 inhibitor, exhibited a broader interaction profile (as shown in Fig. 3a), particularly with the conserved catalytic motif Gly628-X-Ser630-Tyr631-Gly632, which is essential for enzymatic activity [12]. It formed carbon-hydrogen bonds with GLU 206 and conventional hydrogen bonds with SER 630, TYR 547, and TYR 631. These interactions enhance its binding strength and selectivity, particularly through hydrogen-bond contacts with GLU 205 and ARG 125, thereby contributing to its clinical efficacy. By targeting these catalytic residues, Sitagliptin and selected compounds effectively inhibit DPP-4-mediated degradation of glucagon-like peptide-1 (GLP-1), which accounts for over 95% of its inactivation and is a validated therapeutic strategy for Type 2 Diabetes [15].

Table 2
The binding energy of the studied compounds and standards for each target receptor.

Ligand	DPP-4 Binding energy (kcal/mol)	AgIA Binding energy (kcal/mol)	SGLT2 Binding energy (kcal/mol)
Compound 1	-7.6	-6.8	-8.6
Compound 2	-6.4	-7.1	-7.4
Compound 3	-7.6	-6.9	-7.8
Compound 4	-6.6	-7.3	-8.2
Compound 5	-6.2	-7	-7.5
Compound 6	-7	-6.4	-7.2
Compound 7	-7.6	-8.2	-8.5
Compound 8	-6.4	-6.5	-7.8
Compound 9	-6.9	-6.3	-7.7
Compound 10	-6.5	-6.8	-7.5
Acarbose	-7.2	-8.8	-3.1
Empagliflozin	-7.8	-8.5	-10.2
Sitagliptin	-8.3	-8.8	-9.5

For α -glucosidase A (AgIA), Acarbose exhibited strong binding affinity, primarily due to its multiple hydrogen bonding network. It formed seven hydrogen bonds with key residues: ARG 44 (two interactions), ASP 39, MET 86, ALA 151, ASN 153, and CYS 174. These interactions are facilitated by the compound's high oxygen content, which allows it to fit snugly within the enzyme's active site and enhances its aqueous solubility (Fig. 2).

In contrast, compounds 4 and 7 displayed distinct interaction profiles relative to Acarbose, engaging critical catalytic residues within the AgIA binding pocket. Compound 4 formed four hydrogen bonds with GLY 10 (two interactions), TYR 131, and MET 86, along with two π -sigma interactions involving ILE 40 and ALA 85. The predicted contacts with ASP 39 may assist in ligand orientation, as this residue is homologous to ASP 41 in the GH4 enzyme GlvA from *B. subtilis*, where it plays a role in NAD binding and is essential for enzymatic inhibition. Compound 5, although lacking hydrogen bonds, formed three π interactions with MET38, ILE40, and ALA85 at distances of 5.96 Å, 3.77 Å, and 3.71 Å, respectively, suggesting alternative stabilization mechanisms.

For SGLT2 (PDB ID: 8HDH), Empagliflozin, a clinically approved inhibitor, demonstrated the highest binding affinity (-10.2 kcal/mol), forming hydrophobic interactions with VAL 157, PHE 453, LYS 154, VAL 296, and ILE 397 via π -sigma, alkyl, and π -alkyl interactions (Fig. 3c). In addition, five conventional hydrogen bonds were observed with SER 393, GLN 457, HIS 80, LYS 321, and PHE 98, contributing to its high binding stability. Meanwhile, compounds 1 and 7 also showed promising interactions with SGLT2. Compound 1 formed three hydrogen bonds with TYR 290, GLN 457, and THR 153 (Fig. 4a), while compound 7 has five hydrogen bonds with GLN 99, ASN 75, SER 74, LYS 154, and THR 153 (Fig. 4b). Notably, hydrogen bonding with GLN 457 is important for SGLT2 inactivity, as observed in both compound 1 and Empagliflozin, reinforcing their therapeutic potential [16].

Moreover, compound 7 demonstrated similar binding modes with Empagliflozin, including an attractive charge contact with ASP 80, and hydrophobic interactions such as π - π stacking, π -alkyl, and amide- π stacking with PHE 98, HIS 80, and TYR 290. The stacking interaction with TYR 290 is a key aromatic residue at the inner gate, further supporting the compound's inhibitory potential [16]. Collectively, these results highlight compounds 1 and 7 as a promising scaffold for SGLT2 and AgIA inhibition, offering structural insights for the rational design of next-generation antidiabetic agents.

Pharmacokinetics and Drug-Likeness Results

ADMET profiling (absorption, distribution, metabolism, excretion, and toxicity) is integral to contemporary drug development, enabling early predictions of oral bioavailability, organ-specific safety, and metabolism, which are essential for minimizing late-stage clinical failures [17]. *In silico* ADMET modeling offers a cost-effective and time-efficient alternative to conventional experimental methods [18]. Drug-likeness was assessed using key physicochemical descriptors that aligned with Lipinski's and Pizer's rules [19, 20], evaluating parameters such as molecular weight, hydrogen bonding capacity, rotatable bonds, and topological polar surface area (TPSA). Pharmacokinetic predictions focused on consensus lipophilicity (log P, averaged from five predictive models), solubility (logS) in gastrointestinal fluids, blood-brain barrier (BBB) permeability, and interactions with P-glycoprotein (P-gp) and cytochrome P450 (CYP) enzymes [21]. Toxicological endpoints included drug-induced liver injury (DILI), carcinogenicity, mutagenicity, hepatotoxicity, and genotoxicity.

As detailed in Table 5, most studied compounds, including Sitagliptin and Empagliflozin, complied with Lipinski's Rule of Five, supporting their potential for oral administration. Acarbose, however, violates three criteria due to its high molecular weight, numerous

hydrogen-bond donors, and acceptors. According to Pfizer's rule, all studied compounds exhibited TPSA > 75 Å² and had less than five rotatable bonds, which suggests minimal hepatic or systemic toxicity. Quantitative Estimate of Drug-likeness (QED), a composite score ranging from 0 to 1, was used to rank compounds based on multiple molecular properties [22]. QED analysis indicates that compounds with QED > 0.67 are highly drug-like and suitable for drug development; 0.35–0.67 indicate moderate drug-likeness requiring optimization; and 0-0.35 indicate poor drug-likeness. As shown in Table 3 and based on the QED analysis, compounds 4–10 demonstrated high drug-likeness (QED > 0.67), indicating a favorable balance of absorption, permeability, and metabolic traits. Compound 1, 3, Sitagliptin, and Empagliflozin scored in the moderate range (QED 0.35–0.67), suggesting potential for structural optimization. Acarbose, with a very low QED of 0.07, exhibited poor drug-likeness due to its high counts of hydrogen-bond donors and acceptors and large polar surface area, resulting in limited permeability and rendering it less suitable for small-molecule development [22].

Table 3

Drug-likeness and bioavailability of the studied ligands with reference compounds.

Parameters	Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4	Cmpd 5	Cmpd 6	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 10	Sit	Aca	Emp
MW	233.19	222.64	267.63	204.19	188.19	216..24	298.31	278.27	231.26	248.24	407.32	645.61	450.44
nRing	2	2	2	2	2	2	3	2	2	2	3	3	4
nRot	3	2	3	2	2	2	5	5	3	4	4	13	6
HBA	6	4	6	5	4	4	5	7	5	6	5	19	7
HBD	1	1	1	2	1	1	1	1	1	2	1	14	4
TSPA	106.18	63.04	106.18	83.27	63.04	63.04	72.27	90.73	66.28	92.5	77.04	329.01	106.18
SlogP	0.36	1.11	1.02	0.16	0.45	1.07	2.03	0.48	0.52	0.56	2.02	-8.72	1.02
QED	0.47	0.77	0.51	0.68	0.7	0.76	0.73	0.8	0.78	0.77	0.62	0.07	0.67
Lipinski Rule	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Not accept	Accept
Pfizer Rule	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Not accept	Accept	Accept

Sit = Sitagliptin, Arc = Acarbose, Emp = Empagliflozin

Table 4
Pharmacokinetics prediction output of the studied and reference compounds.

Parameters	Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4	Cmpd 5	Cmpd 6	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 10	Sit	Aca	Emp
LogS	-3.19	-3.15	-3.41	-1.79	-2.71	-3.56	-4.4	-2.84	-3.27	-2.3	-3.15	-0	-4.14
LogP	1.4	2.11	1.79	0.84	1.64	2.11	3.1	1.42	1.82	1.67	0.68	-3.27	2.06
Caco-2	1	1	1	1	1	1	1	1	1	1	0	0	0
F20/F30/ F50	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	0/0/0	1/1/1
HIA	1	1	1	1	1	1	1	1	1	1	1	0	1
MDCK	1	1	1	1	1	1	1	1	1	1	0	0	0
BBB	1	1	1	1	1	1	1	1	1	1	1	0	1
Pgp inhibitor	0	0	0	0	0	0	1	0	0	0	0	0	0
Pgp substrate	0	0	0	0	0	0	0	0	0	0	1	0	1
PPB	1	1	1	1	1	1	1	1	1	1	1	0	1
VDss	-0.2	-0.15	-0.18	-0.22	0.01	-0.33	-0.38	-0.25	-1.01	-0.51	0.25	-0.69	0.35
CYP1A2 inhibitor/ substrate	1/1	1/1	1/1	1/0	1/1	1/1	1/.1	1/1	1/1	1/0	0/0	0/0	0/0
CYP3A4 inhibitor/ substrate	0/1	0/1	0/1	0/0	0/1	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/1
CYP2B6 inhibitor/ substrate	0/1	0/1	1/1	0/0	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0
CYP2C9 inhibitor/ substrate	0/1	0/1	1/1	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0
CYP2C19 inhibitor/ substrate	1/1	1/1	1/1	0/0	0/1	1/1	1/1	0/1	1/0	0/0	0/0	0/0	0/1
CYP2D6 inhibitor/ substrate	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
HLM Stability	0	0	0	0	0	0	1	0	0	0	0	0	0
CL (Plasma Clearance)	0	0	0	1	1	1	0	1	1	0	0	0	0
T _{1/2}	-1.04	-1.13	-1.55	-0.47	-0.43	-0.23	-0.94	-0.13	-0.48	-0.76	-0.93	-0.42	-0.79
1 = Poor, 0 = Good Sit = Sitagliptin, Arc = Acarbose, Emp = Empagliflozin													

Lipophilicity and water solubility are critical determinants of ADMET behavior, influencing membrane permeability, oral bioavailability, and the hydrophobicity required for blood transport to facilitate distribution [23]. As shown in Table 4, most compounds, except for 7 and Empagliflozin, exhibited acceptable solubility (logS > -4), indicating favorable dissolution in gastrointestinal (GI) fluids. Compounds with LogP values between 0 and 3 maintained an optimal balance between solubility and permeability, supporting efficient absorption [24]. Exceptions include Acarbose (logS = -0.3; Caco-2 = 0) and Sitagliptin (LogP = 0.68; Caco-2 = 0), which, despite high solubility, exhibited poor permeability due to very high polar surface area and extensive hydrogen-bond donors/acceptors (HBD/HBA). These features hinder passive diffusion, as supported by Caco-2 permeability models that penalize high polarity and low lipophilicity [25–27]. Artursson's protocol (2007) further emphasizes the roles of TPSA, HBD/HBA, and lipophilicity in modulating permeability across epithelial monolayers [28].

All compounds, except Acarbose, failed at least two key oral absorption parameters (e.g., F% and HIA), whereas only the reference drugs exhibited favorable passive permeability in MDCK assays. Distribution analysis revealed minimal blood-brain barrier (BBB) penetration for most compounds, which is generally desirable for non-CNS antidiabetic agents, except Acarbose, which showed favorable BBB permeability [29]. Meanwhile, compound 7 was predicted to have an unfavorable P-gp inhibition, potentially affecting

drug-drug interactions. However, all synthesized compounds (1-10) were classified as non-P-gp substrates, suggesting reduced efflux and improved absorption [30, 31]. Pharmaceutical formulations with P-glycoprotein inhibitory effects are promising approaches for enhancing oral drug absorption and bioavailability [31]. Acarbose also demonstrated an acceptable fraction of plasma protein binding (PPB). All compounds showed volume of distribution at steady state (VDss) within the optimal range (0.85-1.74 L/kg), indicating efficient tissue penetration [29]. VDss estimates how a drug distributes and remains in tissues after entering the bloodstream.

Regarding metabolism, most compounds exhibited minimal inhibition of the major cytochrome P450 enzymes (CYP3A4, CYP2B6, or CYP2D6), thereby enhancing their safety and reducing the risk of drug interactions [32]. Notably, compounds 1, 2, 3, 5, 7, and Empagliflozin were predicted to be CYP3A4 substrates, the most abundant liver enzyme implicated in drug interactions; however, only compound 7 inhibits CYP3A4, increasing the risk of metabolic conflict. Compound 3 also inhibited CYP2B6, which may affect the metabolism of the co-administered drugs. All the compounds except 7 exhibit good liver microsomal stability (HLM = 0), suggesting resistance to rapid hepatic clearance. However, plasma clearance (CL) values for compounds 4, 5, 6, 8, and 9 indicated poor clearance, which could lead to rapid elimination and a reduced duration of action, complicating dosing predictability and safety. Most compounds also possess short half-lives, which may require frequent dosing [33].

Table 5
Toxicity Profile of the studied ligand with reference compounds.

Parameters	Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4	Cmpd 5	Cmpd 6	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 10	Sit	Aca	Emp
DILI	1	1	1	1	1	1	1	1	1	1	1	0	1
hERG 1uM	0	0	0	0	0	0	0	0	0	0	0	0	0
hERG 10uM	0	0	0	0	0	0	0	0	0	0	0	0	1
hERG 30uM	0	0	0	0	0	1	1	0	0	0	0	0	1
hERG 1-10uM	0	0	0	0	0	0	0	0	0	0	0	0	0
hERG 10-30Um	0	0	0	0	0	0	0	0	0	0	1	0	1
Respiratory toxicity	0	0	0	0	0	0	0	0	0	0	0	1	1
Nephrotoxicity	1	1	1	0	1	1	1	1	1	1	0	1	0
Acute dermal toxicity	1	1	1	1	1	0	0	0	1	1	1	0	1
Ames's mutagenesis	1	1	1	1	1	1	1	0	1	1	0	0	1
Mouse carcinogenicity	1	0	1	1	0	1	1	0	1	1	0	0	0
Rat carcinogenicity	1	0	1	1	1	1	1	0	1	1	0	0	1
Rodents' carcinogenicity	1	1	1	1	1	1	1	0	1	1	0	0	1
Micronucleus (Genotoxicity)	1	1	1	1	1	1	1	1	1	1	1	0	1
Reproductive toxicity	0	0	0	0	0	0	0	1	0	0	1	1	1
Mitochondrial Toxicity	1	1	1	1	1	1	1	1	1	1	0	0	0
Acute oral toxicity	1	1	1	1	1	1	1	1	1	1	0	0	1
Repeated dose toxicity	1	1	1	1	1	1	1	1	1	1	1	0	1
FDAMDD (Max Daily Dose Violation)	0	0	0	0	0	0	0	0	0	0	0	0	1
DILI (Drug-Induced Liver Injury), 1 = poor, 0 = good Sit = Sitagliptin, Arc = Acarbose, Emp = Empagliflozin													

Toxicological predictions summarized in Table 5 highlight key safety concerns. All compounds, along with Sitagliptin and Empagliflozin, showed a high probability of drug-induced liver injury (DILI = 1), mainly linked to hepatotoxicity, a leading cause of drug withdrawal [34]. Acarbose, with a pessimistic DILI score of 0, demonstrated a comparatively safer hepatic profile. Furthermore, cardiotoxicity, assessed via hERG channel inhibition (associated with QT prolongation and sudden cardiac death), was minimal at low concentrations (hERG 1 μ M = 0) for most compounds and reference drugs [35]. However, Empagliflozin was predicted to be cardiotoxic at higher concentrations (hERG 10-30 μ M = 1), which is at odds with the reported cardioprotective characteristics of Empagliflozin in various studies, including the prevention of doxorubicin-induced myocardial dysfunction [36, 37].

Genotoxicity predictions revealed potential chromosomal damage (micronucleus = 1) and gene mutation (Ames = 1) in most of the synthesized compounds. Notably, compound 8 (Sitagliptin) and Acarbose showed negative Ames mutagenesis responses (0), suggesting a more favorable genotoxic profile. Several synthesized compounds, particularly compounds 1-7, 9, 10, and Empagliflozin, showed rodent carcinogenicity risk, while Acarbose and Empagliflozin remained negative across all carcinogenicity parameters. Organ-specific toxicity data reveal potential kidney damage in some of the synthesized compounds, consistent with known renal risks associated with Empagliflozin [38]. Reproductive toxicity was predicted for one of the compounds and all reference drugs. Additionally, all synthesized compounds in this series were associated with oxidative stress risks, unlike the reference compounds. Low LD₅₀ values were observed across the series, suggesting a high acute toxicity and potential challenges with chronic dosing (acute oral and repeated dose toxicity) [39].

A cumulative toxicity score (scoring ≥ 5 across specific parameters) identified most of the compounds as high-risk candidates, with Acarbose scoring 3 and classified as moderate risk. Among the novel compounds, compound 8 displayed the most favorable overall profile, combining high intestinal permeability (Caco-2/MDCK positive), acceptable aqueous solubility ($\text{LogS} \approx -2.84$), absence of P-gp efflux liability, and the longest predicted half-life within the series. Its cumulative toxicity score was comparable to that of sitagliptin and substantially lower than that of empagliflozin. Compound 4 showed promising solubility and permeability but was limited by a high toxicity burden. Based on these silico findings, compound 8 is recommended for immediate *in vitro* ADME/Tox validation, followed by targeted medicinal chemistry if toxicity or CYP liabilities are confirmed.

Conclusion

The current study designed and synthesized a series of new 4-amino-1,2,4-triazol-3(4H)-one Schiff base derivatives and evaluated their α -glucosidase inhibitory activities in comparison with the standard drug, acarbose. The results revealed that several derivatives, particularly compounds 8 and 10, exhibited remarkable inhibitory potency, surpassing that of acarbose, while compounds 4 and 5 also demonstrated comparable activity. The variation in biological activity across the series highlights the significant influence of electronic and steric factors associated with different substituents on the Schiff base framework. Molecular docking studies further supported the experimental results, showing strong binding affinities of the active compounds toward the α -glucosidase active site, characterized by key hydrogen-bonding and hydrophobic interactions that stabilize enzyme-inhibitor complexes.

Furthermore, *in silico* ADMET and drug-likeness analyses confirmed that most of the synthesized compounds possess favorable pharmacokinetic profiles, acceptable solubility, and low predicted toxicity, suggesting their suitability as potential oral antidiabetic agents. The convergence of *in vitro* and *in silico* findings demonstrates that structural modification of the triazol-3-one Schiff base scaffold is a viable strategy for developing potent, selective, and safe α -glucosidase inhibitors.

Declarations

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Author contributions

Conceptualization: E.O.Y, D.D.B, E.O.O; Methodology: E.O.Y, D.D.B, E. O. O., D.L.A, E. A; Formal analysis and investigation: E.O.Y, D.D.B, E. O. O., D.L.A, E. A; Writing – original draft preparation: E.O.Y, D.D.B, E. O. O., D.L.A, E. A; Writing – review and editing: E.O.Y, D.D.B, N. P. M; Software and Data curation: D. D. B, N. P. M.

All authors reviewed the manuscript.

Data availability

The computational study data are readily available upon request; however, all data supporting the findings of this study are available within the paper and its Supplementary Information.

Conflict of Interest

The authors declare no competing interests.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

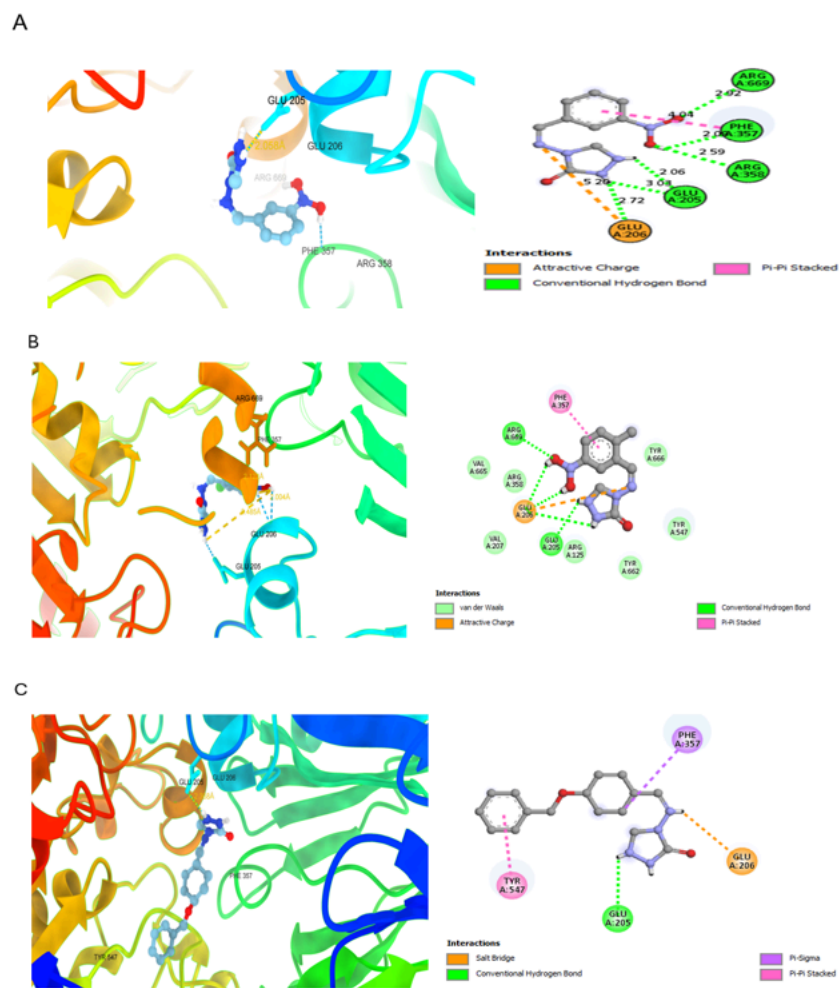


Figure 1

3D and 2D molecular structure of (A) compound 1, (B) compound 3 and (C) compound 7 with DPP-4 (PDB ID: 1NU6)

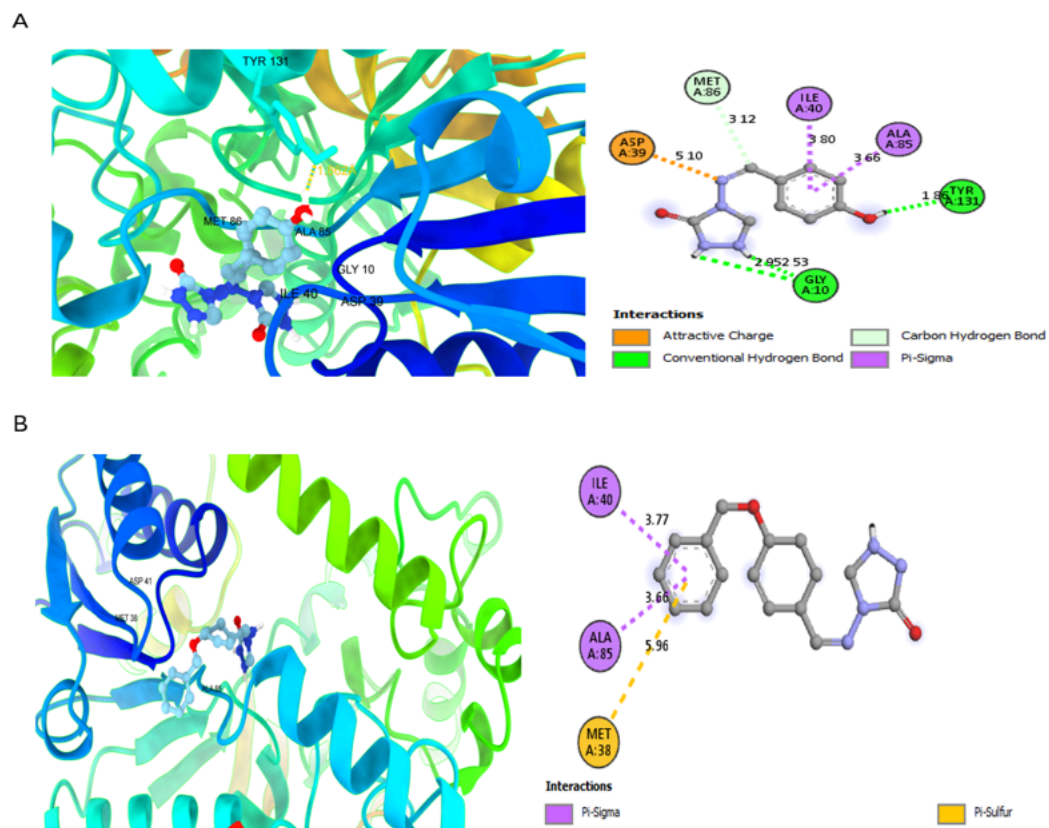


Figure 2

3D and 2D molecular structure of (A) compound 4 and (B) compound 7 with AglA (PDB ID: 10BB)

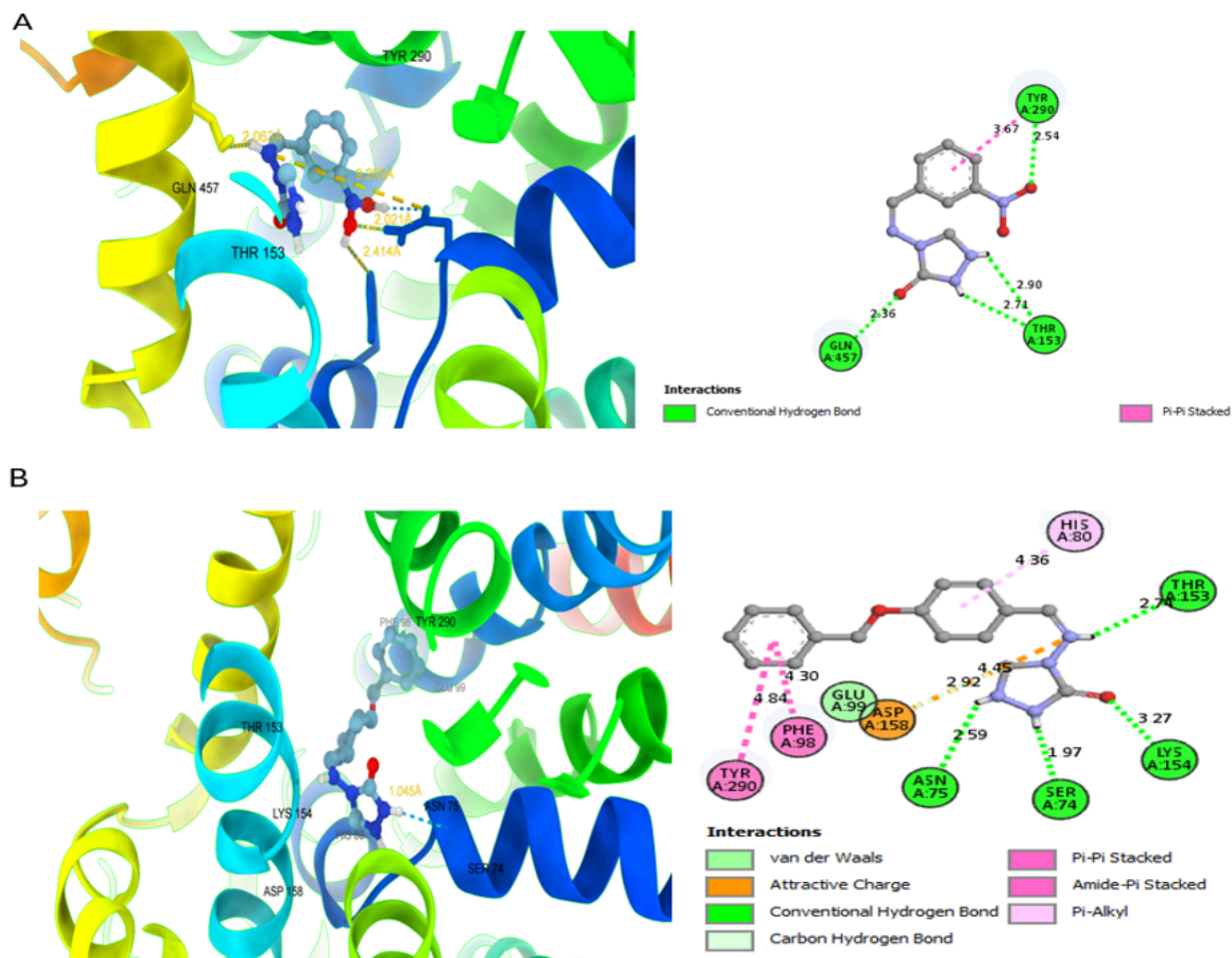


Figure 4

3D and 2D molecular structure of (A) Cmpd 1 and (B) Cmpd 7 with SGLT2 (PDB ID: 8HDH)

Supplementary Files

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