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Biological Evaluation of 2,3-Dimethylnaphthazarin Derivatives as α-Amylase and α-Glucosidase Inhibitors: A Molecular Docking Approach

2,3-Dimetilnaftazarin Türevlerinin α-Amilaz ve α-Glukozidaz İnhibitörleri Olarak Biyolojik Değerlendirmesi: Moleküler Yerleştirme Yaklaşımı

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Abstract

In this study, the antidiabetic properties of various mono- and bis-ester derivatives of 2,3-dimethylnaphthazarin were investigated. The inhibitory effects of these compounds on key enzymes involved in diabetes, particularly α-amylase and α-glucosidase, were evaluated. Furthermore, the binding potentials of these derivatives were analysed using molecular docking. Compounds 10, 13, and 14 exhibited better specific activity against α-glucosidase relative to standard Acarbose but were found to be non-active in inhibiting α-amylase. This disparity indicates a degree of selectivity toward α-glucosidase over α-amylase, suggesting that these compounds may offer a more targeted modulation of postprandial glucose levels while potentially minimizing adverse effects associated with strong α-amylase inhibition. Structural analysis of potent α-glucosidase inhibitors revealed that the presence of nitro and methyl groups as substitutions on the benzene ring were crucial for potency. This outcome was also favoured by data obtained from computational study with the strong hydrogen bonding desirable hydrophobic contacts due to the interactions with the nitro group and Arg400 as well as the methyl substitution and Pro230 residues. Consequently, compounds 10 and 14 emerged as the most promising and selective inhibitors of α-glucosidase, exhibiting IC_{50} values of 217.6 and 245.4 μM, respectively, exhibiting significantly better activity compared to standard Acarbose.

Keywords: ADME, 2,3-dimethylnaphthazarin, diabetes, molecular modelling.

Öz

Bu çalışmada, 2,3-dimetilnaftazarin bileşiklerinin çeşitli tek- ve çift- ester türevlerinin antidiyabetik özellikleri araştırılmıştır. Bileşiklerin diyabette rol oynayan anahtar enzimler üzerindeki, özellikle α-amilaz ve α-glukozidaz inhibitör etkileri değerlendirilmiştir. Ayrıca, bu türevlerin bağlanma potansiyelleri moleküler yerleştirme kullanılarak analiz edilmiştir. 10, 13 ve 14 numaralı bileşikler, standart Akarboza kıyasla α-glukozidaza karşı daha iyi spesifik aktivite sergilerken, α-amilazı inhibe etmede aktif olmadıkları bulunmuştur. Bu farklılık, söz konusu bileşiklerin α-amilaza kıyasla α-glukozidaza karşı belirgin bir seçicilik gösterdiğini ortaya koymakta olup, güçlü α-amilaz inhibisyonuyla ilişkili olumsuz etkileri en aza indirirken, yemek sonrası glikoz seviyelerinin daha hedefli bir şekilde düzenlenmesine katkı sağlayabileceğini düşündürmektedir. Güçlü α-glukozidaz inhibitörlerinin yapısal analizi, benzen halkasında nitro ve metil gruplarının sübstitüent olarak bulunmasının etkinlik için çok önemli olduğunu ortaya koymuştur. Bu sonuç, nitro grubu ve Arg400 ile etkileşimler ve metil ikamesi ve Pro230 kalıntıları nedeniyle arzu edilen güçlü hidrojen bağları içeren hidrofobik temaslar ile elde edilen hesaplamalı çalışma verileriyle de desteklenmiştir. Sonuç olarak, 10 ve 14 numaralı bileşikler, sırasıyla 217.6 ve 245.4 μM'lik IC_{50} değerleri sergileyerek, standart Akarboz'a kıyasla önemli ölçüde daha iyi aktivite gösteren, α-glukozidaz için en umut vadeden ve seçici inhibitörler olarak saptanmıştır.

Anahtar Kelimeler: ADME, 2,3-dimetilnaftazarin, diyabet, moleküler modelleme.

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1. Introduction

Diabetic mellitus (DM) is a widespread, well-known and growing chronic metabolic disorder corresponding with prolonged glucose elevation due to attenuated insulin release, impaired insulin action, or both (Chen et al., 2021; Hameed et al., 2024). Hyperglycemia in DM causes development of diabetic retinopathy, neuropathy, nephropathy, malignancy, cardiovascular and peripheral vascular disease resulting in significantly higher rates of morbidity and death (Patnam et al., 2024; Yu et al., 2024). Prevalence of diabetes is rising at an alarming rate globally; International Diabetes Federation (IDF) estimated that 589 million people over the age of 20 were living with diabetes in 2024 while also projecting that 853 million people will be affected by DM by the year 2050 (Duncan et al., 2025). DM primarily manifests three types: type I Diabetes Mellitus (T1DM), type II Diabetes Mellitus (T2DM), and gestational DM. T2DM is the non-insulin-dependent form and represents most cases globally (Gani et al., 2021; Dhameja et al., 2022). Targeting distinct pathophysiological mechanisms including attenuation of hepatic gluconeogenesis, potentiation of insulin secretion by pancreatic β-cells, reduction of peripheral insulin demand, and inhibition of carbohydrate-digestive enzymes are treatment regimens for T2DM (Gamal et al., 2024).

The enzymes α-glucosidase and α-amylase, classified under EC 3.2.1.20 and EC 3.2.1.1 respectively, are crucial enzymes functioning as central catalysts in gastrointestinal carbohydrate hydrolysis (Hasaninezhad et al., 2020; Wali et al., 2022). Salivary α-amylase triggers the enzymatic digestion of dietary carbohydrates from complex polysaccharides into oligosaccharides within the oral cavity. Pancreatic α-amylase progresses the digestion in the duodenum. α-Glucosidase, expressed in intestinal epithelial cells, further catalyzes the conversion of these oligosaccharides to absorbable monosaccharides (e.g., glucose) that diffuse into the blood stream and cause hyperglycemia — a pathophysiological hallmark of diabetes mellitus (Trinh et al., 2016; Wali et al., 2022). Selective inhibition of α-glucosidase is pharmacologically desirable since it acts as the final step of carbohydrate digestion, thereby allowing a controlled reduction in postprandial glucose absorption without completely blocking the breakdown of polysaccharides. In contrast, excessive inhibition of α-amylase leads to the accumulation of undigested carbohydrates in the intestine, which is commonly associated with adverse gastrointestinal effects such as bloating, flatulence, and diarrhea, limiting clinical tolerability. Given this function, α-glucosidase inhibitors serve as a potent class of antidiabetic agents in mitigating postprandial hyperglycemia when administered alone or in combination regimens. Acarbose, miglitol, and voglibose are clinically established inhibitors in T2DM management (Ali et al., 2017; Akmal & Sasongko, 2023); however, these clinically approved drugs lead to adverse effects on the gastrointestinal tract causing flatulence, diarrhea, and abdominal pain. Hepatotoxicity has also been linked to the chronic use of these drugs due to excessive inhibition of α-amylase (Apostolidis & Lee, 2010; Trinh et al., 2016).

These significant limitations indicate a compelling requirement for the development of novel and more efficacious inhibitors of α-glucosidase and α-amylase enzymes (Etxeberria et al., 2012; Ye et al., 2019). In this regard, studies on the design of new drug candidate compounds and determination of their biological activities will allow for their potential use in the treatment of diabetes.

Naphthoquinone and its various analogues are naturally occurring compounds and have a broad spectrum of bioactivities. For example, one of the naphthoquinones analogues, shikonin and its derivatives exhibit anti-inflammatory, anticancer, antibacterial, antioxidant and antifungal effects (Wang et al., 2012; Guo et al., 2019; Valipour, 2022). Another biologically active naphthoquinone analogue naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) (compound **A**) and its molecular frameworks are commonly observed in many natural products are and reported to show a comprehensive pharmacological profile (Segura-Aguilar et al., 1992; Kyong-Up et al., 1997). Due to exhibiting significant biological activity, interest of scientists in the design and synthesis of new naphthazarin derivatives has increased. For example, studies on breast cancer cells showed that the synthetic imidazole ester derivative of acetyl shikonin (compound **B**) exerted significantly stronger anti-proliferative effects within the first 24 h compared to parent acetyl shikonin, which possesses naphthazarin core structure (Tepe et al., 2023). In another study, various acetyl and benzoyl ester derivatives of naphthazarin were synthesized employing different acyl chlorides (Abadan et al., 2023). In that work, studies on diabetes were conducted using naphthazarin derivatives, and it was reported that the imidazole naphthazarin ester (compound **C**) shown in Figure 1, exhibited an IC_{50} value of 7.4 μM and demonstrated approximately 150-fold better activity than the standard inhibitor Acarbose (Abadan et al., 2023). The synthesis of esterification products of 2,3-dimethylnaphthazarin derivatives has also been reported in the literature (Saglam, 2022). However,

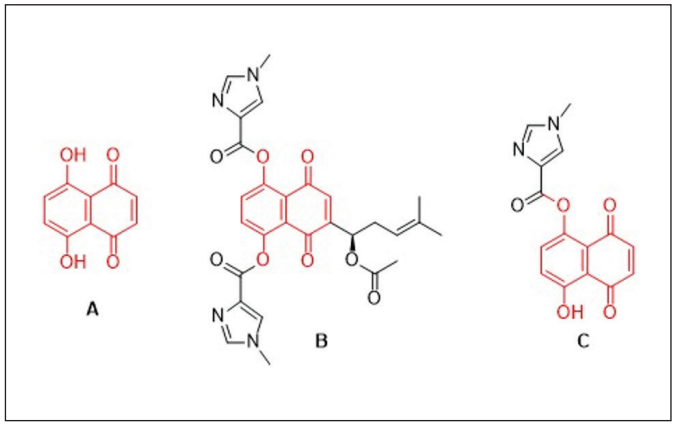


Figure 1. Naphthazarin and representative synthetic bioactive compounds bearing the naphthazarin core structure.

based on current literature, no studies have reported the antidiabetic activities of ester derivatives of 2,3-dimethylnaphthazarin.

2. Material and Methods

2.1. Chemicals

All assay materials, including reagents and standards employed in the bioassays were obtained from Sigma-Aldrich and utilized without further processing. Type VI-B α-amylase from porcine pancreas (EC 3.2.1.1), in lyophilized powder form, 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3), sodium azide, sodium chloride, DMSO and Acarbose were supplied by Sigma-Aldrich (St. Louis, MO). All remaining reagents were of certified high-purity suitable for analytical applications. *p*- Nitrophenol, α-D-glycopyranoside (PNPG) and lyophilized form of α-glucosidase type I derived from *Saccharomyces cerevisiae* (EC 3.2.20) were obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Biological Studies

2.2.1. The α-Glucosidase Inhibition Assay

The inhibitory activity of α-glucosidase was assessed according to the protocol outlined by Schmidt et al. (2012). Succinctly, a 90 µL of phosphate buffer (0.1 M, pH 7.5) containing 0.02% NaN_3, a 10 µL test sample solubilized in DMSO, and 80 µL of enzyme solution (0.05 U/mL well concentration) were applied to each well. The reaction mixture was maintained at 28 ˚C for 10 min prior to PNPG addition, adjusting the final volume to 200 µL (final well concentration of 1.0 mM). A control blank consisting of the enzyme, substrate, and test solvent instead of the sample was used. Absorbance readings were recorded at 405 nm every 40 s for a total duration of 35 min. Microplate photometer (BioTek Power Wave XS) featuring a built-in incubator and operated via the program GEN5 9ver.2.05.20050 was used for the incubation and absorbance measurements. The inhibitory activity of α-glucosidase was assessed by calculating percent inhibition using the following equation:

$$Inhibition\ \% = (Slope\ blank - Slope\ sample) / Slope\ blank * 100 \quad (1)$$

Acarbose served as the positive control in the assay with all experiments carried out in triplicate to ensure reproducibility. Statistical significance was determined using Student's t-test ($p < 0.05$).

2.2.2. The α-Amylase Inhibition Assay

The inhibitory activity of α-amylase was assessed according to the protocol outlined by Okutan et al. (2014) with slight modifications. Succinctly, an 80 µL of phosphate buffer (0.1 M, pH 6.0) containing 0.02% NaN_3, a 20 µL test sample solubilized in DMSO, and 80 µL of enzyme solution (0.05 U/mL well concentration) were applied to each well. Following a 10 min incubation at 37˚C, CNP-G3 was added to commence the reaction, bringing the total volume to 200 µL (final well concentration of 1.0 mM). A control blank consisting of the enzyme, substrate, and test solvent instead of the sample was used. Absorbance readings were recorded at 405 nm for 35 min. A background control containing enzyme, sample, and buffer — excluding the substrate — was used for each compound to eliminate interference from analytes exhibiting absorbance at 405 nm. Microplate photometer (BioTek Power Wave XS) featuring a built-in incubator and operated via the program GEN5 9ver. 2.05.20050 was used for the incubation and absorbance measurements. The inhibitory activity of α-amylase was expressed as percentage inhibition ± SD and calculated using the following equation:

$$Inhibition\ \% = (A\ blank - A\ sample) / A\ blank * 100 \quad (2)$$

Acarbose served as the positive control, and with all assays conducted in triplicate and statistical significance determined by Student's t-test ($p < 0.05$).

2.3. Computational Methods

2.3.1. *In Silico* Molecular Docking

2.3.1.1. Ligand Preparation

Before docking the compounds, we performed intrinsic affinity of reference ligands for commonly crystallized proteins to validate the effectiveness of the docking procedure. X-ray crystallographic coordinates of the α-glycosidase–PRU complex (PDB ID: 3WY1), along with their respective binding sites were utilized for analysis. The 3D structures of the compounds were generated using ChemBioDraw Ultra 14 software and saved in the Protein Data Bank (PDB) file format.

2.3.1.2. Macromolecule Preparation

The docking procedure implemented herein adheres to established methodologies in molecular docking and free energy analysis (Akkus et al., 2022; Kocak & Yildiz, 2022; Akkus et al., 2023; Tasci et al., 2023). Protein models in complex with reference ligands were sourced from the Protein Data Bank (PDB). The α-glucosidase structure used in this study corresponds to PDB entry 3WY1 (Shen et al., 2015). SwissProt was employed to model missing residues within the protein structures (Waterhouse et al., 2018), and non-protein constituents were excluded from the structures via Hermes (Jones et al., 1997).

2.3.1.3. Molecular Docking

Molecular docking is a powerful computational technique that provides detailed insights into binding interactions, often revealing mechanistic information that is difficult to obtain through experimental methods. It offers valuable perspectives into the complex field of molecular recognition. In this study, we explore the potential binding mechanisms of the synthesized compounds with various protein families.

The docking outcomes are shown in parallel with the corresponding experimental IC_{50} values. However, it is important to note that while docking scores are presented, their predictive accuracy remains limited.

The accuracy of PRU self-docking with α-glycosidase was determined by calculating the root mean square deviation (RMSD) between the docked conformation and its crystallographic counterpart. The optimal docking pose of PRU with α-glucosidase yielded an RMSD of 3.25 Å, demonstrating a reasonable alignment with the X-ray crystallographic data.

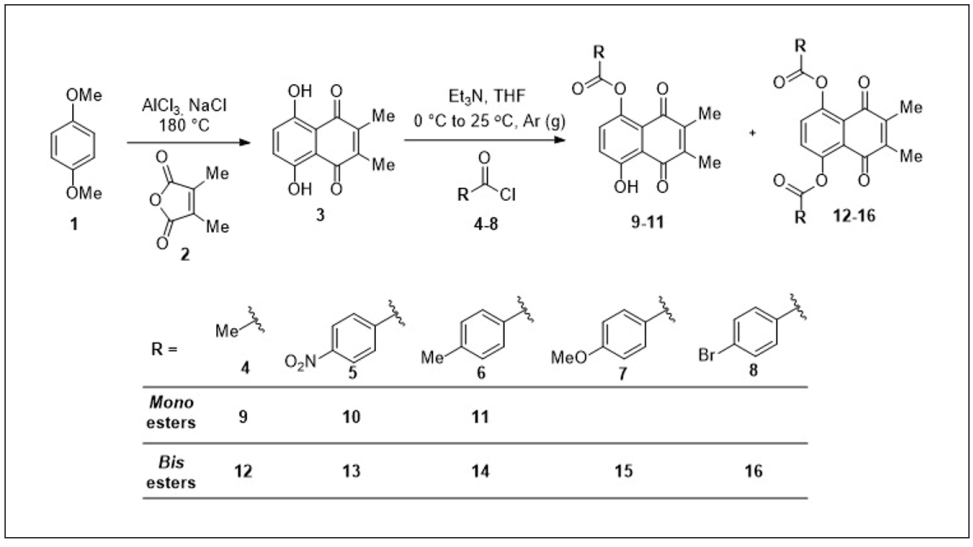
Propka 3.1 was employed to determine the protonation states at pH 7.0. In this analysis, acidic amino acids were modeled with a negative charge, while basic amino acids were modelled with a positive charge. The center of docking grid sphere was defined using the coordinates of the native ligands.

3. Results and Discussion

3.1. Chemistry

3.1.1. Synthesis of the 2,3-Dimethylnaphthazarin Ester Derivatives 9-16

Ester substituted dimethylnaphthazarin derivatives (9-16) were successfully synthesized in two steps which involve double Friedel-Craft acylation followed by an esterification reaction with variety of acyl chlorides. In the first step, treatment of 1,4-dimethoxybenzene 1 and 2,3-dimethyl-



Scheme 1. Synthesis of 2,3-dimethylnaphthazarin and its mono- and bis- ester derivatives.

maleic anhydride 2 with molten aluminum chloride-sodium chloride gave 2,3-dimethylnaphthazarin 3 (Dessolin et al., 2001). Following treatment of 2,3-dimethylnaphthazarin 3 with different acyl chlorides (4-8) (acetyl, *p*-nitro-, *p*-methyl-, *p*-methoxy-, and *p*-bromo-benzoyl chlorides) in the presence of trimethylamine resulted in mono- and bis-ester derivatives of 2,3-dimethylnaphthazarines (9-16) (Scheme 1). The final compound structures were verified by ^{1}H NMR, FTIR, ^{13}C NMR, HRMS techniques and already discussed in detail in our previous study (Saglam, 2022).

3.2. Biological Studies

3.2.1. Evaluation of α-amylase and α-glucosidase Enzyme Inhibitory Activities

The anti-diabetic potential of targeted compounds (9-16) were assessed by evaluating their inhibitory activities against α-amylase and α-glucosidase. Enzyme inhibitory activity tests were conducted to determine the IC_{50} values of the test compounds, indicating their anti-diabetic potential and were compared to Acarbose as the reference standard. IC_{50} concentrations derived from enzyme inhibition experiments are listed in Table 1. Low IC_{50} concentrations indicated potent inhibitory potential of the evaluated compounds and were identified as promising candidates targeting α-glucosidase and α-amylase. Consistent with expectations, the positive control Acarbose exhibited potent inhibition, yielding IC_{50} values of 1033.8 μM for α-glucosidase and 0.05 μM for α-amylase. α-Glucosidase inhibition was assessed through an assay designed to calculate IC_{50} values by testing eight concentration points spanning from 6.75 to 800 μM. Compounds with lower inhibition concentrations than the positive control Acarbose were identified as active and could be potential targets for this enzyme. Among the tested compounds, 9, 11 and 12, as well as 15-16 exhibited inhibition levels below 50% at elevated concentrations, and their IC_{50} inhibition values were higher than 800 μM.

Notably, compound 13 exhibited an IC_{50} value of 452.8 μM, which was markedly lower than that of the positive control Acarbose (IC_{50} = 1033.8 μM), indicating a superior inhibitory potency against α-glucosidase. Structure–activity relationship (SAR) analysis revealed that *p*-nitrobenzene substitutions at the 5*O*- and 8*O*-positions of the 2,3-dimethylnaphthazarin scaffold play a decisive role in modulating inhibitory activity. Interestingly, the mono-*p*-nitrobenzene–substituted derivative at the 5*O*-position (compound 10) demonstrated even more pronounced inhibition, suggesting that reduced steric bulk and optimal orientation within the enzyme active site may enhance binding efficiency.

In addition, the bis-substituted derivative bearing *p*-methylbenzene moieties (compound 14) displayed comparable inhibitory activity, with an IC_{50} value of 245.4 μM, further underscoring the importance of aromatic substitutions in enzyme recognition. These findings suggest that both electronic effects and hydrophobic character introduced by the substituents significantly contribute to enzyme inhibition. It is proposed that these functional groups facilitate the formation of essential hydrogen bonding interactions as well as favorable hydrophobic contacts with key amino acid residues within the catalytic pocket of α-glucosidase, thereby stabilizing the ligand–enzyme complex. Collectively, these results highlight the critical influence of substitution patterns on inhibitory performance and provide valuable insights into the rational design of more potent and selective α-glucosidase inhibitors based on the 2,3-dimethylnaphthazarin framework.

The compounds identified as effective α-glucosidase inhibitors were further evaluated for their inhibitory activity against α-amylase using the same concentration ranges and the reference inhibitor Acarbose for comparison. For all tested derivatives (compounds 9–16), the IC_{50} values against α-amylase were determined to be greater than 800 μM, indicating very weak or negligible inhibitory activity toward this enzyme. In contrast, Acarbose exhibited potent α-amylase inhibition with an IC_{50} value of 0.05 μM. These results clearly demonstrate that the synthesized compounds possess substantially lower α-amylase inhibitory potency compared to the standard drug.

Importantly, the observed activity profile highlights a pronounced selectivity of these derivatives toward α-glucosidase over α-amylase. For compounds 10–11 and 13–14, IC_{50} values could not be achieved for α-amylase inhibition even at the highest tested concentrations, whereas these compounds displayed measurable and significantly stronger inhibition of α-glucosidase, being approximately 2.5–5-fold more potent against it. This selective inhibition pattern is pharmacologically advantageous, as preferential targeting of α-glucosidase may effectively regulate postprandial glucose absorption while minimizing adverse gastrointestinal effects associated with excessive α-amylase inhibition. Overall, these findings further support the potential of the identified compounds as selective α-glucosidase inhibitor candidates.

Table 1. Enzyme inhibition profiles of compounds 9–16 targeting α-amylase and α-glucosidase.

	α-Glycosidase		α-Amylase	
Compound	**IC_{50} μM[a]**	**±SD**	**IC_{50} μM[a]**	**±SD**
9	>800		NT	
10	217.6	14.9	>800	
11	>800		>800	
12	>800		NT	
13	452.8	15.0	>800	
14	245.4	24.5	>800	
15	>800		NT	
16	>800		NT	
Acarbose[b]	1033.8	3.4	0.050275	0.000134

[a]Values are presented as mean ± standard deviation (SD) from three independent measurements ($p < 0.05$). [b]Indicates the positive control. IC_{50} μM NT: Not Tested.

3.3. Computational Analysis

3.3.1. *In Silico* ADME

The comparison of docking studies and the biological assays were also made by evaluation of PLP. Chemscore values and the inhibition concentrations (Table 2). Biological potential and computational scores were consistent apart from compounds 15 and 16, which, despite high docking scores (64.75 and 62.70, respectively), failed to translate into measurable *in vitro* efficacy. The PLP. Chemscore values and biological activity in the case of compounds 13 and 14, were in correlation where higher scores corresponded with enhanced inhibition, indicating the predictive reliability of docking scores for certain structural scaffolds.

3.3.2. Molecular Interaction Analysis of the Ligand Within the Active Site

Molecular docking studies elucidated the potential non-covalent interactions between the ligand and key amino acid residues within the enzyme active site (Figure 2). For compound 10, Arg400 was identified as a critical residue involved in the formation of a conventional hydrogen bond with the nitro substituent, significantly contributing to ligand stabilization. Additionally, the free hydroxyl group of the naphthazarin core established a strong polar interaction in the form of a conventional hydrogen bond with Glu231, which is likely to play an important role in enhancing both ligand affinity and selectivity toward the enzyme's catalytic site. Furthermore, Met302 participated in a π–sulfur inter-

Table 2. α-Glucosidase enzyme inhibitory activities of compounds 9–16.

α-Glycosidase			
Compound	**IC_{50} μM[a]**	**SE**	**PLP. Chemscore**
PRU			55.69
9	>800		42.53
10	217.6	14.9	51.22
11	>800		51,42
12	>800		30.19
13	452.8	15.0	58.15
14	245.4	24.5	62.54
15	>800		64.75
16	>800		62.70
Acarbose[b]	1033.8	3.4	

[a]Values are presented as mean ± standard deviation (SD) from three independent measurements ($p < 0.05$). [b]Indicates the positive control.

action between its sulfur atom and the naphthalene moiety of the ligand, suggesting a contribution to electronic stabilization within the binding pocket. The same residue, along with Val335, was also involved in hydrophobic alkyl and π–alkyl interactions with the methyl substituents on the naphthazarin backbone, thereby supporting the favorable accommodation of the ligand within a predominantly hydrophobic sub-pocket of the enzyme.

3.3.3. Binding Interaction Profile of the Ligand in the Active Site

The 2D interaction map (Figure 3) revealed favorable non-covalent interactions governing ligand binding within the active site. Notably, Arg400 participated in a conventional hydrogen bond with the terminal oxygen atom of the nitro substituent, an interaction that is likely to enhance binding stability and overall affinity of the ligand toward the receptor. A charged residue located on Glu231 amino-acid built the π-anion interaction via aromatic ring system. This electrostatic attraction increased the binding stability of the ligand. A π-donor hydrogen bond with Pro230 and a carbon hydrogen bond was also present, further anchoring the ligand within the binding cavity. Phe397, Lys225, and Ala229 were determined as the sources of hydrophobic attractions due to the formation of π-alkyl interactions. The aromatic fragments of the ligand help to form further stability in the hydrophobic pocket. This interaction played a key role in stabilizing the ligand orientation within the binding pocket, thereby facilitating optimal van der Waals interactions with neighboring amino acid residues.

3.3.4. Molecular Interaction Analysis of the Ligand–Protein Complex

As shown in Figure 4, the key π-anion interaction was detected between the aromatic ring of the ligand and anionic part of the Glu231 residue. Additionally, hydrophobic interactions were also present, including π-alkyl interactions with Lys225, Ala229, Tyr389, and Arg400, as well as alkyl contacts with Pro230 residues. Above mentioned interactions reinforced the ligand's orientation in the nonpolar environment of the protein. However, the presence of an unfavorable bump with Asn301 resulted due to the closer contact with the H atom on the ligand than their allowed van der Waals radii may cause a reduction of affinity due to the possible steric clash or repulsion.

3.3.5. *In Silico* ADMET Properties

The classification of compounds as substrates or non-substrates of permeability glycoprotein (P-gp) plays a pivotal role in shaping their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles. P-gp is essential for protecting the central nervous system (CNS) against harmful foreign substances (Montanari & Ecker, 2015). According to the *in silico* ADMET predictions sum-

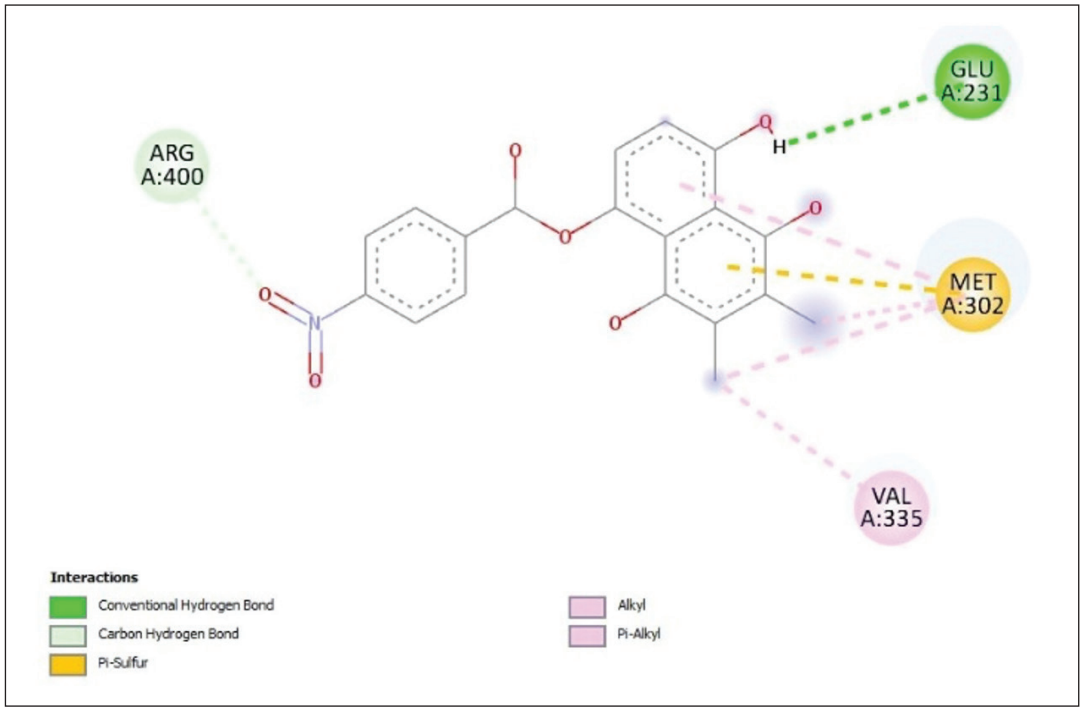


Figure 2. 2D representations of compound 10 interacting with binding site residues of the α-glucosidase enzyme.

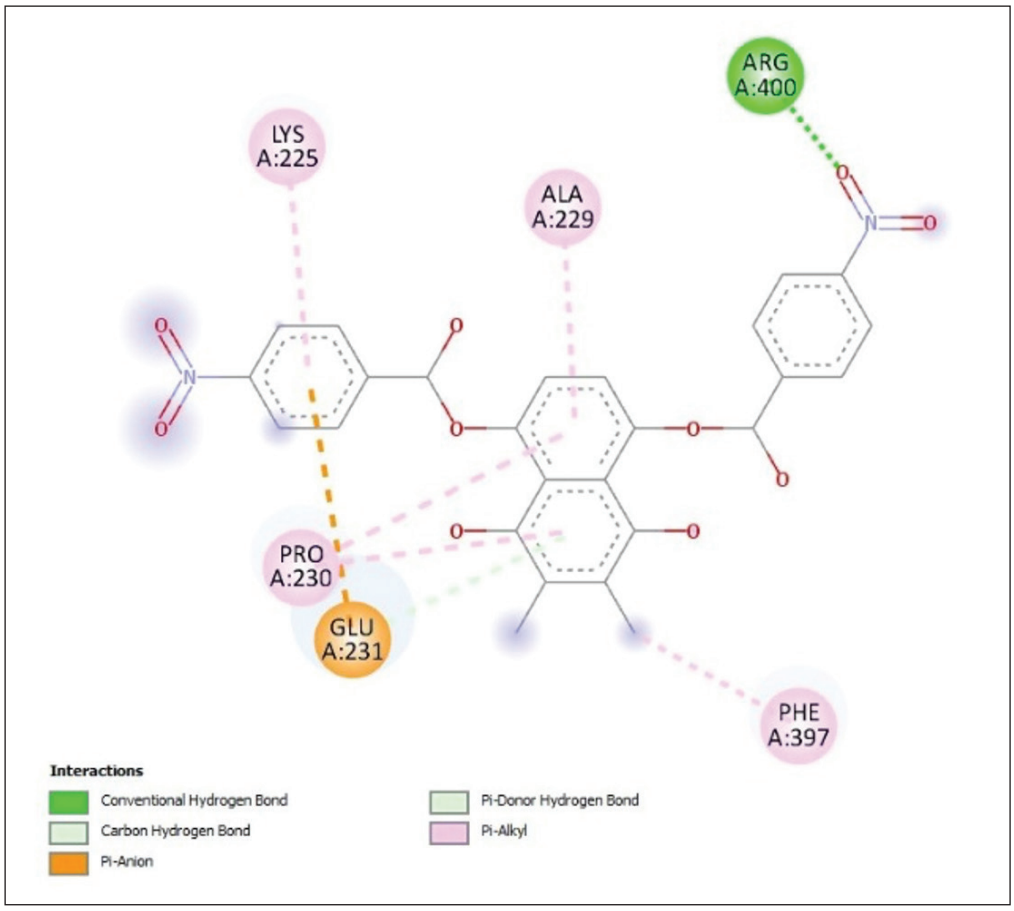


Figure 3. 2D representations of compound 13 interacting with binding site residues of the α-glucosidase enzyme.

marized in Table 3, none of the investigated compounds were predicted to be P-gp substrates, suggesting a lower risk of efflux-mediated limitations on oral bioavailability and multidrug resistance. Consistent with this prediction, none of the compounds were predicted to permeate the blood–brain barrier (BBB), indicating a reduced likelihood of CNS-related side effects.

Cytochrome P450 (CYP) enzymes are key players in the detoxification of numerous pharmaceutical compounds (Testa & Krämer, 2007). Enzymes inhibition can result in clinically relevant drug-drug interactions and toxicity due to buildup of the parent drug or its metabolites (Hollenberg, 2002; Huang et al., 2008). All compounds under investigation are forecasted to be non-inhibitors across the five major CYP isoforms which are CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

3.3.5.1. ADME Profiling and Pharmacokinetic Implications of Compounds 9–16

The predicted ADME properties of compounds 9–16 as shown Table 3 revealed moderate pharmacokinetic behavior in terms of absorption and inhibitions of key enzymes for the metabolism of drugs. The compounds presented high human intestinal absorption (HIA), indicating good oral bioavailability potential. None of the compounds were found to be permeable for BBB suggesting that lower CNS-related side effects would be detected. Some of the cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4, were in target of the compounds. Compounds 10, 11, 14, and 15 inhibit both CYP1A2 and CYP3A4, indicating a potential for drug–drug interactions that should be further evaluated during lead optimization. Additionally, compounds 11, 13, 14, and 15 inhibit CYP2C19 and CYP2C9, which could further complicate co-administration with drugs metabolized by these isoforms. All compounds show no interaction with P-gp substrate, possibly resulting in lower risk of efflux-related bioavailability limitations and multidrug resistance interactions. Skin permeability values were detected within the acceptable limits and the Log Kp values ranged from −6.94 to −5.35 cm/s.

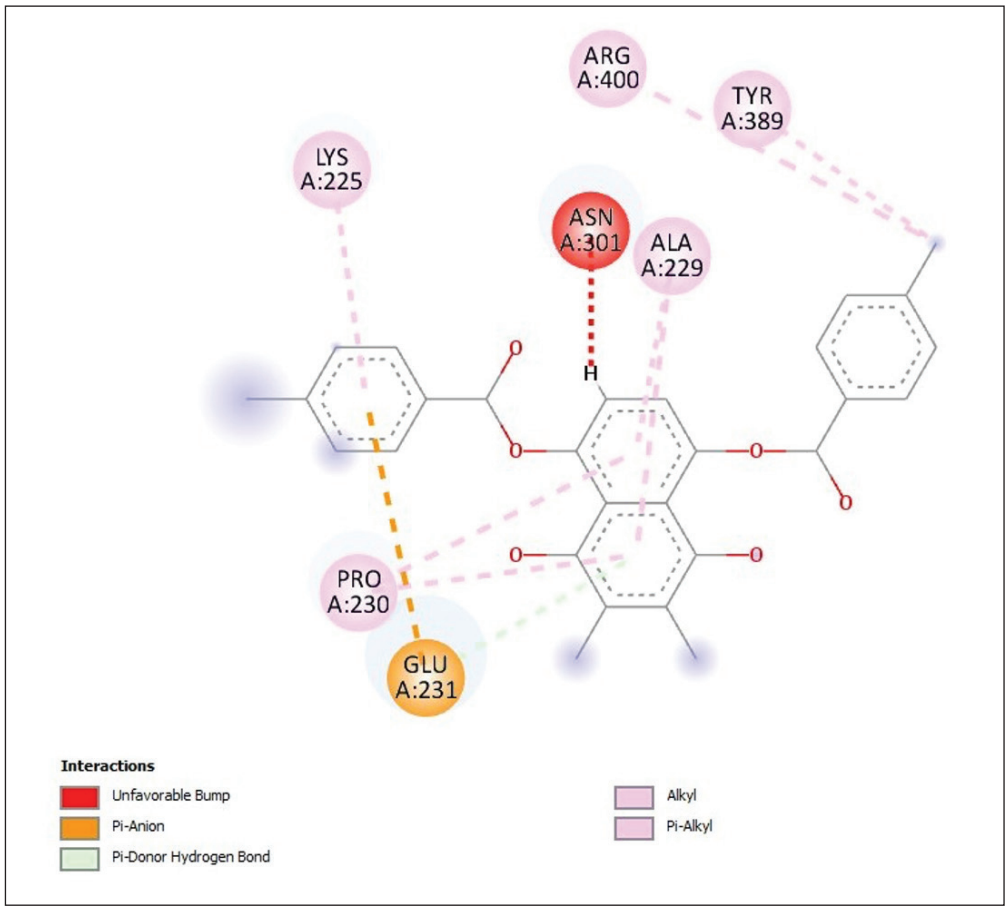


Figure 4. 2D representations of compound 14 interacting with binding site residues of the α-glucosidase enzyme.

Table 3. Computationally derived ADME profiles of compounds 9-16.

Compound	HIA	BBB Permeate	P-gp Substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (Skin permeation cm/s)
9	High	No	No	Yes	No	No	No	No	-6.36
10	High	No	No	Yes	No	Yes	No	Yes	-5.96
11	High	No	No	Yes	Yes	Yes	No	Yes	-5.39
12	High	No	No	Yes	No	No	No	No	-6.94
13	Low	No	No	No	Yes	Yes	No	Yes	-6.49
14	High	No	No	Yes	Yes	Yes	No	Yes	-5.35
15	High	No	No	Yes	Yes	Yes	No	Yes	-5.75
16	High	No	No	Yes	Yes	Yes	No	No	-5.68

HIA: Human gastrointestinal absorption, BBB permeant: blood-brain barrier permeability, P-gp substrate: permeability glycoprotein substrate.

4. Conclusion

In the present study, a series of mono- and bis-ester derivatives of 2,3-dimethylnaphthazarin were synthesized and *in vitro* enzyme inhibition assays were carried out for the systematic evaluation of their antidiabetic potential. The complementary molecular docking analyses were added to support and compare the findings obtained from biological assays. The biological evaluation demonstrated that several derivatives, particularly compounds 10, 13, and 14, exhibited pronounced inhibitory activity against α-glucosidase, surpassing the reference drug Acarbose in terms of specific activity. Among the evaluated compounds, derivatives 10 and 14 emerged as the most promising and selective α-glucosidase inhibitors, with IC_{50} values of 217.6 and 245.4 μM, respectively, demonstrating significantly improved activity compared to Acarbose. In contrast, none of the tested compounds showed meaningful inhibitory effects against α-amylase, indicating a clear selectivity toward α-glucosidase.

This selective inhibition profile is highly desirable, as preferential targeting of α-glucosidase over α-amylase may enable effective modulation of postprandial hyperglycemia while reducing gastrointestinal side effects commonly associated with strong α-amylase inhibition. Structure–activity relationship analysis was created with the data from biological assays and the presence of nitro and methyl substitutions on the benzene was found to be crucial in enhancing α-glucosidase inhibitory potency. Molecular docking studies also supported that substitutional derivatives resulted in better inhibitory activities with the strong hydrogen bonding interactions between the nitro group and Arg400, as well as favorable hydrophobic contacts involving methyl substituents and Pro230. Overall, the combined experimental and computational results suggest that 2,3-dimethylnaphthazarin ester derivatives represent a valuable scaffold for the development of selective α-glucosidase inhibitors and warrant further optimization as potential antidiabetic agents.

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