

Article

Probing Combined Experimental and Computational Profiling to Identify *N*-(benzo[d]thiazol-2-yl) Carboxamide Derivatives: A Path to Potent α -Amylase and α -Glucosidase Inhibitors for Treating Diabetes Mellitus

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Abstract

A novel series of benzothiazole scaffolds were presented to test their in vitro α -amylase and α -glucosidase activities for combating diabetes mellitus, which is one of the most rapidly growing diseases. The tested compounds were elucidated structurally by various spectroscopic techniques like ¹H NMR, ¹³C NMR and HRMS. All compounds exhibited a varied range of inhibitory activities against targeted α -amylase and α -glucosidase enzymes, with IC₅₀ values of 1.58 ± 1.20 to 7.54 ± 3.60 μ M (α -amylase) and 2.10 ± 1.10 to 8.90 ± 4.10 (α -glucosidase), respectively. The obtained results were compared with the standard acarbose drug, with IC₅₀ values of 0.91 ± 0.20 μ M (α -amylase) and 1.80 ± 1.10 μ M (α -glucosidase). Specifically, methyl 2-amino-4-((6-methoxypyridin-3-yl)methoxy)benzo[d]thiazole-6-carboxylate (**5c**) and methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxylate (**6b**) emerged as potent inhibitors of α -amylase and α -glucosidase enzymes. These potent compounds were further screened for in silico molecular docking studies to investigate possible binding interactions with active sites of targeted enzymes, and results obtained demonstrated that potent compounds exhibited stronger binding affinities toward anti-diabetic enzymes compared to the positive control acarbose.

Keywords: benzothiazole; SAR; α -amylase; α -glucosidase; DFT; molecular docking; ADMET properties



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1. Introduction

A number of metabolic diseases, including hyperglycemia, which is defined by an increased blood glucose level brought on by insufficient insulin secretion, metabolic action, or both, which are linked to diabetes mellitus [1,2]. Because it is regarded as a preventable condition, type 2 diabetes is more significant than type-1 diabetes. An imbalance between the release of insulin and the consumption of glucose results in type 2 diabetes. The primary strategy for preventing type 2 diabetes is blood sugar control [3]. With proper dietary

management and medicine, dynamic insulin release can help achieve this goal [4–6]. In 1998, the UK Project Diabetes Research (UKPDS) Group suggested carbohydrate-rich foods, particularly those containing rapidly digestible starches and sugars, which are known to cause a more pronounced increase in postprandial blood glucose. One of the most popular health remedies at the moment is choosing from a list of carbohydrate-rich foods based on their effect on blood sugar. To optimize the effects, nutritional therapy has been found to be effective when combined with various clinical drugs [7–9]. This strategy has the disadvantage of restricting the kinds and amounts of carbohydrate-rich food that can be consumed. Delaying the digestion of dietary starch, the main dietary source of glucose, may also limit the rate at which glucose is absorbed from the small intestine [6]. Because it eliminates the need for testing, modifications, and result sharing, this approach is thought to be more successful than controlling insulin release [5]. Inhibiting enzymes like α -amylase and α -glucosidase, which break down starches into glucose, has become a promising approach for managing blood sugar levels [10–12]. α -Amylase facilitates the breakdown of starch into maltose and glucose by cleaving (1,4)-glycosidic bonds [13,14], while α -glucosidase releases glucose from maltose or sucrose [15,16]. Both enzymes function in the small intestine, with α -amylase also present in saliva. By inhibiting these enzymes, glucose absorption into the bloodstream can be delayed, offering a potential method for controlling type 2 diabetes. Research has focused on discovering α -amylase and α -glucosidase inhibitors that could be used in food or as additives. Evidence [17,18] suggests that some sugar-like phenolic compounds may effectively block α -glucosidase. Much of the research into these inhibitors has centered on phenolic compounds [19,20]. Recent advances in diabetes research highlight diverse therapeutic and diagnostic strategies, including the regulation of selenoproteins as a novel approach to diabetes treatment, the role of astragalus in improving intestinal barrier function and immunity via modulation of gut microbiota in type 2 diabetes. The following have also been investigated: the genetic influence of ICAM1 polymorphisms on diabetes risk, the development of glucose-responsive nanozyme hydrogels for glycemic control and anti-infective diabetic wound healing, and the application of dual-branch attention networks combined with serum Raman spectroscopy for the diagnosis of diabetic kidney disease [21–25].

Designing small organic molecules with specific functional groups that enable highly selective binding to macromolecules is essential for speeding up the drug discovery process [26]. As a result, synthesizing molecules with therapeutic potential has become a key goal in organic and medicinal chemistry research. One approach in developing new, potent small molecules involves combining two or more bioactive heterocyclic structures into a single molecule, creating heterocyclic hybrid compounds that are expected to exhibit enhanced activity with diverse mechanisms of action [27–31].

Over the last decade, benzothiazole derivatives have emerged as important scaffolds due to their remarkable structural diversity, making them highly valuable for the design, optimization, and development of novel pharmaceutical agents [32]. The benzothiazole nucleus is already incorporated into several clinically used drugs, such as *riluzole* for amyotrophic lateral sclerosis, *zopolrestat* for diabetes, and *frentizole*, which functions as an immunosuppressive and antiviral agent (Figure 1) [33]. Literature surveys have further highlighted their broad spectrum of biological activities, including antimicrobial [34,35], antitumor [36,37], anti-parasitic [38], antioxidant [39], anti-inflammatory and anti-diabetic profile [40,41].

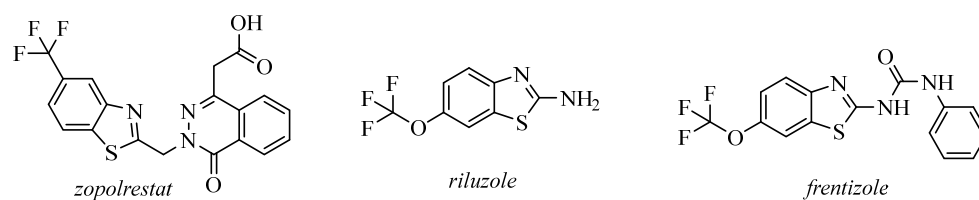


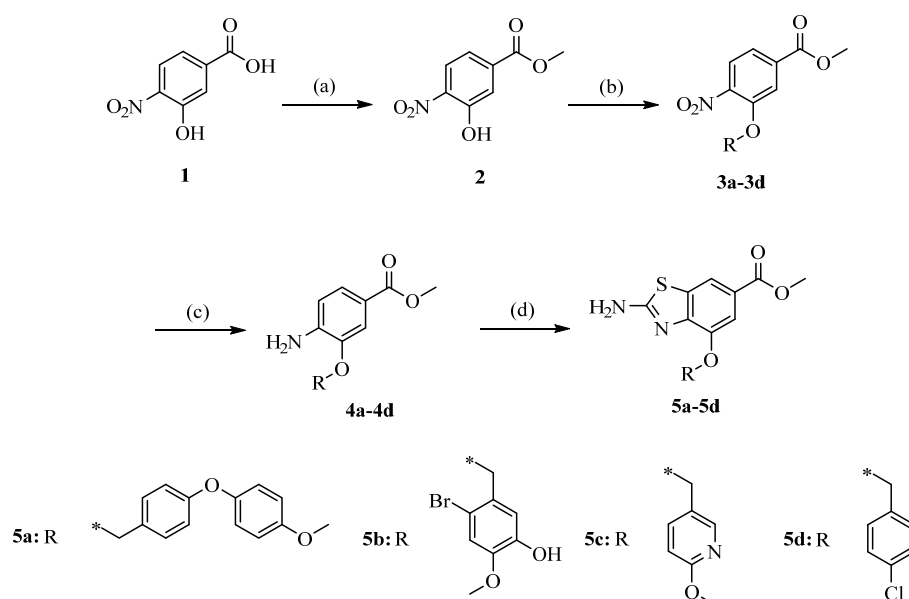
Figure 1. Clinically used benzothiazole derivatives.

In search of exploring new benzothiazole-based therapeutic candidates, the current work aimed to design, synthesize, and evaluate the biological activities of benzothiazole derivatives.

2. Results and Discussion

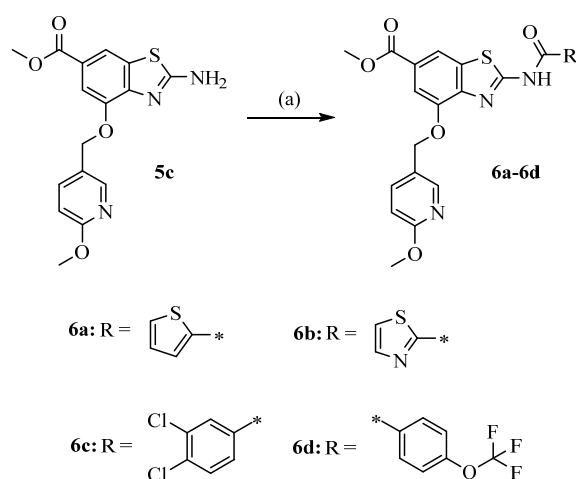
2.1. Chemical Synthesis

As shown in Scheme 1, the compounds **5a–5d** were afforded starting from 3-hydroxy-4-nitrobenzoic acid **1**. Initially, the acid group was changed to an ester group in methanol and sulfuric acid. Then, different substituted alkyl halides were reacted with methyl 3-hydroxy-4-nitrobenzoate **2** to obtain intermediates **3a–3d**. The intermediates **3a–3d** further underwent catalytic hydrogenation to reduce the $-\text{NO}_2$ group into the $-\text{NH}_2$ group as in substrates **4a–4d**. Finally, intermediates **4a–4d** were cyclized with bromine and potassium thiocyanate in acetic acid to offer the targeted benzothiazole-based compounds **5a–5d** in 45–63% yield.



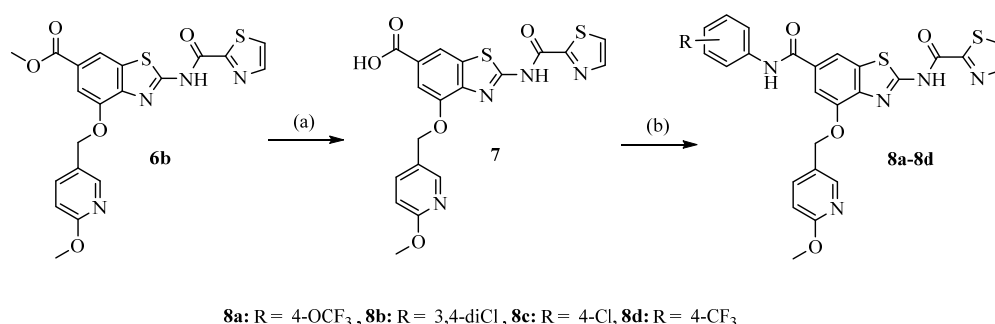
Scheme 1. Synthesis of benzothiazole derivatives (**5a–5d**). **Reagents and conditions:** (a) MeOH, H_2SO_4 , 70°C , 13 h, (b) Corresponding alkyl halide, K_2CO_3 , CH₃CN or DMF, 55°C , 10 h, (c) H_2 , Pd/C, MeOH, rt, 3–6 h, (d) KSCN , Br_2 , CH_3COOH , 15°C , then rt, 17 h, 25% aq. NH_3 .

Scheme 2 outlines the synthesis of compounds **6a–6d** starting from previously afforded compound **5c**. To obtain targeted derivatives **6a–6d**, the $-\text{NH}_2$ group of compound **5c** was condensed with a diversity of corresponding acids using HATU as a condensing agent. DCM was used as the solvent for this reaction. The yield of the synthesized product ranged from 45–58% yield.



Scheme 2. Preparation of compounds (6a–6d). **Reagents and conditions:** (a) Corresponding acid, HATU, DIPEA, DCM, rt, 4h.

Scheme 3 describes the synthesis of derivatives 8a–8d. To obtain compounds 8a–8d, the ester group of compound 6b was converted to an acid group, as in substrate 7, using lithium hydroxide in THF/H₂O. Finally, substrate 7 was treated with various corresponding acids in DCM and DIPEA, followed by adding HATU as a condensing agent to obtain the targeted compounds 8a–8d in 53–67% yield. Formation of all these derivatives were confirmed by NMR studies illustrated in (Figures S1–S6).



Scheme 3. Preparation of compounds (8a–8d). **Reagents and conditions:** (a) LiOH, THF/H₂O, 35 °C, 4 h, (b) Corresponding acid, HATU, DIPEA, DCM, rt, 4 h.

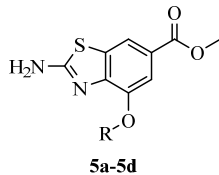
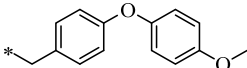
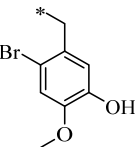
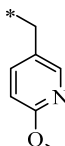
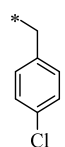
2.2. In Vitro α -Amylase and α -Glucosidase Activity, and Structure-Activity

Relationship (SAR) investigations

Structural modification was initiated by introducing variously substituted alkyl halides in place of the hydrogen atom on the 4-OH group of the benzothiazole scaffold. The impact of these substitutions on α -amylase and α -glucosidase inhibitory activity was observed. The compact inactivity was observed by adding 1-ethyl-4-(4-methoxyphenoxy) benzene group over the oxygen of the –OH group as in analog 5a, likely due to steric hindrance provided by the bulky group, but still this compound exhibited lower potency than acarbose (positive control). With the exception of 1-ethyl-4-(4-methoxyphenoxy)benzene substituent (5a), which is unfavorable to activity, the introduction of functional moieties that are capable of providing better interactions, such as ethylene-2-methoxypyridine (5c), 2-bromo-4-methoxy-5-hydroxy benzyl (5b), or 4-chloro benzyl (5d) moieties, exhibited comparable or somewhat lower potency compared to the standard acarbose drug. In addition, both α -amylase and α -glucosidase potency were sharply increased 3-fold by replacing the 2-bromo-4-methoxy-5-hydroxy benzyl group (5b) with the ethylene-2-methoxypyridine moiety as in compound 5c. The enhanced potency may be owing to the insertion of a

nitrogen heteroatom into the ring with an $-\text{OCH}_3$ group at the 2-position of the pyridine ring, which creates polarity in the ring and helps better binding interactions, and hence improved potency. The comparison of compound **5c** bearing ethylene-2-methoxypyridine moiety with compound **5d** having 4-chlorobenzyl moiety demonstrated that compound **5c** displayed superior potency than its counterpart **5d**. Alternatively, compound **5d** showed 2-fold improved activity for both α -amylase and α -glucosidase relative to compound **5b**, as shown in Table 1. Among these synthesized compounds, was compound **5b** with ethylene-2-methoxypyridine moiety, which showed better binding interactions with the enzyme active site, and hence compound **5b** was selected for further optimization of structure activity relationship investigation (Table 1).

Table 1. Synthesized compounds (**5a–5d**) and their in vitro activity against anti-diabetic proteins.

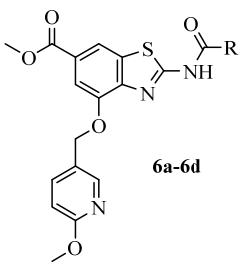
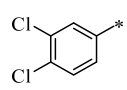
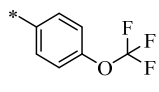
 5a-5d				
Cpds	R	α -Amylase IC ₅₀ $\pm$ SD (μM)	α -Glucosidase IC ₅₀ $\pm$ SD (μM)	
5a		N.D	N.D	
5b		5.64 $\pm$ 3.10	5.78 $\pm$ 2.90	
5c		1.73 $\pm$ 2.50	3.56 $\pm$ 3.80	
5d		3.23 $\pm$ 2.10	5.15 $\pm$ 3.20	
Standard Acarbose drug		0.91 $\pm$ 0.20	1.80 $\pm$ 1.10	

N.D means Not determined.

To further improve the α -amylase and α -glucosidase potency, the ethylene-2-methoxypyridine moiety was retained, and further screening was focused on modifying the *N*-acyl fragment positioned at the 2-position of the benzothiazole scaffold, as presented in Table 2. We screened the influence of incorporating various *N*-acyl substituents (R) on α -amylase and α -glucosidase activities. Notably, it has been reported that the introduction of the thiazole moiety in compound **6b** resulted in an increase in potency against α -amylase and α -glucosidase. Compared to compound **5c**, the analog **6b** showed enhanced activities. In addition, the incorporation of thiophene (**6a**), 3,4-dichlorophenyl (**6c**), or 4-trifluoromethoxyphenyl (**6d**) moieties maintained or decreased the potency. In particular, the analog **6a** with the thiophene moiety showed significantly improved potency compared to analogs **6c** and **6d**. Compared to analog **6c**, analog **6d** showed better α -amylase and α -glucosidase activities, suggesting that the contribution of $-\text{OCF}_3$ phenyl moiety accomplished a large impact on potency. Taken together, explorations of structure–

activity relationships (SARs) showed that the thiazole acetamide substituent at C2 of the benzothiazole scaffold can contribute to improved potency. Hence, further optimization was performed at the ester moiety of the benzothiazole scaffold, while retaining the thiazole acetamide substituent at C2 of the benzothiazole scaffold correspondingly (Figure 2, Table 2).

Table 2. In vitro activities of newly afforded compounds (6a–6d).

			
Cpds	R	α -Amylase IC ₅₀ $\pm$ SD (μ M)	α -Glucosidase IC ₅₀ $\pm$ SD (μ M)
6a		2.69 $\pm$ 2.20	3.21 $\pm$ 1.70
6b		1.58 $\pm$ 1.20	2.10 $\pm$ 1.10
6c		7.54 $\pm$ 3.60	8.90 $\pm$ 4.10
6d		4.10 $\pm$ 1.90	4.70 $\pm$ 1.80
Standard Acarbose drug		0.91 $\pm$ 0.20	1.80 $\pm$ 1.10

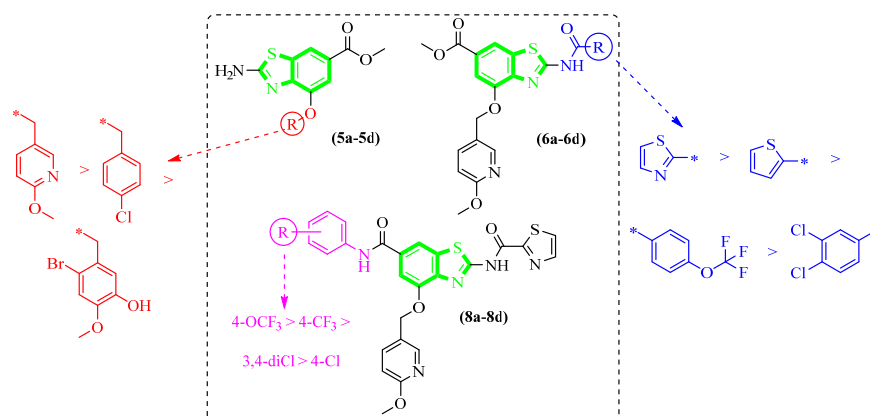
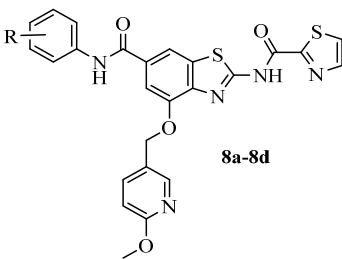


Figure 2. Summary of SAR studies for synthesized compounds.

Contrary to expectations, we observed that modification of the ester group at C6 of the benzothiazole scaffold to variously substituted *N*-phenyl moiety, as in compounds (8a–8d), resulted in a decrease in potency against α -amylase and α -glucosidase proteins. Among them, the analog 8a with a 4-OCF₃ phenyl moiety showed improved activity compared to its counterparts 8b–8d. Compared to analog 8a, analogs 8b (having a 3,4-dichlorophenyl ring) and 8d (4-CF₃ phenyl moiety) showed somewhat lower activity. The analog 8c bearing a 4-chlorophenyl group emerged as the least competitive of α -amylase and α -glucosidase, with IC₅₀ values of 13.65 $\pm$ 4.50 (for α -amylase) and 15.35 $\pm$ 4.80 (for α -glucosidase), respectively. The activity was sharply improved by replacing the 4-Cl group (8c) with either

-OCF₃ (**8a**) or 4-CF₃ groups (**8d**). Moreover, the introduction of an additional Cl group at the 3-position of the phenyl group also improved the potency, as in analog **8b** (Table 3).

Table 3. In vitro activities of newly afforded compounds (**8a–8d**).



Cpds	R	α -Amylase IC ₅₀ $\pm$ SD (μ M)	α -Glucosidase IC ₅₀ $\pm$ SD (μ M)
8a	4-OCF ₃	5.72 $\pm$ 4.10	6.24 $\pm$ 3.60
8b	3,4-diCl	8.73 $\pm$ 3.10	9.68 $\pm$ 4.30
8c	4-Cl	13.65 $\pm$ 4.50	15.35 $\pm$ 4.80
8d	4-CF ₃	7.50 $\pm$ 2.20	7.85 $\pm$ 2.40
Standard Acarbose drug		0.91 $\pm$ 0.20	1.80 $\pm$ 1.10

2.3. Molecular Docking

2.3.1. Docking Studies on α -Amylase

In order to determine the possible interactions of the selected α -amylase inhibitors in this series (compounds **5c**, **6b**, and **8a**) and acarbose with the enzyme active site, docking experiments were performed on the crystal structure of α -amylase (PDB Code: 4W93) are illustrated in Table 4 and Figure 3. The important acidic residues ASP197, GLU233, ASP300, ASP304, and GLU380 are found in the substrate-binding region of the α -amylase active site. These residues of amino acids help break down the substrate by forming hydrogen bonds and electrostatic interactions. Among the catalytic residues are GLU233 and ASP197. The hydrophobic pocket formed by TRP59, TRP62, and PHE63 is located in the cleft region of the active site. Several basic residues, such as ARG195, ARG197, and LYS201, are also present in the active site.

Table 4. Binding energies of selected compounds and reference drug on α -amylase and α -glucosidase.

Compound	α -Amylase	α -Glucosidase
	Binding Energy (kcal/mol)	Binding Energy (kcal/mol)
5c	−5.61	−7.20
6b	−6.98	−8.46
8a	−7.98	−9.60
Acarbose	−7.63	−3.38

Docking studies revealed that the standard drug (acarbose) exhibited vital and stable binding affinities at the active site of α -amylase with a binding energy of −7.63 kcal/mol (Table 4). The binding affinities include the number of hydrogen bonds and important hydrophobic interactions with amino acid residues. With the acidic residues ASP300 and ASP197, acarbose formed a conventional hydrogen bond and a carbon–hydrogen bond at bond lengths of 2.74 Å and 2.51 Å, respectively. These residues are important for the hydrolytic activity of α -amylase. Multiple hydroxyl and oxygen groups of acarbose form strong conventional hydrogen bonds with key residues, including ARG195 (2.21 Å), HIS299 (2.12 Å, 2.58 Å), GLU233 (2.14 Å), HIS201 (2.54 Å), TYR 151 (2.72 Å), and LEU237 (2.35 Å),

which play a critical role in stabilizing the complex. Acarbose's hydrogen exhibited π - σ interaction with residue TYR 62 at a spacing of 2.87 Å. It also exhibited carbon–hydrogen bonds with ASP 236 (2.75 Å), GLU 240 (2.85 Å), and GLU 233 (2.75 Å). These various interactions with active site regions suggest that they can lead to the α -amylase enzyme inhibition.

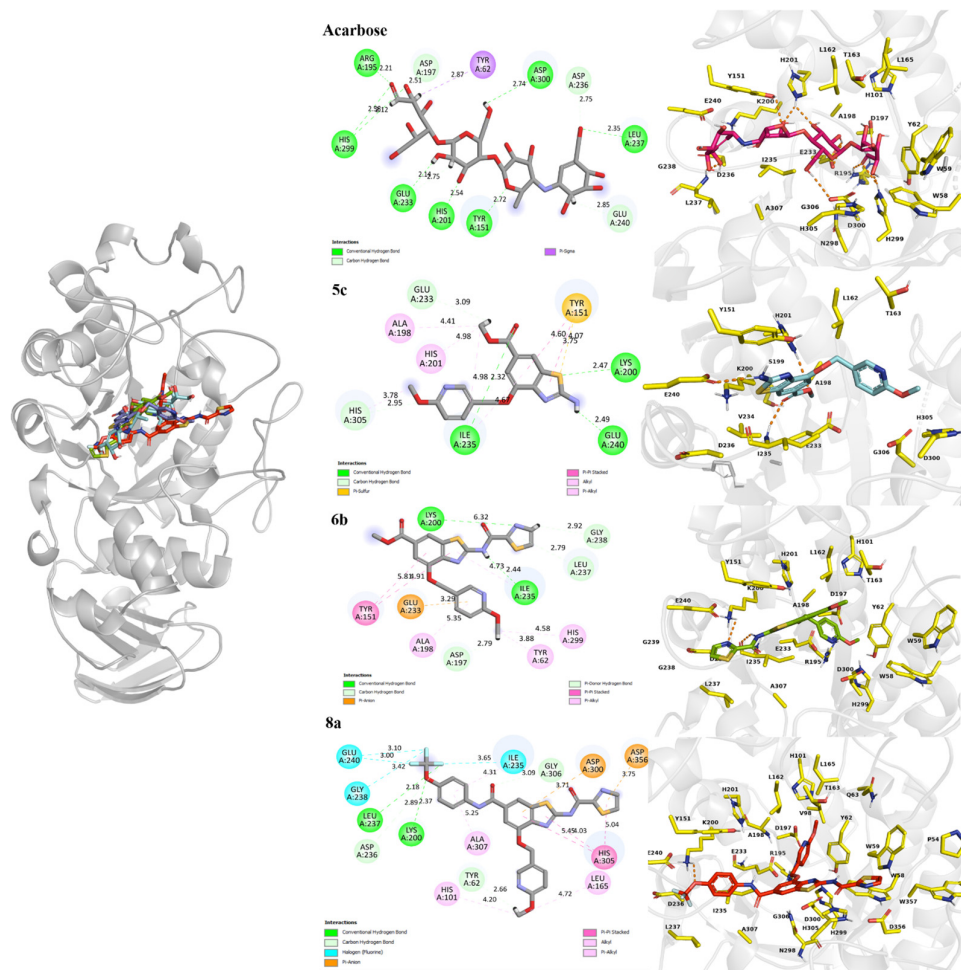


Figure 3. Binding interactions of benzothiazole-based derivatives with amylase (PDB ID: 4W93). The reference inhibitor acarbose is shown alongside representative test compounds (compounds **5c**, **6b**, **8a**).

The compound **5c** exhibited normal binding affinities with the α -amylase, indicated by its binding energy of -5.61 kcal/mol. Various hydrophilic and hydrophobic forces inside the active site stabilize the contact. Key conventional hydrogen bonds are formed with the residues GLU 240 (2.49 Å), LYS 200 (2.47 Å), and ILE 235 (2.32 Å), providing strong, direct polar stabilization to the complex. Additionally, there are notable hydrophobic and aromatic interactions that define the interaction. Sulfur and the aromatic ring of benzothiazole oriented themselves to form a π -sulfur interaction with TYR 151 at a bond distance of 3.75 Å, along with two π - π stacked interactions (4.07 Å, 4.60 Å), which contribute to the binding through aromatic ring stacking. The stability of the complex is further improved by non-polar alkyl interactions with ALA 198 (4.41 Å), ILE 235 (4.98 Å), and π -alkyl interactions with residues HIS 305 (3.78 Å), HIS 201 (4.98 Å), and ILE 235 (4.67 Å). These interactions aid in the ligand's anchoring within the binding site's hydrophobic pockets. While the standard drug depends on extensive hydrogen bonding with catalytic residues, compound **5c** engages in a more varied range of interactions, including π -sulfur, π - π stacking, and π -alkyl contacts with residues, and it may show greater stability in hydrophobic environments, as it is less dependent on strong polar contacts.

A stable interaction profile including a combination of hydrogen bonding, hydrophobic forces, and π -interactions is revealed by the docking analysis of the compound **6b** with α -amylase, with a binding energy score of -6.98 kcal/mol. With bond distances of 2.44 Å and 6.32 Å, respectively, the ligand forms conventional hydrogen bonds with ILE235 and LYS200, which are within the close hydrogen-bonding range and offer substantial stability. A noteworthy interaction of aromatic moiety is observed with GLU233, which contributes to a π -anion interaction at a distance of 3.29 Å. It is a key catalytic residue of α -amylase that helps to break glycosidic bonds, while hydrophobic interactions further enhance the stability of the ligand. The ligand shows π - π stacking with TYR151 (4.91 Å, 5.81 Å), as well as π -alkyl interactions with ALA 198 (5.35 Å), TYR 62 (3.88 Å), HIS 299 (4.58 Å), LEU 237 (4.70 Å), and ILE 235 (4.73 Å). Overall, binding is enhanced by such types of interactions that stabilize the ligand in the hydrophobic cleft. Compound **6b** also exhibited a carbon-hydrogen bond with residues ASP 197 (2.79 Å) and GLY 238 (2.92 Å). Compared to the reference drug, compound **6b** forms π - π -anion and strong π - π stacking interactions, which stabilize the ligand interaction to mimic aromatic sugar binding.

Compound **8a** exhibited excellent binding affinity, which is indicated by its binding energy value of -7.98 kcal/mol. Within the active region of the enzyme, a complex network of interactions stabilizes this strong contact. The fluorine and oxygen atoms of the compound formed key conventional hydrogen bonds with residues LYS200 (2.37 Å) and LEU237 (2.18 Å), providing crucial contacts that anchor the ligand in place. Fluorine atoms have also developed notable halogen interactions with important residues ILE235 (3.65 Å), GLY238 (3.42 Å), and GLU240 (3.00 Å, 3.10 Å), locking the inhibitor into the correct position within the active site. Furthermore, a π -anion interaction with aspartates ASP300 (3.71 Å) and ASP356 (3.75 Å) adds substantial electrostatic stabilization to the complex. Strong aromatic and hydrophobic packing interactions further increase the complex's stability. π - π stacked interactions, potentially with HIS305 (4.03 Å, 5.04 Å, 5.45 Å), contribute to the binding through aromatic ring stacking. Additionally, alkyl interactions with LEU165 (4.72 Å) and π -alkyl interactions with residues ALA307 (5.25 Å), HIS101 (4.20 Å), and ILE235 (4.31 Å) help to cover the ligand entirely within a hydrophobic pocket of the binding site. Three carbon-hydrogen bonds are also observed with residues TYR62 (2.66 Å), ASP236 (2.89 Å), and GLY306 (3.09 Å). The exceptionally high binding affinity is explained by the wide range of different interaction types, including hydrophobic, halogen, electrostatic, and hydrogen bonding forces, giving it more stabilization in the catalytic pocket. So, in comparison to the reference drug acarbose and selected compounds, **8a** shows a stronger binding affinity and a diverse interaction profile, which makes it a more potent and promising inhibitor of α -amylase compared to acarbose.

2.3.2. Docking Studies on α -Glucosidase

The synthesized compounds **5c**, **6b**, and **8a**, and acarbose, were docked on the crystal structure of the α -glucosidase (PDB Code: 3A4A) active site in a comparable procedure to assess their binding affinities (as revealed in Table 4) and interactions (Figure 4). The catalytic triad for substrate catalysis in α -glucosidase is made up of ASP215, GLU277, and ASP352. In addition to the catalytic residues, ASP62, TYR72, and ARG442 are situated close to the active site and hence involved in the catalytic event. With the substrate and the competitive inhibitor of α -glucosidase, the binding site, which is composed of TRP58, PHE301, PHE303, TYR347, TYR387, TYR389, TYR158, HIS280, ASP69, ARG446, ASP409, VAL410, and GLU408, mediates a variety of hydrophilic and hydrophobic interactions.

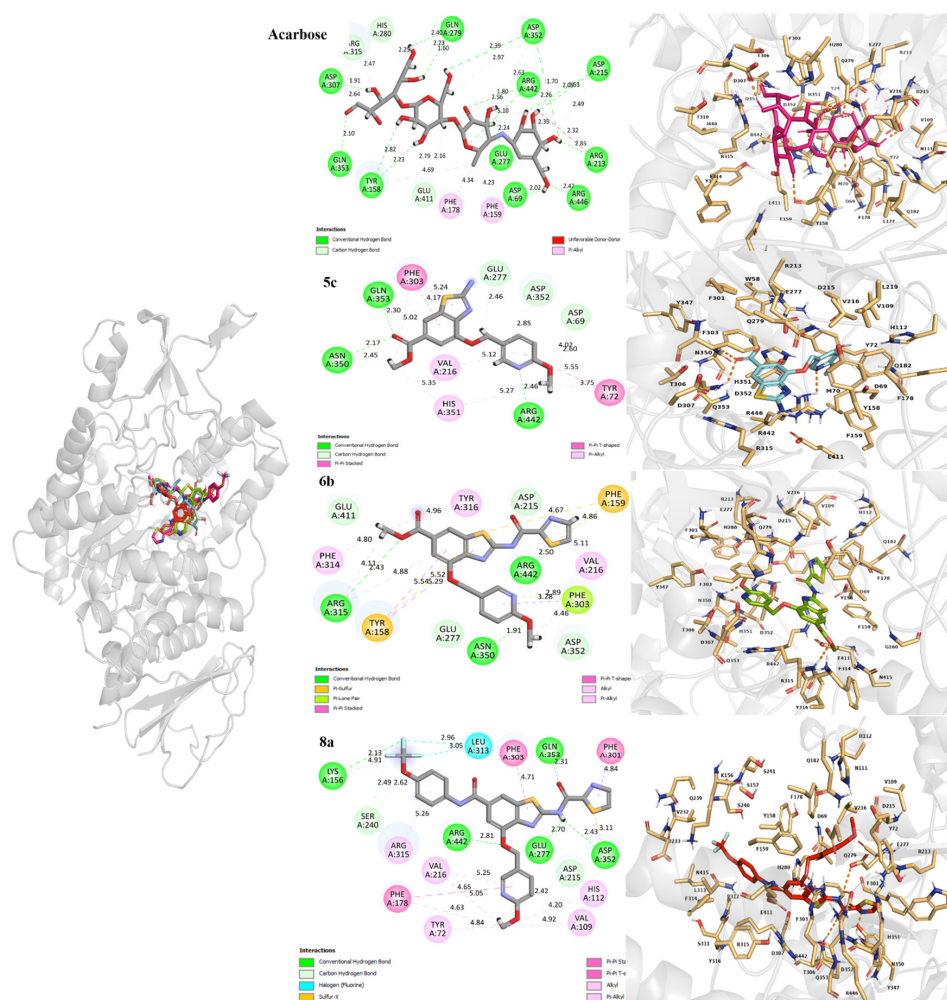


Figure 4. Binding interactions of benzothiazole-based derivatives with glucosidase (PDB ID: 3A4A). The reference inhibitor acarbose is shown alongside representative test compounds (Compounds 5c, 6b, 8a).

Acarbose establishes exceptionally strong and specific binding within the active site of α -glucosidase with a binding energy of -3.38 kcal/mol, achieved through a dense network of hydrogen bonds that directly engage key structural and catalytic residues. Critically, multiple oxygens and hydrogens of acarbose form strong, short-distance conventional hydrogen bonds with catalytic aspartic acids ASP307 (1.91 Å), ASP69 (2.02 Å), ASP215 (1.53 Å, 2.09 Å), ASP352 (1.70 Å, 2.39 Å), GLN279 (1.60 Å, 2.40 Å), GLN353 (2.10 Å), GLU277 (2.24 Å), TYR158 (2.23 Å, 2.82 Å), ARG213 (2.85 Å), ARG446 (2.47 Å), and ARG442 (1.80 Å, 5.10 Å), which aid to perfectly position and secure the acarbose molecule within the pocket. π -alkyl interactions of the ligand with PHE159 (4.23), PHE178 (4.34), and TYR158 (4.69) also contribute to hydrophobic stability. Additionally, acarbose also exhibited carbon–hydrogen bonds with key residues ASP 307 (2.64 Å), ARG 315 (2.47 Å), HIS 280 (2.25 Å), GLU411 (2.16 Å, 2.79 Å), ARG442 (2.39 Å), GLN279 (2.23 Å), ASP352 (2.26 Å, 2.63, 2.97 Å), and ASP215 (2.49 Å). Even though the majority of interactions are quite strong, the existence of one unfavorable donor–donor interaction with ARG213 (2.32 Å) points to a little conflict that is probably counterbalanced by the large number of stabilizing factors. A wide variety of conventional hydrogen bonds and carbon–hydrogen bonds predominate in the binding, creating a tight contact shell that accounts for its strong inhibitory effect.

Compound 5c demonstrates effective and strong binding within the active site of the target enzyme with a binding energy of -7.20 kcal/mol. Certain polar and hydropho-

bic interactions aid in stabilizing the binding pose. Key conventional hydrogen bonds are formed with the residues ARG442 (2.46 Å), ASN350 (2.17 Å), and GLN353 (2.30 Å), providing strong, direct anchoring to the protein backbone. This is complemented by carbon–hydrogen bonds with catalytic residues ASP352 (2.85 Å), GLU277 (2.46 Å), and ASP69 (2.60 Å, 4.02 Å), which additionally solidify the ligand's position. A substantial π - π stacked interaction, potentially with PHE303 (4.17 Å, 5.24 Å), and a π - π T-shaped interaction with TYR72 (5.55 Å) designate strong aromatic stacking, a critical factor for stabilizing the complex within the aromatic-rich active site. Additionally, π -alkyl interactions with residues TYR72 (3.75 Å), VAL216 (5.12 Å), HIS351 (5.27 Å, 5.35 Å), and PHE303 (5.02 Å) significantly contribute to hydrophobic stability. The variety of interaction types, like strong hydrogen bonding, hydrophobic contacts, and precise aromatic stacking, explains the highly favorable binding energy. Compound **5c** binds to the target with over twice the affinity of the reference drug and may show greater stability in hydrophobic environments.

Compound **6b** displays exceptionally strong and potent binding to the target enzyme, as evidenced by its highly favorable binding energy of -8.46 kcal/mol. The enzyme's active region contains a wide and varied network of interactions that enable this strong binding, making it a very promising inhibiting agent. A strong, short-distance conventional hydrogen bond with ASN 350 (1.91 Å), ARG442 (2.50 Å), and ARG315 (2.43 Å) serves as a crucial anchor point that mostly drives the complex's stability. Additional polar contacts with GLU411 (2.83 Å), ASP215 (4.42 Å), GLU277 (2.54 Å), and ASP352 (2.86 Å) complement the ligand, which is positioned precisely. The nitrogen of the pyridine moiety developed a notable π -lone pair interaction with PHE303 (2.89 Å). Prominent π -based interactions further greatly improve binding. The sulfur atoms of the compound exhibited π -sulfur interactions with residues PHE159 (4.67 Å, 4.86 Å) and TYR158 (5.52 Å). While π - π stacked with PHE303 (3.28 Å) and π - π T-shaped interactions with TYR158 (5.29 Å, 5.45 Å) provide substantial stabilization through aromatic stacking. To engage the hydrophobic and aromatic clusters that are frequently present in the active site, these interactions are crucial. Additionally, alkyl and π -alkyl interactions with residues PHE 314 (4.80 Å), TYR316 (4.96 Å), VAL216 (5.11 Å), PHE303 (4.46 Å), and ARG315 (4.11 Å, 4.88 Å) significantly contribute to hydrophobic stability. A complex binding mode is produced by combining a variety of π -interactions (stacked, T-shaped, sulfur, lone pair), strong hydrogen bonds, and a large number of hydrophobic contacts. Unlike standard drugs that rely mostly on hydrogen bonds, compound **6b** combines conventional hydrogen bonds with essential hydrophobic interactions that create a more stable and favorable complex.

With a remarkable binding energy of -9.60 kcal/mol, compound **8a** exhibits a very strong and high-affinity binding contact within the target enzyme's active region. The binding mode is characterized by strong conventional hydrogen bonds with catalytic and structural residues ASP352 (2.43 Å), GLU277 (2.70 Å), GLN353 (2.31 Å), ARG442 (2.81 Å), and LYS156 (2.13 Å), which provide essential polar anchoring to the complex. Carbon–hydrogen bonds provide extra stabilizing forces in addition to this. The occurrence of a halogen bond with LEU313 (2.96 Å, 3.05 Å), which indicates the strategic incorporation of a fluorine atom in the ligand's design, is an important aspect of this interaction, significantly enhancing binding affinity and specificity. Furthermore, the sulfur atom of the compound formed a sulfur-X interaction with ASP352 (3.11 Å). Prominent aromatic and hydrophobic interactions further greatly improve the binding. Interactions like π - π stacked with PHE303 (4.71 Å), PHE301 (4.84 Å), and π - π T-shaped interactions with PHE178 (4.65 Å) are major contributors to binding, creating a favorable environment for the ligand. Additionally, alkyl interactions with residues VAL109 (4.92 Å), VAL216 (5.05 Å), LYS 156 (4.91 Å), and π -alkyl interactions with residues PHE178 (4.63 Å), HIS112 (4.20 Å), TYR72 (4.84 Å), VAL216 (5.25 Å), and ARG 315 (5.26 Å), help the ligand to fit within a hydrophobic pocket. So,

the combination of conventional hydrogen bonds, a unique sulfur-mediated interaction, the halogen bonds, and widespread hydrophobic packing creates a close-to-ideal binding profile. In comparison to acarbose, compound **8a** has exceptionally strong binding affinity and a better interaction profile in terms of hydrophobic interactions.

2.4. Density Functional Theory (DFT) Studies

Density functional theory calculations at the B3LYP/6-31G level were used to characterize the electronic structure of the series. Frontier molecular orbital analysis provided the HOMO, the LUMO, and the band gap ΔE , where $\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$. The HOMO energy reflects the electron-donating capacity of a molecule, while the LUMO energy indicates its ability to accept electrons. Smaller ΔE values generally imply higher chemical reactivity and lower kinetic stability, which in related systems has often coincided with stronger biological activity. In this set, compound **6b** showed the narrowest gap (2.95 eV), followed by **8a** (3.02 eV), suggesting comparatively higher reactivity. Compound **5c** displayed the largest gap (4.4 eV), consistent with greater stability and a lower predicted reactivity. These trends are in line with literature reports that associate reduced band gaps with enhanced bioactivity (Figure 5, Table 5).

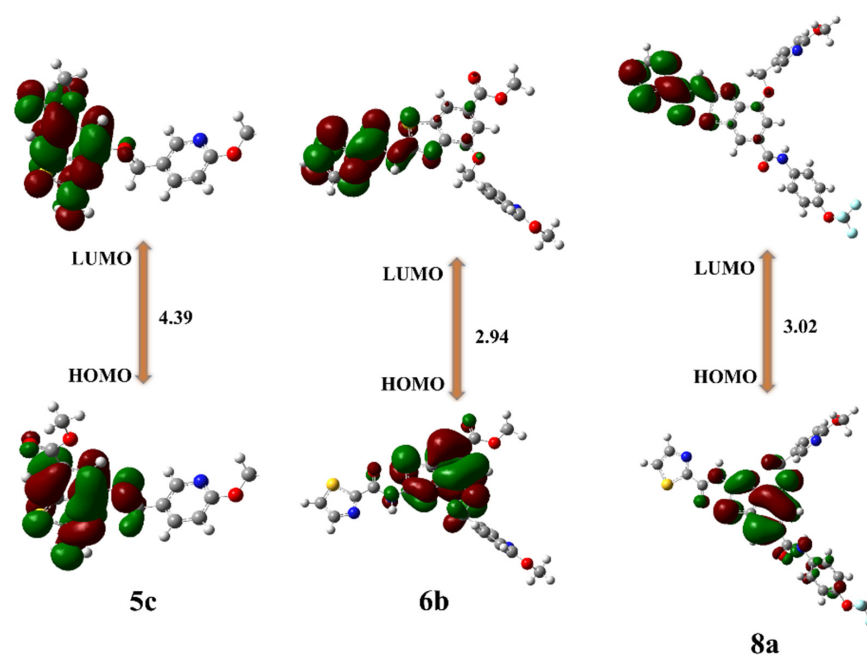


Figure 5. The calculated ground state isodensity surface plots for frontier molecular orbitals (FMOs) for investigated compounds **5c**, **6b**, and **8a**.

Table 5. FMO energy of the synthesized hybrids.

Compound	LUMO (eV)	HOMO (eV)	ΔE (eV)
5c	−5.72	−1.32	4.4
6b	−5.69	−2.74	2.95
8a	−5.85	−2.83	3.02

In FMO analysis, global reactivity descriptors were calculated to determine the molecular properties. The electron affinity (EA) and ionization potential (IP) define the electron-accepting and electron-donating tendencies of synthesized compounds, respectively. Electronegativity (χ) reflects the tendency of a molecule to attract electrons. Chemical potential (μ), hardness (η), softness (S), and the electrophilicity index (ω) were derived from these values to provide a comprehensive picture of the reactivity of compounds. Compounds

6b and **8a** were characterized by relatively low hardness ($\eta \approx 1.5$ eV) and high softness ($S \approx 0.33$ eV⁻¹). The hardness values suggest their strong chemical reactivity. Both exhibited high electrophilicity indices ($\omega = 6.03$ for **6b** and 6.25 for **8a**). The electrophilicity index indicates a greater ability to stabilize upon electron acceptance. In comparison, compound **5c** shows higher hardness (2.19 eV), lower softness (0.22 eV⁻¹), and a lower electrophilicity value (2.83) that is consistent with reduced reactivity. These descriptors show that **6b** and **8a** are excellent candidates for biological interactions, while **5c** is likely to be less active (Table 6).

Table 6. Reactivity indices of the synthesized derivatives.

Compound	EA	IP	χ	μ	η	S	ω
5c	1.32	5.72	3.52	−3.52	2.19	0.22	2.82
6b	2.74	5.69	4.21	−4.21	1.47	0.34	6.03
8a	2.83	5.85	4.34	−4.34	1.51	0.33	6.25

Abbreviations: Electron affinity, (EA); Ionization energy, (IP); Chemical softness, S; Chemical hardness, η ; Chemical potential, μ ; Electronegativity, χ ; Electrophilicity, ω .

Electrostatic potential (ESP) mapping (Figure 6) was further employed to visualize the distribution of electron density and predict possible sites of electrophilic and nucleophilic attack. The ESP surfaces revealed that the electron-rich regions (negative potential, shown in red) were primarily localized around heteroatoms such as nitrogen, oxygen, and sulfur, while electron-deficient regions (positive potential, shown in blue) appeared over hydrogen atoms and selected ring systems. Notably, compounds with broader distributions of high and low potential zones, such as **6b** and **8a**, exhibited stronger electronic polarization, which may facilitate interactions with biological targets. This observation parallels earlier reports, where derivatives displaying more pronounced ESP variations correlated with enhanced cytotoxic potential.

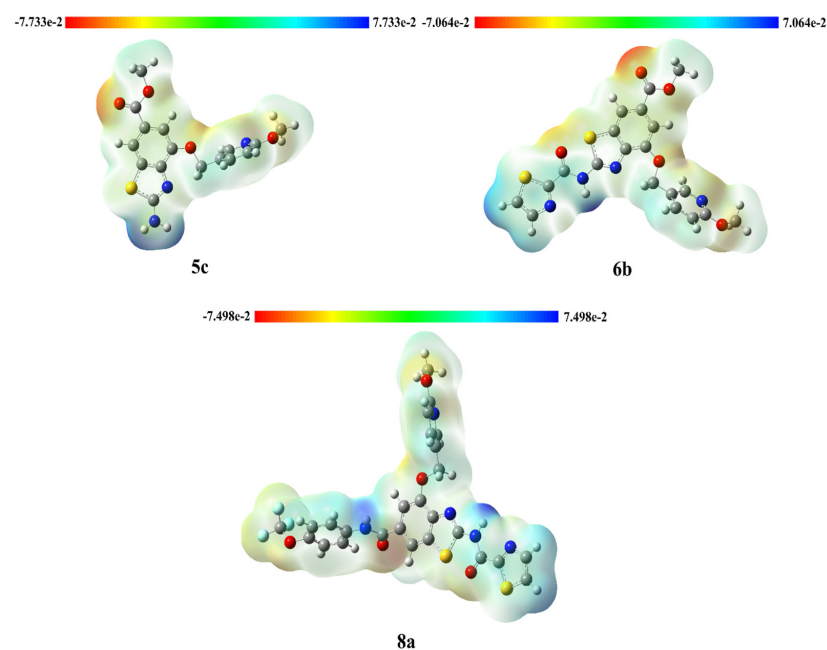


Figure 6. The ESP maps for investigated compounds **5c**, **6b**, and **8a**.

2.5. ADME and Pharmacokinetic Evaluation

Although α -amylase and α -glucosidase are physiologically active in the intestinal lumen and do not require systemic drug absorption for inhibition, ADME and CYP3A4

forecasting have been incorporated as auxiliary methods in early-phase safety assessment and lead optimization. These analyses were applied to assess the possible systemic exposure and metabolic liabilities and off-target risks that can occur due to partial absorption, and to distinguish between the gut-restricted and systemically available candidates.

2.5.1. Absorption

Compound **5c** displayed favorable absorption characteristics, with high gastrointestinal (GI) absorption predicted and no violations across standard drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge). Its moderate lipophilicity (consensus LogP = 2.49) and relatively large topological polar surface area (TPSA = 124.8 Å²) suggest that the molecule is well balanced between solubility and permeability, which supports oral bioavailability. In contrast, **6b** and **8a** showed reduced GI absorption potential. Compound **6b**, despite moderate lipophilicity (LogP = 3.06), had a higher TPSA (169.01 Å²), which likely hinders passive diffusion. Compound **8a** was the least favorable for absorption, with TPSA exceeding 181 Å² and high lipophilicity (LogP = 4.57), both of which predict poor solubility and permeability (Figures 7–9).

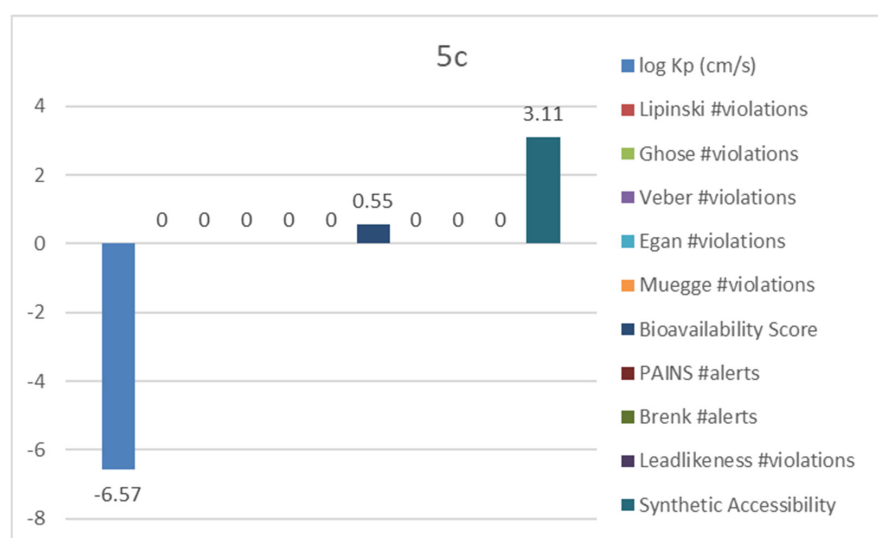


Figure 7. ADME analysis of **5c**.

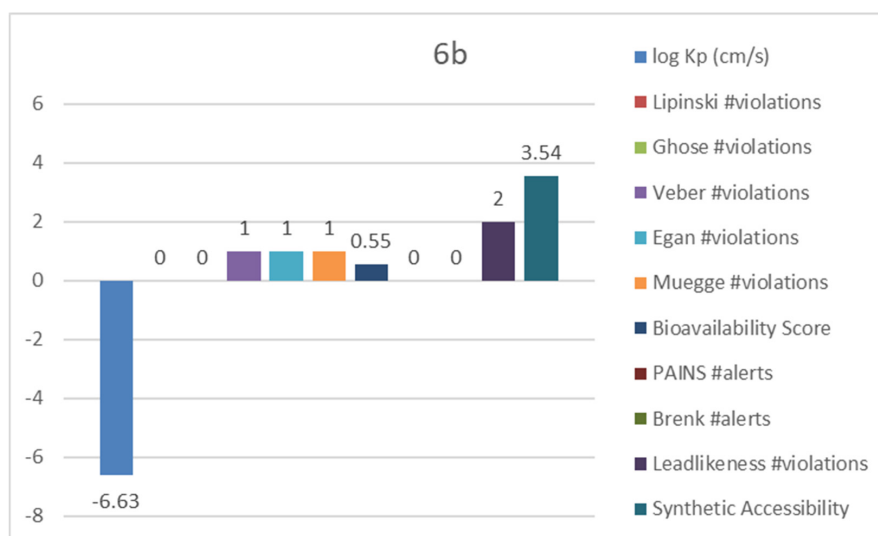


Figure 8. ADME analysis of **6b**.

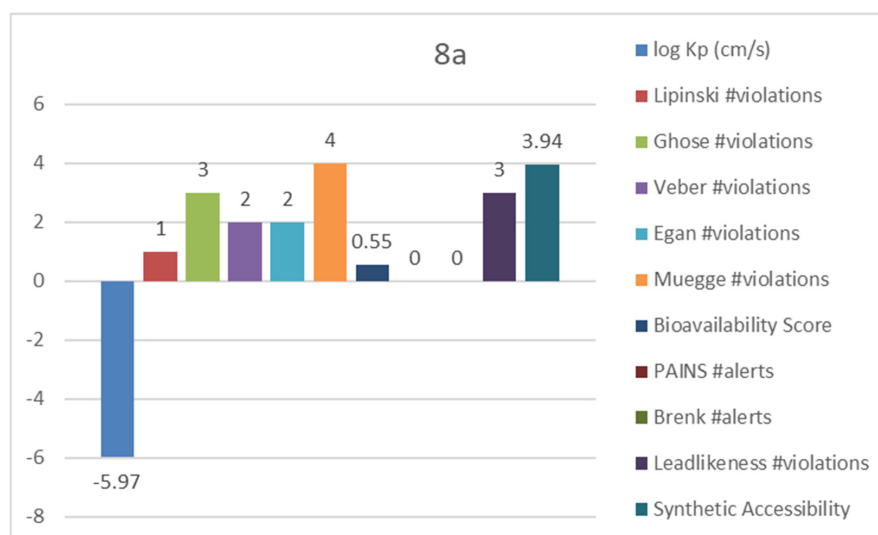


Figure 9. ADME analysis of **8a**.

2.5.2. Distribution

None of the compounds were predicted to cross the blood–brain barrier (BBB), which may limit their application in central nervous system (CNS) disorders. Among them, the moderate size (MW = 345.37 Da) and balanced polarity of **5c** suggest better systemic distribution relative to the larger analogs **6b** (MW = 456.49 Da) and **8a** (MW = 601.58 Da). The absence of BBB permeability, however, indicates that their therapeutic activity would likely be restricted to peripheral targets.

2.5.3. Metabolism

Drug-likeness filters revealed notable differences in metabolic suitability. Compound **5c** passed all filters, indicating good compliance with physicochemical thresholds relevant to metabolism and clearance. Compound **6b** showed single violations under Veber, Egan, and Muegge rules, reflecting its higher polarity and molecular complexity. Compound **8a** demonstrated multiple violations across all major filters (Lipinski, Ghose, Veber, Egan, and Muegge), strongly suggesting metabolic instability and limited oral bioavailability.

2.5.4. Excretion

The predicted skin permeability coefficient (log Kp) values ranged from -6.57 cm/s (**5c**) to -5.97 cm/s (**8a**), with the latter indicating slightly higher transdermal penetration potential. However, the high TPSA and molecular size of **8a** would likely hinder renal clearance, raising concerns about tissue accumulation. By contrast, **5c** exhibited lower molecular complexity and a favorable bioavailability score (0.55), implying more predictable pharmacokinetic handling and better elimination.

2.6. Drug-Likeness and Synthetic Feasibility

All compounds demonstrated acceptable synthetic accessibility, with values ranging between 3.11 (**5c**) and 3.94 (**8a**). Compound **5c** again emerged as the most promising candidate, combining balanced lipophilicity, high GI absorption, absence of rule violations, and low synthetic complexity. In contrast, **8a**, with multiple violations, excessive molecular weight, and polarity, is less likely to serve as a viable drug candidate without structural optimization.

2.7. SMARTCyp Metabolic Soft Spot Prediction

The metabolic liability of compounds **5c**, **6b**, and **8a** was assessed using the SMARTCyp algorithm, focusing on CYP3A4, a major isoform responsible for oxidative clearance of lipophilic heteroaromatic scaffolds. The five most labile sites for each compound are summarized in Table 7.

Table 7. SMART Cyp-predicted CYP3A4 metabolic soft spots of lead compounds.

Compound	Rank	Atom ID	Score	Energy	2D-SASA	Possible Metabolic Pathway
5c	1	N.21	44.8	54.1	48.8	<i>N</i> -oxidation/ <i>N</i> -dealkylation
	2	C.1	51.5	62.2	66.4	Aromatic hydroxylation
	3	C.7	56.9	62.2	25.5	Aromatic hydroxylation
	4	S.22	61.5	70.0	43.9	Sulfoxidation/sulfone formation
	5	C.14	65.2	75.9	66.3	Aromatic hydroxylation
6b	1	C.28	51.5	62.2	66.4	Aromatic hydroxylation
	2	C.22	56.2	62.2	25.5	Aromatic hydroxylation
	3	C.15	60.0	69.4	34.5	Aromatic hydroxylation
	4	S.16	60.5	70.0	49.0	Sulfoxidation
	5	S.8	62.9	70.0	41.0	Sulfoxidation
8a	1	C.38	51.5	62.2	66.4	Aromatic hydroxylation
	2	C.32	55.7	62.2	25.5	Aromatic hydroxylation
	3	C.25	60.4	69.4	34.5	Aromatic hydroxylation
	4	S.26	60.9	70.0	49.0	Sulfoxidation
	5	S.18	63.3	70.0	41.0	Sulfoxidation

2.7.1. Compound **5c**

SMARTCyp ranked the ring nitrogen (N.21) as the most metabolically vulnerable site (Score 44.8; Energy 54.1), indicating a high likelihood of oxidative *N*-dealkylation or *N*-oxide formation. Secondary liabilities were identified on the aromatic carbons C.1 and C.14, suggesting possible aryl hydroxylation pathways. Additionally, the thiazole sulfur (S.22) appeared among the top five hotspots, implying potential sulfoxidation or sulfone formation. These results indicate that **5c** is primarily heteroatom-centered in its metabolic liabilities, with supplementary aromatic soft spots.

2.7.2. Compound **6b**

For **6b**, the highest-ranked soft spots were aromatic carbons C.28 and C.22, strongly suggesting aryl hydroxylation as the primary metabolic clearance route. Additional hotspots included C.15, as well as two sulfur atoms (S.16 and S.8), highlighting possible S-oxidation liabilities. In contrast to **5c**, **6b** is more carbon-centered, with its aromatic moieties predicted to undergo initial metabolic attack before sulfur oxidation pathways become relevant.

2.7.3. Compound **8a**

SMARTCyp predictions for **8a** closely resembled those for **6b**, with C.38 and C.32 identified as the most labile carbons for aromatic hydroxylation. Further soft spots were observed at C.25 and the sulfur atoms S.26 and S.18, consistent with dual liabilities involving aryl hydroxylation and sulfoxidation. Compared with **5c**, compound **8a** displays a more distributed set of carbon- and sulfur-centered metabolic hotspots, which indicates a faster and more complex metabolic profile.

2.7.4. Comparative Analysis

A comparison of the three compounds highlights different patterns of metabolic vulnerability. Compound **5c** is dominated by heteroatom metabolism with *N*-oxidation as the primary route. On the other hand, **6b** and **8a** exhibit carbon-centered liabilities at aromatic carbons, which is supported by secondary sulfur oxidation pathways. This divergence is consistent with the influence of electronic effects and substituent patterns across the series. From a drug-design perspective, **5c** would require protection of its ring nitrogen, while **6b** and **8a** would benefit from strategies aimed at hardening aromatic rings (steric blocks, electron-withdrawing substituents) or mitigating sulfur oxidation through isosteric substitution.

3. Materials and Methods

3.1. Chemicals and Instruments

Sigma Aldrich, Saint Louis, MO, USA, supplied the required chemicals for the synthesis of targeted benzothiazole derivatives. Using thin-layer chromatography, preliminary confirmation was performed to verify the synthesized scaffolds. ^1H and ^{13}C NMR spectra were recorded on a **Bruker Avance spectrometer** (Bruker Avance III, Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600 MHz for ^1H and 150 MHz for ^{13}C , using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). High-resolution mass spectra (HRMS) were obtained using an **electrospray ionization (ESI) source** on an **AB SCIEX Q-TOF mass spectrometer** (AB SCIEX, Concord, ON, Canada). The observed m/z values are reported for the corresponding adduct ions and compared with calculated exact masses.

3.2. General Procedure

3.2.1. Synthesis of Methyl 3-hydroxy-4-nitrobenzoate (**2**)

3-hydroxy-4-nitrobenzoic acid **1** (2.5 g, 13.66 mmol, 1.0 equiv) was dissolved in methanol (20 mL), followed by the addition of sulfuric acid (2.68 g, 27.32 mmol, 2.0 equiv) in sequence. The reaction was stirred overnight at 70 °C. Once the reaction was finished, it was neutralized with 25% ammonia solution (60 mL) to change the pH to 8. The precipitate so obtained was filtered off, excessively washed with water, and dried to afford 1.89 g of methyl 3-hydroxy-4-nitrobenzoate **2** intermediate as a white solid. Yield: 70%. ^1H NMR (600 MHz, DMSO- d_6): δ 12.23 (s, 1H), 8.34 (d, J = 8.6 Hz, 1H), 7.93 (m, 1H), 7.57 (s, 1H), 3.94 (s, 3H).

3.2.2. Synthesis of Targeted Intermediates Methyl

3-((4-(4-methoxyphenoxy)benzyl)oxy)-4-nitrobenzoate (**3a**)

To a solution of methyl 3-hydroxy-4-nitrobenzoate **2** (1.89 g, 9.59 mmol, 1.0 equiv) in MeCN (20 mL) was added 1-(bromomethyl)-4-(4-methoxyphenoxy)benzene (3.37 g, 11.51 mmol, 1.2 equiv) and potassium carbonate (2.65 g, 19.18 mmol, 2.0 equiv), and the resulting residue was put on stirring for 10h at 55 °C to afford 1.82 g of substrate **3a**. Yield: 46%. ^1H NMR (600 MHz, DMSO- d_6): δ 11.84 (s, 1H), 8.29 (d, J = 8.6 Hz, 1H), 7.89 (m, 1H), 7.54 (s, 1H), 7.43–7.34 (m, 4H), 7.13–6.97 (m, 4H), 5.23 (s, 2H), 3.93 (s, 3H), 3.78 (s, 3H). HRMS (ESI) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{20}\text{NO}_7^+$ 410.1240, Found 410.1234.

3.2.3. Synthesis of Intermediate Methyl

3-((2-bromo-5-hydroxy-4-methoxybenzyl)oxy)-4-nitrobenzoate (**3b**)

The intermediate **3b** was synthesized following the same procedure as illustrated for intermediate **3a**, using 4-bromo-5-(bromomethyl)-2-methoxyphenol (3.41 g, 11.51 mmol,

1.2 equiv) for reaction with methyl 3-hydroxy-4-nitrobenzoate **2** (1.89 g, 9.59 mmol, 1.0 equiv) to afford 2.1 g of intermediate **3b**. Yield: 53%. ¹H NMR (600 MHz, DMSO-*d*₆): δ 9.53 (s, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 7.91 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.73 (s, 1H), 7.25 (s, 1H), 6.84 (s, 1H), 5.31 (s, 2H), 3.98 (s, 3H), 3.82 (s, 3H). HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₁₆H₁₅BrNO₇⁺ 412.0032, Found 412.0026.

3.2.4. Synthesis of Methyl 3-((6-methoxypyridin-3-yl)methoxy)-4-nitrobenzoate (**3c**)

The intermediate **3c** was synthesized following the same procedure as illustrated for intermediate **3a**, using 5-(bromomethyl)-2-methoxypyridine (2.33 g, 11.51 mmol, 1.2 equiv) for reaction with methyl 3-hydroxy-4-nitrobenzoate **2** (1.89 g, 9.59 mmol, 1.0 equiv) to afford 1.8 g of intermediate **3c**. Yield: 59%. ¹H NMR (600 MHz, DMSO-*d*₆): δ 8.23–8.09 (m, 2H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.63 (s, 1H), 6.78 (d, *J* = 6.6 Hz, 1H), 5.26 (s, 2H), 3.85 (s, 3H), 3.69 (s, 3H). HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₁₅H₁₅N₂O₆⁺ 319.0930, Found 319.0925.

3.2.5. Synthesis of Methyl 3-((4-chlorobenzyl)oxy)-4-nitrobenzoate (**3d**)

The intermediate **3d** was synthesized following the same procedure as illustrated for intermediate **3a**, using 1-(bromomethyl)-4-chlorobenzene (2.36 g, 11.51 mmol, 1.2 equiv) for reaction with methyl 3-hydroxy-4-nitrobenzoate **2** (1.89 g, 9.59 mmol, 1.0 equiv) to afford 1.73 g of intermediate **3c**. Yield: 56%. ¹H NMR (600 MHz, DMSO-*d*₆): δ 8.18–8.03 (m, 2H), 7.84 (s, 1H), 7.45 (d, *J* = 6.8 Hz, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 5.27 (s, 2H), 3.86 (s, 3H). HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₁₅H₁₃ClNO₅⁺ 322.0482, Found 322.0476.

3.2.6. Synthetic Procedure for Intermediates (**4a–4d**)

Pre-synthesized intermediates **3a–3d** were dissolved in tetrahydrofuran/methanol (3:7, 50mL) under an argon atmosphere; Pd/C (500 mg) was added. The reaction mixture was stirred for 5 h under hydrogen atmosphere at room temperature. Upon completion, the catalyst was filtered off; solvent was removed to get targeted intermediates **4a–4d** in 51–63% (Scheme 1).

3.2.7. Synthetic Procedure for Targeted Compounds (**5a–5d**)

To a solution of intermediates **4a–4d** (1.0 equiv) in acetic acid (20 mL), KSCN (4.0 equiv) was added, and the residue was stirred for 40 min at room temperature. The mixture was then cooled to 10 °C, followed by the addition of bromine (2.0 equiv) in acetic acid dropwise to the solution. The reaction mixture was stirred overnight at room temperature, and then neutralized with 25% aqueous ammonia solution. The combined organic layer was extracted and purified using column chromatography (elution: ethyl acetate: petroleum ether) to afford the targeted compound in 43–54% yield (Scheme 1).

Synthesis of methyl 2-amino-4-((4-(4-methoxyphenoxy)benzyl)oxy)benzo[d]thiazole-6-carboxylate (**5a**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.23 (s, 1H), 7.89 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 6.6 Hz, 2H), 7.23 (s, 2H), 7.19 (d, *J* = 6.2 Hz, 2H), 6.99 (d, *J* = 7.2 Hz, 2H), 5.23 (s, 2H), 3.91 (s, 3H), 3.79 (s, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆): δ 167.66, 166.88, 156.37, 154.80, 153.03, 149.98, 149.65, 147.93, 145.16, 133.91, 131.18, 129.21, 129.11, 128.08, 126.11, 125.52, 117.34, 114.70, 112.32, 105.99, 67.32, 56.26, 52.42. HRMS (ESI) *m/z*: [M + H]⁺ calcd for C₂₃H₂₁N₂O₅S⁺ 437.1165, Found 437.1171.

Synthesis of methyl 2-amino-4-((2-bromo-5-hydroxy-4-methoxybenzyl)oxy)benzo[d]thiazole-6-carboxylate (**5b**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 8.06 (d, *J* = 2.2 Hz, 1H), 7.58 (d, *J* = 2.2 Hz, 1H), 7.12–6.88 (m, 2H), 5.96 (s, 3H), 5.78 (s, 2H), 3.88 (d, *J* = 10.1 Hz, 6H). ¹³C NMR (151 MHz, DMSO-*d*₆): δ 166.76, 165.45, 152.67, 151.78, 147.87, 147.38, 133.19, 129.57, 128.64, 126.73,

121.67, 117.28, 116.32, 112.98, 71.83, 56.73, 52.47. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{17}H_{16}BrN_2O_5S^+$ 438.9957, Found 438.9963.

Synthesis of methyl 2-amino-4-((6-methoxypyridin-3-yl)methoxy)benzo[d]thiazole-6-carboxylate (**5c**).

1H NMR (600 MHz, DMSO- d_6): δ 8.25 (s, 1H), 7.91 (s, 1H), 7.86 (m, 1H), 7.63 (s, 1H), 7.25 (s, 2H), 6.78 (d, $J = 8.2$ Hz, 1H), 5.29 (s, 2H), 3.93 (s, 3H), 3.78 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6): δ 166.76, 165.45, 163.29, 151.75, 148.92, 147.84, 138.74, 128.61, 126.70, 120.79, 116.29, 112.95, 108.97, 71.80, 54.89, 52.44. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{16}H_{16}N_3O_4S^+$ 346.0857, Found 346.0862.

Synthesis of methyl 2-amino-4-((4-chlorobenzyl)oxy)benzo[d]thiazole-6-carboxylate (**5d**).

1H NMR (600 MHz, DMSO- d_6): δ 8.29 (s, 1H), 7.95 (s, 1H), 7.43–7.29 (m, 4H), 7.25 (s, 2H), 5.25 (s, 2H), 3.97 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6): δ 166.79, 165.48, 151.76, 147.85, 135.47, 134.83, 129.98, 129.98, 128.62, 128.56, 128.56, 126.71, 116.30, 112.96, 71.81, 52.45. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{16}H_{14}ClN_2O_3S^+$ 349.0408, Found 349.0414.

3.2.8. Synthetic Procedure for Targeted Compounds (**6a–6d**)

The methyl 2-amino-4-((6-methoxypyridin-3-yl)methoxy)benzo[d]thiazole-6-carboxylate (**5c**) (1.0 equiv) was added to a stirred solution of the corresponding acid (1.0 equiv) in DCM (20 mL), followed by dissolving HATU (0.1 equiv) and DIPEA (3.0 equiv) in sequence. The reaction mixture was heated for 4 h at room temperature. Once the reactants had disappeared, the reaction mixture was washed with water and then extracted with ethyl acetate (50 mL $\times$ 3). The combined organic layer was collected, dried over sodium sulfate, and further purified by silica gel column chromatography (elution with petroleum ether: ethyl acetate) to afford the desired compounds **6a–6d** in 52–64% yield.

Synthesis of methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiophene-2-carboxamido)-benzo[d]thiazole-6-carboxylate (**6a**).

1H NMR (600 MHz, DMSO- d_6): δ 11.37 (s, 1H), 8.30–8.19 (m, 1H), 8.13 (s, 1H), 7.93 (dd, $J = 8.2, 1.8$ Hz, 1H), 7.91 (s, 1H), 7.89 (m, 1H), 7.85–7.73 (m, 1H), 7.63 (s, 1H), 6.78 (d, $J = 8.2$ Hz, 1H), 5.29 (s, 2H), 3.93 (s, 3H), 3.78 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6): δ 166.88, 165.16, 164.62, 159.62, 159.42, 154.30, 153.56, 152.04, 143.86, 143.30, 142.58, 133.34, 128.30, 128.16, 118.60, 115.30, 113.58, 106.28, 65.52, 54.58, 52.42. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{21}H_{18}N_3O_5S_2^+$ 456.0681, Found 456.0688.

Synthesis of methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)-benzo[d]thiazole-6-carboxylate (**6b**).

1H NMR (600 MHz, DMSO- d_6): δ 12.68 (s, 1H), 8.34 (d, $J = 8.2$ Hz, 1H), 8.19 (s, 1H), 7.97 (s, 1H), 7.84 (d, $J = 7.6$ Hz, 1H), 7.81 (m, 1H), 7.65 (s, 1H), 6.79 (d, $J = 8.2$ Hz, 1H), 5.31 (s, 2H), 3.91 (s, 3H), 3.75 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6): δ 169.77, 168.46, 166.79, 166.30, 165.81, 154.76, 151.93, 150.85, 144.67, 141.75, 133.26, 131.62, 129.71, 123.80, 119.30, 115.96, 111.98, 74.81, 57.90, 55.45. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{20}H_{17}N_4O_5S_2^+$ 457.0633, Found 457.0640.

Synthesis of methyl 2-(3,4-dichlorobenzamido)-4-((6-methoxypyridin-3-yl)methoxy)-benzo[d]thiazole-6-carboxylate (**6c**).

1H NMR (600 MHz, DMSO- d_6): δ 8.23 (d, $J = 0.9$ Hz, 2H), 8.10 (d, $J = 2.2$ Hz, 1H), 7.89 (s, 1H), 7.71 (d, $J = 2.2$ Hz, 1H), 7.60 (d, $J = 7.3$ Hz, 1H), 7.45 (d, $J = 7.1$ Hz, 1H), 7.38 (q, $J = 7.1$ Hz, 2H), 5.74 (d, $J = 0.9$ Hz, 2H), 3.99 (s, 3H), 3.87 (s, 3H). **^{13}C NMR** (151 MHz, DMSO- d_6): δ 168.73, 167.42, 166.73, 164.26, 153.72, 150.89, 149.81, 140.71, 137.14, 134.84, 134.25, 131.38, 130.58, 129.76, 128.67, 128.29, 122.76, 118.26, 114.92, 110.94, 73.77, 56.86, 54.41. **HRMS** (ESI) m/z : $[M + H]^+$ calcd for $C_{23}H_{18}Cl_2N_3O_5S^+$ 518.0338, Found 518.0344.

Synthesis of methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(4-(trifluoromethoxy)benzamido)benzo[d]thiazole-6-carboxylate (**6d**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 12.83 (s, 1H), 8.25 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 2H), 7.91 (s, 1H), 7.86 (m, 1H), 7.63 (s, 1H), 7.34 (d, *J* = 7.6 Hz, 2H), 6.78 (d, *J* = 8.2 Hz, 1H), 5.29 (s, 2H), 3.93 (s, 3H), 3.78 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆): δ 167.71, 166.40, 165.74, 164.24, 155.43, 152.70, 149.87, 148.79, 139.69, 129.65, 129.65, 129.56, 128.76, 127.65, 127.37, 121.74, 117.24, 115.39, 115.39, 113.90, 109.92, 72.75, 55.84, 53.39. **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₂₄H₁₉F₃N₃O₆S⁺ 534.0941, Found 534.0947.

3.2.9. Synthesis of 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxylic acid (**7**)

The methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxylate **6b** (1.5 g, 3.29 mmol, 1.0 equiv) was reacted with lithium hydroxide (0.23 g, 9.86 mmol, 3.0 equiv) in THF/H₂O (1:1; 20 mL). The resulting mixture was put on a sand bath and heated at 35 °C for 4 h. After being completed, the mixture was neutralized by adding an aqueous HCl solution to change the pH to 8. Then, excessively washed with water, the precipitate was filtered off, dried over sodium sulfate, and gave 1.10 g of intermediate **7**. Yield: 81%. **¹H NMR** (600 MHz, DMSO-*d*₆): δ 12.73 (s, 1H), 11.13 (s, 1H), 8.31 (d, *J* = 1.6 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.07 (s, 1H), 7.83 (d, *J* = 7.2 Hz, 1H), 7.51 (s, 1H), 6.74 (d, *J* = 7.4 Hz, 1H), 5.21 (s, 2H), 3.76 (s, 3H). **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₁₉H₁₅N₄O₅S₂⁺ 443.0477, Found 443.0484.

3.2.10. Synthetic Procedure for Targeted Compounds (**8a–8d**)

The pre-synthesized intermediate **7** (1.0 equiv) was added to a solution of the corresponding acid (1.0 equiv), being stirred in DCM (20 mL). To this was added HATU (0.1 equiv) and DIPEA (3.0 equiv). The reaction mixture was placed on a sand bath and heated at 25 °C for 4 h. Upon completion, the residue was thoroughly washed with water, and the organic components were extracted with ethyl acetate (50 mL × 3). The organic layer, after separation, was further purified by silica gel chromatography (elution with ethyl acetate: petroleum ether) to obtain the targeted compounds **8a–8d** in 45–57% yield.

Synthesis of 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)-*N*-(4-(trifluoromethoxy)phenyl)benzo[d]thiazole-6-carboxamide (**8a**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 12.69 (s, 1H), 10.43 (s, 1H), 8.34 (d, *J* = 8.2 Hz, 1H), 8.19 (s, 1H), 7.97 (s, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.81 (m, 1H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.65 (s, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 7.03 (d, *J* = 7.8 Hz, 2H), 5.31 (s, 2H), 3.75 (s, 3H). **¹³C NMR** (600 MHz, DMSO-*d*₆): δ 167.04, 162.93, 160.18, 159.62, 158.31, 157.15, 154.30, 152.04, 151.55, 148.41, 148.40, 148.38, 148.36, 147.75, 143.35, 143.30, 142.58, 133.26, 133.20, 133.16, 133.11, 131.00, 128.68, 125.01, 122.92, 120.83, 118.80, 118.74, 116.91, 115.30, 104.62, 65.52, 54.58. **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₂₆H₁₉F₃N₅O₅S₂⁺ 602.0773, Found 602.0780.

Synthesis of *N*-(3,4-dichlorophenyl)-4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxamide (**8b**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 12.78 (s, 1H), 10.45 (s, 1H), 8.36 (d, *J* = 8.2 Hz, 1H), 8.18 (s, 1H), 7.98 (s, 1H), 7.95 (s, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.78 (m, 1H), 7.63–7.49 (m, 2H), 7.44 (s, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 5.33 (s, 2H), 3.77 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆): δ 171.71, 170.40, 168.73, 168.24, 167.75, 156.70, 153.87, 152.79, 146.60, 143.69, 138.76, 135.20, 133.56, 131.75, 131.54, 130.75, 128.97, 125.74, 125.53, 122.18, 121.24, 117.90, 113.92, 76.75, 59.84. **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₂₅H₁₈Cl₂N₅O₄S₂⁺ 586.0169, Found 586.0177.

Synthesis of *N*-(4-chlorophenyl)-4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxamide (**8c**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 12.75 (s, 1H), 10.36 (s, 1H), 8.31 (d, *J* = 8.2 Hz, 1H), 8.15 (s, 1H), 7.98 (s, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.80 (m, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.64 (s, 1H), 7.40 (d, *J* = 7.6 Hz, 2H), 6.77 (d, *J* = 8.2 Hz, 1H), 5.32 (s, 2H), 3.74 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆): δ 170.72, 169.41, 167.74, 167.25, 166.76, 155.71, 152.88, 151.80, 145.62, 142.70, 136.58, 134.21, 133.81, 132.57, 130.66, 129.32, 129.32, 124.75, 122.47, 122.47, 120.25, 116.86, 112.93, 75.76, 58.85. **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₂₅H₁₉ClN₅O₄S₂⁺ 552.0561, Found 552.0567.

Synthesis of 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)-*N*-(4-(trifluoromethyl)phenyl)benzo[d]thiazole-6-carboxamide (**8d**).

¹H NMR (600 MHz, DMSO-*d*₆): δ 12.71 (s, 1H), 10.14 (s, 1H), 8.34 (d, *J* = 8.2 Hz, 1H), 8.19 (s, 1H), 7.97 (s, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.81 (m, 1H), 7.65 (s, 1H), 7.59–7.48 (m, 4H), 6.79 (d, *J* = 8.2 Hz, 1H), 5.31 (s, 2H), 3.75 (s, 3H). **¹³C NMR** (151 MHz, DMSO-*d*₆): δ 163.39, 162.93, 162.56, 160.17, 159.15, 157.15, 153.17, 152.14, 152.04, 151.93, 151.83, 151.53, 147.16, 147.06, 141.74, 141.71, 141.68, 141.65, 141.37, 134.27, 129.46, 128.03, 127.26, 125.82, 125.60, 125.05, 123.63, 122.84, 121.46, 113.09, 105.30, 105.01, 104.72, 104.42, 68.65, 54.61. **HRMS** (ESI) *m/z*: [M + H]⁺ calcd for C₂₆H₁₉F₃N₅O₄S₂⁺ 586.0825, Found 586.0831.

3.3. Assay Protocol for α-Amylase and α-Glucosidase Inhibition

The protocol demonstrated for inhibition of α-amylase and α-glucosidase is similar to that adopted in our previously reported work [42].

3.4. Assay Protocol for Docking Study

Molecular docking analysis was carried out by Autodock 4 tools software (version 1.5.7), Discovery Studio Visualizer, Autodock vina (1.5.7), and Pymol [43–45] to explore all possible orientations, conformations, and binding affinities for the ligands with α-amylase (PDB ID: 4W93) and α-glucosidase (PDB ID: 3A4A) active site [43]. All ligands were converted to PDBQT format using Autodock software to prepare them in an admissible format for docking in AutoDock Vina. A resolution of less than 2 Å, non-mutant type, and the absence of gaps in the residue sequence were the selection criteria used to obtain the target protein structures from the Protein Data Bank (PDB). The coordinates of a co-crystallized ligand found in the protein structure provide a reliable point of reference for determining the grid box center. The grid center is determined by calculating the ligand's geometric center, and the box size is modified to include the ligand and any nearby active site residues. The grid box was generated by entrapping the entire protein within the box to facilitate blind docking. Using AutoDock Tools 1.5.7, the grid box of protein target PDB ID: 4W93 and PDB ID: 3A4A was defined. For PDB ID: 4W93, the centroid of the co-crystallized ligand (*X* = −10.898, *Y* = 3.351, *Z* = −20.613) was chosen as the grid box center. The grid dimensions were adjusted to 50 × 40 × 40 Å with a spacing of 0.45 Å to encompass the binding site and provide enough room for ligand movement. For PDB ID: 3A4A, the centroid of the co-crystallized ligand (*X* = 22.731, *Y* = −8.34, *Z* = 20.661) was chosen as the grid box center. To cover the binding site and provide sufficient room for ligand movement, the grid dimensions were established at 40 × 40 × 40 Å with a spacing of 0.375 Å. As seen in Figure 2, a grid was created around the co-crystallized ligand, and PDBQT files for the ligand and protein target were created. A commercially available reference molecule was used to compare the synthesized compounds' interactions with the receptor active sites after they were docked within the same grid.

Molecular visualization of the docking results was performed, and nonbonding interactions between the docked protein–ligand complexes and the docking pose were analyzed by using BIOVIA Discovery Studio Client 2021. Confirmation indicating the lowest docking score (in kcal/mol) was selected as the lead compound.

3.5. Assay Protocol for ADME Properties

To assess drug-likeness and pharmacokinetic behavior, ADMET properties of the newly synthesized compounds were predicted using SwissADME (<http://www.swissadme.ch>; accessed on 23 March 2025), which also provided synthetic accessibility scores. Pharmacokinetic parameters were further evaluated via the pkCSM platform (<http://biosig.unimelb.edu.au/pkcsml/prediction>, accessed on 23 March 2025). In addition, the potential toxicological risks of the selected sulfonylthioate hybrids were analyzed using the ProTox-II, StopTox, and SMARTCyp Metabolic Soft Spot webserver [46–48].

3.6. Assay Protocol for DFT Study

The calculations of the Density Functional Theory (DFT) were performed with the help of the Gaussian 09 (Rev. E.01) software package. The complete optimization of all molecular geometries was done at the B3LYP/6-31G(d) level of theory without imposing any symmetry constraints. At the same level, frequency calculations were then done to ensure that the optimized structures could represent genuine minima on the potential energy surface (no imaginary frequencies). The ground-state DFT calculations provided frontier molecular orbital energies (HOMO and LUMO), which are suitable for assessing intrinsic electronic properties and global reactivity descriptors of chemical reactivity and biological reactions. Time-dependent DFT (TD-DFT) is a method that offers better accuracy in the calculation of excited-state properties; however, it could not be used in this study, and no electronic excitation or optical transitions were studied. In the calculation of the HOMO and LUMO energies, global reactivity descriptors were calculated using Koopmans' theorem. ESP maps of the molecules were generated to visualize the charge distribution and potential electrophilic and nucleophilic sites. GaussView 6 was used to visualize all the molecular orbitals, ESP surfaces, and graphical analyses [49–52].

4. Conclusions

A novel series of benzothiazole-based derivatives was designed, synthesized, and tested in this research as possible α -amylase and α -glucosidase inhibitors to manage type 2 diabetes mellitus. The structural elucidation was confirmed by analyses of ^1H and ^{13}C NMR spectra and HRMS. The resulting compounds exhibited moderate to strong inhibition of both enzymes, with IC_{50} values of 1.58–7.54 μM and 2.10–8.90 μM for α -amylase and α -glucosidase, respectively. Compounds **5c** and **6b** were the most active in the series and exhibited activity similar to that of the reference drug acarbose. The experimental results were backed by molecular docking studies, which showed positive binding affinities and stable interactions of these compounds in the catalytic sites of the two enzymes. Their increased reactivity was further supported by density functional theory (DFT) analysis, which showed a reduced HOMO-LUMO energy gap and positive global reactivity descriptors. Moreover, ADME and metabolic forecasts showed that methyl 2-amino-4-((6-methoxypyridin-3-yl)methoxy)benzo[d]thiazole-6-carboxylate (**5c**) has a more favorable pharmacokinetic profile, and methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)benzo[d]thiazole-6-carboxylate (**6b**) has a high target affinity but has moderate drug-likeness constraints. In general, the combined experimental and computational findings point to benzothiazole derivatives, especially **5c** and **6b**, as the best lead molecules for further optimization in order to produce new anti-diabetic drugs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules31040751/s1>. Figure S1. ^{13}C NMR of compound methyl methyl 2-amino-4-((4-(4-methoxyphenoxy)benzyl)oxy)benzo[d]thiazole-6-carboxylate (**5a**); Figure S2. ^{13}C NMR of compound methyl 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiophene-

2-carboxamido)benzo[d]thiazole-6-carboxylate (**6a**); Figure S3. ^{13}C NMR of compound 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)-N-(4-(trifluoromethoxy)phenyl)benzo[d]thiazole-6-carboxamide (**8a**); Figure S4. ^{13}C NMR of compound 4-((6-methoxypyridin-3-yl)methoxy)-2-(thiazole-2-carboxamido)-N-(4-(trifluoromethyl)phenyl)benzo[d]thiazole-6-carboxamide (**8d**); Figure S5. ^1H NMR of compound methyl 2-amino-4-((2-bromo-5-hydroxy-4-methoxybenzyl)oxy)benzo[d]thiazole-6-carboxylate (**5b**); Figure S6. ^1H NMR of compound methyl 2-(3,4-dichlorobenzamido)-4-((6-methoxypyridin-3-yl)methoxy)benzo[d]thiazole-6-carboxylate (**6c**).

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