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DESIGN, SYNTHESIS, AND *IN-VITRO* GLYCOLYTIC ENZYME INHIBITION ACTIVITY OF NOVEL BENZIMIDAZOLE DERIVATIVES

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ABSTRACT
A novel series of benzimidazole derivatives (3a-f) was rationally designed and synthesized to explore their potential as α-glucosidase inhibitors for the management of hyperglycaemia. The synthesized compounds were structurally characterized using FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, confirming the presence of key functional groups including hydroxyl (-OH), carbonyl (C=O), and imine (C=N) moieties. The *in vitro* α-glucosidase inhibitory activity was assessed using acarbose as the standard reference drug. Among the tested derivatives, compound 3f exhibited the highest inhibitory activity (IC_{50} = 25.57 ± 3.09 µM), followed by 3c (IC_{50} = 30.35 ± 3.42 µM) and 3a (IC_{50} = 37.32 ± 2.61 µM), all demonstrating superior potency compared to acarbose (IC_{50} = 43.52 ± 2.76 µM). Structure-activity relationship (SAR) analysis suggested that electron-donating substituents enhance inhibitory potential, whereas electron-withdrawing groups reduce activity. To further elucidate the binding interactions, molecular docking studies were performed using the crystal structure of α-glucosidase (PDB ID: 3A4A). The docking scores ranged from -8.93126 to -12.0635 kcal/mol, with compounds 3f (-12.0635 kcal/mol) and 3d (-11.6917 kcal/mol) exhibiting the most favourable binding affinities and stable interactions within the enzyme active site, comparable to the standard drug. These findings highlight the potential of benzimidazole-based scaffolds, particularly compound 3f, as promising candidates for the development of effective α-glucosidase inhibitors for diabetes management.

KEYWORDS: Benzimidazole derivatives; *In-vitro*; α-glucosidase inhibition; hyperglycaemia; Molecular Docking Study.

1. INTRODUCTION

Diabetes mellitus is the most severe and chronic metabolic disorder, characterized by hyperglycemia.[1] According to the International Diabetes Federation (IDF) atlas 2024, 11.11% of the world's population, corresponding to 589 million individuals, suffered from diabetes, and this number is expected to rise to 12.96%, or 853 million, by 2050.[2] The IDF guidelines 2025 acknowledge worldwide inequities in healthcare access, with around 80% of Type 2 diabetes patients living in low-to-middle-income countries.[3] The most common type of Diabetes mellitus includes Type 1 and Type 2 Diabetes mellitus. Type 1 diabetes, also called juvenile-onset diabetes, is an autoimmune disease in which the body's immune system mistakenly destroys the pancreatic beta cells that produce insulin, resulting in insulin insufficiency. Type 2 diabetes mellitus (T2DM) is the most common type of diabetes, which is also called adult-onset diabetes or non-insulin-dependent diabetes mellitus.[4] T2DM makes up 90-95% of all cases of diabetes and mostly affects adults, while it is becoming more common in younger people because of poor eating habits and sedentary lifestyles.[5] It is caused by a combination of impaired insulin secretion and insulin resistance.[6] Various complications are linked to diabetes, including macrovascular conditions like myocardial infarction, stroke, and peripheral artery disease, as well as microvascular conditions like retinopathy, nephropathy, and neuropathy.[7]

One of the therapeutic approaches for the treatment of DM is to control blood glucose level via inhibiting carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase.[8] α-amylase breaks down complex polysaccharides like starch and glycogen into oligosaccharides and disaccharides, which are then further hydrolysed into absorbable monosaccharides like glucose by the α-glucosidase enzyme, found in the brush border cells of the intestine (Figure 1).[9] Inhibiting these enzymes can effectively slow down the absorption of glucose into the bloodstream, thereby it prevents sharp rise of blood sugar levels after a meal and preventing late-onset problems associated with diabetes mellitus.[9,10] Standard α-glucosidase inhibitors such as acarbose, voglibose, and miglitol, etc.[11] Acarbose is

mostly used among these, due to its dual enzyme inhibition.[12]

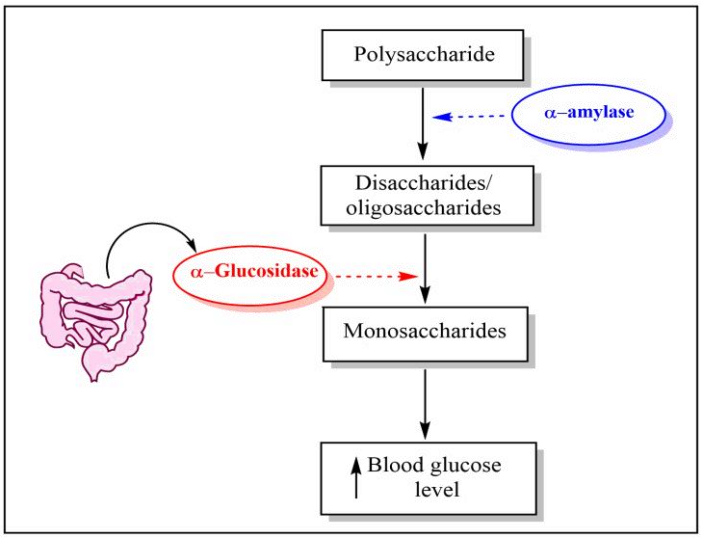


Figure 1: Schematic representation of the mechanism of action of α-glucosidase.

Heterocyclic compounds are universal in nature and play indispensable roles in cellular metabolism and biochemical processes. Among them, benzimidazole constitutes a prominent class of nitrogen-containing heteroaromatic compounds showing extensive biological and pharmacological significance. Structurally, it is characterized by a fused bicyclic system comprising a benzene ring condensed with an imidazole moiety bearing two nitrogen atoms. The presence of these heteroatoms imparts unique electronic distribution, hydrogen-bonding capacity, and amphoteric behavior, which together contribute to its broad spectrum of biological interactions. A wide range of biological activities has been reported, including anti-inflammatory, antimicrobial, antiviral, anticancer, anticonvulsant, antidiabetic, and antioxidant effects.[13,14] These multifaceted activities highlight the versatility of the benzimidazole core as a privileged pharmacophore. Significantly, recent studies have demonstrated that benzimidazole derivatives exhibit significant potential as α-glucosidase inhibitors, owing to their ability to interact effectively with the enzyme active site through hydrogen bonding and π-π interactions. The presence of nitrogen atoms and easily modifiable substituents enhances their binding affinity and inhibitory efficiency.[13-15] Since α-glucosidase plays a crucial role in carbohydrate metabolism by catalyzing the final step of glucose release, its inhibition is an effective strategy for controlling postprandial hyperglycemia. Therefore, the structural features and pharmacological versatility of benzimidazole make it a promising scaffold for the design and development of novel α-glucosidase inhibitors for antidiabetic therapy.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals and reagents, including *o*-phenylenediamine, tartaric acid, 2-bromoethylamine, and different substituted aromatic aldehydes, were of analytical grade and were procured from Loba Chem, HiMedia, and Sigma-Aldrich. The solvents were purified by distillation before use. The completion of the reactions was monitored by thin-layer chromatography (TLC).

2.2 Chemistry

2.2.1 General procedure for the synthesis of 3-(1H-benzo[d]imidazol-2-yl)-2,3-dihydroxypropanoic acid (1)

An equimolar mixture (0.01 mol each) of *o*-phenylenediamine and tartaric acid was dissolved in 10 mL of ethanol. The reaction mixture was refluxed at 90 °C for 4 hours in the presence of a small amount of dilute glacial acetic acid. After completion of the reaction, the mixture was cooled and neutralized using KOH solution. The obtained product was then washed with acetone and dried.[16]

Color: Bright pink; % Yield: 82%; M.P.: 88 ^{o}C; FTIR (cm^{-2}): 3285.45 (O-H), 2601.52 (O-H (COOH)), 1725.12 (C=O), 1621.74 (C=N), 1532.15 (C=C), 1203.14 (C-O), 1085.45 (C-N); 1H NMR: 4.84, 5.15, 5.19, 5.82, 7.15, 7.52, 7.52, 12.17, 13.35; 13C NMR: 76.1, 88.1, 155.1, 122.9, 122.9, 138.8, 141.4, 173.1; Molecular Mass [M^{+}]: 222.06; Elemental Analysis: C, 54.05; H, 4.54; N, 12.61; O, 28.80.

2.2.2 General procedure for the synthesis of 3-(1-(2-aminoethyl)-1H-benzo[d]imidazol-2-yl)-2,3-dihydroxypropanoic acid (2)

An equimolar mixture (0.01 mol) of compound 1 and 2-bromoethylamine was dissolved in 15 mL of acetonitrile. The reaction mixture was refluxed at 100 °C for 4 hours in the presence of K_2CO_3 as a base. After completion of the reaction, the mixture was poured into crushed ice and neutralized by adding 20% H_2SO_4 in ethanol until a

precipitate formed. The solid product was then collected and dried.[17]

Color: white; % Yield: 85%; M.P.: 86 ^{o}C; FTIR (cm^{-2}): 3289.74 (O-H), 3045.92 (Ar C-H), 2862.41 (Alp.C-H), 1729.12 (C=O), 1632.74 (C=N), 1537.96 (C=C), 1216.74 (C-O), 1082.74(C-N); 1H NMR: 1.4, 3.12, 3.98, 4.83, 5.16, 5.18, 5.82, 7.15, 7.18, 7.50, 7.52, 13.34; 13C NMR: 38.4, 48.9, 73.8, 88.3, 109.9, 119.7, 122.9, 122.8, 142.0, 142.8, 166.4, 173.0; Molecular Mass [M^{+}]: 265.11; Elemental Analysis: C, 54.33; H, 5.70; N, 15.84; O, 24.13.

2.2.3 General procedure for the synthesis of 3-(1-(2-(substituted benzylidene) amino) ethyl)-1H-benzo[d]imidazol-2-yl)-2,3-dihydroxypropanoic acid (3a-f)

An aqueous mixture (0.01 mol) of compound 2 and various aromatic aldehydes was prepared, followed by the addition of a few drops of dilute acetic acid in ethanol. The reaction mixture was refluxed at 60-70 °C for 3-4 hours. Upon completion of the reaction, the mixture was poured onto crushed ice, leading to the formation of a precipitate. The resulting solid products were then collected and dried.[18]

2.2.3.1 3-(1-(2-(benzylidene amino) ethyl)-1H-benzo [d]imidazol-2-yl)-2,3-dihydroxy propanoic acid (3a)

Color: white; % Yield: 86%; M.P.: 87 ^{o}C; FTIR (cm^{-2}): 3281.12 (O-H), 3152.45 (Ar C-H), 2864.96 (Alp.C-H), 1727.20 (C=O), 1622.74 (C=N), 1537.96 (Ar C=C), 1452.86 (-CH_2), 1216.74 (C-O), 1082.74(C-N); 1H NMR: 4.01, 4.08, 4.84, 5.15, 5.18, 5.81, 7.15, 7.19, 7.50, 7.52, 7.54, 7.53, 7.57, 7.75, 7.75, 8.74,13.35; 13C NMR: 52.7, 54.0, 73.9, 88.4, 109.9, 119.6, 122.9, 122.8, 129.0, 129.1, 130.9, 136.2, 142.1, 142.8, 160.6, 166.5, 173.0; Molecular Mass [M^{+}]: 353.14; Elemental Analysis: C, 64.58; H, 5.42; N, 11.89; O, 18.11.

2.2.3.2 2,3-dihydroxy-3-(1-(2-(4-nitrobenzylidene) amino) ethyl)-1H-benzo[d]imidazol-2-yl) propanoic acid (3b)

Color: pale yellow; % Yield: 82%; M.P.:88 ^{o}C; FTIR (cm^{-2}): 3289.17 (O-H), 3150.63 (Ar C-H), 2862.06 (Alp.C-H), 1722.96 (C=O), 1627.26 (C=N), 1531.09 (Ar C=C), 1506.45 (-NO_2), 1449.16 (-CH_2), 1222.79 (C-O), 1072.63 (C-N); 1H NMR: 4.01, 4.07, 4.84, 5.15, 5.19, 5.81, 7.16, 7.18, 7.50, 7.51, 8.12, 8.11, 8.32, 8.31, 8.74,13.34; 13C NMR: 52.7, 53.9, 73.8, 88.4, 109.9, 119.7, 122.9, 122.8, 123.9, 123.8, 130.2, 130.3, 142.1, 142.3, 150.1, 160.6, 166.4, 173.0; Molecular Mass [M^{+}]: 398.12; Elemental Analysis: C, 57.28; H, 4.55; N, 14.06; O, 24.10.

2.2.3.3 2,3-dihydroxy-3-(1-(2-(3,4,5-trimethoxy benzylidene) amino) ethyl)-1H-benzo[d] imidazol-2-yl) propanoic acid (3c)

Color: yellow; % Yield: 86%; M.P.:86 ^{o}C; FTIR (cm^{-2}): 3284.74 (O-H), 3149.25 (Ar C-H), 2860.96 (Alp.C-H), 1728.09 (C=O), 1639.27 (C=N), 1509.52 (Ar C=C), 1452.14 (-CH_2), 1320.07 (-OCH3), 1207.19 (C-O), 1069.17 (C-N); 1H NMR: 3.70, 3.82, 3.81, 4.01, 4.08, 4.83, 5.16, 5.18, 5.82, 7.16, 7.19, 7.21, 7.20, 7.50, 7.51, 8.52, 13.35; 13C NMR: 52.7, 53.9, 55.9, 55.8, 60.6, 73.9, 88.3, 103.9, 103.8, 109.9, 119.6, 122.9, 122.8, 134.0, 141.4, 142.1, 142.8, 153.1, 153.0, 160.6, 166.5, 173.0; Molecular Mass [M^{+}]: 443.17; Elemental Analysis: C, 59.59; H, 5.68; N, 9.48; O, 25.25.

2.2.3.4 2,3-dihydroxy-3-(1-(2-(4-isopropylbenzylidene) amino) ethyl)-1H-benzo[d] imi -dazol-2-yl) propanoic acid (3d)

Color: white; % Yield: 82 %; M.P.: 83 ^{o}C; FTIR (cm^{-2}): 3280.14 (O-H), 3141.96 (Ar C-H), 2867.19 (Alp. C-H), 1740.28 (C=O), 1632.45 (C=N), 1518.15 (Ar C=C), 1444.24 (-CH_2), 1322.87 (-OCH3), 1212.25 (C-O), 1070.27 (C-N); ^{1}H NMR : 1.19, 1.18, 4.02, 4.08, 4.83, 4.90, 5.16, 5.18, 5.81, 7.16, 7.18, 7.34, 7.35, 7.50, 7.51, 7.80, 7.80, 8.75, 13.34; ^{13}C NMR:23.2, 23.1, 33.1, 52.7, 53.9, 73.9, 88.4,109.9,119.7,122.9, 122.8, 126.1, 126.0, 128.8, 128.7, 133.6, 142.8, 150.6, 160.6, 166.5, 173.0; Molecular Mass [M^{+}]: 395.18; Elemental Analysis: C, 66.82; H, 6.37; N, 10.63; O, 16.18.

2.2.3.5 3-(1-(2-(2,4-dichlorobenzylidene) amino) ethyl)-1H-benzo[d] imidazol-2-yl)-2,3-dihydroxypropanoic acid (3e)

Color: white; % Yield: 86%; M.P.: 84 ^{o}C; FTIR (cm^{-2}): 3272.45 (O-H), 3198.06 (Ar C-H), 2870.45 (Alp. C-H), 1780.96 (C=O), 1642.56 (C=N), 1550.14 (Ar C=C), 1443.24 (-CH_2), 1304.97 (-OCH3), 1210.74 (C-O), 1075.15 (C-N); 1H NMR: 4.02, 4.08, 4.84, 5.15, 5.18, 5.82, 7.16, 7.18, 7.47, 7.48, 7.50, 7.52, 7.82, 7.83, 8.52, 13.35; 13C NMR: 46.7, 53.9, 73.8, 88.4, 109.9, 119.7, 122.8, 122.9, 128.7, 128.8, 130.5, 130.4, 134.4, 136.5, 142.1, 142.8, 160.6, 166.5, 173.0; Molecular Mass [M^{+}]: 387.10; Elemental Analysis: C, 58.84; H, 4.68; Cl, 9.14; N, 10.84; O, 16.50.

2.2.3.6 2,3-dihydroxy-3-(1-(2-(4-methylbenzylidene) amino) ethyl)-1H-benzo[d] imidazol-2-yl) propanoic acid (3f)

Color: white; % Yield: 85%; M.P.: 86 ^{o}C; FTIR (cm^{-2}): 3221.15 (O-H), 3168.16 (Ar C-H), 2872.18 (Alp. C-H), 1742.16 (C=O), 1696.16 (C=N), 1559.75 (Ar C=C), 1441.45 (-CH_2), 1324.17 (-OCH3), 1263.14 (C-O), 1049.74 (C-N); 1H NMR: 2.40, 4.02, 4.07, 4.83, 5.16, 5.18, 5.82, 7.16, 7.19, 7.20, 7.22, 7.50, 7.51, 7.75, 7.76, 8.74, 13.33; 13C NMR: 21.2, 46.6, 53.9, 73.8, 88.4, 109.9, 119.5, 122.8, 122.9, 128.8, 129.8, 128.9, 129.0, 133.2, 140.6, 142.0, 142.7, 160.7, 166.5, 173.1; Molecular Mass [M^{+}]: 367.15; Elemental Analysis: C, 65.38; H, 5.76; N, 11.44; O, 17.42.

2.3 Biological Activity

2.3.1 Glycolytic Enzyme Inhibition Assay

α-Glucosidase from *Saccharomyces cerevisiae* (1 mg) was dissolved in 20 mL phosphate buffer (pH 7.0) containing bovine serum albumin (BSA, 40 mg) and diluted (1:10) before use. Test compounds (3a-f) were

prepared in DMSO (10 mM) at concentrations of 50-250 µg/mL. The reaction mixture, containing substrate (p-nitrophenyl-D-glucopyranoside), buffer, and test sample, was pre-incubated at 37 °C for 5 min. The reaction was initiated by adding the enzyme solution and further incubated at 37 °C for 15 min. The reaction was terminated using 200 mM sodium carbonate (Na_2CO_3), and the absorbance was measured at 400 nm using a UV-Vis spectrophotometer against a DMSO blank.[19]

2.4 Statistical Analysis

Statistical analysis was executed using GraphPad Prism v.5.00. Data were stated as mean ± SEM. One-way ANOVA followed by Dunnett's post hoc test was applied to limit statistical significance.

2.5 Computational Study

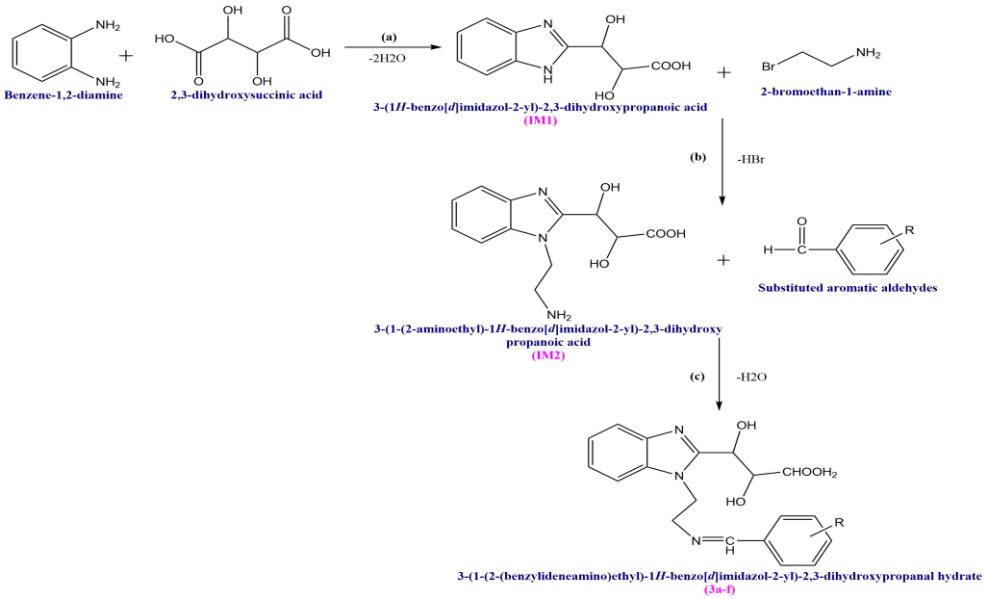
2.5.1 Molecular Docking Study

The 2D structures of designed ligands **(3a-f)** were drawn using ChemDraw 22.2.0 software. and converted into 3D structures in Chem3D with energy minimization using the MM2 force field. The optimized structures were saved in. pdb format.[20] The crystal structure of isomaltase from Saccharomyces cerevisiae (PDB ID: 3A4A) was obtained from the Protein Data Bank.[21] Molecular docking was performed using ArgusLab 4.0 software, and visualization was carried out using PyMOL v.3.0.[22]

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthesis of the target compounds (3a-f) was completed through a multistep synthetic strategy, as illustrated in Scheme 1. The synthesized compounds were successfully obtained in good to excellent yields (82-86%) with distinct physical appearances ranging from bright pink to white and pale-yellow solids, indicating purity and effective structural alterations. The FTIR spectral analysis of compound 1 confirmed the presence of characteristic functional groups, including a broad band at 3285.45 cm^{-1} corresponding to hydroxyl (-OH), a peak at 2601.52 cm^{-1} for carboxylic -OH, 1725.12 cm^{-1} for carbonyl (C=O), 1621.74 cm^{-1} for imine (C=N), 1532.15 cm^{-1} for aromatic (C=C), along with C-O (1203.14 cm^{-1}) and C-N (1085.45 cm^{-1}) stretching vibrations. In compound 2, the introduction of the aminoethyl group was evidenced by additional aliphatic C-H stretching at 2862.41 cm^{-1} along with retained O-H (3289.74 cm^{-1}), aromatic C-H (3045.92 cm^{-1}), C=O (1729.12 cm^{-1}), and C=N (1632.74 cm^{-1}) functionalities. Further structural variation in compounds (3a-f) through Schiff base formation was confirmed by the appearance of a characteristic imine (C=N) band in the range of 1622.74-1642.56 cm^{-1} along with consistent O-H stretching (3221–3289 cm^{-1}) and carbonyl peaks (1722-1780 cm^{-1}). Substituent-specific peaks further validated the structures, such as -NO_2 stretching at 1506.45 cm^{-1} in compound 3b, (-OCH_3) at 1320 cm^{-1} in 3c, and (C-Cl) influence in 3e, while aromatic and aliphatic (C-H) bands appeared in the regions of 3140-3198 cm^{-1} and 2860-2872 cm^{-1}, respectively. The ^1H NMR spectra supported the proposed structures, showing characteristic signals for aliphatic protons (1.18-5.82 ppm), aromatic protons (7.15-8.75 ppm), and a separate downfield signal for the carboxylic proton (13.33-13.35 ppm). Similarly, ^{13}C NMR spectra exhibited signals corresponding to aliphatic carbons (21.2-88.4 ppm), aromatic carbons (103.8-142.8 ppm), imine carbons (160-166 ppm), and carboxylic carbon (173 ppm). The molecular ion peaks and elemental analysis data were in good agreement with the calculated values, further positive the successful synthesis and structural integrity of all compounds. Overall, the spectral data clearly determine the positive formation of benzimidazole-based dihydroxy propanoic acid derivatives with various substituted benzylidene moieties.



Scheme 1. Synthetic strategies of designed benzimidazole derivatives (3a-f).

Compound ID	Substituted Aromatic Aldehydes (R)
3a	H
3b	4-NO_2
3c	3,4,5-$(OCH_3)_3$
3d	4-$CH(CH_3)_2$
3e	4-Cl
3f	4-CH_3

Reaction conditions: (a) Glacial acetic acid, reflux, 90 °C for 4 hours; (b) K2CO3, reflux, 100 °C for 4 hours; (c) Glacial acetic acid, reflux, 60-70 °C for 3-4 hours.

3.2 Biological Activity

3.2.1 Glycolytic Enzyme Inhibition Assay

The α-glucosidase inhibitory activity of the synthesized benzimidazole derivatives (3a-f) was evaluated using IC_{50} values (µM ± SEM). Among the tested compounds, 3f (IC_{50} = 25.57 ± 3.09 µM) showed the highest inhibitory potency, followed by 3c (IC_{50} = 30.35 ± 3.42 µM) and 3a (IC_{50} = 37.32 ± 2.61 µM), all of which were more active than the standard drug Acarbose (IC_{50} = 43.52 ± 2.76 µM) (Table 1). Compound 3d (IC_{50} = 39.31 ± 4.39 µM) exhibited moderate activity, 3e (IC_{50} = 73.68 ± 2.03 µM) and 3b (IC_{50} = 93.85 ± 2.81 µM) exhibited moderately weaker inhibition (Figure 2). The differences in activity can be attributed to the nature of substituents on the aromatic ring. Compounds bearing electron-donating groups, particularly at the para position, enhance binding interactions within the enzyme active site, resulting in improved inhibition. In contrast, electron-withdrawing substituents such as chloro and nitro groups reduce electron density and weaken enzyme-ligand interactions, leading to decreased inhibitory activity. These results highlight the importance of electronic effects in modulating α-glucosidase inhibition.

Table 1: Glycolytic enzyme assay of synthesized compounds (3a-f).

Compound ID	R	IC_{50} value[a]
Acarbose	-	43.52 ± 2.76
3a	H	37.32 ± 2.61
3b	4-NO_2	93.85 ± 2.81
3c	3,4,5-$(OCH_3)_3$	30.35 ± 3.42
3d	4-$CH(CH_3)_2$	39.31 ± 4.39
3e	4-Cl	73.68 ± 2.03
3f	4-CH_3	25.57 ± 3.09

IC_{50} value[a] expressed in µM ± SEM.

Figure 2. Glycolytic enzyme assay of synthesized compounds (3a-f).

3.3 Computational Study

3.3.1 Molecular Docking Study

Molecular docking studies were performed using Argus Lab 4.0 to investigate the binding interactions of the synthesized compounds **(3a-f)** with the active site of α-glucosidase enzyme, using acarbose as the standard drug (Table 2). The docking scores of all six synthesized compounds ranged from -8.93126 to -12.0635 kcal/mol. Among these, two compounds exhibited the highest docking scores. Compounds 3d and 3f showed strong interactions with α-glucosidase enzyme, with binding affinities of -11.6917 and -12.0635 kcal/mol, respectively. A more negative binding energy indicates a more stable ligand-protein interaction. The potent compounds 3d and 3f demonstrated similar amino acid interactions, similar to those of the standard drug, within the active site, suggesting a comparable binding pattern. Notably, the designed compounds demonstrated greater

stability in complex formation with the target compared to the standard drug. Molecular visualization was carried out using PyMol (Figure 3).

Table 2: Molecular docking simulation of the designed ligands with the target protein.

Compound ID	Docking score (kcal/mol)	No. of H-bonds	Bond length of H-bonds (Å)	Amino acid interaction
Acarbose	-6.8542	2	2.213464	543 PHE
			2.264331	523 LYS
3a	-11.0860	4	2.888482	215 ASP
			2.269441	352 ASP
			2.827519	352 ASP
			2.996611	442 ARG
3b	-10.9546	6	2.706886	442 ARG
			2.999321	351 HIS
			2.717993	352 ASP
			2.561682	277 GLU
			2.628434	350 ASN
			2.229363	347 TYR
3c	-8.9312	6	2.911372	335 ARG
			2.202419	335 ARG
			2.642277	335 ARG
			2.656453	519 ASP
			2.662194	520 LEU
			2.661398	521 ASP
3d	-11.6917	3	2.631087	112 HIS
			2.900189	69 ASP
			2.886284	442 ARG
3e	-9.9805	3	2.247459	523 LYS
			2.892515	543 PHE
			2.476712	543 PHE
3f	-12.0635	4	2.487682	442 ARG
			2.993618	442 ARG
			2.635566	352 ASP
			2.604448	277 GLU

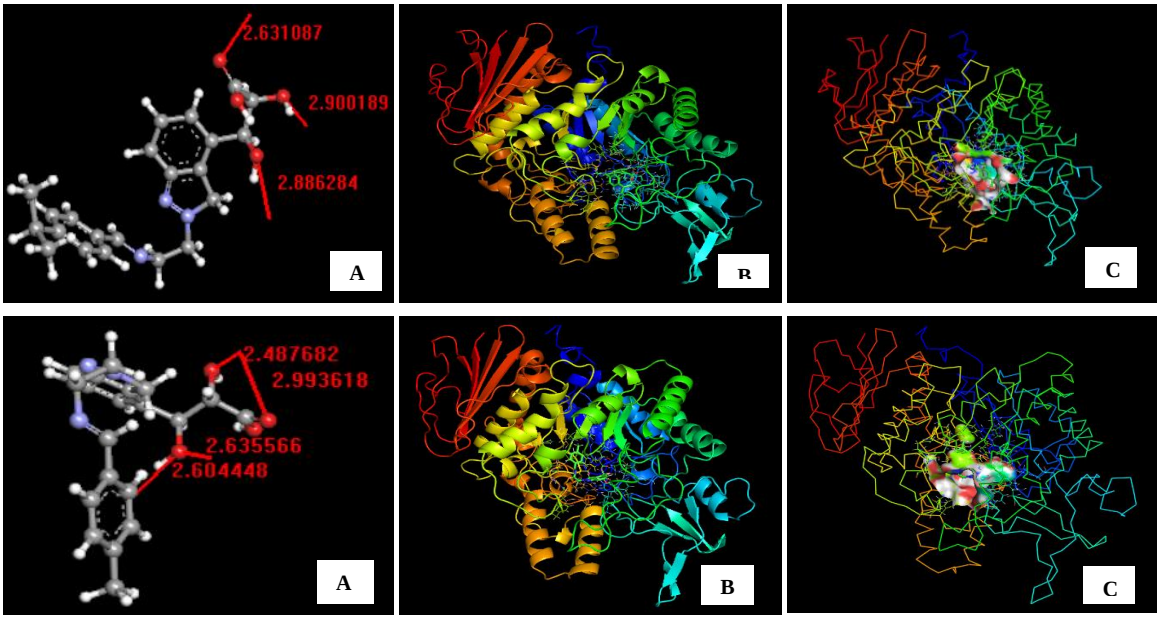


Figure 3: Ligand-protein complex interaction of the designed ligands with the target protein. (A) ligand depicted in the stick form with the hydrogen bond with the key amino acids; (B) Cartoon structure of the protein-ligand complex; (C) Proteins are presented in ribbon structure, and the ligand is covered with the active site solid surface structure.

CONCLUSION

In the present study, a novel series of benzimidazole-based derivatives (3a-f) was effectively designed and synthesized, and their biological potential as α-glucosidase inhibitors was evaluated. Among the tested compounds, 3f emerged as the most potent inhibitor (IC_{50} = 25.57 ± 3.09 µM), followed by 3c and 3a, all of which established superior activity compared to acarbose. The improved activity of these derivatives can be attributed to the presence of electron-donating substituents, which favourably influence protein-ligand interactions within the binding site. Molecular docking simulation further supported the investigational findings, revealing that compounds 3f and 3d showed the most favourable binding affinities (-12.0635 and -11.6917 kcal/mol, respectively). These compounds exhibited stable binding configurations and strong interactions with key amino acid residues, indicating a high degree of compatibility with the enzyme active site. Overall, the study highlights compound 3f as a promising lead molecule with significant α-glucosidase inhibitory potential. These findings emphasize the therapeutic relevance of benzimidazole derivatives and provide a strong structural feature for further optimization toward the development of effective antidiabetic agents targeting postprandial hyperglycaemia.

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