INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, characterized by elevated blood glucose levels. It is one of the major threats to human health in the 21st century disrupting the metabolism of carbohydrates, proteins, and lipids. Obesity, physical inactivity, and oxidative stress are the major factors causing T2DM, which can lead to serious complications such as cardiovascular diseases, retinopathy, nephropathy, and so forth. The International Diabetes Federation (IDF) has reported 425 million patients with diabetes globally in 2017, which is estimated to increase to 629 million by 2045. Two enzymes, α-amylase and α-glucosidase, play key roles in carbohydrate metabolism, thereby increasing blood glucose (hyperglycemia). The inhibition of these enzymes has found an effective way of managing T2DM.

Pakistan is a South Asian country with a population of 207.68 million people (Pakistan Bureau of Statistics, 2017). The national diabetes survey of Pakistan in 1999 reported the prevalence of diabetes as 11%; however, this number has alarmingly increased in the 21st century. The IDF Atlas 2017 has ranked Pakistan at 10 of 221 countries of the world having 7.5 million cases of diabetes aged 20–79 years, while in its recent survey (IDF Atlas 9th Edition 2019), the prevalence of diabetes in Pakistan has reached 17.1% (19 million adults with diabetes), which is 148% higher than the previously reported figures. This alarming situation of growing diabetes crisis needs immense attention.
Benzimidazole is a privileged structure in organic and medicinal chemistry as is evident from its different medicinal candidates such as omeprazole, pantoprazole (proton pump inhibitors), triclabendazole, thiabendazole, albendazole, mebendazole (anthelmintic), benomyl, carbendazim, fuberidazole (fungicide), candesertan, telmisartan (anti-hypertensive), astemizole (antihistamine), bendamustine (anti-cancer), afobazole (anxiolytic), and so forth. New synthetic analogues of benzimidazole have been continuously reported to have a broad spectrum of activities. Our research group has previously reported benzimidazole-based antioxidants (IC$_{50}$: 131.50 ± 12.39; IC$_{50}$: 167.4 ± 7.4), α-glucosidase (IC$_{50}$: 352 μg/mL), cholinesterase (IC$_{50}$: 121.2 μM and 38.3 μM), and carbonic anhydrase inhibitors (IC$_{50}$: 13.3 ± 1.25 μM). In search of exploring new benzimidazole-based medicinal candidates, the present study was aimed at designing, synthesis, and bioactivity assessment of novel benzimidazole derivatives.

**RESULTS AND DISCUSSION**

This work is based on a multistep reaction procedure. The target compounds 7a–m were accomplished using methods, as outlined in Scheme 1. In the first step, 2-nitroaniline 1 was reduced to benzene-1, 2-diamine 2. Then, it was cyclized to 1H-benzo[d]imidazole-2-thiol 3 through CS$_2$. The obtained product 3 was oxidized to 5-nitro-1H-benzo[d]imidazole-2-thiol 4 and then reduced to 5-amino-1H-benzo[d]imidazole-2-thiol 5. Finally, the reaction of 5 with different aromatic aldehydes 6a–m yielded the target compounds 7a–m. The synthesis of all the compounds was monitored by their thin-layer chromatography (TLC) analysis in n-hexane: ethyl acetate/chloroform systems under UV. The structures of all the compounds were confirmed with the help of ESI-HRMS, 1HNMR, and 13CNMR spectroscopy.

Table 1. In Vitro α-Glucosidase Inhibition Activity of the Target Compounds 7a–m

| sample | percent inhibition (0.5 mM) | IC$_{50}$ ± μM (SEM) |
|--------|----------------------------|-----------------------|
| 7a     | 95.68                      | 11.84 ± 0.26          |
| 7b     | 93.25                      | 27.26 ± 0.30          |
| 7c     | 95.74                      | 9.84 ± 0.08           |
| 7d     | 96.61                      | 5.34 ± 0.16           |
| 7e     | 91.35                      | 16.38 ± 0.53          |
| 7f     | 95.00                      | 6.46 ± 0.30           |
| 7g     | 94.86                      | 8.62 ± 0.19           |
| 7h     | 91.70                      | 20.73 ± 0.59          |
| 7i     | 95.41                      | 0.64 ± 0.05           |
| 7j     | 94.73                      | 18.65 ± 0.74          |
| 7k     | 87.64                      | 70.28 ± 1.52          |
| 7l     | 83.20                      | 343.10 ± 1.62         |
| 7m     | 94.00                      | 11.09 ± 0.79          |
| acarbose | 58.49                  | 873.34 ± 1.21        |

In Vitro α-Glucosidase Inhibition Activity of the Target Compounds 7a–m

Figure 1. (A) % Inhibition of 7a–m against α-glucosidase in comparison with acarbose. (B) IC$_{50}$ of 7a–m against α-glucosidase in comparison with acarbose.
BIOLOGICAL EVALUATION

α-Glucosidase Inhibitory Activity of the Target Compounds 7a–m. The in vitro α-glucosidase inhibition potential of the target compounds 7a–m was evaluated using acarbose as a standard antidiabetic drug. As shown in Table 1, all the tested compounds displayed the highest inhibition of the enzyme than acarbose (IC$_{50}$: 873.34 ± 1.21 μM), where 7i was the most active inhibitor (IC$_{50}$: 0.64 ± 0.05 μM) followed by 7d (IC$_{50}$: 5.34 ± 0.16 μM), 7f (IC$_{50}$: 6.46 ± 0.30 μM), 7g (IC$_{50}$: 8.62 ± 0.19 μM), 7c (IC$_{50}$: 9.84 ± 0.08 μM), 7m (IC$_{50}$: 11.09 ± 0.79 μM), 7a (IC$_{50}$: 11.84 ± 0.26 μM), 7e (IC$_{50}$: 16.38 ± 0.53 μM), 7j (IC$_{50}$: 18.65 ± 0.74 μM), 7b (IC$_{50}$: 20.73 ± 0.59 μM), 7b (IC$_{50}$: 27.26 ± 0.30 μM), 7k (70.28 ± 1.52 μM), and finally 7l (IC$_{50}$: 343.10 ± 1.62 μM) which was the last inhibitor in the series.

The comparative % inhibition of α-glucosidase and the IC$_{50}$ values of the target compounds with reference to acarbose are provided in Figure 1a,b. It is clear from the two graphs that the inhibition potential of target compounds 7a–m is much better than that of the reference drug.

Structure Activity Relationship. The target compounds 7a–m are multifunctional benzimidazoles, as shown in Figures 2 and 3. Figure 2 represents their basic skeleton where...
benzimidazole bears a thiol group at C-2 and an imine group at C-5 to which various aromatic substituents are bonded. These compounds bear differences in the substituents at the benzylidene ring except \(7k\) where a thiophene ring bonds the imine carbon. \(7i\) and \(7f\) are 2,4-disubstituted while \(7a, 7b, 7c, 7d,\) and \(7m\) are 4-substituted benzylidene analogues. Furthermore, \(7g\) and \(7h\) are 3,4-disubstituted, \(7e\) is 2,3-disubstituted, \(7j\) is 3,4,5-trisubstituted while \(7l\) is 2-substituted analogue. Lastly, \(7k\) is thiophen-2-ylmethylene analogue as shown in Figure 3. All the substituents present at the 2nd, 3rd, 4th, and 5th position of the benzylidene ring as well as the heterocyclic moiety have greatly enhanced the inhibition potential of the compounds. Overall, the 13 novel compounds evaluated against \(\alpha\)-glucosidase inhibited the enzyme more than the standard inhibitor acarbose (IC\(_{50}\): 873.34 ± 1.21 \(\mu\)M). All these compounds demand further investigation which may help in drug development against type 2 diabetes mellitus.

### MOLECULAR DOCKING STUDIES

**Methodology.** To explore the interaction of the target compounds \(7a-m\) with the enzyme, a homology model of \(S.\) cerevisiae \(\alpha\)-glucosidase (ID AF-P38158-F1) was downloaded from the AlphaFold Protein Structure Database server (https://alphafold.ebi.ac.uk/).\(^{13}\) Ramachandran plot was generated to further validate the quality of the model (https://zlab.umassmed.edu/bu/rama/).\(^{14}\) The allowed regions and disallowed residues were identified. Furthermore, the ProSA-web server was used to compute the stereochemical properties of the enzyme by comparing the model with the reported crystal structure.\(^{15}\) Employing molecular mechanics, the missing hydrogens in the model were added using molecular operating environment (MOE-2020.0109)\(^{16}\) and the partial charges were calculated using the tethered energy minimization implemented in MOE. The enzyme geometry was optimized using the autocorrect protocol of MOE and the bad contacts were removed. The low energy conformations of the enzyme was determined using the AMBER14:ETH force field.\(^{17}\)

**Three-Dimensional Structure of \(\alpha\)-Glucosidase.** The three-dimensional (3D) structure of the \(\alpha\)-glucosidase is presented in Figure 4. The active site and catalytic residues of \(S.\) cerevisiae (PDB ID 3A47) and \(\alpha\)-glucosidase (ASP214, GLU276, ASP349) are conserved for substrate attachment.\(^{18}\) The three domains (A, B, C) of the enzyme are reported in different colors (yellow, blue, red) representing the amino acids.

**Model Validation.** To learn about the general structure of the model, the homology model of the enzyme must be validated. The Ramachandran plot demonstrates good stereo-
chemical characteristics of the model. The model’s residues were in the favorable zone (98.84%), the allowed region was 1.16%, and there was no disallowed area in the 3D model of the enzyme. The AlphaFold Protein Structure alignment with the template expected error was zero in 90% of the model residues. The deviation from the template was calculated in angstrom (Å) (Figure 5).

The overall structural quality of α-glucosidase is depicted in Figure 6, revealing that the model was inside the acceptable quality zone. The small variations were at a positive energy state (enzyme’s unstable areas), but the overall model was in a negative energy window (enzyme’s overall excellent quality). The current model showed that the X-ray structure zone had the same amino acid size, offering excellent quality of the model. The high and low energy areas were highlighted in blue and red, respectively. The entire model was registered in a lower energy state, defining the enzyme structure’s stability.

**Docking Studies on α-Glucosidase.** The docking protocols were validated by transferring the substrate molecule (isomaltose) from PDB ID 3AXH (S. cerevisiae isomaltase) because of high structural similarity and conserved catalytic residues (Asp214, Glu276, and Asp349) with the S. cerevisiae α-glucosidase. The conserved catalytic residues triad for substrate catalysis, where Asp349 maintained the transition state of the substrate molecule while Glu276 and Asp214 acted as nucleophile and proton donors, respectively. The lining of the active site residues includes Arg212, Glu276, Asp214, Tyr71, His348, Asp349, Arg439, Asp68, and Phe177 that form multiple hydrophilic and hydrophobic interactions with the substrate molecule (Figure 7). Redocking was performed, and the reported RMSD between the reported and redocked substrate is 0.729 Å, which appears to be an acceptable range. To explore the selected compound interactions at the atomic level, their stability and the best fit in the active pocket of the α-glucosidase protein molecular docking study was performed using MOE. The target compounds 7a–m were docked in the active pocket residues of the 3D structure of the α-glucosidase, and the docking contacts were examined. All the compounds demonstrated excellent interactions with the active pocket residues. The important active site residues (catalytic residues) involved in the interaction with the compounds were ASP214, GLU276, and ASP349. Similarly, other residues in the surrounding were GLU276, GLU304, and GLY217 which have occupied the 1st, 2nd, and 3rd positions, respectively, in the interaction population (Figure 8).

The 3D interactions of the target compounds 7a–m in the active pocket of the α-glucosidase along with their two-dimensional images are presented in Figure 9 which highlights the various parts of each compound involved with the residues.

**Docking Score, Binding Energy, and Affinity.** The binding energy and affinity were determined using MOE’s implicit solvent technique generalized Born/volume integral (GB/VI). The generalized Born interaction is nonbonded interaction energy between an enzyme and the inhibitor. The target compounds 7a–m showed good binding energy, affinity, and docking score with slight variations (Table 2). The highest binding energy and affinity were reported for 7i as $-69.65$ kcal/mol and $-10.54$ kcal/mol, for 7f as $-63.36$ kcal/mol and $-10.09$ kcal/mol, and for 7d as $-57.02$ kcal/mol and affinity $-9.06$ kcal/mol, respectively. On the other hand, 7j displayed the highest docking score ($-7.34$ kcal/mol) and has formed hydrogen bond interactions with Asp68, His111, Gln181, and Asp214 while 7c, 7e, 7f, 7g, 7h, 7i, and 7m displayed the 2nd highest docking ($-6.1$ to $-6.5$ kcal/mol) followed by 7a, 7b, 7d, and 7k ($-5.7$ to $-5.9$ kcal/mol), making good interactions with the active pocket of the enzyme.
The lowest docking score reported for 7i was $-4.19$ kcal/mol, which had formed a single hydrogen bond with the Gly217 backbone while making two arene hydrogen interactions with Phe157 and Glu276 (Tables 2 and 3). All the compounds showed good negative interaction energies with the active pocket residues and fulfill the criteria of druglike properties. These interactions, binding energies, and affinities support the prominent α-glucosidase inhibition of the target compounds. Compounds having a good IC$_{50}^\alpha$ value from 0.64 to 20 μM include 7c, 7f, 7g, 7h, 7a, 7e, 7h, 7j, and 7m which shows a high docking score greater than $-6$ kcal/mol except for 7d for which the docking score is $-5.86$ kcal/mol. In contrast, the lowest IC$_{50}^\alpha$ value reported for the 7i shows a docking score as $-4.19$ kcal/mol and the lowest binding energy of $-29.98$ kcal/mol. In the case of 7b and 7k, the reported docking score is $-5.7$ kcal/mol, whereas the IC$_{50}^\alpha$ values reported are 27.26 and 70.28 μM, respectively. The results indicate that the docking score values satisfied the in vitro inhibition assay of the α-glucosidase.

**EXPERIMENTAL SECTION**

Solvents and reagents of analytical grade were purchased from Daejung, Alfa Aesar, Merck, and Sigma Aldrich and were used as such. All the reactions were carried out in round-bottom flasks (Pyrex) over a hotplate magnetic stirrer and their progress was monitored by TLC analysis. Melting points were determined on Gallenkamp digital melting point equipment MGB-595-010 M. $^1$H NMR spectra were recorded via a Bruker 600 MHz ASCEND spectrometer. Chemical shifts are given in ppm, with TMS used as an internal standard. Coupling patterns are described as s (singlet); d (doublet); t (triplet); q (quartet); and m (multiplet), and the coupling constants (J values) were reported in Hertz (Hz). $^{13}$C NMR spectra were recorded via a Bruker 150 MHz ASCEND spectrometer. Chemical shifts are given in ppm, with TMS used as an internal standard. ESI-HRMS spectra were recorded on an Agilent 6530 Accurate Mass Q-TOF LC/MS system performed at the University of Nizwa, Oman.

**Synthesis of Benzene-1,2-Diamine 2.** 2-Nitroaniline 1 and zinc dust (0.1:0.2 mol) were taken in methanol and stirred at room temperature. Then, hydrazinium monoformate was added and stirred for 2–5 min. After completion, the reaction mixture was filtered and dried. The product was extracted in ethyl acetate, washed with brine, dried, and preserved for the next reaction.19 Brownish solid; yield: 80%; m.p: 100–103 °C.

**Synthesis of 1H-Benzol[d]imidazol-2-thiol 3.** Equimolar amounts of KOH and CS$_2$ (0.06: 0.06 mol) were taken in ethanol—water (50:40 mL) and stirred. Then, benzene-1,2-diamine (0.06 mol) 2 was added and refluxed for 12 h. After completion, the excess solvent was removed. The product was precipitated in dilute HCl, filtered, dried, and recrystallized.20 Off white crystals; yield: 85%; m.p: 300–303 °C.

**Synthesis of 5-Nitro-1H-benzol[d]imidazol-2-thiol 4.** The previous compound 3 (0.03 mol) was taken with chilling in conc. H$_2$SO$_4$ (8 mL) and a mixture of conc. HNO$_3$ and conc. H$_2$SO$_4$ (1:2) was added dropwise keeping overnight. Then, the reaction mixture was quenched with freezing water and the pH was made 9 by adding 10% NaOH solution. The product precipitated was filtered, washed, and dried.21 Brownish solid; yield: 84%; m.p: 273–275 °C.

**Synthesis of 5-Amino-1H-benzol[d]imidazol-2-thiol 5.** Compound 5 was synthesized in a similar manner as compound 2 using Zn dust and hydrazinium monoformate.19 Off white crystals; yield: 82%; m.p 239–242 °C.

**Synthesis of 5-(Arylideneamino)-1H-benzol[d]-imidazol-2-thiols 7a–m.** The target compounds 7a–m were finally synthesized by refluxing the equimolar amounts of 5 with different aromatic aldehydes 6a–m for 4–6 h catalyzed using 3–5 drops of organic or mineral acids.11 After completion, the product was precipitated in freezing water, filtered, washed, and dried. All the compounds were colored solids, having high melting points and soluble in polar solvents. Their details are given below.

4-(((2-Mercapto-1H-imidazol-5-yl)limino)methyl)phenol 7a. Brownish solid; yield: 87%; m.p: 245–247 °C. $^1$H NMR (600 MHz, DMSO): $\delta_H$ = 12.528–12.510 (s, 2H, Ar=SH, Ar=NH), 8.487 (s, 1H, Ar=N=CH), 7.768–7.755 (d, J = 7.8 Hz, 2H, Ar=H), 7.121–7.107 (d, J = 8.4 Hz, 1H, Ar=H), 7.043–7.029 (d, J = 8.4 Hz, 1H, Ar=H), 6.967 (s, 1H, Ar=H), 6.870–6.858 (d, J = 8.4 Hz, 2H, Ar=H), 4.933 (s, 1H, Ar=OH) ppm. $^{13}$C NMR (150 MHz, DMSO) $\delta_C$ = 165.81, 155.51, 155.47, 147.07, 133.06, 130.59, 130.43, 127.65, 116.62, 115.64, 109.69, 101.27 ppm. ESI-HRMS m/z: 270.06 (M+H)$^+$; calcd: 269.06 (C$_{19}$H$_{17}$N$_2$OS).

5-((4-Chlorobenzylidene)amino)-1H-benzol[d]-imidazol-2-thiol 7b. Light brown solid; yield: 83%; m.p: 280–282 °C. $^1$H NMR (600 MHz, DMSO): $\delta_H$ = 12.611–12.574 (s, 2H, Ar=SH, Ar=NH), 8.683 (s, 1H, Ar=N=CH), 7.953–7.940 (d, J = 7.8 Hz, 2H, Ar=H), 7.580–7.567 (d, J = 7.8 Hz, Ar=H), 7.158–7.119 (q, J = 8 Hz, 2H, Ar=H), 9.369 (s, 1H, Ar=H).
Figure 8. Docking interactions of 7a–m. (A) Compounds interacting with the active site residues of the α-glucosidase are represented by a barcode. Each line shows a single compound interacting with each residue in a row. (B) Overall population of the interacting active pocket residues of the α-glucosidase.

7.012 (s, 1H, Ar−H) ppm. 13C NMR (150 MHz, DMSO) δ C = 168.82, 158.26, 146.03, 135.83, 135.06, 131.13, 130.19, 128.96, 117.05, 109.74, 101.53 ppm. ESI-HRMS m/z: 288.03 (M + H)+; calcd: 287.03 (C12H16Cl). 3-Ethoxy-4-(((2-mercapto-1H-imidazol-5-yl)imino)methyl)-6-methoxyphenol 7e. Deep red solid; yield: 87%; m.p: 250–253 °C. 1H NMR (600 MHz, DMSO): δ H = 12.688–12.634 (s, 2H, Ar−SH, Ar−NH), 8.965 (s, 1H, Ar−N≡CH), 7.103–7.089 (d, J = 8.4 Hz, 1H, Ar−H), 6.899–6.873 (t, J = 7.8 Hz, 1H, Ar−H), 7.236–7.169 (m, 4H, Ar−H), 4.100 (s, 1H, Ar−OH), 3.805 (s, 3H, Ar−OCH3) ppm. 13C NMR (150 MHz, DMSO): δ C = 169.12, 162.42, 150.43, 147.89, 142.87, 133.17, 131.54, 123.90, 119.34, 118.58, 117.08, 115.39, 109.91, 101.66, 55.89 ppm. ESI-HRMS m/z: 300.07 (M + H)+; calcd: 299.07 (C15H12N2O3S).

5-((4-Nitrobenzylidene)amino)-1H-benz[d]imidazole-2-thiol 7c. Red solid; yield: 87%; m.p: 283–285 °C. 1H NMR (600 MHz, DMSO): δ H = 12.672–12.626 (s, 2H, Ar−SH, Ar−NH), 8.863 (s, 1H, Ar−N≡CH), 8.353–8.339 (d, J = 8.4 Hz, 2H, Ar−H), 8.170 (d, J = 7.8 Hz, 2H, Ar−H), 7.103–7.144 (m, 3H, Ar−H) ppm. 13C NMR (150 MHz, DMSO) δ C = 169.08, 157.41, 148.68, 145.41, 141.79, 133.10, 129.50, 124.04, 117.58, 109.79, 101.74 ppm. ESI-HRMS m/z: 299.05 (M + H)+; calcd: 298.05 (C12H16N2O3S).

5-((4-Diethylamino)benzylidene)amino)-1H-benz[d]imidazole-2-thiol 7d. Deep brown solid; yield: 93%; m.p: 280–283 °C. 1H NMR (600 MHz, DMSO): δ H = 12.496–12.479 (s, 2H, Ar−SH, Ar−NH), 8.396 (s, 1H, Ar−N≡CH), 7.709–7.696 (d, J = 7.8 Hz, 2H, Ar−H), 7.103–7.089 (d, J = 8.4 Hz, 1H, Ar−H), 7.016–7.002 (d, J = 8.4 Hz, 1H, Ar−H), 6.933 (s, 1H, Ar−H), 6.733–6.720 (d, J = 7.8 Hz, 2H, Ar−H), 3.421–3.388 (q, J = 7 Hz, 4H, Ar−N(CH3)2) ppm. 13C NMR (150 MHz, DMSO): δ C = 168.30, 158.92, 149.83, 133.09, 130.63, 130.07, 116.52, 110.90, 109.66, 101.09, 43.86, 12.47 ppm. ESI-HRMS m/z: 325.14 (M + H)+; calcd: 324.14 (C13H18N3S).
[51x391]reported in stick representation. Compound with different colored label residues while the rectangle shows the 3D interaction poses of the compounds. Interacting residues are Table 2. Binding Energy, Affinity, and Docking Score of the Target Compounds 7a–m Calculated with the Active Pocket of the α-Glucosidase

| entry | docking score (kcal/mol) | binding energy (kcal/mol) | binding affinity (kcal/mol) |
|-------|--------------------------|---------------------------|-----------------------------|
| 7a    | −5.8895102               | −36.95                    | −6.64                       |
| 7b    | −5.7605391               | −48.66                    | −8.07                       |
| 7c    | −6.0312943               | −47.16                    | −7.82                       |
| 7d    | −5.8632793               | −57.02                    | −9.06                       |
| 7e    | −6.3273244               | −45.27                    | −7.77                       |
| 7f    | −6.4258284               | −63.36                    | −10.09                      |
| 7g    | −6.5642915               | −50.7                     | −8.28                       |
| 7h    | −6.3905635               | −49.06                    | −8.29                       |
| 7i    | −6.1296835               | −69.65                    | −10.54                      |
| 7j    | −7.3497996               | −52.12                    | −9.1                        |
| 7k    | −5.7656335               | −39.56                    | −6.96                       |
| 7l    | −4.1924725               | −29.98                    | −4.8                        |
| 7m    | −6.1924725               | −39.98                    | −7.2                        |

H, J = 8.4 Hz, 1H), 6.985  (s, 1H, Ar−H), 6.895–6.882  (d, J = 7.8 Hz, 1H, Ar−H2), 5.05  (s, 1H, Ar−OH), 4.080–4.070  (q, J = 6 Hz, 2H, Ar−OCH3CH3), 1.363–1.339  (t, J = 7 Hz, 3H, Ar−OCH3CH3) ppm. 13C NMR (150 MHz, DMSO): δC = 168.49, 166.46, 159.29, 153.23, 150.40, 147.30, 147.13, 146.83, 133.28, 133.06, 130.47, 128.73, 127.92, 128.86, 123.89, 116.60, 115.48, 111.92, 111.81, 110.94, 109.86, 109.72, 101.32, 63.86, 63.88, 14.74 ppm. ESI-HRMS m/z: 314.09 (M + H)+; calcd: 313.09 (C16H13N3O5S).

5-((3,4-Dimethoxybenzylidene)amino)-1H-benzo[d]imidazole-2-thiol 7h. Yellowish solid; yield: 92%; m.p: 257–260 °C. 1H NMR (600 MHz, DMSO): δH = 12.563–12.553  (s, 2H, Ar−SH, Ar−NH), 8.539  (s, 1H, Ar−N¼CH), 7.545  (s, 1H, Ar−H), 7.449–7.435  (d, J = 8.4 Hz, 1H, Ar−H), 7.137–7.124  (d, J = 7.8 Hz, 1H, Ar−H), 7.080–7.066  (d, J = 8.4 Hz, 2H, Ar−H), 7.007  (s, 1H, Ar−H), 3.822  (s, 6H, Ar−OCH3) ppm. 13C NMR (150 MHz, DMSO) δC = 168.9, 159.5, 152.1, 149.4, 147.1, 133.4, 131.0, 129.6, 124.2, 117.1, 111.7, 110.1, 109.8, 101.7, 56.0, 55.8 ppm. ESI-HRMS m/z: 314.09 (M + H)+; calcd: 313.09 (C16H13N3O5S).

4-((2-Mercapto-1H-benzo[d]imidazol-5-yl)methyl)benzene-1,3-diol 7i. Brownish solid; yield: 89%; m.p: 268–271 °C. 1H NMR (600 MHz, DMSO): δH = 12.631–12.578  (s, 2H, Ar−SH, Ar−NH), 8.800  (s, 1H, Ar−N¼CH), 7.432–7.418  (d, J = 8.4 Hz, 1H, Ar−H), 7.141–7.111  (m, 3H, Ar−H), 6.391–6.377  (d, J = 8.4 Hz, 1H, Ar−H), 6.280  (s, 1H, Ar−H), 5.00  (s, 2H, Ar−OH) ppm. 13C NMR (150 MHz, DMSO) δC = 168.85, 162.84, 162.28, 161.63, 143.34, 134.35, 133.17, 130.94, 116.70, 112.19, 109.89, 107.82, 102.38, 101.31 ppm. ESI-HRMS m/z: 286.06 (M + H)+; calcd: 285.06 (C14H11N2OS).

5-((3,4,5-Trimethoxybenzylidene)amino)-1H-benzo[d]imidazole-2-thiol 7j. Yellow solid; yield: 94%; m.p: 270–273 °C. 1H NMR (600 MHz, DMSO): δH = 12.608–12.554  (s, 2H, Ar−SH, Ar−NH), 8.571  (s, 1H, Ar−N¼CH), 7.277–7.245  (s, 2H, Ar−H), 7.149–7.136  (d, J = 7.8 Hz, 1H, Ar−H), 7.011–7.087  (d, J = 8.4 Hz, 1H, Ar−H), 7.024  (s, 1H, Ar−H), 3.845–3.723  (s, 9H, Ar−OCH3) ppm. 13C NMR (150 MHz, DMSO) δC = 168.68, 159.18, 153.16, 146.39, 140.22, 133.08,
Table 3. Binding Interactions of the Target Compounds 7a−m with the Active Site Residues of α-Glucosidase

| Entry | Bond Type | Residues | Energy (kcal/mol) | Distance (Å) | Backbone | Frequency |
|-------|-----------|----------|------------------|-------------|----------|-----------|
| 7a    | H         | Arg212   | −0.50            | 2.92        | 1        |           |
| H     | Asn241    | −3.80    | 3.29             | 1           |          |           |
| H     | His279    | −0.70    | 3.99             | 1           |          |           |
| H     | His348    | −2.90    | 2.87             | 1           |          |           |
| IH    | Asp408    | −1.19    | 2.86             | 2           |          |           |
| 7b    | H         | His111   | −2.70            | 4.06        | 1        |           |
| H     | Gin181    | −1.10    | 4.32             | 1           |          |           |
| A     | Arg212    | −1.40    | 4.49             | 1           |          |           |
| A     | Asp214    | −6.80    | 3.05             | 2           |          |           |
| IH    | Glu276    | −24.65   | 2.99             | 5           |          |           |
| H     | Asp349    | −6.20    | 2.86             | 1           |          |           |
| H     | Arg439    | −2.50    | 3.00             | 1           |          |           |
| 7c    | H         | Arg212   | −0.60            | 3.13        | 1        |           |
| A     | His245    | −0.50    | 4.09             | 1           |          |           |
| H     | Glu276    | −6.20    | 2.87             | 1           |          |           |
| H     | Val277    | −0.50    | 3.85             | b-          | 1        |           |
| H     | Glu304    | −2.60    | 2.88             | 1           |          |           |
| H     | His348    | −3.30    | 2.86             | 1           |          |           |
| 7d    | H         | Tyr23    | −3.30            | 3.10        | 1        |           |
| H     | Asp68     | −3.10    | 2.74             | 1           |          |           |
| IH    | Glu276    | −16.66   | 2.96             | 3           |          |           |
| H     | Asp349    | −3.40    | 3.12             | 2           |          |           |
| 7e    | H         | Thr215   | −0.70            | 4.13        | b-       | 1        |
| H     | Glu276    | −5.20    | 2.84             | 1           |          |           |
| H     | Val277    | −2.60    | 3.36             | b-          | 1        |           |
| H     | Glu304    | −2.50    | 3.12             | 1           |          |           |
| 7f    | A         | Phe157   | −0.80            | 3.74        | 1        |           |
| H     | Gly217    | −0.90    | 3.50             | b-          | 1        |           |
| H     | Glu276    | −6.70    | 2.78             | 1           |          |           |

131.69, 130.84, 116.87, 109.76, 106.77, 105.81, 101.33, 60.20, 55.97 ppm. ESI-HRMS m/z: 344.10 (M + H)\(^+\); calcd: 343.10 (C\(_7\)H\(_6\)NO\(_3\)).

5-((Thiophen-2-ylmethylene)amino)-1H-benzo[d]-imidazole-2-thiol 7k. Yellowish solid; yield: 82%; m.p: > 300 °C. \(^1\)H NMR (600 MHz, DMSO): \(\delta_H = 12.590 – 12.550\) (s, 2H, Ar, Ar- NH), 8.816 (s, 1H, Ar-N=CH), 7.781–7.773 (d, \(J = 4.8\) Hz, 1H, Ar=H), 7.686 (s, 1H, Ar-H), 7.025–7.028 (m, 4H, Ar-H) ppm. \(^13\)C NMR (101 MHz, DMSO) \(\delta_C = 168.73, 152.82, 145.82, 142.67, 133.36, 133.08, 130.94, 130.88, 128.26, 116.97, 109.75, 101.40\) ppm. ESI-HRMS m/z: 260.03 (M + H); calcd: 259.02 (C\(_7\)H\(_6\)N\(_2\)S).

5-((2-Methoxybenzylidene)amino)-1H-benzo[d]-imidazole-2-thiol 7l. Yellowish solid; yield: 90%; m.p: 234–237 °C. \(^1\)H NMR (600 MHz, DMSO): \(\delta_H = 12.548 – 12.522\) (s, 2H, Ar, Ar- NH), 8.864 (s, 1H, Ar-N=CH), 8.010–7.998 (d, \(J = 7.2\) Hz, 1H, Ar-H), 7.562–7.499 (t, \(J = 7.2\) Hz, 1H, Ar-H), 7.158–7.130 (t, \(J = 8.4\) Hz, 2H, Ar-H), 7.063–7.049 (d, \(J = 8.4\) Hz, 2H, Ar-H), 6.972 (s, 1H, Ar-H), 3.887 (s, 3H, Ar-OCH\(_3\)) ppm. \(^13\)C NMR (150 MHz, DMSO) \(\delta_C = 168.66, 159.21, 154.39, 147.09, 136.51, 133.08, 133.05, 130.85, 127.77, 126.74, 123.95, 120.71, 120.66, 116.63, 112.75, 112.04, 109.81, 101.38, 55.82 ppm. ESI-HRMS m/z: 284.08 (M + H); calcd: 283.08 (C\(_8\)H\(_6\)N\(_2\)O).

5-((4-Methylbenzylidene)amino)-1H-benzo[d]-imidazole-2-thiol 7m. Off white solid; yield: 88%; m.p: > 300 °C. \(^1\)H NMR (600 MHz, DMSO): \(\delta_H = 12.559\) (s, 2H, Ar- SH, Ar-NH), 8.609 (s, 1H, Ar-N=CH), 7.827–7.815 (d, \(J = 7.2\) Hz, 2H, Ar-H), 7.321–7.308 (d, \(J = 7.8\) Hz, 2H, Ar-H), 7.142–7.128 (d, \(J = 8.4\) Hz, 1H, Ar-H), 7.089–7.085 (d, \(J = 7.8\) Hz, 1H, Ar-H), 7.025 (s, 1H, Ar-H), 2.490–2.368 (s, 3H, Ar-CH\(_3\)) ppm. \(^13\)C NMR (150 MHz, DMSO) \(\delta_C = 168.65, 159.39, 146.58, 141.32, 133.67, 133.05, 129.43, 128.62, 116.80, 109.71, 101.42, 21.20 ppm. ESI-HRMS m/z: 268.09 (M + H); calcd: 267.08 (C\(_8\)H\(_6\)N\(_2\)O).

α-Glucosidase Inhibition Assay. The α-glucosidase inhibition potential of the target compounds 7a–m was evaluated with reference to acarbose.\(^{11}\) The enzyme (0.2 U/mL) taken in phosphate buffer (50 mM, pH. 6.8) was incubated with various concentrations of the compounds at 37 °C for 15 min. Then, \(p\)-nitrophenyl-\(α\)-D-glucopyranoside (0.7 mM) was added as the substrate. The change in absorbance at 400 nm was monitored up to 30 min by a multiplate spectrophotometer, and then, the compound was replaced by DMSO (7.5% final) as the control. All the reactions were performed in triplicate and the percent inhibition was calculated by the following formula:

\[
\% \text{Inhibition} = 100 \left(\frac{\text{OD test well} - \text{OD control}}{\text{OD control}}\right) \times 100
\]

Molecular Docking Simulations. The molecular docking technique was used to explore the atomic-level attachment and ligand fitting of the selected compounds with the α-glucosidase active site. MOE was used to run docking simulations on the target compounds 7a–m. Fast Fourier Transform (FFT) was used to produce their poses near the active site. Using eq 1, the
R-Field electrostatics of the London dG solvation model was chosen for the revised top 30 poses.

$$
\Delta G = c + E_{flex} + \sum_{h-bonds} c_{HB} f_{HB} + \sum_{m-lig} c_{M} f_{M} + \sum_{atom} \Delta D_{i}
$$

(1)

In eq 1, “c” calculates the gain or loss in entropy (rotational/translational), \( E_{flex} \) determines the loss of energy in the ligand, and \( f_{HB} \) detects the defects in the hydrogen bonds. In contrast, \( c_{HB} \) calculates the favorable hydrogen bond energy, whereas \( f_{M} \) determines the metal ligand imperfection and \( c_{M} \) determines the ideal metal-binding energy, whereas \( D_{i} \) estimates the dissolution energy of each atom. The top 30 poses obtained from the London dG algorithm were improved to pick the final pose using the GBVI/WSA dG solvation technique implemented in MOE’s Dock.

$$
\Delta G \approx c + a \left[ \frac{2}{3} \left( \Delta E_{coul} + \Delta E_{vdw} \right) + \Delta E_{vdw} + \beta \Delta S_{weighted} \right]
$$

(2)

In eq 2, the increase or decrease in entropy (rotational/translational), that is, “c” and “a” are constants that depend on the force field chosen, \( \Delta E_{coul} \) calculates Coulomb electrostatics, \( \Delta E_{vdw} \) calculates solvation energies, \( \Delta E_{vdw} \) calculates the van der Waals contributions of the systems, and \( \Delta S_{weighted} \) calculates the exposed surface area. As the crystal structure of the *Saccharomyces cerevisiae*, α-glucosidase is not present. The *S. cerevisiae* isomaltase (PDB ID: 3AXH)\(^{22}\) and *S. cerevisiae* α-glucosidase catalytic residues are conserved. The substrate molecule (isomaltose) co-crystallized in the isomaltase 3D structure was manually docked in the α-glucosidase by aligning the protein sequence and structure, and the RMSD of the protein structure and sequence alignment was reported.\(^{23}\) To validate the docking protocols, the redocking was performed using the substrate molecule with the α-glucosidase active site residues. The superimposed RMSD of the reported substrate and the redocked substrate was calculated in angstrom (Å). The 2D structures of the target compounds 7a–m were drawn using ChemDraw, transformed to 3D, and saved in the MOE database.\(^{24}\) The compounds were washed and the energy was reduced using the MMFF94X force field. Then, docking techniques were used to molecularly attach these compounds with the active pocket of the α-glucosidase. The final refine pose for each compound was saved in the MDB format with the energies calculated in the database.

**CONCLUSIONS**

A series of 13 novel benzimidazoles 7a–m was synthesized, characterized via MS and NMR techniques, and assessed for their in vitro α-glucosidase inhibitory potential. All the compounds showed prominent inhibition of the enzyme (IC\(_{50}\) range: 0.64 ± 0.05 μM to 343.10 ± 1.62 μM) as compared to *acarbose* (IC\(_{50}\): 873.34 ± 1.21) where 7i showed the highest activity (IC\(_{50}\): 0.64 ± 0.05 μM). Molecular docking further supported the effective enzyme inhibition of these compounds in terms of the docking score, binding energy, and affinity. These promising results could be utilized in drug development against type 2 diabetes mellitus.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03854.

Details of the spectroscopic data including ESI-HRMS, \(^{1}\)HNMR, and \(^{13}\)CNMR (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

Sardar Ali — Department of Chemistry, University of Malakand, Chakdara 18800 Khyber Pakhtunkhwa, Pakistan; orcid.org/0000-0001-6514-4003; Email: sardaraliium@gmail.com

Mumtaz Ali — Department of Chemistry, University of Malakand, Chakdara 18800 Khyber Pakhtunkhwa, Pakistan; orcid.org/0000-0001-7573-7953; Email: mumtazphd@gmail.com

**Authors**

Ajam Khan — Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Oman

Saeed Ullah — Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Oman; H. E. J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

Muhammad Waqas — Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Oman; Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra 21120, Pakistan

Ahmed Al-Harrasi — Natural and Medical Sciences Research Center, University of Nizwa, Nizwa 616, Oman; orcid.org/0000-0002-0815-5942

Abdul Latif — Department of Chemistry, University of Malakand, Chakdara 18800 Khyber Pakhtunkhwa, Pakistan

Manzoor Ahmad — Department of Chemistry, University of Malakand, Chakdara 18800 Khyber Pakhtunkhwa, Pakistan; orcid.org/0000-0001-9736-5900

Muhammad Saadq — Department of Chemistry, Bacha Khan University, Charsadda 18800 Khyber Pakhtunkhwa, Pakistan; orcid.org/0000-0002-4963-4341

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03854

**Notes**

The authors declare no competing financial interest.

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