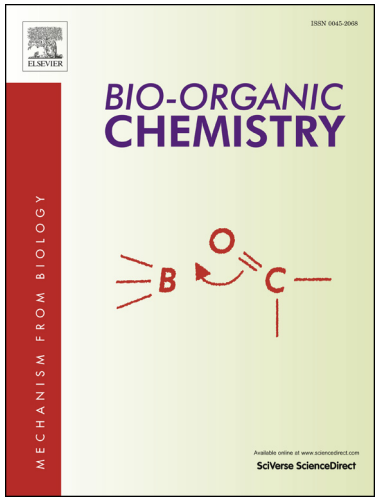

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Synthesis, α-Glucosidase inhibitory, Cytotoxicity and Docking Studies of 2-Aryl-7-methylbenzimidazoles

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Abstract

Benzimidazole analogs **1-27** were synthesized, characterized by EI-MS and ^{1}HNMR and their *α*-glucosidase inhibitory activities were found out experimentally. Compound **25**, **19**, **10** and **20** have best inhibitory activities with $\mathbf{IC_{50}}$ values 5.30 ± 0.10, 16.10 ± 0.10, 25.36 ± 0.14 and 29.75 ± 0.19 respectively against *α*-glucosidase. Compound **6** and **12** has no inhibitory activity against *α*-glucosidase enzyme among the series. Further studies showed that the compounds are not showing any cytotoxicity effect. The docking studies of the compounds as well as the experimental activities of the compounds correlated well. From the molecular docking studies, it was observed that the top ranked conformation of all the compounds fit well in the active site of the homology model of *α*-glucosidase.

Keywords: 4-Methylbenzimidazol; *α*-glucosidase inhibitors; antihyperglycemia; structure-activity relationship, cytotoxicity Docking studies

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1. Introduction

Benzimidazole is a fused heterocyclic compound formed by the merger of benzene and imidazole. This nuclues has a variety of therpeutical potentials which have greatly appealed the medicinal chemists. Anticancer [1], antiinflammatory [2], antimicrobial [3], antioxidant and antiglycation [4], α-Glucosidase [5], β-glucuronidase [6], Carbonic anhydrase [7], antiulcer [8], and antiviral [9] are some of the properties associated with this medicinally important skeleton. Some anticancer drugs based on benzimidazole, such as Bendamustin [10], Hoechst 33258 [11], and nocodazole [12] (Figure-1) are in preclinical or clinical trials.

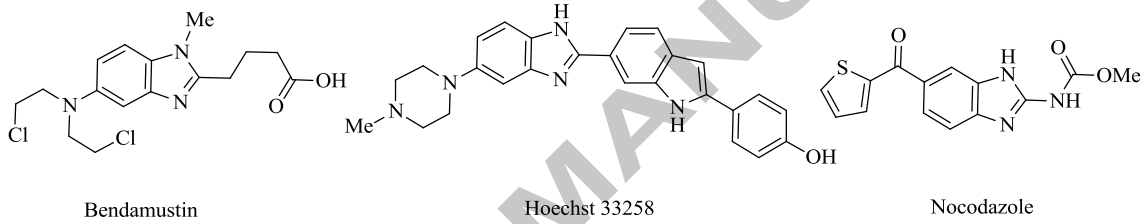


Figure-1. Benzimidazole that had been used in clinical and preclinical stages

α-Glucosidase (EC 3.2.1.20) is a hydrolase enzyme that is found in the the intestinal cells at the brush border surface. The breakdown of carbohydrates into glucose is aided by this enzyme by the hydrolysis mechanism, as the human intestine is only capable of absorbing glucose monomers into the blood stream [13]. Digestion of carbohydrates and processing of glycoprotiens have essentially dependence upon the enzymatic action of α-glucosidase. Therefore diseases like diabetes, cancer, and HIV are susceptable to be cured by inhibitors of α-glucosidase [14-16]. Some of the synthetic α-glucosidase inhibitors (AGI's), such as acarbose, miglitol, and voglibose have gained much attention during the last two decades, in the field of medicinal chemistry [17,18]. These inhibitors deccelerate the digestion process of oligosaccharides and polysaccharides into coressponding monomers and consequently lower the glucose level in blood [19]. These inhibitors, in contrast to the other antihyperglycemic drugs which modulate certain biochemical processes, act locally in the human intestine [20,21]. However, the clinical use of these is associated with side effects such as abdominal discomfort, diarrhea, and flatulence [22,23]. Hence, designing of new α-glucosidase inhibitors with no or minimum side effects is still a reasonable demand. The benzimidazole derivatives showed α-glucosidase [24] as well as antidiabetic [25] activity, therefore we design this project to further investigate the potential of benzimidazole as antidiabetic compounds.

2. Results and Discussion

2.1. Chemistry

In continuous effort to discover potent *enzyme* inhibitors [26], twenty six benzimidazole derivatives were successfully synthesized and purified. The 3-methyl-*o*-phenylenediamine is reacted with various benzaldehydes substituted with hydroxyl group, methyl group, methoxy group, nitro group, chlorine, fluorine on different carbon. The synthesis of benzimidazoles occurred via condensation of arylaldehye and 3methyl-phenylenediamine in the presence of $Na_2S_2O_5$ as oxidizing reagents in DMF (Scheme-1).

(**a**) (**b**) (**1-27**)

Scheme-1. Synthesis of benzimidazole derivatives (**1-27**)

The crude products were further recrystallized from methanol to afford pure compounds. The structures of the benzimidazoles **1-27** were determined using spectroscopic techniques such as NMR, MS and were further confirmed using CHN analysis.

2.2. Enzyme Inhibitory Studies

Survey on structure-activity relationship (SAR) suggests that the variation of different substituents on the aryl part at C-2 is responsible for varied level of α-glucosidase inhibitory potential of 2-aryl-7-methylbenzimidazole **1-27**.

Compound **25** (IC_{50} = 5.30 ± 0.10 μM) showed the highest activity among the series of twenty-seven analogs of 2-aryl-7-methylbenzimidazoles (**1-27**). It was found to be one hundred and sixty two (162) folds more active than the standard drug, acarbose (IC_{50} = 856.45 ± 5.60 μM). The presence of chloro group at C-2′ must have been in best compatibility mode to interact with the enzyme, α-glucosidase. This inhibitory potential of chloro group at C-2′, drastically reduced to twenty-eight (28) folds, when the chloro group moved to C-4′, as in compound **27** (IC_{50} = 145.28 ± 1.82 μM). Activity was further halved when chloro group changed its position from C-4′ to C-3′, as in compound **26** (IC_{50} = 325.02 ± 2.86 μM). This

chlorine substitution trend of *ortho* (C-2′) > *para* (C-4′) > *meta* (C-3′), for the activity was durther supported by the three regio-isomers of fluorinated anolgs, as well. Fluorine at C-2′, as in compound **8** (IC_{50} = 34.42 ± 0.35 μM), was found to have seven-folds lesser activity than its corresponding chlorinated derivative (compound **25**). The *para* (C-4′) fluorinated analog, as in compound **16** (IC_{50} = 135.70 ± 1.20 μM), had a drop in activity upto four-folds. Switching of fluoro group from C-4′ to C-3′, as in compound **9** (IC_{50} = 165.01 ± 1.28 μM), resulted with further decline in activity.

Among the monohydroxylated anaologs, same *o* > *p* > *m* activity trend was followed. Hydroxy at C-2′ as in compound **19** had an IC_{50} value of 16.10 ± 0.10 μM, which lowered to six folds upon movement of hydroxyl from C-2′ to C-4′, as in compound **17** (IC_{50} = 95.60 ± 0.92 μM). Placement of hydroxy group at *meta* (C-3′), as in compound **18** (IC_{50} = 325.69 ± 2.68 μM), resulted in decrement of twenty folds activity with respect to its *ortho* analog (compound **19**).

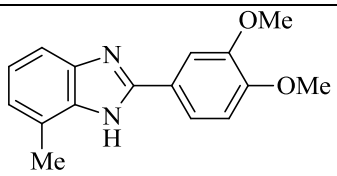
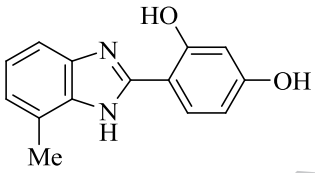
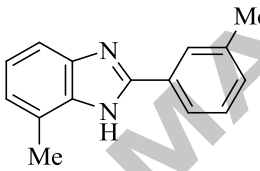
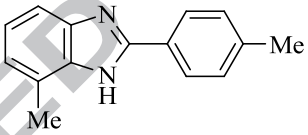
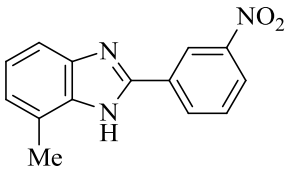
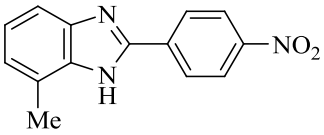
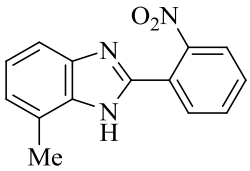
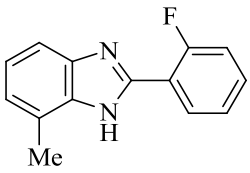
Dihydroxylation at *ortho* (C-2′) and *para* (C-4′), as in compound **2** (IC_{50} = 60.90 ± 0.55 μM), showed the activity intermediate to individual *ortho* (compound **19**) and *para* (compound **17**) anologs of monohydroxy substitutions. Movement of hydroxy from C-4′ to C-3′, as in compound **15** (IC_{50} = 85.60 ± 0.75 μM) slightly lowered the activity. Hydroxy at C-5′ from C-4′, as in compound **13** (IC_{50} = 125.34 ± 1.06 μM) halved the activity. The presence of two hydroxyl groups at C-3′ and C-4′, as in compound **10** (IC_{50} = 25.36 ± 0.14 μM), doubled the activity with respect to compound **2**.

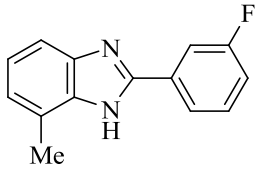
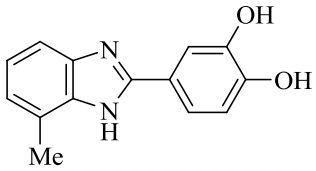
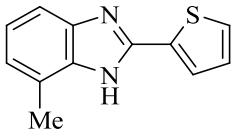
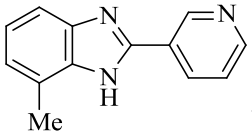
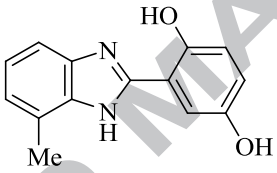
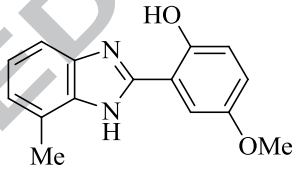
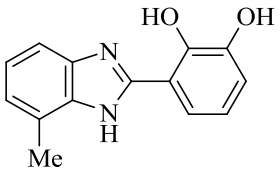
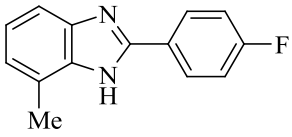
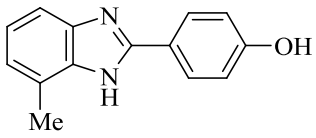
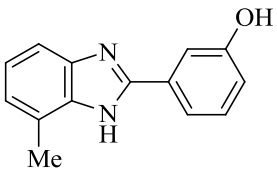
Although merely methoxylation at the aryl part did not show any activity (compounds **1**, **22**, and **23**) but methoxylation along with hydroxylation showed some inhibitory potentials. The *meta* linkage of hydroxy and methoxy, as in compound **20**, showed a good inhibitory potential with IC_{50} value 29.75 ± 0.19 μM. The activity was dropped to 45.20 ± 0.19 μM for the *para* linkage of hydroxy and methoxy, as in compound **14**. A further decline in activity was observed for compound **21** (IC_{50} = 225.80 ± 2.05 μM), which had hydroxy and methoxy substituted *ortho* to each other.

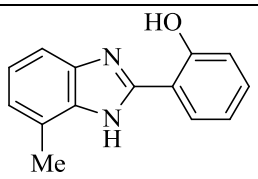
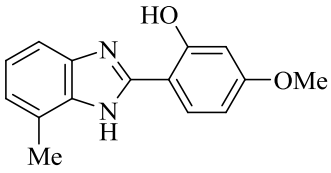
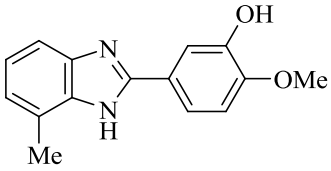
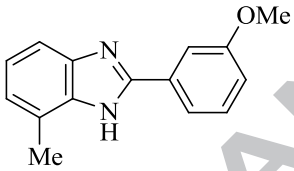
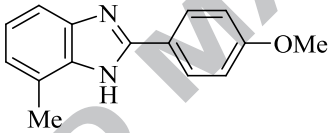
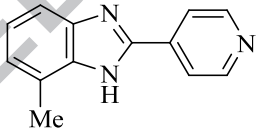
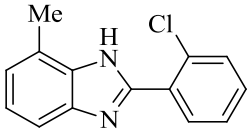
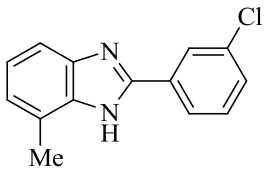
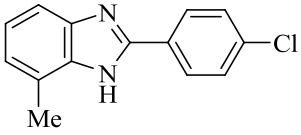
Nitro substitutions at C-3′ (compound **5**) or C-4′ (compound **6**) did not show any activity, however the ortho (C-2′) substitution, as in compound **7** (IC_{50} = 44.62 ± 0.42 μM) showed nineteen folds better activity than the standard drug, acarbose. Methylation at the aryl part (compounds **3** and **4**) was found to be useless to inhibit the enzymatic action of α-glucosidase.

Among the heterocycles, replacement of pyidines with phenyl (compound **12** and **24**) did not show any activity but thiophene redeemed the activity with IC_{50} value of 225.05 ± 2.18 μM. The compounds were not showing any inhibition against cytotoxicity activity.

Table-1. α-Glucosidase inhibitory activity of 2-aryl-7-methylbenzimidazoles (**1**-**27**)

Compounds	Structures	IC_{50} ± SEM[a] [μM]
1		N. A.[b,c]
2		60.90 ± 0.55
3		N. A.[b,c]
4		N. A.[b,c]
5		N. A.[b,c]
6		N. A.[b,c]
7		44.62 ± 0.42
8		34.42 ± 0.35

9		165.01 ± 1.28
10		25.36 ± 0.14
11		225.05 ± 2.18
12		N. A.[b,c]
13		125.34 ± 1.06
14		45.20 ± 0.39
15		85.60 ± 0.75
16		135.70 ± 1.20
17		95.60 ± 0.92
18		325.69 ± 2.68

19		16.10 ± 0.10
20		29.75 ± 0.19
21		225.80 ± 2.05
22		N. A.[b,c]
23		N. A.[b,c]
24		725.50 ± 3.78
25		5.30 ± 0.10
26		325.02 ± 2.86
27		145.28 ± 1.82
	Acarbose (Standard Drug)	856.45 ± 5.60

SEM[a] is the Standard Error of the Mean; N. A.[b] means Not Active, C # the maximum concentration of all inactive compound was tested upto 1 mM.

2.3. Homology modeling and docking simulation

The crystallographic structure for α-glucosidase enzyme has not been solved up-to yet. However, only few homology models have been reported [27-30] so, we developed the 3D model for α-glucosidase by comparative homology modeling technique using the same etiquette as described by Fazal et al. [31]. The primary sequence of α -glucosidase was retrieved from UniProt protein resource data bank (http://www.uniprot.org/) under the access code P53341. Template search was performed by means of MOE Search tools against the PDB data bank implemented in MOE 2010-11. The 1.30 Å resolving crystallographic structure of Saccharomyces cerevisiae isomaltase (PDB code 3AJ7) [32] with 72.4% of sequence identity with the target were selected as the template for modeling. The 3D structure of α-glucosidase for Saccharomyces cerevisiae was built by means of MOE homology modeling tools. The developed 3D model was subjected to energy minimization up to 0.05 gradients. Before docking, ligands and protein were prepared using MOE 2010-11. 3D structure of all synthesized compounds was built using the Molecular Builder program implemented in MOE. Finally, a database was created in which all the ligands were converted into their respective 3D structures and this database was used as input file MOE-docking. Subsequently, the energy of compounds present in the database was minimized up to 0.05 Gradient using MMFF94x force field. Energy minimization of the database was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was accomplish prior to docking using Protonate 3D tools implemented in MOE. Protonation was followed by energy minimization up to 0.05 Gradient using Amber99 force field. The database was docked into the active site of protein using the Triangular Matching docking method and 30 conformations of each Ligand protein complex were generated with docking score (S). Each complex was analyzed for interactions and their 3D images were taken.

2.4. Interaction analysis

Benzimidazole analogs were synthesized and their inhibitory activities were found out experimentally. The highly potent compounds from the series were selected on the basis of IC50, which was determined experimentally. From the molecular docking studies, it was observed that the top ranked conformation of all the compounds fit well in the active site of

the homology model of a-glucosidase. Compounds **25**, **19, 10,** and **20** have best inhibitory activity with IC_{50} values **5.30 ± 0.10, 16.10 ± 0.10, 25.36 ± 0.14** and **29.75 ± 0.19** respectively against *α*-glucosidase and compound **6** and **12** has no inhibitory activity against *α*-glucosidase enzyme. The docking studies of the compounds as well as the experimental activities of the compounds correlated well.

Compound **25** was found with a best fit in the binding pocket of the *α*-glucosidase enzyme with the docking score **-8.7211** and have shown good interactions with the active site residues (**Arg 439**) of the receptor protein. Arg 439 was observed making two arene-cation interaction with the π system of the benziimidazole moiety of the compound. The 3D interactions of the compound with the receptor *α*-glucosidase protein was shown in the **Figure 2a**. The substituted chlorobenzene group showed inertness regarding interaction in this compound and this might be due to the electron withdrawing inductive effect of chlorine which deactivte the aromatic benzene ring. In compound **19** the chlorine is replaced by OH group which is active in bonding formation and thus here this group interacts with the target protein.

Compound **19** showed good docking score of **-8.9503** and **Glu 276** formed hydrogen bonds with the hydrogen atoms of the –OH moiety of the compound. **Arg 439** made an arene-cation interaction with one of the imidazole moiety of the compound. In compound **10** there are two OH group with the substituted moiety and the thus the number of interactions increases as shown in **Figure 2b.**

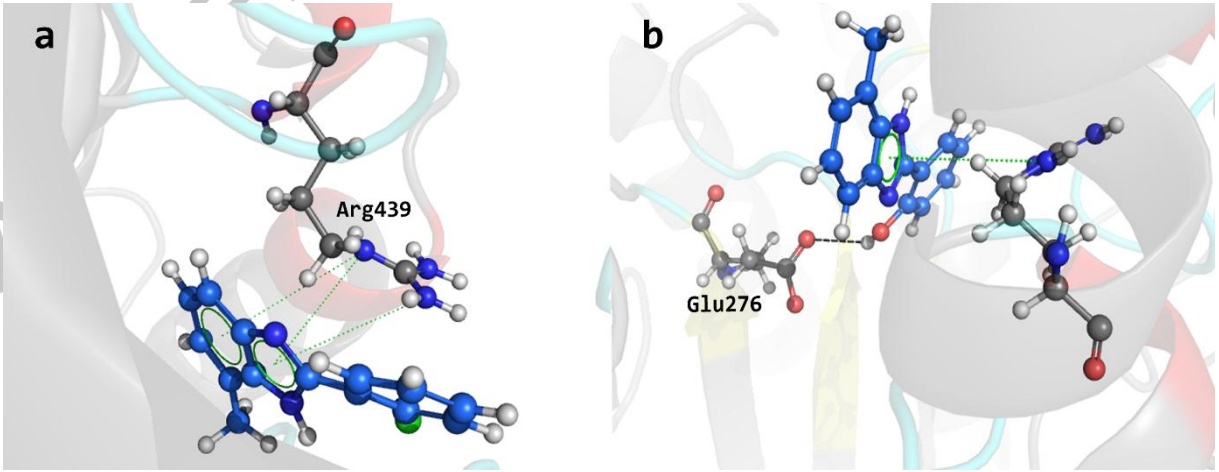


Figure-2. Docking pose of the compound 25 and 19 in the active site of α-glucosidase enzyme.

Compound **10** have good IC_{50} (**25.36 ± 0.14**) as well as good docking score i-e **-11.3224**. This compound had shown four interactions with the **Arg 439**, **Asp 408**, **Arg 312** and **Tyr**

313 as shown in the figure 3. **Arg 312** and **Tyr 313** interact with the hydroxyl group of catechol moiety of the ligand at one end and **Asp 408** with the OH of the nearby atom of catechol group at the other end. **Arg 439**, additionally, made an arene-cation linkage with the the π electronic cloud of the same catechol moiety of the compound (**Figure 3a)**. In compound **20** one of the OH group is replaced by methoxy group which resulted in the non involvement of the remaining OH group in bonding directly. Compound **20** have good IC_{50} (**29.75 ± 0.19**) as well as good docking score i-e **-10.1882**. This compound had shown three interactions with the **Arg 439** and **Phe 177** as shown in the **Figure 3b**. **Arg 439** interacts with the imidazol and methoxyphenol rings of the ligand via arene-cation bonds. **Phe 177** made an arene- arene linkage with the the π electronic cloud of the benzimidazole moiety of the compound. From the above theoritical approach we have observed that the interaction between ligand and protein increases as the polar groups (OH) increase. However their attachement to the compounds may alter the various other drug like properties i.e. TPSA, LogS, LogP, Molecular weight and Toxicity as reflected from their grading by experimental values i.e. $IC_{50.}$ The inactive compounds also showed here a poor behavior in the binding mode. The poor or no binding interaction of these compounds might be attributed to the less active substituted groups like NO_2 and $OCH_{3.}$ The placement of inactive compound **6** in the binding pocket of protein is shown in **Figure 3c** having no interaction.

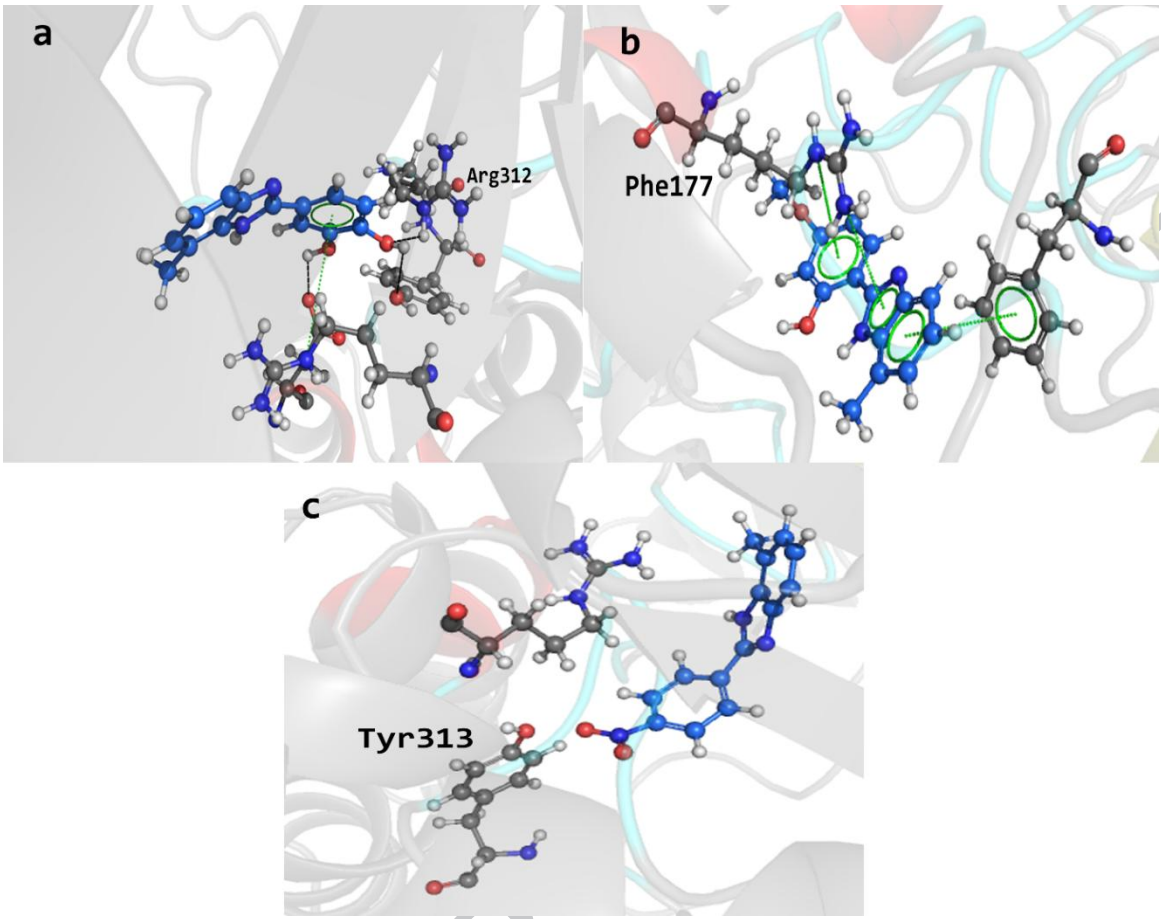


Figure-3. Docking pose of the compound **10**, **20** and **06** in the active site of *α*-glucosidase enzyme.

3. Conclusion

We had synthesized benzimidazole derivatives in this study and evaluated for α-glucosidase inhibition. Substituent effect showed that compounds with hydroxyl and halides are capable of inhibiting the activity at a concentration as low as 16.10 and 5.30 µM, respectively. Molecular docking studies have correlated well with biological inhibitory assay. Analysis of the binding mode clearly shows the increase in hydrophilicity of substituents played the most important role in the activity profile. Further studies on cytotoxicity showed that the compounds do not exhibit cytotoxicity activity.

4. Experimental

4.1. General

NMR experiments had been carried out using UltraShield Bruker FT NMR 500 MHz. Electron impact mass spectra (EI-MS) were recorded on a Finnegan MAT-311A, Germany. CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Reaction progress was monitored using thin layer chromatography (TLC) which was performed on pre-

coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

4.2. Assay for α-Glucosidase Inhibitory Activity

The 135 μL of 50 mM phosphate saline buffer pH (6.8), solution of enzyme (20 μL), and 20 µl of test sample with 70% DMSO was added to 96-well plate. The samples were incubated for 15 minutes. pre- read of the plate was taken after incubation using SpectraMax. substrate (pNPG) (25 μL) was added before final reading was taken at 400 nm on Spectra Max. The percent inhibition was calculated using the following formula:

[(absorbance for control - absorbance for sample)/ absorbance for control] x 100%

4.3. Cytotoxicity activity

The Neutral Red cytotoxicity assay is based on the initial protocol described [33] with some modifications. Briefly, the cells (1x104/well) were seeded in 96-well microtiter plates (Nunc) and allowed to grow for 24 hours before treatment. After 24 hours of incubation, the cells were treated with six different concentrations (0.1-100 μg/mL) of test compounds, in three replicates. The plates were incubated for 72 h at 37°C in a 5% CO_2 incubator. A stock solution was obtained by dissolving the test compounds in DMSO. Further dilution to different tested concentrations were then carried out ensuring that the final concentration of DMSO in the test and control wells was not in excess of 1% (v/v). No effect due to the DMSO was observed. Doxorubicin was used as the positive control. The well containing untreated cells was the negative control. At the end of the incubation period, the media were replaced with medium containing 50 μg/mL of Neutral Red. The plates were incubated for another 3 hours to allow for uptake of the vital dye into the lysosomes of viable and injured cells. After the incubation period, the media were removed and cells were washed with the neutral red washing solution. The dye was eluted from the cells by adding 200 μL of Neutral Red resorb solution and incubated for 30 minutes at room temperature with rapid agitation on a microtiter plate shaker. Dye absorbance was measured at 540 nm using a spectrophotometer ELISA plate reader.

4.4. General procedure for the synthesis of compounds 1-27

In a typical reaction, sodium metabisulfite ($Na_2S_2O_5$) (20 mL) was added to a stirred mixture of 3-methyl-o-phenylenediamine (2.12 mmol) and substituted aromatic aldehydes (2.16

mmol) in *N*,*N*-dimethylformamide (DMF) in 100 mL round bottom flask. The reaction mixture was refluxed for 4 hours at 130°C at atmospheric pressure, the progress of reaction was monitored by TLC. After completion of reaction, reaction mixture was allowed to cool to room temperature. Addition of 30 mL of water resulted in precipitation of a solid material. The solid benzimidazole was filtered and dried overnight. The solid benzimidazole derivatives obtained inhigh yields. Recrystallization from methanol afforded pure products.

4.4.1. 2-(3′,4′-Dimethoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (1)

m.p. 256-257 °C; IR ν cm-1 (KBr disk): 2951 (NH stretch), 1607 (C=N), 1433 (C=C), 1380 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 12.12 (s, 1H, NH), 7.82 (s, 1H), 7.76 (s, 1H), 7.43 (s, 1H), 7.32 (d, 1H, J = 8.0 Hz), 7.14 (d, 1H, J = 8.5 Hz), 7.09 (t, 1H, J = 7.5 Hz), 6.98 (d, 1H, J = 7.5 Hz), 3.90 (s, 3H, OCH3), 3.85 (s, 3H, OCH3), 2.58 (s, 3H, CH3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 153.88, 151.91, 149.19, 138.45, 137.42, 125.16, 124.84, 124.60, 124.32(s), 120.84, 114.27, 113.23, 109.56, 56.82, 18.13; HREI-MS: m/z calcd for $C_{16}H_{16}N_2O_2$ [M]+ 268.1212; Found 268.1216; Anal. Calcd for $C_{16}H_{16}N_2O_2$, C, 71.62; H, 6.01; N, 10.44; Found C, 71.63; H, 5.99; N, 10.46

4.4.2. 2-(2′,4′-Dihydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (2)

m.p. 248-249 °C; IR ν cm-1 (KBr disk): 3234 (NH stretch), 1603 (C=N), 1483 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 13.39 (s, 1H, NH), 12.90 (s, 1H, OH), 10.03 (s, 1H, OH), 7.95 (d, 1H, J = 11.0 Hz), 7.43 (s, 1H), 7.16 (s, 1H), 7.07 (s, 1H), 6.54 (d, 1H, J = 13.0 Hz), 2.57 (s, 3H, CH3), ^{13}C NMR (150 MHz, DMSO-d_6): δ160.52, 158.62, 151.97, 138.25, 137.34 (s), 129.73 (s), 125.09, 124.52, 124.34, 114.38, 109.59, 103.94, 103.03, 19.07 ; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O_2$ [M]+ 240.0899; Found 240.0903; Anal. Calcd for $C_{14}H_{12}N_2O_2$, C, 69.99; H, 5.03; N, 11.66; Found C, 70.01; H, 5.04; N, 11.64

4.4.3. 2-(3′-Methylphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (3)

m.p. 218-219 °C; IR ν cm-1 (KBr disk): 3168 (NH stretch), 1627 (C=N), 1432 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 12.76 (s, 1H, NH), 8.05 (s, 1H), 8.00 (s, 1H), 7.47 (t, 2H, J = 9.0 Hz), 7.31 (d, 1H, J = 7.5Hz), 7.12 (t, 1H, J = 8.0 Hz), 7.00 (d, 1H, J = 7.5 Hz), 2.58 (s, 3H, CH3), 2.51 (s, 3H, CH3), ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.19, 138.45, 137.42, 137.52, 133.48, 131.29, 127.95, 126.50, 125.12, 124.60, 124.28, 124.16, 114.38, 21.25, 18.17; HREI-MS: m/z calcd for $C_{15}H_{14}N_2$ [M]+ 222.1157; Found 222.1163; Anal. Calcd for $C_{15}H_{14}N_2$, C, 81.05; H, 6.35; N, 12.60; Found C, 81.04; H, 6.37; N, 12.58

4.4.4. 2-(4′-Methylphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (4)

m.p. 184-185 °C; IR ν cm-1 (KBr disk): 3623 (NH stretch), 1620 (C=N), 1437 (C=C), 1311 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 12.12 (s, 1H, NH), 8.11 (d, 2H, J = 8.0 Hz), 7.40 (s, 1H), 7.37 (d, 2H, J = 8.0 Hz), 7.10 (t, 1H, J = 7.5 Hz), 7.00 (d, 1H, J = 7.0 Hz), 2.57 (s, 3H, CH3), 2.39 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.67, 139.81, 138.25, 137.64, 131.17, 129.74, 129.74, 126.89, 126.89, 125.06, 124.60, 124.21, 114.51, 21.02, 18.25; HREI-MS: m/z calcd for $C_{15}H_{14}N_2$ [M]+ 222.1157; Found 222.1164; Anal. Calcd for $C_{15}H_{14}N_2$, C, 81.05; H, 6.35; N, 12.60; Found C, 81.04; H, 6.33; N, 12.61

4.4.5. 2-(3′-Nitrophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (5)

m.p. 191-192 °C; IR ν cm-1 (KBr disk): 3219 (NH stretch), 1620 (C=N), 1520 (C=C), 1348 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 12.95 (s, 1H, NH), 9.12 (s, 1H), 8.69 (s, 1H), 8.34 (d, 1H, J = 8.0 Hz), 7.88 (t, 1H, J = 8.5Hz), 7.53 (s, 1H), 2.51 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.12, 149.05, 138.45, 137.54, 134.63, 133.12, 132.08, 125.06, 124.52, 124.14, 124.39, 121.32, 114.37, 18.21; HREI-MS: m/z calcd for $C_{14}H_{11}N_3O_2$ [M]+ 253.0851; Found 253.0847; Anal. Calcd for $C_{14}H_{11}N_3O_2$, C, 66.40; H, 4.38; N, 16.59; Found C, 66.41; H, 4.37; N, 16.57;

4.4.6. 2-(4′-Nitrophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (6)

m.p. 274-275 °C; IR ν cm-1 (KBr disk): 3192 (NH stretch), 1624 (C=N), 1438 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 12.95 (s, 1H, NH), 8.48 (d, 2H, J = 8.5 Hz), 8.44 (d, 2H, J = 9.0 Hz), 7.47 (s, 1H), 7.19 (t, 1H, J = 7.5 Hz), 7.08 (d, 1H, J = 7.5 Hz), 2.61 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.68, 149.13, 138.25, 137.47, 134.86, 127.21, 127.21, 125.72, 125.72, 125.10, 124.68, 124.21, 114.37, 18.25; HREI-MS: m/z calcd for $C_{14}H_{11}N_3O_2$ [M]+ 253.0851; Found 253.0859; Anal. Calcd for $C_{14}H_{11}N_3O_2$, C, 66.40; H, 4.38; N, 16.59; Found C, 66.38; H, 4.39; N, 16.61

4.4.7. 2-(2′-Nitrophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (7)

m.p. 259-261 °C; IR ν cm-1 (KBr disk): 3297 (NH stretch), 1617 (C=N), 1439 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 11.76 (s, 1H, NH), 8.20 (t, 1H, J = 1.5 Hz), 7.63 – 7.58 (m, 1H), 7.48 – 7.36 (m, 3H), 7.19 (t, 1H, J = 7.5 Hz), 7.06 (d, 1H, J = 11.5 Hz), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 150.18, 145.62, 138.31, 137.48, 135.29, 132.21, 129.46, 128.05, 125.10, 124.72, 124.47, 124.36, 114.28, 18.27; HREI-MS: m/z calcd for

$C_{14}H_{11}N_3O_2$ [M]+ 253.0851; Found 253.0856; Anal. Calcd for $C_{14}H_{11}N_3O_2$, C, 66.40; H, 4.38; N, 16.59; Found C, 66.38; H, 4.37; N, 16.58

4.4.8. 2-(2′-Fluorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (8)

m.p. 144-145 °C; IR ν cm-1 (KBr disk): 3097 (NH stretch), 1603 (C=N), 1439 (C=C); 1H NMR (500 MHz, DMSO-*d6*): δ 12.12 (s, 1H, NH), 8.05 (d, 1H, J = 7.0 Hz), 8.00 (d, 1H, J = 7.5 Hz), 7.62 (m, 1H), 7.42 (s, 1H), 7.35 (dt, 1H, J = 2.5 Hz, 6.0 Hz), 7.14 (t, 1H, J = 7.5 Hz), 7.03 (d, 1H, J = 7.5 Hz), 2.57 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.49 (d, J = 262.3 Hz), 147.55, 147.47, 138.36, 137.42, 130.60, 128.82, 126.19, 124.87, 124.65, 124.41, 120.18, 117.68, 114.34, 18.19; HREI-MS: m/z calcd for $C_{14}H_{11}FN_2$ [M]+ 226.0906; Found 226.0912; Anal. Calcd for $C_{14}H_{11}FN_2$, C, 74.32; H, 4.90; N, 12.38; Found C, 74.33; H, 4.91; N, 12.37

4.4.9. 2-(3′-Fluorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (9)

m.p. 174-175 °C; IR ν cm-1 (KBr disk): 3097 (NH stretch), 1612 (C=N), 1426 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 9.81 (s, 1H, NH), 8.39 (s, 1H, OH), 7.64 (d, 1H, J = 2.5 Hz), 7.54 (dd, 1H, J = 1.5 Hz, 1.5 Hz), 7.44 (d, 1H, J = 8.0 Hz), 7.21 (s, 1H), 7.12 (s, 1H), 6.96 (d, 3H, J = 8 Hz), 2.58 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.26 (d, J = 261.3 Hz), 149.17, 138.34, 137.47, 131.68, 130.54, 125.09, 124.52, 124.32, 120.18, 114.86, 114.32, 112.49, 18.14; HREI-MS: m/z calcd for $C_{14}H_{11}FN_2$ [M]+ 226.0906; Found 226.0899; Anal. Calcd for $C_{14}H_{11}FN_2$, C, 74.32; H, 4.90; N, 12.38; Found C, 74.34; H, 4.89; N, 12.36

4.4.10. 2-(3′,4′-dihydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (10)

m.p. 251-253 °C; IR ν cm-1 (KBr disk): 3098 (NH stretch), 1614 (C=N), 1445 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 9.57 (s, 1H, NH), 8.07 (dd, 1H, J = 1.0 Hz, 1.0 Hz), 8.00 (d, 2H, J = 7.5 Hz), 7.90 (m, 1H), 7.79 (m, 1H), 7.43 (m, 1H), 7.16 (t, 1H, J = 7.0 Hz), 7.06 (d, 1H, J = 7.5 Hz), 2.73 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 148.96, 147.61, 138.36, 137.47, 125.09, 124.52, 124.21, 120.51, 119.18, 115.29, 114.34, 113.18, 18.29; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O_2$ [M]+ 240.0899; Found 240.0904; Anal. Calcd for $C_{14}H_{12}N_2O_2$, C, 69.99; H, 5.03; N, 11.66; Found C, 70.01; H, 5.01; N, 11.64

4.4.11. 7-methyl-2-(thiophen-2′-yl)-1*H*-benzo[*d*]imidazole (11)

m.p. 278-279 °C; IR υ cm-1 (KBr disk): 3065 (NH stretch), 1567 (C=N), 1423 (C=C), 1311 (C-N), 718 (C-S); 1H NMR (500 MHz, DMSO-d_6): δ 12.83 (s, 1H, NH), 7.94 (s, 1H), 7.72 (d,

1H, *J* = 4.0 Hz), 7.42 (s, 1H), 7.23 (s, 1H), 7.09 (d, 1H, *J* = 8.0 Hz), 6.99 (s, 1H), 2.55 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 143.95, 139.82, 138.34, 137.42, 130.14, 127.69, 127.39, 124.87, 124.67, 124.21, 114.33, 18.27; HREI-MS: m/z calcd for $C_{12}H_{10}N_2S$ [M]+ 214.0565; Found 214.0565; Anal. Calcd for $C_{12}H_{10}N_2S$, C, 67.26; H, 4.70; N, 13.07; Found C, 67.25; H, 4.71; N, 13.08

4.4.12. 7-methyl-2-(pyridin-3′-yl)-1*H*-benzo[*d*]imidazole (12)

m.p. 234-235 °C; IR ν cm-1 (KBr disk): 3092 (NH stretch), 1618 (C=N), 1437 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 12.99 (s, 1H, NH), 9.37 (s, 1H), 8.69 (d, 1H, *J* = 3.5 Hz), 8.54 (d, 1H, *J* = 7.0 Hz), 7.61 (t, 1H, *J* = 4.5 Hz), 7.48 (s, 1H), 7.15 (t, 1H, *J* = 7.5 Hz), 7.05 (d, 1H, *J* = 7.0 Hz), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 150.02 (s), 149.83 (s), 149.52 (s), 138.45 (s), 137.34 (s), 135.64 (s), 132.13 (s), 128.09 (s), 124.92 (s), 124.42 (s), 124.11 (s), 114.30 (s), 18.27; HREI-MS: m/z calcd for $C_{13}H_{11}N_3$ [M]+ 209.0953; Found 209.0948; Anal. Calcd for $C_{13}H_{11}N_3$, C, 74.62; H, 5.30; N, 20.08; Found C, 74.60; H, 5.31; N, 20.10

4.4.13. 2-(2′,5′-dihydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (13)

m.p. 236-238 °C; IR ν cm-1 (KBr disk): 3082 (NH stretch), 1623 (C=N), 1449 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 9.19 (s, 1H, NH), 7.51 (s, 1H), 7.26 (s, 1H), 7.17 (s, 1H), 6.92 (s, 2H), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 152.68, 152.24, 151.41, 138.28, 137.53, 125.12, 124.71, 124.20, 118.97, 116.73, 116.08, 114.39, 114.28, 18.32; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O_2$ [M]+ 240.0899; Found 240.0910; Anal. Calcd for $C_{14}H_{12}N_2O_2$, C, 69.99; H, 5.03; N, 11.66; Found C, 69.97; H, 5.05; N, 11.64

4.4.14. 2-(2′-hydroxy-5′-methoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (14)

m.p. 264-266 °C; IR ν cm-1 (KBr disk): 3085 (NH stretch), 1601 (C=N), 1442 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 11.89 (s, 1H, NH), 7.68 (s, 1H), 7.48 (d, 1H, *J* = 8.0 Hz), 7.21 (t, 1H, *J* = 7.5 Hz), 7.10 (d, 1H, *J* = 7.5 Hz), 7.01 (d, 1H, *J* = 6.0 Hz), 6.98 (d, 1H, *J* = 9.0 Hz), 3.80 (s, 3H, OCH3), 2.58 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 155.68, 153.20, 151.47, 138.25, 137.54, 125.08, 124.63, 124.41, 117.13, 115.92, 114.79, 114.21, 113.15, 56.03, 18.25; HREI-MS: m/z calcd for $C_{15}H_{14}N_2O_2$ [M]+ 254.1055; Found 254.1048; Anal. Calcd for $C_{15}H_{14}N_2O_2$, C, 70.85; H, 5.55; N, 11.02; Found C, 70.83; H, 5.57; N, 11.03

4.4.15. 2-(2′,3′-dihydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (15)

m.p. 258-259 °C; IR ν cm-1 (KBr disk): 3097 (NH stretch), 1613 (C=N), 1435 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 13.30 (s, 1H, OH), 12.12 (s, 1H, NH), 9.30 (s, 1H, OH), 7.55 (d, 2H, J = 7.5 Hz), 7.50 (d, 1H, J = 7.5 Hz), 7.25 (t, 1H, J = 7.5 Hz), 7.15 (d, 1H, J = 7.0 Hz), 6.96 (d, 1H, J = 7.5 Hz), 6.87 (t, 1H, J = 8.0 Hz), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 151.92, 148.09, 147.56, 138.35, 137.44, 125.00, 124.62, 124.31, 122.19, 120.95, 117.01, 114.31, 112.39, 18.23; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O_2$ [M]+ 240.0899; Found 240.0892; Anal. Calcd for $C_{14}H_{12}N_2O_2$, C, 69.99; H, 5.03; N, 11.66; Found C, 70.01; H, 5.02; N, 11.65

4.4.16. 2-(4′-Fluorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (16)

m.p. 142-143 °C; IR ν cm-1 (KBr disk): 3119 (NH stretch), 1608 (C=N), 1443 (C=C), 1316 (C-N), 1234 (C-F); 1H NMR (500 MHz, DMSO-d_6): δ 12.75 (s, 1H, NH), 8.27 (d, 1H, J = 6.0 Hz), 8.25 (d, 1H, J = 5.5 Hz), 7.42 (t, 3H, J = 9.0 Hz), 7.11 (t, 1H, J = 7.5 Hz), 7.01 (d, 1H, J = 7.0 Hz), 2.57 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.89 (d, J = 262.3 Hz), 149.76, 138.25, 137.47, 129.25, 129.25, 128.69, 125.13, 124.67, 124.21, 116.14, 116.14, 114.51, 18.21; HREI-MS: m/z calcd for $C_{14}H_{11}FN_2$ [M]+ 226.0906; Found 226.0913; Anal. Calcd for $C_{14}H_{11}FN_2$, C, 74.32; H, 4.90; N, 12.38; Found C, 74.34; H, 4.91; N, 12.36

4.4.17. 2-(4′-Hydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (17)

m.p. 246-248 °C; IR ν cm-1 (KBr disk): 3271 (NH stretch), 1597 (C=N), 1492 (C=C), 1375 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 13.07 (s, 1H, OH), 11.63 (s, 1H, NH), 8.16 (d, 2H, J = 9.0 Hz), 7.42 (d, 1H, J = 8.0 Hz), 7.18 (s, 1H), 7.15 (d, 1H, J = 8.5 Hz), 7.09 (d, 1H, J = 7.5 Hz), 2.47 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.71, 149.76, 138.32, 137.48, 126.98, 126.98, 124.79, 124.42, 124.28, 120.92, 116.43, 116.43, 114.28, 18.28; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O$ [M]+ 224.0950; Found 224.0946; Anal. Calcd for $C_{14}H_{12}N_2O$, C, 74.98; H, 5.39; N, 12.49; Found C, 74.97; H, 5.38; N, 12.47

4.4.18. 2-(3′-Hydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (18)

m.p. 239-241 °C; IR ν cm-1 (KBr disk): 3274 (NH stretch), 1593 (C=N), 1494 (C=C), 1382 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 12.92 (s, 1H, OH), 11.91 (s, 1H, NH), 7.86 – 7.84 (m, 2H), 7.53 (t, 1H, J = 8.0 Hz), 7.47 (d, 1H, J = 8.0 Hz), 7.22 (t, 1H, J = 7.5 Hz), 7.17 (s, 1H), 2.62 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 157.78 (s), 149.19 (s), 138.36 (s), 137.48 (s), 132.26 (s), 130.84 (s), 125.09 (s), 124.72 (s), 124.41 (s), 119.25 (s), 117.09 (s),

114.41 (s), 114.34 (s), 18.29; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O$ [M]+ 224.0950; Found 224.0942; Anal. Calcd for $C_{14}H_{12}N_2O$, C, 74.98; H, 5.39; N, 12.49; Found C, 74.99; H, 5.37; N, 12.51

4.4.19. 2-(2′-Hydroxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (19)

m.p. 224-225 °C; IR ν cm-1 (KBr disk): 3272 (NH stretch), 1596 (C=N), 1490 (C=C), 1388.83 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 13.16 (s, 1H, NH), 8.05 (s, 1H), 7.44 (s, 1H), 7.40 (t, 1H, J = 7.0 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.10 (d, 1H, J = 7.5 Hz), 7.05 (t, 2H, J = 8.5 Hz), 2.59 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.62, 152.13, 138.38, 137.24, 131.09, 129.75, 125.13, 124.52, 124.21, 121.01, 118.52, 114.30, 111.36, 18.21; HREI-MS: m/z calcd for $C_{14}H_{12}N_2O$ [M]+ 224.0950; Found 224.0958; Anal. Calcd for $C_{14}H_{12}N_2O$, C, 74.98; H, 5.39; N, 12.49; Found C, 75.02; H, 5.41; N, 12.52

4.4.20. 2-(2′-Hydroxy-4′-methoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (20)

m.p. 208-209 °C; IR ν cm-1 (KBr disk): 3302 (NH stretch), 1637 (C=N), 1407 (C=C), 1288 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 13.20 (s, 1H, NH), 8.04 (d, 1H, J = 8.0 Hz), 7.46 (d, 1H, J = 7.5 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.10 (d, 1H, J = 7.0 Hz), 6.67 (d, 1H, J = 7.2 Hz), 6.61 (s, 1H), 3.82 (s, 3H, OCH3), 2.58 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.56, 160.33, 152.13, 138.25, 137.47, 130.08, 125.10, 124.69, 124.21, 114.33, 108.05, 107.58, 102.81, 56.13, 18.33; HREI-MS: m/z calcd for $C_{15}H_{14}N_2O_2$ [M]+ 254.1055; Found 254.1063; Anal. Calcd for $C_{15}H_{14}N_2O_2$, C, 70.85; H, 5.55; N, 11.02; Found C, 70.82; H, 5.57; N, 11.03

4.4.21. 2-(3′-Hydroxy-4′-methoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (21)

m.p. 261-263 °C; IR ν cm-1 (KBr disk): 3092 (NH stretch), 1602 (C=N), 1437 (C=C);7 1H NMR (500 MHz, DMSO-*d6*): δ 9.39 (s, 1H, NH), 7.67 (s, 1H), 7.66 (d, 1H, J = 8.5 Hz), 7.43 (d, 1H, J = 8.0 Hz), 7.19 (d, 1H, J = 7.5 Hz), 7.16 (d, 1H, J = 8.5 Hz), 7.09 (d, 1H, J = 7.0 Hz), 3.88 (s, 3H, OCH3), 2.58 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.58, 149.08, 148.11, 138.25, 137.46, 125.01, 124.84, 124.52, 124.21, 119.09, 115.16, 114.30, 110.32, 56.71, 18.14; HREI-MS: m/z calcd for $C_{15}H_{14}N_2O_2$ [M]+ 254.1055; Found 254.1061; Anal. Calcd for $C_{15}H_{14}N_2O_2$, C, 70.85; H, 5.55; N, 11.02; Found C, 70.86; H, 5.54; N, 11.01

4.4.22. 2-(3′-Methoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (22)

m.p. 242-243 °C; IR ν cm-1 (KBr disk): 3082 (NH stretch), 1607 (C=N), 1497 (C=C), 1251 (C-N); 1H NMR (500 MHz, DMSO-*d6*): δ 11.89 (s, 1H, NH), 7.82 – 7.80 (m, 2H), 7.56 (t, 1H, *J* = 8.0 Hz), 7.51 (d, 1H, *J* = 8.0 Hz), 7.26 (t, 1H, *J* = 7.5 Hz), 7.19 – 7.15 (m, 1H), 3.90 (s, 3H, OCH3), 2.62 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.59, 149.19, 138.37, 137.46, 131.67, 129.02, 125.07, 124.52, 124.30, 119.41, 114.51, 114.27, 114.01, 56.13, 18.25; HREI-MS: m/z calcd for $C_{15}H_{14}N_2O$ [M]+ 238.1106; Found 238.1109; Anal. Calcd for $C_{15}H_{14}N_2O$, C, 75.61; H, 5.92; N, 11.76; Found C, 75.59; H, 5.90; N, 11.78

4.4.23. 2-(4′-Methoxyphenyl)-7-methyl-1*H*-benzo[*d*]imidazole (23)

m.p. 172-174 °C; IR ν cm-1 (KBr disk): 3197 (NH stretch), 1627 (C=N), 1419 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 12.53 (s, 1H, NH), 8.18 (d, 2H, *J* = 9.0 Hz), 7.46 (d, 1H, *J* = 8.0 Hz), 7.21 (s, 1H), 7.19 (d, 1H, *J* = 8.5 Hz), 7.11 (d, 1H, *J* = 7.5 Hz), 3.87 (s, 3H, OCH3), 2.59 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.69, 149.46, 138.36, 137.49, 126.79, 126.79, 125.43, 124.97, 124.42, 123.31, 114.85, 114.11, 114.11, 56.02, 18.13; HREI-MS: m/z calcd for $C_{15}H_{14}N_2O$ [M]+ 238.1106; Found 238.1158; Anal. Calcd for $C_{15}H_{14}N_2O$, C, 75.61; H, 5.92; N, 11.76; Found C, 75.63; H, 5.91; N, 11.77

4.4.24. 7-Methyl-2-(pyridin-4′-yl)-1*H*-benzo[*d*]imidazole (24)

m.p. 164-165 °C; IR ν cm-1 (KBr disk): 3107 (NH stretch), 1608 (C=N), 1443 (C=C), 1315 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 13.16 (s, 1H, NH), 8.76 (d, 2H, *J* = 5.5 Hz), 8.17 (s, 1H), 8.10 (s, 1H), 7.54 (s, 1H), 7.17 (s, 1H), 7.06 (s, 1H), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d6): δ 150.68, 150.37, 149.52, 140.25, 138.31, 137.27, 125.17, 124.67, 124.19, 123.43, 123.81, 114.40, 18.17; HREI-MS: m/z calcd for $C_{13}H_{11}N_3$ [M]+ 209.0953; Found 209.0957; Anal. Calcd for $C_{13}H_{11}N_3$, C, 74.62; H, 5.30; N, 20.08; Found C, 74.61; H, 5.32; N, 20.10

4.4.25. 2-(2′-Chlorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (25)

m.p. 227-229 °C; IR ν cm-1 (KBr disk): 3092 (NH stretch), 1606 (C=N), 1433 (C=C); 1H NMR (500 MHz, DMSO-d_6): δ 11.58 (s, 1H, NH), 7.86 (d, 1H, *J* = 7.0 Hz), 7.67 (d, 1H, *J* = 8.0 Hz), 7.58 – 7.52 (m, 1H), 7.45 (d, 1H, *J* = 8.0 Hz), 7.16 (t, 1H, *J* = 8.0 Hz), 7.05 (d, 1H, *J* = 7.0 Hz), 2.56 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 147.18, 138.39, 137.47, 132.10, 131.09, 130.73, 130.58, 128.09, 127.66, 124.90, 124.67, 124.32, 114.20, 18.31; HREI-MS: m/z calcd for $C_{14}H_{11}ClN_2$ [M]+ 242.0611; Found 242.0617; Anal. Calcd for $C_{14}H_{11}ClN_2$, C, 69.28; H, 4.57; N, 11.54; Found C, 69.30; H, 4.59; N, 11.51

4.4.26. 2-(3′-Chlorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (26)

m.p. 214-216 °C; IR ν cm-1 (KBr disk): 3092 (NH stretch), 1612 (C=N), 1441 (C=C); 1H NMR (5MHz, DMSO-d_6): δ 11.84 (s, 1H, NH), 8.29 (s, 1H), 8.19 (d, 1H, J = 6.0 Hz), 7.64 (d, 2H, J = 6.5 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.22 (t, 1H, J = 7.5 Hz), 7.12 (d, 1H, J = 7.5 Hz), 2.60 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.07, 138.25, 137.47, 133.78, 132.17, 130.56, 128.41, 128.10, 125.79, 125.03, 124.72, 124.30, 114.51, 18.14; HREI-MS: m/z calcd for $C_{14}H_{11}ClN_2$ [M]+ 242.0611; Found 242.0614; Anal. Calcd for $C_{14}H_{11}ClN_2$, C, 69.28; H, 4.57; N, 11.54; Found C, 69.27; H, 4.56; N, 11.55

4.4.27. 2-(4′-Chlorophenyl)-7-methyl-1*H*-benzo[*d*]imidazole (27)

m.p. 144-145 °C; IR ν cm-1 (KBr disk): 3097 (NH stretch), 1603 (C=N), 1439 (C=C), 1095.34 (C-N); 1H NMR (500 MHz, DMSO-d_6): δ 11.35 (s, 1H, NH), 8.24 (d, 2H, J = 7.0 Hz), 7.69 (d, 2H, J = 8.5 Hz), 7.47 (d, 1H, J = 8.0 Hz), 7.20 (t, 1H, J = 7.5 Hz), 7.10 (d, 1H, J = 7.5 Hz), 2.59 (s, 3H, CH3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 149.67, 138.45, 137.34, 134.57, 134.29, 129.35, 129.35, 128.54, 128.54, 125.80, 124.65, 124.34, 114.41, 18.21; HREI-MS: m/z calcd for $C_{14}H_{11}ClN_2$ [M]+ 242.0611; Found 242.0607; Anal. Calcd for $C_{14}H_{11}ClN_2$, C, 69.28; H, 4.57; N, 11.54; Found C, 69.29; H, 4.55; N, 11.56

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Graphical Abstract

Synthesis, *α*-Glucosidase inhibitory, Cytotoxicity and Docking Studies of 2-aryl-7-methylbenzimidazoles

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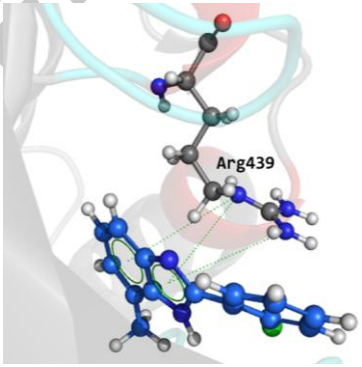

Synthesis of benzimidazole derivatives (**1**-**27**) as *α*-glucosidase inhibitor

Research Highlights

- Synthesis of 27 benzimidazole derivatives
- *In-vitro* α-glucosidase inhibitory activity
- Cytotoxicity
- Docking studies were carried out to confirm binding of active compounds with enzyme