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Original Article

Benzimidazole Derivatives as Multitarget Agents: Synthesis, Characterization, *Invitro* Alpha-glucosidase Inhibition and Antioxidants Activity

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Nitrogen-containing compounds, specifically benzimidazole derivatives, represent a significant class of molecules with diverse biological properties that have garnered considerable attention in medicinal chemistry. In this study, two 2-substituted benzimidazoles were successfully synthesized using a boric acid-catalyzed method. The resulting products demonstrated good yields and sharp melting points. The structures of the synthesized compounds were elucidated using Fourier-transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy, including both proton (^{1}H-NMR) and carbon (^{13}C-NMR) analyses.The compounds were evaluated for their alpha-glucosidase inhibitory activity and antioxidant properties. Both compounds exhibited notable antidiabetic and antioxidant activities. Notably, the compound designated as BL24 demonstrated the highest alpha-glucosidase inhibitory activity and radical scavenging properties. These experimental results indicate that benzimidazoles possess effective antidiabetic and antioxidant potential.

Keywords: Benzimidazole, Antioxidant, Alpha-Glucosidase, Oxidative Stress, Diabetes Mellitus

Diabetes is a global health disorder characterized by hyperglycemia and glucose intolerance, resulting from insulin dysfunction, impaired insulin secretion, or both (1). Individuals with diabetes face an elevated risk of developing several serious, life-threatening health complications, which lead to increased medical care costs, diminished quality of life, and higher mortality rates (2). Prolonged hyperglycemia can cause widespread vascular damage, affecting the heart, eyes, kidneys, and nerves, ultimately resulting in various complications (3). Diabetes is generally classified into three major categories: Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM)—which accounts for 80–90% of all diabetic cases—and Gestational Diabetes Mellitus (GDM) (4).

According to the International Diabetes Federation (IDF) report (10th edition, 2021), approximately 536.6 million people worldwide were affected by diabetes in 2021, with projections indicating an increase to 783.7 million by 2045. In Nigeria, the IDF reported that 3.6235 million individuals suffered from diabetes in 2021, with this number expected to rise to 7.9883 million by 2045, representing an increase of approximately 279.54%. The rate of increase has been significantly higher in low- and middle-income countries compared to high-income countries (5).

Reactive oxygen species (ROS), also referred

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to as oxygen-containing reactive species, include singlet oxygen, hydrogen peroxide, superoxide anion, peroxynitrite, hydroxyl radicals, and hypochlorous acid. An imbalance between ROS generation and antioxidant capacity leads to the accumulation of ROS, causing chemical alterations in DNA, proteins, and lipids, ultimately resulting in oxidative stress and cellular damage (6, 7). Type 2 diabetes is one of several disorders closely associated with oxidative stress (6).

Recently, there has been a growing interest among medicinal chemists in nitrogen-containing heterocyclic compounds, particularly benzimidazole derivatives. These compounds are recognized for their diverse biological properties and have garnered significant attention in medicinal chemistry. The heterocyclic structure of benzimidazole is formed by the fusion of benzene and imidazole, resulting in an aromatic conjugated compound with a molecular weight of 118.14 g/mol that exhibits tautomerization (8).

The design of benzimidazole derivatives as potential antioxidants and antidiabetics is based on their favorable properties, including enhanced stability, bioavailability, and significant biological activity (9). Benzimidazole derivatives are valuable as chemotherapeutic agents due to their isostructural pharmacophore, which resembles naturally occurring active biomolecules (10). Pharmacological applications of benzimidazole analogs have identified potent inhibitors of various enzymes, with therapeutic uses spanning antidiabetic, anticancer, antimicrobial, antiparasitic, analgesic, antiviral, antihistamine, as well as neurological, endocrinological, and ophthalmological applications (9). The structure-activity relationship suggests that methoxy and methyl substitutions on the phenyl ring between the Schiff-base linkage and the benzimidazole moiety enhance α-glucosidase inhibition (4).

Materials and methods

The melting points were measured using a Gallenklamp melting point apparatus and reported as uncorrected values. Fourier-transform infrared (FT-IR) spectroscopy was conducted with an Agilent FT-IR spectrophotometer at Ahmadu Bello University, Zaria, Nigeria. Ultraviolet-visible (UV-Vis) spectroscopy was performed using a JWNWAY 6705 spectrophotometer at Bayero University Kano, Nigeria. Nuclear Magnetic Resonance (NMR) spectroscopy was carried out at the Jodrell Laboratory, NCPH Royal Botanical Gardens, Kew, TW9 3AE, United Kingdom. All chemicals utilized in this study were of analytical grade (Ahmadu Bello University, Zaria, Nigeria).

General synthesis procedure (Boric acid catalyzed)

An equimolar mixture of o-phenylenediamine (0.01 mol), benzaldehyde (0.01 mol), and boric acid (10 mol %) in 20 mL of water was stirred at room temperature for 30 minutes. The completion of the reaction was monitored by thin-layer chromatography (TLC) using a solvent system of ethyl acetate and diethyl ether (7:3). The solid product was collected by simple filtration and washed with water. The crude product was subsequently purified by recrystallization from ethyl acetate (11).

The synthetic route and the proposed reaction mechanism are illustrated in Figure 1 and Figure 2. The mechanism for the formation of benzimidazoles using a boric acid catalyst involves two steps. The first step entails the formation of a Schiff-base intermediate through the condensation of the amine group of o-phenylenediamine with the carbonyl group of the aldehyde. This reaction is initiated by the boric acid catalyst, which protonates the carbonyl group. The second step involves the cyclization of the Schiff-base intermediate, where an attack occurs on the imine carbon, followed by condensation to yield benzimidazoles.

Alpha-glucosidase inhibitory assay method was conducted following the method described by Kim et al. (12), utilizing α-glucosidase extracted from *Saccharomyces cerevisiae*. The method was

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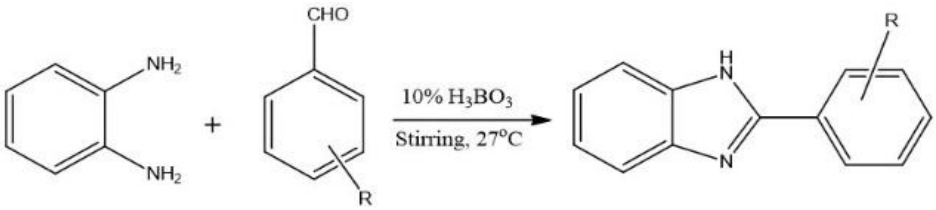


Figure 1. General synthetic route of benzimidazole derivatives.

modified for this study. A total of 50 µL of synthesized compounds at varying concentrations (ranging from 1000 µg/mL to 62.5 µg/mL) was placed in a tube, followed by the addition of 100 µL of α-glucosidase (1000 µg/mL) in 100 mM sodium phosphate buffer (pH 6.9). The mixture was incubated at room temperature for 10 minutes. Subsequently, 50 µL of the substrate solution, p-nitrophenylglucopyranoside (pNPG) (3.0 mM) in 0.02 M sodium phosphate buffer (pH 6.9), was added. This reaction mixture was incubated at 37 °C for 20 minutes. The reaction was then quenched by adding 2 mL of 0.1 M Na_2CO_3. The absorbance of the yellow-colored p-nitrophenol released from pNPG was measured at 405 nm, and the absorbance of the control was also recorded. Acarbose, a standard drug, was used as the positive control. The assay was performed in triplicate, and the results were expressed as the percent inhibition of α-glucosidase activity using the following formula:

Inhibition (%) = [(Absorbance of Control – Absorbance of Sample) / Absorbance of Control] × 100

Antioxidant activity methods

DPPH radical scavenging assay was conducted using a modified method based on Liyana-Pathiranan and Shahidi (13). A 0.135 mM DPPH solution in methanol was prepared, and 1.0 mL of this solution was mixed with 1.0 mL of the compound prepared in ethyl acetate, containing 0.3125 - 0.5 mg of the sample and standard drugs (ascorbic acid). The reaction mixture was vortexed thoroughly and allowed to stand in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability of the plant extract to scavenge DPPH radicals was calculated using the following equation:

$$\text{DPPH Radical Scavenging (\%)} = \frac{\text{OD (Control)} - \text{OD(Sample)}}{\text{OD (Control)}} \times 100$$

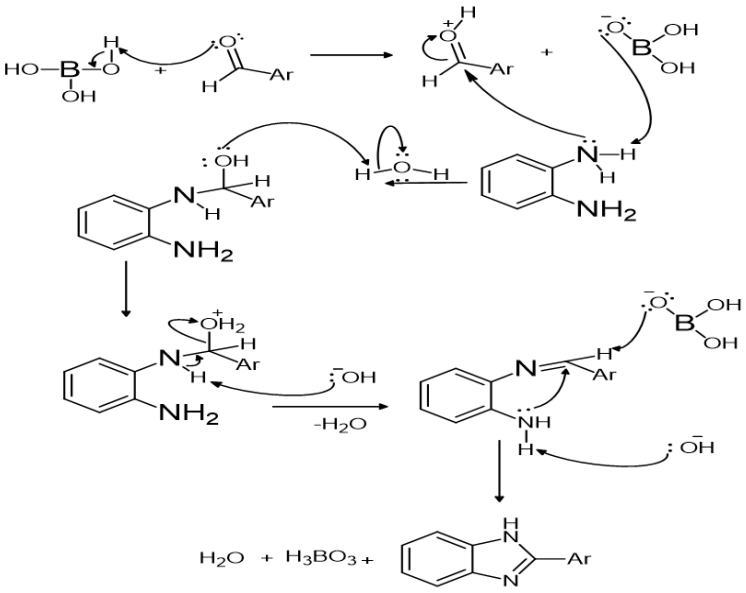
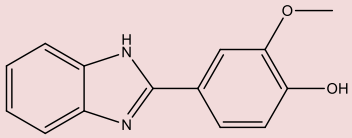
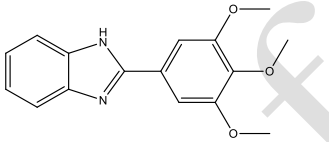


Figure 2. A plausible mechanism for synthesis of 2-substituted benzimidazole derivatives (acid-catalyzed).

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Table 1. Chemical descriptors of the synthesized benzimidazoles

S/No.	Compound Code	IUPAC Name	Chemical Structure (Molecular formula)
1	BL17	4-(1H-benzo[d]imidazol-2-yl)-2-methoxyphenol	 ($C_{16}H_{16}N_2O_3$)
2	BL24	2-(3,4,5-trimethoxyphenyl)-1H-benzo[d]imidazole	 ($C_{14}H_{12}N_2O_2$)

The 50% inhibitory concentration (IC_{50}), defined as the concentration required to scavenge 50% of the radical activity, was estimated using an online server, AAT Bioquest (14).

FRAP scavenging assay

The ferric ion reducing antioxidant power (FRAP) of the synthesized compounds was evaluated according to the method of Yen and Chen (15). A volume of 1.0 mL of the compounds prepared in ethyl acetate and ascorbic acid (0.03125 mg/mL - 1.0 mg/mL) was mixed individually with a solution containing 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide ($K_3Fe(CN)_6$) (1% w/v). The resulting mixture was incubated at 50 °C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). The mixture was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferrous chloride (0.1% w/v). The absorbance was measured at 517 nm against a blank sample. An increase in absorbance of the reaction mixture indicated a higher reducing power of the plant extract (15). The 50% inhibitory concentration (IC_{50}) was estimated using the same online server, AAT Bioquest (14).

Results

Synthesis of 1H-Benzo[d]imidazole Derivatives

The synthesis of two 1H-benzo[d]imidazole derivatives Table 1 was conducted using a boric acid-catalyzed method. The compounds, coded BL17 and BL24, were successfully synthesized with good yields and sharp melting points, as detailed in Table 2. The structures of the synthesized compounds were characterized and confirmed through Fourier-transform infrared (FT-IR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy (both ^1H-NMR and ^13C-NMR).

In Vitro Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibitory activity of the synthesized compounds BL17 and BL24 was evaluated at concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL, as illustrated in Figure 3. Acarbose was used as a

Table 2. Physical properties of the synthesized compounds

Compound ID	Color/Appearance	Melting point (°C)	Yield (%)
BL17	White powder	112-114	68.8
BL24	Yellow powder	102-103	74.4

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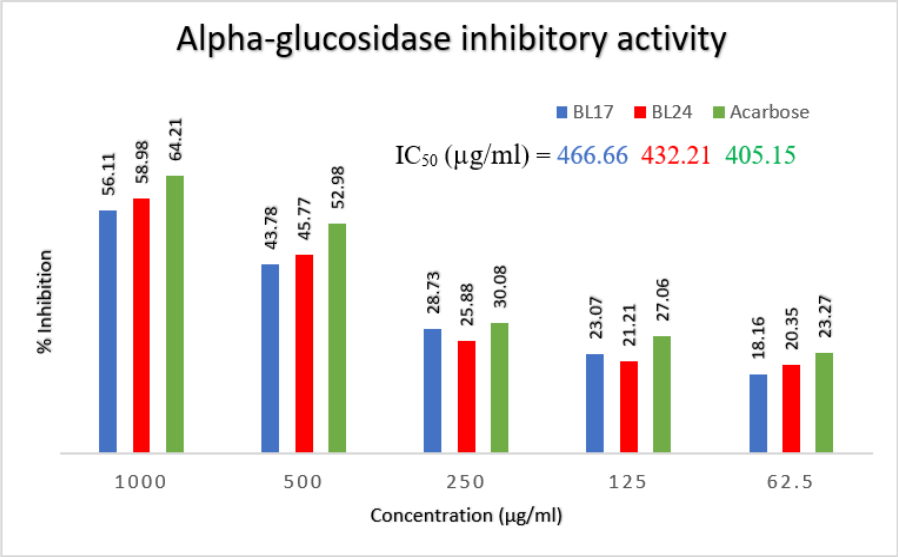


Figure 3. Alpha-glucosidase inhibitory activity of BL17, BL24, and Acarbose.

positive control. The 50% inhibitory concentration (IC_{50}), defined as the concentration required to inhibit 50% of the enzyme activity, was estimated using an online server, AAT Bioquest (14).

Antioxidant evaluation

The antioxidant activity of the synthesized compounds BL17 and BL24 was assessed using DPPH and FRAP scavenging assays. The antioxidant activity at doses of 0.5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL is presented in Figure 4, with ascorbic acid serving as a positive control. The IC_{50} values were calculated using the AAT Bioquest online server (14).

Discussion

The FT-IR spectrum of BL17 exhibited strong stretching vibrations at 3067 cm^{-1} (N-H) and 1599 cm^{-1} (C=N). In contrast, compound BL24 displayed absorption frequencies at 3362 cm^{-1} (N-H) and 1613 cm^{-1} (C=N), indicating the disappearance of the primary amine (-NH_2) from o-phenylenediamine (OPD) and the carbonyl group of the aryl aldehyde. The formation of a secondary amine (-NH) and an imine group (C=N) was corroborated by the observed absorption frequencies. Other notable absorptions included those around 3384 cm^{-1} (O-H stretching), 1300-1450 cm^{-1} (C-N), and 2800-3000

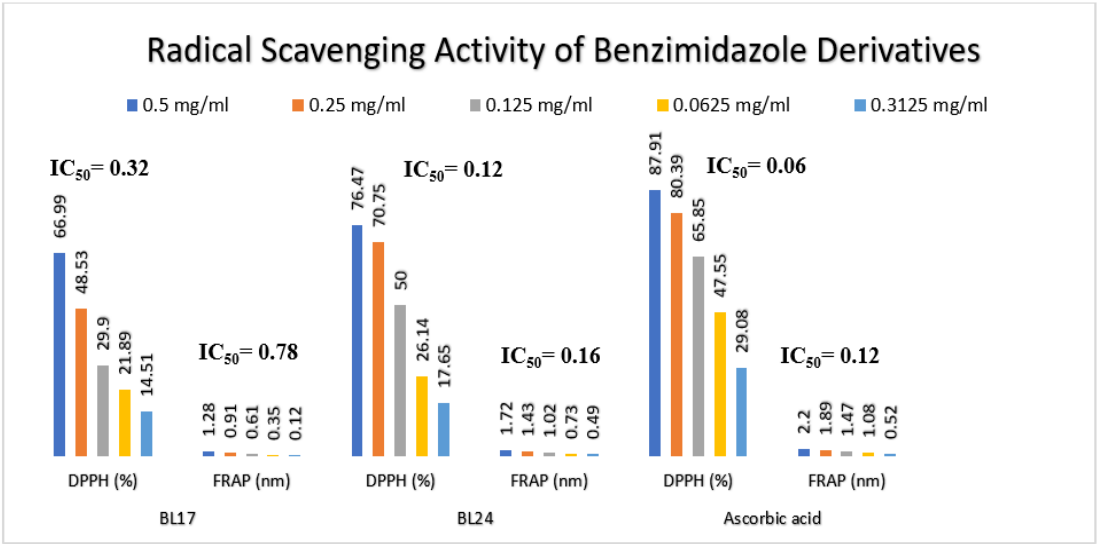


Figure 4. Radical Scavenging Activity of BL17, BL24, and Ascorbic acid.

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cm^{-1} (olefinic carbons of the aromatic ring). These absorption frequencies are consistent with those reported for synthesized benzimidazole derivatives (16-18).

The ^1H-NMR and ^13C-NMR spectra were utilized to elucidate the structures of BL17 and BL24. The ^1H-NMR spectrum of BL17 showed the following chemical shifts: δ, ppm (400 MHz, $CDCl_3$): 8.48 (s, 1H, N-H), 7.86 (d, 2H, J = 4 Hz, Ar-H), 7.54 (s, 1H, Ar-H), 7.07 (d, 1H, J = 8 Hz, Ar-H), 6.93 (d, 1H, J = 8 Hz, Ar-H), 6.79 (dd, J = 12 Hz, J = 8 Hz, 2H), 4.25 (s, 1H, O-H), and 3.87 (s, 3H, $-OCH_3$). The ^13C-NMR spectrum revealed the following chemical shifts: δ, ppm (400 MHz, $CDCl_3$): 55.45, 114.15, 114.51, 115.30, 117.20, 118.51, 127.19, 129.63, 130.36, 137.66, 138.32, 142.02, 157.11 (C=N, imidazole), and 162.08.

For BL24, the ^1H-NMR spectrum presented the following shifts: δ, ppm (400 MHz, $CDCl_3$): 8.45 (s, 1H, N-H), 7.07 (d, 2H, J = 20 Hz, Ar-H), 6.79 (dd, 2H, J = 12 Hz, J = 16 Hz, Ar-H), 6.38 (s, 2H, Ar-H), and 3.96 (s, 9H, $-OCH_3$). The ^13C-NMR spectrum showed: δ, ppm (400 MHz, $CDCl_3$): 55.91, 56.25, 61.03, 103.84, 105.61, 115.41, 116.66, 117.36, 118.57, 127.76, 132.06, 137.31, 141.91, 153.51, 153.74, and 157.41 (C=N, imidazole).

The chemical shift values of 8.48 ppm and 8.45 ppm in the ^1H-NMR spectra of BL17 and BL24, respectively, confirmed the formation of the C-NH bond and the disappearance of the $C-NH_2$ bond. This finding is consistent with the work of Emenike et al., who reported a value of 8.42 ppm for N-H (19). The ^13C-NMR chemical shift values for both compounds appeared in the downfield region around 157 ppm, confirming the formation of C=N (imine) in the benzimidazole structure. This observation aligns with the findings of Welderufael et al., who reported C=N (imine) shifts for synthesized benzimidazole derivatives at downfield regions of 153.1 ppm and 155.3 ppm (20).

The results of the alpha-glucosidase inhibition assay indicated that the inhibitory effects of BL17 and BL24 were comparable to those of the standard drug, acarbose, as shown in Figure 3. The IC_{50} values were estimated to be 466.66 µg/mL for BL17, 432.21 µg/mL for BL24, and 405.15 µg/mL for acarbose. The structure-activity relationship suggests that methoxy and methyl substitutions on the phenyl ring between the Schiff-base linkage and the benzimidazole moiety enhance α-glucosidase inhibition (4). Both synthesized compounds possess methoxy substitutions on the phenyl ring, supporting their inhibitory properties. Notably, BL24, which contains three methoxy groups, exhibited stronger α-glucosidase inhibitory activity (IC_{50} = 432.21 µg/mL) compared to BL17 (IC_{50} = 466.66 µg/mL), which has one methoxy and one hydroxyl group on the phenyl ring. Rahim et al. (21) synthesized a series of benzimidazoles bearing Schiff bases and evaluated their α-glucosidase inhibitory potential, reporting IC_{50} values ranging from 1.10 ± 0.05 µM to 28.30 ± 0.60 µM (21).

Inhibition of alpha-glucosidase is one of the most effective strategies for managing diabetes, as it mitigates the deterioration of pancreatic β-cells caused by oxidative stress (22-24). This evidence supports the notion that the benzimidazole scaffold possesses antidiabetic properties.

In the DPPH assay Figure 4, the IC_{50} values for the two benzimidazole derivatives (BL17 and BL24) and ascorbic acid were found to be 0.32 mg/mL, 0.12 mg/mL, and 0.06 mg/mL, respectively. BL24 exhibited superior scavenging activity compared to BL17; however, both compounds demonstrated slightly lower scavenging activity than ascorbic acid.

The FRAP scavenging assay Figure 4 yielded similar results to the DPPH assay. BL24 (IC_{50} = 0.16 mg/mL) exhibited the highest scavenging activity, followed by BL17 (IC_{50} = 0.78 mg/mL). The positive control, ascorbic acid, demonstrated an IC_{50} of 0.12 mg/mL. A lower IC_{50} value indicates greater overall effectiveness of antioxidant activity (25).

The scavenging properties of the synthesized benzimidazoles align with the findings of Baldisserotto et al., who synthesized 39 aryl

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benzimidazole derivatives and reported their remarkable potency against various free radicals (26). For optimal scavenging activity, positions 3, 6, and 7 should remain unsubstituted (8).

Conclusion

Two 2-substituted benzimidazoles were successfully synthesized, and their structures were elucidated using FT-IR and NMR spectroscopy (^1H-NMR and ^13C-NMR). The spectral data confirmed the formation of 4-(1H-benzo[d]imidazol-2-yl)-2-methoxyphenol and 2-(3, 4, 5-trimethoxyphenyl)-1H-benzo[d]imidazole. The compounds were evaluated for their alpha-glucosidase inhibitory and antioxidant properties, revealing that the benzimidazoles possess potential antidiabetic and antioxidant activities.

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Conflict of interest

The authors declared no conflict of interest.

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