Original Article

New Tetrazole Derivatives As Potent In Vitro Antimicrobial Agents

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Abstract

Conjugated benzimidazole derivatives have been proven in numerous studies to have a variety of biological activities, including antibacterial characteristics. Using methlene linkers, we created and synthesized N-substituted alkylbenzimidazole derivatives in this study that were attached to tetrazole (an1-an5). The prepared molecules were confirmed using FTIR and NMR spectroscopies. The outcomes demonstrated that the synthesized benzimidazole compounds boosted the activity effects on Escherichia coli and Staphylococcus aureus strains. These derivatives included a 5-membered ring with nitrogen and an alkyl chain. (an2) compound shown good action against Staphylococcus Aureus strains whereas (an4 and na5) exhibited good activity against Escherichia Coli strains among the produced compounds.

Keywords: Resistant Bacteria; Benzimidazole; Tetrazole; Alkyl chain; Benzimidazolium Salts.

Introduction

Globally, microbial infections are becoming a more important and difficult issue for human health. Those with neoplastic illnesses, people undergoing organ transplants, and people with impaired immune systems are the most commonly impacted groups by these infections [1]. Due to this circumstance, it is really necessary to create new antibacterial medications using more contemporary chemical groups. Heterocyclic compounds have shown effective as antibacterial agents and have been utilized to treat a number of circulatory, cardiovascular, and central nervous system illnesses [2]. Modern drug research relies heavily on the pharmacophore benzoimidazole scafold, and its derivatives are significant bioactive compounds [3] with advantageous structural features [4]. There is plenty of evidence for this, including an increasing number of benzimidazole-containing synthetic compounds with a wider range of therapeutic properties [4, 5] and efforts to synthesize benzimidazole compounds with preferred geometries carrying particular biological traits [5, 6]. Numerous benzimidazole compounds have been investigated as potential treatments for a variety of infections (disease) or as prescription medications, including antitumor drugs (Pracinostat, Bendamustine), drugs (Astemizole) antihistamine, anthelmintic drugs (Albendazole, Mebendazole), antibacterial drugs (Ridinazole), antihypertensive drugs (Candesartan), and proton pump inhibitors (Ilaprazole, Panto), among others Because of their therapeutic effects as antibacterial [6–10], anticancer [11–13], proton pump inhibitors [14–16], anthelmintics [15, 16], anti-hypertensive [17] drugs, and anti-inflammatory [18–19] agents, benzimidazole derivatives have drawn a lot of interest from the medical world. Tetrazole's wide range of uses in synthetic processes result from its substantial use in medicinal chemistry. Tetrazole scaffold is present in a lot of powerful medications, such as pranlukast, pamiroplast, and candesartan [20]. Because of its identical pKa values and planar delocalized structures, tetrazole is a carboxylic acid analog. The bioavailability of tetrazole derivatives is boosted by their resistance to certain biological degradation processes [21, 22]. Tetrazole derivatives' effectiveness as antifungal [23], anti-HIV [24], anti-cancer [25], hormonal agents [26], antioxidant [27], and

antitubercular [28] agents is influenced by these factors. One might hypothesize that the biological activity of the compound would rise if benzimidazole, an alkyl chain, and tetrazole were all present in the same chemical framework. Building on our past work on the synthesis of tetrazole derivatives [29], we now report on the synthesis of such compounds and an evaluation of their antibacterial activities.

Experimental

The best analytical grade solvents and chemicals, all of which were obtained commercially, were given by Sigma-Aldrich and Fluka. The infrared spectra were recorded by the FT-IR, Bruker ALPHA FT-IR, at University of Kufa. Iranian researchers from University of Shahid Beheshti acquired NMR data in DMSO-d6 using a Bruker instrument (75MHz for 13C NMR, and 300MHz for 1H NMR respectively). The Electro Thermal Melting Point Apparatus, a device created in the UK, is used to calculate melting points.

Synthesis N-Substituted Benzimidazole (n1-n5)

The circular bottom of the flask was filled with benzimidazole (0.03 mol) and KOH powder (0.3809 mol). The abovementioned ingredients were combined, dissolved in 45.0 mL of organic solvent (DMSO), and then agitated at 90°C for two hours. Alkyl bromide, 0.014 mol, was cautiously added dropwise and continuously stirred. After adding the entire amount of alkyl bromide, the mixture was agitated continuously for two hours at 50 °C. After adding 250 mL of broken ice, the flask was withdrawn, and the mixture needed to be violently stirred for 30 seconds. After the combination had been allowed to sit for an hour, it was extracted with distilled water and petroleum ether (3X10 mL), and the petroleum ether was then taken out of the final products. All the substituted benzimidazole were reported in previous study [49].

Synthesis of 1-(cyanomethyl)-3-alkyl-1H-benzo[d]imidazol-3-ium bromide derivatives (a1-a5)

The reported procedures involved adding 20 mmol of prepared N-substituted benzimidazole (n1-n5) rabidly to 25 mL of dioxane as solvent. 2.5 equivalents of the bromoacetonitrile solution were gradually added, in tiny batches, once the reaction's temperature was lowered to below 5 ºC, and the reaction was then allowed to reflux. For an entire day, the reaction mixture was stirred at ambient temperature. The organic solvent was expelled when the chemical reaction was finished, and methanol was used to recreate the crystals.

1-(cyanomethyl)-3-decyl-1H-benzo[d]imidazol-3-ium bromide(a1): Percentage of the Product; 79%, %, FT-IR (KBr, cm^{-1}): 3088($_{aromatic}$C-H), 2953($_{aliph}$C-H), 2855($_{aliph}$C-H), 2214 (-C≡N), 1622 (C=N),1263(C-N). ^{1}H-NMR (ppm), δ 0.82 (t, J = 6.7 Hz, CH_3, 3H), 1.17-1.25 (m, 7 x CH_2, 14H), 1.78 (p, J = 6.9 Hz, 2H), 4.30 (t, J = 7.0 Hz, N-CH_2, 2H), 5.23 (s, N-CH_2-C≡N, 2H), 7.50 -7.21 (m, Ar-H,4H), 9.22 (s, NCHN, 1H), ^{13}C-NMR, δ 144.88(NCHN), 141.65, 133.25, 128.97, 125.07, 121.54, 115.78, (Ar-C), 118.50 (-C≡N),61.14 N-CH_2-C≡N), 44.65(N-CH_2), 32.88, 29.91, 29.77, 29.55, 29.36, 29.18, 28.11, 21.39(8-CH_2), 14.64(8-CH_3).

1-(cyanomethyl)-3-dodecyl-1H-benzo[d]imidazol-3-ium bromide(a2): Percentage of the Product; 84%, FT-IR (KBr, cm^{-1}): 3102($_{aromatic}$C-H), 2966($_{aliph}$C-H), 2845($_{aliph}$C-H), 2198(-C≡N), 1618 (C=N),1265 (C-N). ^{1}H-NMR (ppm), δ 0.84 (t, J = 6.6 Hz, CH_3, 3H), 1.21-1.27 (m, 9 x CH_2, 16H), 1.77 (p, J = 6.8 Hz, 2H), 4.22 (t, J = 7.0 H, N-CH_2z, 2H), 5.25 (s, N-CH_2-C≡N, 2H), 7.54-7.23 (m, Ar-H, 4H), 9.30 (s, NCHN, 1H), ^{13}C-NMR, δ 145.01 (NCHN), 141.35, 134.08, 129.17, 126.74, 121.38, 118.21, (Ar-C),119.14 (-C≡N), 60.28 N-CH_2-C≡N), 44.67(N-CH_2), 32.89, 31.15, 29.96, 29.84, 29.69, 29.45, 29.29, 28.57, 26.45, 22.69(10-CH_2), 14.23 (10-CH_3).

1-(cyanomethyl)-3-tetradecyl-1H-benzo[d]imidazol-3-ium bromide (a3): Percentage of the Product; 79%, FT-IR (KBr, cm^{-1}): 3095($_{aromatic}$C-H), 2974($_{aliph}$C-H), 2855 ($_{aliph}$C-H), 2210(-C≡N), 1630(C=N),1262 (C-N). ^{1}H-NMR (ppm), δ 0.80 (t, J = 6.4 Hz, CH_3, 3H), 1.21-1.27 (m, 11x CH_2, 22H), 1.74 (p, J = 7.0 Hz, 2H), 4.33 (t, J = 7.0 Hz, N-CH_2, 2H), 5.31 (s, N-CH_2-C≡N, 2H), 7.51-7.19 (m, Ar-H, 4H), 9.26 (s, NCHN, 1H), ^{13}C NMR, δ 145.01(NCHN), 142.45, 133.97, 128.84, 125.79, 119.89, 116.81(Ar-C), 119.54(-C≡N),61.87 N-CH_2-C≡N), 43.92(N-CH_2), 32.05, 30.88, 29.98, 29.81, 29.64, 29.42, 29.24, 29.11, 29.00, 28.57, 26.65, 22.75(12-CH_2), 13.98(CH_3).

1-(cyanomethyl)-3-hexadecyl-1H-benzo[d]imidazol-3-ium bromide (a4): Percentage of the Product; 80%, FT-IR (KBr, cm-1): 3105($_{aromatic}$C-H), 2985($_{aliph}$C-H), 2866 ($_{aliph}$C-H), 2205(-C≡N), 1618 (C =N),1252 (C-N). ^{1}H-NMR (ppm), δ 0.87(t, J = 6.5 Hz, CH_3, 3H), 1.20-1.29 (m, 13x CH_2, 26H), 1.75 (p, J = 7.0 Hz, 2H), 4.31 (t, J = 7.0 Hz, N-CH_2, 2H), 5.34 (s, N-CH_2-C≡N,2H), 7.52–7.22 (m, Ar-H, 4H), 9.26 (s, NCHN, 1H), ^{13}C- NMR, δ 144.57(NCHN), 144.14, 134.54, 128.88, 126.754, 122.32, 117.47, (Ar-C), 118.74 (-C≡N),61.11 N-CH_2-C≡N), 44.98(N-CH_2), 31.98, 31.84, 29.90, 29.74, 29.55, 29.41, 29.33, 29.25, 29.20, 29.13, 28.57, 26.77, 24.54, 22.54(14-CH_2), 14.45(CH_3).

1-(cyanomethyl)-3-octadecyl-1H-benzo[d]imidazol-3-ium bromide (a5): Percentage of the Product; 77%, FT-IR (cm^{-1}): 3080($_{aromatic}$C-H), 2944($_{aliph}$C-H), 2850($_{aliph}$C-H), 2212(-C≡N), 1611 (C=N), 1250 (C-N). ^{1}H-NMR (ppm), δ 0.82(t, J = 6.5 Hz, CH_3, 3H), 1.22-1.29 (m, 13x CH_2, 26H), 1.82 (p, J = 7.0 Hz, 2H), 4.31 (t, J = 7.0 Hz, N-CH_2, 2H), 5.30 (s, N-CH_2-C≡N, 2H), 7.61–7.24 (m, Ar-H, 4H), 9.24 (s, NCHN,1H), ^{13}C-NMR, δ 145.35(NCHN), 143.65, 134.15, 128.87, 126.71, 119.89, 116.78 (Ar-C),119.75 (-C≡N), 61.35 N-CH_2-C≡N),45.54(N-CH_2), 31.92, 31.69, 30.56, 29.98, 29.85, 29.71,29.59, 29.40, 29.35, 29.26, 29.17, 29.09, 28.87, 26.67, 26.12, 22.27(16-CH_2), 14.28 (CH_3).

Synthesis of 1,2,3-tetrazole derivatives (an1-an5) [50]

After stirring for 10 minutes, (1.3 eq) nitrile derivatives (a1–a5) were added to (0.48 mmol) sodium azide that had been dissolved in 20 mL DMF, together with (5mol%) $CuSO_4.5H_2O$ and (10mol%) sodium ascorbate. Then, while the TLC plate, dichloromethane: methanol (9:1), and the end point of the chemical reaction were all employed to confirm it, the solution for the end target was allowed to swirl at laboratory temperature.

1-((1H-tetrazol-5-yl)methyl)-3-decyl-1H-benzo[d]imidazol-3-iumbromide(an1): Percentage of the Product; 74%, FT-IR (cm^{-1}): 3094($_{aromatic}$C-H), 2966($_{aliph}$C-H), 2865($_{aliph}$C-H), 1607 (C=N),1254(C-N). ^{1}H-NMR (ppm), δ 0.79 (t, J = 6.7 Hz, CH_3, 3H), 1.17-1.25 (m, 7xCH_2, 14H), 1.71 (p, J = 6.9 Hz, 2H), 4.14 (t, J = 7.0 Hz, N-CH_2, 2H), 5.12 (s, N-$CH_{2\text{-tetrazole ring}}$, 2H), 7.62 -7.21 (m, Ar-H,4H, Ar-H), 9.31 (s, NCHN, 1H), ^{13}C-NMR, δ 146.69 (NCHN), 154.47, (1C, carbons of tetrazole ring), 143.24, 134.65, 129.65, 125.01, 120.77, 117.54 (Ar-C),44.65(N-CH_2), 48.17 N-$\underline{C}H_2$-tetrazole ring), 32.12, 31.25, 30.97, 30.71, 29.98, 29.74, 27.21, 22.45(8-CH_2), 14.35(CH_3).

1-((1H-tetrazol-5-yl)methyl)-3-dodecyl-1H-benzo[d]imidazol-3-iumbromide(an2): Percentage of the Product; 71%, FT-IR (cm^{-1}): 3088($_{aromatic}$C-H), 2984($_{aliph}$C-H), 2869($_{aliph}$C-H), 1625 (C=N),1258(C-N). ^{1}H-NMR (ppm), δ0.81 (t, J = 6.6 Hz, CH_3, 3H), 1.19-1.26 (m, 9xCH_2, 18H), 1.70 (p, J = 6.8 Hz, 2H), 4.15 (t, J = 7.0 Hz, N-CH_2, 2H), 5.11 (s, N-$CH_{2\text{-tetrazole ring}}$, 2H), 7.71-7.27 (m, Ar-H, 4H), 9.30 (s, NCHN, 1H), ^{13}C- NMR, δ 144.98(NCHN), 152.58, (1C, carbons of tetrazole ring), 144.54, 134.28, 129.02, 126.61, 118.65 (Ar-C),44.54 (N-CH_2), 49.27 (N-$\underline{C}H_2$-tetrazole ring), 32.14, 31.48, 31.87, 30.58, 29.95, 29.84, 29.62, 29.42, 27.54, 22.87(8-CH_2), 14.24(CH_3).

1-((1H-tetrazol-5-yl)methyl)-3-tetradecyl-1H-benzo[d]imidazol-3-iumbromide(an3): Percentage of the Product; 74%, FT-IR (cm^{-1}): 3113($_{aromatic}$C-H), 2968($_{aliph}$C-H), 2874($_{aliph}$C-H), 1608 (C=N),1249(C-N). ^{1}H-NMR (ppm), δ0.82 (t, J = 6.4 Hz, CH_3, 3H), 1.20-1.26 (m, 11xCH_2, 22H), 1.70 (p, J = 7.0 Hz, 2H), 4.15 (t, J = 7.1 Hz, N-CH_2, 2H), 5.18 (s, N-$CH_{2\text{-tetrazole ring}}$, 2H), 7.65-7.19 (m, Ar-H, 4H), 9.28 (s, NCHN, 1H), ^{13}C- NMR, δ 145.24 (NCHN), 152.25(1C, carbons of tetrazole ring), 143.98, 134.14, 129.98, 127.47, 119.88, 115.74(Ar-C), 43.17(N-CH_2), 48.17(N-$\underline{C}H_2$-tetrazole ring), 32.35, 31.41, 30.84, 30.30, 29.98, 29.79, 29.70, 29.31, 29.22, 28.14, 26.87, 22.54(CH_2), 13.12(CH_3).

1-((1H-tetrazol-5-yl)methyl)-3-hexadecyl-1H-benzo[d]imidazol-3-iumbromide(an4); Percentage of the Product; 79%, FT-IR (cm^{-1}): 3097($_{aromatic}$C-H), 2945($_{aliph}$C-H), 2868($_{aliph}$C-H), 1612 (C=N),1261(C-N). ^{1}H-NMR (ppm), δ 0.79 (t, J = 6.5 Hz, CH_3, 3H), 1.22-1.29 (m, 13xCH_2, 26H), 1.80 (p, J = 7.0 Hz, 2H), 4.19 (t, J = 7.1 Hz, N-CH_2, 2H), 5.16 (s, N-$CH_{2\text{-tetrazole ring}}$, 2H), 7.64–7.18 (m, Ar-H, 4H), 9.28 (s, NCHN, 1H), ^{13}C- NMR, δ 145.74 (NCHN), 153.78,(1C, carbons of tetrazole ring), 144.25, 134.27, 129.87, 125.82, 119.25, 116.78(Ar-C), 43.74(N-CH_2), 49.14(N-$\underline{C}H_2$-tetrazole ring), 32.14, 31.81, 30.96, 30.45, 30.18, 29.98, 29.84, 29.65, 29.39, 29.21, 29.06, 27.91, 26.78, 22.54(8-CH_2), 14.20(CH_3).

1-((1H-tetrazol-5-yl)methyl)-3-octadecyl-1H-benzo[d]imidazol-3-iumbromide(an5): Percentage of the Product; 82%, FT-IR (cm^{-1}): 3112 ($_{aromatic}$C-H), 2988($_{aliph}$C-H), 2858($_{aliph}$C-H), 1622 (C=N),1254(C-N). ^{1}H-NMR (ppm), δ0.85(t, J = 6.5 Hz, CH_3, 3H), 1.19-1.26 (m, 15xCH_2, 30H), 1.81 (p, J = 7.0 Hz, 2H), 4.16 (t, J = 7.1 Hz, N-CH_2, 2H), 5.17 (s, N-$CH_{2\text{-tetrazole ring}}$, 2H), 7.66–7.26 (m, Ar-H, 4H), 9.33 (s, NCHN, 1H), ^{13}C- NMR, δ 146.24(NCHN), 153.40(1C, carbons of tetrazole ring), 144.80, 134.58, 127.81, 124.56, 121.81, 118.73(Ar-C), 44.57(N-CH_2), 48.74(N-$\underline{C}H_2$-tetrazole ring), 32.28, 31.82, 31.51, 30.97, 30.74, 30.44, 30.19, 29.98, 29.82, 29.60, 29.31, 29.28, 29.12, 28.45, 26.72, 22.47(8-CH_2), 14.24 (CH_3).

Antibacterial Activity

The biological activity (antibacterial activity) of the target products was examined in vitro under controlled conditions using Muller Hinton Agar medium utilizing the disc diffusion technique. The test organisms utilized in this study were grown in nutrient broth for 24 h at 37 °C before being transferred to the Muller Hinton agar dishes in the laminar flow cabinet. Before being used, the freshly created chemicals were first dissolved in sterile plates of filter paper no (6 mm diameter). The created discs were then meticulously placed on top of the previously prepared, contaminated plates and incubated at a predetermined location. Each drug's mm-sized zone of inhibition was discovered after a day of incubation at 37°C. As a check, a disc was filled with dimethylsulfoxide (DMSO). In the assessment of the efficacy, the well-known antibiotic Ciprofloxacin was utilized as a positive drug control [56]. Each exam was administered three times, and the average was the outcome. Compounds having effective zones of inhibition were chosen in order to find the least inhibitory concentration (MIC).

Antifungal activity

The disc diffusion method and the Potato Dextrose Agar Medium were used to determine the in-vitro antifungal activity of the end compounds. The germs utilized in the investigation were injected onto the exterior of a used agar plate. Then, 6 mm-diameter discs of brand-new, sterile filter paper No. 1 were positioned on the plate's surface. As a control, these included exact quantities of the antifungal medication fluconazole (100 mg for the end products). After 72 hours of incubation at 28 °C, the antifungal activity of the used plates was evaluated. A paper disc that had been coated in dimethylsulfoxide (DMSO) was employed as a checkpoint [56]. The prepared nutritive agar medium was autoclaved for 20 minutes at 15 lbs of pressure before being placed into petri plates for setting. Using a sterile cotton swab, a microbial suspension was applied to the surface of the media. Cups were created by punching holes through the surface of the agar with a sterilized cork borer and then scooping up the agar that had been penetrated. Four carved-out cavities or cups were filled with various concentrations of the test chemicals and the reference drug Fluconazole, which served as the control. The plates were kept at room temperature for an hour before being incubated at 37°C for a day. The compound's percentage of inhibition and the diameter of the inhibition zone that had formed around the cavities were calculated after a day of incubation (cups). A solvent control was also used to ascertain the blank's activity.

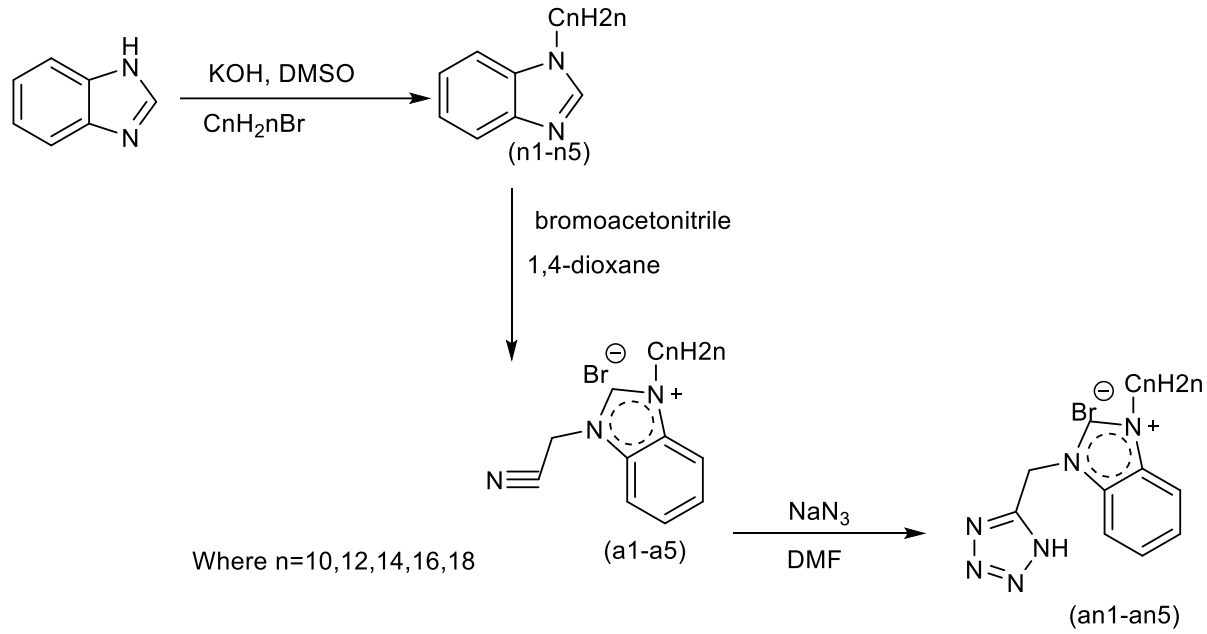
Determination of MIC

In this investigation, the agar streak dilution technique was employed to calculate the substance's MIC. Graded amounts of the test chemicals from standard solution of the manufactured chemical products (100 g/mL in DMSO) were added to a prescribed volume of melted sterile agar (Muller Hinton agar). The created substance was added to a Petri dish with a depth of 3–4 mm and a predetermined volume of the substance-containing medium at (45°C), after which the substance was allowed to crystallize. The microorganism was grown in suspension at a concentration of approximately 105 cfu/mL, added to test plates with chemicals that were serially diluted in DMSO, and then incubated at 37°C. After the incubation time, the MIC values were calculated. Each determination was duplicated three times, with the average serving as the last reading. As a reference, the well-known antibiotic ciprofloxacin (100 g/mL) was used, and as a negative control, DMSO. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test agent at which bacteria or fungi did not readily grow on the lamina [57].

Results and discussion

Chemistry

The multistep synthesis of the benzimidazole derivatives (an1-an5) is shown in Scheme 1. The intermediate-1, 1-alkyl-1H-benzo[d]imidazole, was produced by reacting alkyl bromide with benzimidazole in the presence of a strong base as a catalyst and utilizing DMSO as a solvent (n1-n5). The aforementioned intermediate-1 and bromoacetonitrile were combined to create (intermediate-2). The intermediate-2 reaction with sodium azide in DMF in the presence of CuSO4.5H2O and sodium ascorbate as the reaction's catalyst resulted in the synthesis of end compounds (an1-an5) with respectable yields.



Scheme 1: Tetrazole Derivative Synthesis

Spectral characterization data

Nuclear magnetic resonance (NMR) and infrared (IR) technology were used to confirm the assigned molecular scafolds of the benzimidazole derivatives (proton, carbon). Infrared spectra of the compounds revealed tertiary (-C=C-) bands at 1600 and 1450 cm-1 in addition to the normal (-C=N-) absorption bands between 1608 and 1635 cm^{-1}. The typical peak pattern of prepared N-alkylated benzimidazole is evident between 1500 and 800 cm^{-1}, despite being weaker in N-benzimidazole derivatives (n1-n5). The bending vibrations of the (=C-H) group cause significant bands at 2955-2800 cm^{-1} on the charts of all produced bis-benzimidazolium bromide salts. The appearance of IR stretching vibrations at cm1 in the spectra of several compounds revealed the presence of aromatic (-C=C-) and the peaks at 3112-3068 cm^{-1}. In the generated derivatives, the (-CN) group showed vibrations at 2235-2198 cm^{-1} (a1-a5). Due to the presence of the nitrile group, the final compounds (an1-an5) exhibited both the typical vanishing stretching vibration bands at 2235–2198 cm1 and the distinguishing alkyl chain stretching vibrations at 2840–2980 cm^{-1}. The generated compounds' 1H-NMR spectra have been recorded in dimethyl sulfoxide (DMSO) solvent. Between 7.18 and 7.71 ppm, there were numerous signs that aromatic protons were present. The appearance of the triplet signal at 0.79–0.89 ppm indicated that CH3 was present at the end of the alkyl chain in each of the compounds that were formed. The triplet signal at 4.16-4.24 ppm revealed the existence of the (imidazole ring-N-CH2-) group, whilst the singlet signal at 5.15-5.22 ppm demonstrated the presence of the (tetrazole ring-N-CH2-) group that was connected with the imidazole ring. All of the generated compounds contained (NCHN) in the imidazole ring, which was indicated by singlet signals that emerged between 9.29 and 9.33 ppm. The multiples signal in the range of 1.22-1.85 ppm confirmed the presence of the (-CH2-) group in the alkyl chain, which was promptly attached to the imidazole ring. As mentioned in the

experimental section, 13C-NMR spectral investigations show how the carbon atoms appear in synthetic molecular structures. While the carbon value of the tetrazole ring appeared at 152.55-154.25 ppm, the carbon that bonded to the acidic proton (NCHN) in the imidazole ring caused a signal to show at 144.87-146.07 ppm. However, the signals in the 14.22–33.45 ppm range provided evidence that the generated compounds were attached to the alkyl chain. This information explains how the successful synthesis of end products, such as tetrazole derivatives and their salts.

Evaluation of Anti-Microbial Screening

In this work, fungus, Gram-negative and Gram-positive bacteria, and synthetic drugs were tested in vitro while holding onto the functional groups required for antimicrobial action. It has been demonstrated that gram-positive bacteria are more resistant to manmade chemicals than other types of microbes. The molecule's strong lipophilicity is thought to play a key role in producing the antibacterial activity. The physical property of lipophilicity (hydrophobicity), which controls membrane permeability, solubility rate, and bioavailability, determines how a substance interacts with biological systems. The log P representation of a compound's lipophilicity is considered to be a crucial indicator of potential antibacterial action [58]. The log of the octanol/water partition index is used to express the ratio of hydrophobicity to lipid susceptibility. Controlling the permeability of passive membrane barriers is also essential (i.e. increased logP enhances permeability). While hydrophobic pharmaceuticals (high partitioning coefficients) are preferentially transported to lipophilic compartments like the bilayers of fat cells, hydrophilic drugs (low partitioning coefficients) are preferentially detected in hydrophilic compartments like serum. The Swiss ADME results, shown in Table 1, were used to get the values of log P. SwissADME was also used to compute molar refractivity (MR), a measure of molecular mass, polarizability, and steric characteristics, in order to better understand the activity performance of generated molecules [58, 59]. The final results are shown in Table 1. Molar refractivity is produced by adjusting the molar volume for the refractive index. The link between the antimicrobial data and the values of molar refractivity and logP led to the conclusion that the compounds with greater molar refractivity and logP values had more antibacterial activity. The relationship between antibiotic action and bacterial membrane permeability must be evaluated. The antibacterial activity of the compounds may be related to the formation of bacterial cell walls. This makes sense given that many bacteria depend on their cell walls to survive and that some antibiotics have the ability to kill bacteria by obstructing a mechanism necessary for the manufacture of peptidoglycans. The comparatively thin cell walls of gram-negative bacteria are composed of a few peptidoglycan layers and a second lipid membrane that is made up of lipopolysaccharides and lipoproteins. On the other hand, gram-positive bacteria have thick cell walls that are comprised of several layers of peptidoglycan and teichoic acids. Numerous drugs that are effective against Gram-positive bacteria have been discovered to be ineffective against Gram-negative bacteria as a result of these changes in cell wall composition [58,60].

Table 1: Substituted (-R), Compounds symbol, molar refractivity and log P of prepared products (mr1-mr5).

No Com.	Subs. R	Molar refractivity	Log P
an1	$C_{10}H_{21}$	99.8	3.95
an2	$C_{12}H_{25}$	109.6	4.79
an3	$C_{14}H_{29}$	121.4	5.88
an4	$C_{16}H_{33}$	129.1	6.97
an5	$C_{18}H_{37}$	140.5	6.87

Anti-bacterial activity

Table 2 displays the outcomes of all newly created compounds' anti-bacterial screening. All examined microbes were significantly resistant to the chemical (an5). While some demonstrated strong activity, others of the produced compounds only displayed mild activity. It was discovered that as the alkyl chain's tail contains more carbon atoms, the activity against bacteria rises. Table 2 displays the results of the study on antibacterial activity at a single dose of 100 g/mL.

Table 2: Antibacterial activity expressed as the proportion of selected bacteria that the title compounds (an1-an5) inhibit.

No Com.	Gram-positive		Gram-negative	
	S. aures	B. subtils	E. coli	K. pneumoniae
an1	60	75	55	35
an2	65	70	60	55
an3	75	80	45	70
an4	60	65	50	55
an5	80	85	75	65
Ciprofloxacin	100	100	100	100

Anti-fungal activity

Table 3 lists all of the newly created compounds' anti-fungal screening outcomes. (an5) and (an4) compounds showed discernible action against all tested Fungi. Some of the synthesized compounds showed only modest action, while others showed good activity. It was discovered that as the amount of carbon atoms in the alkyl chain's tail rises, so does the action against bacteria. At a single dose of 100 g/mL, the research's findings on antibacterial activity are listed in Table 2.

Table 3: Results of antifungal activity expressed as the percentage of selected fungi that the title compounds (an1–an5) suppress.

No Com.	A. nigeer	C. albicans
an1	65	45
an2	75	80
an3	45	65
an4	65	55
an5	60	45
Fluconazole	100	100

Conclusion

All attempts to synthesize the benzimidazole compounds (an1-an5) have been successful. To determine how the substituent altered the antibacterial and antifungal properties, a pharmacological investigation was conducted. The biological activity data showed that when compared to the reference medicine, the newly synthesized tetrazole compounds containing imidazole rings displayed better antibacterial than antifungal activity. The findings of the anti-bacterial tests also showed that compound (an5) had the largest antibacterial impact of all the compounds against the tested bacteria, although compounds (an3) and (an4) had only slightly stronger anti-bacterial capabilities than ciprofloxacin. The anti-fungal screening findings demonstrated that the compounds (an4) and (an5) successfully fought the investigated fungus, with particular success against A. niger and C. albicans were susceptible to the molecules in a moderate (an2) and (an3).

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