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Synthesis, molecular docking, *in silico* ADME and antimicrobial activity studies of some new benzimidazole-triazole derivatives

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Abstract

In this study, new benzimidazole-triazole derivatives were synthesized in two steps. First, 4-benzaldehyde derivatives are synthesized by reacting 1,2,4-triazole ring and 4-fluorobenzaldehyde. In the last step, the benzimidazole ring was obtained with *o*-phenylenediamine derivatives under microwave radiation. The structures of synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, infrared spectroscopy, mass spectroscopy, and elemental analysis. Antimicrobial activity of synthesized compounds is associated with six different types of bacteria *(Escherichia coli* ATCC 35218, *E.coli* ATCC 25922, *Klebsiella pneumoniae* NCTC 9633, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Staphylococcus aureus* ATCC 25923), and four different Candida (*C. albicans* ATCC 24433, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019). Synthesized compounds showed weak antibacterial activity. However, 3a, 3b, and 3c compounds against *C. albicans* of the Candida species were found to show promising activity. Given the effect of substituents on antifungal activity, it is seen that the compounds 3a, 3b, and 3c carry chlorine, methyl, and fluoro substituents on the benzimidazole ring attract attention with higher activities. Molecular docking studies of 3a, 3b, and 3c were performed Schrödinger Glide XP against Candidas' sterol 14-alpha demethylase (CYP51), and estimated ADME calculations were analyzed.

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Benzimidazole.

1. Introduction

Antimicrobial resistance (AMR) has become a global problem in recent years. The World Health Organization's Global Antimicrobial Resistance Monitoring System (GLASS) report in 2016-2017 shows that *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and Salmonella species are the most commonly reported resistant bacteria [1]. Due to this increasing antimicrobial resistance, the treatment of microbial diseases has become a challenging process. The conscious use of existing antibiotics and the development of new antimicrobial agents have gained importance against this development of resistance. Therefore, drug development studies have gained a reputation [2].

The benzimidazole core is a vital ring system in medicinal chemistry. This ring system is primarily used to develop compounds belonging to a wide range of therapeutic classes such as antimicrobial, anticancer, antioxidant, antiasthmatic and antiallergic, antiprotozoal, antidiabetic, anti-inflammatory, antiparasitic [3-12]. The benzimidazole scaffold is an important structure that plays a crucial role in drug discovery due to its similarity to the purine ring, which forms the structure of nucleotide bases, is an important pharmacophore group found in the structure of many biologically active compounds [13,14]. The structure of 1,2,4-triazole is another important ring found in the structure of many drugs such as triazolam, alprazolam, etizolam, rizatriptan, fucozole, and efnaconazole. Fluconazole and itraconazole to first generation triazoles; vorikonazole, posakonazole, isavukonazole, albaconazole and ravukonazole are examples of second-generation triazoles [15,16]. Since the benzimidazole ring is a purine analog and antifungal drugs contain a triazole ring, it was thought that the hybridization of these two rings would create significant antifungal activity.

In the light of this information, ten new benzimidazole-triazole derivatives were synthesized, their antimicrobial activity was evaluated, and molecular

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docking against *C. albicans*' sterol 14-alpha demethylase (CYP51) and estimated ADME studies of compounds 3a, 3b, and 3c were performed.

2. Materials and Methods

2.1. Chemistry

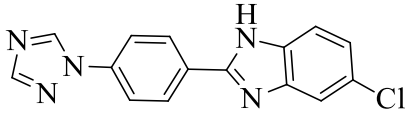
Microwave-assisted 4-(1,2,4-triazol-1-yl) benzaldehyde and 4-(1,2,4-triazol-3-yl)thio) benzaldehyde synthesis (Method A):

1*H*-1,2,4-triazole (1 mol) and 3-mercapto-1*H*-1,2,4-triazole (1 mol) and 4-fluoroenzaldehyde (1.1 mol) were enclosed in the vial with the volume of 30 mL, and 10 mL dimethylformamide (DMF) was added and dissolved. K_2CO_3 (1 mol) was added as a catalyst and heated under the reversing cooler (reflux). The reaction mixture was held in the microwave synthesis reactor for 15 minutes under the temperature of 200 °C and the pressure of 10 bar. At the end of the reaction period, the vial content is poured into the ice water, and the product is preceded, washed with plenty of water, dried, and crystallized from ethanol.

Microwave-assisted 2-(4-(1,2,4-triazol-1-yl) phenyl)-1,3-benzimidazole synthesis (Method B):

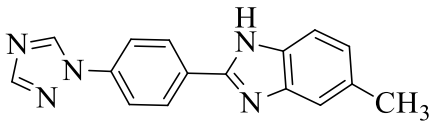
Into the microwave synthesis reactor vial (30 mL), methyl 4-(1,2,4-triazol-1-yl)benzaldehyde (0.03 mol) and 4-(1,2,4-triazol-3-yl)thio)benzaldehyde (0.03 mol), sodium disulfide (0.03 mol) and DMF (10 mL) were added. The reaction mixture was kept in a microwave synthesis reactor at 240 °C under 10 bar pressure for 5 minutes. At the end of this period, the mixture was removed from the reactor, and 5-chloro or 5-fluoro-1,2-phenylenediamine (0,03 mol) was added. The reaction was subjected to microwave irradiation for another 5 minutes under the same conditions. At the end of the reaction period, the product was precipitated by pouring into ice water, filtered, washed with plenty of water, and crystallized from ethanol.

2-(4-(1H-1,2,4-triazol-1-yl)phenyl)-5-chloro-1-benzimidazole (3a)



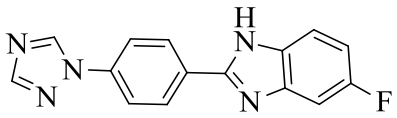
M.p.: 190 °C, yield: 87.5%. IR (ATR) $v_{max}(cm^{-1})$: 3134.33 (N-H voltage band), 1653.00 – 1500.62 (C=C and C=N voltage bands), 839.03 (1.4-dissected non-resynthetic deformation tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.24 (1H, s, Aromatic CH), 7.56-7.64 (4H, m, Aromatic CH), 8.07-8.18 (3H, m, Aromatic CH), 8.34 (1H, s, Aromatic CH), 13.23 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=111.58, 113.23, 118.79, 120.11, 120.70, 122.76, 123.40, 127.82, 128.45, 131.20, 137.80, 144.93, 145.17. Anal Calcd for $C_{15}H_{10}N_5Cl$: C:61.12977; H:3.07789; N:23.76288. Found: C: 61.3534; H:3.0756; N:23.7089. Mass (ES) m/z: 296 [% 100, M+1].

2-(4-(1*H*-1,2,4-triazol-1-yl)phenyl)-5-methyl-1-benzimidazole (3b)



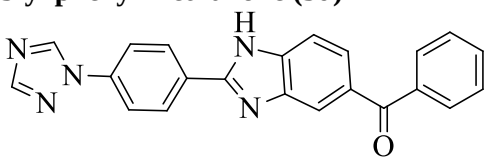
M.p.: 314 °C, yield: 82.6%. IR (ATR) $v_{max}(cm^{-1})$: 3101.54 (N-H voltage band), 1654.92 – 1438.90 (C=C and C=N voltage bands), 839.03 (1.4-dissected non-flaxformation tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 2.43 (3H, s, CH_3), 7.68 (2H, d, J=8.49 Hz, Aromatic CH), 7.95 (1H, s, Aromatic CH), 8.03 (2H, d, J=8.76 Hz, Aromatic CH), 8.17 (2H, d, J=8.49 Hz, Aromatic CH), 8.32 (2H, d, J=8.76 Hz, Aromatic CH), 12.87 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=21.81, 120.04, 124.16, 127.55, 128.10, 129.40, 130.08, 130.20, 130.66, 131.33, 131.72, 133.69, 137.37, 144.80. Anal Calcd for $C_{16}H_{13}N_5$: C:70,0589; H:4,409; N:25,532. Found: C:69,8994; H:4,403; N:25,5091. Mass (ES) m/z: 276 [% 100, M+1].

2-(4-(1*H*-1,2,4-triazol-1-yl)phenyl)-5-fluoro-1-benzimidazole (3c)



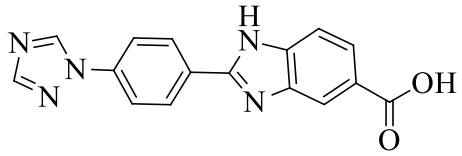
M.p.: 306 °C, yield: 84.4%. IR (ATR) $v_{max}(cm^{-1})$: 3103.46 (N-H voltage band), 1654.92 – 1440.83 (C=C and C=N voltage bands), 837.11 (1.4-dissected non-flax deformation band). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.69 (2H, d, J=8.46 Hz, Aromatic CH), 7.95 (1H, s, Aromatic CH), 8.06 (2H, d, J=8.76 Hz, Aromatic CH), 8.17 (2H, d, J=8.46 Hz, Aromatic CH), 8.33 (2H, d, J=8.76 Hz, Aromatic CH), 13.16 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=120.09, 127.68, 128.27, 129.46, 129.57, 129.75, 130.29, 131.25, 131.61, 131.72, 134.27, 137.64, 144.88. Anal Calcd for $C_{15}H_{10}N_5F$: C:64.74457; H:3.259896; N:25.16805. Found: C:64.85; H:3.25456; N:25,1745. Mass (ES) m/z: 280 [% 100, M+1].

2-(4-(1*H*-1,2,4-triazol-1-yl)phenyl)-1-benzimidazol-5-yl phenyl methanone (3d)



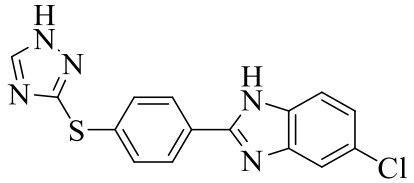
M.p.: 250 °C, yield: is 85%. IR (ATR) $v_{max}(cm^{-1})$: 3116.97 (N-H voltage band), 1653.00 – 1386.82 (C=C and C=N voltage bands),839.03 (1.4-dissected non-resuming deformation tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.59-7.63 (2H, m, Aromatic CH), 7.67-7.70 (2H, m, Aromatic CH), 7.90-7.94 (3H, m, Aromatic CH), 8.05-8.07 (2H, m, Aromatic CH), 8.23-8.26 (3H, m, Aromatic CH), 8.40-8.43 (2H, m, Aromatic CH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=114.80, 117.11, 120.06, 124.41, 127.92, 128.53, 129.41, 129.70, 129.92, 131.20, 134.34, 137.66, 144.89, 152.28, 152.61, 158.81, 162.90, 170.38. Anal Calcd for $C_{22}H_{15}N_5O$: C:72.51666; H:3.872507; N:19,21997. Found: C:72.60; H:3.8691; N:19.22366. Mass (ES) m/z: 366 [% 100, M+1].

2-(4-(1*H*-1,2,4-triazol-1-yl)phenyl) -1-benzimidazole-5-carboxylic acid (3e)



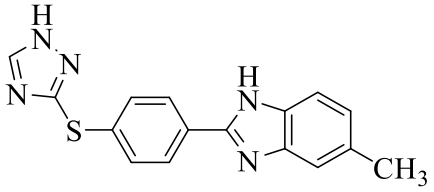
M.p.: 85 °C, yield: 86.7%. IR (ATR) $v_{max}(cm^{-1})$: 3095.75 (N-H voltage band), 1651.07 – 1500.62 (C=C and C=N voltage bands),831.32 (1.4-dissected non-resuming tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.76-7.78 (2H, m, Aromatic CH), 7.95 (1H, s, Aromatic CH), 8.08-8.09 (2H, m, Aromatic CH), 8.20-8.23 (2H, m, Aromatic CH), 8.36-8.39 (2H, m, Aromatic CH), 9.56 (1H, s, OH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=120.14, 122.17, 124.42, 125.43, 127.99, 128.63, 129.94, 130.60, 131.15, 131.72, 132.58, 138.51, 144.97, 162.75. Anal Calcd for $C_{16}H_{11}N_5O_2$ C:63.15552; H:3.312382; N:23.01595. Found: C:63,1855; H:3.3252; N:23.0678. Mass (ES) m/z: 306 [% 100, M+1].

2-(4-((1*H*-1,2,4-triazol-3-yl)thio)phenyl)-5-chloro-1-benzimidazole (3f)



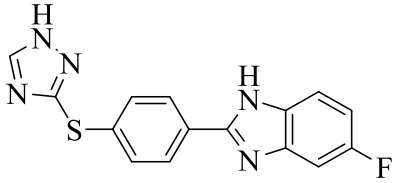
M.p.: 265 °C, yield: 84.3%. IR (ATR) $v_{max}(cm^{-1})$: 3086.11 (N-H voltage band), 1517.98 – 1454.33 (C=C and C=N voltage bands), 842.89 (1.4-dissected non-flaxformation tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.05-7.12 (1H, m, Aromatic CH), 7.42-7.61 (2H, m, Aromatic CH), 8.07 (2H, d, J=8.76 Hz, Aromatic CH), 8.30 (1H, s, Aromatic CH), 8.32 (2H, d, J=8.76 Hz, Aromatic CH), 9.42 (1H, s, NH), 13.16 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 104.95, 110.90, 112.26, 112.73, 120.12, 128.28, 129.49, 138.05, 142.98, 153.10, 153.12, 157.77, 160.80. Anal Calcd for $C_{15}H_{10}N_5SCl$: C:55,13152; H:2.775878; N:21.43119. Found: C:55,20321; H:2.77654; N:21.45458. Mass (ES) m/z: 328 [% 100, M+1].

2-(4-((1*H*-1,2,4-triazol-3-yl)thio)phenyl)-5-methyl-1-benzimidazole (3g)



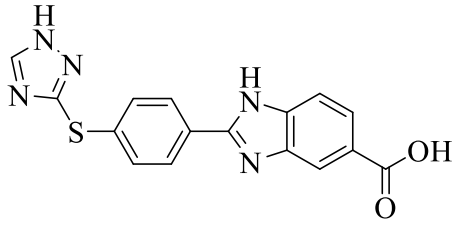
M.p.: 153.7 °C, yield: 84.3%. IR (ATR) $v_{max}(cm^{-1})$: 3116.97 (N-H voltage band), 1606.0 – 1500.26 (C=C and C=N voltage bands), 840.96 (1.4-dissected non-rectal deformation band). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 2.43 (3H, s, CH_3), 7.03-7.06 (1H, m, Aromatic CH), 7.40 (1H, s, Aromatic CH), 7.49 (2H, d, J=8.19 Hz, Aromatic CH), 8.06 (2H, d, J=8.76 Hz, Aromatic CH), 8.32 (2H, d, J=8.76 Hz, Aromatic CH), 9.41 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 21.79, 112.28, 115.94, 120.07, 124.28, 128.11, 128.28, 129.93, 132.06, 137.79, 142.93, 150.33, 152.15, 153.08. Anal Calcd for $C_{16}H_{13}N_5S$: C:62.72633; H:3.947846; N:22.85953. Found: C:62,7212; H:3.9489; N:22.8345. Mass (ES) m/z: 308 [% 100, M+1].

2-(4-((1*H*-1,2,4-triazol-3-yl)thio)phenyl)-5-fluoro-1-benzimidazole (3h)



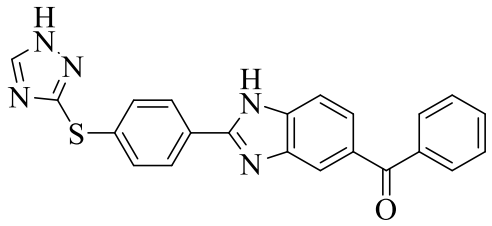
M.p.:. 232.7 °C, yield: 79.4%. IR (ATR) $v_{max}(cm^{-1})$: 3113.11 (N-H voltage band), 1606.70 – 1508.33 (C=C and C=N voltage bands),839.03 (1.4-dissected non-flaxformation tape). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.05-7.12 (1H, m, Aromatic CH), 7.40-7.44 (1H, m, Aromatic CH), 7.59-7.64 (1H, m, Aromatic CH), 8.06 (2H, d, J=8.73 Hz, Aromatic CH), 8.30 (1H, s, Aromatic CH), 8.33 (2H, d, J=8.76 Hz, Aromatic CH), 9.42 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=110.79, 111.12, 112.28, 120.12, 121.90, 128.29, 128.54, 129.46, 138.07, 142.99, 153.13, 157.67, 160.79. Anal Calcd for $C_{15}H_{10}N_5SF$: C:58.05477; H:2.923064; N:22.56754. Found: C:58,025; H:2.9236; N:22,556. Mass (ES) m/z: 312 [% 100, M+1].

2-(4-((1*H*-1,2,4-triazol-3-yl)thio)phenyl)-1-benzimidazole-5-carboxylic acid (3i)



M.p.: 318.8 °C, yield: 78.8%. IR (ATR) v_{max}(cm^{-1}): 3124.68 (N-H voltage band), 2929.87 (C-H voltage band), 1658.7 8 – 1516.05 (C=C and C=N voltage bands),840.96 (1.4-non-plane deformation band that resembles dissectitis). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.67 (1H, d, J=8.46 Hz, Aromatic CH), 7.85-7.87 (1H, m, Aromatic CH), 8.08 (2H, d, J=8.73 Hz, Aromatic CH), 8.21 (1H, s, Aromatic CH), 8.30 (1H, s, Aromatic CH), 8.37 (2H, d, J=8.73 Hz, Aromatic CH), 9.43 (1H, s, OH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=112.24, 120.12, 121.93, 124.21, 124.86, 125.24, 128.62, 129.18, 138.32, 143.03, 152.99, 153.14, 162.80, 168.28. Anal Calcd for $C_{16}H_{11}N_5SO_2$: C:57,13472; H:2.996603; N:20.82177. Found: C:57,304; H:2.9998; N:20.8565. Mass (ES) m/z: 338 [% 100, M+1].

(2-(4-((1*H*-1,2,4-triazol-3-yl)thio)phenyl)-1-benzimidazol-5-yl)phenyl)methanone (3j)



M.p.: 272.6 °C, yield: 82.6%. IR (ATR) v_{max}(cm^{-1}): 3109.25 (N-H voltage band), 1639.49 (C=O voltage band), 1612.49 - 1514.12 (C=C and C=N voltage bands), 839.03 (1.4-non-flax plane deformation band resemsounding to dissectitis). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 7.57-7.58 (2H, m, Aromatic CH), 7.65-7.71 (2H, m, Aromatic CH), 7.75-7.78 (3H, m, Aromatic CH), 7.98 (1H, s, Aromatic CH), 8.07 (2H, d, J=8.79 Hz, Aromatic CH), 8.30 (1H, s, Aromatic CH), 8.37 (2H, d, J=8.76 Hz, Aromatic CH), 9.43 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ=112.52, 120.14, 120.88, 121.98, 124.85, 128.30, 128.65, 128.89, 129.06, 129.94, 131.63, 132.59, 138.39, 138.50, 143.04, 153.15, 153.36, 196.04. Anal Calcd for $C_{22}H_{15}N_5SO$: C:66.6514; H:3.5593; N:17.6654. Found: C:66,605; H:3.5569; N:17.6584. Mass (ES) m/z: 398 [% 100, M+1].

2.2. Antimicrobial activity studies

To recreate microorganism strains, the fungi are removed from -85 °C to petri dishes with Sabouraud Dextrose Agar (Sigma Aldrich), the bacteria were planted in petri dishes containing Mueller Hinton Agar (MHA-Sigma Aldrich) and left to incubate for 24 hours at 37 °C. Bacteria were taken from the colonies, transferred to tubes containing Mueller Hinton Broth (MHB), and fungi were transferred to tubes with RPMI and left to incubate for 24 hours at 37 °C. After incubation, turbidity was adjusted according to McFarland no: 0.5 (10^8 CFU/mL) tube.

The compounds to be tested were weighed at 10 mg and transferred to sterile vials, and 1 mL of pure dimethyl sulfoxide (DMSO) was added to them. Compounds were fully dissolved within the DMSO and became a homogeneous mixture.

Microtitration plates (Brand) with 96 "U" type wells were used for the experiment. Prepared compound mixtures were transferred to the wells respectively in the concentration range of 1000 to 1.95 µg/mL with serial dilution of 100 µL with the help of micropipettes. After all, concentrations are transferred to the wells, 100 µL pipettes are made of microorganism cultures. The last column is divided into microorganism control, and the last row is divided into fattening location control. After these procedures, the lids of the microtitration plates were closed and incubated at 37 °C for 24 hours, and at the end of this period, 20 µL of resazurin solution was added to the wells to better observe the presence or absence of growth in the wells. It was then incubated at 37 °C for 3 hours for coloration. At the end of the incubation period, the lowest concentration at which growth was observed, that is, the minimum inhibitory concentration (MIC) was determined as µg/mL. Experiments were repeated in pairs in parallel. Ketoconazole was used as a standard antifungal agent, and chloramphenicol was used as an antibacterial. [12.] Resazurin, which is used as a metabolic indicator in evaluating test results, was tried by Alamar Blue on many cell types 50 years ago, and its effectiveness has been proven. It is more preferred than other indicators to dissolve easily in water, be stable in cell culture, not toxic, and easily pass through cell membranes. Resazurin is a blue-colored paint that does not give fluorescence. It is reduced by living organisms and transformed into a pink-colored resorufin metabolite that gives fluorescence. Thanks to this feature, it offers the possibility to evaluate the control of reproduction in each well in the microtitration petri dishes as a result of activity studies both by observing the color change in the well and by fluorometric reading [13].

2.3. Molecular docking studies

Sterol 14-alpha demethylase (CYP51) (PDB ID: 5TZ1, resolution 2.00 Å) was selected for molecular docking for possible interactions of 3a, 3b, and 3c compounds with *C. albicans* [17]. Molecular docking studies were carried out with Schrödinger Maestro version 12.8. Heteroatoms found except HEM in enzyme structure were removed and prepared with Protein Preparation Wizard. The compounds were drawn with ChemDraw Professional 17.0 and prepared with LigPrep. Based on the cocrystal VT1 of the 5TZ1 structure, grid box was created x: 70.87, y: 66.28, z: 4.42 coordinates, and 20*20*20 $Å^3$ size by Receptor Grid Generation. Molecular docking analyses were performed with Glide XP [18]. 2D and 3D imaging were performed with Maestro 12.8.

2.4. ADME prediction

Computational prediction of absorption, distribution, metabolism, and excretion (ADME) of compounds 3a, 3b, and 3c, which are the three most active compounds against *C. albicans*, were performed with SwissADME online tools (http://www.swissadme.ch/) [19].

3. Results and Discussion

The structures of 10 original compounds 3a-3j, which were synthesized with microwave-assisted, were elucidated by data on ^{1}H-NMR, IR and mass spectroscopic methods and elemental analysis results. With the microwave synthesis used, reaction times are shortened, efficiency is increased, less resources are used, and it is more environmentally friendly and economical. Spectrum assessments were given under the heading of the relevant spectroscopic method. IR studies of 10 new triazole-derived compounds synthesized within the scope of the project were elucidated with the help of spectroscopic data. When the chemical structures of synthesized compounds are examined, all synthesis compounds have an aromatic ring system. The voltage band bee of the C=C and C=N groups carried by these rings was obtained in the range of 1386.82 – 1654.92 $cm^{-1.}$ Another structure commonly found in all synthesis products is the 1,4-dissected and benzene rings. The specific non-plane deformation bands for this ring were obtained in the range of 831.32 - 842.89 $cm^{-1.}$ Another structure commonly found in all synthesis products is the voltage bands of the N-H group in the range of 3086.11 – 3134.33 cm^{-1}. In the ^{1}H-NMR spectra, the -CH_3 group protons of compounds 3b and 3g on 5th position of benzimidazole ring were observed at 2.43 ppm as a singlet. A broad singlet due to NH proton of the benzimidazole ring was recorded around at 13 ppm. NH proton of the triazole ring was observed around at 9 ppm. The signals belonging to aromatic protons were found at 7.03–8.43 ppm. The mass spectrums of synthesized compounds were drawn by positive ionization technique using electron spray method. Therefore, peaks with a numerical value greater than the calculated molecular weights (molecular ion peaks; M+1 peaks) are expected to be observed in the spectrums. When the spectrums are examined, it is seen that the M+1 peaks obtained by the molecular weights of the compounds are compatible as expected. Percentage analyses of C, H, N elements were performed for the compounds covered by the study. The results indicate a deviation of 0.4% between theoretically calculated element percentages and experimental findings. This finding is an indication that the compounds contain minimal impurities.

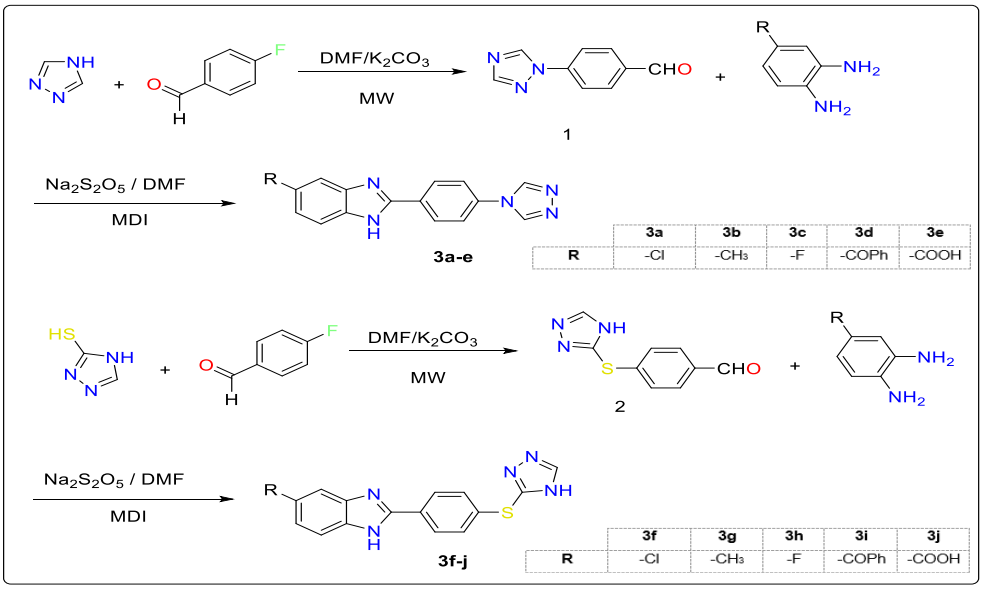


Figure 1. General synthesis pathways of the target compounds

The synthesis of 10 new benzimidazole compounds synthesized as antimicrobial agents was performed in two steps. First, two products were obtained: triazole and 4-fluorobenzaldehyde and 4-(1,2,4-triazol-1-yl)benzaldehyde and 4-(1,2,4-triazol-3-yl)benzaldehyde. The aldehyde derivatives were obtained in the first step were reacted with *o*-phenylenediamine products under microwave radiation, and the benzimidazole ring system was closed. When designing the compounds, five compounds with triazole-related sulfur structures and five compounds that do not carry sulfur were synthesized, and the activities of compounds with two different structures were evaluated. The synthesis pathways of the compounds are given in Figure 1.

The synthesis of the compounds performed was elucidated by various spectroscopic methods. Antibacterial activity results of synthesized compounds were given in Table 1, and antifungal activity results were given in Table 2. *E. coli* ATCC 35218 *(E.coli 1)*, *E. coli* ATCC 25922 *(E.coli 2)*, *K. pneumoniae* NCTC 9633 *(Kp)*, *P. aeruginosa* ATCC 27853 *(Pa)*, *S. typhimurium* ATCC 13311 *(St)*, *S. aureus* ATCC 25923 *(Sa)* bacterial type is used. Chloramphenicol was used as a reference drug. When the results of the activity were evaluated, it was seen that the compounds were ineffective against the types of bacteria tested.

Antibacterial and antifungal activities of synthesized compounds have been tested using microdilution methods reported by the Clinical & Laboratory Standards Institute (CLSI). As a result of microbiological studies, MIC values were obtained by fluorometric measurement using resazurin solution. In this study, a more reliable, repeatable, more standardized spectroscopy method was used in the evaluation of antimicrobial test results instead of the methods of determining the MIC value detected by the eye based on the color change of a particular indicator. For this purpose, the fluorometric method modified from the CLSI method, which is available taking into account the aforementioned properties of resazurin, has been successfully applied as mentioned. According to the antimicrobial results obtained, synthesized compounds were not antibacterially effective in *in vitro* medium conditions (3a-3j for MIC: >1mg/mL).

Table 1. MIC (μg/mL) values for synthesized compounds

Comp.	*E.coli* [1]	*E.coli* [2]	*Kp*	*Pa*	*St*	*Sa*
3a	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3b	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3c	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3d	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3e	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3f	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3g	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3h	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3i	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3j	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
Chloramphenicol	≤1,95 μg/mL	≤1,95 μg/mL	3,9 μg/mL	250 μg/mL	≤1,95 μg/mL	15,62 μg/mL

Escherichia coli ATCC 35218 (E.coli [1]),Escherichia coli ATCC 25922 (E.coli [2]), Klebsiella pneumoniae NCTC 9633 (Kp), Pseudomona aeruginosa ATCC 27853 (Pa), Salmonella typhimurium ATCC 13311 (St), Staphylococcus aureus ATCC 25923 (Sa).

It was also found that compounds other than 3a, 3b, and 3c were again not effective against any *Candida* species. However, compounds 3a, 3b, and 3c show activity only against *C. albicans*. The MIC value of ketoconazole against *C. albicans* was 7.8 mg/mL, while the compounds were 3.9 mg/mL, 7.8 mg/mL, and 3.9 mg/mL, respectively.

Table 2. MIC (μg/mL) values for synthesized compounds

Compound	*Ca*	*Cg*	*Ck*	*Cp*
3a	3.9 μg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3b	7.8 μg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3c	3.9 μg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3d	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3e	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3f	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3g	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3h	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3ı	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
3j	>1mg/mL	>1mg/mL	>1mg/mL	>1mg/mL
Ketoconazole	7,8 μg/mL	≤1,95 μg/mL	≤1,95 μg/mL	≤1,95 μg/mL

Candida albicans ATCC 24433 *(Ca), Candida glabrata* ATCC 90030 *(Cg), Candida krusei* ATCC 6258 *(Ck), Candida parapsilosis* ATCC 22019 *(Cp)*

Antifungal activity test of the compounds was performed against four different *Candida* species: *C. albicans* ATCC 24433 *(Ca)*, *C. glabrata* ATCC 90030 (Cg), *C. krusei* ATCC 6258 *(Ck)*, *C. parapsilosis* ATCC 22019 *(Cp)*. Ketoconazole was used as a reference drug. When we looked at the results of antifungal activity, it was seen that the compounds coded 3a, 3b, and 3c stand out. In particular, compounds with a code of 3a and 3c were twice as effective as the reference drug with a value of 3.9 μg/mL. The 3b compound had an activity equal to ketoconazole with a value of 7.8 μg/mL MIC. When looking at the structures of compounds 3a, 3b, and 3c, the presence of chlorine and fluorine substituent in the benzimidazole ring caused a significant increase in activity. Especially when compared with the activities of sulfur-containing compounds due to triazole, it was seen that the presence of sulfur reduces activity.

Contrary to the current study, in our previous studies [14,15] , it was seen that the sulfur atom was in a bridge position both within the ring system and between the aliphatic carbon chain and the ring system in compounds showing antimicrobial activity. In addition, the fact that the sulfur atom was attached to the acetamide residue in previous studies also explains the differences in the chemotherapeutic effect. As a result of the study, ten new compounds were synthesized in which two ring systems known to be antimicrobial effective were used together. Although synthesized compounds have no antibacterial effectiveness, they appear to give hope in antifungal activity. In future studies, it is aimed to reach more effective compounds with different modifications through this main structure.

Molecular docking studies were carried out with Schrödinger Glide XP against sterol 14-alpha demethylase (CYP51) enzyme of 3a, 3b, and 3c compounds that showed activity against *C. albicans*. CYP51 (PDB ID: 5TZ1) cocrystal VT1 redocking was performed to ensure validation of the molecular docking process and to compare synthesized compounds. The RMSD between the cocrystal natural pose and the redocking docking pose was 1.6393 Å. The RMSD value of less than 3 Å is considered suitable for molecular docking. As shown in Table 3, compounds 3a, 3b, and 3c produced lower docking scores than cocrystal, while VT1 gave a lower glide emodel value than compounds.

Table 3. Glide XP molecular docking interaction energies (kcal/mol) of 3a, 3b, and 3c against *C. albicans'* sterol 14-alpha demethylase (CYP51)

Comp.	Docking score	XP GScore	Glide emodel
3a	-8.749	-9.262	-65.704
3b	-6.077	-6.662	-60.415
3c	-7.518	-7.877	-63.733
VT1*	-6.291	-6.293	-112.080

*VT1: cocrystal of 5TZ1

Protein-ligand interactions and binding poses of the 3a, 3b, and 3c compounds on the CYP51 active site were examined. As shown in Figure 2, an H bond of 2.11 Å between the 3a compound and Met508, pi-pi stacking interactions with Tyr118, Hie377, Phe233, generated hydrophobic interactions with Pro230, Leu376, Tyr64, Phe228, and Tyr505. The 3b compound generated hydrophobic interactions with Tyr118 with pi-pi

stacking, Leu300, Tyr132, Phe380, and Met508. The 3c compound formed hydrophobic interactions with Ser378 (2.33 Å) and Hie377 (1.91 Å), pi-pi stacking with Hie377 and Phe233, Pro230, Leu376, Met508, Leu121, and Ile379.

It's always helpful to do some theoretical calculations so that drug design, research, and development will have fewer problems in terms of ADME. In this direction, the physiochemical, drug-likeness, pharmacokinetics, lipophilicity, and medicinal chemistry parameters of the 3 most active compounds against *C. albicans* were calculated using SwissADME online tools. Details are shown in Table 4. The molecular weight of compounds 3a, 3b, and 3c was between 279 g/mol and 395 g/mol. According to all lipophilicity calculations, compounds 3a, 3b, and 3c were below the logP value. Their solubility in water was moderately soluble to soluble. There was high GI absorption and BBB permeant. It complied with all of the limiting rules of Lipinski, Ghose, Veber, Egan, and Muegge. PAINS, Brenk, Leadlikeness values in all three compounds were suitable and synthetic accessibility was in the easy class.

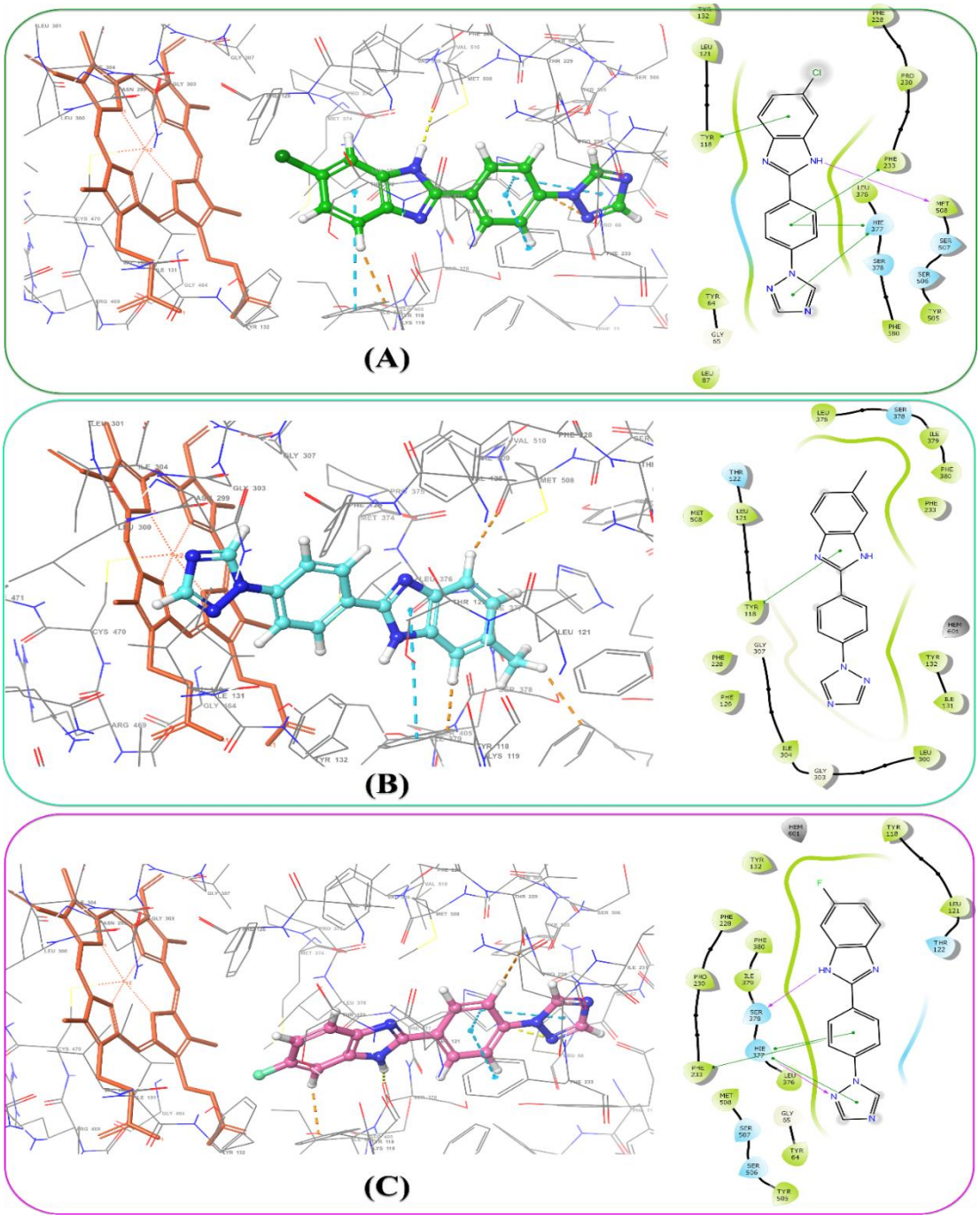


Figure 2. 3D binding poses and 2D schematic protein-ligand interactions of compounds (A) **3a**, (B) **3b**, and (C) **3c** in the active site of *C. Albicans*' sterol 14-alpha demethylase (PDB ID: 5TZ1)

Table 4. Physiochemical, drug-likeness, pharmacokinetics, lipophilicity, and medicinal chemistry parameters of compounds 3a, 3b, and 3c obtained from SwissADME.

Physicochemical Properties			
Formula	$C_{15}H_{10}ClN_5$ (3a)	$C_{16}H_{13}N_5$ (3b)	$C_{15}H_{10}FN_5$ (3c)
Molecular weight	295.73 g/mol	275.31 g/mol	279.27 g/mol
Num. heavy atoms	21	21	21
Num. arom. heavy atoms	20	20	20
Num. rotatable bonds	2	2	2
Num. H-bond acceptors	3	3	4
Num. H-bond donors	1	1	1
Molar Refractivity	81.46	81.41	76.40
TPSA	59.39 Å²	59.39 Å²	59.39 Å²
Lipophilicity			
Log $P_{o/w}$ (iLOGP)	2.17	2.16	2.02
Log $P_{o/w}$ (XLOGP3)	3.52	3.25	2.99
Log $P_{o/w}$ (WLOGP)	3.46	3.12	3.37
Log $P_{o/w}$ (MLOGP)	2.99	2.72	2.87
Log $P_{o/w}$ (SILICOS-IT)	3.08	2.95	2.86
Consensus Log $P_{o/w}$	3.05	2.84	2.82
Water Solubility			
Log *S* (ESOL)	-4.46	-4.17	-4.03
Class	Moderately soluble	Moderately soluble	Moderately soluble
Log *S* (Ali)	-4.45	-4.17	-3.90
Class	Moderately soluble	Moderately soluble	Soluble
Pharmacokinetics			
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	Yes
CYP2C19 inhibitor	Yes	Yes	Yes
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Log K_p (skin permeation)	-5.60 cm/s	-5.67 cm/s	-5.88 cm/s
Druglikeness			
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	Yes	Yes	Yes
Veber	Yes	Yes	Yes
Egan	Yes	Yes	Yes
Muegge	Yes	Yes	Yes
Medicinal Chemistry			
PAINS	0 alert	0 alert	0 alert
Brenk	0 alert	0 alert	0 alert
Leadlikeness	No; 1 violation: XLOGP3>3.5	Yes	Yes
Synthetic accessibility	2.08	2.12	2.21

4. Conclusion

In this study, 10 new triazole bearing benzimidazole derivatives were designed and synthesized, and their structures were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectral analysis, and elemental analysis. It was tested against some gram-positive and negative bacteria and found no significant antibacterial activity. In contrast, in antifungal activity experiments with *C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*, compounds 3a (3.9 µg/mL), 3b (7.8 µg/mL), and 3c (3.9 µg/mL) were used as reference drug ketoconazole (7.8 µg/mL) showed the same or higher activity. In addition, molecular docking of 3a, 3b, and 3c compounds against *C. albicans*' sterol 14-alpha demethylase (CYP51) enzyme was performed with Glide XP, and their binding energies of -9.262 kcal/mol, -6.662 kcal/mol, and -7.877 kcal/mol were obtained, respectively. Theoretical ADME calculations of the 3a, 3b, and 3c were made, and the compounds were found to have good lipophilicity, moderate water solubility, and within the limiting rules of Lipinski, Ghose, Veber, Egan, and Muegge.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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