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Synthesis of new benzimidazole derivatives containing 1,3,4-thiadiazole: their in vitro antimicrobial, in silico molecular docking and molecular dynamic simulations studies

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ABSTRACT

A series of some new benzimidazole-1,3,4-thiadiazoles was synthesized. The structures of target substances were confirmed by using ^{1}H-NMR and ^{13}C-NMR spectroscopy, mass spectrometry and elemental analysis. The synthesized compounds were evaluated for antimicrobial activity against six bacterial strains namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 2942), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213)and four fungal strains namely *Candida albicans* (ATCC 24433), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 22019) and *Candida glabrata* (ATCC 9). Antimicrobial data revealed that compounds 4f and 4i with MIC of < 0.97 µg/mL were found to be most effective against *E. coli*. Among the studied molecules, compounds 4f and 4i showed the best antifungal activity with MIC value of 1.95 µg/mL. Additionally, docking studies were performed towards the most promising compounds 4f and 4i, in the active site of DNA gyrase revealing strong interactions. A molecular dynamics (MD) simulation analysis was also used to investigate the dynamic nature, binding interaction, and protein–ligand stability.

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Benzimidazole; 1, 3, 4-thiadiazole; antimicrobial; molecular docking; molecular dynamic

Introduction

In recent years, microbial infections, particularly those originating in hospitals, have grown increasingly common, necessitating the extensive use of antimicrobial medications [1]. At the same time, widespread usage of these medications has resulted in the emergence of a new condition known as multidrug resistance (MDR). MDR, is the antimicrobial resistance of a microbiological species to at least one antimicrobial drug from three or more antimicrobial classes [2]. Multidrug-resistant gram-positive and gram-

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negative bacteria are a major public health concern around the world, not only because they cause existing treatments to fail, but also because they promote the spread of opportunistic infections [3,4]. Despite the fact that bacterial resistance continues to arise, the rate at which antibiotics are developed is declining at an alarming rate. The pharmaceutical industry's reaction to this problem has been to produce new antibiotics that are effective against these illnesses [5]. As a result, scientists are concentrating their efforts on generating novel compounds and derivatives with a wide range of physicochemical features that promise high activity with few to no adverse effects.

Antibiotics' protective effects have steadily weakened as a result of widespread use and misuse, contributing directly and significantly to the development of drug resistance in bacterial pathogens. As a result of the advent of this circumstance, the development of new antibiotic agents as a single or multi-targeted novel cocktail will help to ensure the efficacy of new compounds [6]. Therefore, heterocyclic frameworks are the preferred structure for developing novel compounds with a wide range of pharmacological activities [7]. In this context, benzimidazoles, which are classified as 'privileged' structures due to their affinity for a variety of enzymes and protein receptors, are an essential heterocyclic family member [8]. At the 4 and 5 locations of the imidazole ring, the benzimidazole molecule is formed by fusing the benzene and imidazole ring systems. They have both acidic and basic characteristics. The NH group is both acidic and basic in this case. Another property is that they are incapable of forming salts [9]. Because of their pharmacological properties, such as anticancer [10,11], antibacterial [12–15], antifungal [16–18], antioxidant [19] and lipase inhibitions [20], the benzimidazole moiety is useful for the development of novel medicinal compounds in the pharmaceutical field.

N-based heterocycles are a particularly significant class since they are found in a wide range of biologically active compounds, including hormones, glycosides, and alkaloids, as well as certain therapeutically used medications [7,21–23]. 1,3,4-thiadiazoles, a five-membered aromatic heterocyclic molecule, are an example of this category. While 1,3,4-thiadiazoles were first developed as antibacterial medicines, they were later shown to have a wide range of biological functions [24,25].

In light of the literature review, the goal of this study was to synthesize a number of compounds with various substituted groups containing 1,3,4-thiadiazoles and 6-methyl-1*H*-benzo[*d*]imidazole ring and their derivatives, as well as to investigate their antifungal and antibacterial (effective against Gram-positive and Gram-negative) activities. Finally, to investigate the medicines' interactions with the probable bacterial targets, molecular docking and molecular dynamics simulations (MD) were used. According to the results of the modelling, these new compounds might be employed as antibacterial agents in the future.

Material and method

Whole chemicals employed in the synthetic procedure were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the obtained compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. ^{1}H-NMR, and ^{13}C-NMR (Nuclear Magnetic Resonance) spectra of the synthesized compounds were registered by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer

(Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet in the NMR spectra. Coupling constants (J) were reported as Hertz. M +1 peaks were determined by Shimadzu LC/MSMS system (Shimadzu, Tokyo, Japan). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany).

Chemistry

Synthesis of sodium metabisulfite salt of benzaldehyde derivative

Methyl 4-formyl benzoate (5 g, 0.03 mol) was dissolved in ethanol. Sodium metabisulfite (6.84 g, 0.036 mol) in ethanol dropped dropwise into the benzaldehyde solution. After the dripping was completed, the reaction contents were stirred at room temperature for one hour. The precipitated product was filtered off.

Synthesis of 4-(5(6)-methyl-1H-benzo[d]imidazol-2-yl)benzoic acid methyl ester (1)

5(6)-methylbenzene 1,2-diamine (0.022 mol) was dissolved in dimethylformamide (DMF) and sodium metabisulfite salt of benzaldehyde derivative (7.09 g, 0.026 mol) was added. At the end of the reaction, the product was precipitated by pouring the reaction contents into ice water. The precipitated product was filtered off and recrystallized from ethanol.

Synthesis of 2-(5(6)-methylphenyl)-1H-benzo[d]imidazole-6-carbohydrazide derivatives (2)

Compound 1 (0.018 mol) and excess of hydrazine hydrate (5 mL) were placed in the same vial and ethanol (15 mL) was added. The mixture were refluxed for 12 h. When the reaction was completed, the mixture was poured into iced water, the product was filtered.

N-substituted-2-(4-(5(6)-methyl-1H-benzo[d]imidazole-2-yl)benzoyl)hydrazine-1-carbothioamide (3a-3k)

The hydrazide derivative compound (1 mmol) was dissolved in 10 ml of ethanol, the appropriate isothiocyanate (1.1 mmol) was added and refluxed for 3 hours. The precipitated product was filtered off.

N-substituted-5-(4-(5-methyl-1H-benzo[d]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4a-4k)

The appropriate thiosemicarbazide derivative was stirred in 10 ml of sulphuric acid (H_2SO_4) in an ice bath. Then it was stirred for another 10 minutes at room temperature, at the end of the time it was poured slowly on ice, adjusted to pH = 8 with aqueous ammonia and filtered. It is washed with water and recrystallized from ethanol.

N-methyl-5-(4-(5-methyl-1H-benzo[d]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4a). Yield: 64%. M.p. 309.2°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.45 (3 H, s, -CH_3), 2.97 (3 H, s, -CH_3), 7.07 (1H, dd, J_1 = 7,56 Hz, J_2 = 1.17 Hz, benzimidazole-C_6), 7.41 (1H, s, benzimidazole-C_7), 7.52 (1H, d, J = 8.19 Hz, benzimidazole-C_4), 7.93 (2 H, d, J = 8.52 Hz, 1,4-disubstituted benzene), 8.23 (2 H, d, J = 8.52 Hz, 1,4-disubstituted benzene), ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 21.80, 31.79, 114.73, 115.68, 124.59, 127.21, 127.49, 128.40,

129.37, 130.92, 132.36, 132.43, 150.37, 155.63, 169.99. HRMS (m/z): $[M+H]^+$ calcd for $C_{17}H_{15}N_5S$: 322.110; found: 322.1121. Anal. calcd. For $C_{17}H_{15}N_5S$, C, 63.53; H, 4.70; N, 21.79. Found: C, 63.34; H, 4.71; N, 21.84.

N-*ethyl-5-(4-(5-methyl-1*H-*benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4b). Yield: 66%. M.p. 294.2°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.22–1.25 (3 H, m, CH_3), 2.45 (3 H, m, CH_3), 3.86–3.91 (2 H, m, CH_2), 7.23–7.27 (1H, m, Aromatic CH), 7.61–7.67 (1H, m, Aromatic CH), 7.92–7.95 (2 H, m, Aromatic CH), 8.01–8.03 (1H, m, Aromatic CH), 8.23–8.27 (2 H, m, Aromatic CH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 15.59, 20.26, 33.04, 115.75, 122.79, 123.67, 125.17, 127.25, 127.64, 127.79, 127.98, 128.47, 130.28, 132.40, 151.89, 165.74. HRMS (m/z): $[M+H]^+$ calcd for $C_{18}H_{17}N_5S$: 336.1277; found: 336.1261. Anal. calcd. For $C_{18}H_{17}N_5S$, C, 64.45; H, 5.11; N, 20.88. Found: C, 64.66; H, 5.10; N, 20.94.

N-*(2-chloroethyl)-5-(4-(5-methyl-1*H-*benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4c). Yield: 62%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.32 (3 H, s, -CH_3), 3.37 (4 H, s, -CH_2), 7.39–7.40 (2 H, m, Aromatic C-H), 7.95–7.97 (1H, m, Aromatic C-H), 8.06–8.08 (1H, m, Aromatic C-H), 8.18–8.31 (3 H, m, Aromatic C-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 20.47, 25.14, 46.13, 114.06, 117.41, 123.97, 126.24, 127.51, 128.88, 130.74, 132.47, 134.92, 136.31, 147.28, 154.58, 169.59. HRMS (m/z): $[M+H]^+$ calcd for $C_{18}H_{16}N_5SCl$: 370.0888; found: 370.0882. Anal. calcd. For $C_{18}H_{16}N_5SCl$, C, 58.45; H, 4.36; N, 18.93. Found: C, 58.60; H, 4.35; N, 18.90.

N-*phenyl-5-(4-(5-methyl-1*H-*benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4d). Yield: 64%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.42 (3 H, s, -CH_3), 7.63 (4 H, br.s., Aromatic CH), 7.97–8.02 (2 H, m, Aromatic CH), 8.10–8.12 (2 H, m, Aromatic CH), 8.25 (2 H, d, *J* = 8.46 Hz, Aromatic CH), 8.29–8.32 (2 H, m, Aromatic CH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 20.78, 108.37, 112.42, 115.12, 118.14, 118.86, 120.01, 121.15, 123.33, 125.72, 128.11, 131.23, 133.20, 134.66, 143.07, 147.02, 150.66, 160.43. Anal. calcd. For $C_{22}H_{17}N_5S$, C, 68.91; H, 4.47; N, 18.26. Found: C, 69.05; H, 4.47; N, 18.30.

N-*(2-chlorophenyl)-5-(4-(5-methyl-1*H-*benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4e). Yield: 69%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.31 (3 H, s, -CH_3), 7.21–7.24 (5 H, m, Aromatic C-H), 7.52–7.55 (1H, m, Aromatic C-H), 8.03 (2H, d, *J* = 8.52 Hz, Aromatic C-H), 8.28–8.32 (3 H, m, Aromatic C-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 25.77, 97.36, 98.92, 103.18, 105.77, 109.72, 112.73, 116.68, 117.41, 117.83, 120.84, 124.16, 126.64, 128.01, 128.84, 129.67, 134.35, 145.78, 150.76, 165.41. Anal. calcd. For $C_{22}H_{16}N_5SCl$, C, 69.09; H, 4.22; N, 18.31. Found: C, 68.90; H, 4.20; N, 18.36.

N-*cyclohexyl-5-(4-(5-methyl-1*H-*benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4f). Yield: 71%. M.p. 233.1°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.23–1.33 (6 H, m, cyclohexyl), 1.58–1.73 (3 H, m, cyclohexyl), 2.02 (2H, br.s., cyclohexyl), 2.30 (3 H, s, CH_3), 7.61 (1H, s, Aromatic C-H), 7.92 (2H, brs., Aromatic C-H), 8.13 (2H, d, *J* = 7.53 Hz, Aromatic C-H), 8.34 (2H, d, *J* = 7.32 Hz, Aromatic C-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 20.68, 24.64, 25.53, 28.37, 32.29, 114.71, 119.93, 124.23, 126.73, 127.69, 128.16, 129.24, 129.85, 132.32, 134.78, 148.52, 154.41, 1168.61. HRMS (m/z): $[M+H]^+$ calcd for C_{22}

$H_{23}N_5S$: 390.1751; found: 390.1747. Anal. calcd. For $C_{22}H_{23}N_5S$, C, 67.84; H, 5.95; N, 17.98. Found: C, 68.09; H, 5.96; N, 18.03.

N-*(2,4-dichlorophenyl)-5-(4-(5-methyl-1*H*-benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4g). Yield: 72%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.28 (3 H, s, -CH_3), 7.29 (3 H, br.s., Aromatic CH), 7.62–7.63 (2H, br.s., Aromatic CH), 8.07–8.11 (2H, m, Aromatic CH), 8.27–8.31 (3 H, m, Aromatic CH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 22.13, 98.50, 100.37, 102.34, 103.90, 106.71, 109.51, 110.34, 114.29, 116.06, 120.22, 123.85, 127.38, 129.77, 132.48, 135.28, 140.37, 147.23, 149.52, 165.93. Anal. calcd. For $C_{22}H_{15}N_5SCl_2$, C, 58.28; H, 3.56; N, 15.45. Found: C, 58.45; H, 3.56; N, 15.40.

N-*isopropyl-5-(4-(5-methyl-1*H*-benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4h). Yield: 74%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.24 (6 H, d, *J* = 6.42 Hz, -CH_3), 2.28 (3 H, s, -CH_3), 3.87–3.91 (1H, m, CH), 7.40–7.43 (1H, m, Aromatic CH), 7.67 (1H, s, Aromatic CH), 7.77 (1H, d, *J* = 8.46 Hz, Aromatic CH), 8.08 (2H, d, *J* = 8.61 Hz, Aromatic CH), 8.30 (2H, d, *J* = 8.58 Hz, Aromatic CH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 21.69, 22.30, 48.37, 113.98, 114.29, 124.55, 127.80, 128.38, 129.15, 130.26, 132.41, 134.11, 136.94, 147.79, 154.46, 168.35. Anal. calcd. For $C_{19}H_{19}N_5S$, C, 65.30; H, 5.48; N, 20.04. Found: C, 65.51; H, 5.47; N, 20.09.

N-*butyl-5-(4-(5-methyl-1*H*-benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4i). Yield: 68%. M.p. 311.7°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.94 (3 H, t, *J* = 7.29 Hz, -CH_3), 1.58 (2H, s, -CH_2), 1.62–1.63 (2H, m, CH_2), 2.35 (3 H, s, CH_3), 3.30–3.33 (2H, m, CH_2), 7.27–7.31 (1H, m, Aromatic CH), 7.57 (1H, s, Aromatic C-H), 7.66 (1H, d, *J* = 8.25 Hz, Aromatic CH), 8.03–8.06 (2H, m, Aromatic CH), 8.24–8.26 (2H, m, Aromatic CH).^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 11.12, 20.99, 25.87, 29.09, 112.84, 115.33, 123.54, 126.35, 127.40, 129.67, 130.92, 134.45, 135.59, 141.72, 148.58, 152.11, 169.72. HRMS (m/z): $[M+H]^+$ calcd for $C_{19}H_{19}N_5S$: 350.1434; found: 350.1432. Anal. calcd. For $C_{19}H_{19}N_5S$, C, 65.30; H, 5.48; N, 20.04. Found: C, 65.47; H, 5.49; N, 20.10.

N-*(4-methoxyphenyl)-5-(4-(5-methyl-1*H*-benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4j). Yield: 70%. Semi-solid. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.33 (3 H, s, -CH_3), 3.75 (3 H, s, -OCH_3), 6.99 (1H, d, *J* = 8.85 Hz, Aromatic CH), 7.12 (2H, br.s., Aromatic CH), 7.39 (2H, br.s., Aromatic CH), 7.76 (1H, dd, J_1 = 2.88 Hz, J_2 = 8.76 Hz, Aromatic CH), 7.86–7.87 (1H, m, Aromatic CH), 7.98 (2H, d, *J* = 8.52 Hz, Aromatic CH), 8.25 (2H, d, *J* = 8.52 Hz, Aromatic CH).^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 20.52, 56.23, 113.13, 115.02, 119.21, 119.62, 120.24, 122.78, 127.30, 127.52, 129.16, 131.50, 132.00, 133.06, 136.75, 149.92, 152.31, 156.75, 165.42. HRMS (m/z): $[M+H]^+$ calcd for $C_{23}H_{19}N_5OS$: 414.1383; found: 414.1383. Anal. calcd. For $C_{23}H_{19}N_5OS$, C, 66.81; H, 4.63; N, 16.94. Found: C, 67.06; H, 4.62; N, 16.92.

N-*isobutyl-5-(4-(5-methyl-1*H*-benzo[*d*]imidazole-2-yl)phenyl)-1,3,4-thiadiazole-2-amine (4k). Yield: 71%. M.p. 314.6°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.94 (6 H, d, *J* = 6.63 Hz, CH_3), 1.88–1.94 (1H, m, CH), 2.43 (3H, s, CH_3), 3.15–3.17 (2H, m, CH_2), 7.03–7.05 (1H, m, Aromatic CH), 7.33–7.53 (1H, m, Aromatic CH), 7.89–7.93 (2H, m, Aromatic CH), 8.11–8.12 (1H, m, Aromatic CH), 8.22–8.25 (2H, m, Aromatic CH).^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm): 20.57, 21.81, 28.07, 52.95, 111.60, 119.01, 123.95, 124.74, 127.15,

127.37, 131.43, 132.17, 132.67, 133.00, 150.48, 155.40, 169.44. HRMS (m/z): $[M+H]^+$ calcd for $C_{20}H_{21}N_5S$: 364.1597; found: 364.1590.

Antimicrobial activity

The antimicrobial activity of final compounds (4a-4k) was screened on eight bacterial and three fungal strains according to the standard procedure of CLSI [26,27] as described in the previous study [28]. The antibacterial activities of the synthesized compounds were tested against *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 2942), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213). *Candida albicans* (ATCC 24433), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 22019) and *Candida glabrata* (ATCC 9) were used to test the antifungal activity of the same compounds. Azithromycin (against bacterial strains) and voriconazole, fluconazole (against candida strains) were used as standard reference drugs.

In silico studies

Using molecular docking methods, our study group was aimed to define the active compounds and explain the binding modes of active compound(s). For this purpose, potential compounds determined from antimicrobial studies were evaluated for their binding with DNA gyrase's active pocket. The X-RAY crystal structure of the protein (PDB ID: 4DUH) was retrieved from the Protein Data Bank (PDB) server (www.pdb.org, accessed 29 October 2021). Schrödinger Maestro (Schrödinger Release 2020) [29] interface was used for the molecular docking study and the enzymes crystals were processed using the Protein Preparation Wizard protocol of the Schrödinger Suite 2020. Compounds were prepared using the LigPrep module [30] (Schrödinger Release. 2020–1) to correctly assign the protonation states as well as the atom types. Bond orders were assigned, and hydrogen atoms were added to the structures. The grid generation was formed using the Glide module (Schrödinger Release 2020–3) [31], and docking runs were performed in standard precision docking mode (SP) [32].

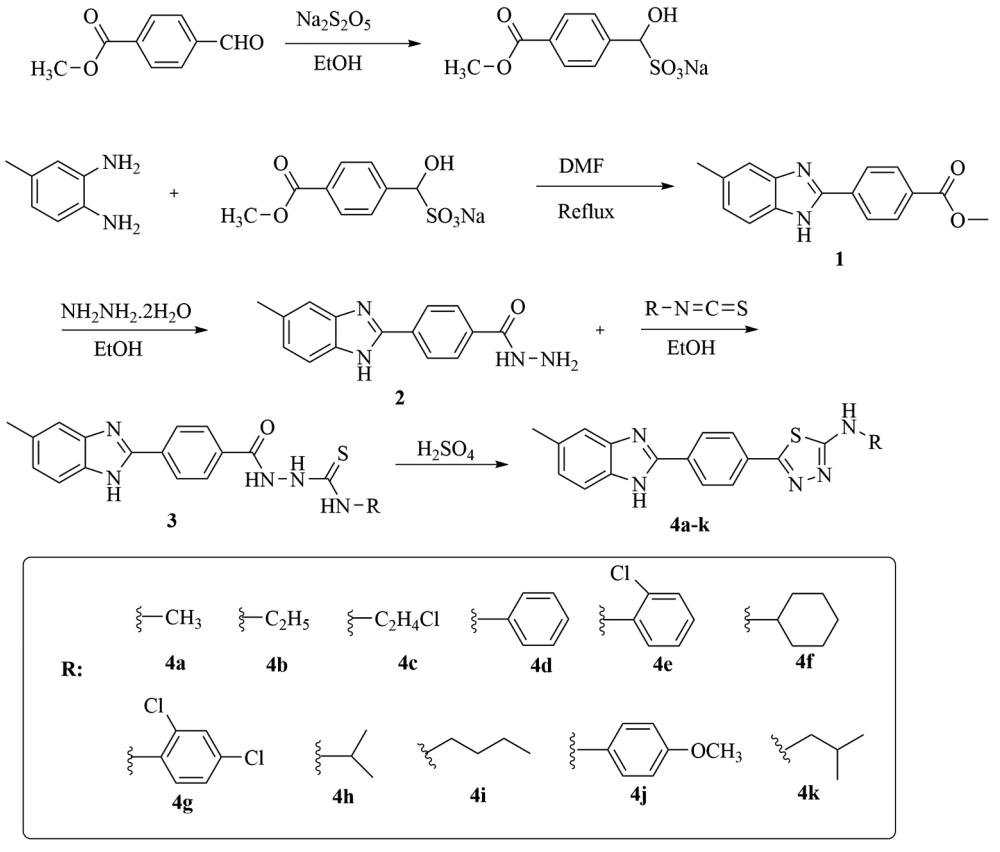
After determining the potential DNA gyrase inhibitor, using molecular dynamic simulation technics, we aimed to determine and clarify the changes in the interactions during the time, since explaining the structure-activity study (SAR) will be more detailed. Thus, an MDS study was performed for 100 ns with the POPE transmembrane model system and 3 points (TIP3P) water model followed by energy minimization of the complex waters as same as bacterial DNA gyrase. The neutralization of the system was achieved using Na+ and Cl− ions and 150 mM NaCl was added. The molecular dynamic simulation was performed following the completion of the system setup. The radius of gyration (Rg), root mean square fluctuation (RMSF), and root mean square deviation (RMSD) values were calculated by the Desmond application (Schrödinger Release 2020).

Results and discussion

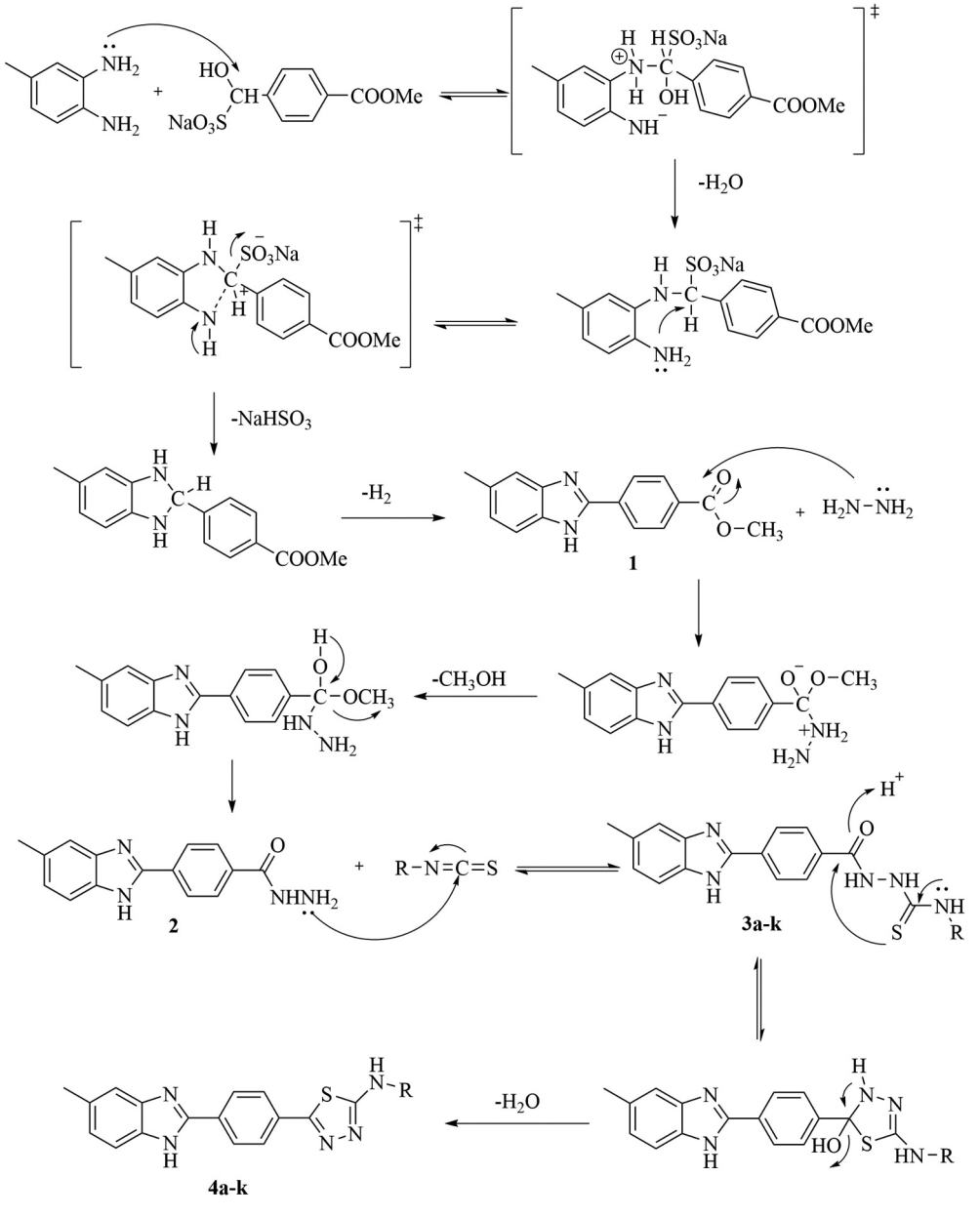
Chemistry

As shown in Scheme 1, The target molecules were synthesized in five steps. First of all, the aldehyde part of the methyl 4-formylbenzoate compound was treated with sodium disulphide in ethanol to obtain the sodium disulphide addition product of the aldehyde. In the second step, as a result of the condensation reaction of benzaldehyde sodium bisulphite product and 1,2-phenylenediamine under reflux the methyl 4-(1*H*-benz[*d*] imidazole-2-yl)benzoate (1) was obtained. In the next step, compound 1 was treated with hydrazine hydrate in ethanol to obtain the 4-(1*H*-benz[*d*]imidazol-2-yl)benzohydrazide (2). The hydrazide derivative compound and the appropriate isothiocyanate derivative in ethanol were refluxed and the precipitated product is filtered off. In the last step, thiadiazole derivatives were obtained by cyclization of the thiosemicarbazide compound in the presence of concentrated sulphuric acid.

A mechanism have been proposed for the preperation of benzimidazoles based on some information in the literature (Scheme 2). The reaction begins with the nucleophilic attack of the amine group on the *o*-phenylenediamine to the carbon atom of aldehyde metabisulfite adduct. One mole of water is eliminated. Subsequently, the resulting alkyl



Scheme 1. General procedure for synthesis of the final compounds 4a-4k.



Scheme 2. Synthesis mechanism of compounds 4a-k.

sulphonate further reacts with the other amine group of *o*-phenylenediamine, resulting in the formation of dihydroimidazole intermediate. Finally, aromatization gives benzimidazole nucleus [33,34]. In the next step, compound 1 was treated with hydrazine hydrate in ethanol to obtain the compound 2 [35]. The hydrazide derivative compound and the appropriate isothiocyanate derivatives in ethanol were reflux and the precipitated product is filtered off. In the last step, thiadiazole derivatives (4a-k) were obtained by cyclization of the thiosemicarbazide compound in the presence of concentrated sulphuric

acid [36,37]. The preference formation of the 1,3,4-thiadiazole ring under acidic condition can be due to the loss of nucleophilicity of *N*-4 as a result of its protonation leading to a comparable increase in the nucleophilicity of the sulphur atom towards the attack of the carbonyl carbon [38–40].

The compounds were crystallized from ethanol for purification. First, ethanol was gradually added to the compound placed in a round bottom flask and boiled under reflux. Then activated charcoal was added to the solution and boiled again. The saturated solution was filtered. The product precipitated in the cooled solution was filtered off.

Antimicrobial activity

The antibacterial activity of the final compounds 4a-4k was evaluated by determining their minimal inhibitory concentration (MIC) against the following strains: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 2942), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Candida albicans* (ATCC 24433), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 22019) and *Candida glabrata* (ATCC 9). The antimicrobial activities were summarized in Tables 1 and 2.

As shown in Table 1, Compounds 4f and 4i show the greatest antibacterial activity against *E. coli* (MIC< 0.97 µg/mL); compounds 4d, 4i and 4k (MIC = 3.90 µg/mL) are the

Table 1. Antibacterial activity of the compounds 4a-4k as MIC values (µg/mL).

Comp.	*A*	*B*	*C*	*D*	*E*	*F*
4a	125	125	250	7.81	15.625	125
4b	125	125	125	7.81	31.25	125
4c	125	125	125	31.25	125	125
4d	62.5	62.5	125	3.90	125	125
4e	125	62.5	125	7.81	125	125
4f	< 0.97	31.25	125	7.81	125	31.25
4g	7.81	62.5	62.5	7.81	62.5	31.25
4h	1.95	15.625	31.25	7.81	31.25	7.81
4i	< 0.97	125	125	3.90	125	250
4j	3.90	125	125	7.81	125	125
4k	3.90	125	125	3.90	125	62.5
Azithromycin	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97

A: *E. coli* B: *K. pneumoniae* C: *P. aeruginosa* D: *E. faecalis* E: *B. subtilis* F: *S. aureus*.

Table 2. Antifungal activity of the compounds 4a-4k as MIC values (µg/mL).

Comp.	*C. albicans*	*C. krusei*	*C. glabrata*	*C. parapsilosis*
4a	1.95	62.5	3.90	62.5
4b	15.625	62.5	3.90	62.5
4c	15.625	62.5	3.90	125
4d	15.625	125	125	125
4e	31.25	125	7.81	125
4f	15.625	125	31.25	125
4g	7.81	62.5	3.90	62.5
4h	1.95	7.81	1.95	7.81
4i	7.81	125	125	62.5
4j	15.625	125	62.5	62.5
4k	3.90	125	62.5	62.5
Voriconazole	3.90	3.90	1.95	3.90
Fluconazole	7.81	7.81	3.90	3.90

most active compounds against *E. faecalis*. Also, compound 4h with 1.95 µg/mL MIC value and compounds 4j and 4k with 3.90 µg/mL MIC value were found to show significant activity against *E. coli*. The target compounds have different arylamine and alkylamine structures at the 5th position of the thiadiazole ring. When the structures of the effective derivatives were examined, it was determined that the antibacterial activities of the compounds carrying the alkyl chain in their structure were higher. In particular, compound 4f with cyclohexyl structure and compound 4i with n-butyl structure were found to be the most effective derivatives against *E. coli*.

As shown in Table 2, the antifungal activity screening results show that compounds 4a and 4h (1.95 µg/mL) were found to be two times more effective than the reference drug voriconazole (3.90 µg/mL), and 4 times more effective than fluconazole (7.81 µg/mL), with a MIC value of 1.95 µg/mL against *C. albicans*. While the compound 4k shows the same activity as voriconazole, it was found to be two times more effective than fluconazole. Moreover, compound 4h, with an MIC of 1.95 µg/mL, shows the same effect as voriconazole on *C. glabrata*, and it shows twice the effectiveness of fluconazole. When the antifungal activity results were evaluated, it was found that those carrying alkylamine group in the 5th position of the thiadiazole ring were more effective. In particular, the compound 4a carrying the methyl group and the compound 4h carrying the isopropyl structure were found to be the most effective in the series.

In silico studies

Determination of the most active compounds against microbe cell lines, it can be suggested that the activation mechanism is related to inhibition *E. coli* DNA-gyrase enzyme according to literature [41–43]. Therefore, the docking study was applied for compounds 4f and 4i against DNA-gyrase subunit B. The best poses were displayed in Figure 1.

Due to the similar localization of both compounds in the active pocket, the interactions between compounds and DNA gyrase enzyme are also similar. The hexyl and *n*-butyl moieties were found near to hinge amino acids (Asp73, Asp74, Gly75, Arg76, and Gly77). In fact, these two substitutions have also similar physicochemical properties such as volume, length, and flexibility, thus, their amine group was bonding with Asp73 via H-bond. On the other hand, benzimidazole nitrogen atoms of both compounds interacted with Arg76 and Glu101 via H-bond. Moreover, there was a π-cation interaction between phenyl and Lys103 for both compounds. But, there is only one difference between compounds 4f and 4i, which is an extra π-cation interaction between the 4f thiadiazol ring and Lys103 residue. To explain the SAR, all these amino acids were identified as key amino acids for inhibition activity of DNA gyrase ATPase enzyme [44] Overall, these features of substitutions can be thinkable as optimal properties for inhibition activity. Because of that, the rigidity of compounds based on them should be remodelled and decreased for further studies, and for this purpose, thiadiazol core may be substituted with nonaromatic carbocyclic or carbon chains. On the other, although the amino acids around the hexyl or butyl groups constitute the hydrophobic pocket, their backbone amine group is suitable for pi-cation connection, hence, it could be sayable that amine-carbon chains or nonaromatic heterocyclics should be designed and synthesized.

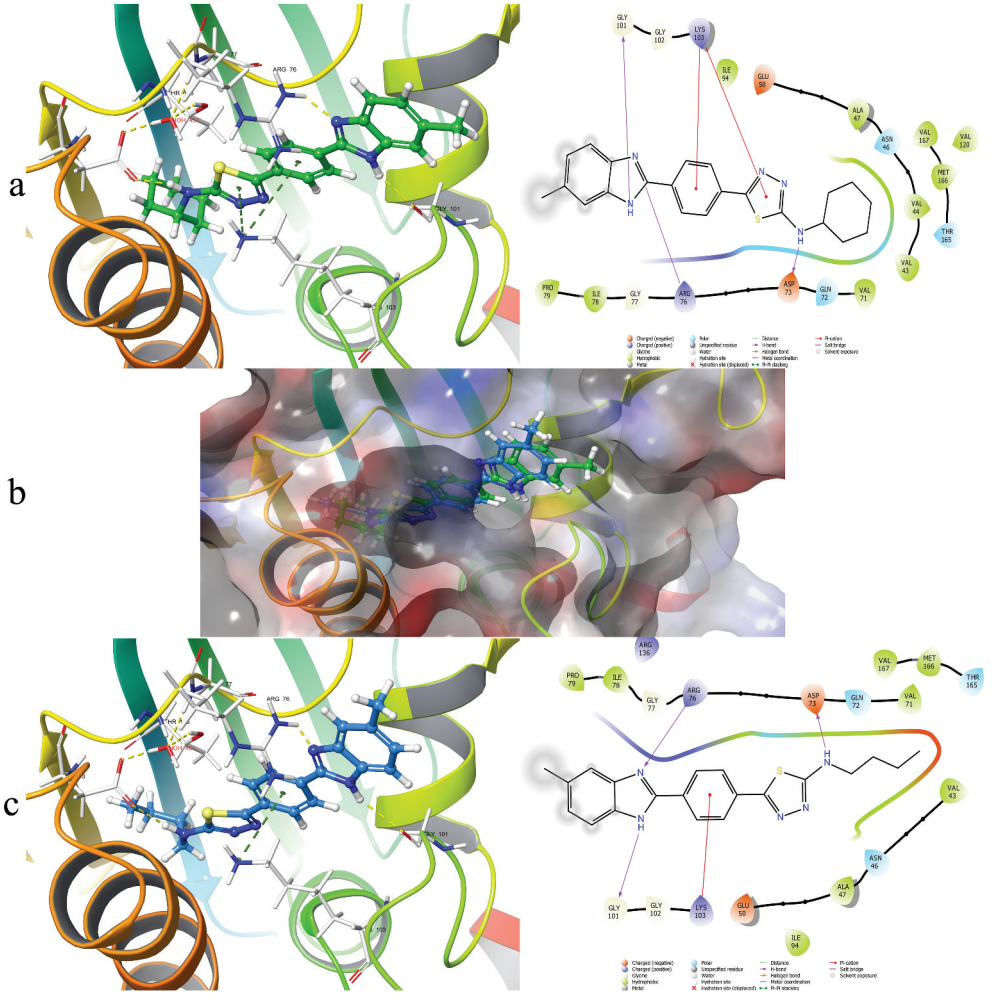


Figure 1. Representation of the active compounds 4f and 4i at DNA-gyrase complex (PDBID: 4DUH). A: 4f as 3D and 2D diagram; B: Superimposed 4f and 4i at DNA-gyrase active pocket; C: 4i as 3D and 2D diagram.

After determination of the best poses, we chose 4f as a pattern molecule for molecular dynamic simulation. Data obtained from MDS were displayed in Figures 2 and 3. According to Figure 2, RMSD values of protein are calculated between 1–2 Å; Rg values have not shown drastic fluctuation; and the fluctuation of the loop region (white area) is reduced due to interactions with 4f as seen in the RMSF plot. These plots were as should be for ligand-small protein complexes [29,45]. Therefore, the 4f-DNA gyrase complex was found stable during simulation.

Three interaction types were observed namely H-bonds, π-π stackings, and π-cation interactions (Figure 3(a)). The interaction types between 4f and Asn46, and Asp73 are direct H-bonds; between 4f and Asn46, Asp73, Asp76, Gly77, Ala90, Gly101, and Arg136 are water-mediated H-bonds; between 4f and Val43, Ala47, Val71, Arg76, Ile78, Pro79, His83, Ile94, Lys103, and Val167 are hydrophobic

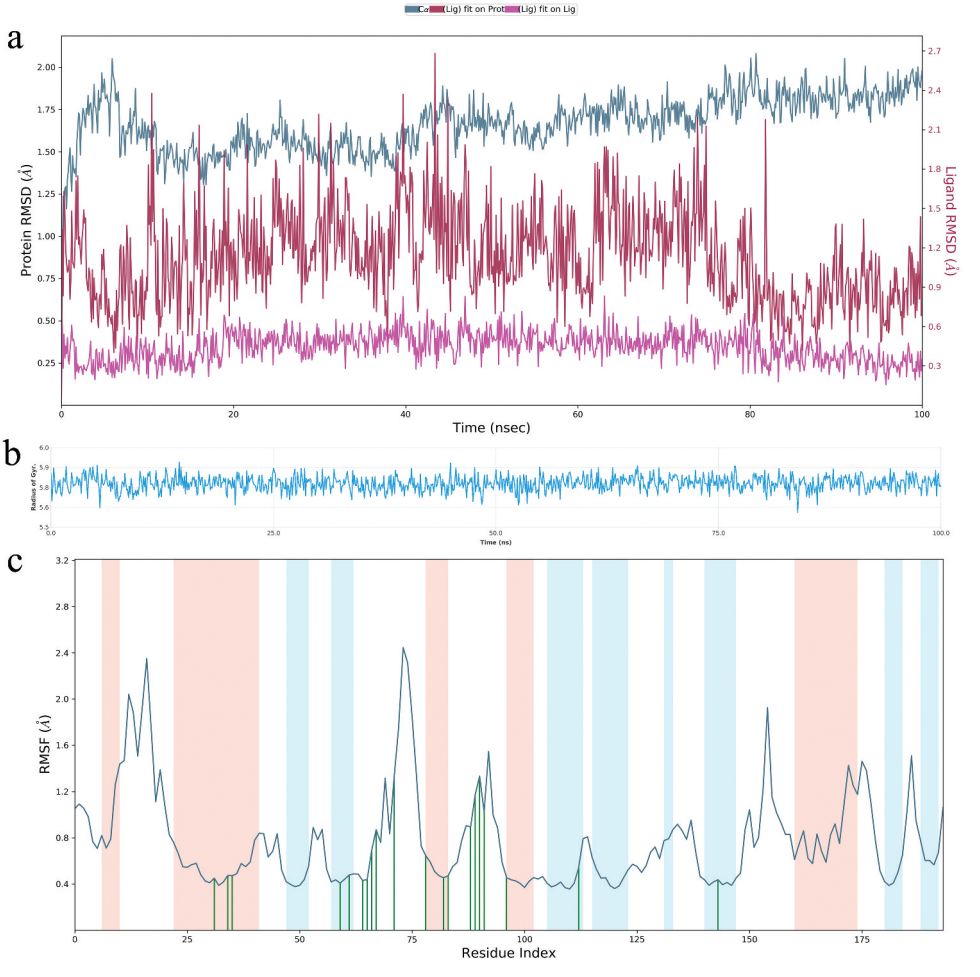


Figure 2. The stability properties of 4f-DNA gyrase complex. A: RMSD plot for protein and ligand B: Rg plot C: RMSF plot of 4f-DNA gyrase complex.

interactions; and between 4f and Gly77 is aromatic H-bond (Figure 3(b)). During the simulation, the interactions with Asp73 and Asp76 are protected. Moreover, the interaction fractions of Gly77 and Arg136 were high, therefore, these 4 amino acids have may major impact on inhibition activity. Indeed, Asp73, Asp76 and Arg136 residues were descripted as key amino acids [44]. On the other hand, solvent exposure was established on the 5/6-methyl benzimidazole ring, thus, this ring can be substituted with bioisosteric groups (OMe, OEt, etc.) which show the hydrophilic property. However, benzimidazole-phenyl-thiadiazol ring system has a good profile to observe DNA-gyrase inhibition, if the system is hybridized with amine-carbon chains or nonaromatic heterocyclics. But the hydrophobic properties of this fragment should be protected which allows entering the active cavity.

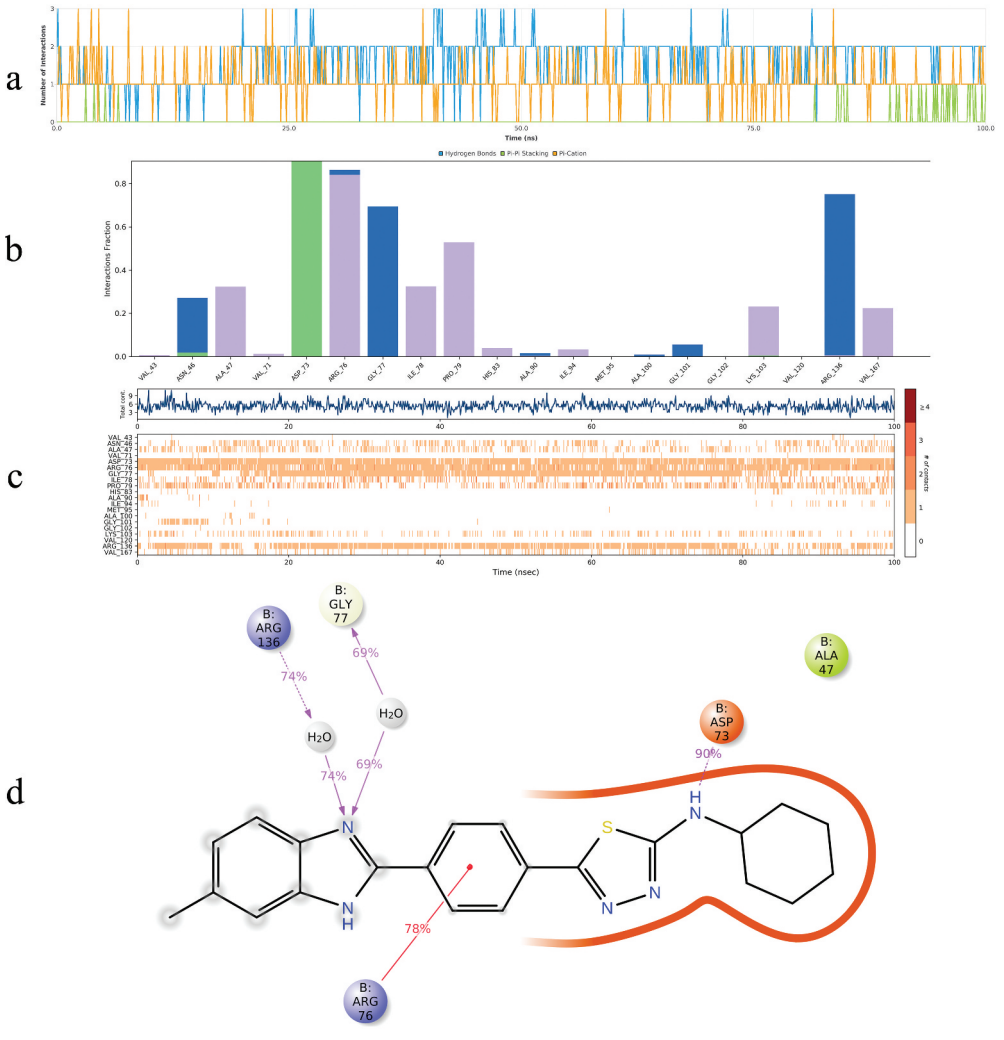


Figure 3. The interaction plots for 4f-DNA gyrase complex during simulation. A: Number of interactions and interaction types versus time plot; B: Plot of interaction fractions versus residues with their interaction types; C: Residue connections versus time plot; D: Contact strength (cut-off = 30%) diagram.

Conclusion

In conclusion, a series of novel benzimidazole-1,3,4-thiadiazole derivatives were efficiently synthesized, structurally characterized and screened for their antimicrobial activity towards six bacterial and four fungal strains. Among this series, compounds 4f and 4i were found to be the most active displaying potent antibacterial activity. Compounds 4a and 4h were found to be the most active displaying potent antifungal activity. The intermolecular interactions of the promising compounds 4i and 4f with microbial target proteins were investigated, which revealed significant binding interaction scores associated with these compounds. Following that, the interaction's stability was assessed using a typical atomistic 100 ns dynamic simulation study.

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