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ORIGINAL RESEARCH

Synthesis, molecular docking and ADME prediction of some new benzimidazole carboxamidines derivatives as antimicrobial agents

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Abstract

In this study, 15 new 1*H*-benzimidazole-5-carboxamidine derivative compounds that could be new antimicrobial agents were synthesized and their antimicrobial activities were determined using the microdilution method. When the activity results were examined, it was observed that the antibacterial effects of the new benzimidazole derivatives were weaker than standard drugs, but some derivatives showed significant efficacy against MRSA and VREF with the value of MIC: 8 µg/ml compared to reference drugs. The antifungal effects of the compounds were found to be weaker compared to the reference drugs. Molecular docking studies of compounds and reference drugs used were performed against PBP4 and the active and allosteric site of PBP2a, and estimated ADME profiles were calculated. In addition, 2D and 3D interactions of N10, one of the most effective antimicrobial compounds compared to reference drugs, were demonstrated in both sites.

Keywords Benzimidazole · Carboxamidine · Antimicrobial activity · ADME prediction · Molecular docking

Introduction

Infectious diseases seriously threaten public health, but the biggest problem in combating infectious diseases is the rapid development of resistance to existing drugs (Blair et al. 2015). Antimicrobial resistance is currently responsible for more than 700,000 deaths annually worldwide, and by 2050 it is estimated that it will reach an estimated economic cost of $100 million and more than 10 million deaths per year (Jansen et al. 2018). Resistance to antimicrobials (antibiotics, antifungals, antivirals, anthelmintics, antimalarials) leads to higher medical costs, treatment failure, increased mortality and morbidity, longer hospital stay, and increased disability. Without effective antimicrobials for the prevention and treatment of infections, many medical procedures such as organ transplantation, cancer treatment, diabetes and surgical operations become a high risk (Spellberg et al. 2013; Ventola 2015; Peters et al. 2019). In recent years, there has been an alarming increase in the incidence of Gram (+) infection. Among these, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VREF) infections are an increasingly important treatment problem in hospital patients (Boucher et al. 2009).

In *S. aureus*, methicillin resistance is realized by "Penicillin Binding Protein" (PBP), which is a different mechanism than penicillinase (beta-lactamase) production. PBPs are responsible for carrying and attaching their peptidoglycan precursors to the cell wall being constructed, and a different PBP called PBP2 or PBP2a is synthesized in MRSAs (Hackbarth and Chambers 1993). Unlike other PBPs, PBP2/2a shows low affinity for antibiotics in beta-lactam structure (Gordon and Lowy 2008). In *E. faecalis*, decreased sensitivity to B-lactam antibiotics is due to the expression of PBP4 (Moon et al. 2018; Rice et al. 2018).

Benzimidazole ring system has an important role to play in many pharmacological activities such as antimicrobial, antiviral, antiparasitic, anthelmintic, antihistamine, antiulcer, antihypertensive, anticancer, antioxidant, spasmolytic, anticonvulsant, antitumor, anti-inflammatory, antiastmatic,

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analgesic, and neuroleptic (Salahuddin et al. 2017). Since the benzimidazole ring system is structural analogs of heterocyclic bases in the structure of nucleic acids, it is thought that they can show their microbiological activities in this way (Oehlers et al. 2004). Therefore, studies on these derivatives have been increased in recent years. Researches to date reveal that the benzimidazole ring system is mostly substituted from the 1st, 2nd, and 5th positions.

Aromatic amidine and diamidine group compounds show strong activity against many bacteria, ameba, protozoa and viruses. Although the mechanism of action of the amidine group compounds has not been adequately illuminated, it has revealed that most of the compounds of this class show antimicrobial effects by binding to the minor cavity in the AT rich region of DNA (Huang et al. 2001). Another mechanism is that it can show its activities by inhibiting Topoisomerase I and/or II enzyme by reversible interaction with DNA (Bell et al. 1991; Cory et al. 1992; Fairley et al. 1993; Tidwell et al. 1993).

In the light of this information, it was aimed in this study to synthesize some new mono cationic benzimidazole carboxamidine derivatives (Fig. 1), to clarify the structures of the compounds using ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy, elemental analysis methods and to investigate in vitro antimicrobial activities compared to clinical reference drugs. Estimation of ADME profiles of all compounds, molecular docking studies and molecular mechanics generalized born surface area (MM-GBSA) calculations were performed with Schrödinger software.

Materials and methods

Chemistry

Chemicals and solvents were purchased from Sigma-Aldrich, Across Organics, Merck, Riedel de Haen and used without further purification. Silica gel 60 HF254 chromatoplates (Merck) for TLC and chloroform:methanol (10:0.5) were used as the mobile phase. UV light of 254 and 366 nm wavelength was used to detect stains. Melting points were determined with Büchi B-540 capillary melting point device and results were given without correction. ^{1}H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance spectroscopy was taken with Varian Mercury 400 MHz High Performance Digital FT-NMR spectrometer (Palo Alto, CA, USA). Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as the solvent, chemical shifts were expressed in ppm according to TMS and coupling constants (J) were given as Hertz. The molecular weights of the synthesized compounds were taken on the Waters 2695 Alliance Micromass ZQ LC/MS spectrometer (Milford, MA, USA) using the ESI (+) method.

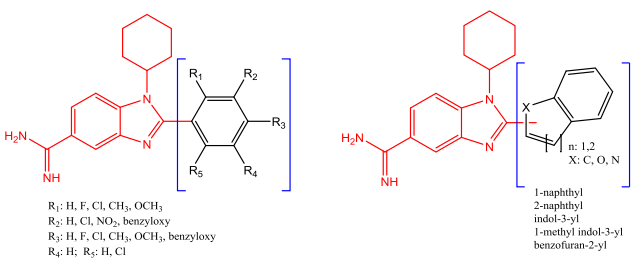


Fig. 1 Designed 1*H*-benzimidazole-5-carboxamidine derivatives

General procedures for preparation of compounds (N1–N15)

It was obtained by reacting **1a**, 4-chloro-3-nitrobenzonitrile and cyclohexylamine in DMF. Dry HCl gas was passed through absolute EtOH cooled in an ice water bath for 30 min and mixed with 1 in a sealed flask for 3 days to obtain the imidate ester (**1b**). All imidate esters was continued their reactions without any purification due to their unstable structure. Imidate esters were left to stir overnight with absolute ethanol passed NH_3 gas to obtain **1c**. Then, the amine group (**1d**) was formed by reducing the nitro group in the compound with H_2/Pd-C. The target compounds **N1**–**N15** were prepared by condensation of **1d** with $Na_2S_2O_5$ adduct of related aldehydes in DMF. A portion of the resulting crude product was purified by column chromatography using a chloromethane:methanol:ammonia (10:1:0.1) solvation mixture, while the other was crystallized from methanol. The HCl salts of the compounds were obtained in a mixture of HCl passed EtOH. List of synthesized compounds (Table S1) and their physical and spectral data were reported below (see also Supplementary Information).

1-cyclohexyl-2-(2,4-dimethylphenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N1): Yield: 35%, M.p. 195–200 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.12–1.20 (m, 4H, –CH_2), 1.28–1.34 (t, H, –CH), 1.56 (d, H, –CH), 1.78 (d, 2H, –CH_2), 2.09 (s, 3H, –CH_3), 2.17 (d, 2H, –CH_2), 2.36 (s, 3H, –CH_3), 3.95–4.04 (t, H, N–CH), 7.17 (d, H, Jo = 8.4 Hz, H-3′), 7.25 (d, 2H, Jo = 7.6 Hz, H-5′, 6′), 7.68 (dd, H, Jo = 8.4 Hz, Jm = 1.6 Hz, H-6), 8.05 (d, H, Jo = 8.4 Hz, H-7), 8.20 (d, H, Jm = 1.2 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 15.22, 24.18, 25.49, 28.02, 30.33, 56.25, 113.18, 120.15, 120.53, 121.87, 123.58, 126.41, 129.19, 136.65, 139.78, 143.75, 145.17, 155.51, 166.41. MS (ESI+) *m/z*: 347.3 (M + H, 100%), 265.2 (100%). Anal. Calcd for $C_{24}H_{26}N_4$·HCl·C_2H_6O: C, 67.19; H, 7.75; N, 13.06. Found: C, 67.35; H, 8.05; N, 13.06.

1-cyclohexyl-2-(2,4-dimethoxyphenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N2): Yield 15%, M.p. 182–185 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.22–1.40

(m, 4H, –CH_2), 1.60 (d, H, –CH), 1.83 (d, 3H, –CH_2 and –CH), 2.20 (m, 2H, –CH_2), 3.79 (s, 3H, OCH_3), 3.86 (s, 4H, OCH_3 and N–CH), 6.70 (dd, H, Jo = 8.4, Jm = 2, H-5′), 6.76 (d, H, Jm = 2, H-3′), 7.35 (d, H, Jo = 8.4, H-6′), 7.70 (d, H, Jo = 8.8, H-6), 8.03 (d, H, Jo = 9.2, H-7), 8.21 (s, H, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 15.15, 24.14, 25.98, 28.05, 30.34, 56.80, 113.27, 119.24, 120.47, 121.00, 122.68, 127.14, 129.19, 135.89, 136.66, 139.79, 142.76, 155.49, 166.04. MS (ESI+) *m/z*: 379.5 (M + H, 100%), 297.4 (100%). Anal. Calcd for $C_{20}H_{20}F_2N_4$·0.5HCl·0.5C_2H_6O: C, 66.82; H, 7.08; N, 13.35. Found: C, 66.81; H, 6.89; N, 13.56.

1-cyclohexyl-2-(2,4-difluorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N3): Yield 8%, M.p. 196–200 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.17–1.40 (m, 3H, –CH_2 and –CH), 1.61 (s, H, –CH), 1.86 (d, 4H, –CH_2), 2.20 (d, 2H, –CH_2), 4.01 (t, H, N–CH), 7.33–7.37 (m, 2H, H-3′,5′), 7.53–7.58 (m, H, H-6′), 7.71–7.77 (m, H, H-6), 8.40 (d, H, Jo = 8.4, H-7), 8.29 (s, H, H-4), 9.04 (s, 2H), 9.34 (s, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.85, 25.12, 31.87, 55.12, 113.60, 116.57, 119.80, 121.53, 122.37, 125.60, 131.01, 136.84, 142.65, 154.15, 160.71, 163.15, 166.01. MS (ESI+) *m/z*: 355.1 (M + H, 75%), 273.4 (100%). Anal. Calcd for $C_{20}H_{20}F_2N_4$: C; 59.24, H; 6.16, N; 12.56. Found: C; 58.95, H; 6.40, N; 12.34.

1-cyclohexyl-2-(2-chlorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N4): Yield: 13%, M.p. 200–202 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.17–1.25 (m, 3H, –CH_2 and –CH), 1.34–1.37 (m, H, –CH), 1.67 (d, H, –CH), 1.82 (3H, –CH_2 and –CH), 2.19 (m, 2H, –CH_2), 3.86–3.90 (t, H, N–CH), 7.15–7.19 (t, H, H-3′), 7.27 (d, H, Jo = 8.4 Hz, H-5′), 7.45 (dd, H, Jo = 7.6 Hz, Jm = 1.6 Hz, H-4′), 7.61–7.64 (m, H, H-6′), 7.77 (dd, H, Jo = 8.8 Hz, Jm =2 Hz, H-6), 8.04 (d, H, Jo = 8.8 Hz, H-4), 8.29 (s, H, H-7). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.71, 25.32, 30.86, 57.08, 113.62, 115.78, 119.81, 121.67, 122.73, 125.65, 131.10, 132.91, 1 36.46, 142.90, 154.15, 160.72, 163.51, 166.01. MS (ESI+) *m/z*: 353.4 (M + H, 90%), 271.3 (100%). Anal. Calcd for $C_{20}H_{21}ClN_4$·1.25HCl·2C_2H_6O·0.5H_2O: C, 68.08; H, 6.00; N, 15.88. Found: C, 68.41; H, 6.29; N:15.45.

1-cyclohexyl-2-(3-chlorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N5): Yield 18%, M.p. 188–190 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.15–1.41 (m, 3H, –CH_2 and –CH), 1.62 (d, H, –CH), 1.87 (d, 2H, –CH_2), 1.92 (d, 2H, –CH_2), 2.24–2.33 (m, 2H, –CH_2), 4.24–4.29 (m, H, N–CH), 7.48–7.69 (m, 4H, H-2′,4′,5′,6′), 7.73 (dd, H, Jo = 8.4 Hz, Jm = 2 Hz, H-6), 8.11 (d, H, Jo = 8.8 Hz, H-7), 8.27 (d, H, Jm = 1.6 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.15, 25.34, 30.86, 57.05, 113.64, 116.59, 119.94, 121.35, 122.48, 125.62, 131.11, 132.51, 136.46, 143.49, 154.51, 160.72, 163.45, 165.01. MS (ESI +) *m/z*: 353.2 (M + H, 100%), 271.1 (100%). Anal. Calcd for $C_{20}H_{21}ClN_4$·HCl·0.5C_2H_6O·0.25 H_2O: C, 58.97; H, 5.94; N, 13.82. Found: C, 58.65; H, 5.67; N, 13.82.

1-cyclohexyl-2-(3,4-dichlorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N6): Yield 23%, M.p. 178–182 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.12–1.20 (m, 3H, –CH_2 and –CH), 1.28–1.34 (t, H, –CH), 1.56 (d, H, –CH), 1.78 (d, 3H, –CH_2 and –CH), 2.17 (d, 2H, –CH_2), 3.84–3.90 (t, H, N–CH), 7.17 (d, 2H, Jo = 8.4 Hz, H-2′, 5′), 7.24 (d, H, Jm = 1.6 Hz, H-6′), 7.68 (dd, H, Jo = 8.4 Hz, Jm = 1.6 Hz, H-6), 8.05 (d, H, Jo = 8.4 Hz, H-7), 8.20 (d, H, Jm = 1.2 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.80, 25.23, 31.86, 56.85, 113.27, 119.24, 121.00, 122.68, 127.14, 129.19, 132.25, 136.66, 139.79, 142.76, 146.87, 155.49, 160.78, 166.04. MS (ESI+) *m/z*: 387.27 (M + H, 100%). Anal. Calcd for $C_{20}H_{20}Cl_2N_4$·3HCl: C, 48.36; H, 4.67; N, 11.28. Found: C, 48.52; H, 5.05; N, 11.02.

1-cyclohexyl-2-(2,4-dichlorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N7): Yield: 23%, M.p. 186–188 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.12–1.20 (m, 3H, –CH_2 and –CH), 1.28–1.34 (t, H, –CH), 1.56 (d, H, –CH), 1.78 (d, 3H, –CH_2 and –CH), 2.17 (d, 2H, –CH_2), 3.75–3.78 (t, H, N–CH), 7.14 (d, 2H, Jo = 8.4 Hz, H-5′, H-6′), 7.24 (d, H, Jm = 1.6 Hz, H-3′), 7.65 (dd, H, Jo = 8.4 Hz, Jm = 1.6 Hz, H-6), 8.14 (d, H, Jo = 8.4 Hz, H-7), 8.25 (d, H, Jm = 1.2 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.18, 25.02, 30.25, 55.80, 113.58, 119.63, 121.02, 122.84, 127.41, 129.15, 130.45, 136.67, 139.92, 142.77, 145.82, 155.87, 166.04. MS (ESI+) *m/z*: 387.4 (M + H, 100%), 305.4 (100%). Anal. Calcd for $C_{20}H_{20}Cl_2N_4$·1.2HCl·0.8C_2H_6O·1.5H_2O C, 55.45, H, 5.60; N, 11.97. Found: C, 55.82; H, 5.31; N, 11.58.

1-cyclohexyl-2-(2,6-dichlorophenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N8): Yield: 17%, M.p. 200–202 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.28–1.36 (m, 3H, –CH_2 and –CH), 1.70 (d, H, –CH), 1.75 (d, 2H, –CH_2), 1.83 (d, 2H, –CH_2), 2.29–2.37 (m, 2H, –CH_2), 4.24–4.30 (m, H, N–CH), 7.64 (d, 2H, Jo = 8.4 Hz, H-3′,5′), 7.80 (dd, H, Jo = 8.4 Hz, Jm = 1.6 Hz, H-6), 7.85 (d, H, Jo = 8 Hz, H-4′), 8.17 (d, H, Jo = 8.4 Hz, H-7), 8.31 (d, H, Jm = 1.2 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.72, 25.41, 30.87, 57.11, 113.69, 120.31, 121.45, 123.92, 128.17, 131.39, 131.73, 137.28, 142.22, 142.79, 154.89, 166.78. MS (ESI+) *m/z*: 387.24 (M + H, 100%), 305.35 (48%). Anal. Calcd for $C_{20}H_{21}N_4Cl_2$·HCl·1.5-C_2H_6O·2H_2O: C, 52.24; H, 5.48; N, 12.19. Found: C, 51.98; H, 5.65; N, 12.52.

1-cyclohexyl-2-(3-nitro-4-chloro)-1*H*-benzimidazole-5-carboxamidine HCl (N9): Yield 7%, M.p. 174–176 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.12–1.21 (m, 3H, –CH_2 and –CH), 1.25–1.32 (t, H, –CH), 1.67 (d, H, –CH), 1.79 (d, 3H, –CH_2 and –CH), 2.21 (d, 2H, –CH_2), 3.86–3.92 (t, H, N–CH), 7.22 (d, 2H, Jo = 8.4 Hz, H-5′, 6′), 7.25 (d,

H, Jm = 1.6 Hz, H-2′), 7.86 (dd, H, Jo = 8.4 Hz, Jm=1.6 Hz, H-6), 8.11 (d, H, Jo=8.4 Hz, H-7), 8.24 (d, H, Jm = 1.2 Hz, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.82, 25.25, 31.89, 57.24, 112.68, 121.20, 121.34, 121.89, 123.92, 129.17, 131.56, 131.87, 132.58, 137.29, 143.68, 145.41, 156.68, 165.76. MS (ESI+) *m/z*: 398.3 (M + H, 100%) 316.3 (50%). Anal. Calcd for $C_{20}H_{20}ClN_5O_2$·2.2HCl·0.75C_2H_6O: C, 50.25; H, 4.68; N, 14.65. Found: C, 49.91; H, 4.87; N, 13.98.

1-cyclohexyl-2-(3,4-dibenzyloxyphenyl)-1*H*-benzimidazole-5-carboxamidine HCl (N10): Yield: 28%, M.p. 104–106 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.20–1.27 (m, 3H, –CH_2 and –CH), 1.31–1.39 (m, H, –CH), 1.60 (d, H, –CH), 1.79–1.85 (t, 3H, –CH_2 and –CH), 2.18–2.27 (q, 2H, –CH_2), 4.24–4.30 (t, H, N–CH), 5.20 (s, 2H, –OCH_2), 5.23 (s, 2H, –OCH_2), 7.18–7.47 (m, 13H, 2′, 5′, 6′, 2″,3″, 4″, 5″, 6″, 2‴, 3‴, 4‴, 5‴, 6‴), 7.70 (d, H, Jo=8.4, H-6), 8.05 (d, H, Jo = 8.4, H-7), 8.25 (d, H, Jo = 1.6, H-4), 9.19 (br, 4H, $NH_{amidine}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.14, 25.37, 30.34, 56.80, 69.92, 70.09, 113.36, 113.94, 115.07, 119.75, 120.90, 121.35, 122.41, 122.57, 127.31, 127.53, 127.75, 127.82, 128.34, 128.36, 136.80, 136.88, 137.17, 142.61, 147.92, 149.71, 155.53, 165.72. MS (ESI+) *m/z*: 531.7 (M + H, 75%), 449.5 (100%). Anal. Calcd for $C_{34}H_{34}N_4O_2$·2HCl·1.4C_2H_6O·H_2O: C, 62.60; H, 7.25; N, 14.13. Found: C, 62.51; H, 7.58; N, 13.85.

1-cyclohexyl-2-(naphthalen-2-yl)-1H-benzimidazole-5-carboxamidine HCl (N11): Yield 18%, M.p. 206–210 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.17–1.42 (m, 3H, –CH_2 and –CH), 1.60–1.74 (d, H, –CH), 1.83 (d, 2H, –CH), 1.98 (d, 2H, –CH_2), 2.28–2.36 (m, 2H, –CH_2), 4.35–4.41 (m, H, N–CH), 7.62–7.68 (m, 2H, H-6′-7′), 7.84 (m, 2H, H-5′-8′), 8.05 (dd, 2H, Jo = 8.8, Jm = 2.4, H-3′,4′), 8.11 (dd, 2H, Jo = 8.0, Jm = 1.2, H-1′-6), 8.19 (d, H, Jm = 1.2, H-7), 8.27 (s, H, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.01, 25.26, 30.50, 56.97, 113.28, 120.15, 121.06, 121.73, 124.68, 125.19, 126.59, 127.21, 127.59, 128.38, 130.37, 131.60, 132.88, 136.75, 142.77, 165.79. MS (ESI+) *m/z*: 369.4 (M + H, 100%), 287.2 (100%). Anal. Calcd for $C_{24}H_{24}N_4$·2.25HCl·C_2H_6O·1.8H_2O: C, 59.03; H, 6.83; N, 10.59. Found: C, 59.48; H, 6.58; N, 11.00.

1-cyclohexyl-2-(naphthalen-1-yl)-1*H*-benzimidazole-5-carboxamidine HCl (N12): Yield: 18%, M.p. 268–272 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.20–1.40 (m, 3H, –CH_2 and –CH), 1.59 (d, H, –CH), 1.82 (d, 2H, –CH_2), 1.95–1.98 (t, 2H, –CH_2), 2.26–2.34 (m, 2H, –CH_2), 4.32–4.39 (m, H, N–CH), 7.61–7.66 (m, 2H, H-6′, 7′), 7.71–7.76 (m, 2H, H-2′, 3′), 8.00–8.11 (m, 4H, H-4′, 5′, 8′, 6), 8.19 (s, H, H-7), 8.25 (s, H, 4), 9.08 (br, 4H, $NH_{amidine}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.26, 25.47, 30.55, 56.98, 113.11, 118.92, 121.31, 125.26, 126.21, 127.63, 128.34, 128.53, 129.30, 132.45, 133.17, 136.13,142.94, 155.00, 164.85, 176.88. MS (ESI+) *m/z*: 369.2 (M + H, 70%), 287.2 (100%). Anal. Calcd for $C_{24}H_{24}N_4$·0.8HCl·C_2H_6: C, 70.38; H, 7.42; N, 12.63. Found: C, 70.54; H, 7.58; N, 13.02.

1-cyclohexyl-2-(1H-indol-3-yl)-1*H*-benzimidazole-5-carboxamidine HCl (N13): Yield 30%, M.p. 222–225 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.26–1.46 (m, 3H, –CH_2 and –CH), 1.66 (d, H, –CH), 1.87 (d, 2H, –CH_2), 1.95 (d, 2H, –CH_2), 2.30–2.51 (m, 2H, –CH_2), 4.61–4.67 (m, H, N–CH), 7.17 (dd, H, Jo = 7.6 Hz, Jm = 2 Hz, H-5′), 7.22–7.26 (m, H, H-4′), 7.55 (d, H, Jo = 8, H-7′), 7.66 (dd, H, Jm = 8, Jm = 2, H-6′), 7.84 (s, H, H-2′), 7.98 (d, H, Jo = 7.6, H-6), 8.04 (d, H, Jo = 8.8, H-7), 8.22 (d, H, Jm = 1.6, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.34, 25.57, 30.55, 56.56, 104.27, 112.05, 112.91, 118.66, 120.22, 121.77, 122.31, 126.57, 127.09, 136.14, 136.94, 143.60, 151.74, 166.29, 175.85. MS (ESI+) *m/z*: 358.5 (M + H, 100%), 276.3 (100%). Anal. Calcd for $C_{22}H_{23}N_5$·2HCl·0.3C_2H_6O·1.2H_2O: C, 58.27; H, 6.32; N, 15.03. Found: C, 58.51; H, 6.54; N, 15.38.

1-cyclohexyl-2-(1-methyl-1H-indol-3-yl)-1*H*-benzimidazole-5-carboxamidine HCl (N14): Yield: 25%, M.p. 225–230 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.15–1.23 (m, 3H, –CH_2 and –CH), 1.87 (d, 2H, –CH_2), 1.94(d, 2H, –CH_2), 2.31–2.39 (m, 3H, –CH_2 and –CH), 3.95 (s, 3H, –N–CH_3), 4.62–4.69 (m, H, N–CH), 7.20–7.23 (t, H, H-5′), 7.29–7.33 (m, H, H-6′), 7.59 (d, H, Jo = 8.4, H-4′), 7.66 (dd, H, Jo = 8, Jm = 1.6, H-7′), 7.85 (s, H, H-2′), 8.00–8.04 (m, 2H, H-6, 7), 8.21 (d, H, Jm = 1.6, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.32, 25.50, 30.53, 32.96, 56.48, 103.34, 110.40, 112.98, 118.70, 120.48, 120.54, 120.61, 121.72, 122.43, 126.88, 130.91, 136.65, 143.60, 151.37, 166.26, 175.65. MS (ESI +) *m/z*: 372.5 (M + H, 100%), 290.2 (100%). Anal. Calcd for $C_{23}H_{25}N_5$·2HCl: C, 61.49; H, 7.27; N, 14.64. Found: C, 61.82; H, 7.58; N, 14.31.

1-cyclohexyl-2-(benzofuran-2-yl)-1*H*-benzimidazole-5-carboxamidine HCl (N15): Yield 12% M.p. 256–258 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.43–1.52 (m, 3H, –CH_2 and –CH), 1.73 (s, H, –CH), 1.92 (d, 2H, –CH_2), 2.01 (d, 2H, –CH_2), 2.28–2.36 (q, 2H, –CH_2), 4.88–4.94 (m, H, N–CH), 7.37–7.41 (m, H, H-5′), 7.46–7.51 (m, H, H-6′), 7.65 (s, H, H-3′), 7.73–7.77 (m, 2H, H-4′, 7′), 7.83 (d, H, Jo=8, H-6), 8.10 (d, H, Jo = 8.8, H-7), 8.25 (d, H, Jm = 1.6, H-4). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 24.19, 25.44, 30.50, 57.35, 104.27, 112.05, 112.91, 118.66, 120.22, 121.77, 122.31, 126.57, 127.09, 136.14, 136.94, 143.60, 151.74, 166.29, 175.85. MS (ESI+) *m/z*: 359.5 (M + H, 85%), 277.4 (100%). Anal. Calcd for $C_{22}H_{22}N_4O$: C, 59.30; H, 6.89; N, 11.53. Found: H, 59.58; H, 6.45; N, 11.18.

Antimicrobial evaluation

In microbiological studies, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *C. albicans* ATCC 10231 standard strains and clinical isolates of these microorganisms known to be resistant to various antibiotics were used. Resistance states of isolates were investigated by Kirby Bauer Disk Diffusion method according to CLSI guidelines and resistant strains were studied (Wayne 2018). The synthesized result was prepared by DMSO for the stock solutions of the compounds. First, microorganisms were passaged by single colony cultivation method for purity and viability control. Bacteria were incubated on SDA plates at 24 °C for 24 h and mushrooms at 35 °C for 24–48 h at 37 °C. The resulting synthesized compounds were diluted in 96-well microplates in liquid medium (MHB or RPMI-1640) to give concentrations of 1024, 512, 256, 128, 64, 32, 16, 8 µg/ml. Reference antimicrobial drugs were diluted in liquid media (MHB or RPMI-1640) in 96-well microplates to achieve concentrations of 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 µg/ml. A bacterial susceptibility test was performed at the Mueller Hinton Broth (MHB) feeder. Bacterial suspensions to be used in the susceptibility test were prepared at a density of 10^6 cfu/ml, diluted with fresh broth from overnight culture at a density of 0.5 McFarland (10^8 cfu/ml). The bacterial suspension at a concentration of 10^6 cfu/ml was inoculated in 10 µl of wells containing 0.1 ml of diluted compounds. After inoculation, there are 10^5 cfu/ml bacteria in the wells. The microplates were incubated at 35 °C for 24 h. Fungal susceptibility testing was performed in RPMI-1640 medium buffered with pH 7 MOPS containing L-glutamine. For inoculation, the yeast suspension adjusted to a McFarland 0.5 concentration (10^6 cfu/ml) was diluted 1/100, followed by 1/20 dilution and inoculated to 0.9 ml in wells containing 1 ml of diluted compounds. After inoculation, there are 5×10^2 cfu/ml yeast in the wells. Microplates were incubated at 35 °C for 24–48 h.

Molecular docking studies

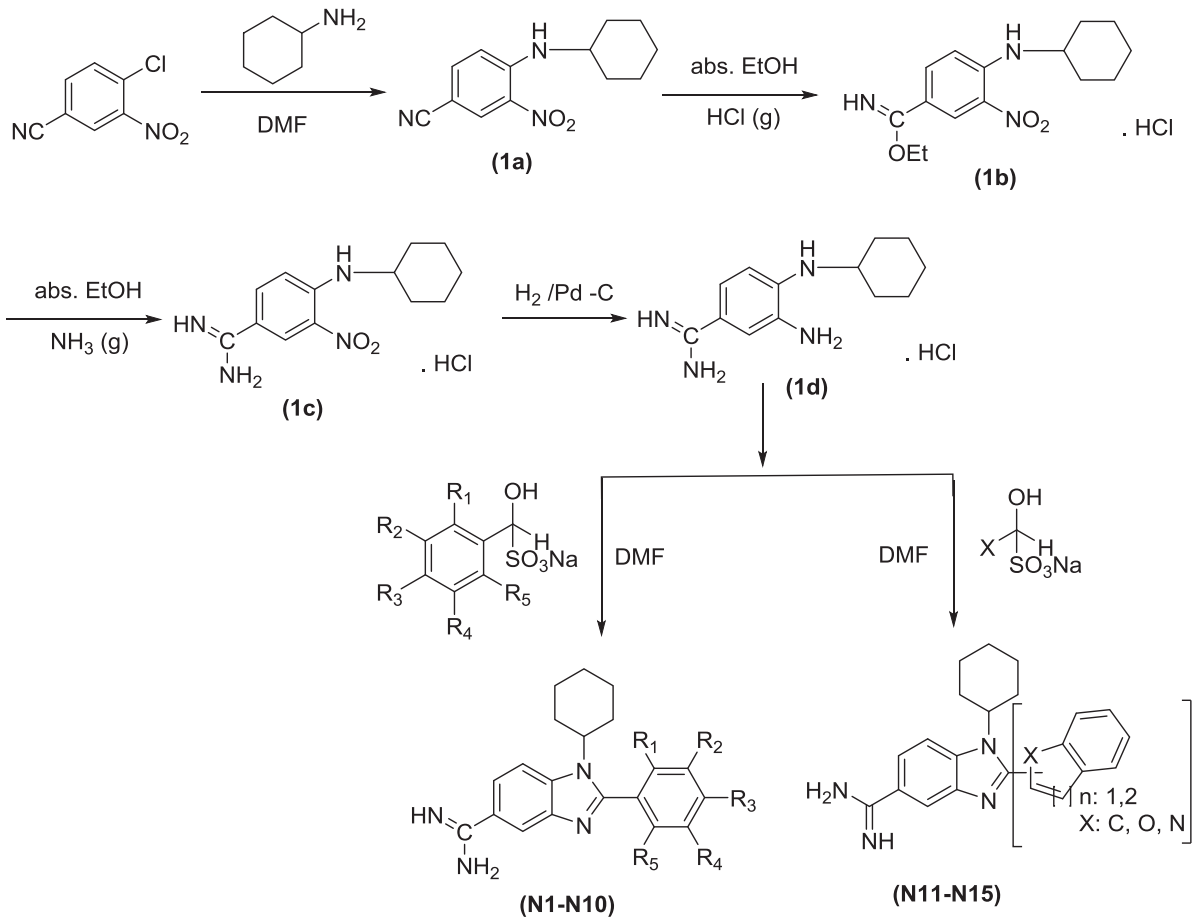
All computerized studies were performed using Maestro 10.5 suit software (Schrödinger 2018). Molecular docking studies were carried out in four stages: ligand preparation, protein preparation, grid creation, and docking. The structure of the compounds was plotted using ChemDraw 17.1. LigPrep module was used to prepare ligands. OPLS3 was preferred for field forces. Ionization was carried out using Epik in the range of possible pH: 7.00 ± 2.00, desalted and tautomer was formed. Protein preparation was carried out in three stages using Protein Preparation Wizard module. Import and Process, Review and Modify, and Refine. Crystal structure of *S. aureus* PBP2a in complex with Quinazolinone (PDB ID: 4CJN at 1.95 Å resolution) and crystal structure of *E. faecalis* PBP4 in the ceftaroline-bound ((PDB ID: 6MKI at 2.98 Å resolution) was imported from protein data bank (https://www.rcsb.org/). Hydrogens were added, non-bonding command with metals, formation of disulfide bonds, deletion of water at 5 Å distance from het groups and preprocess by creating pH: 7.00 ± 2.00 het states using Epik. In the second step, the appropriate chain was selected. Water molecules and other structures other than ligands were deleted. pH: 7.00 ± 2.00 regenerate state was created with S2. In the third step, the Hydrogen bond determination was optimized using PROKA pH: 7.00 with water sample orientation. Finally, protein was prepared by minimizing OPLS3 field forces. Receptor Grid Generation was used to binding site: for the 4CJN allosteric site *x*: 45.05, *y*: 44.42, and *z*: 7.36 coordinates, for the 4CJN active site *x*: -35.89, *y*: −12.57, and *z*: −23.65 coordinates and for the 6MKI active site *x*: 35.24, *y*: 1.34, and *z*: 10.06 coordinates was created in 20 × 20 × 20 Å. Docking with XP (extra precision) was performed using a previously prepared, ligand, protein, and grid box by using Glide Ligand Docking module. Ligands were flexible specials and added Epik states penalties to docking score. Glide emodel and XP Gscore values of all compounds were calculated and two- and three-dimensional ligand–protein interactions were determined (Erol et al. 2020).

MM-GBSA free energy calculations

MM-GBSA is a calculation method used to estimate protein–ligand free energies. Calculations were made using the Schrödinger Prime module. It is calculated by the formula $\Delta G\text{bind} = E\text{complex}$ (minimized) − [Eligand (unbound, minimized) + Ereceptor (unbound, minimized)]. The ligands and protein construct were selected from the XP implantation performed earlier. VSGB was used as the solvent model and OPLS3 was used as the Force Field. MM-GBSA (dG Bind) value of all compounds was calculated (Genheden and Ryde 2015).

Theoretical ADME prediction

The Schrodinger QikPprop module (Release 2018) was used to determine the "absorption, distribution, metabolism, and excretion" profiles of the compounds, and some important parameters were selected. Molecular weight, number of recipient and donor hydrogen bonds, estimated octanol/water distribution coefficient, predicted human oral absorption, Van der Waals surface area, number of violations of Lipinski's five rules, and number of violations of Jorgensen's three defining feature rules were calculated.



Scheme 1 Synthesis pathways of the target compounds

Results and discussion

Chemistry

Target compounds were synthesized in 5 steps according to the method specified in the literature (Alp et al. 2009; Alp et al. 2014; Ates-Alagoz et al. 2006; Goker et al. 2005; Karataş et al. 2012). Compounds **N1**–**N15** were prepared using the methods outlined in Scheme 1. **1a** was prepared by the reaction of 4-chloro-3-nitrobenzonitrile and cyclohexylamine in the presence of DMF. Then, imidate esters (**1b**) from the cyano group were formed by the Pinner reaction method. The obtained and unstable imidate esters were rapidly converted to the amidine group (**1c**) with NH_3/EtOH, and the nitro group was reduced by Pd/C catalyzed hydrogen gas to obtain the amine group (**1d**). The targeted result products (**N1**–**N15**) were obtained by reaction of **1d** with $Na_2S_2O_5$ additive products of aldehydes in DMF. Some of the derivatives were purified by column chromatography (chlorofom: methanol:ammonia (10:1:0.1) solvent mixture), while some were re-crystallized from methanol. After the purity controls of the synthesized compounds (TLC studies) and their melting points were determined, spectral analyzes were performed to prove the structures. ^{1}H-NMR, ^{13}C-NMR, mass spectral analysis, and elemental analysis were found to prove the expected structures. The ^{1}H-NMR and ^{13}C-NMR spectra of the compounds were taken by dissolving in DMSO-d_6. Since these solvents are not 100% deuterated and contain 98–99.8% deuterium, there are also peaks of non-deuterated forms of solvents in the spectra. The peak observed in the range of 3.25 to 3.65 ppm of the compounds dissolved with DMSO-d_6 is due to the non-deuterated DMSO in the solvent. In addition, the peaks observed in the range of 2.20–2.40 ppm in the analysis made with DMSO-d_6 are the peaks from the water in the solvent. 1-Cyclohexylamino-2-substitue-5-carboxamidine compounds have aromatic protons between 6.85 and 8.35 ppm, and $-CH_2$ protons in cyclohexyl are between 1.14 and 1.52 ppm (protons overlapped-net unallocated), and the cyclohexyl –CH of benzimidazole bound to the N atom is 3.84–4.94 ppm was also observed. The methyl protons attached to the phenyl ring at the 2nd position of the benzimidazole are between

Table 1 In vitro antimicrobial MIC values (μg/mL) of synthesized compounds (**N1–N15**) and reference drugs

Compound	A	B	C	D	E	F	G	H	I
N1	256	128	64	64	16	16	16	16	16
N2	128	128	64	64	64	128	128	128	128
N3	128	128	128	128	512	512	64	64	512
N4	32	32	128	128	32	32	32	32	128
N5	128	128	64	64	8	16	32	16	32
N6	32	32	128	128	8	**8**	16	16	32
N7	32	32	128	128	8	**8**	8	**8**	>512
N8	32	32	128	128	8	16	32	32	64
N9	128	128	64	64	16	16	16	16	64
N10	128	128	64	64	8	**8**	8	**8**	16
N11	32	32	128	128	8	**8**	8	**8**	64
N12	128	128	64	512	512	512	512	512	512
N13	128	128	64	64	8	**8**	8	**8**	64
N14	128	128	128	128	8	**8**	32	32	64
N15	128	128	64	64	8	**8**	16	16	16
Vancomycin	–	–	–	–	<0.0625	<0.0625	<0.0625	**>8**	–
Ampicillin	2	2	–	–	2	**>8**	2	**>8**	–
Meropenem	<0.0625	<0.0625	0.5	0.5	<0.0625	**>8**	–	–	–
Ciprofloxacin	<0.0625	<0.0625	1	2	0.5	0.5	2	2	–
Gentamicin	0.25	0.25	0.5	>8	1	2	–	–	–
Amphotericin B	–	–	–	–	–	–	–	–	0.5

Note: A: *E. coli* ATCC 25922, B: *E. coli* isolate, C: *P. aeruginosa* ATCC 27853, D: *P. aeruginosa* isolate, E: *S. aureus* ATCC 29213, F: *S. aureus* isolate (MRSA), G: *E. faecalis* ATCC 29212, H: *E. faecalis* isolate (VREF), I: *C. albicans* ATCC 10231. *E. coli* isolate is susceptible to the tested antimicrobial agents. *S. aureus* isolate is a methicillin-resistance (MRSA) isolate. Antibacterial drugs are not tested against fungi, while antifungal drugs are not tested against bacteria. *P. aeruginosa* is naturally resistant to ampicillin. Gram (−) bacteria used in this study are resistant to vancomycin

Bold values indicates the most effective compounds compared to standard drugs

2.09 and 2.36 ppm, the methoxy protons are singlets, 3.79–3.86 ppm. Among the protons belonging to benzyl-CH_2, the singlet was observed between 5.20 and 5.23 ppm. While the protons belonging to the amidine group were observed as 9.04–9.34 ppm in some spectra, in the form of a large singlet 4H, in some spectra as 2H and 2H singlet peaks, in some spectra it could not be observed.

MASS spectral analysis, electrospray ionization (ESI) method and molecular ion peak was observed as M + H. M + H + 2 was also observed in compounds containing chlorine atoms. The C, H, and N percentages of the synthesized compounds were found, and these findings were compared with the theoretical calculations to determine the purity of the compounds and the amount of water, ethanol and HCl contained in them, and the amount of C, H, and N elements in all compounds were within the limits of ±0.4%.

In vitro antimicrobial activities

Antimicrobial effect determinations of all synthesized compounds (**N1**–**N15**) were determined in vitro by microdilution technique. Vancomycin, ampicillin, meropenem, ciprofloxacin, and gentamicin were used as reference drugs, and amphotericin B against fungi. The antimicrobial effects of compounds and reference drugs in the form of MIC values were determined and are given in Table 1. According to the microbiological results, when the effects of derivatives against *E. coli* and *E. coli* isolate are examined; it was determined that it is sensitive to ampicillin, meropenem, ciprofloxacin, and gentamicin, and reference drugs have a much better effect than benzimidazole derivatives.

Compounds generally had a weak antimicrobial effect against Gram-negative *P. aeruginosa* ATCC 27853 and *P. aeruginosa* isolate (MIC: 64–128 μg/ml—except **N12**). More than half of the compounds against *S. aureus* ATCC 29123 showed good activity with MIC: 8 μg/ml, while showing weaker efficacy compared to reference drugs. The results of the **N6**, **N7**, **N10**, **N11**, **N13**, **N14**, and **N15** derivatives against MRSA (MIC: 8 μg/ml) are quite satisfactory compared to ampicillin and meropenem. The compounds showed a broad range of activity against the Gram-positive bacterium *E. faecalis* ATCC 29212 (MIC: 8–512 μg/ml) compared to the reference drugs. Against VREF; **N7**, **N10**, **N11**, and **N13** derivatives against VREF showed better efficacy than vancomycin and ampicillin with MIC: 8 μg/ml. The antifungal activities of the

compounds against *C. albicans* were in the range of 8–>512 µg/ml and much less effective than amphotericin B.

When the microbiology findings were evaluated in general, it was found that the tested derivatives showed more important antimicrobial activity against Gram-positives than Gram-negatives, and the best antibacterial activity was observed against MRSA and VREF. The methyl substituent of the benzimidazole in the 2nd position, which gave electrons to the ring on the phenyl ring, had no positive effect on activity. In addition, it has been observed that the fluorine substituent has low activity. Instead, it has been found that substitution with electron withdrawal groups such as 4-chlorine and 3,4-dibenzyloxy increases antimicrobial activity. The fact that the chlorine group becomes ortho or meta decreases the activity. Also, the presence of heterocyclic rings at 2nd position of the benzimidazole contributed positively to activity. While 2-naphthyl showed significant antimicrobial activity, 1-naphthyl group did not. While the indol-3-yl substituent is effective against both MRSA and VREF, the anti-VREF activity of the 1-methylindol-3-yl substituent is weaker.

Results of molecular docking

Molecular docking techniques are often used to investigate how drug or drug candidates and enzyme, nucleic acid, and receptor proteins fit together in computer-aided rational drug design. In these docking studies, binding energies to the receptor with a clear three-dimensional structure can be determined and the position of the ligand in the binding site of the receptor can be revived. This can be useful for understanding the type of binding and designing molecule and more compatible ligands that target proteins (Celik et al. 2020). Also, MM-GBSA is a method used to estimate free binding energies, requires less calculation than free energy methods, and is often reported to give more accurate results than the molecular docking scoring function. MM-GBSA is widely used in biomolecular studies such as protein–ligand binding and protein–protein interaction (Wang et al. 2019).

In this study, some of the synthesized compounds exhibited higher activity against MRSA and VREF than reference drugs. As mentioned in the "Introduction", methicillin resistance in *S. aureus* results from a different synthesis of PBP called PBP2 or PBP2a, while decreased sensitivity to β-lactam antibiotics in *E. faecalis* was due to expression of PBP4. So we focused on PBP2a and PBP4 resistance proteins (PDB: 4CJN and 6MKI). In order to ensure the validation of the docking process, the ligands were removed from the protein–ligand complex crystal structure and re-docking was performed on these regions. In 4CJN allosteric site gave RMSD = 0.068 and in the 6MKI active site gave RMSD = 0.053 value. The low of these RMSD values indicates that the docking process predicts fairly accurate binding exposure. According to the results of the docking studies performed in the 4CJN allosteric site, it was concluded that the target compounds may act by binding to the 4CJN allosteric site, since the Glide emodel scores of most compounds were calculated lower than the active site. The calculated Glide emodel, MM-GBSA (dG Bind) and XP Gscore scores of all compounds were given in Table 2. **N10** gave the lowest Glide emodel scores against both MRSA (Glide emodel: −57.901) and VREF (Glide emodel: −82.278).

According to antimicrobial activity results, 2D and 3D interactions of **N10**, one of the most effective compounds (also, Glide emodel score is lowest in both sites), were shown in both sites. In the PBP2a allosteric site, it formed a 1-Å long hydrogen bond between -NH_2 and GLN613 in the amidine group. It also showed hydrophobic interactions with MET641, ALA642, ALA646, TYR446, VAL578, VAL579, TYR588, and PHE617. 2D and 3D interactions of **N10** in this site were shown in Fig. 2.

In the PBP4 binding site, it formed two separate hydrogen bonds between the length of 1.83 and 2.08 Å between the NH_2 and GLN542 in the amidine group, and a hydrogen bond between the 4th position benzyloxy oxygen and THR620. **N10**; it also showed hydrophobic interactions with TYR605, ALA664, PHE638, TYR540, and VAL467 in this region. Figure 3 was showed 2D and 3D interactions of **N10** in the PBP4 active site.

Theoretical ADME prediction

In the process of drug discovery and development, molecules are asked to show high biological activity with low toxicity. ADME parameters can be estimated by in silico methods using the molecular structure. Therefore, in silico studies allow us to learn about the possibility of a compound being a potentially good drug. Access to the therapeutic goal and concentration in the organism is equally important. Early estimation of ADME parameters greatly reduces the rate of pharmacokinetic failure in clinical phases during the discovery phase. It is increasingly accepted to predict ADME parameters in cases where numerous compounds are screened for ADME or access to physical samples is limited. Estimates of ADME properties of all synthesized compounds (**N1**–**N15**) were made with QikProp. According to Lipinski's five rules and Jorgensen's three rules, drug-like compounds should not have more than one violation. Data of the compounds such as molecular weight, logP, % oral absorption, polar surface area, hydrogen donor number, hydrogen acceptor number, and volume are given in Table 3 together with the violation of the third and fifth rules. From the table, it can be seen that **N10** violates the Lipinski rule, but all other compounds comply with these rules.

Table 2 Calculated Glide emodel, MM-GBSA (dG Bind) and XP Gscore values of compounds and reference drugs

Comp.	Allosteric site			4CJN Active site			6MKI Active site		
	Glide emodel	MM-GBSA (dG Bind)	XP Gscore	Glide emodel	MM-GBSA (dG Bind)	XP Gscore	Glide emodel	MM-GBSA (dG Bind)	XP Gscore
N1	−31.147	−44.73	−4.251	−28.268	−32.19	2.316	−46.007	−65.42	−7.025
N2	−37.561	−33.84	−3.637	−33.081	−46.76	−2.483	−45.627	−56.69	−7.251
N3	−36.411	−34.14	−4.130	−35.354	−34.69	−2.628	−45.832	−54.97	−4.544
N4	−40.164	−41.08	−3.863	−40.964	−42.24	−2.624	−52.641	−70.82	−7.697
N5	−38.393	−39.58	−3.960	−41.035	−47.17	−3.048	−50.017	−62.54	−5.382
N6	−39.582	−44.66	−3.974	−34.156	−52.56	−2.347	−54.093	−66.58	−7.504
N7	−37.376	−38.92	−3.143	−36.517	−33.04	−0.439	−50.499	−64.67	−5.033
N8	−39.803	−44.70	−3.599	−42.160	−44.08	−2.925	−57.377	−72.92	−6.663
N9	−39.653	−40.74	−3.329	−40.385	−40.30	−3.155	−53.660	−58.48	−5.185
N10	−57.901	−36.41	−2.931	−50.826	−48.09	−3.615	−82.278	−70.96	−8.093
N11	−39.190	−39.20	−3.344	−23.204	−34.47	−2.978	−54.775	−63.50	−7.678
N12	−35.178	−44.68	−4.411	−34.027	−28.56	−1.940	−53.463	−59.91	−5.437
N13	−40.638	−28.90	−4.004	−29.064	−21.04	−4.275	−51.264	−51.49	−5.435
N14	−33.487	−38.34	−1.843	−27.547	−22.87	−4.352	−46.410	−59.43	−5.541
N15	−40.814	−38.95	−3.695	−21.147	−10.82	−3.615	−49.778	−52.98	−5.017
Vancomycin	−75.607	−57.85	−6.233	−70.037	−30.43	−5.487	−70.737	−95.36	−6.770
Ampicillin	−36.564	−27.08	−3.589	−32.240	−32.61	−3.719	−43.251	−25.30	−4.936
Gentamycin	−31.879	−40.88	−3.410	28.645	−17.92	−2.183	−77.755	−68.84	−2.082
Meropenem	−40.453	−44.19	−4.556	−37.238	−32.54	−6.472	−48.571	−46.11	−5.184
Ciprofloxacin	−32.610	−37.05	−2.678	−47.965	−54.78	−5.998	−46.662	−52.33	−5.216

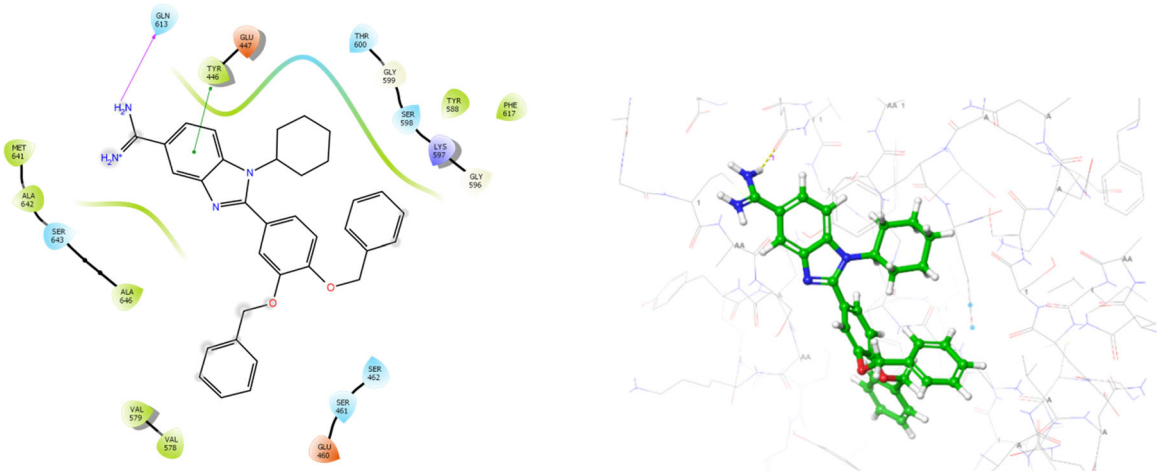


Fig. 2 2D and 3D interaction between **N10** with PBP2a (4CJN) at allosteric site

Conclusions

In this study, a series of 1*H*-benzimidazole-5-carboxamidine derivative compounds that we hope might be a new antimicrobial agent were designed, synthesized and their antimicrobial activities were determined. **N7**, **N10**, **N11**, and **N13** derivatives were observed more effectively compared to reference drugs against MRSA and VREF with MIC: 8 µg/ml. Derivatives showed better antibacterial activity against Gram-positives than Gram-negatives; It was observed that especially the binding of heterocyclic rings to the 2 position of benzimidazole or groups that attract electrons to the

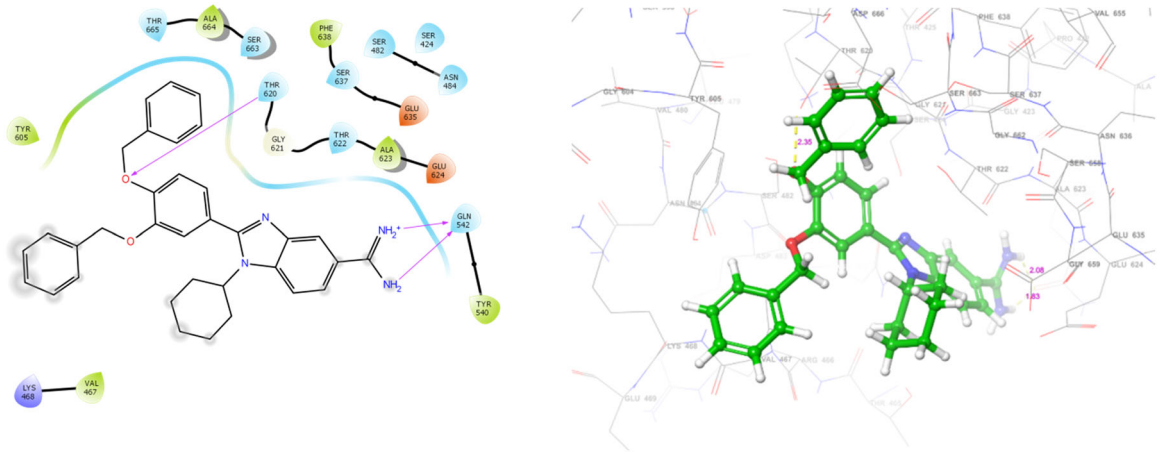


Fig. 3 2D and 3D interaction between **N10** with PBP4 (6MKI) at active site

Table 3 Calculated ADME parameters **(N1–N15)**

Comp.	MW	DonorHB	AccptHB	QPlogPo/w	QPlogS	% HOA	PSA	RuleOfFive	RuleOfThree
N1	336.411	3.000	3.000	3.954	−5.654	100.000	63.589	0	0
N2	378.473	3.000	4.500	3.954	−5.631	100.000	78.638	0	0
N3	354.402	3.000	3.000	4.132	−5.844	100.000	63.707	0	1
N4	352.866	3.000	3.000	4.071	−5.624	100.000	63.836	0	0
N5	352.866	3.000	3.000	4.196	−5.968	100.000	63.561	0	1
N6	387.311	3.000	3.000	4.636	−6.638	100.000	63.608	0	1
N7	387.311	3.000	3.000	4.564	−6.362	100.000	63.838	0	1
N8	387.311	3.000	3.000	4.452	−6.073	100.000	62.805	0	1
N9	397.863	3.000	4.000	3.565	−6.129	82.570	107.732	0	1
N10	**530.668**	**3.000**	**4.500**	**7.602**	**−9.954**	**95.039**	**76.852**	**2**	**1**
N11	368.480	3.000	3.000	4.643	−6.424	100.000	63.511	0	1
N12	368.480	3.000	3.000	4.567	−6.136	100.000	63.597	0	1
N13	357.457	4.000	3.000	3.864	−5.744	95.364	76.216	0	1
N14	371.484	3.000	3.000	4.684	−6.621	100.000	64.809	0	1
N15	358.442	3.000	3.500	4.038	−5.870	100.000	72.567	0	1

Donor HB: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (recommended value: 0–6); acceptor HB: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (recommended value: 2–20); QPlogPo/w: Predicted octanol/water partition coefficient (recommended value: −2–6.5); QPlogS: Predicted aqueous solubility (recommended value: −6.5–0.5); % HOA: percent of human oral absorption; PSA: Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (recommended value: 7–200); RuleOfFive: Number of violations of Lipinski's rule of five. The rules are: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, and accptHB ≤ 10. Compounds that satisfy these rules are considered druglike (the "five" refers to the limits), RuleOfThree: Number of violations of Jorgensen's rule of three. The three rules are: QPlogS > −5.7, QP PCaco > 22 nm/s, and # Primary Metabolites < 7

Bold values indicates the compound that violated the Lipinski rule

phenyl ring significantly increased antimicrobial activity. Molecular docking studies of all compounds and reference drugs were conducted and when the scores of all compounds were examined, a good relationship was found between Glide emodel scores and antimicrobial effect. 2D/3D interactions of **N10**, which is one of the most effective antimicrobial compounds and also has the highest Glide emodel score, were demonstrated in the PBP2a allosteric site and PBP4 active site. According to all these results, the synthesized compounds showed promising antimicrobial activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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