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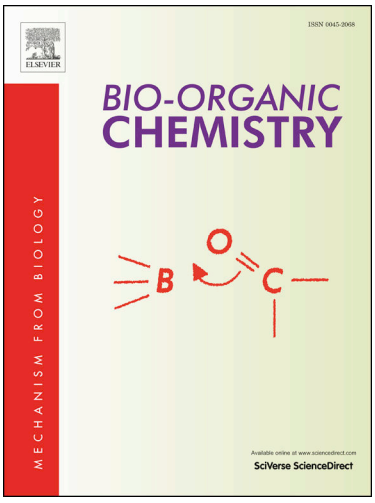

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Synthesis and Molecular Docking Studies of Some Novel Antimicrobial Benzamides

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Abstract

Common use of classical antibiotics has caused to the growing emergence of many resistant strains of pathogenic bacteria. Therefore, we aimed to synthesize a number of *N*-(2-hydroxy-(4 or 5)-nitrophenyl)benzamide derivatives as a new class of antimicrobial compounds. Moreover, our second goal is to predict the interaction between active structures and enzymes (DNA – gyrase and FtsA) in the binding mode. In this study, thirteen *N*-(2-hydroxy-(4 or 5-nitrophenyl)-substituted-benzamides were synthesized and determined for their antimicrobial activity using the microdilution method. According to this work, none of the compounds showed any activity against *Candida albicans* and its clinical isolate. Some of the benzamides (**4N1, 5N1, 5N2**) displayed very significant activity against *Staphylococcus aureus* and MSSA with <4 µg/ml MIC value, even they were found to be more potent than ceftazidime. **4N1** was also found to be more effective than gentamicin against *Enterococcus faecalis* clinical isolate. Molecular docking studies revealed that **4N1**, **5N1**, and **5N2** showed a good interactions with DNA-gyrase. Moreover, **5N1** has interacted with FtsA enzyme in the binding mode, as well. Only compound **5N4** displayed very good activity against *Escherichia coli* ATCC 25922. These findings showed us that **4N1, 5N1, 5N2,** and **5N4** could be lead compounds to discover new antibacterial candidates against multidrug-resistant strains.

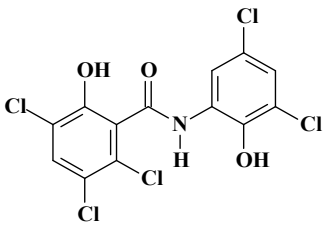
Keywords: Benzamide; Antimicrobial activity; Molecular docking; DNA-gyrase; FtsA

1. Introduction

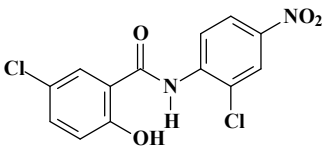
The count of multidrug-resistant bacterial infections such as vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) is enhancing at a worrying percentage and it causes mortality and morbidity in hospitals. Researching of new antibacterial agents is very important by virtue of enhancing resistance of clinically substantial pathogens to know classes of antibiotics [1]. MRSA indicates resistance to β-lactams, methicillin, macrolides, fluoroquinolones, oxazolidinones, glycopeptides, and carbapenems [2], [3], [4], [5], [6]. Development of potent and novel classes of antibacterials having new mechanisms of action play a vital role to alleviate the complications related to multidrug-resistant bacterial infections.

The benzamide compounds are characterized by a relatively wide range of pharmacological properties such as clinically used in the treatment of gastric diseases, intestinal pseudo-obstruction, characterized by anticonvulsant action, antibacterial, anthelmintic, antifungal, antiviral, anticancer, and HDAC (Histone deacetylase) inhibitors [7], [8], [9], [10], [11], [12], [13], [14].

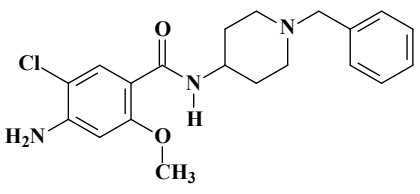
Oxyclozanide [15], an anthelmintic agent which treats liver fluke infection for *Fasciola hepatica*, and an anti-tapeworm drug niclosamide (*N*-(2-hydroxy-5-chlorophenyl)-2-chloro-4-nitrobenzamide) [16] are benzamide derivatives (Fig. 1). It has been recently displayed that niclosamide had anticancer activity [16] and strong *in vitro* and *in vivo* effects against MRSA [17]. Another benzamide drug is clebopride (Fig. 1) which is a dopamine antagonist with antiemetic and prokinetic properties used to treat functional gastrointestinal disorders [18]. In 1999, a natural antibacterial benzamide derivative, 3,4-dihydroxy-6-(*N*-ethylamino)benzamide, has been found in the green pepper (*Piper nigrum* L.) [19]. Moreover, *N*-methyl-3-[2-(2-naphtyl)acetylamino]benzamide called as BAS-118 (Fig. 1) is shown to have selective and strong activity against *Helicbacter pylori*. It was also reported to be effective against clarithromycin- and metronidazole -resistant isolates [20].



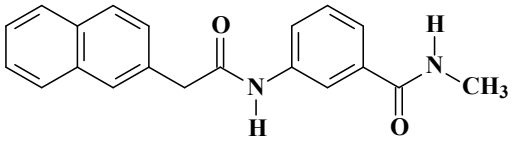
Oxyclozanide



Niclosamide



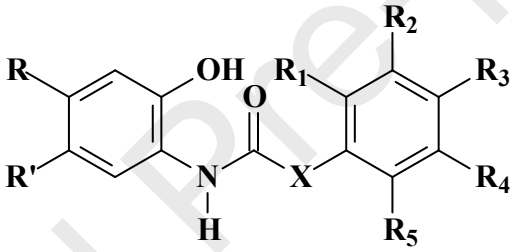
Clebopride



BAS-118

Fig. 1. Some of the benzamide derivatives

Lately, we informed that some substituted benzamide / phenylacetamide / phenoxyacetamide / thiophenoxyacetamide derivatives displayed significantly antimicrobial activities at a MIC values between 1.95 - 250 µg/ml (Fig. 2) [21], [22], [23], [24]. According to these studies, it has been realized that compounds bearing a nitro instead of an amine group attached at the 4th or 5th position of *N*-(2-hydroxyphenyl) of phenylacetamide or benzamide was important for the antibacterial activity [24].



X= –, CH_2, CH_2O, CH_2S; **R** = H, CH_3, NO_2, NH_2; **R´** = H, Cl, CH_3, NO_2, NH_2;
R_1 = H, OCH_3; **R_2**= H, OCH_3; **R_3** = H, Cl, Br, F, CH_3, C_2H_5, NO_2, OCH_3, $C(CH_3)_3$;
R_4 = H, OCH_3; **R_5** = H, CH_3, OCH_3

Fig. 2. Previously synthesized antimicrobial amide derivatives

In 2008, the QSAR study of some phenylacetamides and benzamides against drug-resistant *S. aureus* was done by us [25]. In there, we declared that having benzamide structure instead of phenyl acetamide played more remarkable role for enhancing activity for drug-resistant *S. aureus*.

Since the need of novel antimicrobial agents for the multi-drug resistant microorganisms is the ultimate goal of our ongoing researches, we designed and synthesized a number of *N*-(2-hydroxy-(4 or 5)-nitrophenyl)-substituted-benzamides (see Table 1) as a new group of antibacterial molecules. Their antibacterial activities were evaluated for *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, along with their clinical isolates. Besides, they were

also tested for their antifungal activities on *Candida albicans* and its clinical isolate. All of the antimicrobial results of the tested benzamides was compared to some standard drugs.

In the field of drug discovery and design, molecular docking studies have become a progressively crucial tool in order to understant how to interact a ligand with a protein in the binding mode. The other purpose of this article was to explore the interactions of active compounds against *S. aureus* and its isolate into the DNA-gyrase and FtsA enzymes by using molecular docking studies employing CDOCKER method at the Discovery Studio (DS) 3.5 [26].

2.Materials and Methods

2.1. Chemistry

All of the chemicals were used without further purification and purchased from the commercial venders. Thin layer chromatography (TLC) was applied for monitoring the reactions and checking the purity of the last products using Silica gel 60 F254 (Merck TLC plates) chromatoplates. The mixture of chloroform/methanol (30:1) as a solvent system of TLC was used for all benzamide derivatives. The plates were visualized using UV light. All the melting points were measured on Büchi B-540 capilalry melting point apparatus and are uncorrected. Mass spectra for compounds were taken on a Waters Micromass ZQ by using ESI (+) or ESI (-) method. The ^{1}H (400 MHz) and ^{13}C (100MHz) Nuclear Magnetic Resonance spectroscopy were taken using a Varian Mercury 400 MHz FT-NMR, chemical shifts (δ) were in ppm relative to TMS, and coupling constants (J) were informed in Hertz. Elemental analyses (C, H, N) of compounds were recorded on a Leco CHNS-932. All elemental analyses results of newly synthesized benzamide derivatives were found to be within ± 0.4% of the computed amounts.

*2.1.1. General synthetic procedure of N-(2-hydroxy-(4 or 5)-nitrophenyl)benzamides (**4N1-4N6, 5N1-5N7**)*

The synthesis of all benzamide derivatives were done by using the method shown on the literature [24]. Suitable benzoic acid (0.5 mmol) with thionyl chloride (1.5 ml) were refluxed at 80 °C in benzene (5 ml) for 3 h. Afterwards, the excess thionyl chloride was evaporated *in vacuo*. Then, the residue was solved in ether (10 ml). This solution added during 1 h to a stirred, ice-cold mixture of suitable *o*-aminophenol (2-amino-(4 or 5)-nitrophenol) (0.5 mmol), water (10 ml), diethyl ether (10 ml), and sodiumbicarbonate (0.5 mmol). The mixture was kept stirred at the room temperature overnight, then filtered. Afterwards the residue

was washed by using water, 2 N HCl, water, and ether, respectively, and finally benzamide derivatives were achieved (Scheme 1). The obtained crude benzamides were recrystallized using the ethanol in order to purify. All of the obtained crystals were dried *in vacuo*. Physical and spectral datas of the newly synthesized benzamides were reported below (see also Supplementary Information 1).

4-Butyl-N-(2-hydroxy-4-nitrophenyl)benzamide (**4N1**)

Yield 41 %; M.p. 195-196°C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 0.90 (t, 3H, CH_3); 1.27-1.36 (m, 2H, CH_2); 1.55- 1.62 (m, 2H, CH_2); 2.67 (t, 2H, CH_2); 7.37 (d, 2H, J_o=8.4, 3-H, 5-H); 7.73 (d, 1H, J_m=2.8, 3'-H); 7.79 (dd, 1H, J_m=2.8 and J_o=9.2, 5'-H); 7.89 (d, 2H, J_o=8, 2-H, 6-H); 8.25 (d, 1H, J_o=9.2, 6'-H); 9.49 (s, 1H, OH); 11.13 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 13.718 (CH_3-1C), 21.681 (CH_2-1C), 32.787 (CH_2-1C), 34.646 (CH_2-1C), 109.422, 115.064, 121.335, 127.631, 128.574, 131.286, 133.126, 143.212, 147.098, 148.085, 165.160 (11C-Ar). MS (ESI -) m/z: 313.6 (M - H, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_4 \cdot 0.1H_2O$: C: 64.58, H: 5.802, N: 8.861. Found: C: 64.55, H: 5.860, N: 8.804.

4-(tert-Butyl)-N-(2-hydroxy-4-nitrophenyl)benzamide (**4N2**) [24]

Yield 34 %; M.p. 283-286°C (283-284°C) [24]. MS (ESI +) m/z: 315.3 (M + H, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C: 64.95, H: 5.771, N: 8.911. Found: C: 65.04, H: 5.949, N: 8.865.

4-Ethoxy-N-(2-hydroxy-4-nitrophenyl)benzamide (**4N3**)

Yield 55 %; M.p. 258-259°C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 1.33 (t, 3H, CH_3); 4.10 (q, 2H, OCH_2); 7.04 (d, 2H, J_o=9.2, 3-H, 5-H), 7.70 (d, 1H, J_m=2.8, 3'-H), 7.76 (dd, 1H, J_m=2.4 and J_o=8.8, 5'-H); 7.92 (d, 2H, J_o=8.4, 2-H, 6-H); 8.20 (d, 1H, J_o=8.8, 6'-H); 9.40 (s, 1H, OH); 11.07 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 14.487 (CH_3-1C), 63.474 (OCH_2-1C), 109.422, 114.301, 115.090, 121.245, 125.676, 129.606, 133.287, 143.084, 147.996, 161.672, 164.673 (11C-Ar). MS (ESI +) m/z: 303.4 (M + H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_5$: C: 59.60, H: 4.668, N: 9.267. Found: C: 59.57, H: 4.856, N: 9.258.

4-Butoxy-N-(2-hydroxy-4-nitrophenyl)benzamide (**4N4**)

Yield 53 %; M.p. 200-202°C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 0.91 (t, 3H, CH_3); 1.37-1.47 (m, 2H, CH_2); 1.66-1.73 (m, 2H, CH_2); 4.03 (t, 2H, OCH_2); 7.04 (d, 2H, J_o=9.2, 3-H, 5-H); 7.70 (d, 1H, J_m=2.8, 3'-H); 7.76 (dd, 1H, J_m=2.8 and J_o=8.8, 5'-H); 7.91 (d, 2H, J_o=9.2, 2-H, 6-H); 8.21 (d, 1H, J_o=8.4, 6'-H); 9.39 (s, 1H, OH); 11.08 (s, 1H, NH). ^{13}C-NMR δ ppm

(DMSO-d_6): 13.654 (CH_3-1C), 18.668 (CH_2-1C), 30.581(CH_2-1C), 67.514 (OCH_2-1C), 109.422, 114.340, 115.103, 121.194, 125.669, 129.593, 133.293, 143.071, 147.957, 161.852, 164.666 (11C-Ar). MS (ESI +) m/z: 331.5 (M + H, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_5 \cdot 0.1H_2O$: C: 61.47, H: 5.523, N: 8.434. Found: C: 61.44, H: 5.658, N: 8.522.

N-(2-Hydroxy-4-nitrophenyl)-3,5-dimethylbenzamide (**4N5**)

Yield 64 %; M.p. 268-270°C. ^1H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 2.35 (s, 6H, CH_3); 7.25 (s, 1H, 4-H); 7.56 (s, 2H, 2-H, 6-H); 7.73 (d, 1H, J_m=2.8, 3'-H); 7.78 (dd, 1H, J_m=2.8 and J_o=8.8, 5'-H); 8.23 (d, 1H, J_o=8.8, 6'-H); 9.44 (s, 1H, OH); 11.09 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 20.793 (CH_3-2C), 109.445, 115.061, 121.348, 125.219, 133.075, 133.502, 133.868, 137.922, 143.218, 148.049, 165.492 (11C-Ar). MS (ESI +) m/z: 287.3 (M + H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_4 \cdot 0.1H_2O$: C: 62.53, H: 4.96, N: 9.72. Found: C: 62.39, H: 5.207, N: 9.72.

N-(2-Hydroxy-4-nitrophenyl)-3,5-dimethoxybenzamide (**4N6**) [22]

Yield 43 %; M.p. 265-267°C (259°C) [22]. MS (ESI +) m/z: 319.5 (M + H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_6 \cdot 0.4H_2O$: C: 55.35, H: 4.583, N: 8.606. Found: C: 55.40, H: 4.572, N: 8.624.

4-Ethyl-N-(2-hydroxy-5-nitrophenyl)benzamide (**5N1**) [24]

Yield 49 %; M.p. 264-266°C (254-256°C)[24]. MS (ESI -) m/z: 285.6 (M - H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_4 \cdot 0.2H_2O$: C: 62.14, H: 5.006, N: 9.66. Found: C: 62.18, H: 5.031, N: 10.05.

4-(tert-Butyl)-N-(2-hydroxy-5-nitrophenyl)benzamide (**5N2**) [23]

Yield 33 %; M.p. 288-290°C (287°C)[23]. MS (ESI -) m/z: 313.8 (M - H, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_4 \cdot 0.2H_2O$: C: 64.22, H: 5.833, N: 8.810. Found: C: 64.28, H: 6.039, N: 9.154.

4-Ethoxy-N-(2-hydroxy-5-nitrophenyl)benzamide (**5N3**)

Yield 57 %; M.p. 276-277°C. ^1H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 1.33 (t, 3H, CH_3); 4.09 (q, 2H, OCH_2); 7.03 (d, 2H, J_o=9.2, 3-H, 5-H); 7.06 (d, 1H, J_o=8.8, 3'-H); 7.91-7.97 (m, 3H, 2-H, 6-H, 4'-H); 8.75 (d, 1H, J_m=2.8, 6'-H); 9.43 (s, 1H, OH); 11.58 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 14.491 (CH_3-1C), 63.435 (OCH_2-1C), 114.185, 115.183, 118.574, 121.424, 125.745, 126.461, 129.570, 139.149, 155.395, 161.522, 164. 836 (11C-Ar). MS (ESI -) m/z: 301.7 (M - H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_5 \cdot 0.1H_2O$: C: 59.24, H: 4.706, N: 9.212. Found: C: 59.02, H: 4.772, N: 9.416.

4-Butoxy-N-(2-hydroxy-5-nitrophenyl)benzamide (**5N4**)

Yield 51 %; M.p. 216-218°C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 0.92 (t, 3H, CH_3); 1.38-1.47 (m, 2H, CH_2); 1.66-1.73 (m, 2H, CH_2); 4.03 (t, 2H, OCH_2); 7.02-7.07 (m, 3H, 3-H, 5-H, 3'-H); 7.91-7.97 (m, 3H, 2-H, 6-H, 4'-H); 8.75 (d, 1H, J_m=2.8, 6'-H); 9.43 (s, 1H, OH); 11.59 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 13.645 (CH_3-1C), 18.666 (CH_2-1C), 30.592 (CH_2-1C), 67.474 (OCH_2-1C), 114.223, 115.183, 118.590, 121.424, 125.730, 126.461, 129.555, 139.141, 155.403, 161.689, 164.829 (11C-Ar). MS (ESI -) m/z: 329.8 (M - H, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_5 \cdot 0.1H_2O$: C: 61.47, H: 5.523, N: 8.434. Found: C: 61.52, H: 5.758, N: 8.731.

N-(2-Hydroxy-5-nitrophenyl)-2,4-dimethylbenzamide (**5N5**)

Yield 62 %; M.p. 284-286 °C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 2.30 (s, 3H, 1''-CH_3); 2.39 (s, 3H, 2''-CH_3); 7.02-7.10 (m, 3H, 3-H, 5-H, 6-H); 7.44 (d, 1H, J_o=8, 3'-H); 7.95 (dd, 1H, J_m=2.4 and J_o=8.8, 4'-H); 8.84 (d, 1H, J_m=2.4, 6'-H); 9.36 (s, 1H, OH); 11.59 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 19.596 (CH_3-1C), 20.800 (CH_3-1C), 115.061, 117.927, 121.371, 126.187, 126.446, 127.543, 131.422, 133.091, 135.910, 139.095, 139.804, 155.022, 167.962 (13C-Ar). MS (ESI -) m/z: 285.7 (M - H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_4 \cdot 0.1H_2O$: C: 62.53, H: 4.968, N: 9.72. Found: C: 62.50, H: 4.965, N: 10.07.

N-(2-Hydroxy-5-nitrophenyl)-3,5-dimethylbenzamide **(5N6)**

Yield 65 %; M.p. 284-286 °C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 2.34 (s, 6H, CH_3); 7.07 (d, 1H, J_o=9.2, 3'-H); 7.23 (s, 1H, 4-H); 7.56 (s, 2H, 2-H, 6-H); 7.97 (dd, 1H, J_m=2.8 and J_o=8.8, 4'-H); 8.75 (d, 1H, J_m=2.8, 6'-H); 9.47 (s, 1H, OH); 11.55 (s, 1H, NH). ^{13}C-NMR δ ppm (DMSO-d_6): 20.79 (CH_3-2C), 115.206, 118.635, 121.592, 125.219, 126.294 133.251, 133.906, 137.754, 139.042, 155.555, 165.614 (11C-Ar). MS (ESI +) m/z: 287.2 (M + H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_4 \cdot 0.1H_2O$: C: 62.53, H: 4.968, N: 9.72. Found: C: 62.52, H: 5.196, N: 10.03.

N-(2-Hydroxy-5-nitrophenyl)-3,5-dimethoxybenzamide (**5N7**)

Yield 48 %; M.p. 251-253°C. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm J=Hz): 3.80 (s, 6H, OCH_3), 6.70 (t, 1H, 4-H), 7.06 (d, 1H, J_o=8.8, 3'-H); 7.10 (d, 2H, J_m=2, 2-H, 6-H); 7.98 (dd, 1H, J_m=3.2 and J_o=9.2, 4'-H); 8.65 (d, 1H, J_m=3.2, 6'-H); 9.59 (s, 1H, OH). ^{13}C-NMR δ ppm (DMSO-d_6): 55.571 (OCH_3-2C), 103.741, 105.555, 115.385, 119.610, 121.989, 126.003,

136.076, 139.089, 156.055, 160.460, 165.115 (11C-Ar). MS (ESI +) m/z: 319.4 (M + H, 100%). Anal. Calcd for $C_{15}H_{14}N_2O_6$: C: 56.60, H: 4.433, N: 8.801. Found: C: 56.54, H: 4.500, N: 8.714.

2.2. Microbiology

2.2.1.Microorganisms

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 10231 and their clinical isolates obtained from Trakya University Faculty of Pharmacy Department of Pharmaceutical Microbiology were used as a standard quality control strains.

2.2.2.Microdilution Method

Antimicrobial susceptibility testing was done according to the guidelines of CLSI M100-S28 and M27-A3 standards [27], [28].

Mueller Hinton Broth (MHB) (Merck), Mueller Hinton Agar (MHA) (Merck), Sabouraud Liquid Medium (SLM) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), and RPMI 1640 medium (Sigma) containing L-glutamine buffered with MOPS to pH: 7 were used in here. MHB, MHA, SLM and SDA were autoclaved at 121˚C for 15-20 minutes and RPMI medium was sterilized by filtration through milipore (0.22µm) filter.

A 100 µL of MHB and RPMI-1640 medium with L-glutamine (Sigma) buffered with MOPS (pH:7) were put in each well of the microplates for bacteria and fungi, respectively.

The bacterial suspensions applied for inoculation were arranged at 10^5 CFU/mL by diluting fresh cultures at McFarland 0.5 density. Suspensions of the yeast at McFarland density was diluted 1:100 and 1:20 respectively and $2{,}5x10^3$ CFU/mL were inoculated to the twofold-diluted solution of the benzamide derivatives.

Standard powders of ampicillin (Biomatik), gentamicin sulphate (Sigma), ceftazidime (Sigma), meropenem (Sigma), ciprofloxacin (Sigma), flucytosine (Sigma), azithromycin (Sigma) and clarithromycin (Sigma) were used.

Stock solutions of the tested benzamides were dissolved in dimethyl sulfoxide (DMSO). The solutions of standard antimicrobial drugs were dissolved in suitable solvents recommended by CLSI guidelines.

The stock solutions of the benzamides and reference antibiotics were diluted two-fold in the wells of the microplates so the solution of all the synthesized benzamide derivatives and standard drugs were prepared at 128, 64, 32, 16, 8, 4, 2, 1 µg/ml and standard drugs were

prepared at 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/ml concentrations. All solvents and diluents, pure microorganisms and pure media were used in control wells.

A 10 µl microorganisms inoculum was added to each well of the microplates. Microplates including bacteria were incubated at 37°C for 16–20 hours and microplates including fungi were incubated at 35°C for 24-48 hours. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MICs).

2.3. Molecular Docking Studies

2.3.1. Preparation of the enzymes

The crystal structures of the *Staphylococcus aureus* DNA gyrase (PDB ID: **3G7B**) enzyme which has an important role in the replication of bacterial DNA, *Staphylococcus aureus* FtsA enzyme (PDB ID: **3WQU**) plays crucial role in bacterial cell division were taken from the Protein Data Bank [29], [30]. The protein and ligands were arranged using Accelrys Discovery Studio 3.5 (DSV) software [31]. The target protein was taken, the ligand and all other heteroatoms were extracted and then hydrogens were added. Afterwards, their positions were optimized using the all atom CHARMm force field and the Adopted Basis set Newton Raphson (ABNR) method available in DSV protocol with RMSD (Root Mean Square Deviation) value well within the reliable range of 2 Å. (Supplementary information 2).

The minimized protein was defined as the receptor using the binding site module. The docking area was defined around the small molecules in receptors, which was determined in crystal structure. For this purpose binding spheres were built by CDOCKER module of Discovery Studio 3.5. Binding spheres for **3G7B** (50.354, -2.964, 19.129, with 7,50065 as radius value), **3WQU** (2.104, 31.034, -22.349, with 8,73157 as radius value) were chosen from the active site by using the binding site tools (Supplementary information 3).

2.3.2. Ligand preparation

For validation of docking protocol X-ray ligand were firstly docked to receptors. Reference molecules for whole receptors and interested ligands (**4N1-4N6, 5N1-5N7**) sketched with ChemSketch [32] but hydrogens were added by DSV, All atom CHARMm force field parameterization was assigned, and afterwards minimized by using the ABNR (Adopted Basis

Newton-Raphson) method as defined in section 2.3.1. A simulated annealing molecular dynamics (MD) approach was applied in order to search conformations of the ligands. The ligands were heated to 700K and then annealed to 200 K.

2.3.3. Docking

CDOCKER module of DSV is a docking method which uses a random initial ligand placement and full CHARMm force field [26]. This method provides to hold rigid the proteins while the ligands are flexible during the docking process. The docking parameters were used as below:

Top Hits: 10;

Random Conformations: 10;

Random Conformations Dynamics Step: 1000;

Grid Extension: 8.0;

Random Dynamics Time Step: 0.002.

X-ray ligands were redocked to validate the docking and scoring methodology. The Analyze Ligand Posses subprotocol was applied for scoring of the docked poses. In situ ligand minimization step (ABRN method) and implicit solvent model (GBMV) in DSV were used in order to calculate the binding energies. Having the lowest binding energy of compounds was received as the best-docked conformation into the proteins.

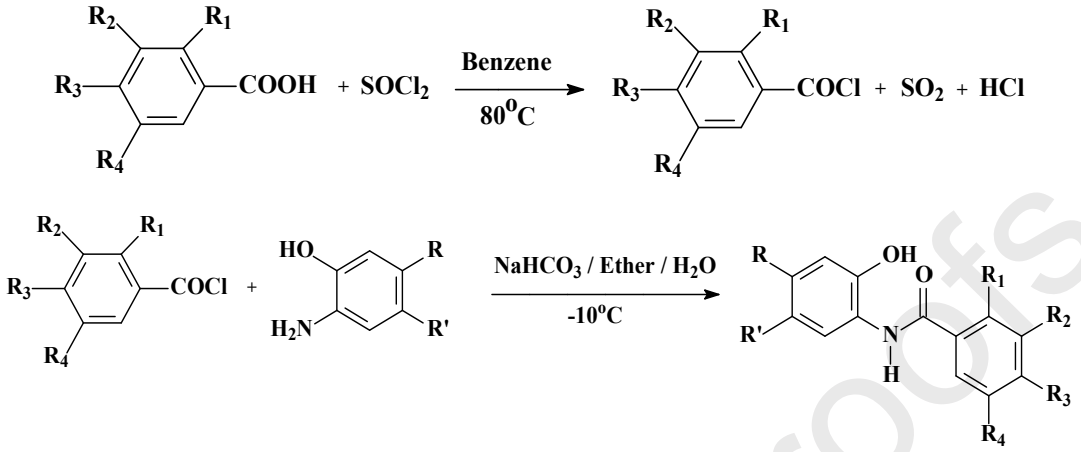
3.Results and Discussion

3.1.Chemistry

In this study, the *N*-(2-hydroxy-(4 or 5)-nitrophenyl)-substituted-benzamides were obtained following synthetic pathway demonstrated in Scheme 1.

The chemical synthesis of the substituted-benzamide derivatives (**4N1-4N6, 5N1-5N7)** was performed by reacting 2-amino-4-nitrophenol or 2-amino-5-nitrophenol with suitable benzoyl chlorides, obtained in turn by reacting benzoic acid derivatives with thionyl chloride. All of the benzamides are new products except **5N1, 5N2, 4N2, 4N6**. The purity of the benzamides was controlled by TLC using solvents system ($CHCl_3$/MeOH 30:1). The UV light was used in order to visualize the plates. Melting points of the synthesized structures were determined and uncorrected. All of the newly synthesized benzamides were promoted by using ^1H-NMR, ^{13}C-NMR, Mass spectral datas and elemental analyses. The results of elemental analyses and all spectral datas were found to be fully compatible with the presented molecules.

The physical, chemical, and spectral results of the newly obtained benzamides **4N1**, **4N3-5** and **5N3-7** were reported in section 2.1.1.



R = **R′** = H, NO_2; **R₁** = H, CH_3; **R₂** = H, CH_3, OCH_3;

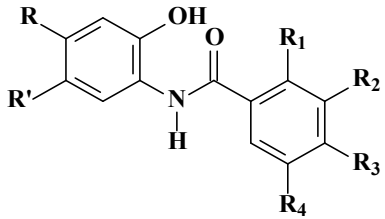
R₃ = H, CH_3, C_2H_5, $C(CH_3)_3$, OC_4H_9, OC_2H_5, C_4H_9; **R₄** = H, CH_3, OCH_3

Scheme 1. The synthetic pathway of benzamide derivatives (**4N1-4N6, 5N1-5N7**)

3.2. In vitro antimicrobial activities

All of the synthesized *N*-(2-hydroxy-(4 or 5)-nitrophenyl)-substituted-benzamides (**4N1-4N6, 5N1-5N7**) were measured for their *in vitro* antibacterial activities against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. coli* isolate (susceptile to the tested antibacterial agents), *P. aeruginosa* isolate (resistant to gentamicin and ceftazidim) as Gram-negative bacteria, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. aureus* isolate (Methicilline Sensitive (MSSA) and resistant to gentamicin), *E. faecalis* isolate (resistant to vancomycin) as Gram-positive bacteria. Moreover, all compounds were also tested against *C. albicans* ATCC 10231 and its clinical isolate for their *in vitro* antifungal activities. Antimicrobial activities were detected as the MIC values by using the two-fold serial dilution technique in Sabouraud dextrose agar and Mueller-Hinton broth for the antifungal and antibacterial effects, respectively. The antimicrobial activities of the synthesized compounds (**4N1-4N6, 5N1-5N7**) were compared to some reference drugs such as gentamicin, ampicillin, ceftazidime, meropenem, ciprofloxacin, azithromycin, clarithromycin for antibacterial, flucytosine for antifungal activity. When the effect of solvent control was taken into consideration, the substances which do not show antimicrobial effect are indicated with (-) sign. Minimum Inhibition Concentrations (MICs) (µg/ml) of antimicrobial agents and the synthesized benzamides were shown at the Table 1.

Table 1: Microbiological results of the synthesized benzamide derivatives (MIC, µg/ml)



Comp. Code	R	R´	R_1	R_2	R_3	R_4	Microorganisms*,**								Fungi*	
							A	B	C	D	E	F	G	H	I	J
4N1	NO_2	H	H	H	C_4H_9	H	-	-	-	-	<4	<4	<4	<4	-	-
4N2	NO_2	H	H	H	$C(CH_3)_3$	H	-	-	-	-	-	-	64	-	-	-
4N3	NO_2	H	H	H	OC_2H_5	H	-	-	-	-	32	-	32	-	-	-
4N4	NO_2	H	H	H	OC_4H_9	H	-	-	-	-	16	-	64	-	-	-
4N5	NO_2	H	H	CH_3	H	CH_3	-	-	-	-	64	-	32	-	-	-
4N6	NO_2	H	H	OCH_3	H	OCH_3	-	-	-	-	-	-	64	-	-	-
5N1	H	NO_2	H	H	C_2H_5	H	64		-	8	<4	<4	64	-	-	-
5N2	H	NO_2	H	H	$C(CH_3)_3$	H	64	64	-	16	<4	<4	64	-	-	-
5N3	H	NO_2	H	H	OC_2H_5	H	-	-	-	-	-	-	-	-	-	-
5N4	H	NO_2	H	H	OC_4H_9	H	<4	-	-	-	<4	-	-	-	-	-
5N5	H	NO_2	CH_3	H	CH_3	H	64	64	-	8	<4	-	64	-	-	-
5N6	H	NO_2	H	CH_3	H	CH_3	-	-	-	-	<4	-	64	-	-	-
5N7	H	NO_2	H	OCH_3	H	OCH_3	64	-	-	-	8	-	64	-	-	-
Gentamicin							0,25	<0,125	0,5	16	0,125	2	4	>16	-	-

Ampicillin	2	4	-	-	0,5	2	0,5	2	-	-
Ceftazidime	<0,125	1	1	>16	4	>16	-	-	-	-
Meropenem	<0,03125	<0,03125	<0,03125	<0,03125	<0,03125	0,5	2	2	-	-
Ciprofloxacin	<0,0156	<0,0156	0,125	<0,0156	0,125	0,125	0,25	0,25	-	-
Azithromycin	-	-	-	-	0,5	0,5	-	-	-	-
Clarithromycin	-	-	-	-	<0,0156	<0,0156	-	-	-	-
Flucytosine	-	-	-	-	-	-	-	-	<0,125	<0,125

*A: *E.coli* ATCC 25922, B: *E. coli* isolate, C: *Pseudomonas aeruginosa* ATCC 27853, D: *P. aeruginosa* isolate, E: *Staphylococcus aureus* ATCC 29213, F: *S. aureus* isolate, G: *Enterococcus faecalis* ATCC 29212, H: *E. faecalis* isolate, I: *Candida albicans* ATCC 10231, J: *C. albicans* isolate *E. coli* isolate is susceptible to the tested antimicrobial agents. *P. aeruginosa* isolate is resistant to gentamicin and ceftazidim. *S. aureus* isolate is a Methicilline Sensitive (MSSA) isolate. It is recommended not to use ceftazidime for this isolate in the guidelines of EUCAST which is compatible with our finding. The isolate is resistant to gentamicin. *E. faecalis* isolate is a VRE isolate which is resistant to vancomycin.

** ATCC strains were used as quality control agents in the experiment.

According to microbiological results, some of the benzamides (**5N1, 5N2, 5N5, 5N7**) exhibited moderate activity against *E.coli* ATCC 25922. Compounds **5N2** and **5N5** showed the activity with 64 μg/ml MIC value against multi-drug resistant *E.coli*. Only, compound **5N4** (4-butoxy-*N*-(2-hydroxy-5-nitrophenyl)benzamide had a noteworthy effect with the MIC value of <4 μg/ml for *E. coli* strain. The structure-activity relationships (SAR) displayed that the position of the nitro group bound to the *N*-(2-hydroxyphenyl)anilide was quite remarkable in the activity against the *E.coli* strain and its clinical isolate. It was seen that 5th position come to the fore especially in increasing this activity. It has been realized that the hydroxy and nitro groups should be located opposite each others. Besides, it has been found that the presence of a butoxy group at the para position of the benzamide is necessary for improving activity, as well. It can be concluded that the para position of benzamide is important and in addition this position should attach to some substituent which can be an electron-donating and bulky group. Even if this compound did not display more activity than the reference drugs, it could be a lead compound for developing new anti-*E.coli* molecules. Although none of the benzamides showed any effect for Gram-negative *P. aeruginosa* ATCC 27853, suprisingly, **5N1, 5N5** displayed more activity at the MIC value of 8 μg/ml than gentamicin and ceftazidime against *P. aeruginosa* isolate. Besides, compound **5N2** had a same activity with gentamicin and more effect (MIC value: 16 μg/ml) than ceftazidime. These results showed us that the position of nitro group had a good role for improving activity against multi-drug resistant *P. aeruginosa*. Therefore, we need to notify that the position of nitro group had to be on the 5th location of anilide in order to develop new anti-*P.aeruginosa* structures. Another important thing is that the benzamide contains an alkyl group at the para position. Nevertheless, the alkyl group should not be bulky, as the *tert*-butyl group decreased the activity one-fold.

Generally, we could say that the tested benzamide derivatives displayed more significant activities against Gram-positives compared to Gram-negatives. While the compounds **4N2, 4N6, 5N3** indicated no effect for Gram-positive bacterium *S. aureus* ATCC 29123, fortunately, **4N1, 5N1, 5N2, 5N4, 5N5** and **5N6** were found to be more effective (MIC value of <4 μg/ml) than ceftazidime. The results of the derivatives **4N1, 5N1, 5N2** against MSSA had very satisfactory, as well. Even, they were found to be more potent than ceftazidime. When we generally take a glance at the MIC values for *S. aureus* it should be considered that NO_2 group on the R' position and bearing a alkyl group(s) or butoxy on the benzamide played a remarkable role for enhancing the potency.

Most of the benzamide derivatives showed moderate activiy against Gram-positive bacterium *E. faecalis* strain. None of them except **4N1** displayed any activity against *E. faecalis*

isolate. Surprisingly, **4N1** (4-butyl-*N*-(2-hydroxy-4-nitrophenyl)benzamide) demonstrated more activity against either *E. faecalis* strain and its drug-resistant isolate compared to gentamicin. It can be noticed that **4N1** would be important for developing new antibacterial structures against multi-drug resistant *E. faecalis*.

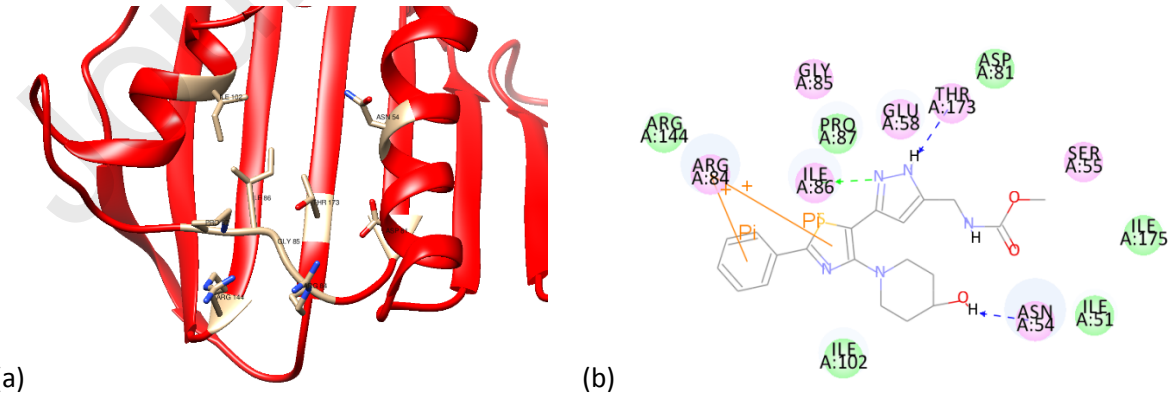
In this study, all of the newly synthesized benzamide had no activity against *C. albicans* strain and its clinical isolate.

3.3. Results of molecular docking

3.3.1. Molecular docking study for DNA gyrase

DNA gyrase is an ATPase that introduces negative supercoils into DNA. The enzyme removes positive supercoils which accumulate in front of replication forks and provide to restore the negative superhelicity of the genome [33]. By this way DNA gyrase effects many different supercoiling-dependent proseses such as DNA replication, regulation of gene expression and chromosome condensation. Due to play an essential role in bacterial life, it makes this enzyme is very important target for drug discovery.

In here, we used the crystal structures of the *Staphylococcus aureus* DNA gyrase (**PDB ID: 3G7B)** enzyme. Binding pocket of **3G7B** (Fig. 3a) was identified with residues such as Asn54, Asp81, Arg84, Gly85, Ile86, Pro87, Ile102, Arg144, Thr173 in previous studies and X-ray crystallographic structure [34], [35]. Our re-docking results of X-ray ligand with the receptor showed similar docking profile, as well (Fig. 3b).



(a) (b)

Fig. 3. (a) The active site of **3G7B**; (b) Re-docking of X-ray ligand of DNA gyrase. Blue and green intermittent lines demonstrates H bonds. Orange lines represents Pi-cation bonds.

All of the docking results and interacted residues of newly synthesized benzamides were shown in Table 2. (Supplementary Information 2).

Table 2. Receptor-ligand interactions between binding pocket of DNA gyrase enzyme (**PDB ID: 3G7B**) and benzamides

Compound Name	Binding Energy (kcal/mol)	Interacted residues (van der Waals contact distance <4 Å)
X-ray ligand	-18,0708	*Thr173[a], Ile86[a], Asn54[a], Arg84[b]*
4N1	-24,4902	*Arg144[a,c], Arg84[b,c], Thr173[a], Gly85[a]*
4N2	-3,07456	*Arg84[b]*
4N3	-13,2136	*Arg144[c], Arg84[b,c]*
4N4	-18,4578	*Arg144[a,c], Arg84[b,c]*
4N5	-13,1006	*Arg144[c], Arg84[b,c]*
4N6	-18,3141	*Ser129[a]*
5N1	-16,5803	*Arg144[a,c], Arg84[a,b,c]*
5N2	-21,4846	*Thr173[a], Arg144[a,c], Glu58[c], Arg84[a,b]*
5N3	-10,9953	*Asp81[a], Arg84[b]*
5N4	-12,1941	*Arg144[a,c], Arg84[b,c], Gly85[a], Asn54[a]*
5N5	-1,50751	*Arg144[a], Thr173[a], Arg84[b]*
5N6	-12,0287	*Arg144[a,c], Arg84[a,c], Glu58[c], Thr173[a]*
5N7	-21,3654	*Ser129[a]*

[a]: H bonds, [b]:pi-cation interactions, [c]: receptor-ligand bumps

Compound **4N1** molecule indicated the best docking results with -24,4902 kcal/mol binding energy. It also constituted bonds with residues as seen in Table 2 and Fig. 4. This structure was found to be more effective than the others against *E. faecalis* and its resistant isolate. Besides, **4N1** showed very significant activity against *S. aureus* and MSSA.

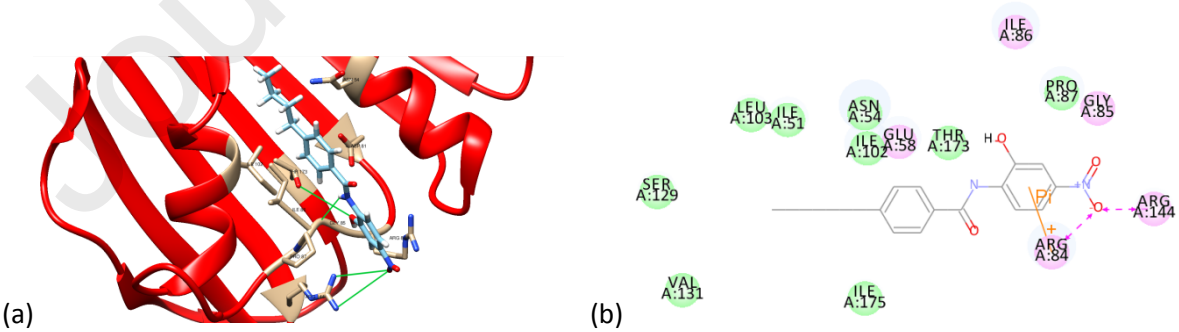


Fig. 4. (a) **4N1** (Blue molecule) were demonstrates in binding pocket. Green lines show H-bonds. Chimera molecular modeling program [36] (b) 2D demonstration of **4N1** molecule. Orange lines show Pi-cation bonds, Pink lines show receptor-ligand bumps. DSV program was used for preparation of figure**.**

Compound **5N2** which was significantly potent *S. aureus* and its isolate has more satisfactory binding energy than the others which is -21,4849 kcal/mol, as well. **5N2** formed H bond with Thr173, Arg144, and Arg84 residues in the binding pocket of **3G7B** (Supplementary Information 3)

FtsA is an actin-like protein, which plays a crucial role with FtsZ protein in bacterial cell division. Tubulin-like FtsZ localize at midcell when the bacterial cell division starts. FtsZ polymerizes into protofilaments by GTP binding [37], [38]. Attachment of FtsZ protein is essential for assembly of the Z ring. Actin-like FtsA is needed both to tether FtsZ to the cell.

In this study, the synthesized benzamides were also docked into FtsA enzyme (**PDB ID: 3WQU**) in order to be able to understand the mechanism of antibacterial for *S. aureus*. Binding pocket of FtsA (Fig. 5a) was identified with residues such as Ser13, Ser15, Lys17, Gly44, Lys77, Glu209, Asp210, Val211, Glu251, Lys254, His 255, Gly325, Ser328 in previous studies and X-ray crystallographic structure [38], [39]. The re-docking result with X-ray ligand (ATP) of receptor also shows similar docking profile as well (Fig. 5b).

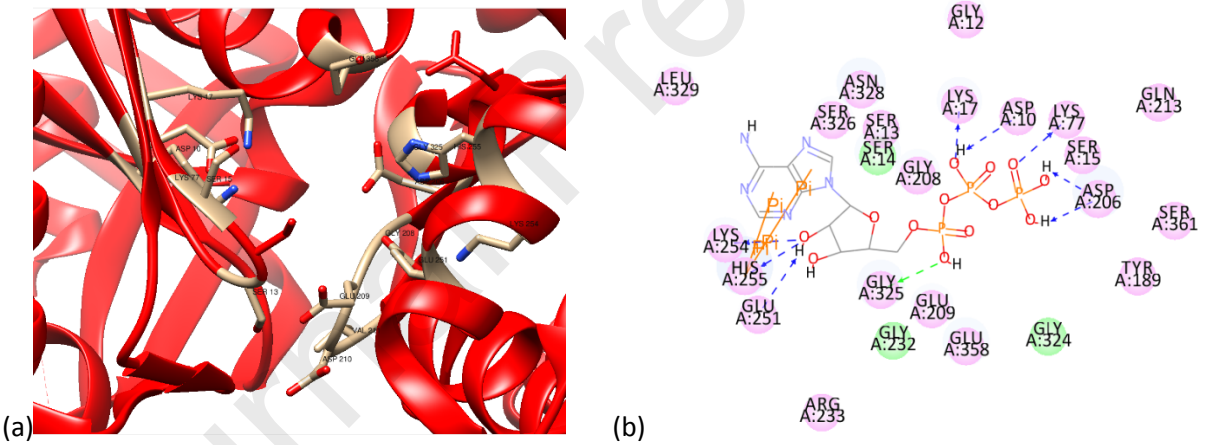


Fig. 5. (a) The active site of **3WQU**; (b) Re-docking of X-ray ligand of FtsA. Blue and green intermittent lines demonstrates H bonds. Orange lines demonstrates Pi-cation and pi-pi interactions.

All of the compounds were docked using CDOCKER method in DSV, as well. The docking results and interacted residues were represented in Table 3. (Supplementary Information 2).

Table 3. Receptor-ligand interactions between binding pocket of FtsA enzyme (**PDB ID: 3WQU)** and benzamides

Compound Code	Binding Energy (kcal/mol)	Interacted residues (van der Waals contact distance <4 Å)
X-ray ligand	-24,267	*Asp10[a], Lys17[a], Lys77[a], Asp206[a], Gly325[a], Glu251[a], His255[a,b], Lys254[a,c]*
4N1	-0,14109	*Lys17[a,c], Lys77[e],Asp185[e], Asp206[e], Gly325[a], Glu358[a]*
4N2	-1,88197	*Ser13[a], Lys77[e], Glu209[a], Asp210[a], Gly325[d]*
4N3	5,9273	*Lys17[a,c], Lys77[e],Asp185[e], Asp206[e], Glu209[a]*
4N4	2,72427	*Asp10[e], Lys17[a,c], Lys77[a,e],Asp185[e], His255[b], Gly325[a]*
4N5	-5,83571	*Ser13[a], Ser14[a], Lys17[a], His 255[e]*
4N6	11,772	*Lys17[a,c], Lys77[e],Asp185[e], Asp206[e], Glu209[a]*
5N1	-13,6264	*Asp10[a], Ser15[a], Lys17[a], Glu209[e], Asp210[a,e], Val211[a]*
5N2	-7,35629	*Lys17[a],His 255[e], Glu358[e]*
5N3	-10,4012	*Lys17[a], Lys254[e], His 255[b,c,e], Glu358[a]*
5N4	-8,97427	*Ser13[a], Lys17[a], Glu209[a,e], Asp210[a], Val211[a], His255[c]*
5N5	3,42188	*Asp10[e], Lys17[a,c], Lys77[e], Asp206[e], Gly208[a], Glu209[a]*
5N6	-14,1632	*Lys17[a,c],Asp206[e],Asp10[e]*
5N7	-4,80481	*Asp10[a], Lys17[a], Glu209[e], Asp210[a,e], Val211[a]*

[a]: H bonds, [b]: pi–pi interactions,[c]:pi-cation interactions, [d]:pi-sigma interactions, [e]: receptor-ligand bumps

The compound **4N1**, which is an effective against *S. aureus, E. faecalis* and their clinical isolates having < 4µg/ml MIC value, has low binding energy. Nevertheless, it formed H bond with Lys17, Gly325, Glu358 residues in the active site of FtsA enzyme (Fig. 6). **5N6** showed the best binding energy among the compounds, which was also potent against *S. aureus* strain. This sturucture formed H bond with Lys17 residue (Fig. 6).

The binding energy of **5N1** which is one of potent benzamides against *S. aureus* and MSSA was also satisfactory. **5N1** formed H bond with Asp10, Ser15, Lys17, Asp210, Val211 residues in the binding pocket of **3WQU** (Supplementary Information 3)

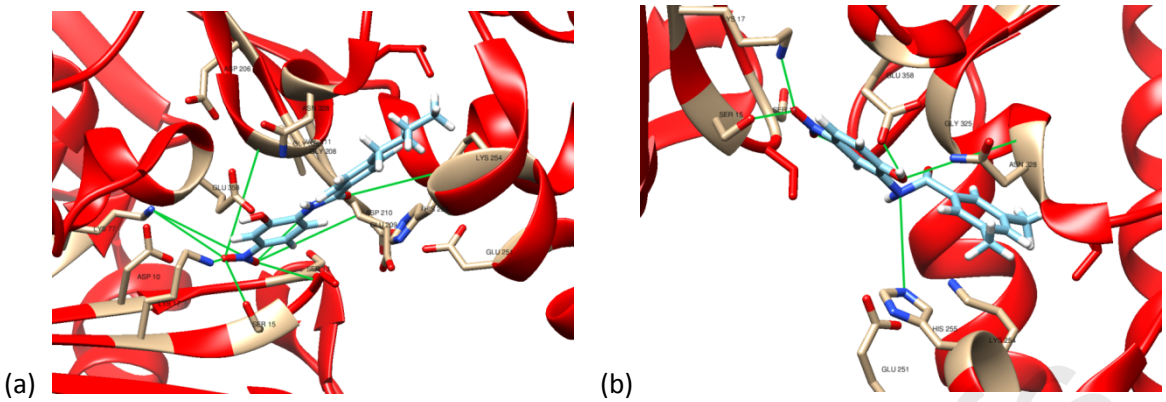


Fig. 6. 4N1 (a) and **5N6 (b)** were demonstrates in binding pocket. Green lines show H-bonds. Chimera molecular modeling program [36] was used for preparation of figure.

4. Conclusion

In the current study, a series of *N*-(2-hydroxy-(4 or 5)-nitrophenyl)benzamide derivatives was designed and synthesized because of increasing multidrug-resistant bacterial infections at a worrying percentage. According to this study, we have noticed that our newly synthesized benzamide derivatives mostly showed significantly antibacterial activity against Gram-positives than Gram-negatives. Specifically, compounds **4N1**, **5N1**, and **5N2** had very important activity against MSSA, even these compounds are found to be more effective than reference drug ceftazidime. The structure-activity relationships revealed that the para position of benzamide played very important role in order to enhance the activity for MSSA. Particularly, having a bulky alkyl group on this position has come to the fore for increasing anti-MSSA. Molecular docking studies demonstrated that the potent compounds **4N1**, **5N1**, and **5N2** showed a satisfactory interactions with DNA-gyrase. **5N1** has also showed good interaction with FtsA enzymes. It can be considered that obtained the anti-MSSA activity of compound **5N1** could be related to these two enzymes. Among the tested compounds only **5N4** was found to be as the best anti-*E. coli* ATCC 25922 molecule.

In conclusion, it could be considered that **4N1**, **5N1**, and **5N2** structures could be lead compounds in the development of new potential anti-*S. aureus* agents, besides the **5N4** could be guide compound for anti-*E. coli*.

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Conflict of interest

The authors declare no conflict of interest

References

[1] Bax R, Mullan N, Verhoef J. The millennium bugs - The need for and development of new antibacterials. Int J Antimicrob Agents 2000;16:51–9. doi:10.1016/S0924-8579(00)00189-8.

[2] Fair RJ, Tor Y. Antibiotics and Bacterial Resistance in the 21st Century. Perspect Medicin Chem 2014;6:PMC.S14459. doi:10.4137/PMC.S14459.

[3] Noble WC, Virani Z, Cree RGA. Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett 1992;93:195–8. doi:10.1111/j.1574-6968.1992.tb05089.x.

[4] Lee H, Ahn S, Hwang NY, Jeon K, Kwon OJ, Huh HJ, et al. Limited Effect of Later-Generation Fluoroquinolones in the Treatment of Ofloxacin-Resistant and Moxifloxacin-Susceptible Multidrug-Resistant Tuberculosis. Antimicrob Agents Chemother 2018;62:e01784-17. doi:10.1128/AAC.01784-17.

[5] Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, et al. Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet 2001;358:207–8. doi:10.1016/S0140-6736(01)05410-1.

[6] Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov 2006;6:29.

[7] Sonda S, Kawahara T, Murozono T, Sato N, Asano K, Haga K. Design and synthesis of orally active benzamide derivatives as potent serotonin 4 receptor agonist. Bioorg Med Chem 2003;11:4225–34. doi:10.1016/S0968-0896(03)00412-7.

[8] Diouf O, Bourhim M, Lambert DM, Poupaert JH, Stables JP, Vamecq J. Anticonvulsant and neurotoxicological properties of 4-amino-N-(2-ethylphenyl)benzamide, a potent ameltolide analogue. Biomed Pharmacother 1997;51:131–6. doi:10.1016/S0753-3322(97)86911-9.

[9] Bala S, Sharma N, Kajal A, Kamboj S. Design, synthesis, characterization, and computational studies on benzamide substituted mannich bases as novel, potential antibacterial agents. Sci World J 2014;2014. doi:10.1155/2014/732141.

[10] Khatiwora E, Mundhe K, Deshpande NR, Kashalkar R V. Anthelmintic activity of transition metal complexes of some benzamides. Der Pharma Chem 2012;4:1264–9.

[11] Kim BJ, Kim J, Kim YK, Choi SY, Choo HYP. Synthesis of benzoxazole amides as novel antifungal agents against Malassezia furfur. Bull Korean Chem Soc 2010;31:1270–4. doi:10.5012/bkcs.2010.31.5.1270.

[12] Jiang Z, Wang H, Li Y, Peng Z, Li Y, Li Z. Synthesis and antiviral activity of a series of novel N-phenylbenzamide and N-phenylacetophenone compounds as anti-HCV and anti-EV71 agents. Acta Pharm Sin B 2015;5:201–9. doi:10.1016/J.APSB.2015.03.013.

[13] Ekblad T, Lindgren AEG, Andersson CD, Caraballo R, Thorsell A-G, Karlberg T, et al. Towards small molecule inhibitors of mono-ADP-ribosyltransferases. Eur J Med Chem 2015;95:546–51. doi:10.1016/J.EJMECH.2015.03.067.

[14] Li Y, Wang Y, Xie N, Xu M, Qian P, Zhao Y, et al. Design, synthesis and antiproliferative activities of novel benzamides derivatives as HDAC inhibitors. Eur J Med Chem 2015;100:270–6. doi:10.1016/J.EJMECH.2015.05.045.

[15] Mrozik H, Jones H, Friedman J, Schwartzkopf G, Schardt RA, Patchett AA, et al. A new agent for the treatment of liver fluke infection (Fascioliasis). Experientia 1969;25:883. doi:10.1007/BF01897937.

[16] Ye T, Xiong Y, Yan Y, Xia Y, Song X, Liu L, et al. The anthelmintic drug niclosamide induces apoptosis, impairs metastasis and reduces immunosuppressive cells in breast cancer model. PLoS One 2014;9. doi:10.1371/journal.pone.0085887.

[17] Rajamuthiah R, Fuchs BB, Conery AL, Kim W, Jayamani E, Kwon B, et al. Repurposing Salicylanilide Anthelmintic Drugs to Combat Drug Resistant Staphylococcus aureus. PLoS One 2015;10:e0124595.

[18] Cuena Boy R, Maciá Martínez M. Extrapyramidal toxicity caused by metoclopramide and clebopride: study of voluntary notifications of adverse effects to the Spanish Drug Surveillance System. Aten Primaria 1998;21:289–95.

[19] Pradhan KJ, Variyar PS, Bandekar JR. Antimicrobial Activity of Novel Phenolic Compounds from Green Pepper (Piper nigrumL.). LWT - Food Sci Technol 1999;32:121–3. doi:10.1006/FSTL.1998.0508.

[20] Kobayashi I, Muraoka H, Hasegawa M, Saika T, Nishida M, Kawamura M, et al. In vitro anti-Helicobacter pylori activity of BAS-118, a new benzamide derivative. J Antimicrob

Chemother 2002;50:129–32. doi:10.1093/jac/dkf106.

[21] Yalcin I, Kaymakcioglu B, Oren I, Sener E, Temiz O, Akin A, et al. Synthesis and microbiological activity of some novel N-(2-hydroxyl-5-substitutedphenyl)benzacetamides, phenoxyacetamides and thiophenoxyacetamides as the possible metabolites of antimicrobial active benzoxazoles. Farmaco 1997;52:685–9.

[22] Aki Sener E, Bingol KK, Temiz-Arpaci O, Yalcin I, Altanlar N. Synthesis and microbiological activity of some N-(2-hydroxy-4-substitutedphenyl)benzamides, phenylacetamides and furamides as the possible metabolites of antimicrobial active benzoxazoles. Farmaco 2002;57:451–6. doi:10.1016/S0014-827X(02)01226-0.

[23] Yildiz Oren I, Aki-Sener E, Ertas C, Temiz Arpaci O, Yalcin I, Altanlar N. Synthesis and microbiological activity of some substituted N-(2-hydroxy-4-nitrophenyl)benzamides and phenylacetamides as possible metabolites of antimicrobial active benzoxazoles. Turkish J Chem 2004;28:441–9.

[24] Ertan T, Yildiz I, Ozkan S, Temiz-Arpaci O, Kaynak F, Yalcin I, et al. Synthesis and biological evaluation of new N-(2-hydroxy-4(or 5)-nitro/aminophenyl)benzamides and phenylacetamides as antimicrobial agents. Bioorganic Med Chem 2007;15:2032–44. doi:10.1016/j.bmc.2006.12.035.

[25] Yildiz I, Ertan T, Bolelli K, Temiz-Arpaci O, Yalcin I, Aki E. QSAR and pharmacophore analysis on amides against drug-resistant S. aureus. SAR QSAR Environ Res 2008;19:101–13. doi:10.1080/10629360701844159.

[26] Wu G, Robertson DH, Brooks III CL, Vieth M. Detailed analysis of grid-based molecular docking: A case study of CDOCKER - A CHARMm based MD docking program. J Comput Chem 2003;24:1549–62.

[27] Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 28th ed. Philadelphia: 2018.

[28] Clinical Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard 3rd ed. Philadelphia: 2008.

[29] Ronkin SM, Badia M, Bellon S, Grillot AL, Gross CH, Grossman TH, et al. Discovery of pyrazolthiazoles as novel and potent inhibitors of bacterial gyrase. Bioorganic Med Chem Lett 2010;20:2828–31. doi:10.1016/j.bmcl.2010.03.052.

[30] Fujita J, Maeda Y, Nagao C, Tsuchiya Y, Miyazaki Y, Hirose M, et al. Crystal structure

of FtsA from Staphylococcus aureus. FEBS Lett 2014;588:1879–85. doi:10.1016/j.febslet.2014.04.008.

[31] Accelrys Software Inc. Discovery Studio 3.5 Client 2012.

[32] ACD/ChemSketch 2001.

[33] Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev 1997;61:377–92.

[34] RSCB Protein Data Bank. Staphylococcus aureus Gyrase B co-complex with Methyl ({5-[4-(4-hydroxypiperidin-1-yl)-2-phenyl-1,3-thiazol-5-yl]-1H-pyrazol-3-yl}methyl)carbamate inhibitor 2009. https://www.rcsb.org/3d-view/3G7B?preset=ligandInteraction&sele=B47. (accessed July 1, 2019).

[35] Durdagi S, Tahir ul Qamar M, Salmas RE, Tariq Q, Anwar F, Ashfaq UA. Investigating the molecular mechanism of staphylococcal DNA gyrase inhibitors: A combined ligand-based and structure-based resources pipeline. J Mol Graph Model 2018;85:122–9. doi:10.1016/j.jmgm.2018.07.010.

[36] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera - A visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605–12. doi:10.1002/jcc.20084.

[37] Mura A, Fadda D, Perez AJ, Danforth ML, Musu D, Rico AI, et al. Roles of the Essential Protein FtsA in Cell Growth and Division in Streptococcus pneumoniae J Bacteriol 2017;199:e00608-16. doi:10.1128/JB.00608-16.

[38] Ragunathan A, Malathi K, Ramaiah S, Anbarasu A. FtsA as a cidal target for Staphylococcus aureus: Molecular docking and dynamics studies. J Cell Biochem 2019;120:7751–8. doi:10.1002/jcb.28049.

[39] RSCB Protein Data Bank. Staphylococcus aureus FtsA complexed with ATP 2014. https://www.rcsb.org/3d-view/3WQU?preset=ligandInteraction&sele=ATP. (accessed July 1, 2019).

Conflict of interest

The authors declare no conflict of interest

Highlights

1- We have described the synthesis of some novel *N*-(2-hydroxy-(4 or 5)-nitrophenyl)benzamides for their antimicrobial activities.

2- Some of the benzamides **4N1 (**4-butyl-*N*-(2-hydroxy-4-nitrophenyl)benzamide), **5N1 (**4-ethyl-*N*-(2-hydroxy-5-nitrophenyl)benzamide), and **5N2 (**4-(*tert*-butyl)-*N*-(2-hydroxy-5-nitrophenyl)benzamide) displayed very significant activity against *S. aureus* and MSSA with <4 μg/ml MIC value. They were also found to have more potent than ceftazidime. Besides, **4N1** indicated more effective than gentamicin against *E. faecalis* clinical isolate. Only 4-butoxy-*N*-(2-hydroxy-5-nitrophenyl)benzamide (**5N4**) displayed very good activity against *E. coli*.

3- Molecular docking studies revealed that **4N1**, **5N1**, and **5N2** showed a good interactions with DNA-gyrase (PID: **3G7B)**. Moreover, **5N1** has interacted with FtsA (PID: **3WQU)** enzyme in the binding mode, as well.

4- These researches showed that the compounds **4N1, 5N1, 5N2, 5N4** could be lead to discover new multidrug-resistant antibacterial candidates.