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RESEARCH ARTICLE

Synthesis, Antimicrobial Activity, and Molecular Modeling Studies of Some Benzoxazole Derivatives

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Abstract: ***Background*:** The need to develop novel antimicrobial agents is apparent as infectious diseases are increasing and resistance is rapidly developing against the drugs used in the treatment.

***Objective*:** This study aimed at the synthesis, antimicrobial susceptibility testing, and computational elucidation of the mechanism of action of benzoxazole derivatives. It also aimed to compare the results obtained in this study with the previous studies by our group. This would pave the way for designing novel molecules with better antimicrobial activity. The other goal was pharmacophore analysis and *in silico* ADMET analysis of them.

***Methods*:** In this study, synthesis, antimicrobial susceptibility testing, molecular docking, pharmacophore analysis, and ADMET prediction were carried out.

***Results*:** The antimicrobial activity studies demonstrated that the synthesized compounds were active against standard strains and clinical isolates at high concentrations. Then, the antimicrobial testing results were compared to similar benzoxazoles tested by our group previously. Benzoxazole derivatives without a methylene bridge between oxazole and phenyl ring were found to be more active than those with the methylene bridge. This was also confirmed by molecular modeling undertaken in this study. The computational results indicated that the antibacterial activity could be achieved by DNA gyrase inhibition. Pharmacophore analysis showed that hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and hydrophobicity features would contribute to the inhibition. In addition, *in silico* ADMET property investigation of the compounds exhibited that they had the desired pharmacokinetics.

***Conclusion*:** Although antibacterial activity by inhibiting DNA gyrase is selective, the synthesized compounds were active at much higher concentrations than the standards. Therefore, in prospective antimicrobial studies, it is better to focus on benzoxazole derivatives without the methylene bridge. Since the compounds had suitable *in silico* ADMET properties, screening them against the other pharmacologic activities should be carried out. It is recommended to support the molecular modeling results with *in vitro* or *in vivo* studies.

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1. INTRODUCTION

Infectious diseases represent an increasing global public health problem [1]. Antibiotics are very important in the treatment of these diseases, but the development of resistance by microorganisms against them is a danger. Indeed, the World Health Organization (WHO) estimated that after 2050, approximately 10 million people will die from antibiotic resistance each year. In those years, deaths due to antibiotic resistance are estimated to surpass deaths from cancer [2]. The rapidly growing global antibiotic resistance crisis is generally caused by multi-drug resistant (MDR) Gram-positive pathogens such as *Streptococcus*, *Enterococcus*, and *Staphylococcus*, and Gram-negative pathogens such as *Escherichia*, *Salmonella*, and some *Pseudomonas* strains. Especially, hospital infections caused by the MDR *Acinetobacter*

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type strains show resistance against many antibiotic agents [3]. In addition, the growing concern with the emergence of MDR strains of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is important [4].

The use of antibiotics has been increasing around the world. It was reported that global antibiotic use has increased by 65% (from 21.1 billion to 34.8 billion DDD (defined daily dose)) between the years 2000 and 2015. Especially, the high increase in developing countries was striking [5]. If countries continue to use antibiotics according to their annual growth rates, the total use will reach 128 billion DDDs by 2030. It is also known that the majority of antibiotics used are broad-spectrum antibiotics [5]. As a result of the widespread and irrational use of antibiotics, treatment failures, mortality, and cost are increasing [6]. When the extensive use of antibiotics is combined with the increase in resistance to these drugs, the need for novel antimicrobial agents is apparent [7, 8].

Computer-aided drug design (CADD) methods have been applied in drug design, discovery, and development. The application is increasing with the increase in biological and chemical data, increase in data storage capacity, increase in identified drug targets, and advance in data processing capacity [9-11]. Such applications reduce the time, cost, and effort required in the drug discovery process [12, 13].

Molecular docking is a structure-based computational method that is used to generate the binding pose and affinity between ligands and targets by predicting their interactions [14]. DNA gyrase is used in the design and optimization of novel compounds that are selectively active against bacteria. DNA gyrase brings the negative superhelix required for chromosome replication. This enzyme is found in bacteria but not in mammals. In addition, it is a well-known target that plays a crucial role in bacterial DNA replication. Thus, DNA gyrase is an important therapeutic target in designing new selective antibacterial agents [15]. Therefore, it was used as a target in the molecular docking studies undertaken in this work.

In silico predictions of ADMET (absorption, distribution, metabolism, elimination, toxicity) properties are important in drug discovery and development. ADMET evaluations have been shifted to the early stages of drug discovery. Early evaluation of ADMET properties reduces the time and expense needed for screening and trial by identifying the best candidates for drug development and rejecting those that are unlikely to be successful. The ultimate goal of *in silico* ADMET modeling is to predict the *in vivo* affinity behavior of drug candidates in the human body [16].

The structure of benzoxazole is similar to adenine and guanine bases. Therefore, it is one of the important heterocyclic structures utilized in drug designing. Benzoxazole scaffold can participate in several energetically preferable interactions with receptors. The oxygen and nitrogen atoms in its structure can act as HBAs (hydrogen bond acceptors). Its aromatic planar structure enables π-π and π-cation interactions. Hydrophobic interactions with receptors are also possible due to its lipophilic character [17]. In addition, as it is open to chemical modifications, its various derivatives can be formed [18].

Among benzoxazole derivatives, 2-substituted ones have been thoroughly investigated so far, so there is extensive data about their structural properties and activities [19]. In this regard, 2-substituted benzoxazole derivatives have been reported to have broad-spectrum pharmacological activity such as antimicrobial [20], anti-inflammatory [21], analgesic [22], antiepileptic [23], antimalarial [24], anti-HIV [25], anticancer [19], topoisomerase inhibitors [26], kinase inhibitors [27], protease inhibitors [28], GSH inhibitors [29] and cyclooxygenase inhibitors [30]. There are 2-substituted benzoxazole structures containing drugs available on the market. The nonsteroidal anti-inflammatory drugs (NSAIDs) flunoxaprofen and benoxaprofen, the antibiotics calcimycin, and boxazomycin A-B, and the muscle relaxant chloroxazone are among the drugs that have a 2-substituted benzoxazole nucleus (Fig. **1**) [20].

Benzoxazole derivatives have antibacterial activities on various Gram-positive and Gram-negative bacteria. Antibacterial activity studies on 2,5-disubstituted benzoxazole derivatives showed that they are active against *Streptococcus faecalis*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* [31, 32]. Similar studies have shown that 2,5-disubstituted benzoxazole derivative compounds are effective on *Bacillus subtilis* and *Staphylococcus aureus* isolates [33]. There are also benzoxazole derivatives that are active against *Enterococcus faecalis* and *Escherichia coli* isolates [34]. Calcimycin and boxazomycin A-B are active drugs against Gram-positive bacteria (Fig. **1**) [35]. Benzoxazole derivatives also have antibacterial activity on *Mycobacterium tuberculosis* [36]. Furthermore, 2-substituted benzoxazole derivatives were found to have antifungal activity against *Candida albicans*, *C. krusei, C. glabrata*, *Trichophyton mentagrophyes, T. rubrum, T. mentagrophytes, Trichoderma viride, Aspergillus niger, A. oryzae, Mucor cirinelloides,* and *Trichomonas* species [37, 38].

These studies showed that benzoxazole derivatives are active against various microorganisms that cause infectious diseases. Therefore, considering the information in the literature, benzoxazole derivatives are expected to have a potent antimicrobial activity with a broad spectrum.

In the light of this information, 2-(4-substituted benzyl)-5-substituted benzoxazole derivatives were synthesized. Antimicrobial activity of the compounds was tested against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis,* and *C. albicans* standard strains and clinical isolates. They were found to be active only at high concentrations (64-512 μg/ml). Then, the antimicrobial activity of the compounds was compared to the activity of the other previously tested benzoxazole compounds without the methylene bridge between oxazole and phenyl ring. Benzoxazole derivatives without the methylene bridge were found to be much more active than those with the methylene bridge. This was also confirmed by molecular docking and pharmacophore analysis performed in this study. Molecular docking results indicated that the benzoxazole derivatives could act as an antimicrobial agent by inhibiting DNA gyrase. Pharmacophore analysis demonstrated that HBA, HBD, and hydrophobicity features were important

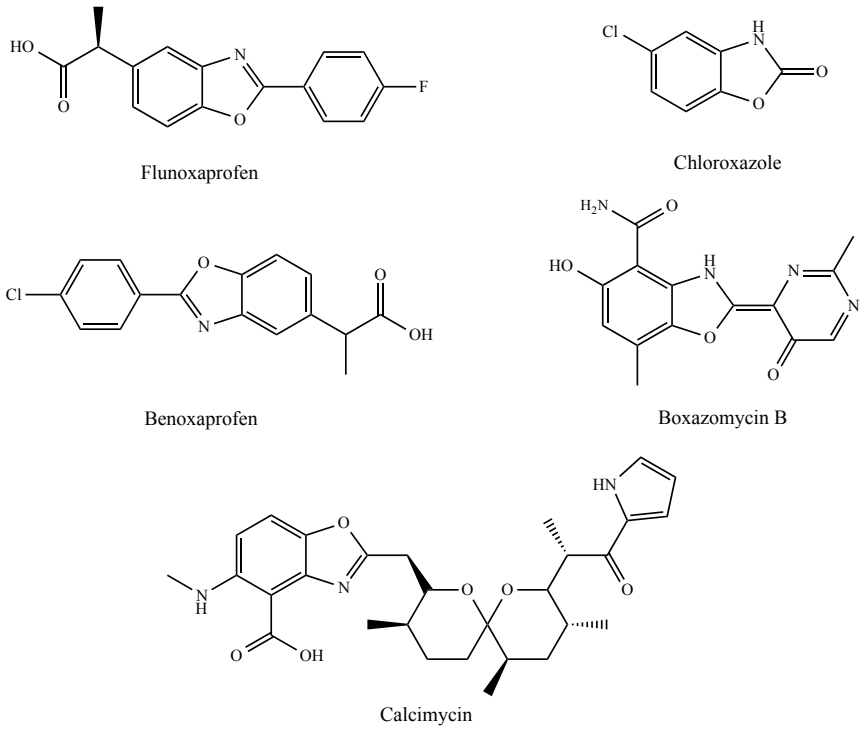


Fig. (1). Benzoxazole ring-containing drugs on the market.

for the inhibition. In addition, the ADMET property of the compounds was predicted. According to *in silico* analysis, the compounds are expected to have good pharmacokinetics.

2. MATERIALS AND METHODS

In this study, synthesis of benzoxazole derivatives, determination of their antimicrobial activities, and molecular modeling studies were carried out.

2.1. Synthesis of 2-(4-Substituted Benzyl)-5-Substituted Benzoxazole Derivatives

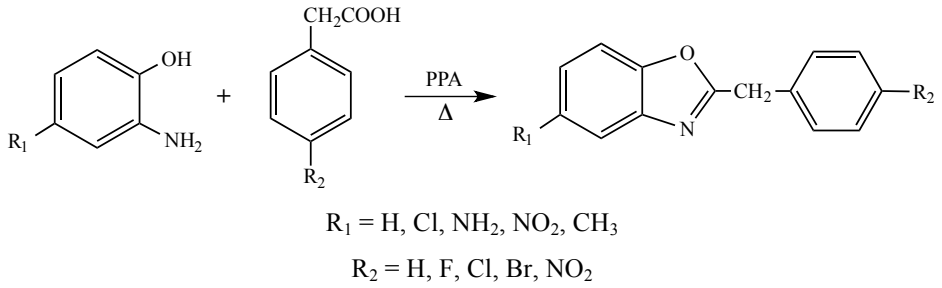
10 mmol 2-amino-4-substituted phenol and 10 mmol 4-substituted phenylacetic acid reacted in the presence of PPA (polyphosphoric acid) at 130°C for about 3 hours. The reaction product was mixed with ice. Then, the mixture was neutralized with 10% NaOH. The settled mixture was filtered. Finally, the end product was cleaned with activated charcoal and recrystallized (Fig. **2**). The reaction progress was followed, and the purity of the end products was checked by TLC (thin layer chromatography) [39]. The structure of the synthesized compounds was also elucidated by mass spectrometry (Waters Alliance ZQ Micromass), NMR (Varian Mercury (Agilent), 400MHz), and elemental analysis (Leco CHNS 932).

2.2. Determination of Antimicrobial Activity

The standard strains *P. aeruginosa* ATCC 27853*, E. coli* ATCC 25922*, S. aureus* ATCC 29213, *E. faecalis* ATCC 29212*, C. albicans* ATCC 10231, and clinical isolates provided from Trakya University Hospital were used. Ampicillin (Sigma), vancomycin (Mayne Pharma), cefotaxime (Sigma), ciprofloxacin (Sigma), gentamicin (Sigma), fluconazole (Sigma), and amphotericin B (Riedel-de Haen) were used as standard antimicrobial agents. Stock solutions of the standards and the synthesized compounds were prepared with appropriate solvents.

Antibacterial sensitivity testing was carried out according to CLSI M100-S16 principles [40]. Antifungal sensitivity testing was performed according to CLSI M27-A3 principles [41]. MHA, MHB, SDA, SLM, and RPMI-1640 medium with L-glutamine buffered to pH 7 with MOPS, supplied by Merck and Sigma, were used for microbial cultures. Bacterial isolates were subcultured in MHA plates and incubated overnight at 37^{o}C, and fungi were subcultured in SDA plates at 35^{o} C for 24-48 h. The pure ones were transferred to MHB and SLM for bacteria and fungi, respectively. Then, they were incubated overnight. After incubation, the bacterial suspensions used for inoculation were prepared at 10^5cfu/ml by diluting fresh cultures at McFarland 0.5 density (10^8 cfu/ml). Similarly, yeast suspensions were prepared at McFarland 0.5 density. Then, the working suspension ($2.5x10^3$ cfu/ml) was prepared by diluting the stock solution at 1:50, followed by 1:20.

Sensitivity testing was conducted with MHB for bacteria and RPMI-1640 medium for fungi. Solution of the synthesized compounds and standards were prepared at 512, 256, 128, 64, 32, 16, 8, 4 µg/ml. After dilution, a 10 µl bacterial or fungal inoculum was added to each well. The plates were incubated at 37^{o}C for bacteria and 35^{o}C for fungi in a humid chamber, and MIC endpoints were read after incubating for 24 h. The lowest concentration of the compound that inhibits the visible growth completely was fixed as minimum inhibitory concentration (MIC). All tests were made in 3 replicates. Solvents, microorganisms, and media were used as controls.



R_1 = H, Cl, NH_2, NO_2, CH_3

R_2 = H, F, Cl, Br, NO_2

Fig. (2). Synthesis of 2-(4-substituted benzyl)-5-substituted benzoxazole derivatives.

2.3. Molecular Modeling Studies

2.3.1. Molecular Docking

The structure of DNA gyrase B with PDB ID of 4DUH was retrieved from PDB. The crystal structure from *E. coli* is DNA gyrase B in complex with a small inhibitor and has a resolution of 1.50 Å [42]. Using AutoDock tools, the GRID box was adjusted in a manner that covers the ligand bound to the protein used. Then, the protein was prepared by adding polar hydrogens and assigning Gasteiger charges. The ligands drawn with ChemDraw ultra 12.0 were first optimized with the Avogadro program [43, 44]. Then, ligand preparation was completed by adding polar hydrogens and assigning Gasteiger charges. As all the parameters were ready, the AutoDock Vina program was run by giving an appropriate command to the command prompt [45]. Visualization of docking results was done with Discovery Studio 3.5 [46]. To validate the docking undertaken, the ligand bound to the 4DUH protein was redocked with the crystal structure. In addition, the docking results were compared to docking results of a standard drug, ciprofloxacin, which is a well-known DNA gyrase inhibitor.

2.3.2. Pharmacophore Analysis

Pharmacophore analysis was performed with the HipHop method of Discovery Studio 3.5. The ligands used were optimized with a CHARMM force field. A set of known DNA gyrase inhibitors (ciprofloxacin, emodin, pyridine carboxamide, and epicatechin) were picked as a training set for use in pharmacophore model generation. Ciprofloxacin was considered as a reference compound, and the principal value of 2 and MaxOmitFeat value of 0 were specified for it. Hypotheses were generated with Common Feature Pharmacophore Generation. In building the pharmacophore hypotheses, HBA, HBD, hydrophobicity, and aromatic ring were chosen as pharmacophore features. Among the hypotheses built, the most suitable one was selected for further evaluation. The results obtained from mapping the synthesized compounds to the hypothesis were also analyzed.

2.3.3. Prediction of ADMET Properties

ADMET properties of the compounds were predicted with Discovery Studio 3.5. Among the parameters related to pharmacokinetic properties, PSA-2D (polar surface area-2 dimensional) that expresses partial absorption and AlogP98 (atomic logarithmic partition coefficient) that expresses lipophilicity were estimated. Metabolism was predicted using the CYP2D6 enzyme among CYP enzymes. The toxicity of the compounds was estimated by hepatotoxicity and Ames toxicity. In addition, the solubility, BBB (blood-brain barrier) permeability, and PPB (plasma-protein binding) were estimated [47].

3. RESULTS AND DISCUSSION

3.1. Synthesis of Benzoxazole Derivatives

In this study, 23 benzoxazole derivatives were resynthesized. Two of the synthesized compounds (6,13) were synthesized for the first time in this study. The remaining compounds were synthesized by our group previously [19, 48-51]. Herein, the compounds were synthesized again to conduct antimicrobial activity studies.

3.1.1. 2-(4-Nitrobenzyl)-5-aminobenzoxazole (6)

Yield: 70.5%; mp: 196-197℃; ^{1}H-NMR (DMSO-d_6): δ ppm = 4.20 (2H, s, CH_2), 5.01 (2H, s, NH_2), 6.56 (1H, dd, J = 8.8 Hz, J = 2.0 Hz), 6.76 (1H, d, J = 2.0 Hz), 7.30 (1H, d, J = 8.8 Hz), 7.37-7.40 (4H, m, phenyl); MS (m/z): 270.96 $[M + H]^+$ (%62), 311.96 $[M + H + 41(CH_3CN)]^+$ (%100); theor. elem. calc. for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61; pract.: C, 61.96; H, 4.16; N, 15.71.

3.1.2. 2-(4-Fluorobenzyl)-5-chlorobenzoxazole (13)

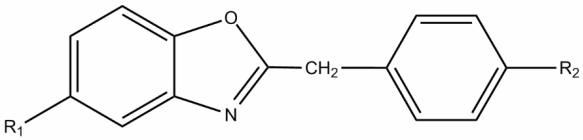
Yield: 65%; mp: 74-75℃; ^{1}H-NMR (DMSO-d_6): δ ppm = 4.20 (2H, s, CH_2), 7.20-7.49 (4H, m, phenyl), 7.56 (1H, d, aromatic), 7.62 (1H, d, aromatic), 7.80 (1H, d, aromatic); MS (m/z): 262.74 $[M + H]^+$ (%100), 264.73 (%32), 263.74 (%15.3), 265.74 (%4.9), 264.74 (%1.3); theor. elem. calc. for $C_{14}H_9ClFNO$: C, 64.26; H, 3.47; N, 5.35; pract.: C, 65.01; H, 3.51; N, 5.30.

3.2. Antimicrobial Activity of Synthesized Compounds

The synthesized compounds were found to be active only at much higher concentrations than the standard drugs (Table **1**).

The antimicrobial activity studies conducted showed that the synthesized compounds were active against Gram-positive and Gram-negative bacteria at 128-512 μg/ml concentrations. Similarly, it was determined that they had antifungal activity at 64-128 μg/ml concentrations. Though the compounds were active against clinical isolates, the concentration was high compared to the standard drugs used (Table **1**). Then, the antimicrobial activity of the synthesized compounds was compared to the activity of their benzoxazole derivative analogs without the methylene bridge between the

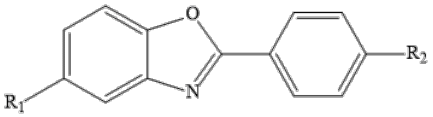
Table 1. Antimicrobial activity of the synthesized compounds (MIC in µg/ml).



Compounds	R_1	R_2	S.a.	S.a.*	E.f.	E.f.*	E.c.	E.c.*	P.a.	P.a.*	C.a.	C.a.*
1	CH_3	NO_2	256	512	256	128	256	256	128	256	128	128
2	CH_3	Cl	256	256	256	128	256	256	128	256	128	128
3	CH_3	F	256	512	256	128	256	256	128	256	128	128
4	CH_3	Br	256	512	256	128	256	256	128	256	128	128
5	CH_3	H	256	512	256	256	256	256	128	256	128	128
6	NH_2	NO_2	256	512	256	256	256	256	128	256	128	128
7	NH_2	Cl	256	512	256	256	256	256	128	256	128	128
8	NH_2	F	256	512	256	256	256	256	128	256	128	128
9	NH_2	Br	256	512	256	256	256	256	128	256	64	128
10	NH_2	H	256	512	256	256	256	256	128	128	64	128
11	Cl	NO_2	256	512	256	256	256	256	128	256	128	128
12	Cl	Cl	256	512	256	128	256	256	128	128	64	128
13	Cl	F	256	256	256	128	256	256	256	256	128	128
14	Cl	Br	256	512	256	128	256	256	256	256	128	128
15	Cl	H	256	512	256	128	256	256	256	256	128	128
16	H	NO_2	256	512	256	256	256	256	256	256	128	128
17	H	Cl	256	512	256	256	256	256	256	256	128	128
18	H	Br	256	256	256	256	256	256	256	256	128	128
19	NO_2	NO_2	256	512	256	256	256	256	256	256	128	128
20	NO_2	Cl	256	512	256	256	256	256	256	256	128	128
21	NO_2	F	256	512	256	256	256	256	256	256	128	128
22	NO_2	Br	256	512	256	256	256	256	256	256	128	128
23	NO_2	H	256	512	256	128	256	256	256	256	128	128
Ampicillin	-	-	0.5	>16	2	>16	8	>16	-	-	-	-
Vancomycin	-	-	0.5	2	1	>8	-	-	-	-	-	-
Gentamycin	-	-	0.25	>16	4	>8	0.5	>8	0.5	>8	-	-
Ciprofloxacin	-	-	0.5	>16	2	>4	0.02	>2	0.13	>2	-	-
Cefotaxime	-	-	1	>16	-	-	0.13	>8	8	-	-	>4
Fluconazole	-	-	-	-	-	-	-	-	-	-	0.13	-
Amphotericin-B	-	-	-	-	-	-	-	-	-	-	0.5	0.5

The representation is: **S.a.**:*Staphylococcus aureus* ATCC 29213, **S.a.***: *S. aureus* isolate, **E.f.**: *Enterococcus faecalis* ATCC 29212, **E.f.***: VRE isolate, **E.c.**: *E.coli* ATCC 25922, **E.c.***: *E.coli* isolate, **P.a.**: *P. aeruginosa* ATCC 27853, **P.a.***: *P. aeruginosa* isolate, **C.a.**: *Candida albicans* ATCC 10231, **C.a.***: *C. albicans* isolate.

Table 2. Antimicrobial activity of benzoxazole derivatives (MIC in µg/ml) [54, 55].



Compounds	R_1	R_2	*S.aureus*	*S.faecalis*	*E.coli*	*P.aeroginosa*	*C.albicans*
3’	CH_3	F	25	25	12.5	12.5	25
6’	NH_2	NO_2	25	25	25	12.5	12.5
7’	NH_2	Cl	100	50	100	100	50
8’	NH_2	F	25	25	25	12.5	25
9’	NH_2	Br	25	25	25	12.5	25
10’	NH_2	H	25	25	25	12.5	12.5
11’	Cl	NO_2	25	25	12.5	25	25
12’	Cl	Cl	25	50	25	25	25
17’	H	Cl	100	100	100	100	200
18’	H	Br	200	200	200	200	200
20’	NO_2	Cl	12.5	12.5	25	25	12.5
21’	NO_2	F	100	-	50	50	50
22’	NO_2	Br	6.25	12.5	12.5	12.5	12.5
23’	NO_2	H	12.5	100	12.5	12.5	12.5
Ampicillin	-	-	0.39	0.39	1.56	>400	-
Amoxicillin	-	-	0.39	0.39	1.56	>400	-
Erythromycin	-	-	25	1.56	50	25	-
Chloramphenicol	-	-	12.5	6.25	25	25	-
Haloprogin	-	-	-	-	-	-	3.24
Clotrimazole	-	-	-	-	-	-	6.25

oxazole and phenyl ring. There was a significant difference between the activity of the two groups. Benzoxazole derivatives without a methylene bridge were found to be more active than the ones with the methylene bridge (Table **2**). According to previous research by our group, there were derivatives that had potent antibacterial activity against similar bacterial species at concentrations as low as 6.25 µg/ml (Table **2**). In another previous study by our group, the antimicrobial activity of similar 5-ethylsulphonyl substituted benzoxazole derivatives with and without methylene bridge was tested. Two compounds without the methylene bridge were found to be the most active against *B. subtilis* at 7.8125 µg/ml concentration [33]. In another antimicrobial activity study on 5-substituted-2-(4-tertbutylphenyl) benzoxazole derivatives, one of the derivatives without the methylene bridge was found to be more active than the standard drugs against *E. coli* at 8 µg/ml [52]. Furthermore, all of the benzoxazole derivatives in the pharmaceutical market, except calcimycin, are without the methylene bridge (Fig. **1**). Even the natural benzoxazole compound, caboxamycin, which was found to be active against various bacteria species, is without the methylene bridge [53].

3.3. Molecular Modeling

3.3.1. Molecular Docking

Molecular docking of DNA gyrase B with the ligands was carried out using AutoDock Vina. The binding mode of the ligands was found to be good (Fig. **3**). The redocking of the ligand bound to the 3D structure used resulted in a binding mode, which is good and similar to the reference drug (Fig. **3b**). The relatively active compounds against the microorganisms used in the susceptibility testing were also found to have a common interaction pattern with the reference drug (Fig. **3c-d**). Furthermore, the binding energies of the reference drug, bound ligand, and active compounds were similar (Table **3**).

Molecular docking of the synthesized compounds with a B subunit of DNA gyrase was performed to elucidate the

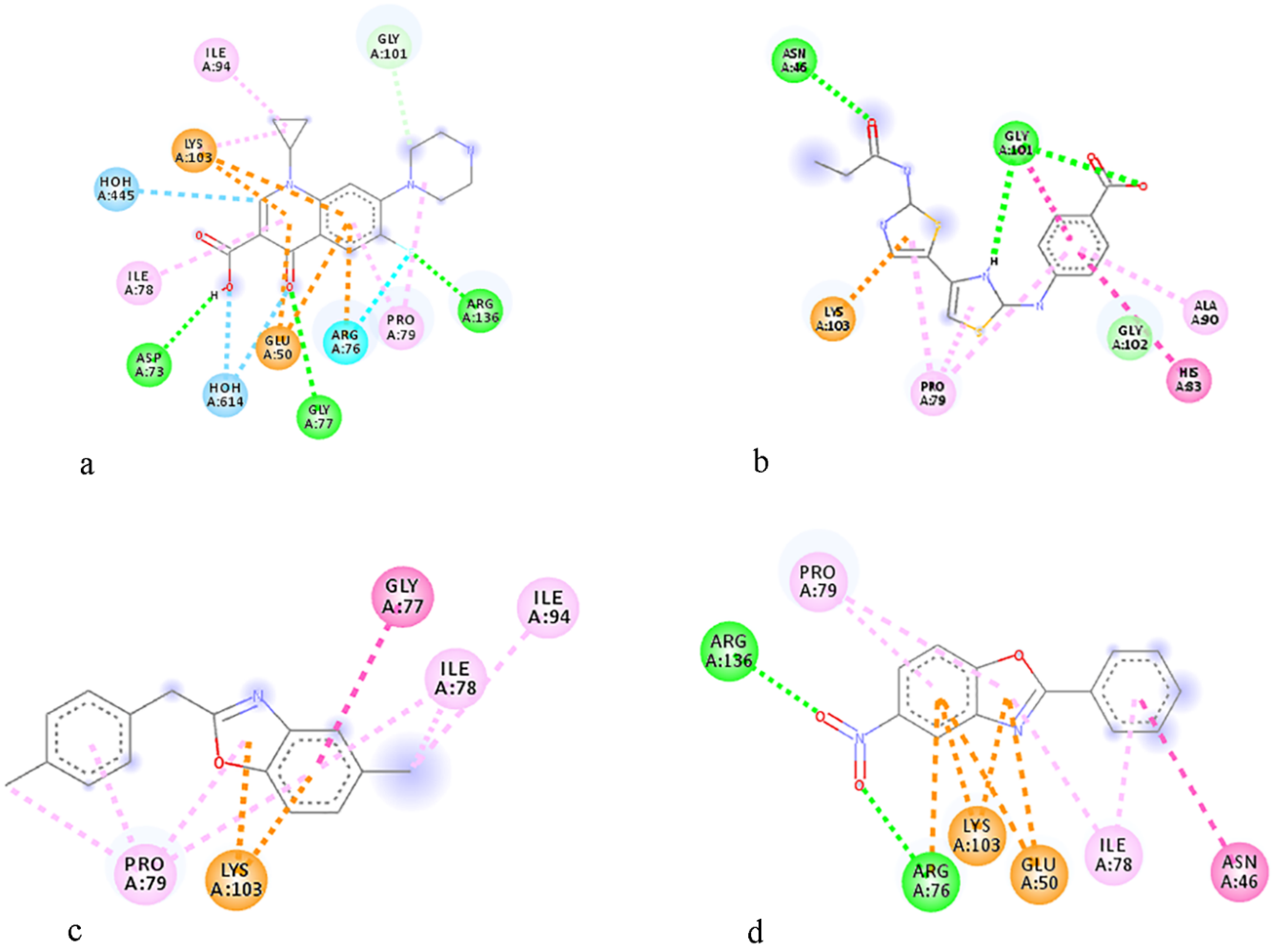


Fig. (3). Binding profile of DNA gyrase B with: **a**) ciprofloxacin, **b**) bound ligand, **c**) compound 12 (with methylene), and **d**) compound 22' (without methylene). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

mechanism of action. The reason behind the use of the DNA gyrase B subunit as a target is that ATP binding site is located in this unit. Furthermore, this subunit has been thoroughly investigated due to its functional value [56].

Redocking with the ligand bound to the DNA gyrase structure used was performed to validate the molecular docking results. The binding mode obtained was good and similar to the results of the standard drug as well as the active molecules (Table **3**, Fig. **3**). The interaction pattern and the binding energy similarity detected approved the quality of docking carried out (Table **3**).

In the interaction of the most active compound (22'), two conventional hydrogen bonds were detected between its nitro group and the residues in the ATP binding site (Arg76, Arg136). Pi-cation/anion interactions were formed with Glu50, Arg76, and Lys103. Furthermore, hydrophobic interactions were detected with Asn46, Ile78, and Pro79. Superimposition of the docked 22' and ciprofloxacin demonstrated the structural fitting between them. In addition to this, they were found to be inside the binding pocket of the enzyme (Fig. **4a**). The role of the conserved water molecules in the interaction of the ligands with the enzyme was also investigated. W445 and W614 were found to play a role in the binding of ciprofloxacin to the enzyme (Table **3**).

In the antimicrobial activity testing, in general, compounds with high electronegative substituents at the 5th position of the benzoxazole ring (R_1) had relatively better efficacy (Tables **1** and **2**). This was also observed in the docking results as there were interactions with substituents at this position. Two conventional hydrogen bonds were formed with the nitro group of compound 22' at R_1. Compound 12 had less antimicrobial activity and also weaker interactions compared to compound 22'. This might result from the orientation of the hydrogen bond acceptors (O,N) in the benzoxazole moiety (Fig. **4b**). The two HBA atoms were oriented near the HBA region of the binding pocket of the enzyme. Had these atoms oriented in the HBD region of the binding pocket, the possibility of forming hydrogen bonds would have been higher. In addition to this, this molecule had weaker interaction since its 4-substituted phenyl group was out of the binding pocket (Fig. **4b**). Visual inspection of the docked molecule's 3D structure in the binding pocket exhibited the distance between the hydrogen bond exchange regions of the ATP binding site and the electronegative substituents of the molecule (Fig. **4b**). This result clearly

Table 3. Comparison of the interactions of the reference drug (ciprofloxacin), bound ligand, and some selected active compounds.

Ligands	Binding Energy (kcal/mol)	Conventional Hydrogen Bonds	Other Amino Acid Interactions
Ciprofloxacin	-9.2	Asp73, Gly77, Arg136, W445, W614(2)	Glu50(2), Arg76(2), Ile78, Pro79(2), Ile94, Gly101, Lys103(3)
Bound ligand	-9.0	Asn46, Gly101(2),	Pro79(3), His83, Ala90, Lys103
12 (with methylene)	-7.5	-	Gly77, Ile78(2), Pro79(4), Ile94, Lys103 (2)
22' (without methylene)	-9.4	Arg76, Arg136	Asn46, Glu50(2), Ileu78(2), Pro79(2), Lys103(2)

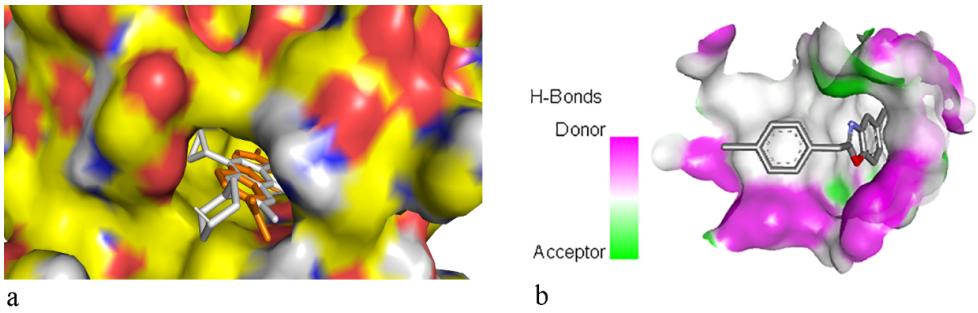


Fig. (4). Structural analysis of docking results: **a**) superimposition of docked 22' (orange) and ciprofloxacin (gray), **b**) the conformation of docked 12 with hydrogen bond donor and acceptor regions in the binding pocket. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

showed the role of the structural difference in binding and thus the activity of the ligands.

A previous experimental study showed that Lys103 residue played an important role in the binding of ATP, the natural substrate, to DNA gyrase. It was revealed that DNA gyrase-inhibitor crystal complex did not form in the absence of the interaction at Lys103 [42]. In this study, the reference drug, bound ligand, and active compounds were found to have at least one interaction with Lys103 (Table **3**). In this way, a crucial interaction in the binding of a compound to DNA gyrase was achieved. In the same study, X-ray crystallography revealed hydrophobic interactions with residues at Ile78 and Ile94 [42]. In the docking study, it was detected that all of the active compounds and the standard exhibited a similar interaction at these residues (Table **3**). In another experimental study, hydrophobic interactions of DNA gyrase with residues at Ile78, Pro79, and Ile94 were detected by X-ray crystallography [57]. Similarly, the docking results revealed that the active compounds and the standard have hydrophobic interactions at these residues (Table **3**). The main residues involved in the binding of inhibitors to the ATP binding site of the A-domain of DNA gyrase B are Asn46, Asp73, and Arg136 [57, 58]. In this computational study, the standard and the most active compound were found to meet the interactions at two of these residues (Table **3**). In short, the experimental study data validated the results obtained from this study.

Molecular docking results demonstrated that the standard drug, bound ligand, and active molecules interacted with the target very well. In addition, it was revealed that their interactions with DNA gyrase were similar to each other (Table **3**). Hence, it can be said that the active benzoxazoles exhibit their antibacterial activity by inhibiting DNA gyrase. Benzoxazole derivatives without a methylene bridge were found to be more active than those with the methylene bridge. The docking results exhibited that compound 22' (without methylene bridge) had two hydrogen bonds, but compound 12 (with methylene bridge) had only hydrophobic and electrostatic interactions with the target. This result approved the experimental antimicrobial study results in this study and previous studies.

3.3.2. Pharmacophore Analysis

Pharmacophore analysis was performed with known DNA gyrase inhibitors using the HipHop method. HBA, HBD, and hydrophobicity features were found to be important in DNA gyrase inhibitory activity (Fig. **5a**). The standard inhibitors used to fit with the pharmacophore model built (Fig. **5b**). Furthermore, it was revealed that the methylene bridge in the synthesized compounds was not among the essential features of the inhibition (Fig. **5c**). Thus, the suggestion that benzoxazole derivatives without this bridge are more active is compatible with the pharmacophore analysis.

3.3.3. ADMET Properties

ADMET properties of the compounds were predicted with Discovery Studio 3.5. The result was evaluated based on PSA-2D, AlogP98, CYP2D6, BBB, PPB, and mutagenicity (Table **4**). Through *in silico* ADMET investigations, the pharmacokinetic properties of the synthesized compounds were found to be good. PSA-2D values were below one

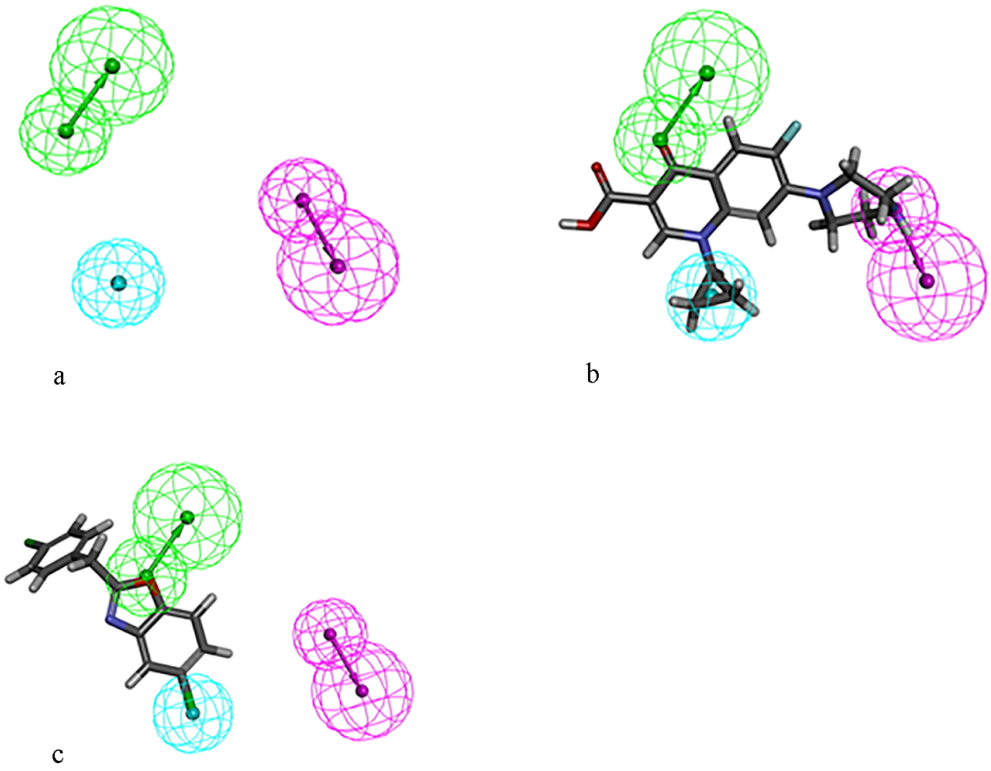


Fig. (5). a) Hypothesis generated using DNA gyrase inhibitors (green-HBA, pink-HBD, sky blue-hydrophobicity), **b**) mapping of ciprofloxacin with the hypothesis, **c**) mapping of compound 12 with the hypothesis. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 4. Predicted ADMET properties of the synthesized compounds.

Compounds	AlogP98	PSA-2D	S	BBB	L	CYP2D6	T	H	PPB	AM
1	4.207	61.62	-5.549	0.171	1	-3.433	F	3.260	3.878	M
2	4.466	23.815	-5.671	0.849	0	0.712	T	2.161	7.608	N
3	4.007	23.815	-5.259	0.708	0	0.604	T	4.606	6.973	N
4	4.55	23.815	-5.749	0.875	0	-1.425	F	2.653	4.370	N
5	3.801	23.815	-5.014	0.644	1	-0.950	F	-0.072	4.485	M
6	2.974	88.16	-4.679	-0.63	3	-4.373	F	4.057	0.238	M
7	3.233	50.355	-4.632	0.048	1	-0.434	F	3.500	5.120	N
8	2.774	50.355	-4.22	-0.09	2	-0.370	F	6.078	4.072	M
9	3.317	50.355	-4.71	0.074	1	-2.571	F	3.905	1.852	N
10	2.568	50.355	-3.806	-0.16	2	-2.894	F	1.400	1.463	M
11	4.385	61.62	-5.763	0.226	1	-0.317	F	4.960	5.804	M
12	4.644	23.815	-5.8	0.904	0	2.048	T	2.790	7.134	N
13	4.185	23.815	-5.387	0.763	0	3.614	T	6.300	8.844	N
14	4.728	23.815	-5.877	0.93	0	1.257	T	4.274	5.928	N
15	3.979	23.815	-5.143	0.699	1	1.820	T	1.396	6.011	N
16	3.72	61.62	-5.047	0.021	1	-2.968	F	3.163	2.689	M

(Table 4) Contd….

Compound	AlogP98	PSA-2D	S	BBB	L	CYP2D6	T	H	PPB	AM
17	3.979	23.815	-5.157	0.699	1	1.216	T	2.148	6.228	N
18	4.063	23.815	-5.234	0.725	0	-1.055	F	2.633	3.273	N
19	4.126	99.426	-5.837	-	4	-3.130	F	3.635	1.701	M
20	4.385	61.62	-5.731	0.226	1	-0.249	F	3.356	6.039	N
21	3.926	61.62	-5.318	0.084	1	-0.589	F	5.485	5.349	M
22	4.469	61.62	-5.808	0.252	1	-2.820	F	3.455	1.971	N
23	3.72	61.62	-5.001	0.021	1	-2.296	F	0.807	2.707	M

In Table 4 the representation is: S: solubility, BBB: blood-brain barrier, L: BBB level, T: predicted CYP2D6 metabolism, H: hepatotoxicity, PPB: plasma-protein binding, AM: predicted TOPKAT Ames mutagenicity, T: true, F: false, M: mutagenic, N: non-mutagenic.

hundred (Table **4**). This means the synthesized compounds are expected to have good oral absorption or membrane permeability [59]. AlogP98 values were estimated to be below five (Table **4**). This implies that the compounds have an ideal lipophilic property [60]. BBB permeability analysis demonstrated that compounds 2, 3, 4, 12, 13, 14, and 18 had a high possibility of crossing the barrier, compound 19 could be the least likely to cross, and the remaining compounds had a moderate ability of crossing (Table **4**). The study demonstrated that compounds 2, 3, 12, 13, 14, 15, and 17 might be substrates for the CYP2D6 enzyme (Table **4**). This suggests that these compounds can be metabolized in the liver and also be toxic to the enzyme. PPB results showed that all compounds could be bound to plasma proteins highly (Table **4**). Therefore, this implies that they have good bioavailability and do not bind much with carrier proteins in the blood [61]. Ames mutagenicity predictions indicated that compounds 2, 3, 4, 7, 12, 13, 14, 15, 17, 18, 20, and 22 were not mutagenic, whereas the rest might be mutagenic (Table **4**) [47]. These findings will be crucial in prospective studies on these compounds.

CONCLUSION

In this study, 2-(4-substituted benzyl)-5-substituted benzoxazole derivatives were synthesized. Antimicrobial activity testing was performed. The compounds were found to be active only at much higher concentrations than the standard drugs. The antimicrobial activity detected in this study was compared to the susceptibility tests undertaken in our previous research. Benzoxazole derivatives without the methylene bridge were found to be more active than analogs with this bridge. Furthermore, this result was supported by molecular modeling.

Through molecular docking, it was indicated that the active benzoxazole derivatives could act as antimicrobial agents by inhibiting DNA gyrase. Pharmacophore analysis demonstrated that HBA, HBD, and hydrophobicity features would be essential for antimicrobial activity by the inhibition of this enzyme. The *in silico* ADMET property analysis implicated that the compounds would have good pharmacokinetics. Thus, molecular modeling results in this study elucidated important characteristics of the compounds.

Based on the analysis in this study, it is recommended to deal with benzoxazole derivatives without the methylene bridge for antimicrobial efficacy. In addition, in antimicrobial agent designing, it is advised to include HBA, HBD, and hydrophobicity features to bring selective antimicrobial potency by DNA gyrase inhibition. From the molecular modeling, the synthesized compounds are expected to have suitable pharmacokinetics as novel drug candidates. Therefore, looking for the other pharmacologic activity of the compounds is meaningful.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available within the article.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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