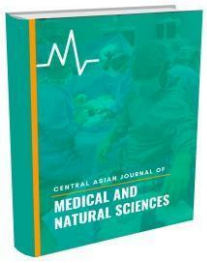

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*Article*

# Synthesis of New 1,3,4-Oxadiazole Derivatives: Molecular Docking Simulation and Biological Activity Study Against Resistant Bacteria with ADME Properties Analysis

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**Abstract:** In this research, we synthesized two new compounds from 1,3,4-oxadiazole derivatives namely compound 5 and compound 7. The biological activity of these two compounds against six different types of bacteria viz. Pseudomonas aeruginosa (MDR), Escherichia coli 44, Escherichia coli 41, Klebsiella pneumoniae 49, Klebsiella pneumoniae 6 and Acinetobacter baumannii 17 were evaluated with the help of antibacterial activity assay. The results indicated that compound 5 exhibited high activity against some bacterial species relative to the standard antibiotic, imipenem. Compound 7 had excellent activities against multi-drug-resistant bacteria. Simulation of the two compounds using Molecular Docking simulations through the molecular simulation program was also done, which gave excellent results indicating strong and possible interactions of the two compounds with bacterial targets. The results indicate that compound 5 has promising potential in treating bacterial infections, and these results are an important step towards the development of new antibiotics with higher efficacy. The compounds were characterized using spectroscopic techniques: FT-IR, 1H-NMR, 13C-NMR and Mass spectra which confirmed the chemical structures of the compounds.

**Keywords:** 1,3,4-Oxadiazole Derivatives, Antibacterial, Molecular Docking, β-lactamase, DNA Gyrase (Gyr-A)

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## 1. Introduction

The impact of microbial infections extends worldwide, where excessive use of antimicrobial drugs has led to the development of microbial resistance. This development causes disease organisms to become resistant to most antimicrobial agents [1]. The prevalence of antibiotic resistance has increased, creating an urgent need to develop new drugs to combat this resistance [2]. On the other hand, preventing oxidative stress is extremely important to reduce the risk of diseases, such as cancer and cardiovascular disease. However, powerful antioxidants with superior bioavailability are needed. The activity of heterocyclic moieties arouses great interest due to their antibacterial ability and their antioxidant and anticancer effects. Among these parts, azole groups containing nitrogen or sulfur, such as 1,3,4-oxadiazole/thiadiazole, are of interest to researchers and scientists due to their potential antimicrobial [3], [4], antifungal [5], [6], and anticancer activities [7], [8], antimalarial [9], antioxidant [10], [11], anti-inflammatory [12], and anticonvulsant [13].

Heterocyclic structures constitute a large part of naturally occurring biologically active molecules and are essential for the synthesis of new molecules with pharmacological and physiological activity. Many recently discovered antibacterial agents contain at least one heterocyclic ring. According to this perspective, the synthesis of new heterocyclic compounds containing nitrogen atoms, such as oxadiazole groups, is a prominent area in medicinal and pharmaceutical chemistry. These groups consist of a heterocyclic ring composed of two carbon atoms, two nitrogen atoms, and one oxygen atom (5). Due to the wide variety of medicinal properties of 1,3,4-oxadiazole compounds, most studies have focused on this isomer, while other less studied isomers (1,2,3-, 1,2,4-, and 1,2,5-oxadiazoles) have been addressed less [14], [15]. 1,3,4-oxadiazole compounds contain a -N=C- bond and exhibit pharmacologically active components [16]. Alkyl substitutions on the oxadiazole ring, compared to phenyl substitutions at the fifth position with electron-withdrawing or electron-donating groups, have been shown to enhance biological activity [17]. In the field of drug discovery and medicinal chemistry, 1,3,4-oxadiazole compounds and their derivatives have been used in a wide range of applications, exhibiting important biological activities such as antibacterial, antidiabetic, antituberculous, antifungal, anticonvulsant, antioxidant, and anticancer activities [18]. Some drugs containing a 1,3,4-oxadiazole ring, such as formamizole, are used as antibacterial agents, while raltegravir and zibutentan, which also contain a 1,3,4-oxadiazole ring, are used in antiviral and chemotherapy treatments [19]–[22].

We have synthesized new derivatives, namely compounds 5 and 7 and the antibacterial activity of all the synthesized compounds was evaluated against the following bacterial isolates: Pseudomonas aeruginosa (MDR), Escherichia coli 44, Escherichia coli 41, Klebsiella pneumoniae 49, Klebsiella pneumoniae 6 and Acinetobacter baumannii 17.

## 2. Materials and Methods

### Synthesis of 2-hydroxybenzohydrazide (2)

28 ml of methyl salicylate (1) (0.22 mol) was mixed with 22 ml of hydrazine hydrate (0.44 mol) in 40 ml of ethanol, and the mixture was then refluxed for 10 hours. The reaction was followed using TLC. The solution was concentrated to half its volume, allowed to cool to room temperature, and then dried, and recrystallized with ethanol, yielding compound (2) in the form of colorless needles. The yield of 2 was 86%, m.p. 143–145°C and IR as reported previously in literature [23]. 1H-NMR (DMSO-d6) δ:6.85-7.85 (4H, Ar-H), 5.81 (2H, s, -NH2), 10.37 (1H, s, -OH), 13.94 (1H, broad s, -NH). MS 194. Anal. CHN: calcd C 49.73, H 3.12, N 14.44, found C 49.77, H 3.14, N 14.47.

Synthesis of 2-(5-sulfanyl-1,3,4-oxadiazol-2-yl) phenol (3).

(25.8 g, 0.17 mol) of compound (2) was dissolved with (0.9 g, 0.17 mol) of potassium hydroxide in 60 ml of ethanol. The solution was cooled in an ice bath to temperature (0-5), then (14 ml, 0.17 mol) of CS2 was added to it using an adding funnel with continuous stirring for 10 minutes. The mixture was stirred for a period of (46 hours) until the H2S gas emission stopped and was detected using paper moistened with lead acetate. The reaction was followed by TLC. After the reaction was completed, the solution was poured into ice water and acidified with hydrochloride solution (10% HCl), then the formed crystals were filtered and recrystallized using absolute ethanol to give the compound crystals (3) [24]. Recrystallized from ethanol to yield 81%, m.p. 202°C, FT-IR (KBr) cm-1: 3347 (C-OH Ar), 3102 (NH), 3069 (C-H Ar), 1612 (C=N, ring), 1309 (C-O-C, ring), 1185 (C=S), 1515 (C=C Ar),. 1H-NMR (DMSO-d6) δ:7.5-8.02 (4H, Ar-H), 10.03 (1H, s, -OH), 12.97 (1H, broad s, -NH). MS-ESI m/z: 194 [M]+. CHN: calcd C 49.73, H 3.12, N 14.44, found C 49.77, H 3.14, N 14.47.

**Synthesis of 2-(5-hydrazinyl-1,3,4-oxadiazol-2-yl) phenol (4)**

20 g of compound (3) (0.1 mol) was mixed with 10 ml of hydrazine hydrate (0.2 mol) in 40 ml of ethanol, and the mixture was then refluxed for 36 hours. The reaction was followed using TLC. The solution was concentrated to half its volume, allowed to cool to room temperature, and then recrystallized with absolute ethanol, resulting in the product of white crystals of the compound (4) [23].Recrystallized from ethanol to yield 75%, m.p. 193°C, FT-IR (KBr) cm-1: 3316 (C-OH Ar), 3192-3113 (NH), 1616 (C=N, ring), 1294 (C-O-C, ring), 1586 (C=C Ar),. 1H-NMR (DMSO-d6) δ: 7.3-7.99 (4H, m, Ar-H), 5.81 (2H, s, -NH2), 9.89 (1H, broad s, -OH), 13.95 (1H, broad s, -NH). MS-ESI m/z: 192.2 [M]+.

**Synthesis of N'-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]benzohydrazide (5)**

In ethanolic solution, 2-(5-hydrazinyl-1,3,4-oxadiazol-2-yl) phenol (4) (4 g) (0.02 mol) was refluxed with 2.6 ml of methyl salicylate (1) (0.02 mol). The whole solution was refluxed for 7 h. The completion of the reaction was identified by TLC. After the reaction was complete, the excess ethanol as a solvent was distilled off and the residue mixture was stirred for 20 min on ice. After filtrating, washing with ice-cold water, drying, and recrystallizing in ethanol, the precipitates were collected [24]. Recrystallized from ethanol to yield 84%, m.p. 228 °C, FT-IR (KBr) cm-1: 3300 (C-OH Ar), 3193 (NH), 1636 (C=O), 1533 (C=N, ring), 1321 (C-O-C, ring), 1499-1481 (C=C Ar). 1H-NMR (DMSO-d6) δ: 7.47-7.63 (5H, m, Ar-H), 7.96-8.01 (4H, m, Ar-H), 9.89 (1H, broad s, -OH), 14.3 (1H, broad s, -NH-C=O), 8.19 (1H, s, -NH). 13C-NMR: δ 110.4 (1C, s, C-Ar), 133.7 (1C, s, C-Ar), 126.7 (1C, s, C-Ar), 127.3 (2C, s, C-Ar), 128.1 (1C, s, C-Ar), 128.5-128.7 (4C, C-Ar), 131.3 (1C, s, C-Ar), 133.7 (1C, s, C-Ar), 155.3 (1C, s, C-Ar), 165.7 (1C, s, C=O), 164.8 and 171.9 (2C, s, C-ring). MS-ESI m/z: 296 [M]+.

**Synthesis of 2-(5-{(2E)-2-[(2Z)-2-bromo-3-phenylprop-2-en-1-ylidene]hydrazinyl}-1,3,4-oxadiazol-2-yl) phenol (7)**

In ethanolic solution, 2-(5-hydrazinyl-1,3,4-oxadiazol-2-yl) phenol (4) (4 g) (0.02 mol) was refluxed with 4.22 g of (2Z)-2-bromo-3-phenylprop-2-enal (6) (0.02 mol). The whole solution was refluxed for 24 h. The completion of the reaction was identified by TLC. After the reaction was complete, the excess ethanol as a solvent was distilled off and the residue mixture was stirred for 20 min on ice. After filtrating, washing with ice-cold water, drying, and recrystallizing in ethanol, the precipitates were collected [24]. Recrystallized from ethanol to yield 84%, m.p. 255 °C, FT-IR (KBr) cm-1: 3450 (C-OH Ar), 3113 (NH), 1579 (C=N, ring), 1375 (C-O-C, ring), 1597 (C=C Aliphatic), 1502-1485 (C=C Ar). 1H-NMR (DMSO-d6) δ: 7.48-7.59 (5H, m, Ar-H), 7.97-8.02 (4H, m, Ar-H), 9.88 (1H, broad s, -OH), 8.11 (1H, s, -N=CH), 7.7 (1H, s, -C=CH), 14.30 (H, broad s, -NH). 13C-NMR: δ 110.4 (1C, s, C-Ar),116.5 (1C, s, C-Ar), 126.7 (1C, s, C-Ar), 127.3 (2C, s, C-Ar), 128.1 (1C, s, C-Ar), 128.5-128.7 (4C, C-Ar), 131.3 (1C, s, C-Ar), 155.3 (1C, s, C-Ar), 148.9 (1C, s, C=C-Br), 149.7 (1C, s, C=N), 164.8 and 171.9 (2C, s, C-ring). MS-ESI m/z: 385 [M]+.

**Antibiotic Susceptibility Test**

Disc diffusion methods were used to determine the sensitivity of isolates (Pseudomonas aeruginosa (MDR), Escherichia coli 44, Escherichia coli 41, Klebsiella pneumoniae 49, Klebsiella pneumoniae 6 and Acinetobacter baumannii 17). Antibiotics (Ipenem and Ciprofloxacin) by using Muller Hinton agar, as mentioned. The isolation of bacteria attended to test their sensitivity to antibiotics compounds (5 and 7) by transferring a small number of pure colonies at the age of 24 hours from the McConkey media in (3) ml of the physiological solution the density was measured by comparing with a fixed turbid solution standard (Mcfrland 0.5). Spread 0.1 ml of the bacterial suspension using a cotton swab on the surface of the Muller Hinton agar media. plates Left for 5 minutes at room temperature. Transfering of several antibiotic disks by sterile forceps, ranging from 3 to 5 tablets per plate, according to the size of the plate, the plate was incubated at 37 ° C for 24 h. The results were read by observing the inhibiting zones

formed by the disk and explained that the bacteria, sensitive, media, or resistant according to standard specifications [25].

**Docking studies**

The docking simulations were performed using the open-source programs AutoDock Chimera and AutoDock Vina, with DSV software used to visualize the interaction. The synthesized compounds 5 and 7 were docked with β-lactamase enzyme (PDB ID:8J6Y), an enzyme that breaks down the β-lactam ring of antibiotics such as penicillin, reducing their antibacterial activity, and DNA gyrase (Gyr-A) enzyme (PDB ID: 3LPX), an enzyme that relieves tension in DNA during the replication process by introducing cuts in the double strands to rewrap them. The enzymes were downloaded from the Protein Data Bank (RCSB). A docking grid box was constructed to determine the active binding site, and four independent dockings were performed to confirm the results. Gasteiger charges and minimum energy were used in the calculations. The main binding modes of the compounds were studied based on their docking scores with the target β-lactamase and DNA gyrase (Gyr-A) using Chimera, with a focus on identifying the amino acids essential for small molecule binding and studying their molecular interactions.

## 3. Results and Discussion

**Chemistry**

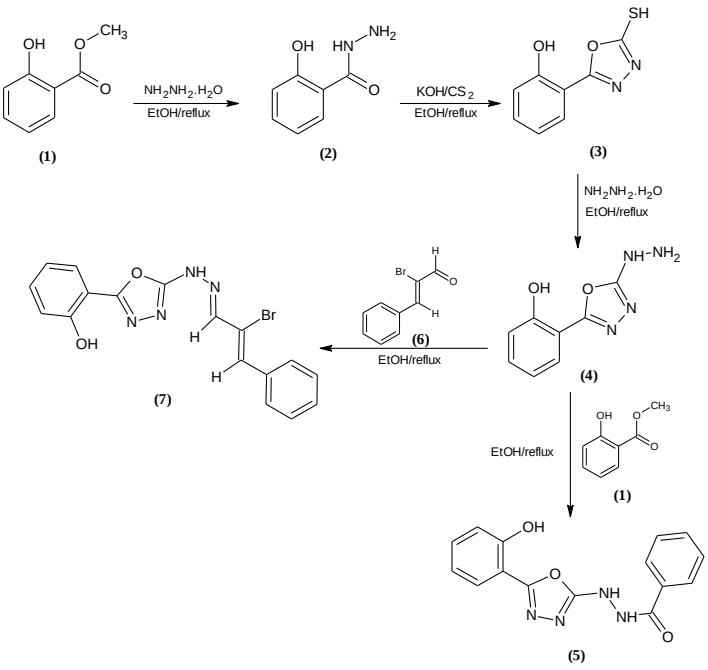
The final products 5 and 7 were synthesized by a combined route, as shown in (Scheme 1). Hydrazide 3, used as starting material for the synthesis, was obtained in 90% yield by heating ester 2 with 64% hydrazine. The product shows characteristic IR bands at 3404 cm-1 for the OH group, 3267 cm-1 for the NH group, and 1635 cm-1 for the CO-N stretching.

To prepare oxadiazole 3, hydrazide 2 reacts with carbon disulfide in an ethanolic medium in the presence of KOH, the compound 5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2(3H)-thione 3 is formed in high production. The weak band with no stretching SH at 2550–2600 cm−1 and the appearance of a strong C=S stretching band at 1177 cm-1 were adopted in the IR spectrum of compound 3 with its thione conformation, excluding the tautomeric thiol structure [26]. The infrared spectrum of the compound also showed 3 bands at 1309 cm-1 (for the C–O–C stretch of the oxadiazole ring), 1515 cm-1 (C=C), 1606 cm-1 (C=N), 3102 cm-1 (NH) and 3347 cm-1 (OH). For 1H-NMR, it revealed the presence of exchangeable singlets at 10.03 ppm and 12.97 ppm, which fit well with the oxadiazole OH proton and phenol NH proton, respectively. While the SH proton which is normally expected to be present in the range of 3.5-6.5 ppm was absent. The 1H-NMR also showed at 7.5-8.02 ppm, indicating the presence of four aromatic protons. The mass spectrum of compound 3 is characterized by the appearance of a molecular ion peak at m/z= 194.

Then 2-(5-hydrazinyl-1,3,4-oxadiazol-2-yl) phenol 4 was prepared by reacting hydrazine hydrate 2 with compound 3 in an ethanolic medium, compound 2-(5-hydrazinyl-1,3,4-oxadiazole). -2-yl) phenol 4 is formed in high yield. The product shows characteristic IR bands at 3316 cm-1 for the OH group, 3192-3113 cm-1 for the NH group, 1616 cm-1 for the (C=N) ring, 1294 cm-1 for the (C-O-C) ring, 1586 cm-1 for the (C=C) aromatic. In the 1H-NMR spectrum of the compound 2-(5-hydrazinyl-1,3,4-oxadiazol-2-yl) phenol 4, we notice the appearance of aromatic proton signals (phenol ring protons) within the range of 7.3-7.99 ppm. We notice the appearance of a broad signal of the proton attached to the phenol group -OH, which usually appears at 9.89 ppm. We also notice the appearance of a broad signal for the proton attached to the hydrazine-NH group, appearing at 13.95 ppm. Finally, we notice the appearance of a broad signal for the protons of the amine group -NH2 in the hydrazine group, appearing at 5.81 ppm. The mass spectrum of compound 4 is characterized by the appearance of a molecular ion peak at m/z= 192.2.

Then N'-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]benzohydrazide 5 was prepared by reacting compound 4 with methyl salicylate 1 in an ethanolic medium, compound N'-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]benzohydrazide 5 is formed in high yield. The product shows characteristic IR bands at 1636 cm-1 for the carbonyl amide (-N-C=O), 3300 cm-1 for the OH group, 3124-3193 cm-1 for the NH group, 1533 cm-1 for the (C=N) ring, 1321 cm-1 for the (C-O-C) ring and 1499-1481 cm-1 for the (C=C) aromatic. In the 1H-NMR spectrum of the compound N'-[5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]benzohydrazide 5, we notice multiple badges in the range of 7.47-8.01 ppm, representing the aromatic protons in the two benzene rings. We notice a broad singlet signal at 9.89 ppm indicating the protonation of the phenol group. We also notice the appearance of a broad singlet signal for the proton (-NH-C=O) at 14.3 ppm and a broad singlet signal for the amine protons (-NH-) at 8.19, indicating the protons of the amine group in hydrazine. The mass spectrum of compound 5 is characterized by the appearance of a molecular ion peak at m/z= 296.

Then 2-(5-{(2E)-2-[(2Z)-2-bromo-3-phenylprop-2-en-1-ylidene] hydrazinyl}-1,3,4-oxadiazol-2-yl) phenol 7 was prepared by reacting compound 4 with (2Z)-2-bromo-3-phenylprop-2-enal 6 in an ethanolic medium. The product shows characteristic IR bands at 3450 cm-1 for the OH group, 3113 cm-1 for the NH group, 1579 cm-1 for the (C=N) ring, 1375 cm-1 for the (C-O-C) ring, 1597 cm-1 for the (C=C) aliphatic and 1502-1485 cm-1 for the (C=C) aromatic. The 1H-NMR spectra of 2-(5-{(2E)-2-[(2Z)-2-bromo-3-phenylprop-2-en-1-ylidene]hydrazinyl}-1,3,4-oxadiazol-2-yl)phenol was distinguished with appear (7.48-8.02 ppm, m, 10H) due to protons of aromatic rings and proton double bond (-C=CH-), (8.11 ppm, 1 H) due to proton of azo methane group (-N=CH-), (9.88 ppm, s,1H) due to OH group, (14.30 ppm) due to NH proton. The mass spectrum of compound 7 is characterized by the appearance of a molecular ion peak at m/z= 385.




**Scheme 1.** Synthesis of 1,3,4-oxadiazole derivatives, compound 5 and compound 7.

**Antimicrobial activity**

Our current study is considered a small part of the ongoing efforts, the purpose of which is to develop new organic chemical compounds that have antibacterial activity, especially against drug-resistant bacterial strains.

In this research, the effectiveness of compound 7 and compound 5 against six types of resistant bacteria (Pseudomonas aeruginosa (MDR), Escherichia coli 44, Escherichia coli 41, Klebsiella pneumoniae 49, Klebsiella pneumoniae 6 and Acinetobacter baumannii 17) was evaluated at two different concentrations (50% and 100%) as shown (Fig.1) and (Table 1).

The effectiveness of compound 7 against the bacteria Pseudomonas aeruginosa (MDR) was evaluated at concentrations (50% and 100%). It was found that compound 7 did not show any anti-Pseudomonas aeruginosa (MDR) effect at concentrations (50% and 100%). Compound 5 inhibition diameter is about 23 mm at 50% and 25 mm at 100% proved to be most active. This shows that compound 5 is extremely active against multi-drug resistant (MDR) Pseudomonas aeruginosa. The assessment of compounds 7 and 5 compared to Escherichia coli strains 44 and 41 shown no detectable-antibacterial activity at concentrations of (50% and 100%) proposing their ineffectiveness against these bacterial-strains. Also when tested against Klebsiella pneumoniae 49 compound 7 shown no antibiotic-activity at the similar concentrations. Compound 5 was observed to have an antagonistic activity with an inhibition zone of 14 mm at 50% concentration whereas there was no inhibition-activity at (100%) concentration. Such fluctuation reflects complex underlying interactions that should be investigated additionally. Compound 7 however was observed to possess activity against Klebsiella pneumoniae 6 with inhibition zones of (12 mm) at 50% concentration and (15 mm) at 100% concentration. Compound 5 was not active against Klebsiella pneumoniae 6 at 50% and 100% concentration. In the case of Acinetobacter baumannii [17] compound 7 was found to have excellent antibacterial activity with inhibition zones of 13 mm (50% concentration) and 17 mm (100% concentration). Encouragingly, compound 5 was found to be even more active against A. baumannii, with inhibition diameters 18 mm (50%) and 20 mm (100%), which suggests it as a potential candidate for new antibiotic development against this pathogen[17].

The results totally show a strong difference in antibacterial-activity between compound 5 and compound 7 in bacterial strains. Compound 5 was highly active against Pseudomonas aeruginosa (MDR) and Acinetobacter baumannii (17), a sign of broad inhibitory action making it an effective antibacterial candidate in new development. Compound 7 displayed less activity against these disease-causing agents than compound 5, a sign of the superior therapeutic activity of the latter (17).

Compound 5 likely utilizes its antibacterial effects through multiple mechanisms, including by inhibition of the Gyr-A enzyme, essential for bacterial DNA replication and cell division thereby blocking bacterial growth [27], [28] and also suppression of β-lactamase-activity which protects β-lactam antibiotics from degradation and improves their efficacy [29], [30] and by disruption of "bacterial membrane integrity" through interactions with membrane lipids which leading to increased permeability, loss of cellular components and osmotic imbalance. These multi-target actions suggest that compound 5 may be particularly effective against resistant bacterial strains, including Pseudomonas aeruginosa (MDR) and Acinetobacter baumannii (17).


*Pseudomonas aeruginosa* *Escherichia coli 44* *Escherichia coli 41*


*Klebsiella pneumoniae 49* *Klebsiella pneumoniae 6* *Acinetobacter baumannii 17*

**Figure 1.** The effectiveness of compounds against resistant bacteria (W= Comp. 5 and Y= Comp. 7).

The antibacterial activity of compounds 5 and 7 was assessed against Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumonia bacteria with "Imipenem and Ciprofloxacin" serving as reference-antibiotics. These reference drugs are well documented in scientific literature for their efficacy against these bacterial strains [31].

Among the tested compounds, Compound 5 exhibited superior activity compared to Compound 7, consistent with earlier results. Against P. aeruginosa, Compound 5 revealed strong antibacterial-effects and producing inhibition zones of (23 mm and 25 mm) comparable to those of Imipenem and Ciprofloxacin. This suggests that Compound 5 has potent antibacterial properties similar to conventional antibiotics.

Compound 7 exhibited moderate-activity against K. pneumonia with (14 mm) inhibition-zone at a (50%) concentration. While this indicates some antibacterial-potential its effectiveness remains lower than that of the reference-antibiotics. In addition we find that compound 5 showed serious effectiveness with inhibition diameter (18 and 20 mm) against Acinetobacter baumannii bacteria which indicates that it is an antibacterial compound and is similar to the antibiotics used. Through this comparison, compound 5 can be classified as an antibacterial compound with strong effectiveness against Pseudomonas aeruginosa and Acinetobacter baumannii bacteria. The strong antibacterial potency of Compound 5 puts it on par with existing antibiotics like Imipenem and Ciprofloxacin, suggesting it as a effective cure for bacterial infection.

In their test against Pseudomonas aeruginosa and Acinetobacter baumannii, Compound 5 exhibited strong inhibitory activity comparable to the reference antibiotics. The big inhibition-zones observed indicate the compound would be most probably bactericidal in action, killing bacteria and not merely inhibiting their growth. This is also supported by the long-time observation that compounds with large inhibition diameters tend to exhibit lethal action against bacterial cells.

In addition, Compound 5 has promise in combating antibiotic-resistant strains, particularly against challenging-to-battle pathogens like P. aeruginosa. Since this bacterium is best known for resistance mechanisms, the observed activity signifies that Compound 5 is capable of breaching resistance, which is a wanted quality in a candidate for development. In all, Compound 5 can be classed as bactericidal compound (due to its highly active growth inhibition activity) and resistance-breaking agent (as it is active against resistant bacteria).

**Table 1.** Antibacterial Effects of Compounds 5 and 7 on Different Bacterial Strains.

| Type of Bacteria | inhibition diameter | | | |
|---|---|---|---|---|
| | **Compound 7 at 50%** | **Compound 7 at 100%** | **Compound 5 at 50%** | **Compound 5 at 100%** |
| *Pseudomonas aeruginosa (MDR)* | - | - | 23 mm | 25 mm |
| *Escherichia coli 44* | - | - | - | - |
| *Escherichia coli 41* | - | - | - | - |
| *Klebsiella pneumoniae 49* | - | - | 14 mm | - |
| *Klebsiella pneumoniae 6* | 12 mm | 15 mm | - | - |
| *Acinetobacter baumannii 17* | 13 mm | 17 mm | 18 mm | 20 mm |

The symbol "-" indicates no antibacterial effect in that case.

**Molecular docking studies**

In this study, two compounds, compound 5 and compound 7, were evaluated by molecular docking techniques to examine their inhibitory capacity to β-lactamase enzyme (PDB ID:8J6Y) and DNA Gyrase (Gyr A) enzyme (PDB ID: 3LPX). β-lactamase enzyme is known to play a pivotal role in bacterial resistance to β-lactam antibiotics, making it targeting a key focus in the development of novel therapies to combat bacterial resistance [32]. According to the simulation results using AutoDock Vina software against β-lactamase enzyme (PDB ID:8J6Y), compound 5 showed a strong negative binding energy of -8.2 kcal/mol (Table 2), indicating a stable and efficient interaction with the active site of β-lactamase. In (Fig. 2) compound 5 forms five hydrogen bonds with amino acids SER125, SER65, ASN127, and ASN165, which enhances its stability within the active site. In addition, electrostatic interactions with GLU161 were observed, which enhances the electrostatic bonding between the compound and the enzyme. Hydrophobic interactions with VAL211 were also observed, which further enhanced the stability of the compound, as the nonpolar regions of the compound and the enzyme interact to reduce exposure to the aqueous medium. However, an unfavorable interaction with the amino acid LYS68, a positive-positive interaction, was observed, which may negatively affect the stability of the compound at the active site. However, the overall effect of this interaction appears to be insufficient to significantly reduce the efficacy of the compound. Compound 7, on the other hand, recorded a negative binding energy of -8.1 kcal/mol, which also indicates a strong and stable interaction with the β-lactamase enzyme. In (Fig. 2) compound 7 is characterized by the formation of four hydrogen bonds with the amino acids SER125, SER65, and ASN165. These bonds play a vital role in enhancing the stabilization within the active site, which increases the efficacy of the compound as an enzyme inhibitor. In addition, the compound exhibits hydrophobic interactions with ASN127 and VAL211, which contribute to the overall stability of the compound within the active site, similar to compound 5. The results show that both compounds exhibit strong negative binding energies, indicating their ability to bind to and inhibit the active site of β-lactamase. Compound 5 exhibits a more diverse interaction pattern, combining hydrogen bonds, electrostatic and hydrophobic interactions, while compound 7 focuses on hydrogen bonds and hydrophobic interactions only. Finally, we conclude that compounds 5 and 7 have promising interaction properties that make them strong candidates for the development of effective inhibitors of β-lactamase. Both compounds exhibit strong negative binding energies and diverse non-covalent interactions with amino acids in the active site of the enzyme. These interactions contribute significantly to the stabilization of the two compounds, which increases the likelihood of their success as effective inhibitors against antibiotic-resistant bacteria. These results encourage further research to confirm

the biological efficacy of these compounds in living systems and explore their potential for development into new therapeutics.

DNA Gyrase, a type of topoisomerase II, is a prime target in antibacterial drug design. This enzyme plays a vital role in reducing the stresses that arise from DNA winding during replication by introducing and rejoining double-strand breaks in DNA. Targeting this enzyme is an effective step in inhibiting bacterial replication, making it an attractive drug target [33]. GyrA was chosen as the target of the study because it plays a vital role in bacterial DNA replication, making it an effective target for developing novel inhibitors to combat antibiotic-resistant bacteria [34]. The active region of GyrA, to which the drug can bind, contains important sites including the cleavage site, where DNA cleavage and rejoining occur, and the DNA binding site, which contains acidic residues that help in recognizing and binding DNA [35].

According to the simulation results using AutoDock Vina software against DNA Gyrase (Gyr A) enzyme (PDB ID: 3LPX), compound 5 and compound 7 showed high negative binding energy (-8.3 kcal/mol and -7.8 kcal/mol) respectively, indicating a strong binding between compound 5 and GyrA enzyme compared to compound 7 (Table 2). These results support the hypothesis that compound 5 can be an effective inhibitor of the enzyme, thus hindering its vital function in bacterial DNA replication. Molecular analysis of the interaction of compound 5 and compound 7 with GyrA enzyme, as shown (Fig. 3), reveals a complex dynamic of the binding between the compound and the amino acids within the active site. Compound 5 appears to interact primarily with amino acids SER97, ARG91, GLN94, ALA117, VAL90, GLN267, and PHE96, and compound 7 primarily interacts with amino acids THR 451, ILE458, ALA455, ILE411, and LEU410. The results indicate the presence of two stable hydrogen bonds between compound 5 and amino acids SER97 and ARG91. Also, two hydrogen bonds exist between compound 7 and amino acid THR 451. Hydrogen bonds are essential for the stability of the compound within the active site, as they help maintain the optimal position of the compound for interaction with the enzyme. Such bonds are a good indicator of the binding strength and efficacy of the compound as a potential inhibitor of the enzyme. The unfavorable interactions observed between compound 5 and amino acids GLN94 and ARG91 indicate the presence of electrostatic repulsion between these molecules. These interactions may lead to a decrease in the stability of the compound within the active site or change the position of the compound, which may negatively affect the compound's activity as an inhibitor. However, the presence of these interactions does not eliminate the possibility of enzyme inhibition, especially if the hydrogen bonds are strong enough to compensate for the negative effect of these interactions. Also, the presence of van der Waals interactions between compound 5 and amino acids such as VAL268 and also between compound 7 and amino acids GLU 440, GLY 414, TRP 415, ILE 385, ALA 386, ALA 452 and ALA 455. These bonds are considered relatively weak compared to hydrogen bonds, but they play an important role in the stability of the compound within the active site. These interactions act as a complement to stronger bonds such as hydrogen bonds and contribute to enhancing the binding of the compound to the enzyme by improving the overall molecular stability. Also, there are hydrophobic interactions between compound 5 and amino acids ALA117 (Pi-Alkyl), VAL90 (Pi-Sigma), GLN267 (Amide-Pi Stacked) and PHE96 (Pi-Pi T Shaped) and between compound 7 and amino acids ILE411 (Pi-Sigma), ILE458 (Pi-Alkyl), ALA455 (Pi-Alkyl) and LEU410 (Pi-Alkyl).

The active site that compound 5 binds to is shown in (Fig.3) as being close to the cleavage site in the GyrA enzyme. This site is where DNA is cut and reattached, making it crucial for the process of DNA replication. Inhibiting this site will disrupt the vital function of the enzyme, which will ultimately stop the bacterial cell from replicating and thus kill it. As for compound 7, it appears to be close to the DNA binding site. The DNA binding site in the Gyrase A enzyme works to bind and stabilize the bacterial DNA during the replication process, which helps relieve the tension resulting from the DNA winding.

When the DNA binding site is inhibited, the DNA replication process will be hindered, which leads to the cessation of bacterial growth and reproduction, making it unable to divide. In analyzing the effectiveness of our compounds against the Gyrase A enzyme, it is shown that compound 5 and compound 7 target different sites in the enzyme, which affect its vital function differently. Compound 5 binds close to the cleavage site in the Gyrase A enzyme, while compound 7 binds close to the DNA binding site in the enzyme. Inhibition of the cleavage site is better than inhibition of the DNA binding site because the cleavage site is the primary site where the enzyme cuts and re-binds DNA, a vital process that cannot be compensated for or carried out indirectly [36]. Therefore, inhibition of this site has a direct and strong effect on the enzyme's replication capacity, causing rapid and effective cessation of bacterial growth. In contrast, inhibition of the DNA binding site, although it hinders DNA stability, may not be as effective as the direct effect of the cut site in disrupting the vital function of the enzyme. Accordingly, compound 5 is better than compound 7 because it targets the cut site that directly contributes to disrupting the primary activity of the Gyrase A enzyme, leading to a stronger and more effective effect in eliminating bacteria. This is what was observed through scientific experiments in the laboratory, and these results make compound 5 a strong candidate for the development of new antibacterial drugs targeting this enzyme. Although there are some unfavorable interactions, the strong hydrogen bonds and van der Waals interactions contribute to the stability of the compound within the active site.

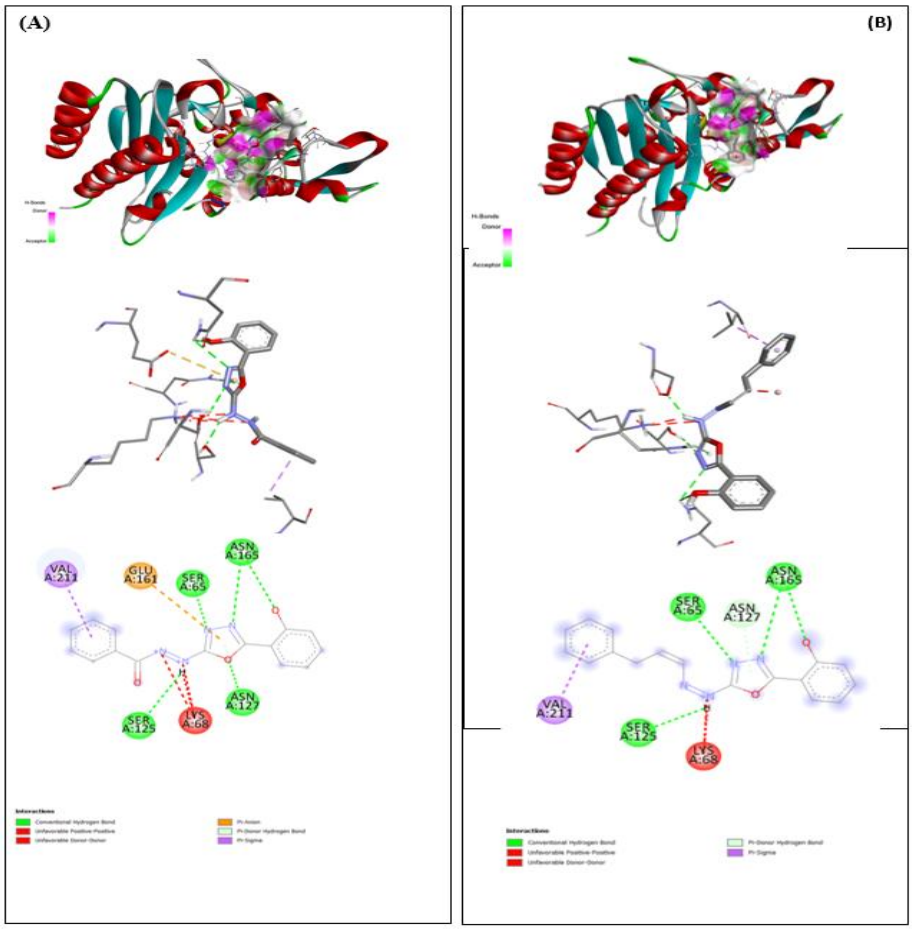



**Figure 2.** Molecular docking interactions of compounds 5 and 7 at the binding region of β-lactamase enzyme (PDB ID:8J6Y): (A) 2D and 3D interaction of compound 5, (B) 2D and 3D interaction of compound 7.

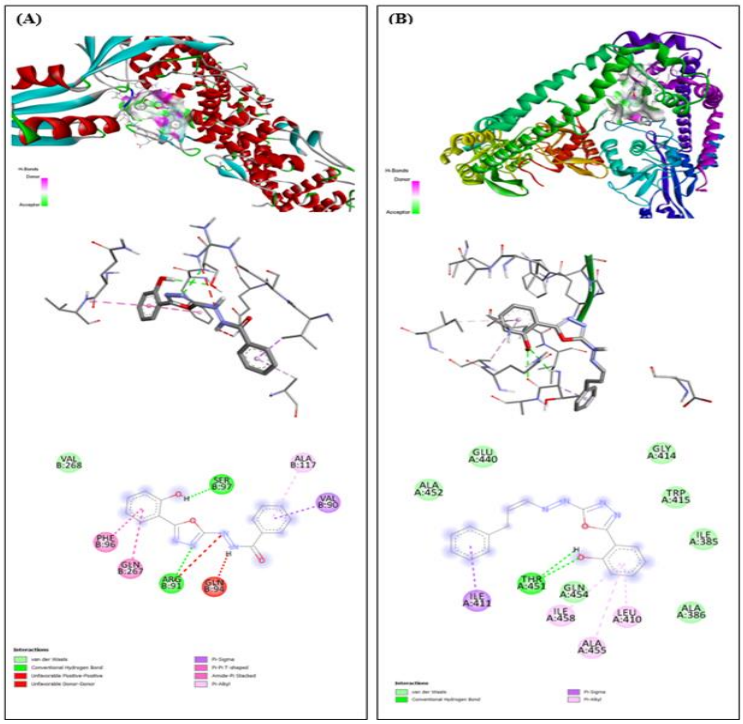
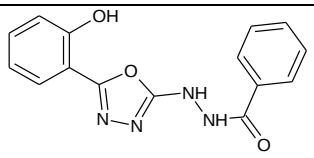
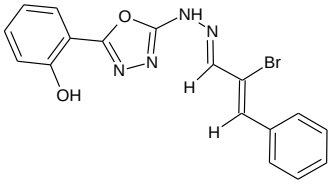



**Figure 3**. Molecular docking interactions of compounds 5 and 7 at the binding region of DNA Gyrase (Gyr A) enzyme (PDB ID: 3LPX): (A) 2D and 3D interaction of compound 5, (B) 2D and 3D interaction of compound 7.

**Table 2.** Molecular Docking Scores of Compounds 5 and 7 at the binding region of β-lactamase enzyme (PDB ID:8J6Y) and DNA Gyrase (Gyr A) enzyme (PDB ID: 3LPX).

| No. | Compound Structure | β-lactamase enzyme | | | DNA Gyrase (Gyr A) enzyme | | |
|---|---|---|---|---|---|---|---|
| | | Score (ΔG) | Distance | Type | Score (ΔG) | Distance | Type |
| 1 |  Comp. 5 | -8.2 | 2.133<br>2.305<br>2.207<br>2.917<br>2.818<br>4.543<br>3.952 | Hydrogen bond<br>Hydrogen bond<br>Hydrogen bond<br>Hydrogen bond<br>Hydrogen bond<br>Electrostatic<br>Hydrophobic | -8.3 | 2.793<br>2.554<br>3.478<br>4.694<br>4.230<br>4.829 | Hydrogen bond<br>Hydrogen bond<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic |
| 2 |  Comp. 7 | -8.1 | 2.089<br>2.258<br>2.789<br>2.851<br>3.610 | Hydrogen bond<br>Hydrogen bond<br>Hydrogen bond<br>Hydrogen bond<br>Hydrophobic | -7.8 | 2.931<br>3.009<br>3.574<br>4.889<br>5.121<br>5.051 | Hydrogen bond<br>Hydrogen bond<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic<br>Hydrophobic |

**ADME Study**

The pharmacokinetics of compounds 5 and 7 were studied using the SwissADME [37], [38] simulation site, which provides advanced analytical tools for evaluating physicochemical and pharmacological properties (absorption, distribution, metabolism, and excretion) as shown in (Table 3). The results obtained from these simulations reflect the compliance of the compounds with the standards used in drug development.

Compound 5 has a heavy atom count of 22. Its chemical properties show 5 rotatable bonds and 3 donor hydrogen bonds, which provide suitable flexibility. The compound has moderate water solubility with a LogS of -3.52, reflecting a good balance between water and lipid solubility, with a harmonic LogP of 1.92. The compound shows high intestinal absorption but is unable to penetrate the brain barrier. According to Lipinski's rules, the compound complies with the rules, as there are no violations. It also complies with the rules of Ghose, Veber, Egan and Muegge, which indicates its potential use as an effective drug.

Compound 7 has a bromine atom, which may add unique properties. It has high lipid solubility, with a LogP of 3.59, but low water solubility with a LogS of -5.15, which can be challenging in pharmaceutical dosage formulation. The compound also shows high intestinal absorption, making it suitable for oral use, but it carries a higher risk of drug interactions, being an inhibitor of several CYP enzymes. For the five rules, compound B complies with Lipinski, Ghose, Veber, and Egan, but violates Muegge's rule due to its solubility of less than 4.5.

When comparing compounds 5 and 7, we find that both compounds have promising chemical and physical properties that make them potential candidates for the treatment of antibiotic-resistant bacteria. However, when analyzing the pharmacokinetic data for each, we notice important differences that affect their potential use in different environments within the body.

For compound 5, the data showed that it has high GI absorption but does not cross the blood-brain barrier (BBB). This means that the compound may be effective in treating bacterial infections in the body, but it cannot be used effectively to treat infections affecting the central nervous system or brain tissue. The compound is not a substrate for P-gp, which increases the likelihood that it will remain at the target site for a longer period, improving its efficacy. It also does not show any violations of Lipinski's rule,

which enhances its potential development as a potential drug. Compound 7 also has high absorption in the gastrointestinal tract but, like compound 5, is unable to cross the blood-brain barrier (BBB). However, the compound has a higher lipophilicity, which may make it more effective in lipid environments such as cell membranes. It is also an inhibitor of several enzymes from the CYP450 family (CYP1A2, CYP2C19, and CYP2C9), which may lead to drug interactions if used with other drugs metabolized by these enzymes. Despite this, the compound shows strong pharmacological properties such as good absorption in the gastrointestinal tract and not being a substrate for P-gp, which enhances its potential as an antibacterial. However, the compound shows one caveat based on Brenk analysis, which suggests that some chemical considerations were involved in its design. Based on these data, compounds 5 and 7 could be useful for treating resistant bacteria outside the central nervous system. Their inability to cross the blood-brain barrier limits their use for treating infections affecting the brain or nervous system. However, with their ability to inhibit Gyrase and β-lactamase, and other good properties such as good absorption and the absence of negative effects from P-gp, these compounds could be considered effective options for combating antibiotic-resistant bacteria in other areas of the body.

**Table 3.** Pharmacological properties of compounds 5 and 7 via SwissADME.

| **Comp. No.** | **5** | **7** | **Comp. No.** | **5** | **7** |
|---|---|---|---|---|---|
| **physiochemical Properties** | | | **Water Solubility** | | |
| Num. heavy atoms | 22 | 24 | Log S (ESOL) | -3.52 | -5.15 |
| Num. arom. heavy atoms | 17 | 17 | Solubility | 8.97e-02 mg/ml ; 3.03e-04 mol/l | 2.72e-03 mg/ml ; 7.07e-06 mol/l |
| Fraction Csp3 | 0 | 0 | Class | Soluble | Moderately soluble |
| Num. rotatable bonds | 5 | 5 | Log S (Ali) | -4.29 | -5.8 |
| Num. H-bond acceptors | 5 | 5 | Solubility | 1.51e-02 mg/ml ; 5.10e-05 mol/l | 6.12e-04 mg/ml ; 1.59e-06 mol/l |
| Num. H-bond donors | 3 | 2 | Class | Moderately soluble | Moderately soluble |
| Molar Refractivity | 78.77 | 97.04 | Log S (SILICOS-IT) | -5.37 | -6.35 |
| TPSA | 100.28 Å² | 83.54 Å² | Solubility | 1.27e-03 mg/ml ; 4.28e-06 mol/l | 1.73e-04 mg/ml ; 4.50e-07 mol/l |
| | | | Class | Moderately soluble | Poorly soluble |

| Pharmacokinetics | | | Drug likeness | | |
|---|---|---|---|---|---|
| GI absorption | High | High | Lipinski | Yes; 0 violation | Yes; 0 violation |
| BBB permeant | No | No | Ghose | Yes | Yes |
| P-gp substrate | No | No | Veber | Yes | Yes |
| CYP1A2 inhibitor | Yes | Yes | Egan | Yes | Yes |
| CYP5c19 inhibitor | No | Yes | Muegge | Yes | Yes |
| CYP5c9 inhibitor | No | Yes | Bioavailability Score | 0.55 | 0.55 |
| CYP5d6 inhibitor | No | No | | | |
| CYP3A4 inhibitor | No | No | | | |
| **Medicinal Chemistry** | | | **Lipophilicity** | | |
| PAINS | 0 alert | 0 alert | Consensus Log $P_{o/w}$ | 1.92 | 3.59 |
| Brenk | 0 alert | 1 alert: imine_1 | | | |
| Leadlikeness | Yes | No; 2 violations: MW>350, XLOGP3>3.5 | | | |
| Synthetic accessibility | 2.95 | 3.68 | | | |

## 4. Conclusion

This study has successfully synthesized two new compounds from 1,3,4-oxadiazole derivatives, namely compounds 5 and 7. Both compounds demonstrated significant antibacterial activity against a variety of bacterial strains, with compound 5 showing particularly high activity against certain bacteria compared to the reference antibiotic, imipenem, while compound 7 exhibited notable activity across multiple bacterial types. Spectroscopic techniques (FT-IR, 1H-NMR, 13C-NMR and Mass spectra) confirmed the chemical structures of the compounds, and molecular docking simulations indicated strong potential interactions with bacterial targets. These results suggest compound 5 as a promising candidate for treating bacterial infections and highlight important steps towards developing new, more effective antibiotics.

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