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An in Silico Molecular Docking and in Vitro Investigation of the Bioactivity of Amoxicillin Derivatives Against Staphylococcus Aureus and Escherichia Coli

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Abstract

Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. We developed an amoxicillin drug via modification with different aldehyde compounds. Evaluation of the bioactivity of synthesized derivatives against Staphylococcus aureus and Escherichia coli. The derivatives A1 – A4 exhibited more excellent biological activity than the parent drug, and derivative A2 had the most significant biological activity by higher zone inhibition. Derivative 2 tested as against prostate cancer PC3, and the IC50 rates of derivative A2 give (5.75– 100) at 24 hours and (2.45 – 100) at 48 hours. The ligand showed multiple interactions with the enzyme's active site amino acids with residues (ASP49, HIS116, and PHE104), where a hydrophobic interaction was observed with residues (ALA53 and ILE78) and other amino acids in the target's pocket forms.

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1. Introduction

Amoxicillin, often known as penicillin A, is a widely used and safe antibiotic. Amoxicillin is often preferred over beta-lactam antibiotics because the body absorbs amoxicillin more readily. This antibiotic is a bacteriolytic ß-lactam medication with a moderate spectrum of activity [1]. Amoxicillin is prescribed to treat various infections caused by microorganisms susceptible to its effects. Amoxicillin exhibits activity against a broad spectrum of Gram-positive bacteria [2] and a narrow spectrum of Gram-negative bacteria. Amoxicillin and its derivatives are highly significant in various research studies, including pharmaceutical and biological investigations [3, 4]. The development of bacterial resistance to beta-lactam antibiotics has become a significant global concern, presenting an increasing danger to public health [5]. There have been reports of pigs in Spain who have shown a reduced ability to be affected by or are entirely resistant to certain types of antibiotics called extended-spectrum cephalosporins [6]. Recently, antimicrobials have been used more extensively as preventive and curative medicines to fight against infections [7, 8]. The development and natural selection of bacteria and the widespread use of antimicrobial drugs have led to a concerning rise in antimicrobial resistance (AMR). In addition, it has been projected that the mortality rate caused by antimicrobial resistance (AMR) will rise to 10 million by 2050 [9]. Therefore, the attention has been chiefly redirected towards alternate therapeutic approaches to combat the threat of antimicrobial resistance (AMR) and the commonly used antibiotics [10]. In this study, we modified amoxicillin to increase biological activity against the breast cancer MCF-7 cell line.

2. Materials and Methods

2.1. Synthesis of Azomethine-amoxicillin (A1-A3)

The Schiff bases were synthesized by modifying a similar approach, employing substituted benzaldehydes such as 2,4-dihydroxybenzaldehyde, 4-hydroxy-2-bromobenzaldehyde, 4-hydroxy-benzaldehyde and 4-hydroxy-2-chlorobenzaldehyde with 3 drops of glacial acidic acid and amoxicillin trihydrate as starting materials. Dissolve substituted benzaldehydes (1.1 mmol) in absolute ethanol (15 mL) and mix with amoxicillin trihydrate (0.41 g, 1.0 mmol). The solution was stirred using a magnetic stirrer and refluxed for 3 hours. The resulting solution, which had a yellow to brown

colour [11, 12]. The solids were filtration and multiple washes with distal water to produce A1-A4.

2.2. General method for determination of the antibacterial activity of amoxicillin derivatives (A1-A4)

The synthesized derivatives (A1-A4) were evaluated for their antibacterial properties using the cup-plate agar diffusion method, with the inhibition zone measured in millimetres. The synthesized derivatives were tested for comparative antibacterial activity using Amoxicillin at various concentrations (0.1, 0.001 and 0.00001 ppm) as the reference medication [13, 14]. The derivatives were tested for antibacterial activity against both microorganisms, *Staphylococcus aureus* and *Escherichia coli*. These microorganisms were isolated from infected wounds, nose swabs, urinary tract infections, and surgical theatres. The testing was conducted using Muller Hinton agar. The sterilized agar media were placed into Petri dishes and let to harden. Bacterial cultures had been uniformly applied to the medium layer employing a sterilizing square ring. A pre-sterilized the material tube with an outer diameter of twelve millimeters was used to form holes. The synthetic substances had been systematically placed into holes employing a small pipette at various percentages (0.1, 0.001, and 0.00001 ppm). These were thereafter let to disseminate for an hour. DMSO was used as a solvent for the various compounds, but sterile water from distillations was only employed because the reagent for pure amoxicillin. The dishes had been incubated at 37°C for two days. The size of the inhibitory zone around the vessels was measured in millimeters post-incubation [15].

2.3. Cytotoxicity evaluation of inhibitor using MTT Assay

The damaging effect of derivatives (A2) was evaluated using the MTT assay kit (Intron Biotech) [16]. The procedure was conducted by the instructions supplied by the manufacturing business. The cells, distributed at a density of 4.5 x 10^5, were grown in wells of 96 with an average culture that measured 200 µL per hole. The dishes were then covered with clean parafilm, which was carefully stirred, and after that incubated for a day at 37 °C using a 5% CO2 level.2- Following the incubation period, the medium was extracted, and 200 µl of a diluted form of derivative via 20, 40, 80, 160, and 320 µg/mL. Following the organic synthesis, 10 µL of the MTT mixture was added to every sample. The dishes were incubated for another 4 hours at 37°C with a CO_2 dosage of 5%. The culture medium was then removed, and one hundred milliliters of DMSO dissolution liquid was added to every dish and let run for five minutes. The absorbing capacity was measured using a reader for ELISA at an intensity of 575 nm.

2.4. Docking Experiment

The molecular docking studies using Autodock tools. The cryptographic 3D structure of DNA gyrase B was retrieved from the protein data bank (PDB ID: 3G7E). Both of receptor and ligand were saved in pdbqt file extension. The grid box value for the receptor was parametrized with size dimensions (40 x 40 x 40) for x, y and z, respectively, with centre values (37.891, 14.219 and 14.697) Å. The ligand was positioned within the protein's binding site using the identical coordinates of the co-docked ligand. The Autodock vina settings were configured to their default values, resulting in 10 postures for the DNA gyrase B with the ligand. The stances were ranked according to their rating values, and the pose with the highest score was chosen. The docking findings were shown using Discovery Studio (version 21.1.0.2) to generate 2D and 3D images [17, 18].

3. Results and Discussion

In this study, we modified amoxicillin with a different aldehyde to synthesized new azomethine-amoxicillin derivatives. The lone pair at the nitrogen atom of primary amine attacks the carbon of the carbonyl group and the presence of glacial acidic acid that works as a catalyst. In FTIR spectroscopy, the disappearance of two peaks of the primary amine of amoxicillin and a new azomethine group is indicated to be synthesized. Imine derivative (A1): Color: yellow, Yield: 75%. FTIR (cm^{-1}): 3337 (OH), 3050 (C-H starching of aromatic ring), 1652 (azomethine), 1604 (C=C of aromatic ring), as shown in Figure 1. Imine derivative (A2): Color: brown, Yield: 71%. FTIR (cm^{-1}): 3325 (OH), 3045 (C-H starching of aromatic ring), 1631 (azomethine), 1598 (C=C of aromatic ring), as shown in Figure 2. Imine derivative (A3): Color: Light brown, Yield: 77%. FTIR (cm^{-1}): 3347 (OH), 3057 (C-H starching of aromatic ring), 1656 (azomethine), 1602 (C=C of aromatic ring), as shown in Figure 3. Imine derivative (A4): Color: Brown, Yield: 72%. FTIR (cm^{-1}): 3341 (OH), 3050 (C-H starching of aromatic ring), 1633 (azomethine), 1601 (C=C of aromatic ring), as shown in Figure 4.

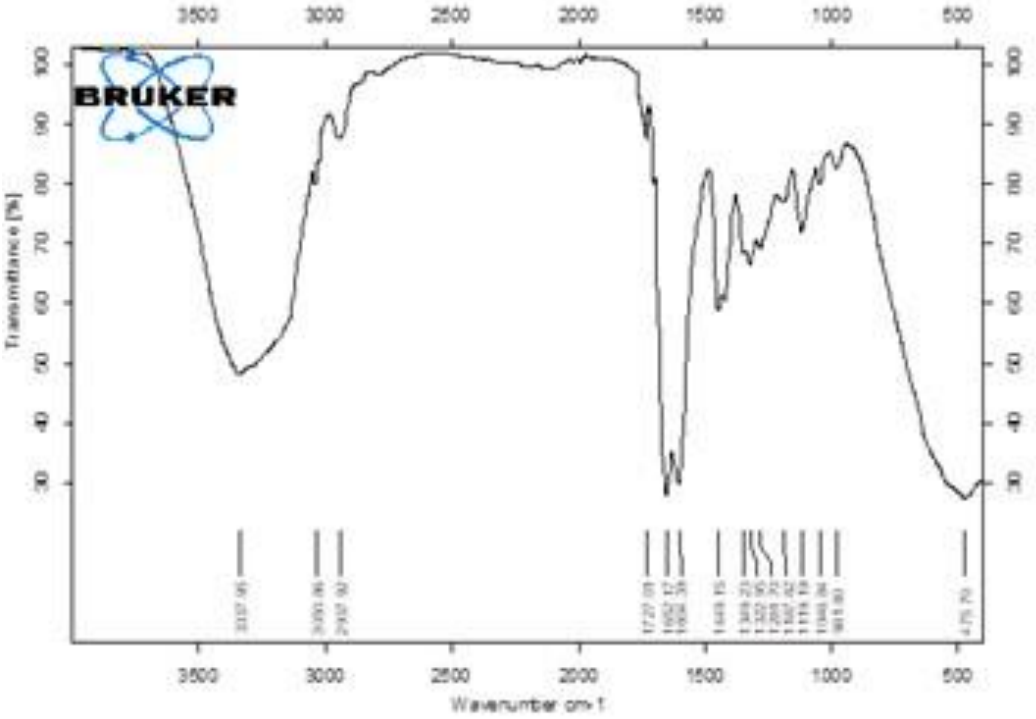


Figure 1. FTIR of derivative A1.

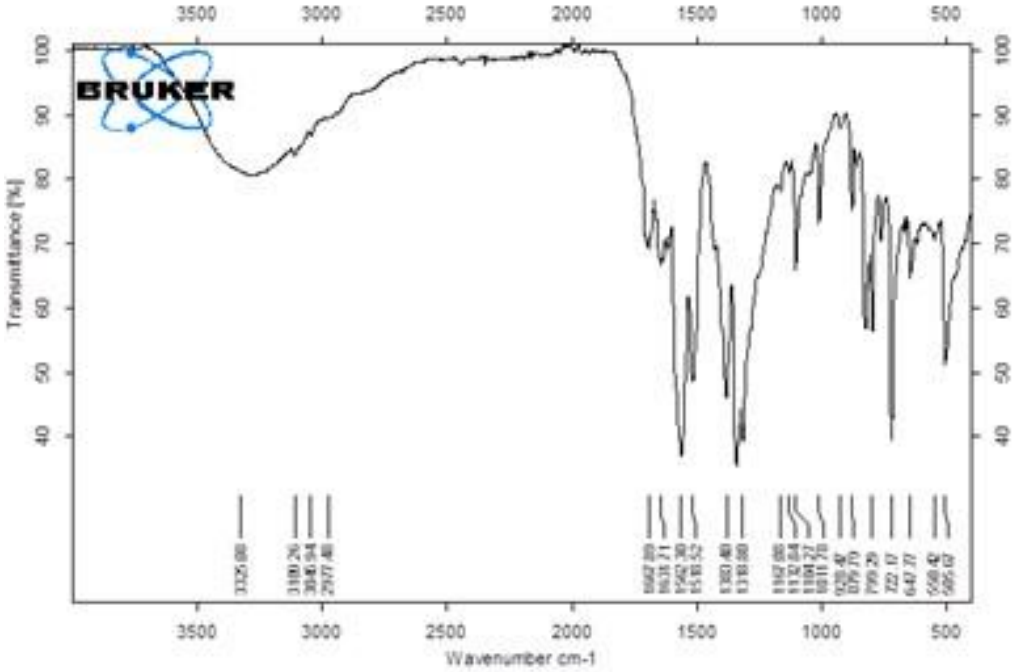


Figure 2. FTIR of derivative A2.

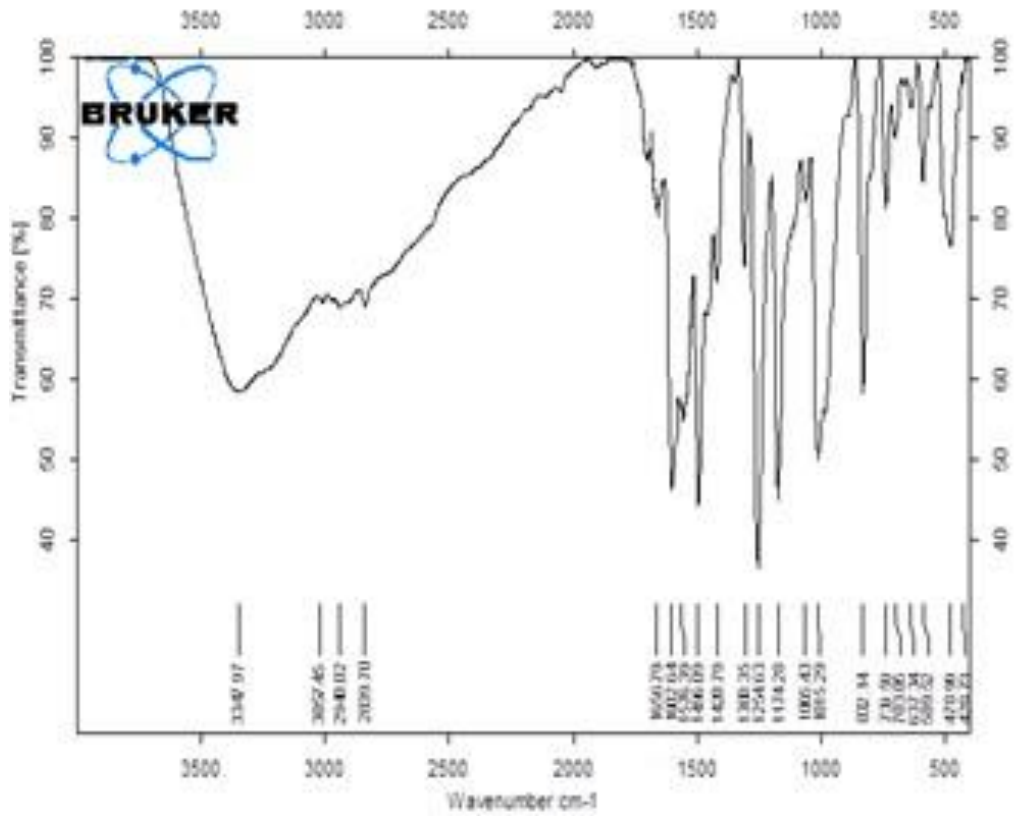


Figure 3. FTIR of derivative A3.

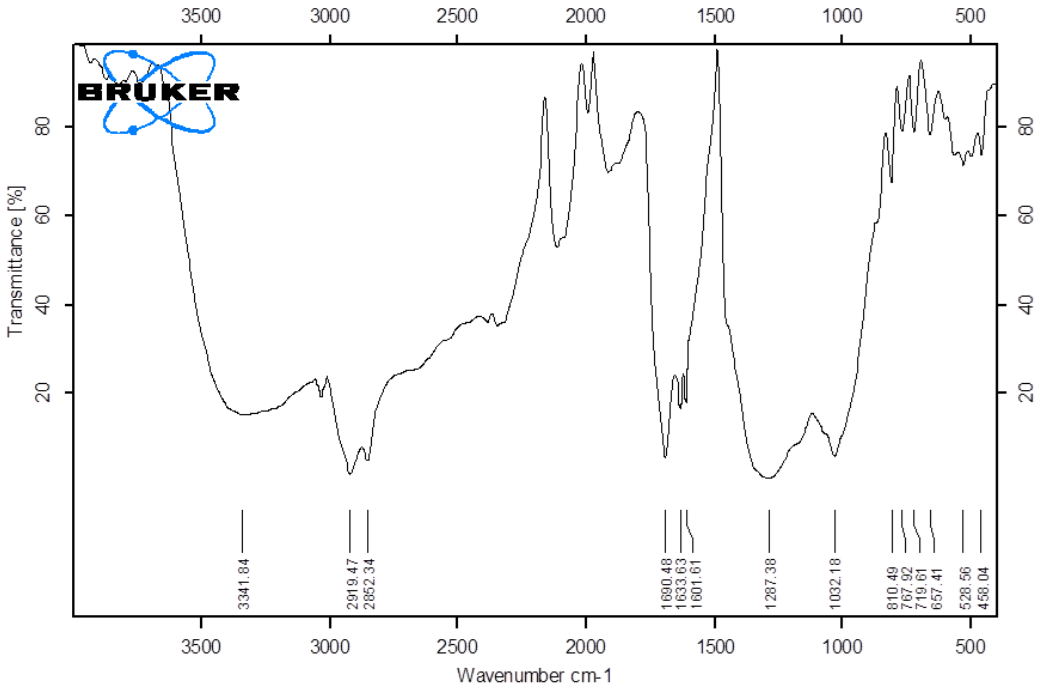


Figure 4. FTIR of derivative A4.

3.1. Antibacterial studies

The *in vitro* bactericidal properties of all produced compounds (A1-A3) with the parent medication (Amoxicillin trihydrate) have been screened. Upon analyzing the data on derivatives (A1-A3) inhibition zone against *Staphylococcus aureus* in Figures 5 and 6, several significant findings emerge. Firstly, derivatives A1 and A2 exhibited greater biological activity than the parent drug. Furthermore, the potency of these compounds increased as their concentration increased. Conversely, compound A3 demonstrated the opposite trend. Furthermore, compound A2 has a superior level of biological activity compared to compounds A1 and A3. However, when presenting the results of our activity against *Escherichia coli* in Figures 7 and 8, we made some significant observations: All compounds (A1-A3) have antibacterial solid action at all concentrations, and this inhibitory effect becomes more pronounced as the concentration of chemicals increases. The derivative, A2, exhibits superior activity compared to the other derivatives.

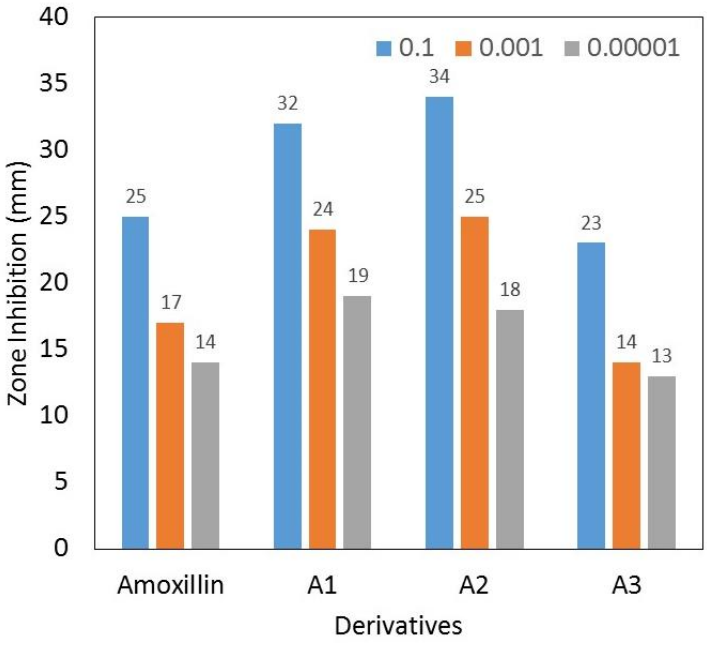


Figure 5. Derivatives activity (A1-A3) against *Staphylococcus aureus* in different dosages.

Figure 6. Biological activity of amoxicillin with derivatives A2 and A3 against *Staphylococcus aureus*.

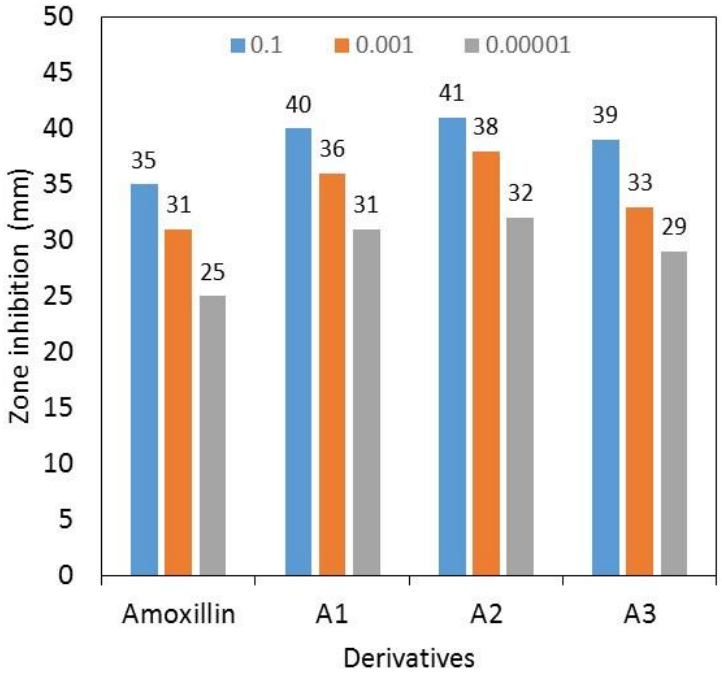


Figure 7. Derivatives activity (A1-A3) against *E. Coli* in different dosages.

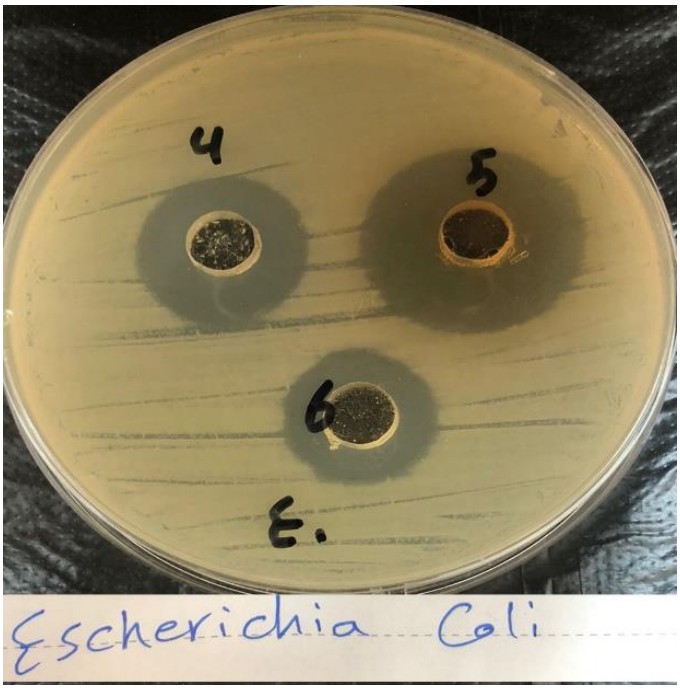


Figure 8. Biological activity of amoxicillin with derivatives A1 and A2 against *E. Coli*.

3.2. Viability Assay MTT

Using the MTT technique, the derivative (A2) was cytotoxic to the cell lines PC3. After 24 and 48 hours, the cell viability was evaluated and treated with various doses of each produced compound. Figure 9 shows the outcome of derivative (A2) (5.75–

100) at 24 hours and (2.45 – 100) at 48 hours. All results obtained are shown in Table 1.

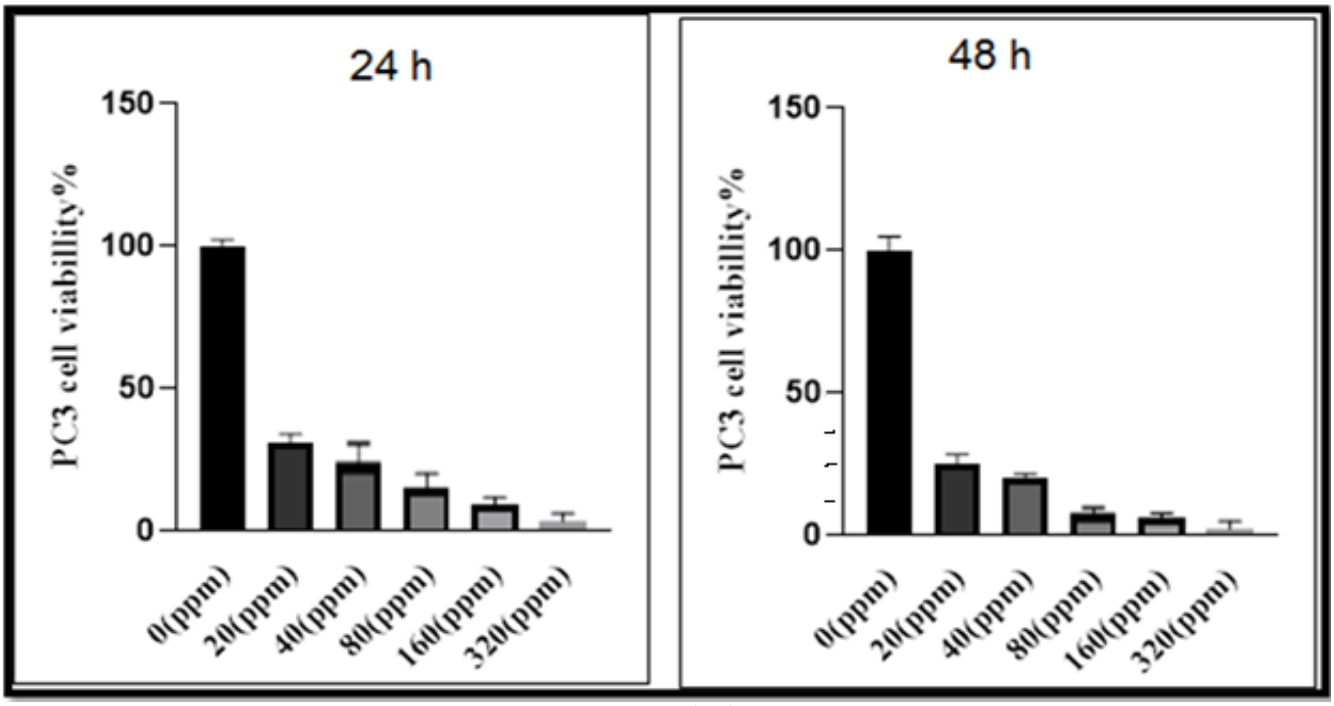


Figure 9. Effect of derivative (A2) on PC3 cell viability.

Table 1. The IC50 rates of derivative (A2) induced PC3 cells.

Dosage (PPM)	At 24 hours		At 48 hours	
	Mean	SD	Mean	SD
0	100	3.926078	100	3.976158
20	33.9864	3.254361	25.7661	3.853372
40	25.7854	3.754403	19.2438	1.196574
80	18.8601	2.211905	9.4427	1.853744
160	10.0826	2.482727	7.4535	1.143206
320	5.7522	2.364386	2.4573	0.530876

3.3. Study of Molecular Docking

DNA gyrase is a topoisomerase enzyme that plays a significant role in DNA topology and is subsequently considered an essential target for developing anti-bacterial drugs. Amoxicillin demonstrated a binding strength of -7.11 kcal/mol via DNA gyrase B (PDB ID: 5D7R) and formed contacts with Arg84, Ser55, Glu58, Ile86, and Ile179 residues as depicted in figures 10 and 11 [19]. The synthesized amoxicillin derivative's docking performance with DNA gyrase (ID:3G7E) is shown in Figures 12 and 13. The ligand showed multiple interactions with the enzyme's active site amino acids with a binding score (-7.9 kcal/mol); the synthesized derivative forms a hydrogen bonding with residues (ASP49, HIS116, and PHE104) where a hydrophobic interaction was observed with residues (ALA53 and ILE78) and other amino acids in target's pocket forms a van der Waals interaction, as shown in Table 2.

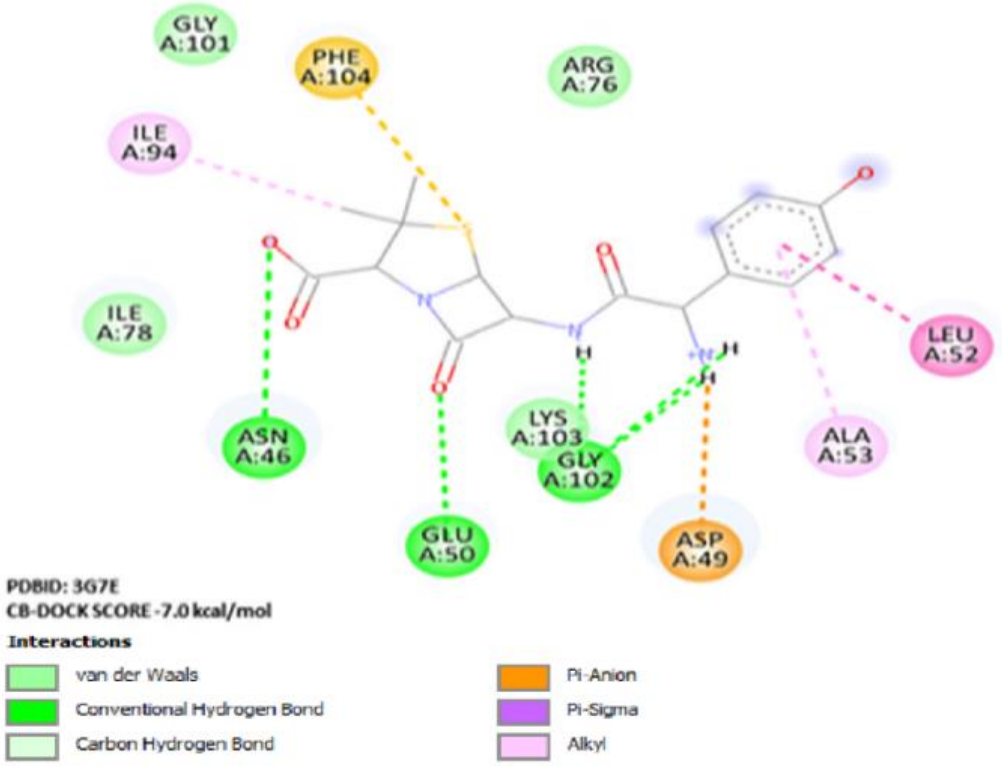


Figure 10. 2D molecular docking of amoxicillin with DNA gyrase (ID:3G7E).

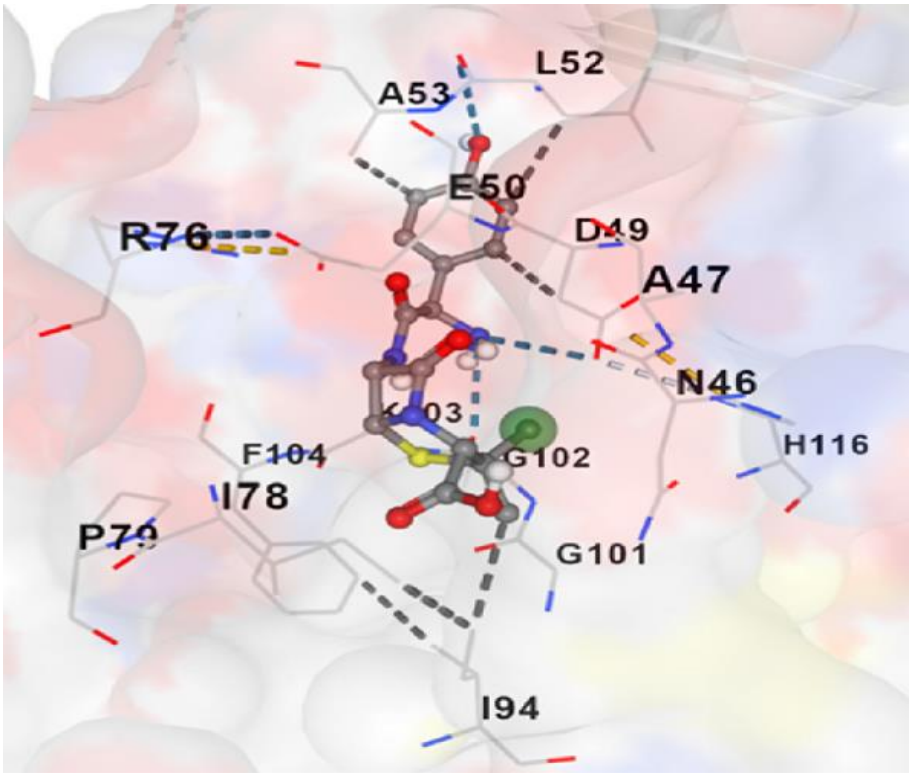


Figure 11. 3D molecular docking of amoxicillin with DNA gyrase (ID:3G7E).

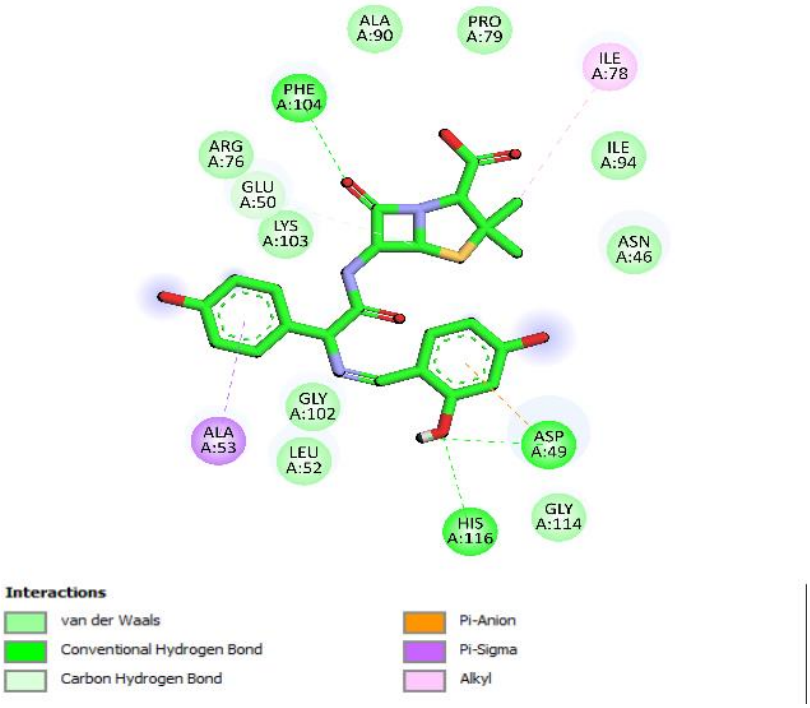


Figure 12. 2D molecular docking of derivative (A1) with DNA gyrase (ID:3G7E).

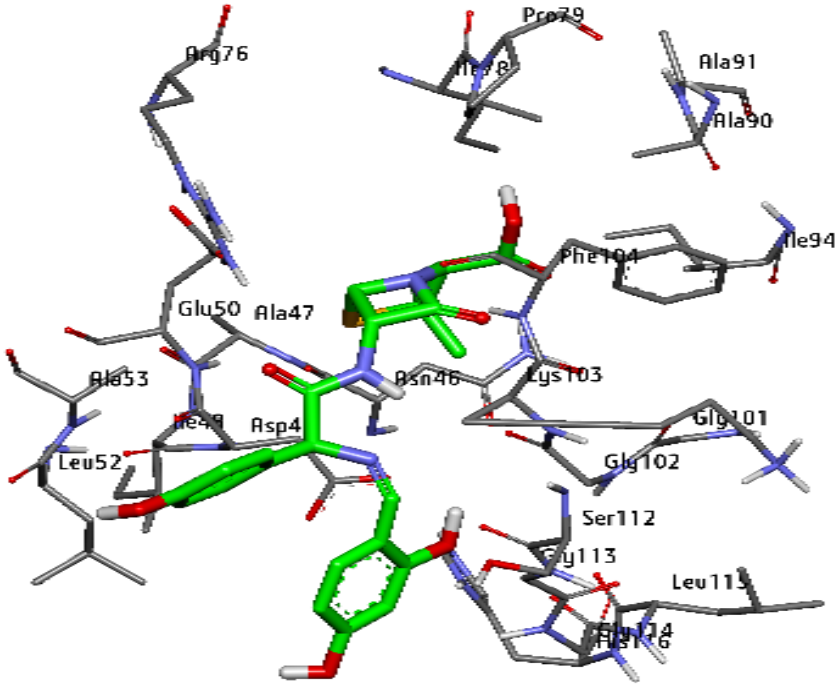
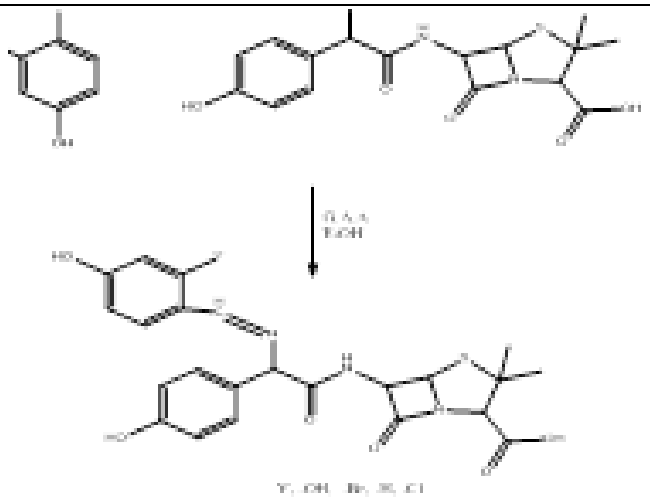


Figure 13. 3D molecular docking of derivative (A1) with DNA gyrase (ID:3G7E).



Scheme 1. Routs of amoxicillin derivatives (A1-A3).

Table 2. Types of bonding between amoxicillin and derivative A1 with target enzyme.

Compound	Van der Waals bond	Hydrogen bond	Pi anion	Alkyl
Amoxicillin	ASN: A46 GLY: A102	LYS: A103 ARG: A76 GLU: A: 50	PHE: A104 ASP: A49	ILE: A94
A1	ALA: A90 PRO:79 GLU: A50 ARG:76 LYS: A 103 LEU: A52 GLY: A114 ASN: A46 ILE: A94	PHE: A104 ASP: A49 HIS: A116		

4. Conclusions

Amoxicillin derivatives (A1-A3) were synthesized, and the evaluation of antimicrobial activity, including amoxicillin and its derivatives against Staphylococcus aureus and Escherichia coli, was tested in vitro. The findings demonstrated that certain derivatives exhibit superior antibacterial properties in comparison to the efficacy of the original drug. The derivative A2 has the most extensive biological activity by higher zone inhibition. Conversely, derivative 2 tested as against prostate cancer PC3, and the IC50 rates of derivative A2 give (5.75– 100) at 24 hours and (2.45 – 100) at 48 hours. The derivatives have halide atoms and hydroxide groups that differ from other derivatives. The ligand showed multiple interactions with the enzyme's active site amino acids with a binding score (-7.9 kcal/mol).

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Conflicts of Interest: According to the authors, a conflict of interest is absent.

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