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STUDYING MOLECULAR MODELLING OF THE CHOLESTEROL ABSORPTION INHIBITOR EZETIMIBE AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

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As is known, ezetimibe is the only drug classified as a cholesterol absorption inhibitor, working by preventing the cholesterol transporter protein Niemann-Pick C1-Like 1 (NPC1L1) from functioning. This compound is a derivative of Azetidinone and substituted β-lactam. However, its antibacterial activity has not yet been fully established. The β-lactam ring constitutes an important focus for proving antibacterial activity. Therefore, in this research, we studied the molecular modeling of ezetimibe and compared it with other β-lactam antibiotics to predict its binding pattern and energy. To achieve this, we utilized penicillin-binding protein 4 (PBP4) in Staphylococcus aureus.

We also evaluated the antibacterial activity of ezetimibe using the agar-well diffusion method against multiple bacterial strains, including both gram-negative and gram-positive bacteria. Subsequently, we determined the minimum inhibitory concentration (MIC) values for ezetimibe on sensitive strains. Oxacillin and amoxicillin were used as controls for comparison.

The results confirmed that ezetimibe bound to the target receptor, and its binding energy (-138.018 kcal/mol) was higher than that of most β-lactam antibiotics. Ezetimibe did not show any activity against Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, or Proteus mirabilis. On the other hand, the results demonstrated that ezetimibe exhibited antibacterial activity against Staphylococcus aureus ATCC 33591, Staphylococcus aureus ATCC 43300, two of its clinical isolates, Escherichia coli ATCC 8739, and one of its clinical isolates. These findings were consistent with the MIC test results, with MIC values ranging between 128 and 256 µg/ml.

In conclusion, ezetimibe has a strong affinity for the PBP4 enzyme and shows promising potential as a treatment against bacterial infections. Thus, This study suggests developing various drug dosage forms of ezetimibe to repurpose its usage as antibacterial agent and to perform clinical trails for these pharmaceutical formulations to select the most appropriate dosage and delivery locations

Keywords: *Ezetimibe, β-lactam, Molecular modelling, Antibacterial activity, Minimum inhibitory concentration (MIC), Penicillin binding protein 4*

INTRODUCTION

Bacterial infections significantly impact public health, as they can cause disease in any part of the body. The source of the disease can either be the bacterium itself or the body's response to it. The response of bacteria to antibiotics varies between types, such as gram-positive and gram-negative bacteria[1]. Unfortunately, bacterial resistance to antibiotics is a growing concern and one of the major health-related challenges of this century. This is largely due to the emergence and spread of multidrug-resistant bacteria (MDR), which are often isolated in hospital environments[2].

β-lactam antibiotics are considered essential drugs for treatment, as they target and inhibit bacterial cells by binding to penicillin-

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binding proteins (PBPs)[3]. The amide bond of the β-lactam ring is ruptured, forming an irreversible covalent bond with the serine residue at the PBPs active site[3]. Bacteria contain numerous PBPs, which catalyze various steps in bacterial cell wall biosynthesis, including transglycosylation, transpeptidation, DD-carboxypeptidation, and endopeptidation[4]. PBPs are classified based on molecular weight into high molecular weight (HMW) and low molecular weight (LMW) PBPs, both of which are further divided into subgroups A, B, and C depending on their structure[4,5]. Recent research has shown that penicillin-binding protein 4 (PBP4) in *Staphylococcus aureus* is a low molecular weight transpeptidase involved in cross-linking peptidoglycan strands in the bacterial cell wall[4].

Previous studies have reported that ezetimibe has activity against *Mycobacterium tuberculosis*, where it was able to effectively reduce the intracellular growth of *Mtb* and dormant *Mtb* induced by hypoxia[6]. In addition, ezetimibe has shown a synergistic effect with azoles against cutaneous leishmaniasis[7]. It is characterized by the presence of a β-lactam ring, which has raised controversy about its potential antibacterial activity[8]. However, no comprehensive studies have yet been conducted on its antibacterial activity.

Therefore, the aim of this study is to confirm ezetimibe's effectiveness against gram-positive and gram-negative bacteria. This study involves binding the compound to the target protein responsible for inhibiting peptidoglycan biosynthesis using molecular modeling. Molecular modeling is a powerful approach for determining and analyzing the 3D structure of molecules[9]. It is widely used to simulate molecular behavior and has become increasingly prevalent in various research applications[10]. As such, it is an important tool for clarifying the mechanisms of drug action and their interactions with receptors.

MATERIALS AND METHODS

Equipment and Chemicals

Ezetimibe was supplied by Barakat Company for Pharmaceutical Industries, Aleppo, Syria. The culture media used was obtained from HiMedia Company, dimethyl sulfoxide (DMSO) was sourced from Fischer (Germany), and the autoclave (Witeg, Germany), laminar flow cabinet (Daihan, Korea), and incubator (Carbolite) were utilized in the experiments.

Protein Preparation

The 3D structure of the target protein, penicillin-binding protein 4 (PBP4), in complex with cefoxitin, was obtained from the Protein Data Bank (https://www.rcsb.org/) with the code ID: 7KCY. It has a resolution of 1.85 ºA and consists of 431 amino acids[11].

Validation of the Docking Method

The validation of the docking method was performed by re-docking the reference ligand (cefoxitin) with the active pocket of the protein, which has a volume of 141.312 Aº and a polar area of 423.68 Å². Re-docking was accepted if the root mean square deviation (RMSD) value was less than 2.0 ºA[12]. The binding of cefoxitin with the active site of PBP4 is shown in **Fig. 1**.

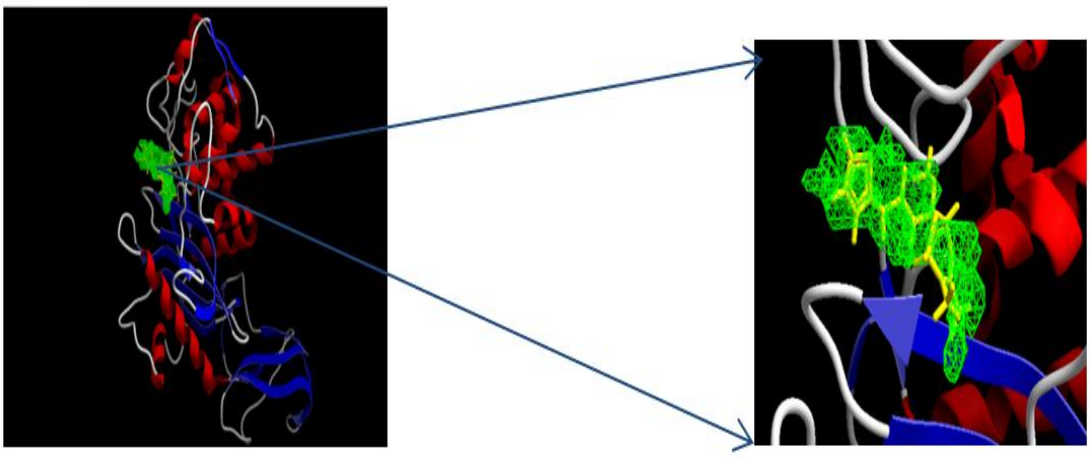


Fig. 1: the binding of cefoxitin with the active site of PBP4.

Ligand Preparation

The 3D structures of the β-lactam compounds used for comparison, along with ezetimibe, were downloaded from the NCBI PubChem database in SDF format. These formats were then converted and saved in MOL2 format using the Marvin Sketch software. Ligand preparation included converting all settings to "Always," ensuring that the software corrected any errors related to bond lengths and atomic positions.

Protein-Ligand Docking

Molecular docking of the compounds was performed using Molecular Virtual Docker Version 2011.4.3, applying the MolDock Score function and the MolDock Optimizer algorithm. The parameters used included a maximum of 2000 iterations, a population size of 50, a scaling factor of 0.50, and a crossover rate of 0.90.

The docking results were determined by the following energy equation:

$E_{SCORE} = E_{inter} + E_{intra}$

E_{score} is the total energy from external ligand interaction plus internal ligand interaction[13].

E_{inter} is the sum of energy consisting of the protein-ligand interaction and cofactor-ligand interaction[14].

$$E_{inter} = \sum_i \sum_j \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4r_{ij}^2} \right]$$

E_{PLP}: Refers to the piecewise linear potential, which uses two different parameters: one for assessing van der Waals interactions between atoms and another for hydrogen bonds. It describes the electrostatic interactions between charged atoms.

4r(Dr): Dielectric constant, convert the electrostatic energy units to Kcal/mol when multiplying by 332.0.

E_{intra} is the sum of energy dependent on the chemical structure of the ligand, such as torsional strain (sp^2 - sp^2), steric, and electrostatic interactions[14].

$$E_{intra} = \sum_i \sum_j \left[E_{PLP}(r_{ij}) + \sum_{flexiblebonds} A[1 - \cos(m\theta - \theta_0)] + E_{clash} \right]$$

θ: Torsional angle of the connection.

$E_{PLP}(r_{ij})$: calculates all energies involving pairs of ligand atoms except for those linked by two bonds.

$\sum_{flexiblebonds} A[1 - \cos(m\theta - \theta_0)]$: is the torsional energy.

E_{clash}: is related to spatial issue of heavy atoms.

The use of both the terms E_{inter} and E_{intra} is necessery to determine the total binding energy of compounds

In-vitro Antibacterial Activity

The procedures for both tests—agar well diffusion and minimum inhibitory concentration (MIC)—were conducted according to the guidelines provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI)[15-16].

Agar Well Diffusion Method

This method was used to evaluate the antibacterial activity against several bacterial strains, including Gram-positive and Gram-negative bacteria, comprising 6 typical bacterial strains and 24 clinical isolates from Aleppo University Hospital **(Table 1)**.

Bacterial suspensions were prepared, and their turbidity was adjusted to achieve a bacterial density of 1.5×10^8 CFU/mL, equivalent to the 0.5 McFarland standard. Mueller Hinton agar (MHA) was prepared and autoclaved at 121 ºC for 20 minutes, with a depth of 4-5 mm in the plates. The bacterial strains were cultured in Petri dishes, using 5 strains of each bacterial type, grown separately. Three wells were made in each Petri dish and filled with 50 µl of ezetimibe solution (1000 µg/mL), and a disk of oxacillin (20 µg) was placed as a control. The ezetimibe solution was prepared by dissolving 1 mg of ezetimibe in 1 mL of DMSO and sterilized by filtration through a 0.22 µm membrane filter. All plates were then incubated in a microbiological incubator at 37 ºC for 24 hours.

The results were recorded, analyzed, and evaluated by measuring the inhibition zone diameters to assess antibacterial activity. The test was repeated for all strains of *Escherichia*

coli using amoxicillin as a positive control. Four wells were prepared, one of which was filled with an amoxicillin solution (10 µg), prepared by dissolving 0.5 mg of amoxicillin in 1 mL of distilled water, then diluted to 50 mL. The test was conducted in triplicate, and the average result of the measurements was recorded.

Table1: The reference and isolated bacterial strains used in the bacterial study.

Strain number	Reference and isolated strains
1	*Staphylococcus aureus* ATTC 33591
2	*Staphylococcus aureus* ATTC 43300
3	*Staphylococcus aureus* isolate1
4	*Staphylococcus aureus* isolate2
5	*Staphylococcus aureus* isolate3
6	*Escherichia coli* ATCC8739
7	*Escherichia coli* isolate1
8	*Escherichia coli* isolate2
9	*Escherichia coli* isolate3
10	*Escherichia coli* isolate4
11	*Klebsiella pneumonia* ATTC 13885
12	*Klebsiella pneumonia* isolate1
13	*Klebsiella pneumonia* isolate2
14	*Klebsiella pneumonia* isolate3
15	*Klebsiella pneumonia* isolate4
16	*Pseudomonas aeruginosa* ATCC 9027
17	*Pseudomonas aeruginosa* isolate1
18	*Pseudomonas aeruginosa* isolate2
19	*Pseudomonas aeruginosa* isolate3
20	*Pseudomonas aeruginosa* isolate4
21	*Proteus mirabilis* ATCC 88
22	*Proteus mirabilis* isolate1
23	*Proteus mirabilis* isolate2
24	*Proteus mirabilis* isolate3
25	*Proteus mirabilis* isolate4
26	*Acinetobacter baumannii* ATCC19606
27	*Acinetobacter baumannii* isolate1
28	*ıetobacter baumannii* isolate2
29	*Acinetobacter baumannii* isolate3
30	*Acinetobacter baumannii* isolate4

Minimum Inhibitory Concentration (MIC)

This test was conducted on strains sensitive to ezetimibe: *Staphylococcus aureus* ATCC33591, *Staphylococcus aureus* ATCC43300, *Staphylococcus aureus* isolate 1, *Staphylococcus aureus* isolate 2, *Escherichia coli* ATCC8739, and *Escherichia coli* isolate 1. The test was performed using 96-well microplates (8×12). A series of concentrations of ezetimibe and the control compounds were prepared. The initial concentration (4096 µg/mL) of each compound was made as follows: 40.90 mg of ezetimibe was dissolved in 1 mL of sterile DMSO, then diluted with 9 mL of phosphate buffer (pH 7.4). Similarly, 40.96 mg of oxacillin and amoxicillin (control compounds) were each dissolved in 1 mL of sterile DMSO and diluted with 9 mL of sterile water.

Each well was filled with 100 µL of Mueller Hinton broth (MHB), prepared and autoclaved at 121 ºC for 20 minutes. Then, 100 µL of the compound solution was added to the first well and mixed thoroughly by aspirating and dispensing several times. 100 µL was then transferred to the next well and mixed, continuing this process to obtain a two-fold serial dilution of the tested compounds, ranging from 2048 µg/mL to 16 µg/mL. Afterward, 100 µL of bacterial suspension was added to each well, except for the negative control wells, ensuring a final bacterial density of 1.5×10^6 CFU/mL[15]. The microplates were incubated at 37 ºC for 24 hours.

After incubation, 20 µL of triphenyltetrazolium chloride dye solution (0.1%) was added to each well, and the microplates were incubated again at 37 ºC for 90 minutes[17].

RESULTS AND DISCUSSION

Results

Molecular Docking

In this research, the molecular docking method was used to evaluate the potential antibacterial activity of ezetimibe, so we docked ezetimibe and compared it with β-lactam antibiotics. According to the molecular docking results, ezetimibe efficiently bound to PBP4, and its MolDock score (-138.018) is higher than amoxicillin (-120.334), penicillin G (-122.511), ampicillin (-123.018), ticarcillin (-125.114), meropenem (-129.45), cefuroxime (-133.055), and cefepime (-134.514), but lower than tigemonam (-139.73), oxacillin (-140.859), and cefazolin (-143.714), as shown in **Table 2**. The results highlighted that ezetimibe had a good interaction with PBP4, and its polar surface area was suitable for the size of the target pocket. On the other hand, the β-lactam carbonyl oxygen of ezetimibe formed two hydrogen bonds with the amino acid residues Ser262 and Tyr291, and the phenolic hydroxyl oxygen formed five hydrogen bonds with the

amino acid residues Ser75, Asn141, Lys78, and Thr180. The hydroxyl group formed a hydrogen bond with the amino acid residue Asn138, and the methylene group formed a hydrogen bond with the amino acid residue Ser116. Van der Waals interactions with the amino acid residues were as follows: the hydroxyl and methylene group of ezetimibe formed Van der Waals interactions with the amino acid residue Ser116, the carbon of phenol formed two Van der Waals interactions with the amino acid residues Ser75 and Asn141, and fluorine formed three Van der Waals interactions with the amino acid residues Asp264, Arg186, and Gly261.

Table 2: Binding energy results of ezetimibe and other β-lactam antibiotics.

Compound	MolDock score Kcal/mol
Ezetimibe	-138.018
penicillin G	-122.511
amoxicillin	-120.334
Ampicillin	-123.018
Oxacillin	-140.859
Ticarcillin	-125.114
Cefipim	-134.514
Cefazolin	-143.714
cefuroxime	-133.055
meropenem	-129.45
Tigemonam	-139.73

Oxacillin formed hydrogen bonds with the amino acid residues as follows: the β-lactam carbonyl oxygen of oxacillin formed a hydrogen bond with the amino acid residue Ser262, the nitrogen of the β-lactam and the hydroxyl group formed two hydrogen bonds with the amino acid residue Ser75, and the oxygen of the carbonyl group formed a hydrogen bond with the amino acid residue Ser116. The oxygen of the hydroxyl group formed two hydrogen bonds with the amino acid residues Ser139 and Lys259, and the oxygen of the carbonyl group formed a hydrogen bond with the amino acid residue Thr260. Van der Waals interactions were as follows: the carbon of the carbonyl and the oxygen of the hydroxyl group formed two Van der Waals interactions with the amino acid residue Ser75, the β-lactam carbonyl oxygen and methyl group formed two Van der Waals interactions with the amino acid residue Ser262, the carbon of the benzene ring formed a Van der Waals interaction with the amino acid residue Ser263, and the oxygen of the hydroxyl group formed a Van der Waals interaction with the amino acid residue Thr260. Most β-lactam antibiotics bind to these and other amino acids, as summarized in **Table 3** and **Fig. 2**.

Table 3: H-bond,Van der waals interactions and bond length obtained for ezetimibe and compared compounds with PBP4.

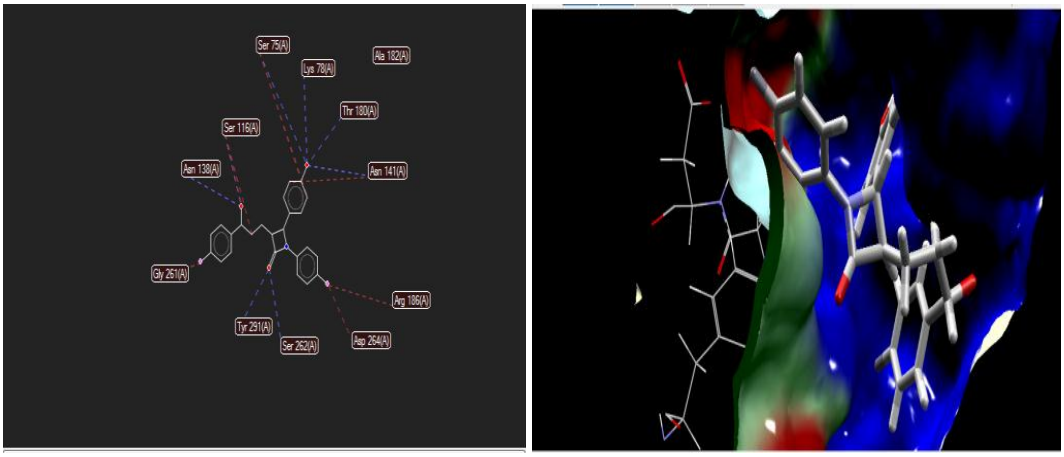
Ligand	H-bond Interactions	Bond length (A)	Van DER Waals Interactions	Bond length (A)
ezetimibe	(Ser262) –O2 (Ser75) –O4 (Ser116) –O3 (Asn141) –O3 (Asn138) –O3 (Thr180) –O4 (Tyr291) –O2 (Lys78) –O4	3.19 2.96 3.10 3.10 2.74 3.24 3.09 3.14	(Ser116) –O3 (Ser116) –C11 (Ser75) –C21 (Asn141) –C21 (Gly261) –F1 (Asp264) –F (Arg186) –F	3.06 2.47 2.37 2.87 3.10 3.03 2.90
penicillin G	(Ser262) –O1 (Ser262) –O2 (Ser75) –N5 (Ser139) –O3 (Ser116) –O4 (Thr260) –O3 (Asn141) –O4	2.89 3.33 2.95 3.45 2.77 2.86 3.37	(Ser262) –O1 (Ser75) –N5 (Ser75) –C10 (Ser116) –C7 (Ser116) –S (Phe241) –C12 (Leu115) –O4	2.47 2.74 3.14 3.18 2.97 3.09 3.20

Table 3: Continued.

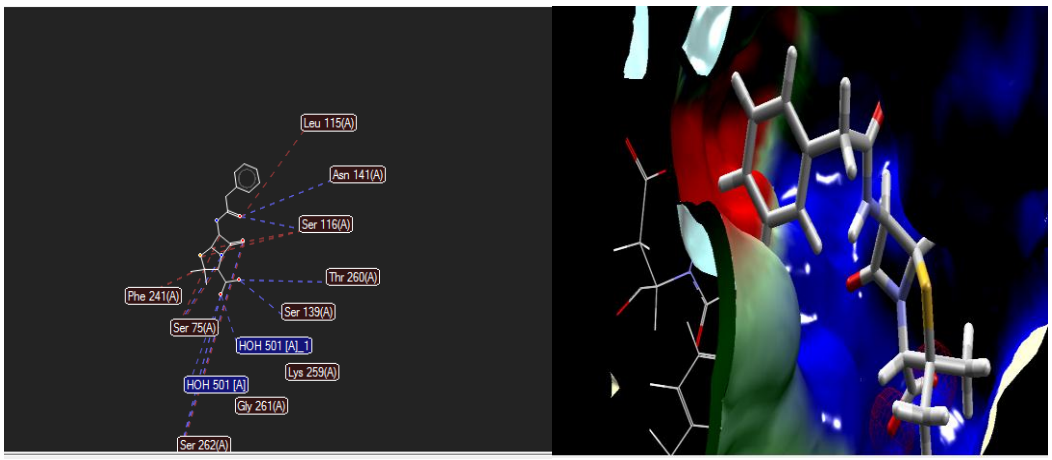
amoxicillin	(Ser262) –O1 (Ser75) –N6 (Ser139) –O3 (Lys259) –O3 (Thr260) –O2 (Asn141) –O4 (Asp264) –O5	2.92 3.10 3.10 2.87 2.60 3.14 2.74	(Ser 262) –O1 (Ser262) –C15 (Ser75) –C13 (Ser75) –C12 (Ser116) –N7 (Ser139) –O3	2.53 2.59 3.38 3.03 3.18 3.00
ampicillin	(Ser262) –O1 ((Ser75) –N5 (Ser116) –O4 (Ser139) –O3 (Asn141) –O4 (Thr260) –O3	2.88 2.94 2.77 3.44 3.37 2.86	(Ser262) –O1 (Ser75) –C11 (Ser75) –C12 (Ser75) –C8 (Ser116) –S (Phe241) –C13	2.46 3.14 2.75 3.18 2.96 3.09
oxacillin	(Ser262) –O1 (Ser75) –N6 (Ser116) –O4 (Ser139) –O2 (Thr260) –O3 (Lys259) –O2	3.17 3.10 2.76 2.95 2.87 2.62	(Ser262) –O1 (Ser262) –C15 (Ser75) –O2 (Ser75) –C13 (Ser263) –C26 (Thr260) –O2	2.44 2.53 2.75 3.11 2.84 2.85
ticarcillin	(Ser262) –O1 (Ser75) –N8 (Ser116) –O5 (Lys259) –O4 (Thr260) –O3 (Asn141) –O5	2.85 3.18 2.82 3.10 2.60 3.34	(Ser262) –O2 (Ser262) –C16 (Ser75) –C13 (Ser75) –C14 (Ser139) –O4	2.51 2.38 3.18 2.82 2.92
Cefipim	(Ser262) –O3 (Ser262) –N8 (Ser75) –N7 (Thr260) –O4	3.15 3.29 3.10 3.16	(Ser262) –C18 (Ser75) –C13 (Phe241) –N9 (Glu297) –O2 (Asn141) –C16	3.11 2.17 3.03 3.10 2.93
cefazolin	(Ser262) –O3 (Ser262) –N14 (Ser262) –N8 (Ser75) –N7 (Ser75) –O3 (Ser139) –O4 (Tyr291) –N13 (Tyr291) –N10 (Thr260) –O5	2.94 2.86 2.60 3.19 3.10 2.92 3.10 2.76 3.30	(Ser75) –O3 (Ser262) –O3 (Ser139) –O4 (Ser75) –C17	2.62 3.13 3.19 2.53
cefuroxime	(Ser262) –O1 (Ser262) –O4 (Ser75) –O4 (Ser75) –O8 (Ser75) –N12 (Lys78) –O4 (Thr180) –O4 (Tyr291) –O1 (Tyr291) –N10 (Tyr291) –N11 (Asn141) –O4 (Asn141) –O2	3.12 3.47 3.33 2.80 3.10 2.60 2.62 3.32 3.02 3.25 3.10 3.05	(Ser262) –C15 (Ser75) –C24 (Tyr291) –O1 (Asn141) –O2 (Leu115) –C18	3.12 2.98 3.00 2.92 2.85

Table 3: Continued.

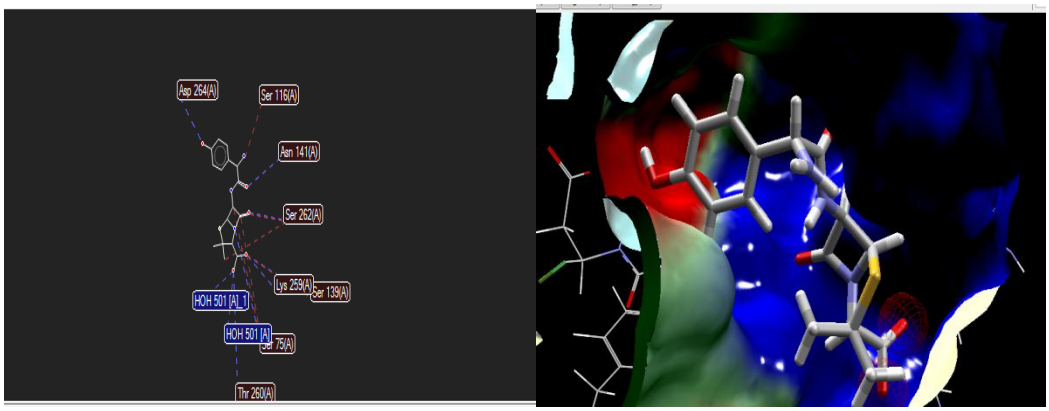
meropenem	(ser262) –O2 (Ser75) –N6 (Ser116) –O4 (Ser139) –O4 (Lys78) –O4 (Asn141) –O4	2.60 2.98 2.78 3.11 2.88 3.03	(Ser262) –C9 (Ser262) –C15 (Ser75) –C14 (Ser75) –O4 (Ser139) –O1	3.11 3.04 3.12 2.52 3.11
tigemonam	(Ser262) –O6 (Ser75) –O9 (Ser116) –O3 (Ser116) –O8 (Ser116) –O10 (Ser139) –O2 (Ser139) –O5 (Thr260) –O7 (Tyr291) –O4 (Asn141) –O10 (Lys78) –O10 (Lys259) –O5	3.14 2.61 3.11 3.12 2.99 3.44 2.92 2.79 3.19 2.67 3.30 2.58	(Ser262) –C19 (Ser262) –C20 (Ser116) –N12 (Ser116) –O3 (Ser75) –O5 (Leu115) –N14 (Thr260) –O5	2.83 2.88 3.07 2.90 2.60 3.11 2.79



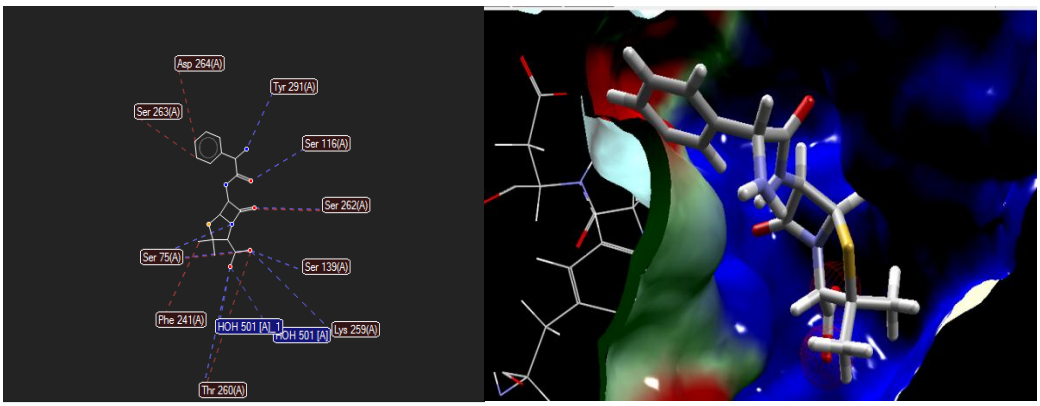
ezetimibe



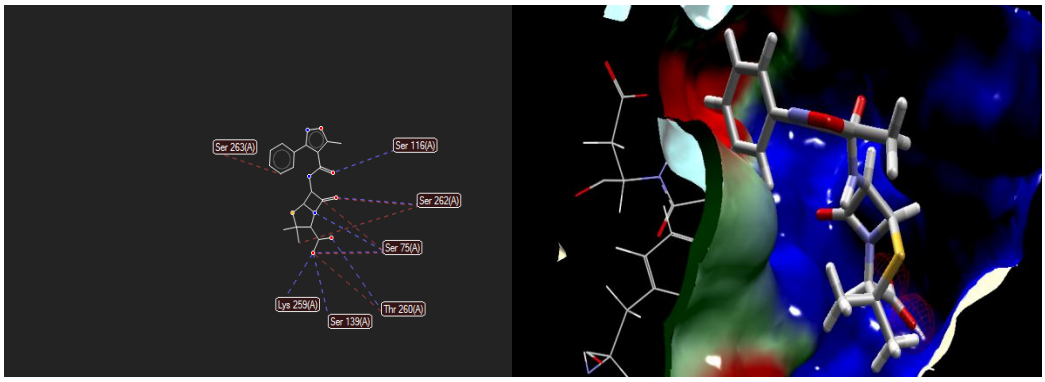
penicillin G



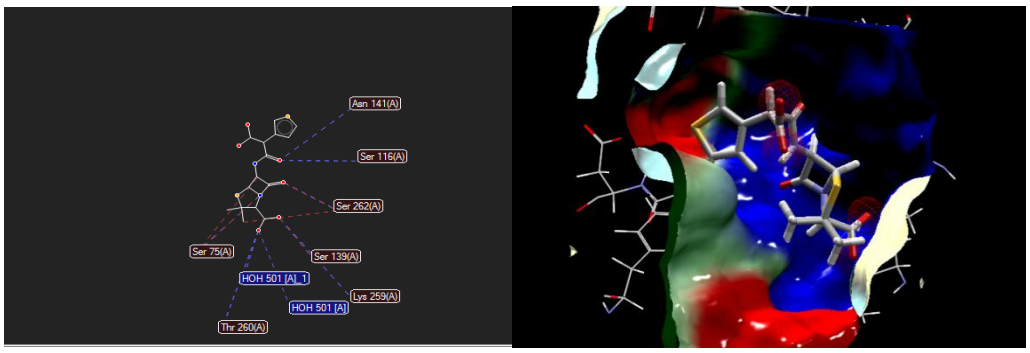
amoxicillin



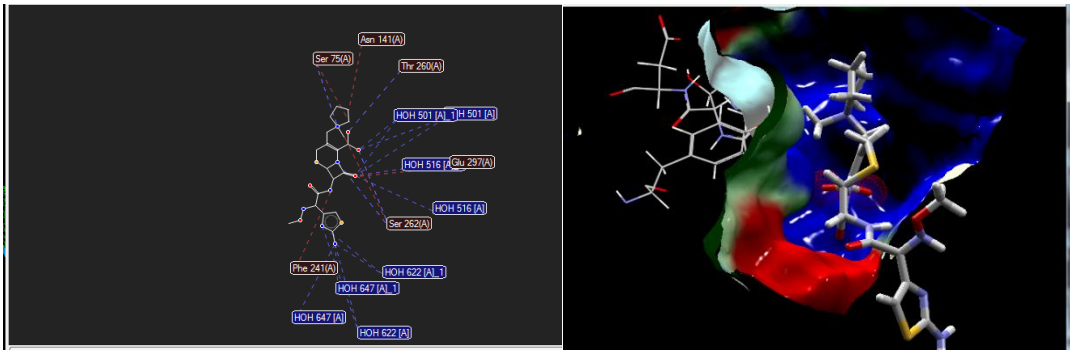
ampicillin



oxacillin

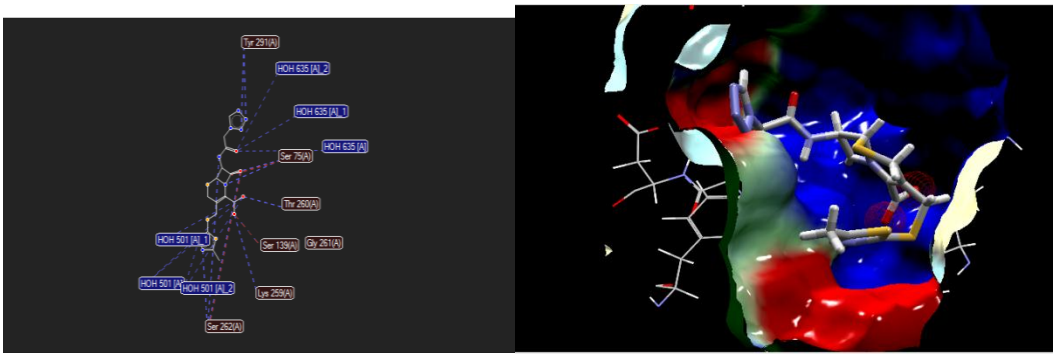


ticarcillin

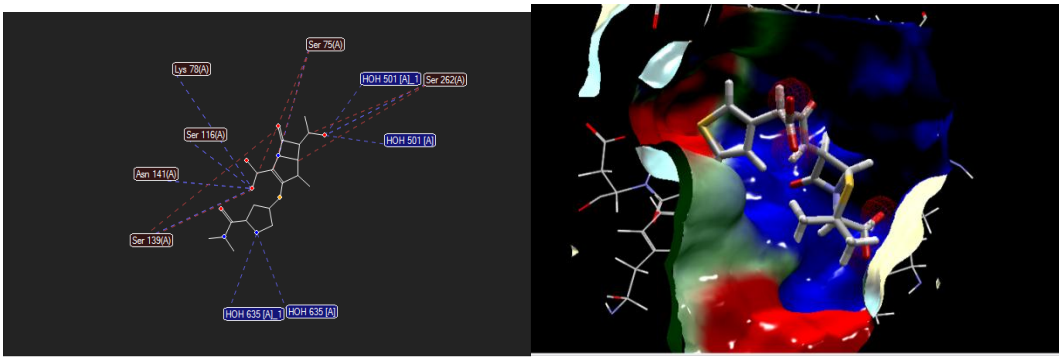


cefipim

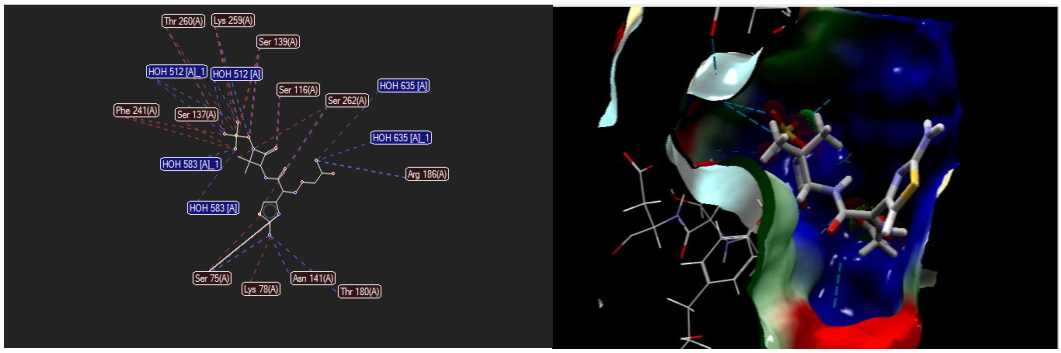
cefuroxime



cefazolin



meropenem



tigemonam

Fig. 2: the interactions between compounds and amino acid residues.

As a result, the β-lactam ring of most antibiotics exhibited interactions with the active site Ser75, which plays an important role as a nucleophile in the catalytic mechanism, and Ser262, while ezetimibe exhibited interactions with Ser75 via the hydroxyl group of the phenol and the carbon of the phenol ring, and with Ser262 via the β-lactam ring. Thus, ezetimibe appears to be able to enter the active site of the target. Actually, to prove the activity of ezetimibe against bacteria, it is important to perform in vitro antibacterial tests.

In-vitro Antibacterial Activity

After incubating at 37 ºC for 24 hours, the antibacterial activity was evaluated and compared with two antibiotics, as shown in **Fig. 3 and Fig. 4**.

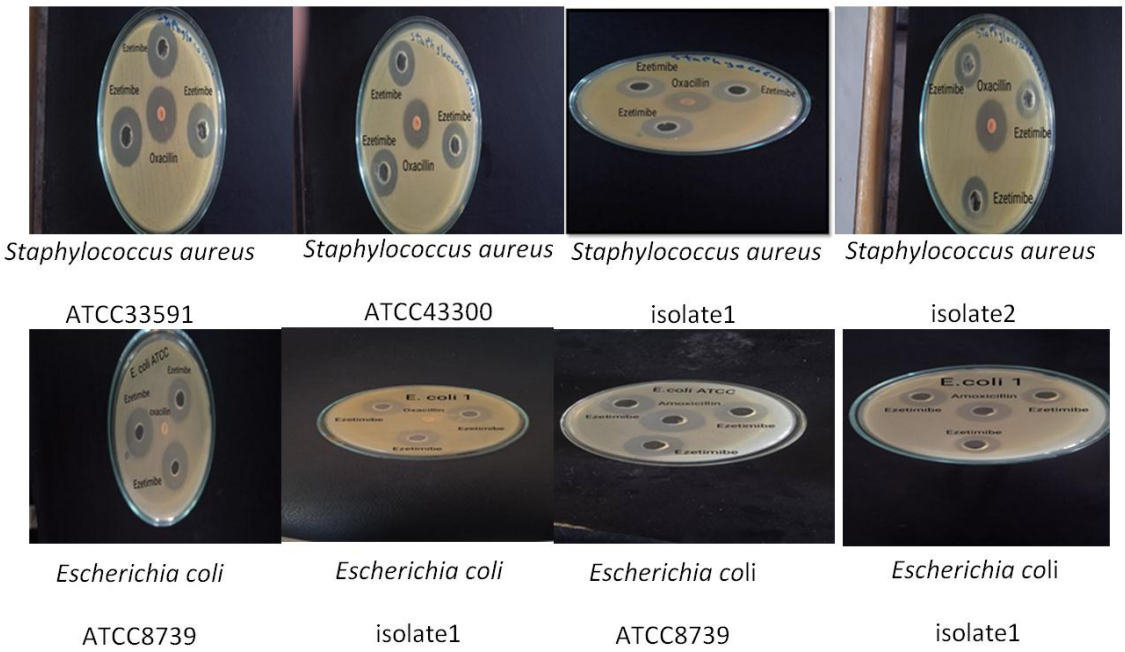


Fig. 3: Zone of growth inhibition diameters (mm) of ezetimibe and compounds compared against *Staphylococcus aureus* and *Escherichia coli.*

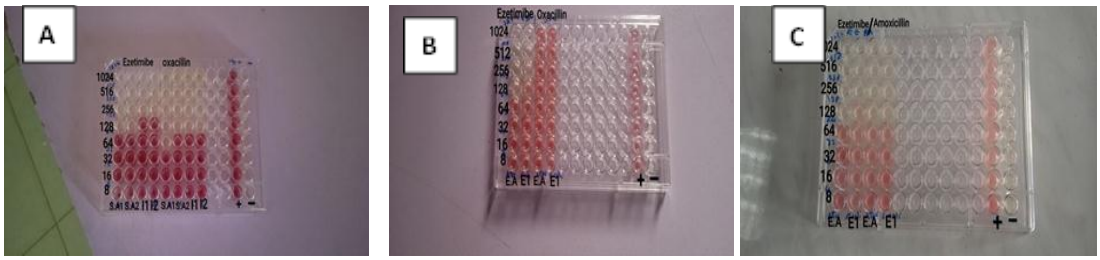


Fig. 4: (A) MIC test of ezetimibe against S.aureus strains using oxacillin as compared compound ,(B) MIC test of ezetimibe against *Escherichia coli* strains using oxacillin as compared compound,(C) MIC test against *Escherichia coli* strains using amoxicillin as a positive compared.

S.A1: *Staphylococcus aureus ATCC3359 1,* S.A2: *Staphylococcus aureus ATCC43300* ,I1*: Staphylococcus aureus Isolate1,I2: Staphylococcus aureus2* ,E.A: *Escherichia coli ATCC8739,E1: Escherichia coli Isolate 1*.

Antibacterial Activity Results Against *Klebsiella pneumoniae* Ezetimibe and oxacillin showed no activity against *Klebsiella pneumoniae* ATCC13885 and any of its clinical isolates.

Antibacterial Activity Results Against *Pseudomonas aeruginosa* Ezetimibe and oxacillin showed no activity against *Pseudomonas aeruginosa* ATCC9027 and any of its clinical isolates.

Antibacterial Activity Results Against *Proteus mirabilis* Ezetimibe and oxacillin showed no activity against *Proteus mirabilis* ATCC88 and any of its clinical isolates.

Antibacterial Activity Results Against *Acinetobacter baumannii*
Ezetimibe and oxacillin showed no activity against *Acinetobacter baumannii* ATCC19606 and any of its clinical isolates.

Antibacterial Activity Results Against *Staphylococcus aureus* **Tables 4 and 5** show the antibacterial activity results against clinical isolates and reference *Staphylococcus aureus*.

Table 4: Zone of inhibition diameters for ezetimibe and oxacillin against *staphylococcus aureus*.

Zone of inhibition diameters (mm)					
Compound	*Staphylococcus aureus* ATCC(33591)	*Staphylococcus aureus* ATCC(43300)	*Staphylococcus aureus* Isolate1	*Staphylococcus aureus* Isolate2	*Staphylococcus aureus* Isolate3
Ezetimibe	20mm	19mm	15mm	11mm	--
Oxacillin	24mm	21mm	21mm	20mm	21mm

(--)There is not any antibacterial activity**.**

Table 5: MIC values for ezetimibe and oxacillin against *staphylococcus aureus*.

MIC values are measured in µg/ml		
Strain	oxacillin	ezetimibe
Staphylococcus aureus ATCC(33591)	64	128
Staphylococcus aureus ATCC(43300)	128	128
Staphylococcus aureus isolate1	128	256
Staphylococcus aureus isolate2	128	256

Ezetimibe showed good activity against *Staphylococcus aureus* ATCC(33591), intermediate activity against *Staphylococcus aureus* ATCC(43300) and *Staphylococcus aureus* isolate1, and weak activity against *Staphylococcus aureus* isolate2. It couldn't show activity against *S. aureus* isolate3. The zones of growth inhibition diameters for ezetimibe were 20 mm, 19 mm, 15 mm, and 11 mm, and the MIC values were 128 µg/ml, 128 µg/ml, 256 µg/ml, and 128 µg/ml against *Staphylococcus aureus* ATCC(33591), *Staphylococcus aureus* ATCC(43300), *Staphylococcus aureus* isolate1, and *Staphylococcus aureus* isolate2, respectively.

Oxacillin showed higher activity than ezetimibe against the five strains studied, with the zones of inhibition diameters for oxacillin being 24 mm, 21 mm, 21 mm, 20 mm, and 21 mm against *Staphylococcus aureus* ATCC(33591), *Staphylococcus aureus* ATCC(43300), *Staphylococcus aureus* isolate1, *Staphylococcus aureus* isolate2, and *Staphylococcus aureus* isolate3, respectively. The MIC values were 64 µg/ml, 128 µg/ml, 128 µg/ml, and 128 µg/ml against *Staphylococcus aureus* ATCC(33591), *Staphylococcus aureus* ATCC(43300), *Staphylococcus aureus* isolate1, and *Staphylococcus aureus* isolate2, respectively.

Antibacterial Activity Results Against *Escherichia coli*

The antibacterial activity against clinical isolates and reference *Escherichia coli* is shown in **Tables 6 and 7**.

Table 6: Zone of inhibition diameters for ezetimibe, oxacillin and amoxicillin against *Escherichia coli*.

Zone of inhibition diameters (mm)					
Compound	*Escherichia coli* ATCC(8739)	*Escherichia coli* isolate1	*Escherichia coli* isolate2	*Escherichia coli* isolate3	*Escherichia coli* isolate4
ezetimibe	18mm	14mm	--	--	--
amoxicillin	21mm	20mm	24mm	23mm	--
oxacillin	--	--	--	--	--

(--)There is not any antibacterial activity.

Table 7: MIC values for ezetimibe and amoxicillin against *Escherichia coli*.

MIC values are measured in µg/ml			
Strain	ezetimibe	oxacillin	Amoxicillin
Escherichia coli ATCC(8739)	128	+	128
Escherichia coli isolate1	256	+	128

(+) the value is greater than 1024µg/ml.

Ezetimibe showed intermediate activity against *Escherichia coli* ATCC(8739) and *Escherichia coli* isolate1, but failed to show any activity against other strains.

The zones of growth inhibition diameters for ezetimibe were 19 mm and 15 mm, and the MIC values were 128 µg/ml and 256 µg/ml against *Escherichia coli* ATCC(8739) and *Escherichia coli* isolate1, respectively. Moreover, oxacillin failed to show activity against *Escherichia coli* strains, but by repeating the experiment, we found that amoxicillin showed good activity against *Escherichia coli* ATCC(8739), *Escherichia coli* isolate1, *Escherichia coli* isolate2, and *Escherichia coli* isolate3, with zones of growth inhibition diameters of 22 mm, 21 mm, 25 mm, and 24 mm, respectively. MIC values were 128 µg/ml against *Escherichia coli* ATCC(8739) and *Escherichia coli* isolate1.

It is worth noting that the results of both tests were consistent, and although ezetimibe had antibacterial activity against some *Staphylococcus aureus* and *Escherichia coli* strains, it was not better than that of amoxicillin and oxacillin.

Conclusion

This research aimed to study ezetimibe as an antibacterial. We can conclude that ezetimibe may represent a potential treatment option against bacterial infections and may reduce the possibility of bacterial infections in patients with high cholesterol who are taking ezetimibe as a cholesterol-lowering agent.

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REFERENCE

1. S. L. Gorbach, "Bacterial Infections:Overview", ***Elsevier eBooks,*** 273-282 (2008).
2. A.D.Sharma and I.Kaur, "Targeting bacterial Penicillin binding proteins (PBPs) by using eucalyptus essential oil", ***AJMAP***, 8(3), 25(2022).
3. K. M. Kumar, P. Anitha, V. Sivasakthi, S. Bag, P. Lavanya, A. Anbarasu and S. Ramaiah, "In silico study on Penicillin derivaties and Cephalosporins for upper respiratory tract bacterial pathogens", ***3Biotech***, 4(3), 241-251(2013).
4. E. Sauvage, F.Kerff, M. Terrak, J. A. Ayala, P. Charlier, "The Penicillin-binding proteins: structure and role in peptidoglycan biosynthesis", ***FEMS Microbiol Rev***, 32(2), 234-258(2008).
5. H. Öztürk, E. Ozkirimli and A.Özgür,"Classification of Beta-Lactamases and Penicillin Binding Proteins Using Ligand-Centric Network Models", ***PloS One***, 10(2), e0117874 (2015).
6. F. Tasi, C. Kuo, A. B. Lin, M. Chein, H. Ho, T. Wei, C. Wu and Y. Lu, "Potential effect of Ezetimibe against Mycobacterium tuberculosis infection in type II diabetes", ***Respirology***, 22(3), 559-566(2016).
7. V. V. Andrade-Neto, E. F. Cunha-Júnior, M. M. D. Canto-Cavalheiro, G.C.Atella, T. De Almeida Fernandes, P. R. R. Costa, E. C. Torres-Santos, " Antileishmanial Activity of Ezetimibe: Inhibition of Sterol Biosynthesis, In Vitro Synergy with Azoles, and Efficacy in Experimental Cutaneous Leishmaniasis", ***ACC***, 60(11), 6844-6852(2016).
8. C. L. Bristow and R. Winston, "Alphataxin, an orally available small molecule, decreases LDL levels in mice as a surrogate for the LDL-Lowering activity of Alpha-1 antitrypsin in humans", ***Front Pharmacol***, 12,٦٩٥٩٧١ (2021).
9. M. J. Forster, "Molecular modelling in structural biology", ***Micron***, 33(4), 365-384(2002).
10. K. I. Ramachandran, G. Deepa and K. Namboori, "Computational chemistry and molecular modeling: principles and applications", ***In Springer eBooks***, (2008).
11. J. Alexander and N.Strynadka,"Crystal structure of S.aureus Penicillin-binding protein4 (PBP4) with cefoxitin", 06-30, (2021).
12. C. S. Prsakoeswa, D. Purwanto, A. Endaryanto, C. Rosita and S. Prakoeswa, "Molecular Docking, Pharmacokinetics, and toxicity prediction of Epigallocatechin-3-Gallate (EGCG) on IKK receptor in photoaging prevention", ***IJFMT***, 14(2), 1467-1473 (2020).
13. M. E. Gondokesumo and I. M. Kurniawan, "Molecular docking study of sappan wood extract to inhibit PBP2A enzyme on methecillin-resistance staphylococcus aureus(MRSA)", ***JBCPP***, 30(6), (2019).
14. S. P. Singh and B.K.Konwar, "Molecular docking studies of quercetin and its analogues against human inducible nitric oxide synthase", ***SpringerPlus,*** 1(1), 69(2012).
15. G. Kahlmeter, D. Brown, F. Goldstein, A. MacGowan, J. Mouton, I. Odenholt, A. Rodloff, C. Soussy, M. Steinbakk, F. Soriano and O. Stetsiok, "European Committee on Antimicrobial Susceptibility Testing(EUCAST) Technical Notes on antimicrobial susceptibility testing", ***CMI,*** 12(6**),** 501-503(2006).

16. L. B. Reller, M. Weinstein, J. H. Jorgensen and M. J. Ferraro, "Antimicrobial susceptibility Testing: A review of general principles and contemporary practices", ***Clin Infect Dis***, 4(11), 1749-1755(2009).

17. A. Veiga, M. Da Graça T Toledo, L. S. Rossa, M. Mengarda, N. C. Stofella, L. J. Oliveira, A. G. Gonçalves and F. S. Muraka, "Colorimetric microdilution assay:Validation of a standard method for determination of MIC, IC50%, and IC90% of antimicrobial compounds", ***J Microbiol Methods,*** 162, 50-61(2019).

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دراسة النمذجة الجزيئية لمثبط امتصاص الكوليسترول الايزيتيميب وتقييم فعاليته المضادة للجراثيم

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كما هو معروف، فإن الايزيتيميب هو الدواء الوحيد المصنف كمثبط لامتصاص الكوليسترول، حيث يعمل على تثبيط امتصاص الكوليسترول من الأمعاء من خلال منع البروتين الناقل للكوليسترول Niemann-Pick C1-Like1 (NPC1L1)من أداء عمله. يعد الايزيتيميب من مشتقات الآزيتيدينون والبيتالاكتام المستبدلة وعلى الرغم من ذلك إلا أن فعاليته المضادة للجراثيم ليست واضحة بعد. وبما أن حلقة البيتالاكتام تشكل محوراً هاماً لإثبات الفعالية المضادة للجراثيم لذلك قمنا في هذا البحث بدراسة النمذجة الجزيئية للايزيتيميب والمقارنة مع صادات البيتالاكتام الأخرى وذلك للتنبؤ بنمط وطاقة الارتباط حيث تم استخدام البروتين الرابط للبنسيللن٤ الموجود في المكورات العنقودية الذهبية. هذا وقد تم أيضاً دراسة الفعالية المضادة للجراثيم باستخدام طريقة الانتشار من الآبار ضد عدة سلالات جرثومية تعود لجراثيم إيجابية وسلبية الغرام. بعد ذلك، تم تحديد قيم MIC باستخدام اختبار التركيز المثبط الأصغري على السلالات الحساسة، كما تم استخدام الأوكزاسيللين والأموكسيسللين كمركبات مرجعية .

أظهرت النتائج أن الايزيتيميب ارتبط مع المستقبل الهدف وكانت طاقة ارتباطه (١٣٨.٠١٨- كيلوكالوري/مول) أعلى من طاقة ارتباط معظم صادات البيتالاكتام المقارنة. كما لم يظهر المركب قيد الدراسة والمركبات المرجعية فعالية تجاه أي من جراثيم الكليبسيلا الرئوية و الزائفة الزنجارية و المتقلبة الرائعة Acinetobacter baumannii بينما أظهرت النتائج أن الايزيتيميب لديه فعالية ضد المكورات العنقودية الذهبية (ATCC33591) والمكورات العنقودية الذهبية (ATCC43300) و اثنين من العزالات السريرية للمكورات العنقودية الذهبية و الايشريكية القولونية (ATCC8739) وواحدة من العزالات السريرية للايشريكية القولونية . هذه النتائج توافقت مع نتائج اختبار التركيز المثبط الأصغري حيث كانت قيم MICللايزيتيميب تتراوح بين ١٢٨و٢٥٦ميكروغرام/مل. بالاستنتاج نجد أن الايزيتيميب لديه ألفة جيدة للأنزيم PBP4 ويحمل نتائج واعدة كعلاج ضد الأخماج الجرثومية. بذلك تقترح هذه الدراسة تطوير أشكال جرعات دوائية مختلفة للايزيتيميب لإعادة استخدامه كعامل مضاد للجراثيم وإجراء تجارب سريرية لهذه الصيغ الصيدلانية لاختيار الجرعات ومواقع التوصيل الأكثر ملائمة.