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Integrating molecular docking and molecular dynamics simulation approaches for investigation of the affinity and interactions of the piperine with Class D β-Lactamase

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ABSTRACT

Introduction: The study explores the potential of piperine, a natural compound with diverse medicinal effects, as a potential inhibitor targeting OXA-10 class D β-lactamase enzymes, as a potential solution to the drug resistance caused by β-lactamase-producing organisms, which contributes to millions of deaths and morbidity cases worldwide each year.

Materials and Methods: Through the utilization of molecular approaches such as molecular docking and molecular dynamics simulation, this study investigated the binding sites and binding energy of class D β-lactamase in the presence of piperine. These analyses were conducted using Autodock 4.2.2 software and the GROMACS 2019.6 program, applying the AMBER99SB force field.

Results: Molecular docking findings and interactional analysis studies of molecular dynamics simulations indicated suitable hydrogen bonds and van der Waals interactions of piperine with OXA-10. These findings suggest that targeting β-lactamase using piperine as an inhibitor analog could provide a good pathway to deal with multi-drug resistance.

Conclusion: By applying calculation-based methodology, including molecular docking and molecular dynamics simulation, this study suggested that piperine, renowned for its various medicinal properties, can serve as an inhibitor or has the potential to act as an inhibitor targeting the OXA-10 class D β-lactamase enzyme.

Keywords: Molecular docking, Molecular Dynamics Simulation, Piperine, Class D β-Lactamase.

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1. Introduction

Resistance of microorganisms to antibiotics poses a significant global health challenge and threatens the well-being of humanity (1). Multi-drug resistance occurs when microbes employ transmogrify mechanisms to shield themselves from the effects of antibiotics, and therefore the drug could not be any longer effective in the treatment of infection (2). Drug resistance is a natural process that occurs over a period of time through genetic remodeling in microorganisms, which is associated with overuse and misuse of antibiotics that enhance the virulence and pathogenicity of diseases. It is also estimated that resistance to drugs was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths (3, 4).

In addition to death and disability, antibacterial resistance has substantial economic burdens. According to World Bank estimates, this could trigger US$11 trillion in additional healthcare costs by 2050 (4). Although only a small percentage of bacteria are responsible for causing diseases, this minor fraction contributes significantly to a wide range of illnesses that disrupt the hemodynamics and physiology of the human organs and put the physical and mental health of a person at risk or danger (5). In simpler terms, this process can lead to the damage of the body's cells either directly by the invading organism or indirectly through the body's immune response. For the prevention and treatment of bacterial infections caused by exposed organisms, β-lactam antibiotics are recommended. These antibiotics play a crucial role in managing such infections (6). Initially, β-lactam antibiotics primarily targeted gram-positive bacteria. However, recent advancements have led to the creation of broad-spectrum β-lactam antibiotics that are effective against a wider range of organisms, including gram-negative bacteria. This expanded spectrum of activity has significantly enhanced their utility in combating bacterial infections (7). β-lactam antibiotics are those that contain a 4-member nitrogen-containing β-lactam ring at the core of their structure. This ring mimics the shape of the terminal D-Ala-D-Ala peptide sequence that serves as the substrate for cell wall transpeptidases (8). The β-lactam ring is key for the activity of these drugs that are pointed and inhibit cell membrane production by binding the enzymes involved in the synthesis (9). These enzymes are embedded in the cell wall and, as a group, are referred to as penicillin-binding proteins (PBPs) (10). Bacterial species may contain between 4 and 6 different types of PBPs. The PBPs involved in cell wall cross-linking (i.e., transpeptidases) are often the most critical for survival (11).

Destruction of β-lactams by β-lactamase enzyme-producing bacteria is a major mechanism of resistance (12). B-lactamases hydrolyze the β-lactam ring, and the altered structure of the drug can no longer bind to PBPs and inhibit cell wall synthesis (13). For example, staphylococcal resistance to penicillin (13) Resistance of Enterobacteriaceae and Pseudomonas aeruginosa to several penicillins, cephalosporins, and aztreonams (14) β-lactamases are divided into four classes (A, B, C, and D) based on their amino acid sequences in the Ambler classification (15). The classes are defined as follows: Class A includes extended-spectrum BLs (ESBLs) and Klebsiella pneumoniae carbapenems (KPCs) (16). Class B includes the MBLs (NDM, IMP, and VIM) (17). Class C includes OXA-10(18). Class D includes the oxacillinases (OXAs) (19).

To avoid the development of resistance, β-lactamase inhibitors are administered with β-lactam antibiotics, so the action of β-lactamase is inhibited (20). This tends to widen the spectrum of antibacterial activity (21). For instance, tebipenem is used in the form of tebipenem-peroxyl (22), clavulanate combined with amoxicillin (Augmentin) (23), sulbactam combined with ampicillin (Unasyn) (24), or cefoperazone (Sulperazon) (25), tazobactam combined with piperacillin (Zosyn, Tazocin) (26), enmetazobactam combined with cefepime (Exblifep) (27), and avibactam combination with ceftazidime (Avycaz, Zavicefta) (28). Natural compound inhibitors of β-lactamase offer advantages such as fewer side effects and lower costs compared to synthetic substances (29). Therefore, identifying natural products as novel β-lactamase inhibitors could be an interesting way, as over the past several decades, natural compounds have been increasing in developing new selective compounds against different.

Piperine (PIP), an alkaloid omnipresent in foods and beverages, is currently one of the compounds of interest for showing numerous pharmacological benefits found in black pepper and long pepper, and it has been shown to have positive health effects (30). BioPerine, a PIP extract found in supplements, may help improve nutrient absorption (31), blood sugar levels (32), fight cancer (33), alleviate inflammation (34), enhance cognitive function (35), act as an antioxidant (36), improve the bioavailability of many other drugs and supplements, treat metabolic syndrome, hypertension, Parkinson's disease, Alzheimer’s disease, cerebral stroke, cardiovascular diseases, kidney diseases, inflammatory diseases, rhinopharyngitis, etc. (37).

Given the significance of identifying β-lactamase inhibitors for the treatment of infections, as well as the importance of natural compounds and the comprehensive and valuable medicinal properties of PIP, this study aims to investigate the inhibitory behavior of PIP on class D β-lactamase utilizing computational molecular docking and molecular dynamics simulation techniques. However, multiple studies have been performed in this field with other classes of β-lactamases with different natural components, but in this investigation, we targeted finding the inhibitory effect of PIP on OXA-10, which had not been studied under computational techniques before (38–43).

2. Materials and Methods

2-1. Preparation of the enzymes and piperine structures

The structure of Class D β-lactamase OXA-10 with PDB codes (4S2O) was obtained from the RCSB protein data bank (44). The 3D structure of Piperine with CID 638024 code was downloaded from the PubChem database in sdf format and converted to pdb format using OpenBabel (45).

2-2. Computational methods

Calculation-based methodology enables us to obtain information about the molecular interaction and activity of macromolecules that is otherwise challenging to achieve through in vivo, in vitro, or other experimental studies. Computer simulations, particularly for diverse combinations, prove more cost-effective than conducting various types of empirical studies. Typically, experts in the field of biological architecture carry out these endeavors, provided they possess a high degree of accuracy and extensive knowledge. This approach relieves the substantial burden imposed by experimental methods, resulting in significant time and financial savings.

In today's context, pharmaceutical giants worldwide exhibit keen interest in adopting computational methodologies for drug design and drug discovery. Molecular docking, a crucial technique in computational drug design, involves using computer calculations to identify the optimal protein-binding site for ligand attachment. It also determines the ligand's most favorable tendency within the protein-binding site, facilitating effective interactions between the two compounds. As a result, the application of molecular docking allows analysis of binding free energy, hydrogen bonds, and functional groups that contribute to stronger ligand-protein interactions (46).

2-3. Molecular docking

To investigate the interactions and binding affinity between piperine and OXA-10 β-lactamase enzymes, we employed a docking technique using Autodock 4.2.2 software (47). Initially, water molecules and co-crystal ligands were present in PDB files, and hydrogen atoms were removed while Gasteiger charges were added to prepare the system for docking. Subsequently, energy minimization of the enzyme was conducted using the GROMACS 2019.6 package with the AMBER99SB force field. The enzyme's active sites were identified based on the co-crystal ligand reported in the enzyme's PDB file (48). A grid box with dimensions of 60×60×60 points and a grid point spacing of 0.375 Å was selected. Finally, we performed 200 docking calculations, involving 25 million energy evaluations using the Lamarckian genetic algorithm (LGA). The lowest binding energy conformation within the most populated cluster was chosen as the optimal docking pose for further investigations.

2-4. Molecular dynamic simulation

MD simulation was employed to study the behavior of the enzyme both in its free form and when complexed with piperine. The enzyme was placed in a cubic box solvated with a water tip3p model, using the GROMACS 2019.6 program and the AMBER99SB force field. Parameters for Piperine were generated using the Python-based ACPYPE tool (49).

To achieve physiological ion concentration (0.15 M), an appropriate number of Na+ or Cl- ions were added to neutralize system charges. The energy minimization process utilized the steepest descent method initially. Subsequently, the energy-minimized systems underwent equilibration through a 1 ns simulation in the NVT ensemble at 310 K and 1 bar. Once well-equilibrated, a 100 ns MD run was performed with a time step of 2 fs. The resulting simulated trajectories provided insights into the molecular structure of the enzyme, ligand, and intermolecular interactions. System analysis included plots for root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and hydrogen bond analysis.

3. Results and Discussions

Figure 1 illustrates the binding position of piperine within the active site of the OXA-10 β-lactamase enzyme. The figure highlights interactions with key residues including Ser67, Gln113, Val114, Ser115, Val117, Leu155, Leu155, Thr206, Gly207, Phe208, Ser209, and Arg250. These interactions involve van der Waals forces. Additionally, the carbonyl groups of

piperine form a hydrogen bond with the amine group of Arg250. Table 1 presents the binding energies and inhibition constants of piperine with OXA-10. Notably, the OXA-10/Piperine system exhibits suitable binding energy, suggesting high affinity between Piperine and the enzyme. This compound may serve as an inhibitor for OXA-10 β-lactamase.

3-1. Molecular dynamic simulation

3-1-1. Analysis of the root mean square deviation (RMSD)

The root mean square deviation (RMSD) provides insights into the stability and structural changes within both the free enzyme and the enzyme-ligand systems. Figure 2 illustrates the RMSD for the free protein and its interaction with the ligand. Notably, the OXA-10 enzyme reaches equilibrium within approximately 80 ns, and the OXA-10/piperine complexes reach equilibrium within a 60 ns period of time. Interestingly, the structural fluctuations of OXA-10 were enhanced upon complexation with piperine, indicating the instability of the complexes. Additionally, Table 2 presents the average MD parameters over the last 30 ns. Remarkably, the binding of piperine to OXA-10 leads to a shift in the average RMSD from 0.174±0.042 nm in the free form to 0.262±0.087 nm in the complexed form.

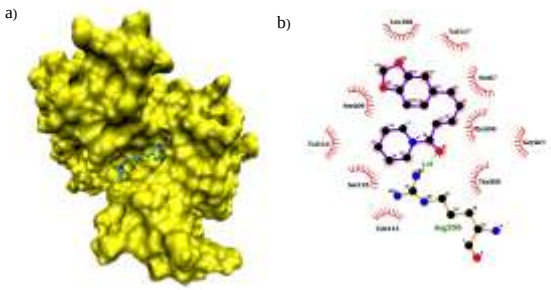

Fig. 1 The best docking pose and molecular interactions of the Piperine and the residues of the enzyme. The C, N and O atoms are indicated in black, blue and red respectively. Hydrogen bonds are identified by green drops and hydrophobic interactions are shown by red curves with spokes radiating towards the ligand atoms they interact. The atoms in contact are shown with spokes radiating back. Figures provided by VMD1.9.3 and Ligplot+ programs

3-1-2. Analysis of the root mean square fluctuation (RMSF)

RMSF analysis reveals the fluctuations and flexibility of individual residues in different regions of the enzyme within both the free and bound states. Figure 3 illustrates the RMSF for the free protein and its interaction with the ligand. Notably, the binding of piperine to OXA-10 results in reduced fluctuation within the system. Specifically, the OXA-10 residues exhibit a minimum RMSF of 0.06 nm across all complexes and a maximum of 0.45 nm. Furthermore, according to Table 2, the mean RMSF value mildly diminished in the presence of piperine, indicating that the bound state of the OXA-10 enzyme experiences minimally less conformational fluctuation compared to its free form.

Table 1. The obtained docking results, binding energies, and inhibition constants predicted by the AutoDock program

System	ΔG binding (KCal/mol)	Ki (μM)
OXA-10-Piperine	-6.1	36.26

Table 2. The average and standard deviations of RMSD, Rg, RMSF, and SASA for free and complex enzymes during the last 30ns

System	Mean RMSD (nm)	Mean Rg (nm)	Mean RMSF (nm)	Mean SASA (nm)
Free OXA_10	0.174±0.042	1.789±0.0158	0.119±0.073	120.884±2.177
OXA-10/Piperine	0.262±0.087	1.814±0.023	0.118±0.081	124.459±2.488

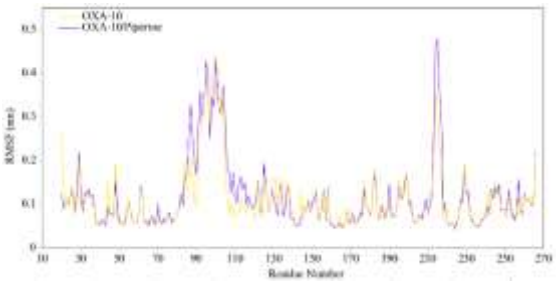

Fig. 3. RMSF plots of free and bound enzymes.

3-1-3. Analysis of the radius of gyration (Rg)

The calculation of Rg provides insight into the protein's shape and how tightly it is packed during the simulation period when it is combined with a ligand. The Rg of the unbound enzyme and the enzyme-piperine complexes are displayed in Figure 4. As per this figure, the OXA-10 enzyme achieves equilibrium around 80 ns, and for complexed systems, it takes a 90 ns period of time. The system's third structure is uncondensed when piperine is combined with OXA-10. Table 2 shows the average Rg value over the final 30 ns of the simulation period. The rg average count of OXA-10 rises in the presence of piperine, indicating that the enzyme shape is uncompressed and the structural density of the enzyme diminished due to its binding with piperine.

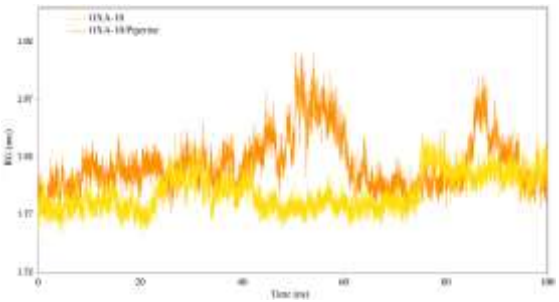

Fig. 4. RG plots of free and bound enzymes as a function of time

3-1-4. Analysis of the solvent-accessible surface area (SASA)

The SASA analysis reveals the portion of the enzyme's surface that solvent molecules can access over the duration of the simulation. The SASA diagrams are depicted in Figure 5. This figure indicates that the average SASA for the enzyme has risen due to its interaction with piperine,

which is a result of piperine making contact with an enzyme residue in a cavity on the OXA-10 surface. As per Table 2, the average SASA value has slightly increased when piperine binds to OXA-10, suggesting that the enzyme's surface available to water molecules has expanded into a complex form.

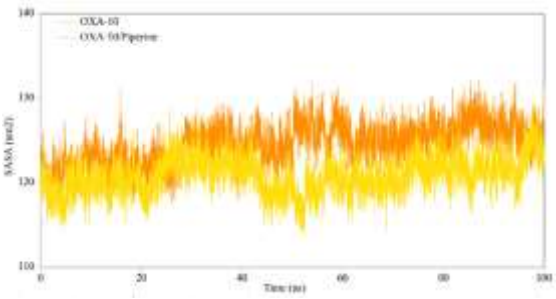

Fig. 5. SASA plots of free and bound enzymes as a function of time.

3-1-5. Hydrogen bond analysis

The examination of the number of hydrogen bonds between the enzyme and ligand indicates the stability of the complexes throughout the simulation period. Figure 6 illustrates the count of hydrogen bonds between piperine and the enzyme during the 100 ns simulation period. The maximum number of hydrogen bonds formed between piperine and OXA-10 during the simulation was 2, demonstrating the stability of the complexes. Piperine exhibits the strongest binding affinity to this enzyme. Figures 7 and 8 depict the hydrogen bonds between the enzyme-enzyme and enzyme-solvent for the unbound and bound enzymes during the simulation period, respectively. The average count of hydrogen bonds between enzyme atoms has slightly decreased in the presence of piperine, while the hydrogen bonds between the OXA-10 enzyme and solvent molecules have increased when piperine is present.

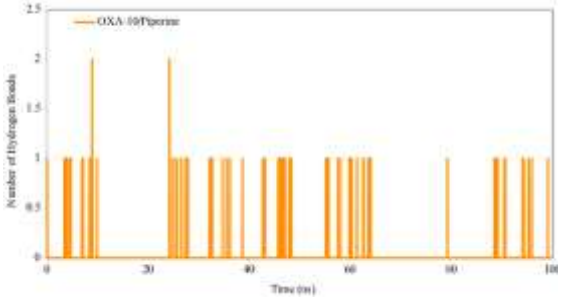

Fig. 6. Time dependence of the number of hydrogen bonds between piperine and enzyme during the simulation time.

4. Conclusions

In this particular investigation, molecular docking and dynamic simulation approaches were applied to discover the inhibitory activity of piperine on class D β-lactamases. Molecular docking analysis showed a suitable binding energy of -6.1 via a hydrogen bond and a proper binding pose within the active site of the OXA-10 enzyme. Molecular dynamic simulation validated the docking result and provided more details about the interaction of piperine with OXA-10.

Table 3: The average and standard deviations of intramolecular enzyme and enzyme-solvent hydrogen bonds during the last 30 ns

System	Enzyme-Enzyme	Enzyme-Solvent
Free OXA-10	195.450±6.915	486.769±13.281
OXA-10/Piperine	193.845± 7.775	488.639±14.059

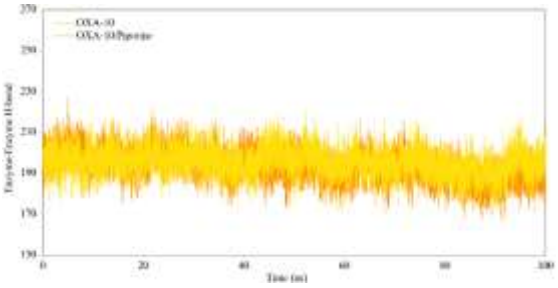

Fig. 7. Enzyme-enzyme hydrogen-bond plots of free and bound enzymes as a function of time

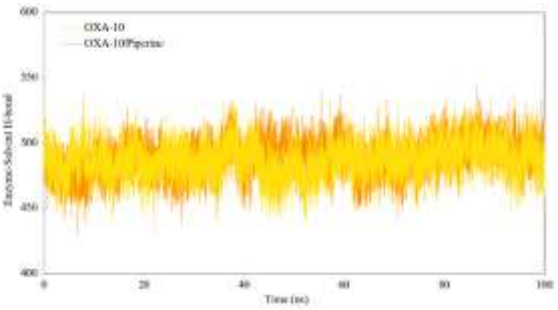

Fig. 8. Enzyme-solvent hydrogen bond plots of free and bound enzymes as a function of time.

Molecular dynamic simulation validated the docking result and provided more details about the interaction of piperine with OXA-10. The RMSD analysis of the system shows the structural instability of OXA-10 as complexed with piperine, and the mean RMSD increased when a complex system was formed. The RG plot of this analysis demonstrates the decompression of OXA-10 in the presence of piperine, which is a good indicator for disturbing the structural compactness of OXA-10 by piperine. The RMSF of the system also shown to be decreased when piperine binds to OXA-10, which explains why the system in complex form has less fluctuation in residue than the free form of OXA-10. SASA analysis also reveals the surface expansion of OXA-10 due to the binding of piperine. The H-bond analysis confirms the above information obtained from molecular docking and dynamic simulation. In comparison to previous research performed to find the inhibitory effect of piperine on class D β-lactamase, the outcome of this project displays that piperine has less inhibitory activity on class D β-lactamase. However, to prove this, more in vivo and in vitro experimental investigation is required, which hopefully this study can provide a starting point for such investigations.

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