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

Allah Nawaz Safi[1], Mohammad Sayed Behrad[2], Sayed Hussain Mosawi[1*], Abdul Musawer Bayan[1]

1. Medical Sciences Research Center, Ghalib University, Kabul, Afghanistan.
2. Thoracic and Cardiovascular Surgery Department, Kabul University of Medical Science, Kabul, Afghanistan.

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*Corresponding Author:
Sayed Hussain mosawi
Address: Medical Sciences Research Center, Ghalib University, Kabul, Afghanistan.

sayedhussain.mosawi@ghalib.edu.af

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ABSTRACT

Background: Antibiotic resistance is a significant health concern, as bacteria produce enzymes that inhibit antimicrobial drug activity, increasing disease generation. This study investigates the inhibitory effect of berberine on β-lactamase enzyme activity and antibiotic effectiveness.

Methods: Molecular docking was utilized to find the binding pose and binding affinity of a new inhibitory ligand with the AmpC enzyme using Autodock software version 4.2.2. MD simulations were performed in free form and complicated to understand the stability of the protein-ligand docked complex.

Results: The molecular docking result indicated the proper interaction between berberine and the AmpC β-lactamase enzyme with a suitable binding pose and binding energy of -6.55 kcal/mol. The MD simulation of systems verifies the docking result, which shows stable hydrogen bonds of berberine with AmpC and good equivalence between RMSD, RMSF, SASA, etc.

Conclusion: This paper reveals that berberine, which is a natural ingredient with multiple medicinal characteristics, can be applied as a potential inhibitor of class C β-lactamase AmpC. Hence, the outcome of the calculations performed provides valuable data to design new inhibitors with therapeutic potential in order to control the β-lactamase activity.

Keywords: Molecular docking Molecular dynamics simulation, Berberine, Class C β-Lactamase.

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1. Introduction

Antibiotic resistance is a problem that can be significantly alleviated by using herbal preparations. Several herbal compounds can target a variety of bacteria, fungi, and occasionally bacilli due to their broad-spectrum antimicrobial effects (1). The potential research of different aggressive antibody types is enabled by this comprehensive activity, which is advantageous in the antibacterial resistance environment. It is difficult for bacteria to promote the degradation of plant compounds because they often have different mechanisms of action (2). Herbal compounds can act on multiple targets simultaneously, contrasting with some common antibiotics that target specific bacterial signaling pathways. This makes it difficult for bacteria to acclimate and become resistant (3). In addition, the antimicrobial effects of common antibiotics and herbal compounds can be added, and attrition mechanisms can be influenced by coexistence (4).

Numerous studies have shown that co-administration of herbal medicines and antibiotics can improve research results and reduce the emergence of resistance. In addition, the accumulation and openness of biofilms are disturbed by the anti-biofilm activity of plant compounds (5). By being absorbed into biofilms, herbal active ingredients can improve the ability of antibacterial research in bacteria that are aggressive to antibiotics. In addition, some plant compounds have the ability to regulate certain reactions that control the body's defenses (6).

Berberine, a naturally occurring compound found in various plants such as goldenseal, barberry, and Oregon grape, has achieved great success in treating various health conditions in traditional medicine, particularly Chinese and Ayurvedic practices (7). The comprehensive study of berberine examined its potential health benefits in treating chronic diseases such as diabetes, cardiovascular disease, and gastrointestinal disease. These effects are thought to be controlled by various mechanisms, such as activation of cellular signaling pathways and access to metabolic processes (8). The acceptance of berberine also extends to the area of antibacterial resistance, as it appears to have an affinity for multidrug-resistant bacteria. Antibiotic resistance is an acute general health problem characterized by a change in bacterial mechanisms that causes antibiotics to become less effective or ineffective in treating infections (9). Berberine has been shown to increase the ability of specific antibiotics to bind to drug-resistant bacteria. This was confirmed by studies enriching berberine with tetracycline, gentamicin, and erythromycin, consistent with additional antibacterial activity (10). This suggests that berberine may contribute to a positive antibacterial effect by counteracting the effects of conventional antibiotics. Bacterial address pumps are mechanisms by which bacteria expel antibiotics from their cells, thus shortening biological processes (11).

By inhibiting the address pumps, berberine can slow down the intracellular absorption of antibiotics and thus influence their antimicrobial effect against drug-resistant bacteria. In addition, berberine has been shown to provide an opportunity for gene announcement in bacteria, including genes associated with antibacterial resistance (12). Studies suggest that berberine may downregulate certain wear-and-tear genes, shortening the wear-and-tear of bacteria caused by antibiotics. In addition, berberine has been shown to inhibit the alteration of wear-and-tear genes in bacteria, potentially resulting in the spread of antibacterial resistance (7). Considering the importance of rare β-Lactamase inhibitors in the treatment of inflammation, as well as the importance of natural compounds and the comprehensive therapeutic effect of piperine, this study aims to investigate the inhibitory behavior of piperine in the liver of various class C inhibitors of β-Lactamase using computational methods.

2. Material and methods

2-1. Computational methods

Computational methodologies acquiesce for the accretion of advice that is backbreaking to access through beginning means. The use of computer simulations for assorted combinations proves to be more cost-effective compared to administering class tests of any kind. Generally, the biologic architecture endeavor is agitated out by clay experts, provided they acquire an aerial amount of accurateness and all-encompassing knowledge. This access alleviates the abundant accountability imposed by beginning methods, leading to cogent reductions in time and banking resources. In contemporary times, biologic giants beyond the apple accept apparent a agog absorption in the acceptance of computational methodologies for biologic design.

Atomic advancing stands as an invaluable address for investigating the interactions among ligands and proteins, a basic axiological apparatus in computational biologic design. Atomic advancing entails employing computer calculations to determine the optimal protein-bound armpit for ligand attachment as well as the most favorable acclimatization of the ligand to the protein-bound armpit to facilitate acceptable interactions between the two compounds. Consequently, through the appliance of the atomic advancing method, one can analyze the bounden chargeless energy, hydrogen bonds, and anatomic groups that accord with the enactment of added almighty ligand-protein interactions.

2-2. Selection of enzyme and ligand structures

The PDB file of class C β-lactamase (4HEF) was downloaded from the RCSB protein database (13) and the three-dimensional structure of berberine was obtained from the PubChem database with the CID C15H10O5 in the SDF format retrieved and converted to the PDB format using Open Babel software (14).

2-3. Molecular docking

To investigate the interactions and binding affinity between berberine and the AmpC β-lactamase enzymes, docking techniques were applied to evaluate them using Autodock 4.2.2 software (15, 16). Originally, the water molecules and co-crystal ligands were in pdb files, with hydrogen atoms removed and Gasteiger charges added to the system to prepare it for docking (17, 18). Energy minimization of enzymes was performed using the GROMACS 2019.6 package using the AMBER99SB force field (19). The active sites of the enzyme were determined by the co-crystal ligand specified in the PDB file of the enzyme, and then the grid array with dimensions of 60 × 60 × 60 points and a grid point spacing of 0.375 Å was selected. Finally, 200 docking calculations consisted of the 25 million energy assessments performed using the Lamarckian Genetic Algorithm (LGA) method. In the end, the conformation with the lowest binding energy in the most highly populated cluster was selected as the best docking position and used for further studies.

2-4. Molecular dynamic simulation

The agitator was subjected to atomic dynamics simulations in chargeless anatomy and in circuitous with berberine in a solvated cubic box application, the tip3p baptize atom archetypal application, and the GROMACS 2019.6 affairs active on a Linux Kubuntu 2020. Berberine force acreage was generated using the Python-based ACPYPE (AnteChamber Python Parsing Interface) apparatus (29). Sufficient ions were added to abrogate the system. The antecedent solvation systems are scaled bottomward, applying the steepest coast adjustment to annihilate actual ample forces. Then, the apish systems were counterbalanced at 310 K and 1 bar by active 1ns simulations in the nvt and npt ensembles. After equilibration of the continued system, an MD run was performed with a time footfall of 2 fs for a simulation time of 200 ns. The atomic anatomy

of the enzyme, the ligand, and the final intermolecular interactions were advised application apish trajectories.

3. Results and discussion

3-1. Molecular docking

Binding energy and the inhibition constants of Berberine with the AmpC are displayed in Table 1. This table indicates that the AmpC/Berberine system has the lowest binding energy, with a mean ΔG binding of -6.55 Kcal/mol and an inhibition constant of 15.79 µM. Figure 1 exhibited the binding pose and interaction of Berberine with the key residues of AmpC β-lactamase in its active site. This diagram shows the main amino acids in the active site of AmpC as follows: Ser90, Gln146, Tyr177, Tyr294, Thr343, Gly344, Ser345, Thr346, Asn347, Arg376 and Asn373, some of which interact with Berberine through van der Waals interactions. It is visible that the carbonyl group of Berberine formed two H-bond complexes with the carboxyl group of Ser90 and the amino group of Asn347, respectively. Findings indicate that Berberine has a high affinity for AmpC, which can potentially be an inhibitor of class C β-lactamase.

Table 1: The binding energies and inhibition constants of AmpC/Berberine

System	ΔG binding (KCal/mol)	Ki (µM)
AmpC/Berberine	-6.55	15.79

3-2. MD simulation

3-2-1. Analyses of RMSD (root mean square deviation)

The root mean square deviation (RMSD) of the trajectory obtained in MD simulations shows us the stability measure of the docked complexes. Figure 2 exhibits the AmpC RMSD of 100 ns for the simulated system. At the initial system running, there was a slight fall and elevation in the RMSD of the protein, but after the binding of the ligand to Ampc, a subsequent gradual decrease occurred and the system got equilibrated. As shown in this figure, the AmpC enzyme reached equilibrium at 85 ns for the free system and 80 ns for the bond system. As ligands bind AmpC, the deviation of protein decline shows stabilization of AmpC in the presence of berberine. The averages of the MD parameters for free and complex systems represented in Table 2 last 20 ns indicate that Berberine binding causes significant stabilization. As with the binding of berberine to AmpC, the average number of RMSD shifts from the free form of 0.139±0.026 nm to 0.120±0.013 nm.

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

RMSF depicts the conformational flexibility residues of the protein-ligand complexes. Figure 3 displays the protein RMSF and the ligand RMSF, respectively. As visible in this figure, the fluctuations were not high, and the RMSF for the AmpC residues was observed to be a minimum of 0.06 Å for all the complexes and a maximum of 0.5 Å. For the ligand-protein RMSF, the atoms showed the minimum fluctuation for residues in the AmpC active site compared to the free form. As shown in Table 2, the mean RMSF value in the presence of berberine for the enzyme is mildly elevated, indicating that the bound state of the AmpC enzyme has a relatively low conformational fluctuation compared to the free form of the enzyme.

3-2-3. Analysis of RG (radius of gyration)

RG is an important parameter that yields information about structural compactness, the third structure of the enzyme, and its deviation in the presence of ligand. The radius of gyration (Rg) of the AmpC and ligand complexes were found to be between 1.76 and 1.85 nm initially, as represented in figure 4.

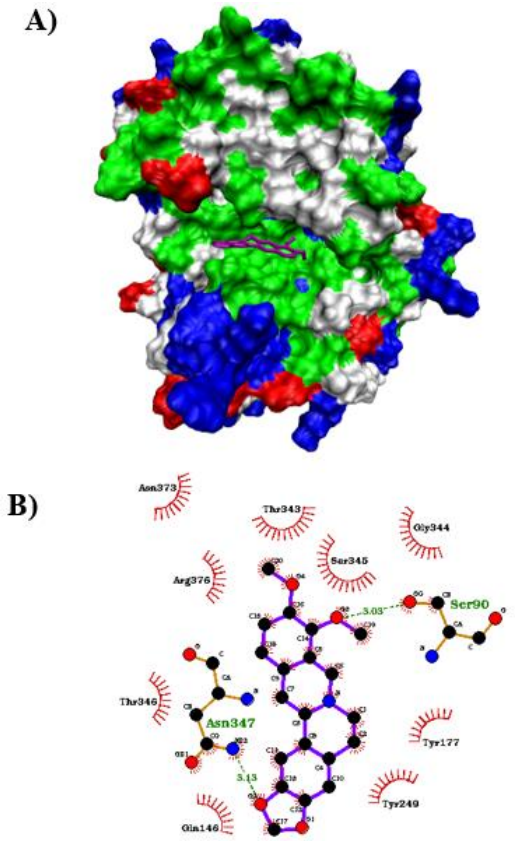

Figure 1. Binding mode of Berberine with docked AmpC (A) Ribbon model: Berberine, Surface model: Berberine, (B) Ligplot showing interactions between Berberine residues and AmpC; the residues in green are involved in hydrogen bonding and the black in hydrophobic interactions. The atoms in contact are shown with spokes radiating back. Figures are provided by the VMD1.9.3 and Ligplot+ programs.

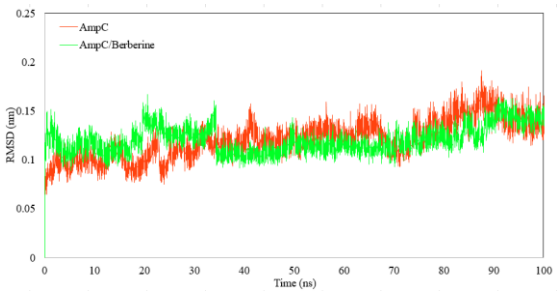

Figure 2. The time evolution of average RMSD during 100 ns MD simulations.

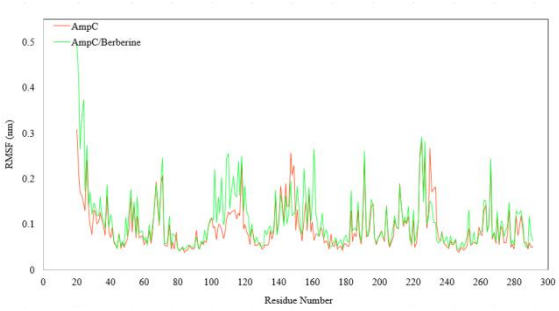

Figure 3. The average RMSF for a 100-ns period of MD simulations.

The Rg values were stabilized after 78 ns for both free and complex systems. RG for complexed systems shows less fluctuation during simulation time, which shows the 3rd structure of AmpC has been compacted as bound to Berberine. Table 2 represents the average amount of R_g during the last 20 ns of simulation time. The average number of AmpC has decreased with the presence of berberine, which shows the compression of the enzyme's 3rd structure due to binding to berberine.

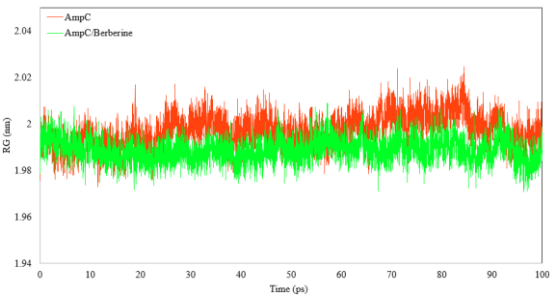

Figure 4. The average RG for a 100-ns period of MD simulations.

2-3-4. Analysis of SASA (solvent-accessible surface area)

SASA plays the reachable surface of the enzyme to its solvent in free and complex form during the periodic time of simulation. Figure 5 exhibits the SASA diagram, as the results indicate the minimum 150 nm to maximum 160 nm SASA, and both systems have reached equilibrium in 80 ns. As berberine binds to AmpC, the SASA stabilizes more than free form. According to Table 2, the average amount of SASA produced by the binding of berberine to AmpC had decreased, which shows the

surface of the enzymes for water molecules was extended in complex form.

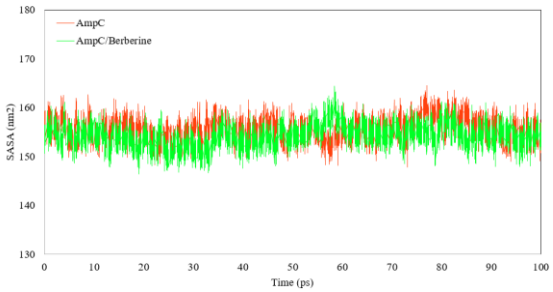

Figure 5. The average SASA for a 100-ns period of MD simulations.

3-3-5. Analysis of the number of hydrogen bonds

Studying the H-bond between enzyme-enzyme, enzyme-solvent, and enzyme-ligand enables us to find more information about the binding affinity and interactions of systems. The number of contacts formed by Berberine with AmpC residues is presented in Figure 6. The maximum number of H-bonds formed by berberine with AmpC residues was 3 in the MD simulations study, which shows the stability of complexes and shows that berberine has a binding tendency to this AmpC. Enzyme-Solvent and Enzyme-Enzyme hydrogen bonds for free and bound are exhibited in Figures 7 and 8, respectively, as these figures show no significant variation or alteration in Enzyme-Solvent for both. The average number of enzyme-solvent and enzyme-enzyme hydrogen bonds is shown in Table 3 for systems during the last 20 ns. The findings in this table suggest that the average number of hydrogen bonds between enzyme atoms in the presence of Berberine is slightly elevated, and the hydrogen bonds between AmpC enzymes and solvent molecules are diminished in the presence of Berberine. The binding affinity of the berberine with AmpC in the MD simulation study confirms the result.

Table 3: The average and standard deviations of intramolecular enzyme and enzyme-solvent hydrogen bonds during the last 20 ns

System	Enzyme-Enzyme	Enzyme-Solvent
Free AmpC	195.650±6.915	654.630±15.157
AmpC/Berberine	191.060±6.600	646.072±15.711

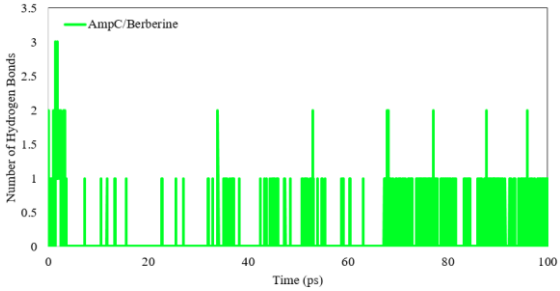

Figure 6. The number of hydrogen bonds between Berberine and AmpC for a 100-ns period of MD simulations.

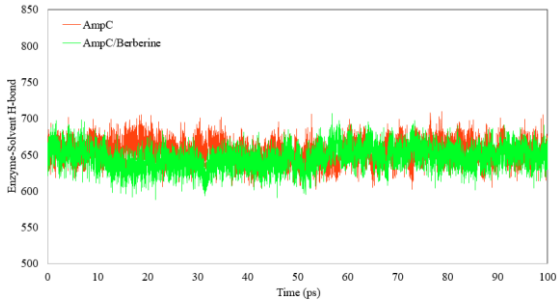

Figure 7. The enzyme-solvent H-bond for a 100-ns period of MD simulations.

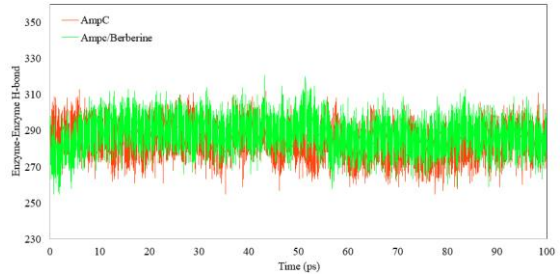

Figure 8. The enzyme-enzyme H-bond for a 100-ns period of MD simulations

4. Conclusion

This investigation is based upon a novel molecular technique to clarify a natural compound inhibitor (berberine) for AmpC combined with the analysis to validate the findings by applying molecular docking and MD simulation. Docking result, marking the suitable binding energy and significant hydrogen bond interaction. A MD simulation

study was executed to verify the stability of the berberine inside the active site of the AmpC, as presented in different structural analyses of MD simulations, such as RMSD, RMSF, RG, and SASA; furthermore, the interactional analysis study indicated a stable hydrogen bond between berberine and AmpC. Therefore, it is clear that this identified inhibitor will serve as a better initiating tip for further experimental studies of β-lactamase inhibitors in the drug design and discovery process.

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