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Integrating molecular docking and molecular dynamics simulation studies of afzelechin as a potential inhibitor of acyl-homoserine lactones (AHLs) synthase

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ABSTRACT

Introduction: Misuse of antimicrobial agents and inadequate treatment methods lead to recurrent infections due to microorganism resistance, multi-drug resistance, and biofilm formation. Acyl-homoserine lactones propagate resistance and establish a stable microbial population. Natural derivatives like afzelechin can regulate biofilm formation and show promise as non-toxic agents against biofilms. Combining these with antibiotics could improve outcomes.

Method: The study used molecular docking to analyze the interaction and binding affinity of afzelechin with AHL synthase, using Autodock 4.2.2. The configuration with the most favorable binding energy was chosen for further study. MD simulations were conducted using AMBER99SB force field within GROMACS 2019.6 software.

Result: The molecular docking analysis revealed that afzelechin fits appropriately within the active site of AHL synthase, exhibiting favorable binding energy. Subsequent molecular dynamics (MD) simulations corroborated these findings, with evaluations of root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), solvent-accessible surface area (SASA), and radius of gyration (RG), among other parameters, confirming the stability and suitability of the interaction.

Conclusion: This study reveals that afzelechin, a compound with various therapeutic properties, shows promise as an inhibitor against the AHL synthase enzyme. The results of the analysis offer significant insights that could assist in the development of novel inhibitors aimed at managing biofilm formation and antibiotic resistance.

Keywords: Acyl-homoserine lactone synthase, Afzelechin, Molecular Docking, Molecular Dynamic Simulation

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1. Introduction

Multi-drug resistance of microorganisms (bacteria, fungi, parasites, and viruses) created universal health challenges or important public health concerns (1). Bacteria, which are the most abundant among these microorganisms, correspondingly have the highest biofilm formation (2). Nearly every species of bacteria, particularly those that are pathogenic, possesses the capability to develop biofilms (2). These bacterial biofilms present a significant health issue due to their extreme resistance to a wide range of medications, including standard antibiotics (3). Furthermore, the potency of current antimicrobial agents has diminished, and some of these microorganisms have become nearly impossible to treat due to the growing problem of pathogen resistance (4). This issue could be attributed to factors such as the intense stress caused by indiscriminate antibiotic use and the lack of effective strategies (5).

Bacterial biofilms represent a specific type of enduring bacterial infection (6). These biofilms are complex structures that house a variety of microorganisms, surrounded by a matrix, often composed of exo-polysaccharides (6). This matrix facilitates their adhesion to both inorganic surfaces such as glass, plastic, and rocks and organic surfaces like cuticles, mucous membranes, and skin. The formation of biofilms could potentially trigger drug resistance and inflammation, leading to chronic infections in the hosts (7). Recently, due to the escalation in antibacterial drug resistance, there has been a surge in biofilm formation by a group of medically significant bacteria known as ESKAPE, which includes Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species. This has resulted in high mortality rates (8).

Quorum sensing (QS) is a communication method used by bacteria to coordinate actions within their community (9). This process plays a crucial role in the growth and development of bacterial biofilms (9). It's also associated with interactions between cells, which are influenced by factors like the creation, exchange, and recognition of tiny signaling molecules among bacteria, or QS serves as a communication system among bacteria, enabling various processes (10). These include the formation of biofilms, the expression of virulence factors, the production of secondary metabolites, and the modification of stress responses (11). It also encompasses mechanisms for bacterial competition, such as secretion systems (11). Once a certain signal threshold of autoinducer or signaling molecules is reached, specific virulence traits in bacteria are regulated in response to the immediate environment (12). These virulence traits are known to play a role in diseases caused by pathogenic bacteria. Secretion systems (SSs) are ubiquitous and can be found in both Gram-negative and Gram-positive bacteria (13). They play a crucial role in bacterial communication and have global roles that contribute to pathogenesis and virulence (13).

The communication between bacterial cells through QS relies on autoinducers (14). These autoinducers involve the production, secretion, and detection of small molecules (15). There are several main types of quantum sensing (QS) systems that have

been identified and studied (16). These include the N-acyl-homoserine lactone (AHL) systems found in Gram-negative bacteria, the 4-quinolone systems also in Gram-negative bacteria, which use a hydrophobic signal, the AgrD peptide systems in Gram-positive bacteria, and the Al2/LuxS systems, which are present in both Gram-negative and Gram-positive bacteria (17). It has been observed that the AHL quorum-sensing mutant, which forms a thin biofilm, is more susceptible to antibiotics and sterilization solutions (18). The phenotype can be complemented by introducing a functional lasl or adding an appropriate AHL (19). In contrast, Gram-positive bacteria utilize autoinducing peptides (AIP) as their autoinducers (20). AHL signals are produced by enzymes from the LuxI family (ALH synthase) and are identified by signal receptor-transcriptional regulators from the LuxR family (21). There exist signals that are chemically different yet related, and these are specific to certain systems (21). For centuries, herbal remedies have been utilized in traditional medicinal systems and by indigenous healers to address a variety of ailments due to their easy accessibility, low cost, and the lower side effects they pose (22).

Afzelechin (AZC) is a flavonoid found in Bergenia ligulata, a resilient plant native to the Himalayas and belonging to the Saxifragaceae family, and is typically found in the rocky areas of Northern India (23). It is known to have various medicinal properties, acting as antibacterial (24), antiviral (25), antipyretic (26), anti-inflammatory (27, 28), anti-neoplastic (26), anti-diabetic activity (29), diuretic (30), antilithic activity (26), anti-bradykinin activity, laxative effects, and others (31). Regarding challenges brought by multi-drug resistance and the formation of biofilm related to QS and the essence of finding a natural compound along with antibiofilm to increase the efficacy of the treatment, this study targeted exploring the inhibitory effect of afzelechin on AHL synthase in gram-negative bacteria with the utilization of molecular docking and molecular dynamic simulation.

2. Materials and Methods

2.1 Small-Molecule Preparation

The 3rd structure of AHL synthase with the PDB 1KZF code was downloaded from the RCSB Protein Data Bank (32). Also, the ligand afzelechin, detected by its CID number 442154, was downloaded from the PubChem database in SDF format and converted into PDB format utilizing the OpenBabel software (33, 34).

2.2 Computational Techniques

At the initial stages of drug design, computational strategies employ modern technology to gain a deeper comprehension of chemical systems through virtual analysis, enhancing physical experiments. Molecular docking, a computational approach, is utilized to predict how small molecules or macromolecules will bind to a receptor and interact at the molecular level. This technique also enables the classification of these molecules based on a ranking system determined by specific scoring functions.

Docking protocols involve several assumptions and often do not account for receptor flexibility, casting doubt on the dependability of the resultant protein-ligand complexes. Pairing with the more precise, albeit expensive, molecular

dynamics (MD) techniques significantly augments docking. MD simulations can precede docking, as they generate a range of "new" and more diverse protein conformations from the processed trajectory data, which can then serve as docking targets. Alternatively, MD can be applied after docking to refine the structures of the resulting complexes, compute more nuanced interaction energies, and shed light on the mechanism of ligand binding (35).

2.3 Molecular Docking

The interaction between afzelechin and AHL synthase was analyzed through molecular docking using the AutoDock 4.2.2 software. Flexibility in the docking process was preserved by allowing all torsion angles in the afzelechin molecules to rotate freely. The AHL synthase was prepared by adding polar hydrogens via the ADT's hydrogen module. Subsequently, the enzyme's energy was minimized using the GROMACS 2019.6 software and the AMBER99SB force field (36).

Docking parameters were set using the empirical free energy function and the Lamarckian genetic algorithm, with settings that included up to 25 million energy evaluations, a starting population of 200 individuals placed at random, and a grid box measuring 60×60×60 points with a spacing of 0.375 Å between points. The most favorable docked positions, indicated by the lowest binding energy within a highly clustered population, were chosen for further molecular dynamic simulations.

2.4 Molecular dynamics simulation

Molecular dynamics (MD) simulations were executed utilizing the GROMACS 2019.6 software alongside the AMBER99SB force field (35). The MD simulations incorporated complexes of 1KZF bound with afzelechin. To achieve charge neutrality, Cl counterions were introduced in place of water molecules. The complexes underwent energy minimization through the steepest descent method within the GROMACS suite. The ACPYPE tool, which is Python-based, was employed to derive parameters for Afzelechin (36). An initial 1 ns simulation was carried out under the NVT ensemble conditions at 310 K and 1 bar pressure. This was followed by an extensive 100 ns MD simulation, progressing at a 2 fs time step. The simulation trajectories provided an intricate portrayal of the enzyme's molecular framework, the ligand, and their interactive dynamics.

3. Result and Discussion

3.1 Molecular docking

Molecular docking studies for the enzyme/ligand system indicate that the afzelechin is appropriately placed within the active site of the 1KZF enzyme. Figure 1 displays the interaction of the ligand afzelechin with key residues within the active site of 1KZF, which include Trp34, Tyr54, Tyr9, Leu12, Ser44, Asp48, Met42, val67, Ser66, Arg68, and Arg100. The ligand's carboxyl group generated hydrogen bonds with the carboxyl atoms of Ser44, Ser66, Arg100, and Asp48. Furthermore, the carboxyl group of afzelechin is observed to establish hydrogen bonds with the carbonyl groups of Arg100.

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

System	ΔG binding (KCal/mol)	Ki (μM)
AHLs synthase/Afzelechin	-7.03	7.00

The outcome of molecular docking allows us to suggest a general mode of binding for the afzelechin and identify the residues that are crucial for ligand specifications. Based on these results, the 1KZF-Afzelechin complexes were chosen for MD simulations. The goal of these simulations is to develop more accurate models of the ligand-receptor interaction under conditions that closely resemble the natural environment. Table 1 presents the binding energies and inhibition constants of afzelechin with AHL synthase. The 1KZF-Afzelechin system demonstrates optimal binding energy, indicating a high affinity between afzelechin and the enzyme, implying that afzelechin could act as an inhibitor of AHL synthase.

3.2 Molecular dynamic simulation

Analysis of the root mean square deviation (RMSD)

The RMSD analysis is used to understand the structural changes of a system over the simulation period. The RMSD of the free enzyme and its interaction with the afzelechin are exhibited in Figure 2. Remarkably, the free AHL synthase enzyme and complex system both reached equilibrium in 65 ns. The structural fluctuations of AHL synthase are observed to be enhanced in the presence of afzelechin, suggesting that the complex form has more structural instability compared to the free form. In addition, the average molecular dynamics parameters displayed in Table 2 for the last 30 ns indicate that the binding of afzelechin to AHL synthase causes the average RMSD to rise from 0.270±0.026 **nm** in its free form to 0.284±0.034 **nm** in the presence of afzelechin.

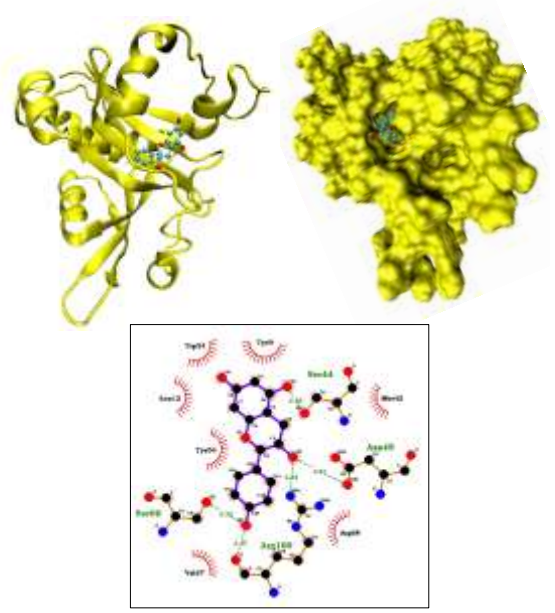

Fig. 1: The best docking pose and molecular interactions of the Afzelechin and the residues of the AHL synthase. The C, N, and O atoms are indicated in black, blue, and red, respectively. Hydrogen bonds are identified by green drops, and hydrophobic interactions are shown by red curves with spokes radiating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

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

The RMSF measurement plot was used to investigate the protein residue alteration in

enzymes for both systems, in free form and in the presence of ligand.

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

System	Mean RMSD (nm)	Mean Rg (nm)	Mean RMSF (nm)	Mean SASA (nm)
1KZF	0.270±0.026	1.735±0.012	0.129±0.054	114.483±2.318
1KZF/Afzelechin	0.284±0.034	1.712±0.016	0.131±0.055	113.672±2.629

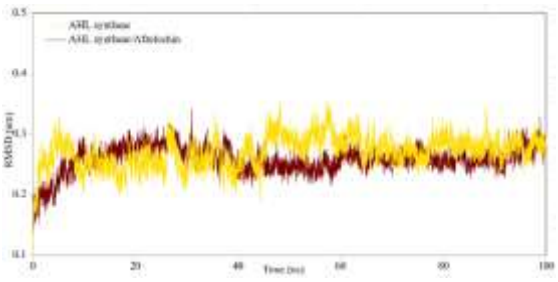

Fig. 2. RMSD plots of free and bound enzyme as a function of time.

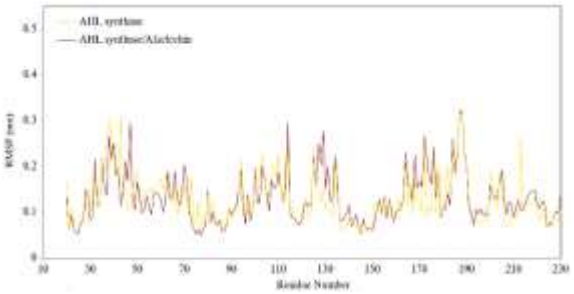

Fig. 3. RMSF plots of free and bound enzyme.

In addition, this analysis also detects the rigidity and flexibility of the molecular base in the whole dynamic simulation. Figure 3 depicts the RMSF values for both free enzymes and in the presence of afzelechin. According to this plot, the RMSF value for AHL synthase had been raised when the afzelechin was present. Additionally, the residues of AHL synthase show a minimum RMSF value of 0.05 nm for both complexes and a maximum reaching 0.32 nm. Moreover, table 2 confirms that the average RMSF value is enhanced when afzelechin forms complexes with enzymes, from 0.129±0.054 nm to 0.131±0.055. Proposing that the complex of AHL synthase with afzelechin undergoes conformational alteration rather than a free-form state.

3-4. Analysis of the radius of gyration (Rg)

The Rg plot shows the structural integrity of the molecule's structure. Figure 4 displays the RG of a free and complex system.

According to this plot, the AHL synthase enzyme reaches equilibrium near 85 ns for both systems. The interaction between afzelechin and AHL synthase leads to structural compression and increases structural condensation. As shown in Table 2, the average radius of gyration (Rg) value in the last 30 nanoseconds of the simulation decreased from 1.735±0.012 free form to 1.712±0.016 complex form, respectively.

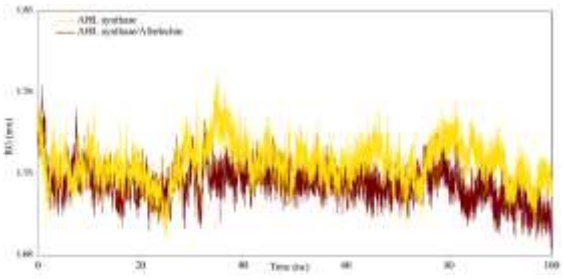

Fig. 4. RG plots of free and bound enzyme as a function of time

Analysis of the solvent-accessible surface area (SASA)

SASA analysis explains the accessible surface area of macromolecules in free form and the complex state in the solvent, in addition to their molecular interactions and stability during simulation time. Figure 5 displays SASA diagrams. According to this figure, the average of SASA for the enzyme has declined in the presence of afzelechin, giving the idea that the available area on the surface of the enzyme is being covered by afzelechin and reducing the attachment of the enzyme's surface molecule to the solvent. According to Table 2, the average SASA value has reduced when afzelechin binds to the AHL synthase surface from 114.483±2.318 to 113.672±2.629, respectively, which implies that the enzyme's surface interaction with water molecules has become reduced when a complex system forms.

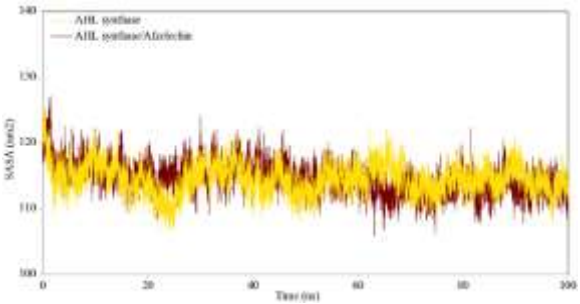

Fig. 5. SASA plots of free and bound enzyme as a function of time.

Hydrogen bond analysis

H-bond study allows us to find molecular interactions and those H-bonds that are important for the functionality, structural integrity, and stability of enzymes, specifically in performing molecular dynamic simulations. H-bonds between afzelechin and the AHL synthase are displayed in Figure 6 throughout the 100 ns of simulation time. The maximum number of hydrogen bonds that were noticed between afzelechin and AHL synthase was six, denoting the structural stability of complexes. Figures 7 and 8 demonstrate the hydrogen bonding schema for enzyme-enzyme and enzyme-solvent interactions, respectively, for free and complex systems during the simulation. The average h-bond value between the enzyme's atoms was raised in the presence of afzelechin from 159.949 ±6.682 free state to 163.554±6.997 complex form, while there's a major reduction in hydrogen bonding between the AHL synthase enzyme and the solvent molecules when afzelechin is introduced, a shift from 436.110±13.525 to 420.258±13.899, respectively, due to the solvent molecules attached to the enzyme by afzelechin.

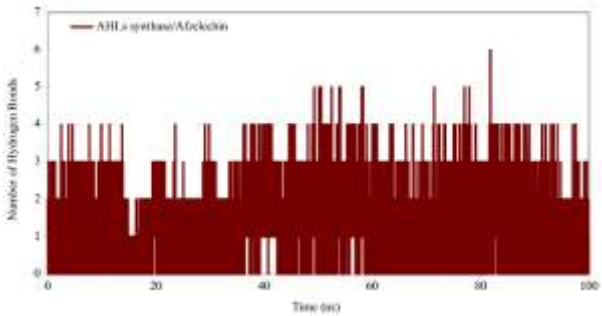

Fig. 6. Time dependence of the number of hydrogen bonds between Afzelechin and enzyme during the simulation time.

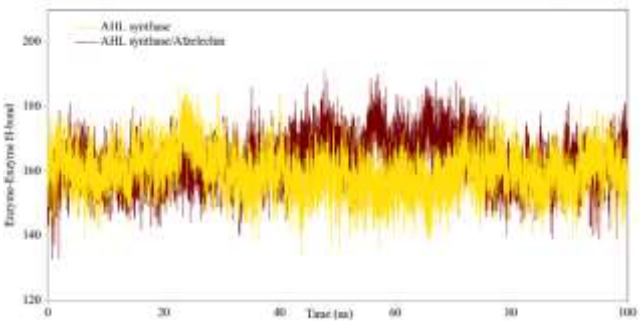

Fig. 7. Enzyme - Enzyme hydrogen-bond plots of free and bound enzyme as a function of time.

Table 3. The average and standard deviations of intra molecular enzyme and enzyme-solvent hydrogen bonds during last 30 ns

System	Enzyme-Enzyme	Enzyme-Solvent
Free 1KZF	159.949 ±6.682	436.110±13.525
1KZF/Afzelechin	163.554±6.997	420.258±13.899

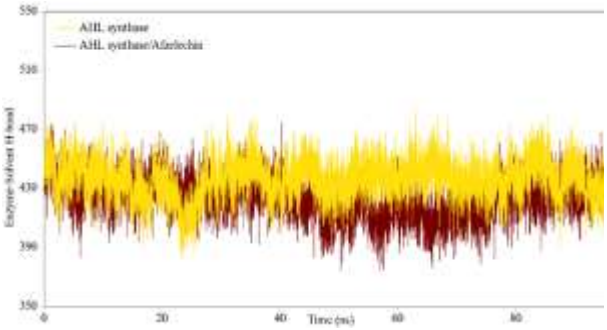

Fig. 8. Enzyme-Solvent hydrogen bond plots of free and bound enzyme as a function of time.

4. Conclusions

Molecular docking and dynamic simulation studies were conducted to explore the inhibitory effects of afzelechin on AHL synthase. The binding energy, or free energy, of afzelechin to bind AHL synthase was -7.22 kcal/mol through hydrogen bonding. This result shows that afzelechin has a strong binding affinity to ALH synthase. The molecular dynamics simulation confirms the above result by providing more details about molecular interactions. The RMSD analysis demonstrated minor structural instability of AHL synthase in the presence of afzelechin. The RG analysis shows that AHLs synthesize structure, compactness, and condensation, while Afzelechin makes interactions with it. On the other hand, the RMSF analysis showed an increasing residue fluctuation when afzelechin was bound to AHL synthase. The SASA also shows a reduction in the surface area of molecules in water due to afzelechin binding. H-bond analysis reveals the enhancement in enzyme-enzyme hydrogen in the presence of afzelechin and the decrease of hydrogen bonds between enzyme-solvent while afzelechin is present. Concerning the high binding affinity and molecular interaction of afzelechin with AHL synthase, it is revealed that afzelechin could be a unique structure for inhibiting AHL synthase and a good reference for designing this molecule along with antibiotics against biofilm formation and antibacterial resistance and employing this through in vivo and in vitro experimental studies.

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