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Research Article

Rigid and Flexible Docking Study with ADME Evaluation of Hesperetin Analogs as LecB Inhibitors in *Pseudomonas aeruginosa*

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Abstract

Infections by *Pseudomonas aeruginosa* pose a considerable challenge in treatment due to its major virulence factor, LecB. LecB plays a significant role in bacterial adherence, infections, biofilm formation, and suppression of the host immune response. This study aimed to assess the affinity and interactions of five hesperetin analogs against LecB in *P. aeruginosa*. The investigation utilized the molecular docking method, employing a combination of rigid and flexible docking. The docking results successfully identified hesperetin 7-O-glucoside and hesperetin 7-O-rhamnoside demonstrating superior binding energies compared to MJO as the reference ligand. Rigid docking indicated binding energies of hesperetin 7-O-glucoside and hesperetin 7-O-rhamnoside were -7.0 and -7.2 kcal/mol, respectively. Flexible docking revealed lower binding energies for both compounds, reaching -10.3 kcal/mol. These top-performing compounds exhibited interactions with critical residues strategically positioned in LecB's substrate-binding site in *P. aeruginosa*. Notably, hesperetin 7-O-rhamnoside displayed an excellent ADME profile, a favorable safety profile, and met Lipinski's rules. This research unveils the potential antibacterial activity of hesperetin analogs and provides an opportunity for experimental verification of these compounds as antibacterial drugs against *P. aeruginosa*.

Keywords: Flexible docking, hesperetin, LecB, lectin, *Pseudomonas aeruginosa*

Studi Penambatan Molekul Kaku dan Fleksibel serta Evaluasi ADME Analog Hesperetin sebagai Penghambat LecB *Pseudomonas aeruginosa*

Abstrak

Infeksi oleh *Pseudomonas aeruginosa* merupakan tantangan besar dalam pengobatan karena faktor virulensi utamanya, LecB. LecB berperan penting dalam adherensi bakteri, infeksi, pembentukan biofilm, dan penekanan respons kekebalan tubuh inang. Penelitian ini bertujuan untuk menilai afinitas dan interaksi dari lima analog hesperetin terhadap LecB pada *P. aeruginosa.* Penelitian ini menggunakan metode penambatan molekul dengan kombinasi *rigid* dan fleksibel *docking.* Hasil penambatan molekul berhasil mengidentifikasi hesperetin 7-O-glucosida dan hesperetin 7-O-ramnosida menunjukkan energi pengikatan lebih baik dibandingkan dengan MJO sebagai ligan referensi. *Rigid docking* menunjukkan energi pengikatan hesperetin 7-O-glucosida dan hesperetin 7-O-ramnosida berturut-turut sebesar -7,0 dan -7,2 kkal/mol. Hasil fleksible *docking* mengungkap energi pengikatan yang jauh lebih rendah sebesar -10,3 kkal/mol untuk kedua senyawa tersebut. Kedua senyawa terbaik ini berinteraksi dengan residu krusial yang terletak strategis di situs pengikatan substrat LecB pada *P. aeruginosa*. Secara signifikan, hesperetin 7-O-ramnosida menunjukkan profil ADME yang sangat baik, memenuhi profil keamanan, serta memenuhi aturan Lipinski. Penelitian ini mengungkapkan potensi aktivitas antibakteri dari analog hesperetin serta memberikan peluang untuk verifikasi eksperimental dari senyawa ini sebagai obat antibakteri terhadap *P. aeruginosa*.

Kata Kunci: Fleksibel docking, hesperetin, LecB, lektin, *Pseudomonas aeruginosa*

1. Introduction

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium belong to the family Pseudomonaceae.[1] This bacterium can survive in natural and artificial environments using its influential binding factors, like flagella, pili and biofilm which are vary widely among individuals.[2,3] This bacterium can grow in a wide range of temperatures, widespread in nature and also common among humans.[4] Although it is naturally common among humans, this bacterium is known as an opportunistic human pathogen.[2,4]

P. aeruginosa has been reported causing a variety of infections in humans. It has become one of the leading cause of nosocomial infections.[5,6] This opportunistic pathogen is related to the hospital-acquired infections, such as intensive care unit infections, bloodstream infections, ventilator-associated pneumonia, urinary infections, burn wound infections and surgical or transplantation infections.[2,4,5] In many cases, it causes diseases with chronic infection and high mortality rate especially in immunocompromised patients, such as genetic disorder cystic fibrosis patients, cancer patients and chronic obstructive pulmonary patients.[7,8,9]

P. aeruginosa infections become one of difficult infections to treat. This bacterium produces virulence factors in the form of molecules which play important roles in bacterial pathogenesis, such as bacterial attachment to host tissues, biofilm formation and reduction of host defenses.[10] The important virulence factor which is produced by this bacterium is lectin, especially lecB. LecB (also called PA-IIL) is soluble protein which has high affinity to fucose.[11,12,13] LecB is noncovalently linked to carbohydrate ligands of the bacterial cell surface through the porin OprF. LecB contributes in bacterial adherence and infections, biofilm formation and suppresses host immune respons.[12,13,14,15] The formation of biofilm gives this bacterium an extra ability to survive in many environmental conditions that do not support its growth. Once a compact biofilm has been formed, it will provide a great opportunity for this bacterium to continue its infections such as suppresses host immune respons.[16,17] Sponsel et al., (2023) found that lecB can mitigate an immune response by inhibiting immune cell migration across endothelial barriers.[13] In addition, *P. aeruginosa* with lower lecB has been reported less pathogenic and shows less biofilm formation.[11,12,14] Therefore, lecB has been identified as a potential drug target for *P. aeruginosa* infections.

The emergence of drug-resistant strains of *P. aeruginosa* poses a significant challenge in clinical settings, particularly in the treatment of nosocomial infections. The identification of lecB as a potential drug target provides a promising avenue for the development of novel antibacterial agents. By targeting lecB, it may be possible to disrupt crucial virulence mechanisms of P. aeruginosa, ultimately enhancing the efficacy of antimicrobial therapies.[18,19]

Hesperetin has been reported to possess antibacterial effects. For instance, it has an effect against the important human pathogen *Helicobacter pylori*, inhibiting the growth of both reference strains and clinical isolates of the bacterium.[20] A study compared the antibacterial activity of hesperetin, hesperidin, and hesperidin glucoside. Hesperetin displayed the strongest effect against both Gram-positive (*Staphylococcus aureus, Bacillus cereus*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria compared to the other two compounds. The minimum inhibitory concentration (MIC) values for hesperetin were generally lower, indicating its higher potency.[21] Based on these facts, hesperetin has the potential to study the development of antibacterial especially against *P. aeruginosa* further. Thus, computational approaches are needed to evaluate the molecular mechanism, interaction and to estimate the affinity of some hesperetin derivatives against lecB from *P. aeruginosa*.

The proposed computational study combines the rigid and flexible docking approaches. Rigid docking facilitates the initial exploration of ligand binding modes within the protein's binding site residue,

which is treated rigidly, while flexible docking considers protein flexibility and ligand-induced conformational changes, thereby enhancing the accuracy of binding affinity predictions.[22] This integrated computational strategy not only provides insights into key structural features governing ligand-protein interactions but also facilitates the rational design of novel antibacterial agents targeting *P. aeruginosa* infections.

This research represents an advancement in the field of bacteriology and drug discovery, offering a rational approach for the development of novel therapeutics against *P. aeruginosa* infections. By leveraging computational techniques, we aim to accelerate the identification of the potential of hesperetin derivatives as antibacterial agents, ultimately contributing to combating multidrug-resistant bacterial pathogens and improving patient outcomes.

2. Method

2.1. Tools

Asus Laptop with Windows 10 Home specifications 64-bit, Intel(R) Core (TM) i5-8250U CPU @ 1.60GHz 1.80 GHz, 8.00 GB RAM, NVIDIA GeForce 940MX graphics card, and 1TB HDD.

2.2. Materials

The software used in this research includes MGL-Tools®, AutoDock Tools v1.5.6, AutoDock Vina v.1.1.2, Discovery Studio Visualizer®, Notepad++®, Protein Data Bank (https://www.rcsb.org/), PubChem database (https://pubchem.ncbi.nlm.nih.gov/), SwissADME (https://www.swissadme.ch/), and KNApSAcK database (https://knapsack3d.sakura.ne.jp/).

2.3. Procedure

2.3.1. Target and Ligands Preparation

The target protein employed in the research is lectin B (LecB) (PDB ID 8AIY),[23] obtained from the protein data bank website. The target protein was subsequently prepared using Discovery Studio Visualizer by removing bound ligands and water molecules from the protein structure. Finally, hydrogen atoms were added to the polar groups of the target protein, along with Kollman charges with assistance of AutoDock Tools v1.5.6.[24]

The ligands utilized in this study are five hesperetin analog compounds obtained from the KNApSAcK database. These ligands were subsequently prepared by adjusting them to their maximum torsional conformation, adding hydrogen atoms, and applying Gasteiger charges using AutoDock Tools v1.5.6.[25]

2.3.2. Molecular Docking Simulation

The molecular docking process was conducted using AutoDock Vina v.1.1.2,[26] employing a cubic grid with dimensions of 25 x 25 x 25 Å with coordinate aligned to the center position of MJO (reference ligand) which complexed in LecB.[23] The molecular docking method was validated by redocking the reference ligand onto LecB. The docking method is considered reliable if the re-docked conformation of the reference ligand exhibits a Root Mean Square Deviation (RMSD) value of ≤ 2 Å when compared to its crystallographic conformation.[27]

The docking process utilized the rigid docking method, followed by flexible docking. Amino acid residues such as Asn21, Ser23, Thr25, Thr45, Val69, Asn70, Glu95, Asp96, Thr98, Asp99, Asn100, Asp101, Tyr102, Asn103, and Asp104 at the substrate-binding site of LecB were made flexible during the simulation. The evaluation of molecular docking was based on selecting ligand conformations with the most favorable binding free energy (ΔG). The optimal conformation of ligand-protein complexes was visualized using the Discovery Studio Visualizer software v17.2.0.16349.

2.3.3. Pharmacokinetics and Drug-likeness Prediction

We conducted an evaluation of the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of hesperetin analogs, considering their potential use in pharmaceuticals. In our research, we employed the SwissADME webserver to predict key parameters, including lipophilicity,

aqueous solubility, pharmacokinetic properties, and drug-likeness criteria.[28]

3. Result

3.1. Rigid and Flexible Docking

A docking simulation was conducted to assess the potential of hesperetin analog as an antibacterial agent by regulating LecB from *P. aeruginosa*. The docking process used in this study was meticulously executed, yielding a RMSD value of 0.615 Å by the redocking procedure of the MJO inhibitor (N-(beta-L-fucopyranosyl)-biphenyl-3-carboxamide) on LecB (Figure S1). This precision in docking ensured a reliable interaction between the hesperetin analogs against LecB.

Upon a comprehensive analysis of the docking results, a notable correlation emerged between the compounds' binding energies and their potential as antibacterial agents.[29] Here, we docked the hesperetin analog into the LecB catalytic region following the location of the MJO inhibitor, which has crystallized with LecB from *P. aeruginosa*. In total, this study used five compounds from hesperetin analog (Figure 1).

We applied two stages of docking, starting with rigid docking and finally with the flexible docking procedure to increase accuracy in assessing compound affinity and conformation by using various precision levels from the docking procedure's scoring function. In this simulation, the rigid docking approach prevented the receptors from moving throughout the simulation. Furthermore, the flexible docking approach was used to analyze the binding energy of the ligand based on applying flexibility to the receptor's residue to clearly define the interaction between the ligand and receptor.[30] Compounds that consistently displayed the lowest binding energy from both procedures were considered potential candidates for binding to LecB.

Based on the results of rigid docking, hesperetin 7-O-rhamnoside showed a binding energy of -7.2 kcal/mol, equivalent to MJO (native ligand). Additionally, other hesperetin analogs exhibited more positive binding energies ranging from -6.5 to -7.0 kcal/mol (Table 1). Meanwhile, the binding energy obtained from flexible docking results showed an increase in binding energy, simultaneously revealing the better potential

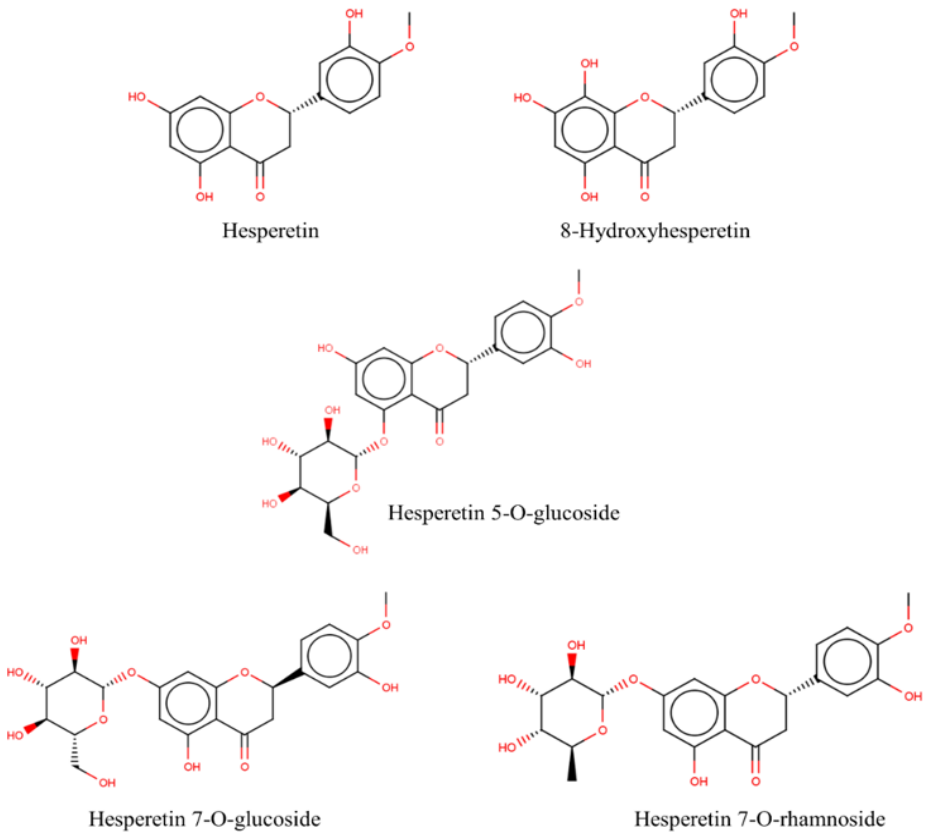

Figure 1. Structures of hesperetin analog.

Table 1. Summary of binding energies of the hesperetin analogs against LecB from *P. aeruginosa.*

Compounds	Binding Energy Rigid Docking (Kcal/mol)	Binding Energy Flexible Docking (Kcal/mol)
MJO (Native ligand)	-7.2	-8.5
Hesperetin	-6.5	-8.5
8-Hydroxyhesperetin	-7.0	-9.4
Hesperetin 5-O-glucoside	-7.0	-9.6
Hesperetin 7-O-glucoside	-7.0	-10.3
Hesperetin 7-O-rhamnoside	-7.2	-10.3

of hesperetin analogs compared to MJO. Specifically, hesperetin 7-O-glucoside and hesperetin 7-O-rhamnoside had the lowest binding energy of -10.3 kcal/mol (Table 1). This indicates a higher affinity of compounds from the hesperetin analog towards binding to LecB, signifying their potential as antibacterial agents against *P. aeruginosa*.

The interaction analysis from rigid docking results of MJO formed hydrogen bond (H-bond) interactions with specific residues on the LecB catalytic site, including Asn21, Ser22, Ser23, Glu95, Asp96, Asp99, and Asp104 (Figure 1). Simultaneously, its benzene rings established hydrophobic interactions with Ser23 and Val69. Interaction changes occurred in these compounds when using the flexible docking procedure. The hydroxyl and carbonyl group of this compound contributed to the H-bond formation with Asn21, Ser23, Asp99, Asp101, and Asp104 residues. In addition, new hydrophobic interactions with Asp96 and Thr98 were observed (Table 2).

In this study, we delved into the molecular interactions of two promising compounds through both rigid and flexible docking procedures from the hesperetin analogs, namely hesperetin 7-O-glucoside and hesperetin 7-O-rhamnoside. Hesperetin 7-O-glucoside showed four H-bonds at residues Asn21, Ser23, Asp96, and Thr98 when applying rigid docking (Figure 2A). The simulation continued with flexible docking, and the result showed that the carbonyl and the hydroxy group from this compound maintained their H-bond with Thr98 and formed three new H-bonds with residues

Table 2. Metformin and Glimepiride data accuracy test

Compounds	Rigid Docking		Flexible Docking	
	Hydrogen Bonds	Hydrophobic Interactions	Hydrogen Bonds	Hydrophobic Interactions
MJO (Native)	Asn21, Ser22, Ser23, Glu95, Asp96, Asp99, Asp104	Ser23, Val69	Asn21, Ser23, Asp99, Asp101, Asp104	Asp96, Thr98
Hesperetin	Thr45, Glu95, Asp99, Asn103	Asp101	Asp96, Asp104	-
8-Hydroxyhesperetin	Gly97, Asp96, Asp101, Asp104	-	Ser23, Glu95, Asp96, Asp99, Asp104	-
Hesperetin 5-O-glucoside	Gly24, Arg72, Asp99, Asp104	-	Gly24, Asp99, Asp104	-
Hesperetin 7-O-glucoside	Asn21, Ser23, Asp96, Thr98	Thr45	Asn70, Arg72, Thr98, Asp99	Asp96
Hesperetin 7-O-rhamnoside	Asn70, Thr98, Asp101, Asn103	Val69	Asn21, Ser23, Thr98	Asp96

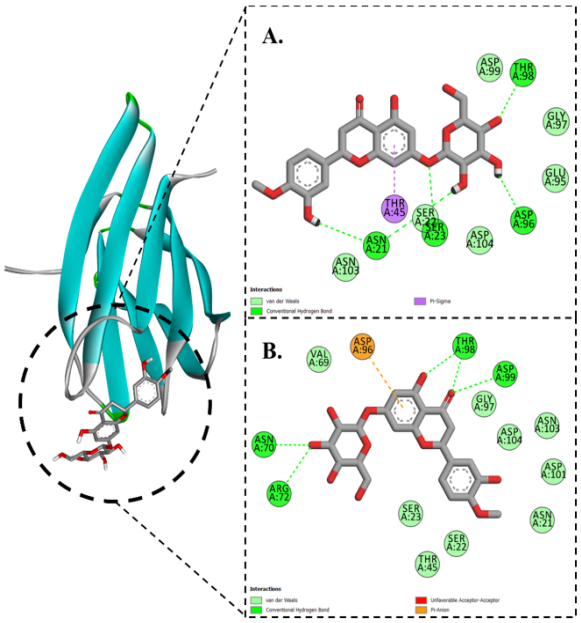

Figure 2. Molecular interaction of hesperetin 7-O-glucoside with LecB. (A) interaction from rigid docking and (B) interaction from flexible docking process.

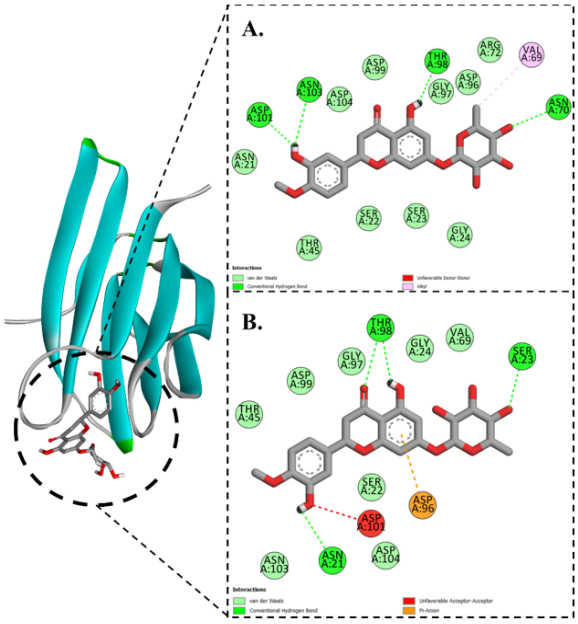

Figure 3. Molecular interaction of hesperetin 7-O-rhamnoside with LecB. (A) interaction from rigid docking and (B) interaction from flexible docking process.

Asn70, Arg72, and Asp99 (Figure 2B). Our investigation revealed intriguing H-bond patterns that shed light on the potential therapeutic implications of these compounds. Interestingly, the hydrophobic interaction of the benzene ring of this compound changed from Thr45 to Asp96.

Hesperetin 7-O-rhamnoside showed four H-bond during the rigid docking procedure. The hydroxy group of this compound formed four H-bonds with Asn70, Thr98, Asp101, and Asn103 residues (Figure 3A). Meanwhile, its methyl group included hydrophobic interactions with the Val69 residue. Uniquely in the flexible docking procedure, the carbonyl and the hydroxy group of these compounds exchanged H-bond interactions with residues Asn21 and Ser23. Interestingly, the hydrophobic interaction of the benzene ring of this compound changed from Val69 to Asp96, as observed in hesperetin 7-O-glucoside (Figure 3B).

Overall, other analog compounds such as hesperetin, in rigid docking simulations, exhibited hydrogen bonds with Thr45, Glu95, Asp99, and Asn103. However, the flexible docking approach uncovered additional interactions with Asp96 and Asp104, emphasizing the dynamic nature of these binding events. Additionally, 8-hydroxyhesperetin displayed distinct H-bond interactions with Gly97 and Ser23 in both rigid and flexible docking. Further analysis of hesperetin 5-O-glucoside revealed its unique binding patterns with Gly24, Arg72, Asp99, and Asp104 in rigid docking, with subtle differences observed in flexible docking (Table S1).

3.2. Pharmacokinetics Evaluation

We have examined the ADME properties results from the hesperetin analogs (Table S2). Interestingly, all the identified compounds exhibited notably good solubility within values ranging from -3.62 to -2.81. Solubility plays a pivotal role in drug development, determining how effectively a compound can dissolve in bodily fluids and subsequently be absorbed by the body. This attribute denotes the potential of these compounds in the drug development process, as it can enhance their effectiveness and ability to navigate various stages, from formulation to successful absorption within the body.31,32 Unfortunately, only hesperetin and 8-hydroxyhesperetin demonstrated high gastrointestinal (GI) absorption, suggesting that these compounds may be readily absorbed

through the gastrointestinal tract. This finding could affect their bioavailability and bodily distribution.[33]

Four hesperetin analog compounds exhibit distinct interactions with the enzymes CYP1A2, CYP2C9, and CYP3A4, which are part of the critical cytochrome P450 (CYP) family. These enzymes play a crucial role in metabolism and eliminating drugs from the body. Inhibition of these enzymes can lead to significant alterations in drug metabolism, potentially resulting in elevated drug concentrations within the body.34,35 Surprisingly, hesperetin 7-O-rhamnoside shows very interesting results, where this compound is estimated not to inhibit the activity of the body's metabolism enzymes.

3.3. Drug-likeness Properties

In particular, the Lipinski's rules prediction from these analogs is presented in Table S3. Interestingly, hesperetin, 8-hydroxyhesperetin, and hesperetin 7-O-rhamnoside show properties that meet Lipinski's criteria. Lipinski's criteria provide an essential framework for assessing the prospect of molecules becoming orally active drugs, specifically concerning solubility and absorption.[36] Furthermore, the Lipinski properties results establish the best compounds as prospective candidates for oral drug development, exhibiting improved bioavailability and thereby enhancing their potential for efficacy and safety when administered orally.

4. Discussion

Molecular docking has emerged as a valuable strategy for identifying potential lead compounds, providing a rapid and cost-effective means of screening a compound library.[37] In our investigation, we utilized molecular docking simulations, combining rigid and flexible docking studies to explore the potential of hesperetin analogs as antibiotic drug candidates against LecB from *P. aeruginosa*. Hesperidin, a citrus flavonoid belonging to the flavanone group, possesses a wide variety of biological activities and is abundantly found in citrus fruits such as lemons, sweet oranges, bitter oranges, citrons, clementines, and mandarins.[38]

LecB from *P. aeruginosa* has been previously shown to be implicated in biofilm formation, although its exact mechanism of action remains poorly understood.[15] In the present study, we investigated whether specific LecB ligands might efficiently interfere with biofilm formation and suppress host immune responses based on docking results.[13] Our docking results revealed that hesperetin 7-O-glucoside exhibited interactions with specific residues in LecB, including Asn70, Arg72, Thr98, Asp99, and Asp96 . Similarly, hesperetin 7-O-rhamnoside demonstrated interactions with Asn21, Ser23, Thr98, and Asp96 from LecB. In this complex, the glucoside and rhamnoside groups are anchored to the binding site and coordinated by acidic side chains from the local chain LecB (Glu95, Asp96, Asp99, Asp101, and Asp104).[39] This binding mode is identical to that reported for free fucose in LecB.[40]

In-depth analysis of the flexible docking results indicates that these compounds interact with residues such as Asn70, Arg72, Thr98, Asp99, Asp96, Asn21, and Ser23, strategically positioned in the substrate-binding site of LecB. This observation aligns with the idea that effective inhibition of bacterial virulence factors often involves disrupting key protein-protein interactions.[41] Our simulations clearly suggest that these compounds indeed prevent biofilm formation and, furthermore, disrupt existing biofilms. These findings highlight a potential binding affinity between these compounds and LecB, emphasizing their candidacy for further exploration as antibiotic agents.

These molecular interactions provide valuable insights into the nuanced behavior of these compounds, laying the foundation for future drug design and development efforts. The study underscores the importance of considering rigid and flexible docking strategies to delve into the intricacies of ligand-receptor binding, crucial for advancing our understanding of potential therapeutic applications. By targeting LecB at the molecular level and effectively preventing

biofilm formation, these compounds have the potential to play a crucial role in the management and treatment of infectious diseases, especially in the context of various mutations associated with antibiotic resistance in *P. aeruginosa*.

5. Conclusion

In conclusion, our docking studies highlight the promising potential of Hesperetin 7-O-glucoside and hesperetin 7-O-rhamnoside as lead candidates for antibiotics against LecB from *P. aeruginosa*. Hesperetin 7-O-rhamnoside exhibited a favorable ADME profile and conformed to safety standards. This analog of hesperetin also satisfies the Lipinski rule criteria, affirming its appropriateness for oral drug administration. Moving forward, a comprehensive investigation involving both computational and experimental approaches is warranted to assess these compounds' effectiveness and suitability for further drug development endeavors.

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