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In Silico Analysis Of The Efficacy Of B-Lactamase Inhibitors Against Staphylococcus Aureus 1BLH Protein

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Abstract:
Background*: Bacterial resistance, particularly in strains of Staphylococcus aureus, poses a significant challenge in both veterinary and human medicine. The production of β-lactamases by these bacteria is one of the primary reasons for the ineffectiveness of β-lactam antibiotics. The use of β-lactamase inhibitors emerges as a promising strategy to restore the efficacy of these antibiotics.*
Materials and Methods*: This study conducted an in silico analysis of various β-lactamase inhibitors against the 1BLH protein of S. aureus. The crystal structure of the protein was obtained from the RCSB Protein Data Bank and used for docking simulations, where five established inhibitors (Clavulanic Acid, Sulbactam, Tazobactam, Avibactam, and Relebactam) were tested. Docking experiments were conducted using the Dockthor server. The interactions between the ligands and the protein were analyzed using tools such as Visual Molecular Dynamics (VMD) and PyMOL.*
Results*: The findings indicated that all ligands formed multiple hydrogen bonds with the 1BLH protein, suggesting strong interactions. Relebactam stood out by exhibiting a π-π interaction in addition to hydrogen bonds, indicating potentially superior affinity. The analysis revealed that the Ser202 residue plays a critical role in the interactions between the inhibitors and the β-lactamase.*
Conclusion: *The in silico analysis demonstrated the effectiveness of β-lactamase inhibitors against the 1BLH protein of S. aureus, with Relebactam showing characteristics that may enhance its efficacy as an inhibitor. These findings provide a solid foundation for future research and may expedite the development of new therapies against resistant bacterial infection.*
Key Word*: β-lactamase; inhibitors; DockThor; S. aureus.*

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I. Introduction

Various bacteria, including *Staphylococcus aureus*, produce enzymes known as β-lactamases, which confer resistance to beta-lactam antibiotics[1].These enzymes function by breaking the β-lactam ring present in these antibiotics, rendering them ineffective against bacterial infections[2].The emergence of β-lactamase-producing strains poses a significant public health challenge, highlighting the need for effective inhibitors to counteract their activity[3].

β-lactamase inhibitors are compounds designed to neutralize these enzymes, restoring the effectiveness of β-lactam antibiotics[4].These inhibitors fall into two main categories: irreversible inhibitors, which form a permanent bond with the enzyme, and reversible inhibitors, which bind temporarily[5,6]. The strategic combination of these inhibitors with antibiotics has proven to be a promising approach in overcoming bacterial resistance[7].

The rapid evolution of bacterial resistance mechanisms makes the development of new antimicrobial agents increasingly challenging[8].Combining traditional antibiotics with β-lactamase inhibitors offers a potential solution, but a detailed molecular-level understanding of these interactions is essential for optimizing their use[9,10]. This complexity underscores the importance of innovative approaches in drug design and evaluation[11].

Computational tools, such as molecular modeling and docking techniques, have become invaluable in drug discovery[12]. These methods allow researchers to simulate and visualize interactions between compounds and their biological targets, facilitating the identification of promising inhibitors and providing insights into their binding mechanisms[13]. By leveraging these in silico techniques, the drug development process can be both accelerated and refined[14,15].

This study aims to conduct an in silico analysis of various β-lactamase inhibitors against the 1BLH protein of *S. aureus*. We hypothesize that specific inhibitors will exhibit strong binding affinity and effectively inhibit the enzymatic activity of 1BLH. The findings from this research could contribute to the development of more effective therapeutic strategies against resistant bacterial strains.

II. Material And Methods

Acquisition of Protein 1BLH

The protein 1BLH, a β-lactamase from *S. aureus*, was obtained from the RCSB Protein Data Bank. Its crystal structure was determined through X-ray diffraction, with a resolution of 2.30 Å. The protein was analyzed in complex with the phosphonate ligand (FOS) and downloaded in PDB file format[16].

Physicochemical Properties of 1BLH

The physicochemical properties of β-lactamase were predicted using the Pepstats database (https://www.ebi.ac.uk/jdispatcher/seqstats/emboss_pepstats) and the ProtParam tool of ExPASy (https://web.expasy.org/protparam/).

Preparation of Proteins and Ligands

The crystal structure of the β-lactamase enzyme was obtained from the Protein Data Bank (PDB), specifically entry 1BLH, chosen for its accurate representation of the enzyme's inhibition site with a specific ligand, [n-(Benzylcarbamoyl)aminomethyl]phosphate (FOS). To assess the inhibitory potential, five established β-lactamase inhibitors - Clavulanic Acid, Sulbactam, Tazobactam, Avibactam, and Relebactam - were retrieved from PubChem in SDF format and converted to MOL2 format[17].

Docking Simulations

Docking experiments were conducted using the Dockthor server (https://docthor.lncc.br/v2/), a specialized platform for simulating protein-ligand interactions. The 1BLH protein was loaded, and its inhibition site was defined based on previous crystallographic findings, yielding coordinates X: 3.77, Y: -9.31, and Z: -9.74. The ligand preparation involved optimizing their geometric states to ensure accurate docking simulations. Each ligand was docked to the enzyme's inhibition site, and binding scores were meticulously recorded to evaluate their potential as inhibitors[18].

Visualization and Analysis of Docking Results

The resulting docking poses were analyzed using Visual Molecular Dynamics (VMD) and PyMOL, allowing for a detailed examination of how the ligands interacted with the enzyme. VMD provided dynamic visual representations of binding interactions, while PyMOL offered high-resolution static images, enabling a closer inspection of hydrogen bonds, hydrophobic interactions, and the overall positioning of the ligands within the active site.

2D Interaction Analysis

To complement the structural analysis, the docking results were further examined using PoseView (//proteins.plus/help/poseview). This tool generated two-dimensional interaction diagrams, illustrating critical interactions such as hydrogen bonds and π-stacking. A comparative study of these diagrams highlighted the key molecular interactions responsible for the inhibitory activity of eugenol and carvacrol in relation to well-characterized inhibitors.

III. Result

The physicochemical properties of the protein are summarized in Table 1. The aliphatic index, which measures the volume occupied by aliphatic side chains (such as alanine, isoleucine, leucine, and valine), is 87.28. This value suggests good thermal stability; the higher the index, the greater the stability at elevated temperatures. The instability index, which considers values below 40 as indicative of stability, yielded a result

of 30.55, confirming the protein's stability. The Grand Average of Hydropathy (GRAVY) is calculated by summing the hydropathy values of all residues and dividing by the total number of amino acids. For the protein 1BLH, the GRAVY is -0.615, indicating that the protein is hydrophilic.

Table no 1: Physicochemical Properties of 1BLH.

Properties	1BLH
Sequence Length	257
Molecular Weight	28794.15
Isoelectric Point	10.1155
Aliphatic Index	87.28
Molecular Formula	$C_{1293}H_{2103}N_{341}O_{392}S_3$
Total Number of Atoms	4132
GRAVY	-0,615
Instability Index	30,55

Table no 2 presents the results of the docking performed on the Dockthor platform. Regarding van der Waals energy (vdW Energy), the co-crystallized ligand with 1BLH exhibited the highest energy at 10.455, while relebactam showed the lowest at -9.455. In terms of electrostatic energy (Elect. Energy), the co-crystallized ligand with the protein had the lowest energy at -67.467, whereas sulbactam had the highest at -21.145. Finally, for total energy (Total Energy), the avibactam ligand displayed the most negative value at -30.469 compared to the other ligands.

Table no 2. Molecular docking results of inhibitors against β-lactamases 1BLH using DockThor server.

Compound	Affinity	Total Energy	vdW Energy	Elect. Energy
FOS	-6.601	-18.664	10.455	-67.467
Ácido Clavulânico	-6.218	-23.680	-4.107	-25.083
Sulbactam	-6.595	-23.678	-7.434	-21.145
Tazobactam	-6.753	-25.285	-5.539	-30.231
Avibactam	-6.314	-30.469	-6.041	-33.036

Table no 3 shows the amino acid residues involved in the interaction between beta-lactamase inhibitors and the 1BLH protein. The most frequent interactions are hydrogen bonds (H-bond), and one such interaction noted among all ligands was with the Ser202 residue. π-π and hydrophobic (HI) interactions were the least frequent.

Table no 3. Relationship of amino acid residues and types of intermolecular interactions through DockThor and PoseView analysis.

Compound	Ser39	Tyr72	Ser83	Ser97	Ser183	Ser202	Gln204	Arg211
FOS	H-bond		H-bond		HI	H-bond		
Clavulanic Acid		HI		H-bond		H-bond	H-bond	H-bond
Sulbactam	H-bond			H-bond		H-bond	H-bond	
Tazobactam	H-bond					H-bond	H-bond	H-bond
Avibactam	H-bond			H-bond		H-bond		H-bond
Relebactam		π-π		H-bond		H-bond		H-bond

The set of figures in Figure 1 illustrates in 2D the types of interactions between the ligands and the amino acid residues, generated by the Poseview server. All ligands, including FOS (Figure 1A), Clavulanic Acid (Figure 1B), Sulbactam (Figure 1C), Tazobactam (Figure 1D), and Avibactam (Figure 1E), formed multiple hydrogen bonds (H-bond), which were a common interaction among them. In addition to the hydrogen bonds, Relebactam (Figure 1F) stood out by exhibiting a less common interaction, the π-π interaction, while also forming several hydrogen bonds. The interaction with the Ser202 residue was common to all ligands. While hydrogen bonds were a shared characteristic among all ligands, the π-π interaction of Relebactam represents a particularity that may influence its effectiveness in interactions with the protein.

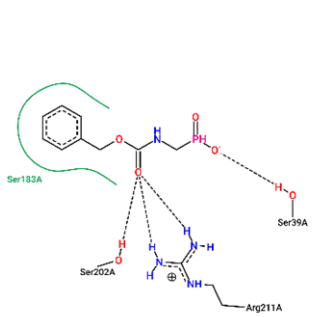

Figure 1A

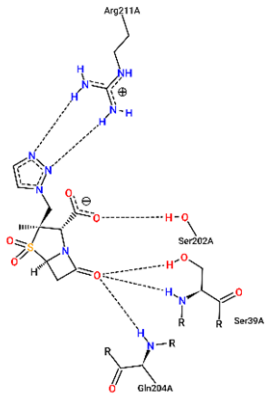

Figure 1B

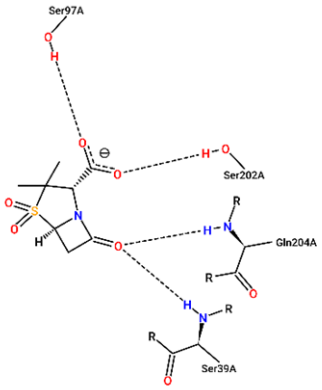

Figure 1C

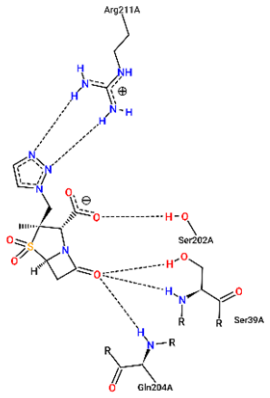

Figure 1D

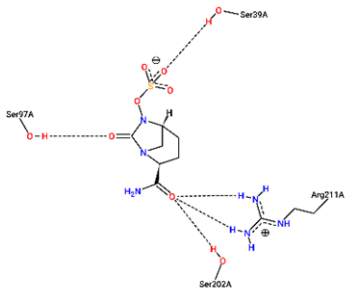

Figure 1E

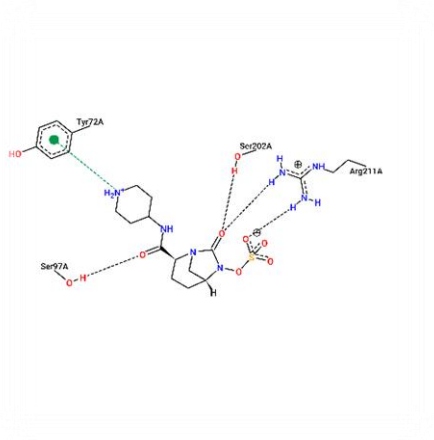

Figure 1F

IV. Discussion

Bacterial resistance, particularly in strains such as *S. aureus*, poses a significant challenge in both veterinary and human medicine. The production of β-lactamases by these bacteria is one of the primary reasons for the ineffectiveness of β-lactam antibiotics[19]. In this context, the use of β-lactamase inhibitors emerges as a promising strategy to restore the efficacy of these antibiotics[20]. The in silico analysis conducted in this study provides valuable insights into the interactions between various inhibitors and the 1BLH protein, a representative model of β-lactamase.

The stability and hydrophobicity of bacterial proteins are essential for antimicrobial resistance. Stable proteins can maintain their functions in adverse conditions, while hydrophobicity may influence interactions with drugs, affecting membrane permeability and the effectiveness of antimicrobial agents[21,22].

The PoseView server was utilized to create visual representations of the molecular interactions within the protein-ligand complexes. It generated two-dimensional diagrams that highlight specific contacts, such as hydrogen bonds, hydrophobic interactions, and π-π interactions, while also illustrating the spatial arrangement of the involved residues and atoms. These results are essential for structural analysis and the interpretation of interaction mechanisms[23,24].

The docking simulations revealed that all tested ligands, including FOS, Clavulanic Acid, Sulbactam, Tazobactam, and Avibactam, formed multiple hydrogen bonds with the protein. These bonds are crucial as they indicate a strong interaction between the inhibitors and the active site of the β-lactamase, suggesting that these compounds have the potential to inhibit enzymatic activity. The presence of hydrogen bonds indicates that the ligands are favorably positioned within the active site, which is essential for the inhibitor's effectiveness.

Notably, Relebactam distinguished itself by exhibiting a π-π interaction in addition to hydrogen bonds. This less common interaction may provide Relebactam with an additional advantage in terms of affinity and specificity for the protein, potentially enhancing its effectiveness as an inhibitor. The π-π interaction is often associated with stronger and more stable interactions between ligands and biological targets, which can be a determining factor in selecting inhibitors for the development of new therapies[25,26].

Furthermore, the common interaction with the Ser202 residue across all ligands suggests that this residue plays a critical role in the binding of inhibitors to β-lactamase. Identifying key residues like Ser202 can guide future investigations into structural modifications of the inhibitors aimed at optimizing their efficacy[27,28].

V. Conclusion

This study demonstrated the effectiveness of in silico analysis in evaluating β-lactamase inhibitors against the 1BLH protein from *S. aureus*. The results indicated that all ligands formed multiple hydrogen bonds, signifying a strong interaction with the enzyme. Relebactam stood out by exhibiting a π-π interaction, suggesting potentially superior affinity. Furthermore, the identification of the Ser202 residue as a common interaction point among all ligands highlights its significance in the development of effective inhibitors. These findings provide a solid foundation for future research and may expedite the development of new therapies against resistant bacterial infections.

References

[1]. George CRR, Lahra MM, Nguyen T, Gatus B. Disc Test For Detecting Staphylococcus Aureus Strains Producing Type A And Type C B-Lactamases. Microbiol Spectr. 2023 Aug 17;11(4):E0022023.
[2]. Wang G, Wen J, Tian Z, Zhou H, Peng X, Zhang P, Li Z. Piceatannol And Its Analogues Alleviate Staphylococcus Aureus Pathogenesis By Targeting B-Lactamase Biofilms And A-Hemolysin. Sci Rep. 2025 Feb 14;15(1):5551.
[3]. Bush K, Bradford PA. Epidemiology Of B-Lactamase-Producing Pathogens. Clin Microbiol Rev. 2020 Feb 26;33(2):E00047-19.
[4]. Oliveira-Tintino CDM, Tintino SR, Justino De Araújo AC, Dos Santos Barbosa CR, Ramos Freitas P, Araújo Neto JB, Begnini IM, Rebelo RA, Silva LED, Mireski SL, Nasato MC, Krautler MIL, Barreto HM, Ribeiro-Filho J, De Menezes IRA, Coutinho HDM. Efflux Pump (Qaca, Qacb, And Qacc) And B-Lactamase Inhibitors? An Evaluation Of 1,8-Naphthyridines Against Staphylococcus Aureus Strains. Molecules. 2023 Feb 15;28(4):1819.
[5]. Christaki E, Marcou M, Tofarides A. Antimicrobial Resistance In Bacteria: Mechanisms, Evolution, And Persistence. J Mol Evol. 2020 Jan;88(1):26-40.
[6]. Le Terrier C, Nordmann P, Buchs C, Poirel L. Effect Of Modification Of Penicillin-Binding Protein 3 On Susceptibility To Ceftazidime-Avibactam, Imipenem-Relebactam, Meropenem-Vaborbactam, Aztreonam-Avibactam, Cefepime-Taniborbactam, And Cefiderocol Of Escherichia Coli Strains Producing Broad-Spectrum B-Lactamases. Antimicrob Agents Chemother. 2024 Apr 3;68(4):E0154823.
[7]. Blanco-Martín T, Alonso-García I, González-Pinto L, Outeda-García M, Guijarro-Sánchez P, López-Hernández I, Pérez-Vázquez M, Aracil B, López-Cerero L, Fraile-Ribot P, Oliver A, Vázquez-Ucha JC, Beceiro A, Bou G, Arca-Suárez J; GEMARA/SEIMC-CIBERINFEC Study Group On The Activity And Resistance Mechanisms To New B-Lactams And B-Lactamase Inhibitors (PROTECT). Activity Of Cefiderocol And Innovative B-Lactam/B-Lactamase Inhibitor Combinations Against Isogenic Strains Of Escherichia Coli Expressing Single And Double B-Lactamases Under High And Low Permeability Conditions. Int J Antimicrob Agents. 2024 May;63(5):107150.
[8]. Sawa T, Kooguchi K, Moriyama K. Molecular Diversity Of Extended-Spectrum B-Lactamases And Carbapenemases, And Antimicrobial Resistance. J Intensive Care. 2020 Jan 28;8:13.
[9]. Xuan J, Feng W, Wang J, Wang R, Zhang B, Bo L, Chen ZS, Yang H, Sun L. Antimicrobial Peptides For Combating Drug-Resistant Bacterial Infections. Drug Resist Updat. 2023 May;68:100954.
[10]. Agarwal V, Tiwari A, Varadwaj P. An Extensive Review On B-Lactamase Enzymes And Their Inhibitors. Curr Med Chem. 2023;30(7):783-808.
[11]. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J. B-Lactamases And B-Lactamase Inhibitors In The 21st Century. J Mol Biol. 2019 Aug 23;431(18):3472-3500.
[12]. Chio H, Guest EE, Hobman JL, Dottorini T, Hirst JD, Stekel DJ. Predicting Bioactivity Of Antibiotic Metabolites By Molecular Docking And Dynamics. J Mol Graph Model. 2023 Sep;123:108508.
[13]. Pestana-Nobles R, Aranguren-Díaz Y, Machado-Sierra E, Yosa J, Galan-Freyle NJ, Sepulveda-Montaño LX, Kuroda DG, Pacheco-Londoño LC. Docking And Molecular Dynamic Of Microalgae Compounds As Potential Inhibitors Of Beta-Lactamase. Int J Mol Sci. 2022 Jan 31;23(3):1630.
[14]. Horishny V, Geronikaki A, Kartsev V, Matiychuk V, Petrou A, Pogodin P, Poroikov V, Papadopoulou TA, Vizirianakis IS, Kostic M, Ivanov M, Sokovic M. Synthesis, Biological Evaluation And Molecular Docking Studies Of 5-Indolylmethylen-4-Oxo-2-Thioxothiazolidine Derivatives. Molecules. 2022 Feb 5;27(3):1068.

[15]. Ho KH, Su SC, Lee KR. Molecular Docking And Simulation Of The Interaction Of Sulbactam With Acinetobacter Baumannii Baesr And Adesr. Biochem Biophys Res Commun. 2021 Nov 26;580:81-86.
[16]. Chen CCH, Rahil J, Pratt RF, Herzberg O. Structure Of A Phosphonate-Inhibited B-Lactamase: An Analog Of The Tetrahedral Transition State/Intermediate Of B-Lactam Hydrolysis. J Mol Biol. 1993;234(1):165-178.
[17]. Schrödinger, LLC. The Pymol Molecular Graphics System, Version 1.2r3pre. 2010. Disponível Em: Http://Www.Pymol.Org/.
[18]. Guedes IA, Pereira Da Silva MM, Galheigo M, Krempser E, De Magalhães CS, Correa Barbosa HJ, Dardenne LE. Dockthor-VS: A Free Platform For Receptor-Ligand Virtual Screening. J Mol Biol. 2024;436(17):168548.
[19]. Abebe AA, Birhanu AG. Methicillin Resistant Staphylococcus Aureus: Molecular Mechanisms Underlying Drug Resistance Development And Novel Strategies To Combat. Infect Drug Resist. 2023 Dec 14;16:7641-7662.
[20]. Vrancianu CO, Gheorghe I, Dobre EG, Barbu IC, Cristian RE, Popa M, Lee SH, Limban C, Vlad IM, Chifiriuc MC. Emerging Strategies To Combat B-Lactamase Producing ESKAPE Pathogens. Int J Mol Sci. 2020 Nov 12;21(22):8527.
[21]. Lei J, Sun L, Huang S, Zhu C, Li P, He J, Mackey V, Coy DH, He Q. The Antimicrobial Peptides And Their Potential Clinical Applications. Am J Transl Res. 2019;11(7):3919-3931.
[22]. Luong HX, Thanh TT, Tran TH. Antimicrobial Peptides - Advances In Development Of Therapeutic Applications. Life Sci. 2020;260:118407.
[23]. Fricker P, Gastreich M, Rarey M. Automated Generation Of Structural Molecular Formulas Under Constraints. J Chem Inf Comput Sci. 2004;44:1065-1078.
[24]. Stierand K, Maaß P, Rarey M. Molecular Complexes At A Glance: Automated Generation Of Two-Dimensional Complex Diagrams. Bioinformatics. 2006;22(14):1710–1716.
[25]. Saral Sariyer A. Three New Inhibitors Of Class A B-Lactamases Evaluated By Molecular Docking And Dynamics Simulations Methods: Relebactam, Enmetazobactam, And QPX7728. J Mol Model. 2022 Mar 4;28(4):76.
[26]. Khademi Dehkordi M, Hoveida L, Fani N. Structure-Based Virtual Screening, Molecular Docking, And Molecular Dynamics Simulation Approaches For Identification Of New Potential Inhibitors Of Class A B-Lactamase Enzymes. J Biomol Struct Dyn. 2024 Jul;42(11):5631-5641.
[27]. Kumar KM, Anbarasu A, Ramaiah S. Molecular Docking And Molecular Dynamics Studies On B-Lactamases And Penicillin Binding Proteins. Mol Biosyst. 2014 Apr;10(4):891-900.
[28]. Khademi Dehkordi M, Hoveida L, Fani N. Structure-Based Virtual Screening, Molecular Docking, And Molecular Dynamics Simulation Approaches For Identification Of New Potential Inhibitors Of Class A B-Lactamase Enzymes. J Biomol Struct Dyn. 2024 Jul;42(11):5631-5641.