MOLECULAR DOCKING APPROACH TO INHIBIT CYSTEINE DESULFURASE SufS FROM *S. AUREUS*

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This study presents a molecular docking approach to identify potential inhibitors of cysteine desulfurase SufS, a key enzyme in the Fe–S cluster biogenesis pathway of Staphylococcus aureus. Bidentate and tridentate ligands were evaluated using molecular docking simulations for their binding affinity to the active binding site of the SufS protein isolated from Staphylococcus aureus, aiming to inhibit the further formation of the Fe-S cluster. As a result of docking studies, the compounds exhibiting the best possible inhibiting capacity were identified and ranked by the best docking score. These ligands can form stable hydrogen bonds with the amino acids' residues ASP131 and HIS127 within the catalytic binding site. The concluding findings highlight SufS as a selective antibacterial target and offer structural insights for the design of new anti-staphylococcal agents.

Keywords: docking simulations; cysteine desulfurase; Fe-S clusters

1. Introduction

The cysteine desulfurase SufS represents an essential protein of *Staphylococcus aureus* (*S. aureus*) involved in the biogenesis of iron-sulfur (Fe-S). *S. aureus* organisms express the SUF-like pathway [1] leading to Fe−S clusters biogenesis, important for various essential cellular functions [2]. This paper reports the results of a molecular docking approach based on the premise that this protein is a therapeutic target whose inhibition may facilitate the identification of potential specific antibiotics for the treatment of *S. aureus* infections.

Staphylococcus aureus is one of the most prevalent human pathogens, responsible for a wide range of infections, from mild skin and soft-tissue lesions to severe systemic diseases such as pneumonia, endocarditis, and sepsis [3]. Its high adaptability and the alarming increase in antibiotic-resistant strains, notably

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methicillin-resistant *S. aureus* (MRSA), make the discovery of new therapeutic targets a pressing priority in modern microbiology [4]. Among its vital metabolic systems, Fe–S cluster assembly plays a central role in maintaining redox homeostasis, enzymatic catalysis, and DNA repair [5].

Cysteine desulfurase SufS catalyzes the initial step in the sulfur mobilization process, removing sulfur atoms from cysteine to form a persulfide intermediate on a catalytic cysteine residue [6]. This sulfur is subsequently transferred to scaffold proteins such as SufU or SufE for Fe–S cluster assembly [7]. By mediating this essential step, SufS ensures the continuous supply of sulfur, which is necessary for numerous metalloenzymes that drive critical cellular functions, including respiration and DNA synthesis [8]. Consequently, the inhibition of SufS leads to the disruption of Fe–S cluster biogenesis, followed by a collapse of cellular metabolism and impaired bacterial survival [9]. Given its indispensable role in the SUF pathway and absence of closely related homologs in humans, SufS represents a highly attractive and selective molecular target for the development of new anti-*Staphylococcal* agents [10].

Cysteine desulfurases belong to a conserved family of pyridoxal 5′-phosphate (PLP)-dependent enzymes that catalyze the mobilization of sulfur from L-cysteine, leading to the formation of a protein-bound persulfide intermediate on an active-site cysteine residue [11]. This persulfide sulfur is then delivered to various acceptor proteins involved in Fe–S cluster biogenesis, thionucleotide synthesis, and tRNA modification [12]. Within the SUF system of *S. aureus*, SufS operates in concert with accessory proteins such as SufU or SufE, which enhance its activity and facilitate sulfur transfer to downstream scaffold complexes [13]. Structural and mechanistic studies have revealed that the SufS active site contains a conserved lysine residue forming a Schiff base with PLP, essential for catalysis [14]. The enzymatic cycle proceeds through a two-step ping-pong mechanism, generating alanine as a byproduct and transferring the sulfur atom to form the persulfide [15]. Understanding this mechanism at the molecular level provides key insights into how selective inhibitors could be designed to disrupt sulfur trafficking and cripple Fe–S cluster assembly in pathogenic bacteria [16].

Based on current knowledge, the ligands evaluated through molecular docking simulations on SufS from *S. aureus* have not been previously reported. The results of this study provide additional and complementary data that may contribute to a better understanding of the inhibition mechanisms and support strategies for the identification of selective inhibitors.

2. Molecular docking simulations methodology

Molecular docking simulations were performed using the CLC Drug Discovery Workbench software, following a protocol previously validated in

similar studies for a variety of ligands [17, 18]. The reference ligand used in this work is the compound encoded "882" [19], and the methodology for docking simulations, previously applied and validated for the same compound [20] includes the following steps: preparation of the ligand, preparation of the target protein by removing co-factors and water molecules, and setting the active binding site and the binding pocket. The simulation results are expressed as molecular docking score (PLANTSPLP scoring function) and RMSD (root mean square deviation). By the docking score, the binding affinity between the protein and the investigated ligand during molecular docking simulations is predicted. The hydrogen bonds formed by the ligand with the surrounding amino acids from the interaction group within the active binding site are identified and measured. The docking score is used to identify and rank potential binding modes, and finally, to order ligands according to their binding affinity.

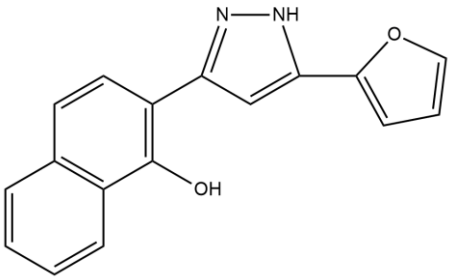
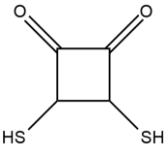
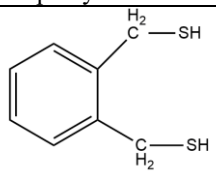
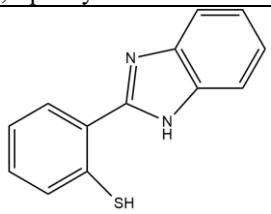
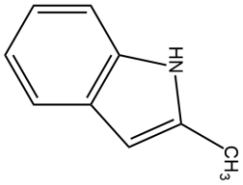
3. Results and Discussions

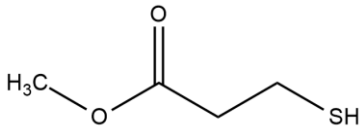
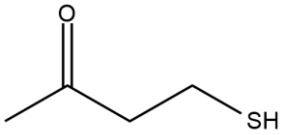
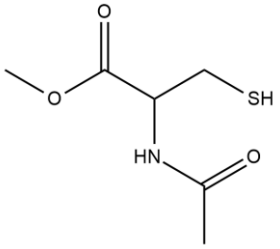
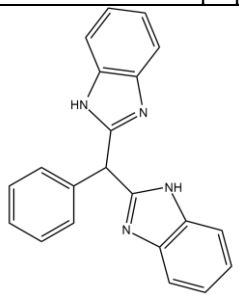
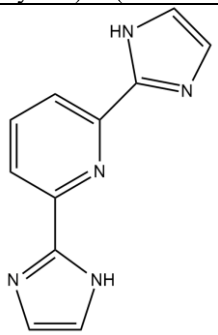
The investigated ligands, belonging to the bidentate and tridentate series [21], which were previously analyzed for their druggability properties using Density Functional Theory (DFT) predictions [22], were subject to docking into the active site of the cysteine desulfurase fragment (PDB ID: 8D8S) [10]. Their structures and docking results in terms of the docking score and root mean square deviation (RSMD) are given in **Table 1**. The best pose of each ligand is reflected in the docking score, as a measure of the binding affinity and conformational stability of each ligand–protein complex. The calculated scores obtained for the investigated ligands are compared with the score of the reference molecule "882", which was confirmed previously by experimental studies to show inhibitory activity against *Staphylococcus aureus* [19].

A more negative score indicates stronger binding, while a less negative score indicates weaker binding. The docking process involves fitting ligands into a protein's binding site, scoring the interaction, and returning the best-scoring binding mode for each ligand along with its score. Taking into account the obtained values, the ligands can be ranked according to the strength of the complex they form, in the following order: L2.10 (- 49.04) > "882" (- 45.52) > L2.5 (- 33.89) > L3.0 (- 31.77) > L2.6 (- 27.80) > L2.3 (- 26.07) > L2.9 (- 21.87) > L2.2 (- 20.29) > L2.7 (- 19.87) > L2.8 (- 19.71). Thus, the stronger affinity toward the SufS catalytic pocket shows the bidentate ligand L2.10, even greater than the reference ligand ("882"), followed by L 2.5 and the tridentate ligand L3.0

Table 1

Results of molecular docking simulations for the selected ligands in complex with cysteine desulfurase (PDB ID: 8D8S) isolated from *Staphylococcus aureus*

Ligand (code)	Structure	Docking Score/RMSD
"882"	 2-(1*H*-benzo[d]imidazole-2-yl)benzenethiol	- 45.52 / 0.19
L2.2	 3,4-dimercaptocyclobutane-1,2-dione	- 20.29 / 0.06
L2.3	 1,2-phenylendimethanethiol	- 26.07 / 0.42
L2.5	 2-(1*H*-benzo[d]imidazole-2-yl)benzenethiol	- 33.89 / 0.20
L2.6	 2-methyl-1H-indole	- 27.80 / 0.09

L2.7	 Methyl 3-mercaptopropanoate	- 19.87 / 0.29
L2.8	 4-mercaptobutan-2-one	- 19.71 / 0.08
L2.9	 Methyl 2-acetamido-3-mercaptopropanoate	- 21.87 / 0.77
L2.10	 2,2'-(phenylmethylene)bis(1H-benzo [d] imidazole)	- 49.04 / 1.61
L3.0	 2,6-di(1H-imidazol-2-yl)pyridine	- 31.77 / 0.93

For each ligand, the hydrogen bonds formed with key amino acid residues within the SufS active site were identified and measured, showing that the most common interactions involve residues ASP131, HIS127, GLU271, PRO272,

and ILE273. These hydrogen bonds significantly influence the docking score values, which reflect the binding strength of the ligands to cysteine desulfurase. The results are analyzed and compared with those for the reference molecule, "882", proven by experimental studies to have inhibitory activity on *Staphylococcus aureus* [19]. The hydrogen bonding details are listed in **Table 2**. These interactions are consistent with previously reported structural studies of the SufS–SufU complex (PDB ID 8D8S), in which the catalytic residue CYS364 and the surrounding acidic cluster (ASP131, GLU271) are essential for sulfur transfer activity. The ligands forming multiple hydrogen bonds with these residues are therefore expected to have the highest inhibitory potential.

Table 2

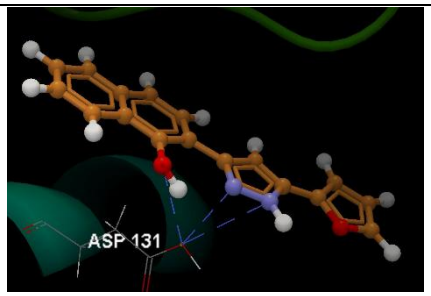
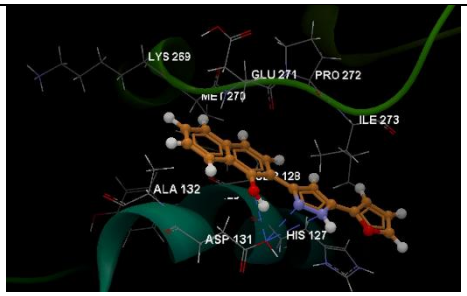
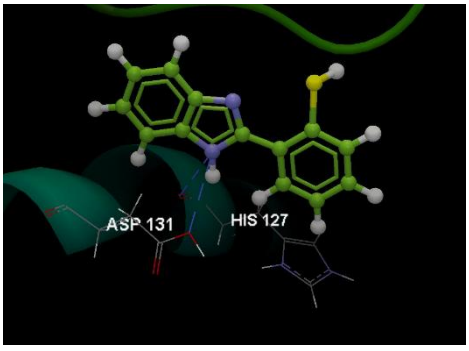
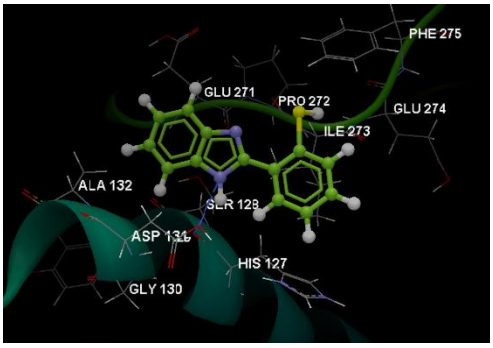
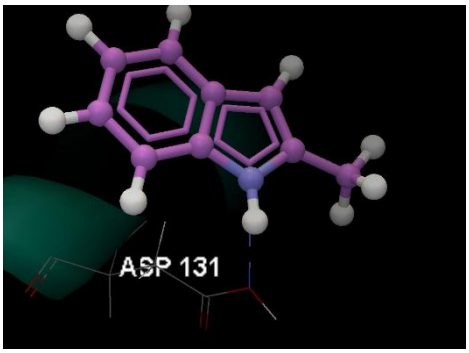
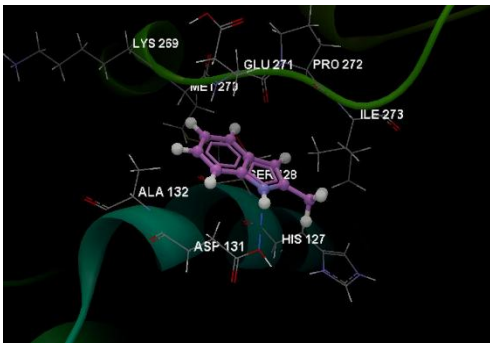
Results of molecular docking simulations performed on cysteine desulfurase (PDB ID: 8D8S) isolated from *Staphylococcus aureus*

Ligand (code)	Hydrogen bonds (Å)	Amino acids residues that interact in the active site
"882"	N sp^2 – O sp^3 ASP131 (3.266) N sp^2 – O sp^3 ASP131 (2.600) O sp^3 – O sp^3 ASP131 (3.025)	LYS269, GLU271, PRO272, ILE273, MET270, ALA132, SER128, ASP131, HIS127, TYR129
L2.5	N sp^2 – O sp^2 HIS127 (3,166) N sp^2 – O sp^3 ASP131 (3,241)	PHE275, GLU274, ILE273, PRO272, SER128, HIS127, ALA132, ASP131, GLY130
L2.6	N sp^2 – O sp^3 ASP131 (2.935)	LYS269, MET270, GLU271, PRO272, ILE273, ALA132, SER128, HIS127, ASP131
L2.10	N sp^2 – O sp^3 ASP131 (3,047) N sp^2 – O sp^2 HIS127 (3,009) N sp^2 – O sp^2 ASP131 (2,811)	PHE275, PRO272, ILE273, GLU271, SER128, TYR129, HIS127, ALA132, ASP131, ARG160
L3.0	N sp^2 – O sp^3 ASP131 (3,138) N sp^2 – O sp^3 ASP 131 (2,636)	PRO272, ILE273, GLU271, MET270, ALA132, SER128, HIS127, ASP131, LYS161, ARG160

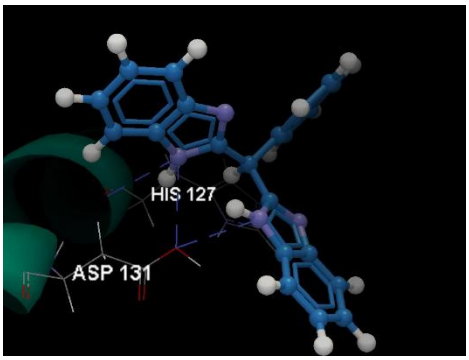
Table 3 illustrates the best binding poses of each docked ligand within the active site of the cysteine desulfurase protein fragment encoded as 8D8S, for which the highest docking scores were obtained. The corresponding figures depict the hydrogen-bonding interactions together with the interactions involving amino acid residues.

Table 3

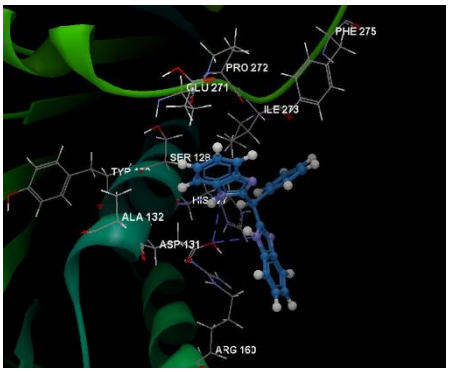
Best pose of investigated ligands in complex with cysteine desulfurase PDB ID: 8D8S, their interactions and the interacting amino acids group residues

Ligand (code)	Hydrogen bonds	Amino-acid interaction group
"882"	 Hydrogen bonds between ligand "882" and ASP131 in the binding site of cysteine desulfurase PDB ID: 8D8S	 Amino acid residues interacting with ligand "882" in the active site of cysteine desulfurase PDB ID: 8D8S
L2.5	 Hydrogen bonds between ligand L2.5 and amino-acid residues ASP131 and HIS127 in the binding site of cysteine desulfurase PDB ID: 8D8S	 Amino acid residues interacting with ligand L2.5 in the active site of cysteine desulfurase PDB ID: 8D8S
L2.6	 Hydrogen bonds between ligand L2.6 and amino-acid residue ASP131 in the binding site of cysteine desulfurase PDB ID: 8D8S	 Amino acid residues interacting with ligand L2.6 in the active site of cysteine desulfurase PDB ID: 8D8S

L2.10

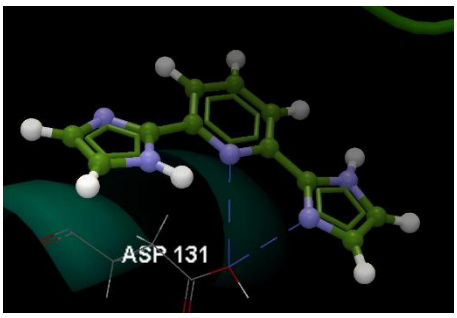


Hydrogen bonds between ligand L2.10 and amino-acid residues ASP131 and HIS127 in the binding site of cysteine desulfurase PDB ID: 8D8S

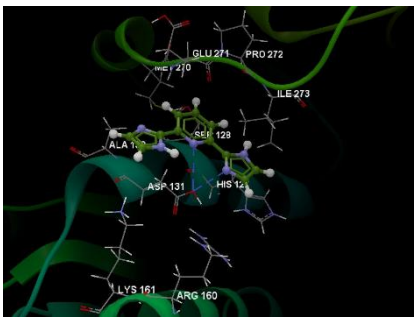


Amino acid residues interacting with ligand L2.10 in the active site of cysteine desulfurase PDB ID: 8D8S

L3.0



Hydrogen bonds between ligand L3.0 and amino-acid residue ASP131 in the binding site of cysteine desulfurase PDB ID: 8D8S



Amino acid residues interacting with ligand L3.0 in the active site of cysteine desulfurase PDB ID: 8D8S

The calculated physicochemical parameters of all investigated ligands, as key features for druggability, are given in **Table 4**: the total number of atoms, the molecular weight ($g \cdot mol^{-1}$), the number of violations of Lipinski's Rule of Five (ROF) [23]; the number of hydrogen bond donors (HBD), the number of hydrogen bond acceptors (HBA), and the octanol–water partition coefficient (logP). None of the compounds show deviations from Lipinski's rule (mass < 500 $g \cdot mol^{-1}$, HBD < 5, HBA < 10, logP < 5). Paying attention to their predicted values for logP, a good balance between hydrophobic and hydrophilic character shows the ligands "882", L2.10, L2.6, and L2.5, exhibiting the same good docking score; thus, they have, in addition, a good oral bioavailability, making them suitable structures for drug formulations.

Table 4

Ligand properties calculated using the CLC Drug Discovery Workbench software

Ligand	Nr. atoms	Mass	ROF	HBD	HBA	logP
"882"	33	276.29	0	2	4	3.53
L3.0	25	211.22	0	2	5	0.53
L2.10	41	324.38	0	2	4	4.49
L2.9	22	177.22	0	1	4	0.38
L2.8	14	104.17	0	0	1	0.17
L2.7	15	120.17	0	0	2	0.34
L2.6	19	131.17	0	1	1	2.45
L2.5	26	226.30	0	1	2	3.30
L2.4	24	218.34	0	0	0	3.85
L2.3	20	170.29	0	0	0	2.09
L2.2	12	148.20	0	0	2	0.08

4. Conclusions

Following molecular docking simulations, the compounds with the highest affinity for cysteine desulfurase (8D8S fragment) isolated from *S. aureus* were L2.10, even greater than the reference ligand ("882"), L2.5, and the tridentate ligand L3.0. The molecular docking results demonstrated that these ligands interact favorably with the active site of cysteine desulfurase (8D8S fragment), supporting their potential as selective inhibitors of the SUF-like pathway.

These structures also possess suitable properties for druggability; therefore, the premises of the studies were accomplished and confirmed. Their common pattern, the imidazole ring, is a five-membered heterocycle present in many biological molecules. Imidazole derivatives are considered promising chemical compounds with potential therapeutic activity against several pathogens.

The identification of the imidazole ring as a common structural motif among the most active ligands highlights the importance of this heterocyclic scaffold in the design of antimicrobial agents targeting Fe-S cluster biosynthesis. Thus, our findings lay the groundwork for developing new therapeutic strategies against *S. aureus* by targeting the SUF-like pathway and designing strong ligands that can inhibit the key SufS protein, thereby preventing Fe-S cluster formation.

Since the SUF-like pathway plays an essential role in Fe-S cluster biosynthesis, targeting SufS may represent a promising strategy for overcoming antimicrobial resistance and developing novel antibiotics with selective mechanisms of action. The present findings encourage the exploration of imidazole-derived compounds as a broader platform for the discovery of inhibitors against sulfur mobilization enzymes in other clinically relevant pathogens.

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