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# *IN SILICO* INVESTIGATION OF INDENOIMIDAZOLE DERIVATIVES AS PROMISING ARSENAL AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS THROUGH STAPHYLOCOCCAL PROTEIN A INHIBITION

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**ABSTRACT**
Methicillin-resistant Staphylococcus aureus (MRSA) poses a significant challenge in healthcare due to its intricate nature and resistance to traditional antibiotics. This study delves into addressing this issue by investigating indenoimidazole derivatives' inhibitory effects on Staphylococcal protein A (SpA). Through rigorous methodology incorporating structure based virtual screening, four derivatives were optimized and identified as having superior efficacy compared to Linezolid. The findings showed that these derivatives had better binding affinities (-11.2 to -11.7 Kcal/mol) and good Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) free energies (ranging from -5.48 to -8.41 Kcal/mol). Among these, Compound 3 (3-(2,4-dihydroxybenzylidene) amino)-3a,8a-dihydroxy-2-thioxo-1,3,3a,8a-tetrahydroindeno[1,2-d] imidazol-8(2H)-one) has the highest binding affinity (-11.7 Kcal/mol) and the most favorable Gibbs free energy profile (-8.41 Kcal/mol). The pharmacokinetic evaluation revealed that all compounds possess acceptable absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles, aligning with the Lipinski rule of five for safety. Molecular dynamics simulations showed that Compound 3 maintains stable interactions and structural integrity over time, exhibiting minimal Root Mean Square Deviation and Fluctuation (RMSD and RMSF), consistent Solvent Accessible Surface Area (SASA), and a higher number of stable hydrogen bonds (H-bonds) compared to Linezolid. By presenting specific inhibitory values against SpA, this research contributes significantly to MRSA therapeutic strategies, suggesting indenoimidazole derivatives, particularly Compound 3, as viable candidates for further drug development analysis, offering hope in the ongoing battle against antibiotic resistance.

**KEY WORDS**: Methicillin resistant staphylococcus aureus, Staphylococcal protein A, Indenoimidazole, Structure based virtual screening, Molecular dynamics.

## 1.0 INTRODUCTION

The increasing challenge of Staphylococcus aureus resistance, especially to multiple drugs, has emerged as a major concern in modern healthcare.[1,2] Prior to the invention of antibiotics, S. aureus infections had a mortality rate that was higher than 80%.[3] The introduction of penicillin, the first beta-lactam antibiotic, in the 1940s represented a momentous advancement, greatly enhancing patient results.[4] However, the effectiveness of penicillin has declined over time, as current data indicates that over 95% of S. aureus strains have developed resistance to it.[5,6] The development of methicillin in 1959 was aimed at addressing this resistance but accidentally resulted in the rise of methicillin-resistant Staphylococcus aureus (MRSA).[7] Discovered in 1961, MRSA has since emerged as a steadily increasing threat in the realm of infectious diseases.[8]

The heightened virulence of MRSA is largely due to various factors that bolster its antibiotic resistance.[9] A major contributor to this resistance is Staphylococcal protein A (SpA), found in approximately 90% of MRSA strains.[10] SpA undermines the host's immune response, particularly by binding to the Fc region of IgG immunoglobulins.[11] This interaction disrupts crucial immune functions like opsonization and phagocytosis, which are key in pathogen identification and elimination.[12] Consequently, SpA's ability to evade these immune processes not only enhances MRSA's survival but also aggravates the severity of infections it causes. This evasion mechanism significantly challenges the effectiveness of traditional antibiotics in treating

MRSA-related infections, making it a formidable pathogen in hospital settings where it causes serious infections affecting the respiratory system, bones, heart, surgical sites, and blood.[13,14] The presence of other virulent factors, such as Panton-Valentine Leukocidin and Alpha Toxin, further contributes to its pathogenicity.[15]

Nitrogen-based heterocycles, particularly those incorporating the imidazole ring, have garnered attention for their therapeutic potential against cancer, microbial infections, and inflammation.[16] Imidazole rings can interact with various drugs through hydrogen bonds, hydrophobic forces, and van der Waals forces, enhancing their biological activity.[17] Among these, fused imidazole derivatives, such as indenoimidazole compounds, are pivotal in drug discovery due to their versatile medicinal applications.[18,19,20] Several studies have utilized different strategies to modify these compounds to enhance their biological effectiveness and reduce potential resistance, utilizing their unique chemical structures to combat MRSA resistance.[21,22,23] This highlights their potential as potent therapeutic agents against antibiotic-resistant infections.[24] Investigations into these derivatives for pharmaceutical development reveal promising antibacterial and antimicrobial properties.[25]

The intricate nature of MRSA resistance underscores the need for innovative therapeutic targets and approaches to tackle infections caused by drug-resistant Staphylococcus aureus.[26] By targeting SpA, the aim is to determine the inhibitory impact of indenoimidazole derivatives on SpA, which constitutes approximately 90% of all MRSA strains, thereby enhancing the host immune response and disrupting the bacterial immune defense mechanism. A thorough understanding of the drug's pharmacokinetics and emerging pharmacodynamic parameters is essential in developing effective treatment strategies against antibiotic-resistant pathogens. The study utilizes structure-based virtual screening to identify potential therapeutic targets and improve treatment safety by mitigating drug toxicity. The insights gained from this research are instrumental in advancing our understanding of MRSA pathogenesis and guiding the development of new therapeutic strategies.

## 2.0 MATERIAL AND METHODS

### 2.1 Preparation of target protein

The essential information on Staphylococcal protein A was obtained via the Protein Data Bank (PDB) (https://www.rcsb.org) and utilized for this study. We capitalized on the homo-domain structure of Staphylococcal protein A as a receptor, with particular focus on its domain C (PDB ID: 4NPE) with a resolution of 1.42 Å, as elucidated by.[27] The Biovia Discovery Studio software version 2021 (http://www.accelrys.com) was employed to ensure the absence of unexpected interactions during virtual screening, a crucial step in our methodology.

### 2.2 Determination of 4NPE active site and molecular docking validation

Our approach also included the use of the CASTp 3.0 web server, a renowned tool for predicting the active site of proteins, which facilitated precise, site-specific docking and opened avenues for new discoveries.[28] The efficacy of Linezolid, an FDA approved drug known for its interaction with MRSA, was validated in our study. We used molecular docking to test how well it could bind and interact with the C-domain site target of Staphylococcal protein A. To enhance the accuracy of our virtual screening, we utilized consensus scoring, employing advanced software tools like iGEMDock.[29] and AutoDock Vina.[30] These tools helped us refine our results by reducing the incidence of false positives and negatives.

### 2.2 Preparation of ligands

The focus of our in-silico investigation was a class of drugs known as indenoimidazole and its analogues used as potential treatments for Staphylococcus aureus. linezolid was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and four indenoimidazole derivatives were central to our study (unpublished data). The 3D structures of these derivatives were precisely modified using ACD/Chemsketch version 2018 (http://www.acdlabs.com/) and saved in PDB format. To convert these compounds, including linezolid, into PDBQT format, we used the open-babel tools.[31] This makes it possible for us to study how each of these compounds interacts with the targeted protein.

### 2.3 Molecular docking study

A key tool for molecular docking simulation efficiency was the Perl scripting language. Because of its ability to carry out effective, high-throughput testing of many ligands, Perl was chosen.[32] The efficiency of Perl in handling numerous ligands at once served as a guide for this decision. We initiated the process by converting the ligands, initially in SDF format, into PDBQT format using Open Babel tools.[33] The Discovery Studio software was instrumental in refining the protein structure, a process that involved removing extraneous elements like ligand groups and water molecules, following protocols established in recent studies. After that, we changed the protein by incorporating polar hydrogen atoms and applying Gasteiger charges through the use of Autodock Tools, which is an important step to make sure the molecular docking is correct.[34] This setup allowed us to focus on particular binding sites on the protein for meticulous examination. The protein's structure was converted into the form of pdbqt using Perl for the purpose of conducting docking simulations. In addition, a configuration file was created to fine-tune the simulation parameters, such as the number of exhaustivenesses and modes. During the docking

process, we utilized Perl scripts and a command-prompt environment to manage the list of ligand files and initiate the docking procedure. The docking results, including intermolecular energies and internal ligand energies, were analyzed to identify the most favorable binding locations. We prioritized ligands based on their binding energies, with lower values indicating more favorable binding modes. A threshold for RMSD values was set to ensure accuracy in pose alignment.[35]

Version 2.1 of iGEMDOCK enabled the setup of the binding site through the importation of the PDB X-ray crystallographic structure downloaded earlier into the software's "prepare binding site" feature. A model molecule of the bound ligand inside the protein was used to make a grid with an 8.0 Angstrom binding site radius. This helped find the protein's active site. The ligands, in their PDB format, were imported and then processed through the "prepare compounds" feature. The docking precision parameters were adjusted to ensure accuracy, with the population size, number of solutions, and number of generations set to 800, 10, and 80, accordingly.[36]

**2.4 MM-GBSA**

The MM-GBSA technique is a widely used method for calculating the free energy involved in the binding of small molecules to large biological structures.[37] Following molecular docking investigations, the Prime MM-GBSA tool in Maestro version 12.5 was used to assess the relative binding free energies of four compounds in comparison to a standard drug. The MM-GBSA assessment offers insights into the different energy components that influence binding free energy.[38]

**2.5 Pharmacokinetic (ADMET) studies**

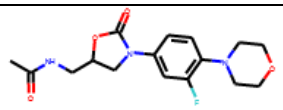
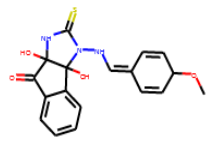
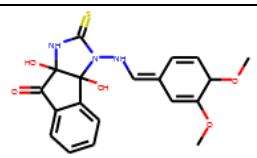
ADMET investigations constitute a vital aspect of drug discovery and development. These studies evaluate the pharmacokinetic and toxicological characteristics of potential drug candidates, offering indispensable insights to enhance drug efficacy and safety. ADMET studies were conducted on four indenoimidazole derivatives and linezolid drug utilizing AdmetSAR web server. The test ligand was uploaded, and the software generated results encompassing physiochemical properties, medicinal chemistry aspects, and toxicity information.[39]

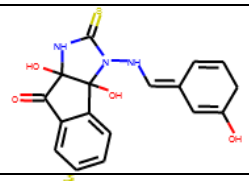
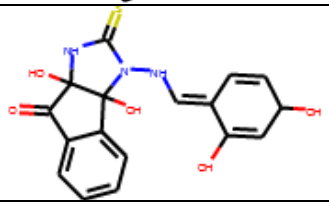
**2.6 Molecular Dynamic (MD) Simulation**

MD simulations were comprehensively conducted in order to assess the structural stability of the protein-ligand complexes, as outlined by.[40] According to research from.[41] MD simulations are a potent tool for examining atomic-level changes within these complexes under dynamic conditions. We employed the GROMOS54a7 force field and SPC water model for all simulations. The topologies and parameter files of the lead compounds and the linezolid were created using the Swiss Param server, adhering to the methodology suggested by.[42] For the simulations, we solvated the protein complexes in a triclinic box using simple point charges (SPC), maintaining a specific volume and distance from the nearest protein atom. The systems were neutralized with counterions ($Cl^-$ ions) through the 'genion' tool and minimized to remove steric clashes. We then analyzed the post-simulation trajectories, focusing on metrics such as RMSD, RMSF, SASA, and H-bond, to evaluate the stability and dynamics of the complexes.[43]

**3.0 RESULTS AND DISCUSSION**

**Table 1: Provides information about four novel compounds (identified as Compound 1, Compound 2, Compound 3, and Compound 4) and a standard drug (Linezolid), presenting data on each ligand name, 2D structures, and molecular weights.**

| Compound Identifier | Ligand Name | 2-D structure | Molecular weight (g/mol) |
|---|---|---|---|
| Standard Drug | Linezolid |  | 337.35 |
| Compound 1 | a,8a-dihydroxy-3-((4-methoxybenzylidene)amino)-2-thioxo-1,3,3a,8a-tetrahydroindeno[1,2-d]imidazol-8(2H)-one |  | 371.090 |
| Compound 2 | -3a,8a-dihydroxy-3-((3,4-dimethoxybenzylidene)amino)-2-thioxo-1,3,3a,8a-tetrahydroindeno[1,2-d]imidazol-8(2H)-one |  | 377.313 |

| | | | |
|---|---|---|---|
| Compound 3 | 3-((2,4-dihydroxybenzylidene)amino)-3a,8a-dihydroxy-2-thioxo-1,3,3a,8a-tetrahydroindeno[1,2-d]imidazol-8(2H)-one |  | 357.080 |
| Compound 4 | -3a,8a-dihydroxy-3-((3-hydroxybenzylidene)amino)-2-thioxo-1,3,3a,8a-tetrahydroindeno[1,2- d]imidazol-8(2H)-one |  | 373.070 |

### 3.1 Structure Based Virtual Screening

#### 3.1.1 Molecular docking

The investigative process included a comprehensive screening of the four indenoimidazole analogues and the standard drug, assessing various critical aspects to ascertain their medicinal viability.

**Tables 2: shows the binding affinities and hydrogen bond interactions of various ligands with the Staphylococcal protein A. The four ligands (Compound 1, Compound 2, Compound 3, and Compound 4) showed higher binding affinity compared to linezolid, indicating their potential effectiveness for the treatment of Staphylococcus aureus, particularly MRSA.**

| Compound Identifier | Binding affinity (AutoDock Vina) Kcal/mol) | Binding affinity (iGEMDOCK) Kcal/mol) | No. of H-bonds | Amino-acid Interaction |
|---|---|---|---|---|
| Linezolid (Standard Drug) | -10.4 | -7.5 | 2 | GLN10, LYS35 |
| Compound 1 | -11.2 | -8.5 | 3 | ASN3, PHE5 (Twice) |
| Compound 2 | -11.2 | -8.9 | 3 | ASN3, PHE5 (Twice) |
| Compound 3 | -11.7 | -8.9 | 4 | ASN3, PHE5 (Twice), LYS35 |
| Compound 4 | -11.6 | -8.6 | 1 | PHE5 |

Our molecular docking studies underscored the importance of conventional hydrogen bonds in defining the affinity of the protein for each ligand. A key observation was that a lower conformational energy requirement for molecular structure alteration correlates with a stronger attraction to a specific protein. Utilizing five ligands, we conducted docking studies on Staphylococcal protein A, with linezolid serving as the standard. Among these, one ligand demonstrated an advantageous docking position, as discerned through detailed analysis of protein-ligand interactions. The selection of the optimal ligand hinged on its highest hydrogen bond count and exceptional binding affinity, as detailed in Table 2. To corroborate these findings, two molecular docking software tools (AutoDock Vina and iGEMDOCK) were employed. Compound 3, the ligand with optimal properties, displayed a binding affinity of -11.7 Kcal/mol in AutoDock Vina. Its interaction with the SpA receptor involved forming four hydrogen bonds at specific amino acid residues, as illustrated in Figure 2. Table 2 also elucidates the hydrogen bond configurations and amino acid interactions of the other drug candidates. Compounds 1, 2, and 4, while exhibiting higher binding affinities than linezolid, varied in their hydrogen bond formations and binding sites. Notably, linezolid formed two hydrogen bonds at a distinct amino acid position, resulting in a binding affinity of -10.4 Kcal/mol. The congruence of results from iGEMDOCK and AutoDock Vina, especially for the top-performing compounds, significantly reduced the likelihood of false positives or negatives in identifying compounds with substantial inhibitory potential.

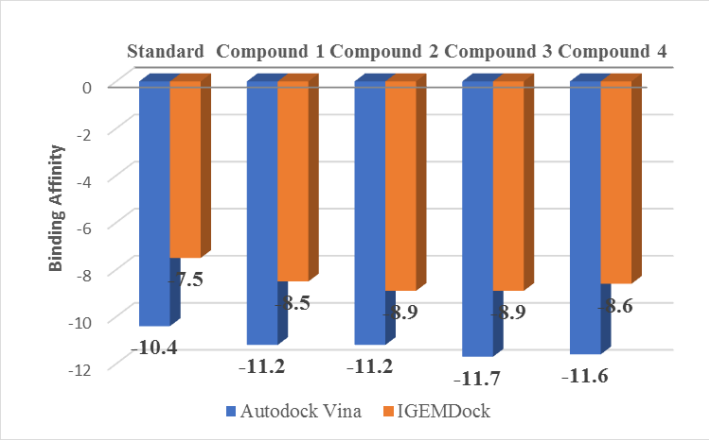
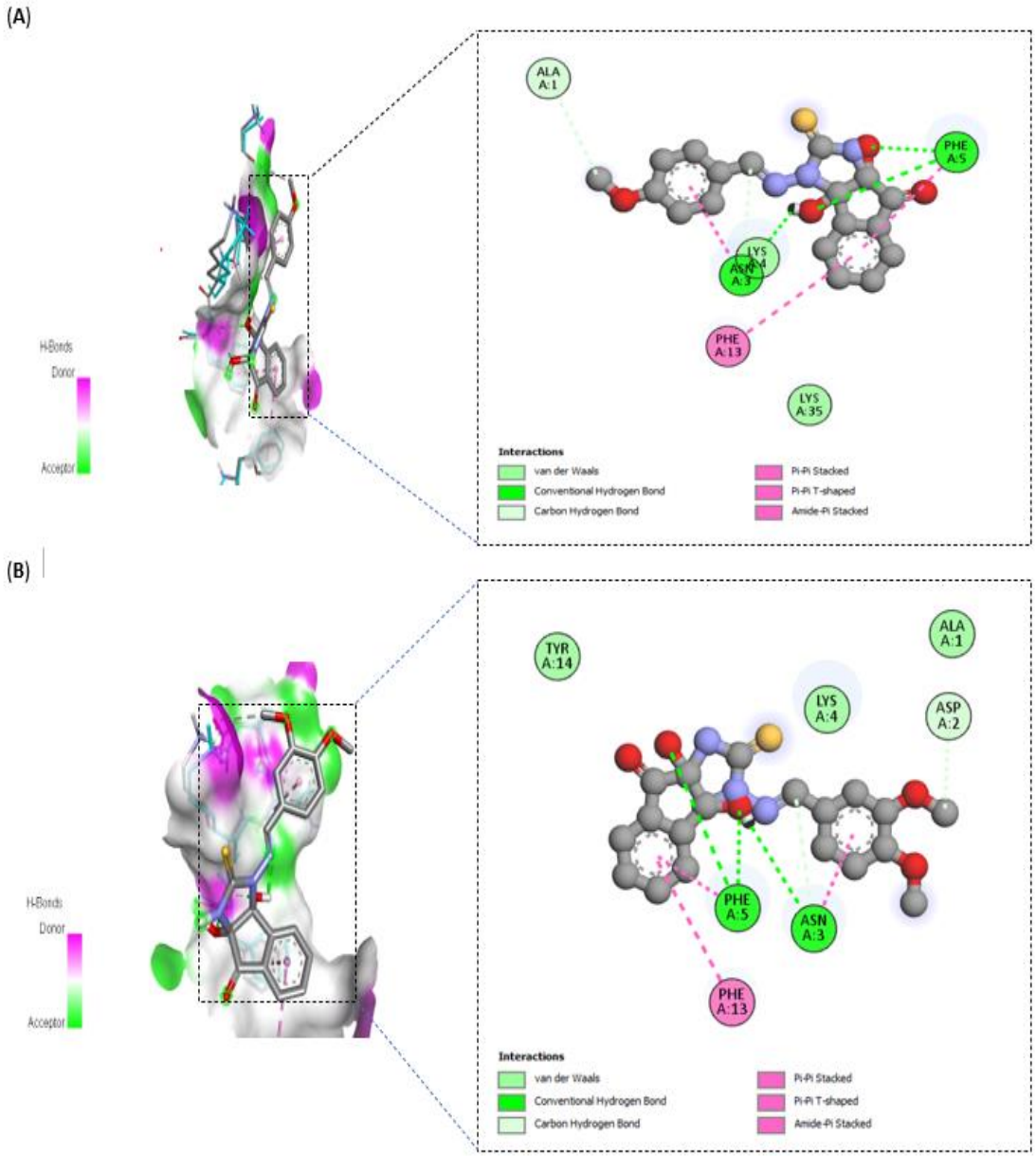



**Figure 1: The Bar chart showing the Binding affinities of the four compounds and the standard in Auto-dock vina and IGEM Dock.**



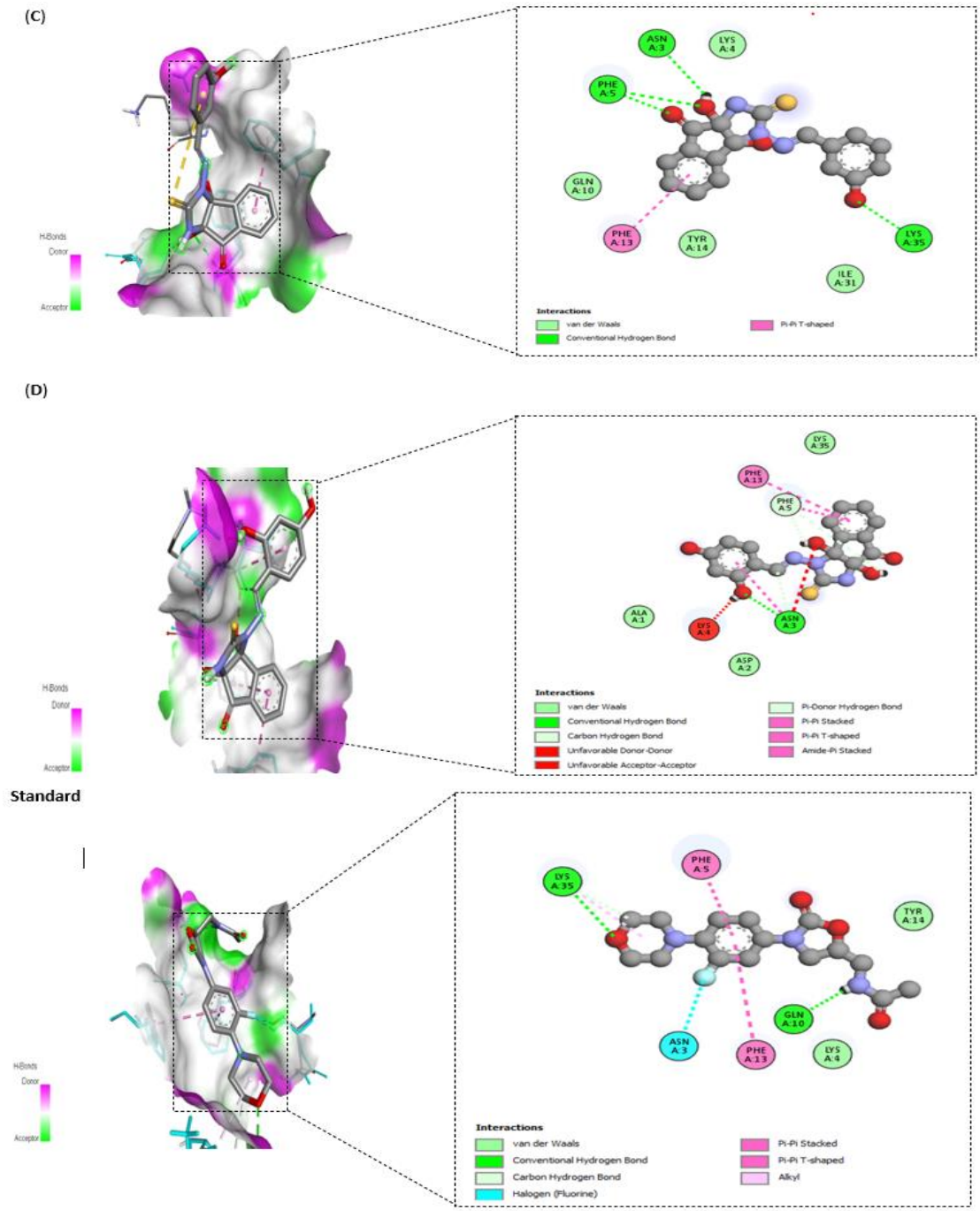



**Figure 2: Molecular interaction between the four compounds and the standard at the active site of Staphylococcal protein A (4NPE) (A) Compound 1 (B) Compound 2 (C) Compound 3 (D) Compound 4, (Standard) Linezolid. The structures were rendered using Biovia Discovery Studio 2021.**

These results were methodically compared and ranked based on their average position, using relative rank as the key criterion rather than binding affinity values. Consequently, four compounds emerged as potential SpA inhibitors, with Compound 3 attaining the highest score.

### 3.1.2 MM-GBSA

To understand the molecular interactions driving the binding of ligands to a target protein, we conducted MM-GBSA calculations. These calculations shed light on the different energy components that contribute to the binding free energy, as summarized in Table 3 and Figure 3.

**Table 3: MM-GBSA binding free energies of Linezolid, Compound 1, Compound 2, Compound 3, and Compound 4.**

| Ligands | H-bond (Kcal/mol) | Vander Waal Forces (Kcal/mol) | Polar Solvation (Kcal/mol) | Overall Binding Energy (Kcal/mol) |
|---|---|---|---|---|
| Linezolid | -1.96 | -21.65 | 35.32 | 11.71 |
| Compound 1 | -2.62 | -16.70 | 11.79 | -7.53 |
| Compound 2 | -3.37 | -15.78 | 12.03 | -7.12 |
| Compound 3 | -3.36 | -14.08 | 9.03 | -8.41 |
| Compound 4 | -4.16 | -14.52 | 13.70 | -5.48 |

Among the analyzed ligands, including the standard drug, the dominant factor influencing binding energy was identified as van der Waals interactions. These interactions, which include all non-covalent forces except hydrogen bonds, are necessary for the ligand to be placed correctly in the binding site. Notably, Compound 3 and Linezolid had strong non-covalent interactions, with values of -22.08 Kcal/mol and -21.65 Kcal/mol, respectively. This made their ligand-receptor complexes much more stable. Hydrogen bonding also plays a pivotal role in stabilizing these complexes, crucially affecting both specificity and binding strength. Linezolid had a hydrogen bond energy of -1.96 Kcal/mol, but the other four compounds had higher hydrogen bond energies, ranging from -2.6 Kcal/mol to -4.16 Kcal/mol, which means they were better at binding. The energy associated with polar solvation, reflecting the cost of desolving both ligand and receptor for interaction, showed that positive values could detract from otherwise favorable binding interactions. Linezolid has the highest polar solvation energy of 35.32 Kcal/mol. This shows how these kinds of energetic costs can lower the overall binding energy by canceling out positive interactions like hydrogen bonds and van der Waals forces.

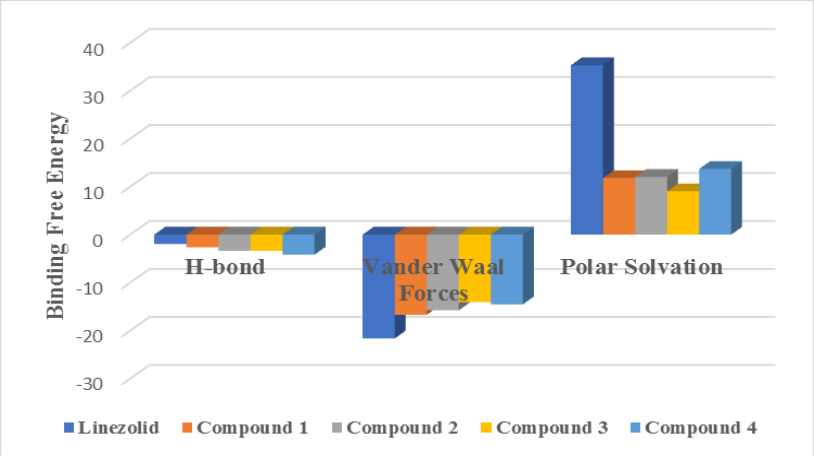



**Figure 3: The Bar chart showing the Binding free energies of the four compounds and linezolid in Maestro 12.5 Overall, the interplay of these energy components varies across different ligands, affecting their binding affinity to the target. Compound 3 emerged as the most effective, achieving the optimal balance among these interactions with the most negative overall binding energy of -8.41 Kcal/mol. This indicates a highly stable complex formation, underscoring its potential efficacy as a therapeutic agent through enhanced potency and target specificity.**

### 3.1.3 Drug likeness Prediction

The evaluation of these compounds was guided by critical benchmarks, including the count of rotatable bonds and adherence to the Lipinski rule of five, along with various other medicinal chemistry filters, such as lead similarity. According to.[44] study, the rule of five sets specific criteria for H-bond donors and acceptors, molecular weight, and lipophilicity, ensuring a drug's favorable pharmacokinetic profile. Adhering to these criteria, with limits set for molecular weight, log P value, hydrogen bond acceptors, and donors, was imperative to enhance the likelihood of achieving acceptable oral bioavailability.

**Table 4: Drug likeness properties of Linezolid, Compound 1, Compound 2, Compound 3, and Compound 4.**

| Ligands | Molecular weight | H-bond donor | H-bond acceptor | Log p | Inference |
|---|---|---|---|---|---|
| Compound ID | ≤ 500 | ≤ 5 | ≤ 10 | ≤ 5 | MEET R05 |
| Linezolid | 337.35 | 1 | 6 | 0.7 | + |
| Compound 1 | 371.4 | 4 | 6 | 0.436 | + |
| Compound 2 | 401.4 | 4 | 7 | 0.7595 | + |

| | | | | | |
|---|---|---|---|---|---|
| Compound 3 | 357.3 | 5 | 6 | 0.697 | + |
| Compound 4 | 373.3 | 6 | 7 | 0.5827 | + |

### 3.1.4 ADMET Evaluation

To understand the pharmacokinetics of a compound, several parameters were evaluated, collectively known as ADMET. These parameters were crucial for assessing the safety and efficacy of a compound before it can be considered for clinical use. The ADMET profiling of compounds using admetSAR provided insights into how drug candidates are absorbed and distributed.

**Table 5: Showing ADMET profiling of the drug candidates and linezolid.**

| Ligands | Absorption and Distribution | | | | | Metabolism | | | | | Excretion and Toxicity | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound Identifier | Water solubility | Caco2 permeability | Human Intestinal absorption | P-glycoprotein Iinhibitor | BBB permeability | CYP2D6 | CYP3A4 | CYP1A2 | CYP2C19 | CYP2C9 | Total Clearance | Acute oral toxicity | AMES toxicity | Carcinogenicity | HERG inhibitor | Hepatoxicity |
| Linezolid | | | | | | | | | | | | | | | | |
| Compound 1 | | | | | | | | | | | | | | | | |
| Compound 2 | | | | | | | | | | | | | | | | |
| Compound 3 | | | | | | | | | | | | | | | | |
| Compound 4 | | | | | | | | | | | | | | | | |

= Good/Pass = Average/Moderate = potential toxicity

All compounds, including linezolid, show good human intestinal absorption (+) indicating their effectiveness in systemic uptake and passage through the intestinal barrier but are negatively marked for water solubility, indicating a challenge in dissolving in the gastrointestinal tract, which could be mitigated through formulation strategies. All of the compounds including linezolid, with the exception of compound 4, are non-inhibitors P-glycoprotein, suggesting they might have less interaction with drug efflux mechanisms, which can be beneficial for maintaining therapeutic levels. Moreover, these drug candidates showed a limited ability to traverse the blood-brain barrier (BBB), suggesting a reduced risk of central nervous system penetration with the exception of linezolid which have the tendency to cross the BBB thereby causing potential CNS side effects. Regarding metabolism, the non-inhibition of cytochrome P450 enzymes by all compounds mitigates the potential for drug interactions and bioaccumulation, underscoring their consistent pharmacological profiles.

Toxicological testing revealed moderate levels of acute oral toxicity for all compounds including linezolid, indicating a reduced likelihood of serious side effects at standard therapeutic doses. Notably, all compounds were non carcinogenic and non-inhibitors of HERG, thereby minimizing the risk of severe cardiovascular side effects. However, all drug candidates, except compound 1, and compound 3, exhibited signs of AMES toxicity, raising concerns about DNA mutations. Moreover, signs of hepatotoxicity were observed in all compounds, suggesting potential liver toxicity. Conclusively, Compound 2 stands out among the novel compounds for favorable ADMET profile, with good systemic uptake and no mutagenicity risk, suggesting a reduced likelihood of severe side effects at standard therapeutic doses.

### 3.1.5 Molecular Dynamic Simulation

The decision to exclusively subject Compound 3 to a 50 ns molecular dynamics simulation among four indenoimidazole derivatives was based on its superior performance in multiple evaluations. Compound 3 showcased the best molecular docking affinity and the most favorable MM-GBSA binding free energy, suggesting it has a more robust and stable interaction with the target protein than its counterparts. Additionally, it displayed favorable pharmacokinetic properties, complying with the Lipinski rule of five and exhibiting a promising ADMET profile, essential for predicting a compound's drug viability. These factors underscored Compound 3's potential as a drug candidate, leading to a 50 ns molecular dynamics simulation to further investigate its structural stability and dynamic interaction with the target protein. This simulation employed structural metrics such as RMSD, RMSF, SASA, and intermolecular hydrogen bonds to analyze the behavior

of protein-drug complexes, highlighting Compound 3's suitability for further drug development efforts.

### 3.1.5.1 RMSD

In accordance with the RMSD plot depicted in Fig. 4a, the complex_compound 3 RMSD fluctuates around 0.1nm with a little variation near 0.3nm, and the fluctuation peaks downward as the simulation comes to a conclusion. The equilibration and stability of compound 3 over time (30 ns to 50 ns) show that this compound has superior stability compared to linezolid (40 ns to 50 ns). The complex RMSD plot result further supports the hypothesis stated by[45] that a lower RMSD equates to a lesser structural deviation, which translates to stronger stability. Also, in Fig. 4b, the RMSD plot illustrates the equilibration and stability of the protein in the lysosome. The graph plot results indicate its stability over time (20 ns to 50 ns) and also fluctuate about 0.1nm and 0.3nm.

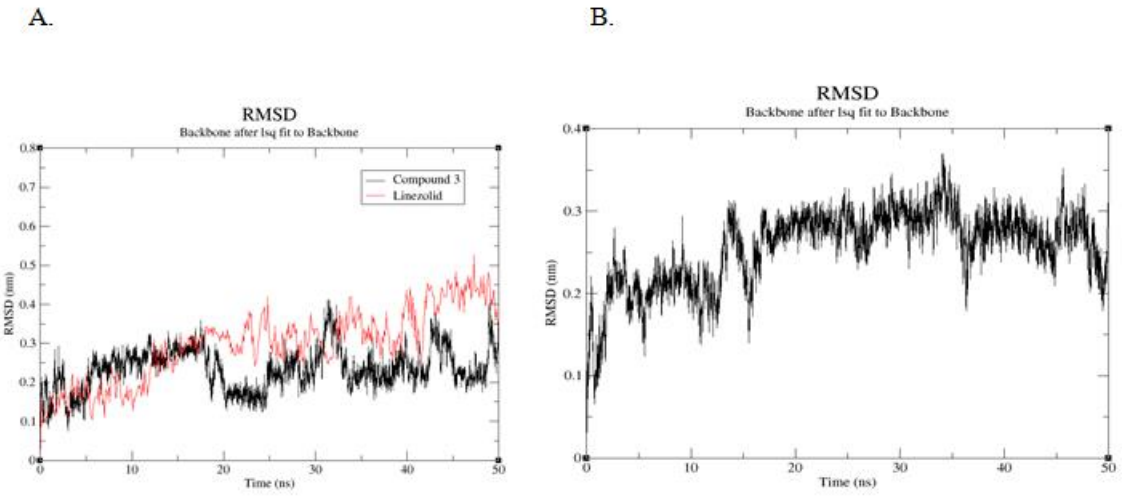



**Fig. 4: Showing the RMSD graph of (A.) Protein in complex with Linezolid and Compound 3 and (B) Protein in water.**

### 3.1.5.2 RMSF

Assessing the flexibility of the protein backbone is crucial, and this was achieved by measuring the RMSF following realignment of the protein within a reference frame. The analysis of root mean square fluctuation (RMSF) values in certain residue ranges provides valuable information about the regions of the protein that exhibit greater flexibility, whereas lower RMSF values indicate areas of higher rigidity, as discussed by. [45] The data from the RMSF analysis (Fig 5a) underlined that the protein_ compound 3 combination completely fits into the active areas of the protein, resulting in a stable complex compared to linezolid. Similarly, as depicted in Figure 5b, the protein in lysosome confirms RMSF value of our compound, hence reinforcing the claim that compound 3 demonstrates a higher RMSF value compared to linezolid.

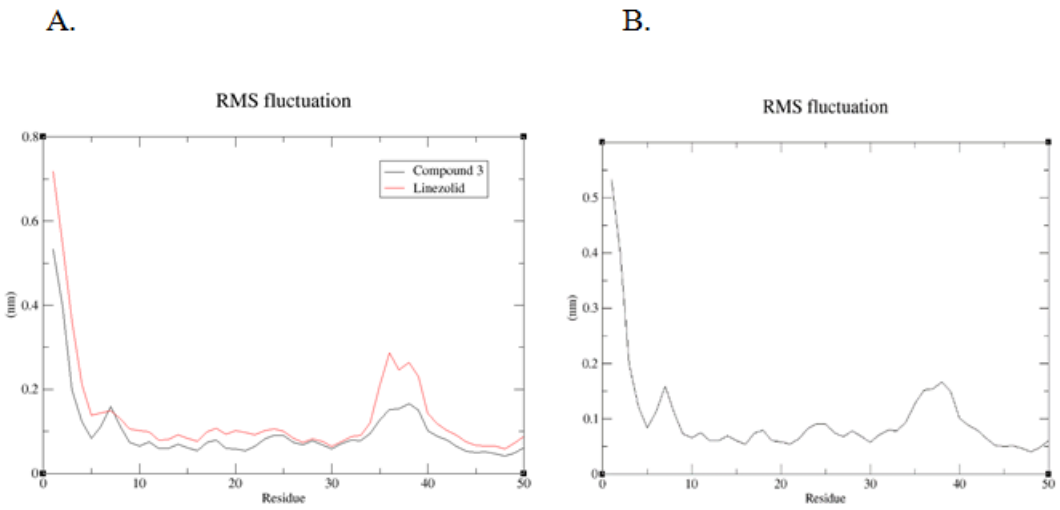



**Fig. 5: Showing the RMSF graph of (A.) Protein in complex with Linezolid and Compound 3 and (B) Protein in water.**

### 3.1.5.3 SASA

The SASA is illustrated in Figures 6a and 6b. The plot reveals that atoms exhibit a stiff and condensed structure, and they are more stable in the vicinity of the solvent's surface area. There is no substantial difference in the SASA plot of compound 3 and linezolid. For compound 3, the value initially dips below 50 nm², but then achieves a consistent level of 45 nm² until the completion of the experiment. Likewise, linezolid decreases below 35 nm² and is stable down to the end of the simulation.

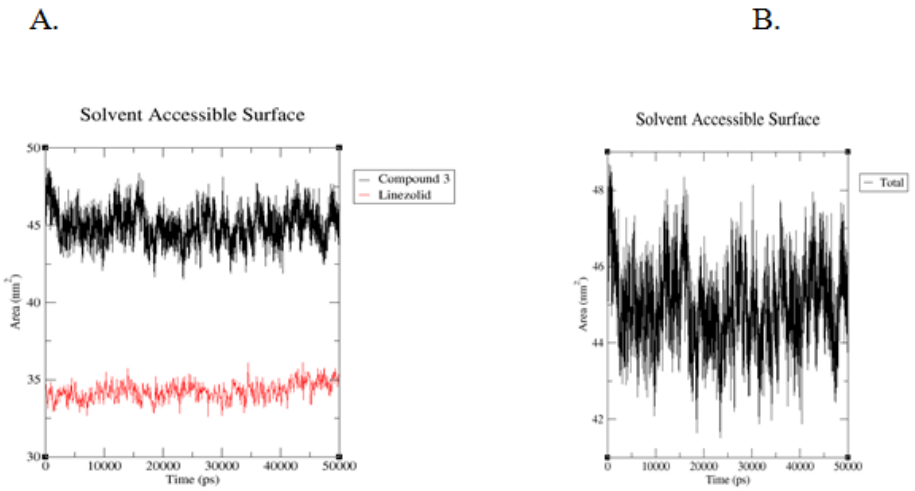



**Fig. 6: Showing the SASA plot of (A.) Protein in complex with Linezolid and Compound 3 and (B) Protein in water.**

### 3.1.5.4 H-bond interaction

Hydrogen bond sites and numbers were evaluated to determine the stability of the protein-ligand complexes during the 50 ns molecular simulation. The g_hbond function in GROMACS was utilized to ascertain the hydrogen bond amount and dispersion of protein-ligand complexes, as done by.[46] Fig. 7 illustrates the intra-protein hydrogen bond graphs for the protein-ligand complexes. The intermolecular hydrogen plot result confirms that the protein-compound 3 combination is stable with a minimum of four hydrogen bonds until the end of molecular simulation, unlike linezolid, which only has two hydrogen bonds.

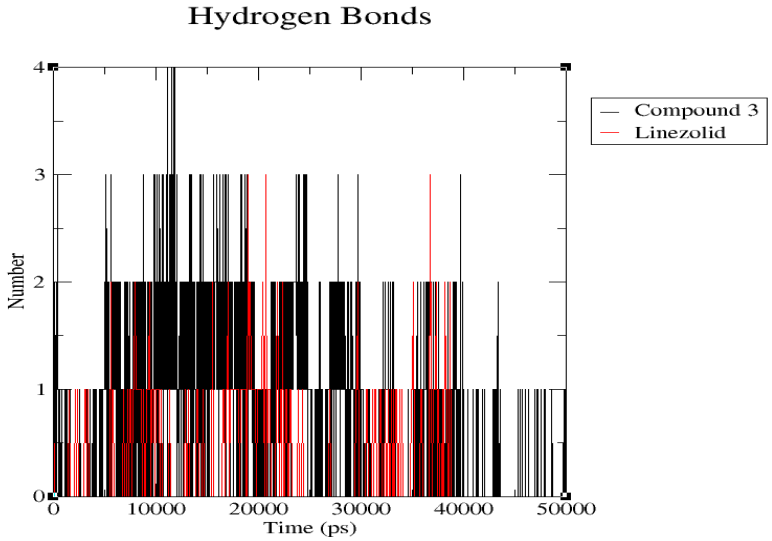



**Fig. 7: Showing the H-bond graph of protein in complex with linezolid and Compound 3.**

## 4.0 CONCLUSION

This study demonstrates a significant advancement in the quest for novel MRSA treatments by highlighting the potential of indenoimidazole derivatives as potent inhibitors of SpA, effectively tackling the issue of MRSA infections. The integration of molecular docking, mechanics, ADMET, and dynamics simulations facilitated the identification of promising compounds with superior binding affinities and good pharmacological profiles. Compound 3 exhibits

exceptional binding affinity, forms strong hydrogen bond interactions, and possesses favorable ADMET properties, making it a highly promising candidate for further investigation. The molecular dynamics simulation also confirms that this binding was in a stable state, establishing the foundation for conducting synthesis, *in vitro*, and *in vivo* experiments of this compound to confirm its effectiveness and safety as a new therapeutic agent against MRSA. This holistic approach not only offers a blueprint for future antimicrobial drug discovery but also reaffirms the critical need for innovative strategies in combating antibiotic resistance. The progress of its clinical evaluation may indicate a major breakthrough in treating antibiotic-resistant infections, thereby highlighting the importance of in silico methods in the discovery and development of drugs.

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