Computational Target-Based Screening of Anti-MRSA Natural Products Reveals Potential Multitarget Mechanisms of Action through Peptidoglycan Synthesis Proteins

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ABSTRACT: Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading causes of bacterial infections in both healthcare and community settings. MRSA can acquire resistance to any current antibiotic, which has major implications for its current and future treatment options. As such, it is globally a major focus for infection control efforts. The mechanical rigidity provided by peptidoglycans in the bacteria cell walls makes it a promising target for broad-spectrum antibacterial drug discovery. The development of drugs that can target different stages of the synthesis of peptidoglycan in MRSA may compromise the integrity of its cell wall and consequently result in the rapid decline of diseases associated with this drug-resistant bacteria. The present study is aimed at screening natural products with known in vitro activities against MRSA to identify their potential to inhibit the proteins involved in the biosynthesis of the peptidoglycan cell wall. A total of 262 compounds were obtained when a literature survey was conducted on anti-MRSA natural products (AMNPs). Virtual screening of the AMNPs was performed against various proteins (targets) that are involved in the biosynthesis of the peptidoglycan (PPC) cell wall using Schrödinger software (release 2020–3) to determine their binding affinities. Nine AMNPs were identified as potential multitarget inhibitors against peptidoglycan biosynthesis proteins. Among these compounds, DB211 showed the strongest binding affinity and interactions with six protein targets, representing three stages of peptidoglycan biosynthesis, and thus was selected as the most promising compound. The MD simulation results for DB211 and its proteins indicated that the protein-ligand complexes were relatively stable over the simulation period of 100 ns. In conclusion, DB211 showed the potential to inhibit six proteins involved in the biosynthesis of the peptidoglycan cell wall in MRSA, thus reducing the chance of MRSA developing resistance to this compound. Therefore, DB211 provided a starting point for the design of new compounds that can inhibit multiple targets in the biosynthesis of the peptidoglycan layer in MRSA.

1. INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most critical etiological agents of both community and hospital-acquired infections.1,2 Strains of β-lactams-antibiotic-resistant bacteria are dangerous and comparatively expensive compared to infections associated with other related infectious agents. For example, a study by Thampi and colleagues investigated the cost analysis of 435 patients admitted with S. aureus bacteremia and reported a total direct medical cost of $12,078. The authors further established that patients affected with MRSA had 1.32 times higher direct costs than those affected with methicillin-susceptible S. aureus (MSSA).3

MRSA-associated infections are responsible for more than 11,000 deaths annually in the United States, and globally, it is one of the leading causes of mortality and morbidity, especially in developing countries.3,4 In addition, the World Health Organization has also included MRSA in the “serious level” among bacteria that globally threaten human health.5 MRSA has evolved with a broad range of strategies to overpower the defense mechanisms of the human host during colonization, causing infections that can result in death.6

Most of the widely used antibiotics against MRSA were designed to block the steps involved in the biosynthesis of peptidoglycan. Peptidoglycan (PPC) is the primary component of the cell wall located outside the cytoplasmic membrane where they confer robustness and flexibility to the cell envelope.7,8 The steps involved in the biosynthesis of peptidoglycan start with the production of UDP-N-acetylmuramyl-pentapeptide (UDP-NAM-pentapeptide) and UDP-N-
acetylglucosamine (UDP-NAG) through a series of sequential reactions catalyzed by the Mur ligases (MurA–MurF). Mur ligases are found in the cytoplasm of the bacteria cell, where they play their essential catalytic roles.9 The second step is the membrane-associated phase, where UDP-GlcNAc is broken down to form UDP-MurNAc by the action of MraY and MurG. In the third step, UDP-MurNAc-pentapeptide is formed by the modification of disaccharide pentapeptide following the addition of the pentaglycine chain to the lysine residue of the pentapeptide. This process is catalyzed by the action of homologous peptidyl transferases specifically, FemA, FemB, and FemX. The last step of this complex process is catalyzed by penicillin-binding proteins (PBPs), and this is when the newly synthesized disaccharide pentapeptide is transferred to the outside of the cell membrane to form linear peptidoglycans.9–11 Figure 1 provides the schematic representation of S. aureus peptidoglycan biosynthesis pathway. All the processes of production of the PPC are essential for the survival of the bacteria,12 and this implies that any of these processes can be explored as potential drug targets in the search for new and effective anti-MRSA drugs.

Over the years, many antibiotics that have been developed for clinical use were designed as single-target drugs. Common examples of these agents include antibacterial like rifampicin, fosfomycin, trimethoprim, sulfonamides, fusidic acid, and popular anticancer agents like carboplatin, adriamycin, and fluorouracil.13,14 Interestingly, this “one-drug, one-target” paradigm has been widely accepted because of its high specificity and fewer off-target side effects, which might result in adverse effects.7,9,15 However, recent developments in drug discovery have shown the limitations of drugs designed with this approach, especially their tendency to promote drug resistance and inability to effectively treat complex diseases and multi-drug-resistant bacterial infections. A recent study on the trend of drug research and development has revealed that multitarget tyrosine kinase inhibitor, imatinib, has a greater anticancer effect than gefitinib which is active against a single target.16 In addition, complex diseases such as cancer, Alzheimer’s disease, and atherosclerosis among others are becoming incurable with the use of single-targeted therapies.13 In an investigation into strategies that can facilitate the development of new therapeutic leads, Oldfield and Feng17 identified compounds that modulate multiple targets as the most promising and logical option to prevent the rise in antibiotic resistance. A recent study has also reported the use of in silico approaches to identify diosmin as a potent inhibitor against multiple protein targets of SARS-CoV-2.18 Since one of the main causes of antibiotic resistance is a mutation in the protein target, the development of multitarget inhibitors can be a potential way to circumvent this bottleneck in antimicrobial drug development.17,19 From an evolutionary perspective, targeting multiple independent pathways appears less likely to allow bacteria to evade antimicrobial drugs and therefore less opportunity to develop drug resistance over time.20

Previous in vitro studies have identified hundreds of natural products with activity against MRSA, but the interaction profiles of most of these compounds with proteins or enzymes (potential drug targets) in MRSA are not known.21 Exploration of the potential of these anti-MRSA natural products to inhibit multiple proteins (targets) in the biosynthesis of the peptidoglycan cell wall might provide good starting points for the design of multitarget, resistant-defiant anti-MRSA lead compounds. In this study, a set of 262 anti-MRSA natural compounds, retrieved through literature search, were screened by docking against key proteins (targets) in the biosynthesis of peptidoglycan cell wall in MRSA. The most promising potential multitarget inhibitors were then selected for molecular dynamics (MD) simulation studies. The identification of compounds that can target different stages of the biosynthesis of peptidoglycan cell wall may result in the
development of a new class of antibiotics against the MRSA bacteria.

2. METHODOLOGY

2.1. Software and Hardware. All the in silico studies were carried out using the software MODELER 10.0 program, Schrödinger Suite (Schrödinger Release 2020–3: Maestro, Schrödinger, LLC, New York, NY, 2020; version 12.2) DataWarrior, and Cytoscape version 3.8.2. The study was performed on a Windows Operating system, with Intel (R) Core (TM) i5–7200U CPU @ 2.50GHX × 64 processors and 8 GB memory. The procedures involved in the studies are described below.

2.2. Data Collection and Preparation. The data sets were made up of the 111 anti-MRSA natural products reported in our previous study. To update the data set, a further literature search, using the same keywords and search strategies described in the study was used to identify other recent studies that reported natural products with activities against MRSA. In all, a sum of 262 anti-MRSA natural products (AMNs) sourced from plants, marine organisms and microorganisms was used in this study. The Simplified Molecular Input Line Entry System (SMILES) structures of the AMNs and their respective bioactivity data were retrieved and stored as text files (Table S1).

2.3. Ligand Preparation. The combined text file of the AMNs was imported and prepared using the LigPrep module of Schrödinger Maestro (Schrödinger Release 2000–3). LigPrep uses a set of rules to correct chiralities, tautomers, and ring conformations in ligands, reducing downstream computational errors. The OPLS3e force field was selected for energy minimization of the ligands because it has the benefit of accurately predicting protein–ligand binding affinities based on past performance. The protonation states were set to a physiologic pH range of 7 ± 2 using the Epik ionization program. All other parameters were kept at default.

2.4. Proteins Involved in the Biosynthesis of Peptidoglycan. The PPC in S. aureus was identified from literature. These include FemA, FemB, MraY, MurA−G, and PBP2 which have been linked with the four stages of peptidoglycan production in S. aureus. The three-dimensional (3D) X-ray crystal structures of FemA, MurB, MurE, and PBP2 were retrieved from the Protein Data Bank (PDB) and then inspected for their active site/binding pockets alongside their cocryrstallized ligands. The 3D crystal structures of the remaining identified proteins were predicted using homology modeling as described below.

2.5. Homology Modeling. The prediction process for homology modeling reported by Webb and Sali was used to search for a suitable template, target-template alignment, model building, and model refinement. The amino acid sequence of the identified proteins without 3D structures was obtained from the UniProt database and subjected to a BLASTp search against the PDB. The templates obtained were selected for each sequence based on sequence similarity (target-template sequence identity) and downloaded from the PDB. Using the MODELER 10.0 program, the target sequences were aligned with the downloaded template sequences and used to build as well as refine the 3D protein models. The best model was selected based on the least discrete optimized protein energy (DOPE) value. The quality and accuracy of the predicted models were further analyzed using SAVES (https://saves.mbi.ucla.edu/), VoroMQA (http://bioinformatics.lit.software/voromqa), and ProSA (https://prosa.services.came.sbg.ac.at/prosa.php) web servers. Each model was also visually inspected to ensure that it comprised the correct portion of the molecule under study.

2.6. Preparation of Target Proteins. The protein preparation step was performed with the protein preparation wizard panel on the Schrödinger Maestro platform (Schrödinger Release 2020–3). Each of the downloaded and modeled 3D protein structures was imported into the Maestro workspace. To ensure structural correctness, missing hydrogen atoms were added, charges, and bond orders were assigned to the crystal structure while heteroatoms and water molecules were deleted. The molecules were energy minimized to relieve steric clashes by applying the default constraint of 0.3 Å root-mean-square-deviation (RMSD) and the OPLS3e force field.

2.7. Grid Generation and Molecular Docking. Before molecular docking, the grid generation panel was used to define a grid box for the binding pocket using the centroid of the cocryrstallized ligands for both MurB and MurE. The binding pockets of the other proteins (without cocryrstallized ligands) were identified using the Computed Atlas of Surface Topography of proteins (CASPt) online server (radius 1.4 Å). The grid boxes were constructed with a size of 20 Å, and the Cartesian xyz-coordinates for the proteins are as follows: FemA (x = 27.42, y = 50.93, z = 82.95), FemB (x = 29.83, y = 50.95, z = 81.15), MraY (x = 163.15, y = 134.05, z = 259.88) MurA (x = 2.80, y = −3.85, z = −32.54), MurB (x = 180.02, y = 148.85, z = 163.35), MurC (x = 41.40, y = 19.36, z = 8.93), MurD (x = 9.92, y = 80.98, z = −1.25), MurE (x = −21.43, y = 3.72, z = 6.34), MurF (x = 15.36, y = 46.78, z = 54.39), MurG (x = −3.54, y = 5.97, z = 6.63), and PBP2a (x = 20.15, y = 30.82, z = 86.37). Next, the molecular docking calculation was set up with the ligand docking panel of the Glide module (using the extra-precision (XP) docking) on the Schrödinger Maestro suite. The output was submitted to a remote cluster machine (https://users.chpc.ac.za/), where the virtual screening was performed. The binding free energy values (kcal/mol), were obtained from the calculation.

2.8. Prime MM-GBSA Calculations. To estimate the relative binding free energy of the interaction, the protein–ligand complexes obtained from the ligand docking were submitted for rescoring using Molecular mechanics combined with Generalized Born Surface Area (MM-GBSA) calculations. The Surface Generalized Born (SGB) model and the variable dielectric (VD) and Optimized Potentials for Liquid Simulations of the Extending Force Field Coverage for Drug-Like Small Molecules (OPLS3e) force field were selected for the analysis while other parameters were kept constant. A more negative value of MM-GBSA free energies implies a more substantial BE. The prioritization of the protein–ligand complexes was based on their MM-GBSA score.

2.9. Molecular Dynamics. The most promising multi-target docked complexes based on the Glide-based docking score and binding interactions were subjected to 100 ns molecular dynamics simulations (MDs) to determine the stability of the interactions. The simulation was carried out by using the Desmond module of Schrödinger suites with the OPL3e force field. Each of the selected protein–ligand interactions was solvated with a predefined three-site transferable intermolecular potential or transferable intermolecular potential 3-point (TIP-3P) water model, and the boundary
condition was made an orthorhombic box shape. The box volume was further minimized, and the overall charge of the system was neutralized by adding Na and Cl ions at a concentration of 0.15 M to mimic physiological conditions. The simulations were run under the Isothermal−Isobaric (NPT) ensemble where temperature and the atmospheric pressure of the system were kept constant at 310 K and 1.013 bar, respectively, with an energy of 1.2. The simulation process was written out on a script and submitted to run on a remote cluster machine (https://users.chpc.ac.za/). The simulation interaction diagram tool of Maestro was used to analyze the trajectories generated after the successful simulations. Finally, the stability of the ligand−protein interaction was evaluated with root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and ligand−protein contacts.

3. RESULTS AND DISCUSSION

This study computationally investigated natural products with reported in vitro anti-MRSA activities as promising multitarget inhibitors of different stages of peptidoglycan biosynthesis. The data sets consist of 262 AMNPs which were obtained from a systematic review as well as 11 protein targets that are key in peptidoglycan biosynthesis. The comprehensive details of the data collection and preparations have been discussed in the “Methodology” section. We present the results and implications of the findings from this study below.

3.1. 3D Model Prediction of Proteins Involved in Biosynthesis of Peptidoglycan

The resolved 3D structures of FemA, MurB, MurE, and PBP2 were retrieved from the RCSB PDB (https://www.rcsb.org/). The 3D structures of the remaining identified proteins were predicted by homology modeling and subsequently validated using different online platforms. The results for the protein structure prediction generated five models for each of the proteins. According to

| proteins | gene ID | DOPE value | ERRAT score (%) | residues in the most favored region (%) | verify 3D (%) | VoroMQA score |
|----------|---------|------------|----------------|------------------------------------------|-------------|---------------|
| FemB     | Q2FYR1  | −36486.84  | 94.02          | 90.01                                    | 80.44       | 0.478         |
| MraY     | Q2FZ93  | −44653.64  | 67.41          | 90.30                                    | 66.04       | 0.380         |
| MurA     | Q2FWD4  | −48761.46  | 78.10          | 92.90                                    | 97.85       | 0.552         |
| MurC     | Q2FXJ0  | −46051.24  | 66.20          | 88.90                                    | 75.06       | 0.393         |
| MurD     | A7X1C4  | −51966.39  | 91.86          | 94.20                                    | 98.63       | 0.539         |
| MurF     | A0A0H2WWP1 | −47688.48   | 65.77          | 86.90                                    | 88.50       | 0.433         |
| MurG     | Q2FYL5  | −38118.98  | 68.96          | 88.40                                    | 81.74       | 0.379         |

Figure 2. Visualization of the 9 top-ranked AMNPs against the different proteins. The pink square markers represent the targets while the red round markers represent the AMNPs. A total of 51 AMNPs were profiled for the 11 targets. The AMNPs connected to their targets by green edges represent compounds that showed the potential to inhibit not more than two stages of the cell wall synthesizing process. All other edges indicate compounds with a strong affinity for at least three stages of the cell wall synthesizing process.
the study by Webb and Sali, the best models were selected based on the least Discrete Optimized Protein Energy (DOPE) value as described in Table 1.

3.2. Quality Assessment and Validation of the Modeled Structures. The program VERIFY3D graph uses a score profile to evaluate the fitness of a modeled structure with the amino acid sequence. A score above zero corresponds to an acceptable environment for the modeled protein. The result of the present study shows that 66.04–98.63% of amino acid residues of the various structures had an average 3D/1D profile score of at least 0.2 (Table 1). The reliability of the modeled structures was also evaluated by using the ERRAT program. An ERRAT score of more than 50% is likely to provide a high-quality model. As shown in Table 1, the ERRAT scores of 65.77–95.02% (all above 50%) were obtained for the models. This implies that the modeled structures are of good quality.

Ramachandran plot analysis also revealed that all the modeled structures have more than 80% residues in the most favorable regions (Table 1) and not more than 1.7% in the disallowed region for each of the modeled structures (Figure S1). Furthermore, quality assessment using the VoroMQA web server revealed a score greater than the 0.3 thresholds across the proteins. The Z-score of all the 3D modeled structures (Figure S2) was also in the range of the native proteins of similar size, implying that they are less erroneous and highly reliable structures. The Z-score checks the 3D model structures for potential errors by measuring the deviation of the total energy of a structure with the energy distribution obtained from random conformations. A Z-score outside the range of scores for native proteins indicates a highly erroneous structure. Overall, all the evaluation and validation tools showed that the selected models are of good and reliable quality. Therefore, they were selected to be utilized for docking calculations below.

3.3. Molecular Docking Simulation. A molecular docking simulation was successfully carried out to evaluate the binding affinity of the 262 AMNPs to the various peptidoglycan proteins. The binding energies (a measure of binding affinity) of the ligands against all the proteins were obtained. Profiling and prioritization of the binding energies based on the MM-GBSA values revealed the top 9 compounds for each of the 11 proteins (Figure 2).

Although none of the compounds showed promising binding affinity for proteins from all the four stages of peptidoglycan syntheses, however, nine ligands including DB145, DB150, DB200, DB211, DB269, DB278, DB307, DB335, and DB350 were prioritized as multitarget compounds due to their high affinity for at least one representative protein from any three of the stages of peptidoglycan biosynthesis. This suggests that the possible mechanisms of action for these compounds might be through binding with multiple proteins to inhibit the peptidoglycan biosynthesis pathways.

Figure 3 describes the chemical structures of these compounds while their estimated binding affinities using the docking scores, ensemble-average MM-GBSA, hydrogen bond
Table 2. Binding Energies (kcal/mol) and Intermolecular Bonds of the Selected Ligands with the Active Sites of the Cell Wall Proteins

| ligands | receptors | dock score (kcal/mol) | XP G-score (kcal/mol) | MM-GBSA ($\Delta$Gtotal kcal/mol) | no. of the H-bonds and the participating residues of other bonds and the participating residues |
|---------|-----------|-----------------------|-----------------------|-----------------------------------|-------------------------------------------------|
| DB145   | FemA      | −6.601                | −6.601                | −45.822                           | FemA, Tyr77, Thr152, Ser153, Asn228 |
|         | FemB      | −7.743                | −7.743                | −54.455                           | Arg34, Tyr70, Thr152, Ser153, Asn228 |
|         | MrA       | −6.192                | −6.192                | −59.339                           | Asn102 |
|         | MurA      | −4.635                | −4.635                | −46.493                           | Asp126, Thr144 |
|         | MurB      | −13.483               | −13.483               | −69.224                           | Val199, Gly81, Ser82, Asn83, Arg225 |
|         | MurC      | −8.756                | −8.756                | −59.742                           | His263, Tyr260, Asn267, Lys296, Arg318 |
|         | MurE      | −8.639                | −8.639                | −66.193                           | Ala150, Asn151, Thr152, Arg187, Glu382, Arg383, Lys470 |
|         | MurF      | −10.531               | −10.531               | −69.345                           | Ile31, Asn337, Thr340, Leu366 |
|         | FemA      | −7.23                 | −7.23                 | −72.039                           | Lys33, Tyr38, Leu153 |
|         | FemB      | −5.042                | −5.042                | −49.513                           | Arg34, Asp37, Pro63, Thr152 |
|         | MrA       | −5.00                 | −5.00                 | −53.468                           | Asn88, Glu91, Thr145 |
|         | MurC      | −7.673                | −7.673                | −71.866                           | Thr113, Ser114, Asn267, Gly294, Glu319, Tyr260 |
|         | MurE      | −7.735                | −7.735                | −59.663                           | Thr111, His205, Glu382, Arg383, Tyr462, His468 |
|         | MurG      | −6.199                | −6.199                | −56.023                           | Ser263, Glu268, Asp289, Glu290 |
|         | MurB      | −8.667                | −9.9                  | −60.864                           | Ser82, Ser143, Gly153 |
|         | MurC      | −6.801                | −7.145                | −56.103                           | Asn267, Glu259, Lys296 |
|         | MurF      | −8.72                 | −9.064                | −74.392                           | Asn49, Asn140, Ser338, Thr340 |
|         | MrA       | −6.783                | −7.127                | −63.071                           | Asn166 |
|         | PBP       | −3.729                | −4.073                | −30.305                           | Thr446 |
|         | FemA      | −9.867                | −10.761               | −57.477                           | Pro151, Glu154, Thr328 |
|         | MurA      | −5.89                 | −6.544                | −47.405                           | Cys118, Asp126, Glu141, Thr144 |
|         | MurD      | −7.775                | −8.429                | −56.924                           | Asn114, Lys116, Asn139, Glu158, Asp183 |
|         | MurE      | −10.641               | −11.535               | −57.132                           | His181, Asp207, Tyr351, Gly640 |
|         | MurF      | −10.17                | −11.064               | −71.140                           | Ile31, Asn140, Asn143 |
|         | PBP       | −7.481                | −9.335                | −42.626                           | Glu460, Thr582, Asp586, Ser643 |
|         | FemA      | −12.073               | −12.101               | −46.549                           | Lys33, Glu36, Asp221, Arg228, Val252 |
|         | MurG      | −10.958               | −10.986               | −62.08                            | His14, Gly198, Lys200, Ser263, Glu319, Lys385 |
|         | MrA       | −6.069                | −6.098                | −30.822                           | Glu447, Glu457, Gly603, Lys851, Glu853 |
|         | MurB      | −3.376                | −4.608                | −45.724                           | Arg94, Arg123, Thr197 |
|         | MurC      | −10.805               | −12.037               | −63.968                           | Asn80, Tyr77, Val199, Leu197 |
|         | MurD      | −6.359                | −6.702                | −56.974                           | His110, Glu259 |
|         | PBP       | −5.885                | −6.228                | −52.169                           | Ser263, Glu268 |
|         | FemA      | −3.233                | −3.576                | −29.708                           | Thr446, Glu447 |
|         | MurA      | −4.585                | −4.597                | −43.807                           | Asn30, Asp37, Tyr70 |
|         | MurB      | −1.17                 | −1.182                | −46.382                           | Asp126 |
|         | MurC      | −6.772                | −7.826                | −66.334                           | Asn49 |
|         | MurD      | −5.114                | −5.167                | −52.321                           | Ser196, Ser263 |
|         | FemA      | −2.396                | −2.835                | −59.167                           | Leu153 |
|         | MurA      | −3.056                | −3.882                | −58.176                           | Asp224, Asn228 |
|         | MurB      | −1.758                | −2.197                | −48.771                           | Cys118 |
|         | MurC      | −3.58                 | −4.406                | −56.035                           | Asp126, Glu141 |
|         | MurD      | −4.626                | −5.066                | −56.231                           | Asn139 |
|         | MurE      | −3.471                | −3.911                | −56.907                           | Thr28 |
|         | MurG      | −1.879                | −2.705                | −54.320                           | Thr164, Asn264 |

\(^{a}\)Amino acid residues with superscripts represent the number of hydrogen atoms participating in the interaction. Amino acids residues with numbers represent the total residues involved in the interaction.

interactions, pi–pi stacking interactions, pi–cation, and the salt bridge formation are shown in Table 2.

Based on the free binding energies (BE), DB211 was identified as the most promising multitarget compound. As shown in Table 2, the Glide score of DB211 was $-10.761$ kcal/mol for FemA, $-5.44$ kcal/mol for MurA, $-8.429$ kcal/mol for MurD, $-11.535$ kcal/mol for MurE, $-11.064$ kcal/mol for MurF, and $-9.335$ kcal/mol for PBP. These proteins also
showed MM-GBSA values ranging from $-42.626$ to $-71.140$ kcal/mol across the proteins.

The binding interactions displayed in Figure 4 reveal that the docked DB211 interacted with binding site residues, Pro151, Gln154, and Try328 from FemA (six hydrogen bonds). Two ammonium groups from the ligand also formed two pi−cation interactions with the amino acid residues, Phe149 and Phe224. Similarly, each of the negatively charged residues, Asp126 and Glu141 from the binding sites of MurA, exhibited 2 hydrogen bonds, and a salt bridge interaction with the positively charged ammonium ion from the ligand DB211, while the residues Cys118 and Thr144 formed two hydrogen bond interactions. The docking interactions between DB211 and MurD revealed the involvement of the amino acid residues, Asn114, Lys116, Asn139, Glu158, and Asp183, in hydrogen bond interactions. In addition, Glu158 and Asp183 residues were involved in the formation of two salt bridges with the positively charged ammonium ion from the ligand. The complex DB211−MurE produced five hydrogen bonds via the residues, His181, Asp207, Tyr351, and Glu460. Apart from hydrogen bonds interactions, an ammonium ion participated in a salt bridge interaction with the negatively charged Asp207, and there was also the formation of pi−cation with the positively charged residue, His353 form MurE. MurF used the amino acids, Ile31, Asn140, and Asn143 to interact with DB211 through four hydrogen bonds. The interaction was also supported by a salt bridge bonding that was formed with the ligand by the residue, Asp32. Finally, DB211 interacted with the PBP protein by forming six hydrogen bonds with the positively charged ammonium ions from the protein, and positively charged ammonium ions from the ligand.

The hydrogren bond is essential for determining the binding strength between protein−ligand complexes. A hydrogen bond distance of more than 3.5 Å can decrease the binding affinity by producing unstable ligand−protein interactions in the binding pocket.\textsuperscript{34} The salt bridge interactions also play an important role in stabilizing the protein structure.\textsuperscript{36}
ingly, the hydrogen bond distances observed in the findings above were all within the required cutoff of 3.5 Å (Figure 4). The involvement of other noncovalent bonds including the salt bridge interactions implies that DB211 may have a strong binding affinity and stability for driving potent binding with its respective targets. Further investigations were carried out to establish the binding conformation and binding stability of the ligand in the active sites of the respective peptidoglycan targets using molecular dynamics simulation.

### 3.4. Molecular Dynamic Simulation

The use and importance of molecular dynamic simulation in various fields are well established most especially in the study of biomolecular interactions as well as the physical movements of macromolecules such as proteins. The dynamic behavior of the candidate compound (DB211) in the binding pockets of the proteins was investigated over the course of the 100 ns simulation period. The MD parameters including the RMSD, RMSF, and radius of gyration (rGyr) were accessed to ascertain the stability and structural adjustment of DB211 with the selected proteins (Figure 5 and Table 3).

The RMSD of DB211 in the binding domain of the proteins, FemA, MurA, D−F, and PBP is depicted in Figure 5. The complexes MurA−DB211, FemA−DB211, and MurF−DB211 were more stable with mean RMSD values of 2.65 ± 0.25 Å, 3.50 ± 0.53 Å, and 3.84 ± 0.48 Å, respectively. The observed deviation in the DB211−MurD complex was between 20 and 40 ns simulation time after which stability was attained with a 4.56 ± 0.91 Å mean RMSD value. A little rise in RMSD value was noticeable in the MD simulation of the complexes MurE−DB211 and MurD−DB211 (4.06 ± 1.79 Å and 4.56 ± 0.91 Å, respectively). With an RMSD value of 3.50 ± 0.79 Å, the PBP−DB211 complex is ranked the most stable among the three complexes with lower RMSD values. The RMSD is a measure of atomic distances or coordinates, such as the C-alpha protein backbone. The MD trajectory analysis of the complexes showed that the observed interactions from the docking were relatively stable over the simulation period. A reduced RMSD value indicates less deviation from the initial structures of the complexes and good stability. The potential inhibitory activity of DB211 can therefore be hypothesized upon binding to these proteins.

The RMSF of the C-alpha backbones of the proteins in complex with DB211 were measured to evaluate the conformational flexibility of the proteins upon ligand binding.

![Figure 5. MDs analysis of the proteins and the selected compound (DB211) over a course of 100 ns. (A) RMSD of the selected compound (DB211) in the proteins’ active sites. (B) RMSF of the selected compound (DB211) in the proteins’ active sites. (C) Radius of gyration (rGyr) of the complexes.](image-url)

| Protein   | RMSD (Å) ± SD | RMSF (Å) ± SD | rGyr (Å) ± SD |
|-----------|---------------|---------------|---------------|
| FemA      | 3.50 ± 0.53   | 1.62 ± 0.80   | 4.58 ± 0.19   |
| MurA      | 2.65 ± 0.25   | 1.38 ± 0.78   | 4.70 ± 0.08   |
| MurD      | 4.56 ± 0.91   | 1.82 ± 0.83   | 4.50 ± 0.12   |
| MurE      | 4.06 ± 1.79   | 2.41 ± 1.26   | 4.70 ± 0.09   |
| MurF      | 3.84 ± 0.48   | 1.84 ± 0.92   | 4.43 ± 0.14   |
| PBP       | 3.58 ± 0.79   | 2.18 ± 1.06   | 4.72 ± 0.06   |

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The average atomic fluctuations of each of the protein against the residual index is in the order MurA (1.38 ± 0.78) < FemA (1.62 ± 0.80) < MurD (1.82 ± 0.83) < MurF (1.84 ± 0.92) < PBP (2.18 ± 1.06) < MurE (2.41 ± 1.26) (Figure 5B and Table 3). Overall, the results imply that the residues in the ligand-binding regions of FemA, MurD, MurF, and PBP were stable and found within their average values upon ligand recognition during the entire simulation.

The rGyr is associated with the conformational changes and expansion of the systems’ structure during MDs. A larger rGyr value indicates more elongation, while a lower value describes more compactness in the system. In this study, rGyr was used to estimate the compactness of the proteins in complex with DB211. Figure 5C and Table 3 represent the results of the rGyr values for the complexes over the 100 ns simulation period. The increase in these values is in the order of MurF < MurD < FemA < MurA, MurE < PBP (Table 3). The high rGyr values observed in this study (4.4–4.8 Å) may be a result of the conformational changes of the proteins induced by DB211 which could hinder the normal function of the proteins, FemA, MurA, −F, and PBP in biosynthesis of the peptidoglycan cell wall.

4. CONCLUSION

In the present study, virtual screening and docking calculations were used to identify potential AMNPs that can serve as a multitarget inhibitor of peptidoglycan proteins. Based on the docking results, compounds DB145, DB150, DB200, DB211, DB269, DB278, DB307, DB335, and DB350 were prioritized because of their strong binding affinity for at least two of the proteins involved in the peptidoglycan cell wall biosynthesis pathway. Among them, DB211 was identified as the most promising compound as it shows strong free binding energy and interactions with six protein targets that are representative of three stages of peptidoglycan cell wall biosynthesis. The MD simulation further confirmed the stability of the DB211–protein complexes and the potential conformational changes induced by the ligand, DB211. Overall, DB211 may be a hit compound with the potential to disrupt the normal functions of these proteins that are involved in the biosynthesis of the peptidoglycan cell wall. This study provided insight into the development of a highly efficient multitarget lead compound. Our future work will use DB211 as a template to find analogues with better binding affinities for the identified proteins and optimizes such analogues into lead compounds. In vitro and in vivo assays will be used to ascertain the potency and multitarget effects of DB211 and its structurally related analogues on the peptidoglycan proteins.

ASSOCIATED CONTENT

Data Availability Statement

All data can be found within the manuscript and the Supporting Information. Web servers such as CASTp (http://sts.bioe.uic.edu/), Open access software such as MODELER 10.0 program, DataWarrior (v 5.5.0), and Cytoscape (v 3.8.2), as well as commercial software, Schrödinger drug discovery suite (Schrödinger Release 2020–3 v 12.2) were used in the study.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05061.

Author Contributions

S.O.O.: conceptualization, methodology, software, writing-original draft, editing. A.O.F.: conceptualization, methodology, software, writing-original draft, reviewing and editing. G.J.W.: conceptualization, supervision, reviewing and editing. G.J.W.: reviewing and editing.

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Notes

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ABBREVIATION

MRSA Methicillin-resistant Staphylococcus aureus
AMNPs Natural products with known in vitro activities against MRSA
PPC Peptidoglycan
MD Molecular dynamic
BE Binding energy
MSSA Methicillin-susceptible Staphylococcus aureus
PBPs Penicillin-binding proteins
SMILES Simplified molecular input line entry system
PDB Protein Data Bank
DOPE Discrete optimized protein energy
RMSD Root-mean-square-deviation
CASTp Computed atlas of surface topography of proteins

List of the ligands, natural products with known in vitro activities against MRSA (AMNPs) as compiled from the literature; Ramachandran plot analysis of the modeled 3D structures; plot showing the overall model quality, Z-scores (PDF)
MM-GBSA Molecular mechanics combined with Generalized Born Surface Area
XP Extra precision
VD Variable dielectric
TIP-3P Three-site transferable intermolecular potential
RMSF Root-mean-square fluctuation

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