Virtual Screening of neisserial heparin binding antigen to elucidate the promising candidates against *Neisseria meningitidis* infection

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ABSTRACT

Background: *Neisseria meningitidis*, or meningococcus, is a bacterial pathogen causing severe infections, notably bacterial meningitis. Antibiotics, such as ceftriaxone and cefotaxime, play a crucial role in treating meningococcal infections. However, antibiotic resistance varies globally. This study aimed to investigate the potential therapeutic strategies against *N. meningitidis* by identifying bioactive compounds from marine sponges targeting neisserial heparin-binding antigen (NHBA) using virtual screening.
Methods: The NHBA receptor complex (PDB ID: 5O1R) and ligands, including marine sponge-derived compounds, were obtained. Protein preparation involved removing ligands and water molecules. Molecular docking used PyRx, converting structures to .pdbqt format. Protein-ligand interactions were analyzed, and binding energies were evaluated. Enrichment analysis was performed using ShinyGO 0.77 for biological processes (BPs), molecular functions (MFs), and KEGG signaling pathways.
Results: Virtual screening identified alisiaquinone C as a potential inhibitor with favorable binding affinity to NHBA. 2D and 3D visualizations illustrated dynamic interactions, and ShinyGO analysis revealed associated pathways.
Conclusion: This study contributed to developing anti-meningococcal agents by exploring marine sponge-derived compounds targeting NHBA. Alisiaquinone C emerged as a noteworthy candidate with high binding affinity to the NHBA receptor. The integration of computational tools and enrichment analysis provides insights into potential therapeutic pathways, emphasizing the relevance of natural products in drug discovery. The potential alisiaquinone C warrants further investigation for infectious disease therapeutics.

Keywords: *Neisseria meningitidis*, neisserial heparin-binding antigen, bioactive compounds, virtual screening

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INTRODUCTION

Neisseria meningitidis, commonly known as meningococcus, is a bacterium that can cause serious infections in humans [1]. It is a leading cause of bacterial meningitis, which is the inflammation of the protective membranes covering the brain and spinal cord. Additionally, *N. meningitidis* can cause bloodstream infections (septicemia) [2]. The bacterium is classified into multiple serogroups based on the characteristics of its outer membrane proteins, with serogroups A, B, C, W, X, and Y being the most clinically significant [3].
Antibiotics play a crucial role in inhibiting *N. meningitidis*, the bacterium responsible for causing meningococcal disease [4]. Effective antibiotic treatment, such as ceftriaxone and cefotaxime, is vital in preventing the progression of the infection, reducing complications, and limiting the spread of the bacterium [5,6]. These third-generation cephalosporins are commonly used as first-line antibiotics for the treatment of meningococcal infections [7]. They have a broad spectrum of activity and are effective against *N. meningitidis* [8]. However, resistance rates vary geographically and by bacterial species [9]. Some regions report higher levels of cefotaxime resistance, possibly due to its earlier and wider use. Ceftriaxone still exhibits lower resistance overall, likely due to its pharmacokinetic advantages.
This study aimed to contribute to the development of potential candidates in therapeutic strategies against *N. meningitidis* infections by identifying bioactive compounds from marine sponges that target neisserial heparin binding antigen (NHBA). The virtual screening approach allows for the efficient screening of a large number of compounds, accelerating the drug discovery process. The findings from this research could pave the way for the development of novel anti-meningococcal

agents with improved efficacy and reduced adverse effects, addressing a critical need for effective treatments against this life-threatening pathogen.

MATERIALS AND METHODS

Materials

The neisserial heparin-binding antigen (NHBA) receptor complex, identified with the ID code 5O1R [10], was obtained from the Protein Data Bank Repository (PDB), and the corresponding files were downloaded in .pdb format. Simultaneously, a 3D conformer file of the antibiotic ceftriaxone was generated, derived from the original ligand 1,2-ethanediol (EDO) within the 3D protein structure. Moreover, 23 ligand files, encompassing compounds like 2,2-bis(6-bromo-1H-indol-3-yl)ethanamine, 3,5-dibromo-2-(2,4-dibromophenoxy)phenol, 4-hydroxybenzoic acid, 5-epi-Ilimaquinone, 6-hydroxyavarol, alisiaquinol, alisiaquinone A, alisiaquinone B, alisiaquninone C, clethric acid, hamigeran B, manoalide, manzamine A, etachromin A, motualevic acid A, motualevic acid E, motualevic acid F, norlichexanthone, oroidin, psammaplin A, stachybogrisephenone B, stelletin A, and tirandamycin, were sourced from PubChem [11] and saved as .sdf format.

Preparation of proteins and virtual screening

After eliminating initial ligands and water molecules using Discovery Studio Visualizer [12], the protein's .pdb files undergo crucial steps in preparation for molecular docking with PyRx [13]. These steps encompass obtaining the protein structure in a compatible format, loading it into PyRx, excluding water molecules, adding missing residues when required, introducing hydrogen atoms, assigning atom types, optimizing the structure, and ultimately saving the prepared protein structure.

Molecular docking analysis

The PyRx software was employed to convert the protein and ligand into .pdbqt format for subsequent molecular docking simulations. To conduct these simulations, follow these steps: Begin by retrieving the protein structure from a database like the Protein Data Bank (PDB) and importing it into PyRx. Prepare the protein by removing water molecules, addressing any missing residues, and introducing hydrogen atoms. Optionally, refine accuracy by assigning atom types and optimizing the structure. Save the prepared protein structure in PDB or PDBQT format. Subsequently, obtain the ligand structure from a chemical database or generate it computationally, ensuring compatibility with PDB or SDF formats. Load the ligand into PyRx, add necessary hydrogen atoms, assign atom types, and convert to PDBQT format.

Protein and ligand interaction analysis

To conform to .pdb file standards, the integration of protein and ligand docking data was carried out. The PyRx program played a crucial role in this data integration process, ensuring a uniform and coherent representation for subsequent analyses. Additionally, PyMOL was employed for three-dimensional structure visualization, allowing for a detailed exploration of spatial arrangements, binding interfaces, and conformational changes [14]. Furthermore, the binding energy (ΔG) value was utilized to quantify the strength of interaction between the ligand and the target in molecular docking. Inhibition constants (Ki) were also calculated to determine how tightly a ligand binds to a target receptor using the formula $Ki = e^{-RT/\Delta G}$.

Protein-protein interaction network

To comprehend the protein-protein interaction network within the KEGG pathway and explore gene functional enrichment, biological processes, molecular functions, and cellular components were incorporated into gene ontology (GO). The ShinyGO 0.77 web server (http://bioinformatics.sdstate.edu/go/) was employed for this purpose, aiming to predict potential therapeutic pathways. Through this analysis, the identified proteins interacting with these compounds were unveiled, presenting opportunities for the exploration of novel treatment options.

RESULTS

Protein and ligand interaction

The analysis employed a PyRx gridbox, which functions as a user-friendly interface for configuring a customized receptor docking gridbox for molecular docking purposes. Figures 1 and 2 depicted the binding energy and inhibition constant of the interaction between the neisserial heparin-binding antigen (NHBA) receptor complex and the ligand inhibitors. Among the 23 compounds examined, alisiaquinone C exhibited the most favorable binding affinity to the NHBA receptor. To validate the docking results, the original ligand 1,2-ethanediol (EDO) extracted from the 3D structure of the protein-ligand complex was utilized.

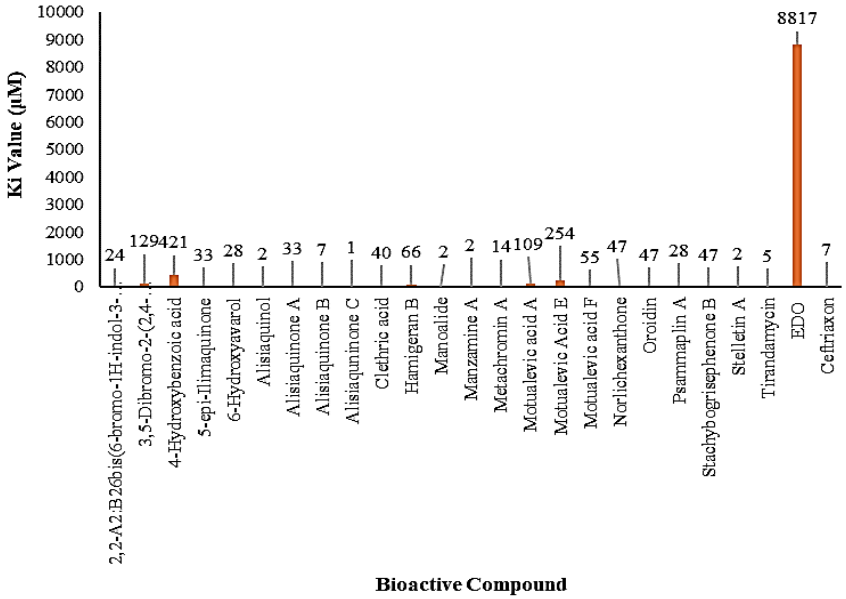

Figure 1. The inhibition constant (Ki) value of marine sponge-derived natural compounds with the neisserial heparin-binding antigen (NHBA) receptor.

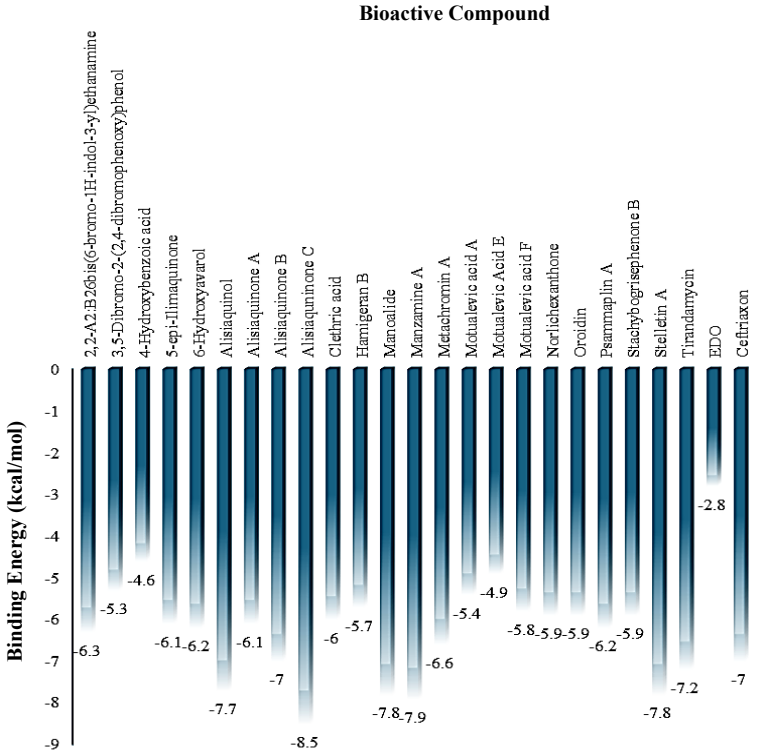

Figure 2. The binding energy evaluation of natural compounds obtained from marine sponges with the neisserial heparin-binding antigen (NHBA).

Figure 3 presents a 2D visualization of receptor-ligand interactions, performing the complexes between diverse entities. Figure 3A illustrates the dynamic interaction between the receptor of the neisserial heparin-binding antigen (NHBA) and alisaquinone C. This interaction involves six hydrogen bonds with Tyr-407, Gly-416, Gly-415, Arg-408, Lys-414, His-327. In Figure 3B, a detailed representation unfolds, revealing the interplay between the NHBA receptor and the

antibiotic agent ceftriaxon, with six hydrogen bonds involving Lys-414, His-327, Thr-328, Tyr-407, Gly-416, Phe-417. Meanwhile, Figure 3C captured the nuanced interaction between the NHBA receptor and ligand EDO. This provides a comprehensive perspective on the molecular relationships within the system, incorporating four hydrophobic interactions with Glu-322, Ala-421, Val-419, Leu-324. Furthermore, Figure 4 showed The 3D image of interactions complexes between: A. neisserial heparin binding antigen (NHBA) receptor and alisaquinone C, B. NHBA receptor and ceftriaxon drug, C. NHBA receptor and the original ligand.

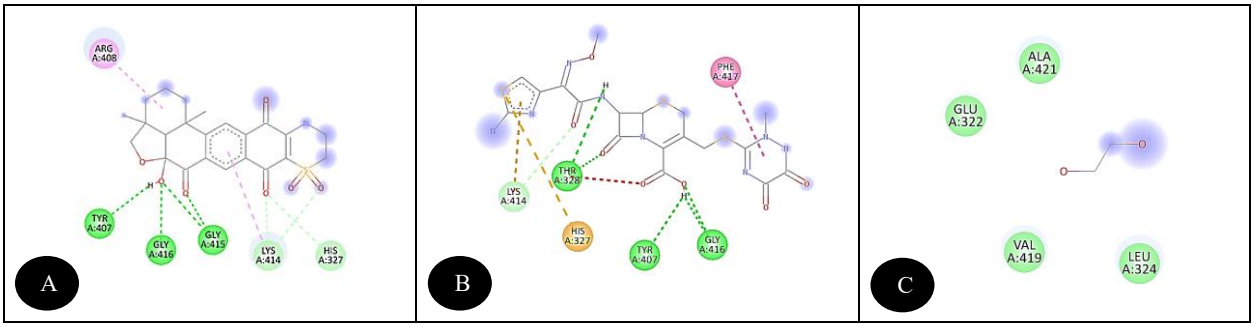

Figure 3. The 2D image of interactions complex between: A. neisserial heparin binding antigen (NHBA) receptor and alisaquinone C, B. NHBA receptor and ceftriaxon drug, C. NHBA receptor and the original ligand.

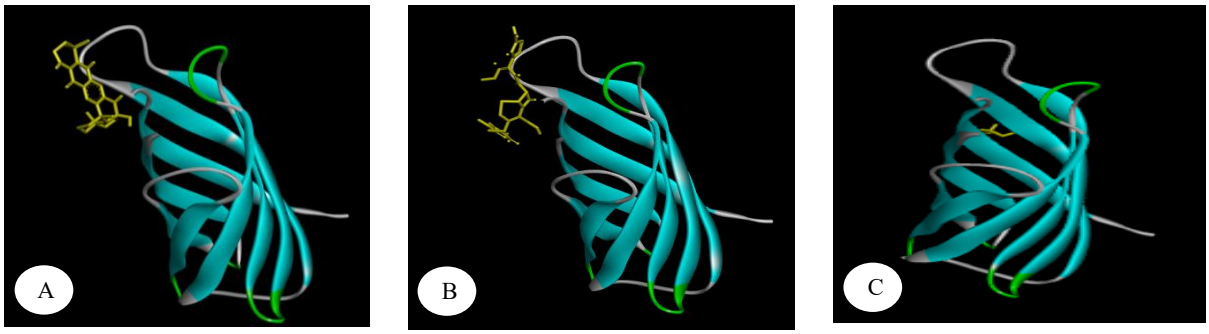

Figure 4. The 3D image of interactions complex between: A. neisserial heparin binding antigen (NHBA) receptor and alisaquinone C, B. NHBA receptor and ceftriaxon drug, C. NHBA receptor and the original ligand.

Protein-protein interaction network

The identified core target proteins underwent subsequent enrichment analysis utilizing the ShinyGO 0.77 protein-protein interaction network. Our emphasis was on enriching the analysis across biological processes (BPs), molecular functions (MFs), cellular components (CCs), and signaling pathways. The interactive network plot visually depicted the relationships within enriched pathways. Nodes connecting two pathways shared 20% or more genes by default. Darker nodes indicated more significantly enriched gene sets, while larger nodes represented larger gene sets. Thicker edges denoted more overlapped genes (Figure 5).

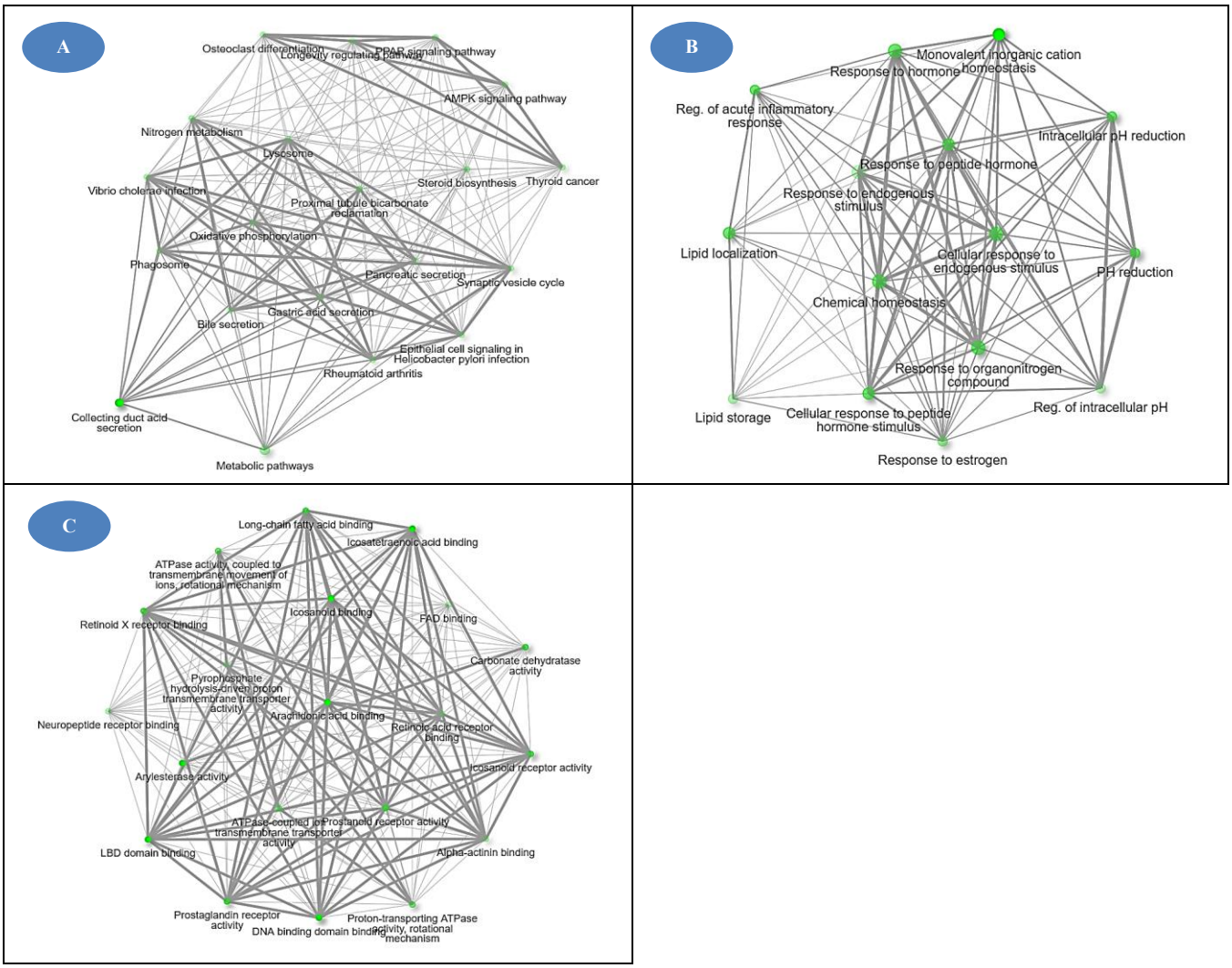

Figure 5. The enriched networks of A. KEGG Pathways, B. Biological Processes (BPs), and C. Molecular Functions (MFs).

DISCUSSION

The exploration of targeting the neisserial heparin-binding antigen (NHBA) in combatting *Neisseria meningitidis* infection presents a promising avenue for the development of novel therapeutic strategies [15]. The assessment of binding energy and inhibition constants between the NHBA receptor complex and various ligand inhibitors, as depicted in Figures 3 and 4, sheds light on potential candidates for intervention. Among the diverse compounds scrutinized, alisiaquinone C emerged with the most favorable binding affinity to the NHBA receptor, suggesting its potential as an effective inhibitor against Neisseria meningitidis. The validation of the docking results, utilizing the original ligand 1,2-ethanediol (EDO) extracted from the 3D structure of the protein-ligand complex, enhances the credibility of the findings. This step ensures that the molecular interactions observed are consistent with known ligand-receptor relationships, bolstering the reliability of alisiaquinone C as a promising candidate for further investigation.

The 2D visualization of receptor-ligand interactions in Figure 3 provides a detailed insight into the molecular dynamics of the system. Figure 3A highlights the dynamic interaction between the NHBA receptor and alisiaquinone C, involving six hydrogen bonds with key residues such as Tyr-407, Gly-416, Gly-415, Arg-408, Lys-414, and His-327. Figure 3B unfolds the intricate interplay between the NHBA receptor and the antibiotic agent ceftriaxone, forming six hydrogen bonds with specific residues including Lys-414, His-327, Thr-328, Tyr-407, Gly-416, and Phe-417. Meanwhile, Figure 3C captures the nuanced interaction between the NHBA receptor and ligand EDO, providing a comprehensive perspective on the molecular relationships within the system. This interaction involves four significant hydrophobic interactions with Glu-322, Ala-421, Val-419, and Leu-324. The 3D images of interaction complexes in Figure 4 further complement the understanding, offering a spatial depiction of the binding relationships between the NHBA receptor and key ligands: alisiaquinone C, ceftriaxone, and the original ligand. These visualizations provide a structural context for the observed interactions,

aiding in the interpretation of the binding modes and the potential therapeutic relevance of the identified compounds.

To contextualize these findings, it is imperative to consider related studies that emphasize the importance of NHBA in *Neisseria meningitidis infection*. Vaccine candidates against serogroup B meningococcus emphasize the significance of NHBA in the development of potential interventions [16]. Additionally, A universal vaccine for serogroup B meningococcus, further highlighting the relevance of NHBA as a potential target [17].

The enrichment analysis using ShinyGO 0.77 chart in Figure 5 adds another layer to the discussion, focusing on the biological processes (BPs), molecular functions (MFs), cellular components (CCs), and signaling pathways associated with the identified target proteins. This comprehensive approach provides insights into the broader functional implications of targeting NHBA and offers potential clues for therapeutic intervention. The multi-faceted approach, combining binding energy assessments, molecular dynamics analyses, and enrichment studies, positions alisiaquinone C as a promising candidate for targeting NHBA in combatting *N. meningitidis* infection. The structural insights provided by the 2D and 3D visualizations, along with the enriched pathway analyses, contribute to a comprehensive understanding of the molecular landscape, paving the way for further exploration and potential drug development.

CONCLUSION

This research advanced the development of anti-meningococcal agents through the exploration of compounds derived from marine sponges that target NHBA. Notably, Alisiaquinone C surfaced as a promising candidate, exhibiting significant binding affinity to the NHBA receptor. The incorporation of computational tools and enrichment analysis yielded insights into potential therapeutic pathways, underscoring the importance of natural products in drug discovery. The considerable potential exhibited by Alisiaquinone C necessitates further investigation for its applicability in infectious disease therapeutics.

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