IN SILICO ANALYSIS OF MANZAMINE A AS A POTENTIAL INHIBITOR OF HEPATITIS C VIRUS NS5B POLYMERASE

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Abstract

Background: Bioactive compounds isolated from marine sponges such as the genera *Haliclona* and *Pellina* have garnered significant attention for their diverse pharmacological, have shown notable antiviral activities. This study employed molecular docking techniques to elucidate the potential interactions between 20 bioactive compounds from marine sponges and hepatitis C virus polymerase. Results from the molecular docking simulations provide valuable insights into the binding modes of manzamine A with the HCV polymerase receptor, exploring potential hydrogen bonding, hydrophobic interactions, and other molecular forces contributing to the stability of the ligand-protein complex. **Methods:** The retrieval and preparation of crystal structures or reliable models of HCV polymerase receptors as target proteins. The protein structures are optimized, and ligand-binding sites are identified. 20 Ligands were subjected to ligand preparation, ensuring its three-dimensional conformation is suitable for docking simulations. The docking studies, conducted using computational tools, focus on understanding the binding affinity, orientation, and potential interactions between 20 bioactive compounds and the target protein. **Results:** Among the compounds tested, manzamine A exhibited the most favorable binding affinity to the receptor, showcasing its superiority over the ribavirin antiviral drug. **Conclusion:** The comprehensive molecular docking analysis underscores the notable binding affinity of Manzamine A with the hepatitis C virus polymerase receptor, surpassing that of the ribavirin antiviral drug. The detailed insights provided into the structural interactions of manzamine A, ribavirin, and ligand 698 contribute to our understanding of potential antiviral mechanisms. These findings lay the groundwork for further experimental validations and highlight manzamine A as a promising candidate for future antiviral drug development.

Keywords: Hepatitis C virus ; marine sponges ; computational docking ; manzamine A ; drug discovery

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global health concern, affecting an estimated 71 million people worldwide [1]. It is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC), accounting for nearly 1.5 million deaths annually [2]. The World Health Organization (WHO) has set a goal of eliminating HCV as a public health threat by 2030, with a target of reducing HCV-related deaths by 90% and reducing new HCV infections by 80% [3].

In silico studies have emerged as a powerful tool for identifying potential inhibitors against hepatitis C virus (HCV), offering a rapid and cost-effective approach to drug discovery [4]. By leveraging computational methods, the vast libraries of compounds can be screened to pinpoint molecules with promising antiviral activity. This approach has yielded several promising drug candidates. One promising drug candidate is spongosine, a compound derived from marine sponges. Studies have shown that spongosine exhibits inhibitory effects against HCV replication. Its antiviral activity involves interference with the viral life cycle, particularly during the stages of viral RNA replication [5]. Other studies showed that avarol, which is derived from the marine sponge *Dysidea avara*, has also demonstrated inhibitory effects against HCV replication [6].

The aim of this study is to utilize molecular docking techniques to identify potential inhibitors of HCV from marine sponges. Marine sponges have long been recognized as a rich source of bioactive compounds with diverse pharmacological properties. Given the antiviral potential of marine natural products and the limitations of current HCV treatment options, exploring marine sponges as a source of novel HCV inhibitors holds significant promise.

Molecular docking simulations have also been employed to elucidate the binding interactions between potential inhibitors and HCV proteins, providing valuable insights into their mechanisms of action [7]. This approach could simulate the

interaction between a ligand and a target protein. By evaluating the binding affinity and energy of the ligand-protein interaction, molecular docking can predict the likelihood of a compound inhibiting the target protein and its potential efficacy as a therapeutic agent. In this study, we will employ molecular docking to screen a library of marine sponge-derived compounds against HCV proteins known to play crucial roles in viral replication. The docking results will then be analyzed to select the most promising compounds for further investigation. The successful identification of HCV inhibitors from marine sponges could lead to the development of novel and effective therapeutic agents against this prevalent and debilitating disease.

MATERIALS AND METHODS

Materials

The receptor complex of hepatitis C virus polymerase receptor was obtained from the Protein Data Bank Repository (PDB) with the identifier: 2JC1 [11]. The corresponding files, in .pdb format, were downloaded. Additionally, a 3D file conformer of the commercial drug ribavirin and 23 ligand files including: 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; tirandamycin were downloaded from PubChem [8]. These ligand files were in .sdf format.

Protein Preparation and Virtual Screening

The .pdb files of the protein, after removing the initial ligands and water molecules with Discovery Studio Visualizer. Preparing a protein structure for molecular docking with PyRx involves several crucial steps to ensure the protein's suitability for docking simulations [9]. These steps include obtaining the protein structure in a compatible format, loading it into PyRx, removing water molecules, adding some missing residues if necessary, adding hydrogen atoms, assigning atom types, optimizing the structure, and saving the prepared protein structure. By following these steps, researchers can ensure that their protein structures are adequately prepared for accurate and reliable docking simulations.

Molecular Docking

The protein and ligand were prepared using PyRx Tools software and converted into .pdbqt format. To perform molecular docking simulations, follow these steps: Begin by obtaining the protein structure from a database like the Protein Data Bank (PDB) and load it into PyRx. Prepare the protein by removing water molecules, adding missing residues, and incorporating hydrogen atoms. Optionally, assign atom types and optimize the structure for enhanced accuracy. Save the prepared protein structure in PDB or PDBQT format. Next, obtain the ligand structure from a chemical database or create it computationally, ensuring compatibility with PDB or SDF formats. Load the ligand into PyRx, add hydrogen atoms if needed, assign atom types, and convert to PDBQT format if necessary. Save the prepared ligand structure in a suitable format. These processed structures are then ready for subsequent docking simulations [11]. The evaluation of the interaction strength between the ligand and the target in molecular docking involved the utilization of the binding energy (ΔG) value. To determine inhibition constants (Ki), it was necessary to calculate the affinity with which a ligand binds to a target receptor, employing the following formula: $Ki = e^{-RT/\Delta G}$.

Protein and Ligand Interaction

The formation of protein and ligand docking data was done to adhere to .pdb files. The PyRx program was employed for the data integration process to ensure a standardized and coherent representation for subsequent analyses. Moreover, PyMOL was utilized for a structured 3D visualization, enabling a meticulous exploration of spatial arrangements, binding interfaces, and conformational changes.

RESULTS

Protein and Ligand Interaction

The analysis involved a gridbox on Pyrx which functions as a graphical user interface for defining the receptor docking gridbox tailored for molecular docking. The binding energy and inhibition constant of the complex between the hepatitis C virus polymerase receptor and the ligand inhibitors are shown in Figure 1 and Figure 2. The binding affinity among 23 compounds, manzamine A showed the most favorable binding to the hepatitis C virus polymerase receptor. The original ligand (ligand 698) obtained from protein-ligand complex 3D structure was used to validate the docking.

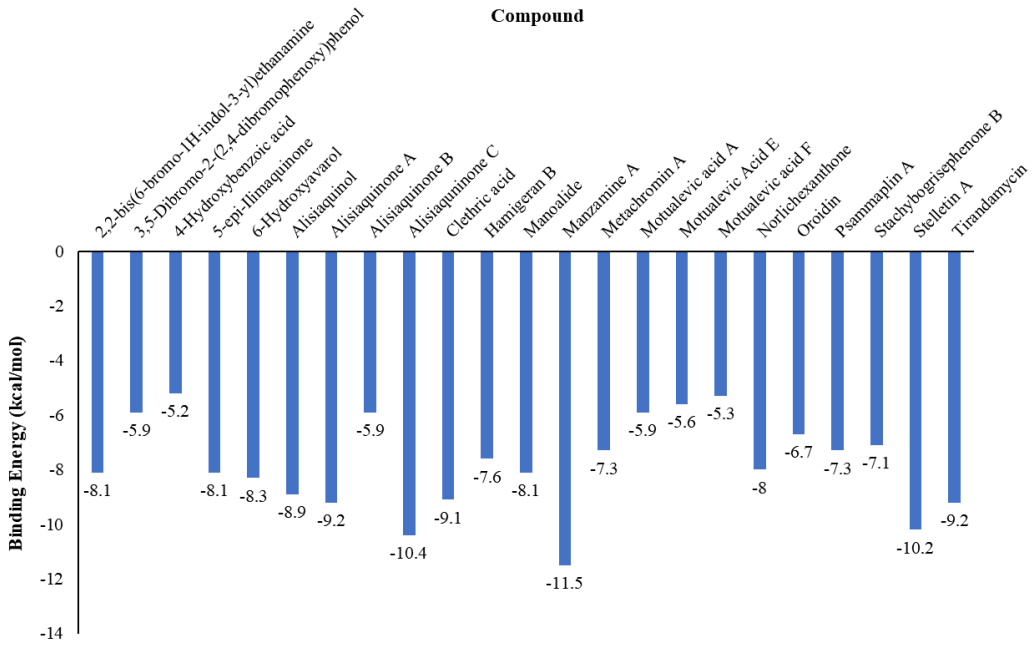

Figure 1: Binding energy between natural compounds from marine sponges and the hepatitis C virus polymerase receptor.

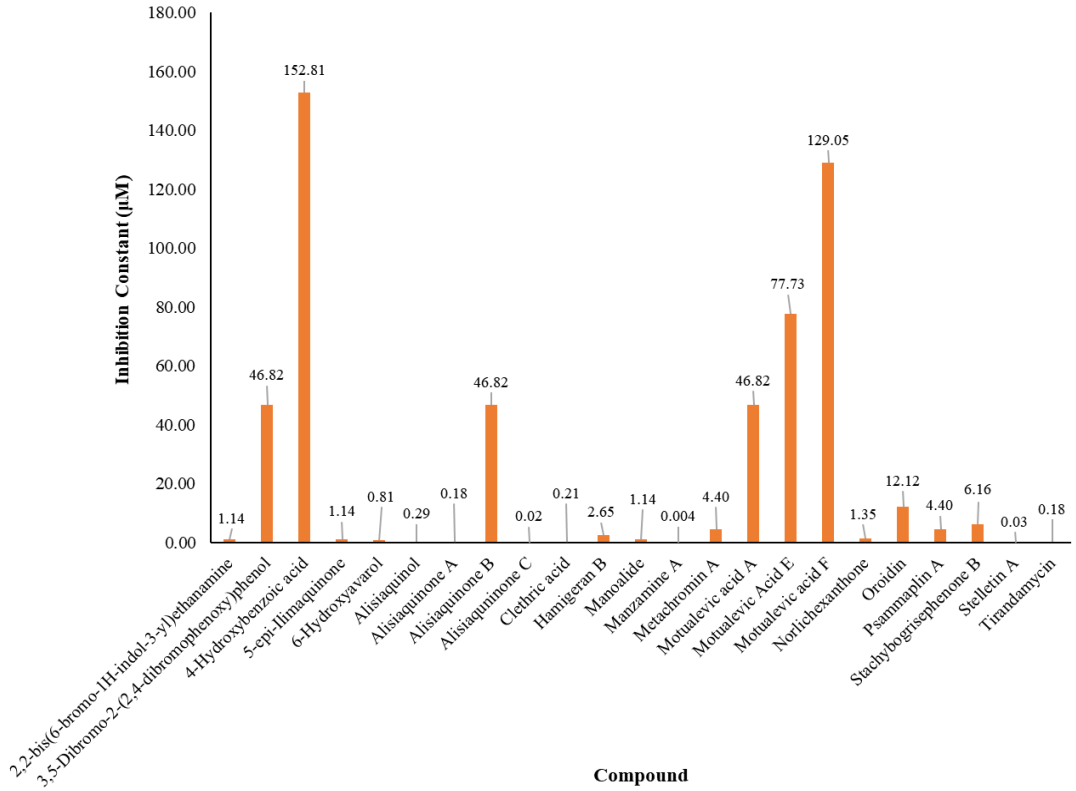

Figure 2: Inhibition constant between natural compounds from marine sponges and the hepatitis C virus polymerase receptor.

The insightful of 2D visualization of receptor-ligand interactions was presented in Figure 3, which has intricated interaction complexes between various entities. Specifically, panel A depicts the dynamic interaction between the receptor of the hepatitis C virus polymerase and manzamine A. The interactions involved hydrophobic interactions with four amino acids of Ala-97, Ile-160, Phe-162, Val-405 and one hydrogen bond with Gln-446. In panel B, a detailed representation unfolds, showcasing the interplay between the receptor of the hepatitis C virus polymerase and the pharmaceutical agent ribavirin by involving three hydrogen bonds with Ser-407, Trp-397 and Val-144. Lastly, panel C captures the nuanced interaction between the hepatitis C virus polymerase receptor and ligand 698, providing a comprehensive perspective on the molecular relationships within the system by involving four hydrophobic interactions with Arg-386, Cys-366, Leu-384, Met-414, and two hydrogen bonds with Tyr-448 and Gly-449. This visual depiction provides insight into the structural relationships and potential binding configurations within these pivotal molecular interactions, shedding light on the molecular dynamics of the hepatitis C virus polymerase and its interactions with distinct compounds.

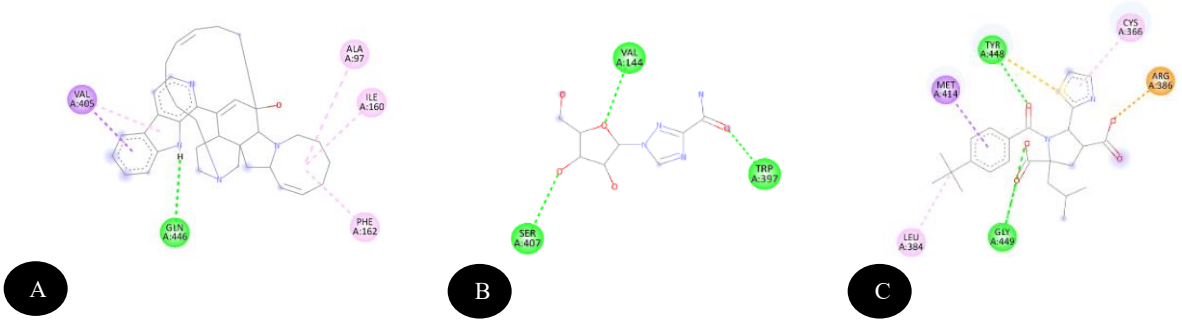

Figure 3: The 2D visualization of interactions complex between: A. hepatitis C virus polymerase receptor and the manzamine A; B. hepatitis C virus polymerase receptor and ribavirin drug; C. hepatitis C virus polymerase receptor and ligand 698.

Figure 4 exhibited a three-dimensional (3D) visualization of interaction complexes involving: the receptor of the hepatitis C virus polymerase and manzamine A, the receptor of the hepatitis C virus polymerase interacting with the antiviral drug ribavirin and the hepatitis C virus polymerase receptor in association with ligand 698. This graphical representation provides a comprehensive view of the spatial arrangements and structural interplays within these significant molecular interactions, offering valuable insights into the dynamic relationships of the hepatitis C virus polymerase with distinct molecular entities.

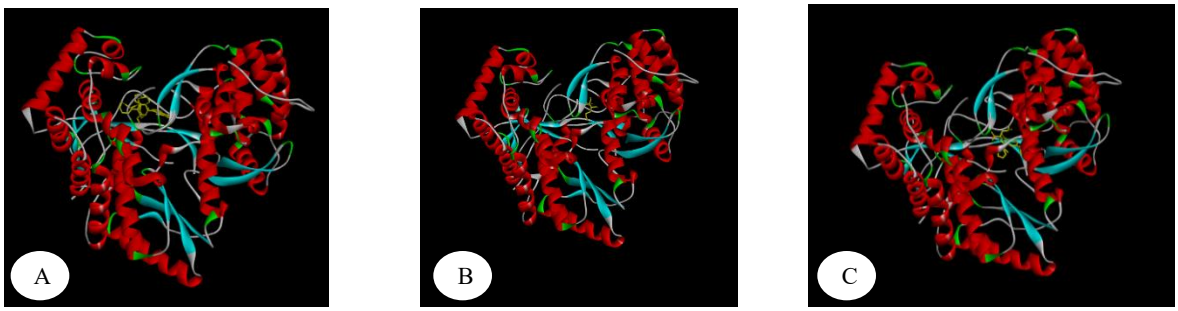

Figure 4: The 3D visualization of interactions complex between: A. hepatitis C virus polymerase receptor and the manzamine A; B. hepatitis C virus polymerase receptor and ribavirin drug; C. hepatitis C virus polymerase receptor and ligand 698.

DISCUSSION

Bioactive compounds derived from marine sponges have demonstrated promising antivirus potential such as: Spongosine, isolated from marine sponges, which has shown inhibitory effects against Hepatitis C Virus (HCV) replication, making it a potential candidate for antiviral drug development [10]. Moreover, aptamine, found in marine sponges, exhibits antiviral activity and has been explored for its potential in inhibiting the replication of human immunodeficiency virus (HIV) [11]. Another bioactive

compounds isolated from marine sponges is discodermolide has shown antiviral properties, including inhibition of HIV-1 replication, and Halichondrin B has demonstrated potent antiviral activity against herpes simplex virus type 1 (HSV-1) [12].

Our study performed that manzamine A has the highest binding affinity to HCV polymerase receptor. Manzamine A is a β-carboline alkaloid isolated from several marine sponges, including *Xestospongia ashmorica*, *Amphimedon* sp. and *Haliclona* sp [13]. It is a potent inhibitor of glycogen synthase kinase-3β (GSK-3β), an enzyme involved in various cellular processes, including cell growth, differentiation, and apoptosis [14]. Manzamine A has demonstrated promising antitumor and neuroprotective activities in preclinical studies [15, 16].

A comparison study with ribavirin was also conducted, and it showed that ribavirin has lower binding affinity than manzamine A. Ribavirin is a synthetic guanosine nucleoside analog that exhibits antiviral activity against a wide range of viruses, including hepatitis C virus (HCV), respiratory syncytial virus (RSV), influenza virus, and hantaviruses [17]. It is primarily used in combination with other antiviral agents to treat chronic HCV infection. Ribavirin is a broad-spectrum antiviral agent with proven efficacy in the treatment of chronic HCV infection and other viral infections. It is generally well-tolerated, but it can cause some side effects, including flu-like symptoms, anemia, and teratogenicity.

Due to its promising antiviral activity, manoalide is considered a potential candidate for the development of novel HCV therapeutics. Ongoing research is focused on optimizing the structure of manoalide to improve its potency, selectivity, and pharmacokinetic properties. Further research is needed to fully elucidate the mechanism of action of manoalide against HCV and to evaluate its safety and efficacy in clinical trials. However, the potential of manoalide as a novel HCV therapeutic agent is promising, and continued research efforts are warranted.

CONCLUSION

This study demonstrates the potential of manzamine A as a novel antiviral agent for the treatment of hepatitis C virus (HCV) infection. Manzamine A exhibited a higher binding affinity to the HCV polymerase receptor than ribavirin, a current antiviral drug for HCV treatment. These findings suggest that manzamine A may be a more effective antiviral agent for HCV than ribavirin. Further studies are needed to fully evaluate the safety and efficacy of manzamine A in vitro and in vivo.

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