BIOACTIVE COMPOUNDS DERIVED FROM MARINE SPONGES AS A POTENTIAL INHIBITOR OF THYMIDYLATE SYNTHASE OF *HELICOBACTER PYLORI*

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Abstract

Background: *Helicobacter pylori*, a gram-negative bacterium, is a notorious human pathogen responsible for chronic gastritis, peptic ulcers, and gastric cancer. Given its prevalence worldwide and the serious health implications associated with its infection, there is a crucial need for effective therapeutic strategies. This study focused on the physicochemical properties analysis of ligands using Lipinski rules of five, essential in drug development. Additionally, the examination of protein-ligand interactions with virtual screening and molecular docking provides insights into potential antibacterial agents. The inclusion of bioactive compounds derived from marine sponges, have potentials for its broad-spectrum antibacterial activities, highlights its potential against thymidylate synthase, a crucial enzyme for bacterial DNA replication. **Methods:** The physicochemical properties analysis involves Lipinski's criteria and utilizes the PubChem database. The preparation of the receptor for molecular docking includes crucial steps to ensure the appropriateness of the protein structure. Virtual screening and molecular docking are conducted using PyRx Tools software, offering a comprehensive understanding of the interactions between ligands and the thymidylate synthase receptor. **Results:** Physicochemical properties analysis revealed adherence to Lipinski rules for a majority of ligands. The examination of protein-ligand interactions, especially with norlichexanthone, demonstrated promising antibacterial activity. The study contributed valuable insights into potential alternative treatments for *Helicobacter pylori* infections. **Conclusion:** Norlichexanthone's favorable binding affinity to thymidylate synthase, its broad-spectrum antibacterial activity, and potential for reduced side effects position it as a compelling candidate for further development as an alternative treatment option against *H. pylori*. The study emphasizes the urgency of finding effective therapeutic interventions and encourages future research for in-depth validation in vivo and clinical settings.

Keywords: *Helicobacter pylori* ; marine sponge ; molecular docking ; norlichexanthone ; bioactive compounds

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INTRODUCTION

Helicobacter pylori, a widespread bacterium, colonizes the human stomach, with prevalence varying significantly globally across regions and populations. Influential factors include socioeconomic conditions, sanitation, age, and geographical location [1]. The bacterium is found worldwide, yet prevalence differs widely, particularly in developing countries such as Africa, Latin America, and parts of Asia, where infection rates tend to be higher than in developed countries. *H. pylori* infection commonly occurs in childhood. In less optimal sanitation conditions of developing countries, individuals are more likely to acquire the bacterium at a young age, while in developed countries with better sanitation practices, acquisition may be delayed until later in life. Lower socioeconomic status is associated with higher *H. pylori* prevalence. Overcrowded living conditions, limited access to clean water, and poor sanitation contribute to the bacterium's transmission [2].

Several studies have significantly contributed to the understanding of combating the *Helicobacter pylori* infection using marine sources such as marine sponges to find new antibiotic potentials. Marine sponges hold immense potential as a source of bioactive compounds with diverse therapeutic applications [3]. The discovery of prodigiosins from a marine sponge-associated actinomycete and their remarkable gastroprotective effects against HCl/ethanol-induced gastric lesions highlights the potential of marine sponges as a source of natural medicines [4]. Additionally, the identification of numerous bioactive compounds from marine sponges, including anti-cancer, anti-inflammatory, and antiviral agents, further underscores their value as a drug discovery treasure trove [5]. The successful determination of the

absolute configurations of two promising anti-*Helicobacter pylori* agents isolated from the marine sponge-derived fungus *Aspergillus niger* paves the way for the development and optimization of these antibacterial agents for the treatment of *Helicobacter pylori* infections, a major cause of peptic ulcers [6, 7].

In our endeavor to comprehend and counteract *Helicobacter pylori* infections, our study sought to reveal the binding interactions between potential therapeutic agents and specific bacterium receptors in silico. The primary objective was to identify and characterize the key receptors implicated in *H. pylori* pathogenesis, establishing the foundation for targeted interventions. Utilizing molecular docking simulations, to predict the binding affinity, orientation, and stability of diverse molecules, spanning from drugs to natural compounds, with the targeted receptor. Through elucidating the molecular intricacies of these interactions, the goal was to streamline the drug discovery process and bolster the development of effective treatments for *H. pylori* by offering valuable insights into the potential therapeutic efficacy of candidate compounds and aiding in the rational design of interventions against *H. pylori* infections.

MATERIALS AND METHODS

Materials

The complex of thymidylate synthase receptor was sourced from the Protein Data Bank Repository (PDB) under the accession number of 3AH5 [11], and the corresponding files were acquired in .pdb format. Moreover, a 3D file conformer of the commercial drug flavin adenine dinucleotide and 20 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; and tirandamycin, were sourced from PubChem [12]. These ligand files were obtained in .sdf format.

Physicochemical Properties Analysis of Ligand

The physicochemical properties analysis of ligands using Lipinski rules of five involves Lipinski's criteria, including molecular weight , hydrogen bond donors, hydrogen bond acceptors, rotatable bond and lipophilicity using PubChem database [8].

Preparation of Receptor

After eliminating initial ligands and water molecules using Discovery Studio Visualizer, the receptor's .pdb files are readied for molecular docking with PyRx. This process encompasses essential steps to guarantee the protein's appropriateness for docking simulations. These steps involve acquiring the protein structure in a suitable format, importing it into PyRx, excluding water molecules, supplementing missing residues when required, appending hydrogen atoms, assigning atom types, optimizing the structure, and ultimately saving the prepared protein structure.

Virtual Screening with Molecular Docking

The PyRx Tools software was utilized to prepare the protein and ligand, which were then converted into .pdbqt format. To conduct molecular docking simulations, follow these steps: Start by acquiring the protein structure from a database like the Protein Data Bank (PDB) and importing it into PyRx. Prepare the protein by eliminating water molecules, adding any missing residues, and introducing hydrogen atoms. Optionally, assign atom types and optimize the structure for improved accuracy. 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 hydrogen atoms, assign atom types, and convert it to PDBQT format [9].

Visualization of Receptor-Ligand Interaction

The generation of docking data for protein-ligand interactions was conducted to conform to .pdb files. The PyRx program was utilized for the integration of data, ensuring a uniform and consistent representation for subsequent analyses. Additionally, Discovery Studio was employed for systematic 3D visualization, enabling a detailed exploration of spatial arrangements, binding interfaces, and conformational changes [10]. Moreover, the binding energy (ΔG) value was utilized to quantify the intensity of interaction between the ligand and the target in molecular docking.In assessing inhibition constants (Ki), the process entailed evaluating the degree of affinity exhibited by a ligand for a specific target receptor. This assessment was conducted through the application of the formula $Ki = e^{-RT/\Delta G}$.

RESULTS

Physicochemical Properties Analysis

The physicochemical properties of the ligands were listed on Table 1. These properties showed no violation of Lipinski Rules on 13 ligands, while the other 10 ligands have only 1 violation.

Table 1. Physicochemical properties of the 23 ligands

Compound	Molecular Weight	Log P	Hydrogen Bond Acceptors (HBA)	Hydrogen Bond Donors (HBD)	Rotatable Bonds	Violation of Lipinski Rules
2,2-bis(6-bromo-1H-indol-3-yl)ethanamine	433.140	4.39	3	4	3	0
3,5-Dibromo-2-(2,4-dibromophenoxy)phenol	501.791	7.19	2	1	2	1
4-Hydroxybenzoic acid	138.121	1.42	3	2	1	0
5-epi-Ilimaquinone	358.4712	6.38	4	1	3	1
6-hydroxyavarol	330.47	5.6	3	3	2	1
Alisiaquinol	354.396	3.1	5	3	0	0
Alisiaquinone A	352.4	2.6	5	1	0	0
Alisiaquinone B	382.4	2.8	6	1	1	0
Alisiaquninone C	457.5	2.1	8	2	0	0
Clethric acid	504.7	4	6	5	3	0
Metachromin A	358.5	5.5	4	1	6	1
Hamigeran B	365.3	4.9	3	1	1	0
Manoalide	416.5	3.5	5	2	7	0
Manzamine A	548.8	5.7	4	2	1	1
Motualevic acid A	439.2	6	3	2	13	1
Motualevic acid E	382.13	6.6	2	1	11	1
Motualevic acid F	421.2	6.6	3	1	12	1
Norlichexanthone	258.23	2.8	5	3	0	0
Oroidin	389.05	2	3	4	4	0
Psammaplin A	664.4	4.8	10	6	13	1
Stachybogrisephenone B	338.74	3.7	6	3	4	0
Stelletin A	462.6	7	4	0	3	1
Tirandamycin	417.5	2.6	7	2	4	0

Protein and Ligand Interaction

The examination utilized the PyRx gridbox, serving as a graphical user interface for customizing the receptor docking grid specifically designed for molecular docking. Figure 1 and Figure 2 illustrated the binding energy and 3D interactions within the complex involving the thymidylate synthase receptor and the ligand inhibitors and inhibition constant. Among the 23 compounds assessed for binding affinity, norlichexanthone exhibited the most favorable binding to the thymidylate synthase receptor. To validate the docking process, the original ligand (flavin adenine dinucleotide) obtained from the 3D structure of the protein-ligand complex was employed as a reference.

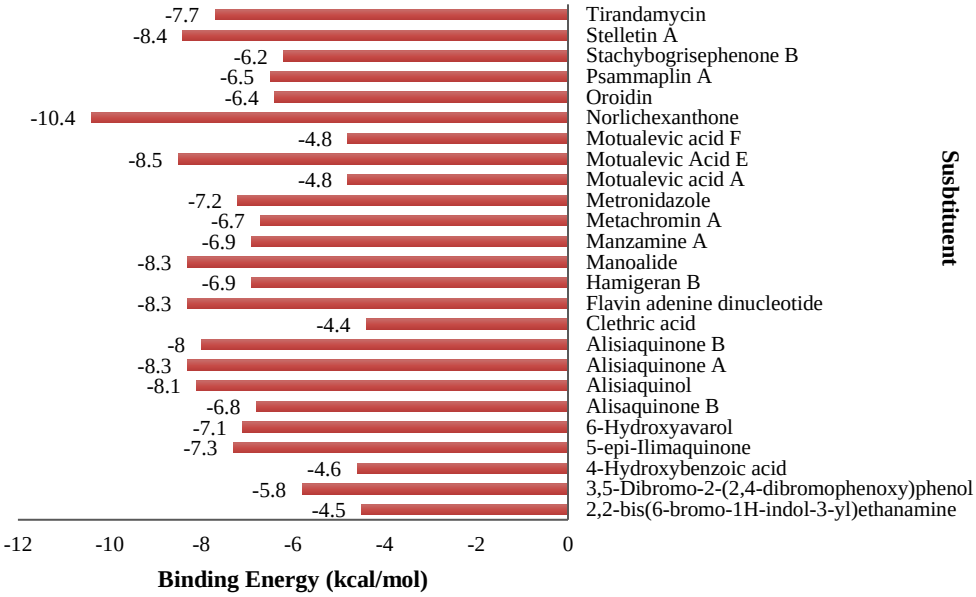


Figure 1: The binding energy of natural compounds derived from marine sponges with the receptor of the thymidylate synthase receptor.

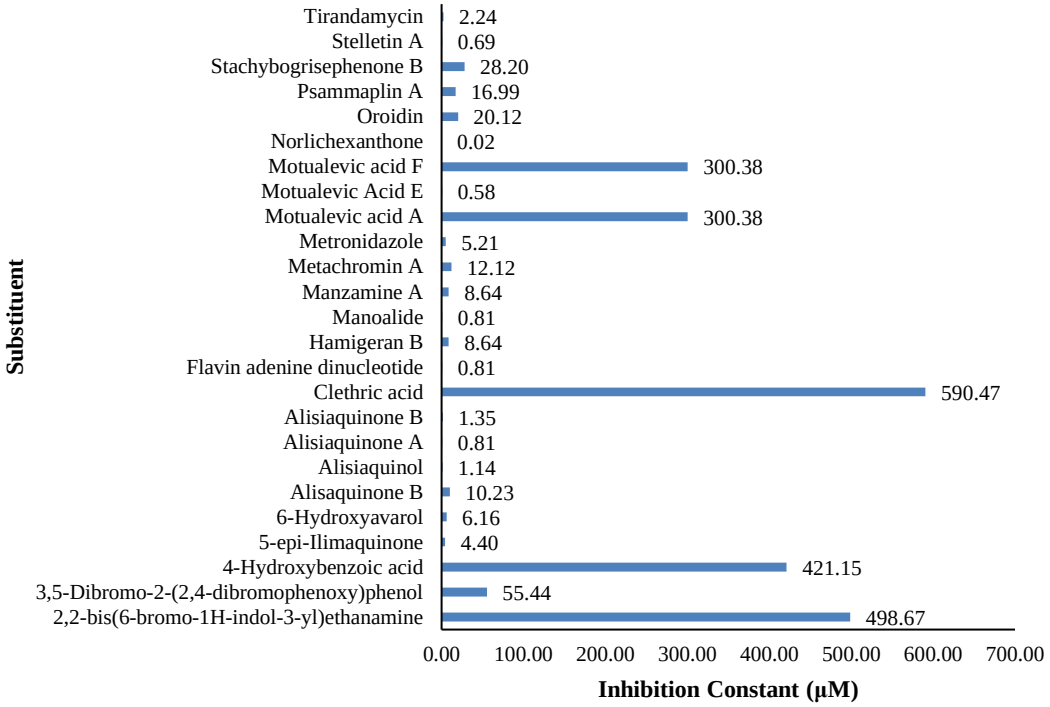
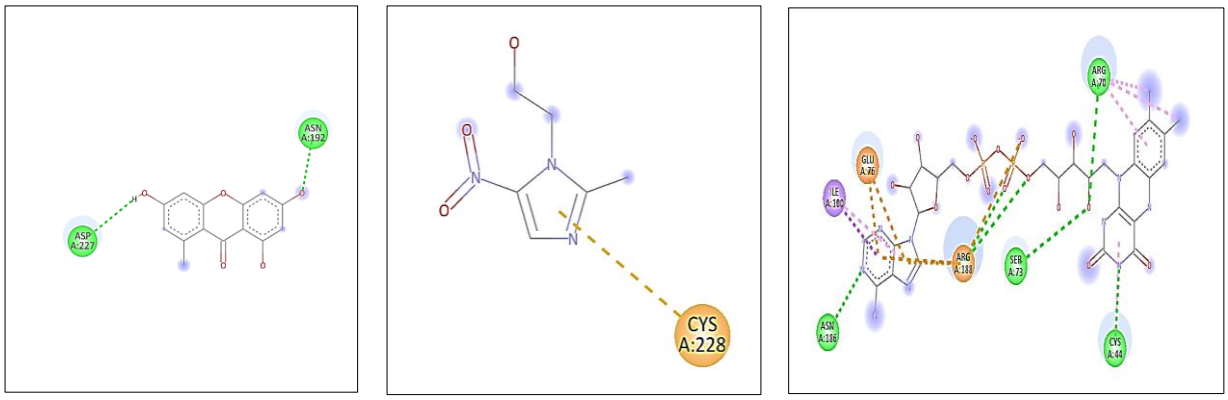


Figure 2: The binding energy of natural compounds derived from marine sponges with the receptor of the thymidylate synthase receptor.

Figure 3 depicted a detailed 2D visualization of receptor-ligand interactions, showcasing the intricate complexes formed between various entities. Panel A depicts the dynamic interplay between the thymidylate synthase receptor and norlichexanthone, highlighting hydrogen bonds with Asp-227 and Asn-192. Panel B provides a magnified view of the interaction between the receptor and the commercial antibiotic metronidazole, demonstrating a hydrophobic interaction with Cys-228. Finally, Panel C captures the intricate interplay between the thymidylate synthase receptor and ligand flavin adenine dinucleotide, revealing three hydrophobic interactions with Ile-100, Glu-76, Arg-188, and four hydrogen bonds with Asn-186, Ser-73, Arg-70, Cys-44. This comprehensive visualization provides valuable insights into the structural relationships and potential binding configurations within these crucial molecular interactions, illuminating the molecular dynamics of the hepatitis C virus polymerase and its interactions with distinct compounds.



A **B** **C**

Figure 3: The 2D complex interactions between the thymidylate synthase receptor and three ligands: A. norlichexanthone, B. metronidazole, and C. the original ligand (flavin adenine dinucleotide).

Figure 4 presented a three-dimensional (3D) visualization of interaction complexes involving the thymidylate synthase (TS) receptor and three distinct ligands: norlichexanthone, the antibiotic drug metronidazole, and flavin adenine dinucleotide (FAD). This comprehensive graphical representation provides a detailed view of the spatial arrangements and structural interplays within these significant molecular interactions. It offers valuable insights into the dynamic relationships of the TS receptor with distinct molecular entities, shedding light on the intricate mechanisms underlying ligand recognition and binding.

A **B** **C**

Figure 4: The 3D complex interactions between the thymidylate synthase receptor and three ligands: A. norlichexanthone, B. metronidazole, and C. the original ligand (flavin adenine dinucleotide).

DISCUSSION

Norlichexanthone has demonstrated potent antibacterial activity against a wide range of bacterial species, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and multidrug-resistant's *Mycobacterium tuberculosis* [11]. Its effectiveness against these resistant strains highlights its potential as an alternative treatment option for infections caused by these difficult-to-treat pathogens. Norlichexanthone has been isolated from several species of marine sponges, including *Ircinia oros* [12], and *Penicillium* strain-associated with marine sponge [13]. The precise mechanism of action of norlichexanthone remains under investigation, but studies suggest it primarily targets thymidylate synthase (TS), an enzyme essential for DNA replication in bacteria [14]. By inhibiting TS, norlichexanthone disrupts DNA synthesis, halting bacterial growth and replication.

On the other side, metronidazole is a synthetic nitroimidazole antibiotic that has been widely used for over 50 years to treat a variety of infections caused by anaerobic bacteria [15]. These bacteria thrive in oxygen-deprived environments, such as deep wounds, abscesses, and the gastrointestinal tract. Metronidazole's efficacy against anaerobic bacteria stems from its unique mechanism of action, which disrupts DNA replication and inhibits bacterial growth. Metronidazole is primarily used to treat infections caused by anaerobic bacteria, including intra-abdominal infections such as peritonitis, appendicitis, cholecystitis; pelvic infections such as bacterial vaginosis, trichomoniasis; gastrointestinal infections: *Pseudomembranous colitis*; antibiotic-associated diarrhea; dental infections such as gingivitis, periodontitis and brain abscesses. However, metronidazole is generally well-tolerated, but it can cause some side effects, including gastrointestinal disturbances causing nausea, vomiting, diarrhea, and metallic taste. Central nervous system effects such as headache, dizziness, vertigo. Skin reactions, for example rash and flushing. Allergic reactions such as anaphylaxis.

Helicobacter pylori is a gram-negative bacterium that infects the stomach and is responsible for chronic gastritis, peptic ulcers, and gastric cancer [16]. Norlichexanthone, a natural product found in the mangosteen fruit, has been shown to have antibacterial activity against *H. pylori* in vitro. Molecular docking studies have been conducted to investigate the potential binding modes of norlichexanthone to *H. pylori* targets. Thymidylate synthase (TS) is an essential enzyme for DNA replication in bacteria. Norlichexanthone has been shown to inhibit TS in *H. pylori*, suggesting that it may disrupt DNA synthesis and prevent bacterial growth. Molecular docking studies have identified the TS binding site for norlichexanthone and have shown that it binds with high affinity.Therefore, molecular docking studies suggest that norlichexanthone has the potential to be a novel antibacterial agent for the treatment of *H. pylori* infections. Further studies are needed to confirm these findings in vivo and to evaluate the safety and efficacy of norlichexanthone in clinical trials.

CONCLUSION

Molecular docking studies have demonstrated that norlichexanthone exhibits promising potential as a novel antibacterial agent against thymidylate synthase (TS), a crucial enzyme involved in DNA replication in bacteria. Norlichexanthone's favorable binding affinity to TS suggests its ability to effectively inhibit TS activity, thereby disrupting bacterial DNA synthesis and hindering bacterial growth. Additionally, norlichexanthone's broad-spectrum antibacterial activity and potential for reduced side effects due to lower dosages make it an attractive candidate for further development as an alternative treatment option for combating bacterial infections. Further in-depth studies are warranted to validate these findings in vivo and to thoroughly evaluate the safety and efficacy of norlichexanthone in clinical settings.

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