



Original article

In-silico investigation of a novel inhibitors against the antibiotic-resistant *Neisseria gonorrhoeae* bacteriaHani Mohammed Ali ^{a,b}^a Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80141, Jeddah 21589, Saudi Arabia^b Princess Najla bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz, P.O. Box 80141, Jeddah 21589, Saudi Arabia

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ABSTRACT

Antibiotics are drugs that are used to treat or prevent bacterial infections. They work by either killing or stopping bacteria from spreading. Nevertheless, it appeared in the last decade, Antibiotic-resistant bacteria are bacteria resistant to antibiotics and cannot be controlled or killed by them. In the presence of an antibiotic, they can live and even reproduce. The *Neisseria gonorrhoeae* bacteria is appearing to be a multidrug-resistant pathogen. Many factors contribute to antibiotic resistance, including unfettered access to antimicrobials, incorrect drug selection, misuse, and low-quality antibiotics. Here, we investigated in-silico docking screening and analysis for ten natural marine fungus extracted compounds. The resulted data were examined for the best binding affinity, toxicity, and chemical interactions. The most superior compound was elipyrone A with six hydrogen bonds, -8.5 of binding affinity, and preferable results in the SWISS-ADME examination. It is well known that "Declining corporate investment and a lack of innovation in the development of new antibiotics are weakening efforts to battle drug-resistant illnesses," according to the World Health Organization (WHO). So, we extended our effort to predict a new natural compound to overcome the resistance of this bacteria.

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1. Introduction

Bacterial infection occurs when a hazardous strain of bacteria multiplies on or within the body and condition occurs when the equilibrium between bacterial pathogenicity and host resistance is disrupted. Antibiotics have been used to treat infectious disorders caused by bacteria since the 1940 s (Chopra and Roberts, 2001). These drugs significantly lower the risk of tuberculosis and pneumonia-related illness and mortality. On the other hand, widely and longtime usage of antibiotics developed resistance to them, rendering these therapies ineffective (Aslam et al., 2018). Antibiotic resistance arises when a medication's ability to kill bacteria is compromised. As a result, even in the presence of the antibiotic, the organisms continue to multiply and cause infection. Some bacteria are naturally resistant to antibiotics, but the majority become resistant due to a genetic mutation or the acquisition of

resistance genes from other bacteria (Fair and Tor, 2014). Unfortunately, when bacteria mate, their resistance genes are passed along. Bacteria can develop many resistance characteristics over time, making them resistant to various medications (Reygaert, 2018). All microorganisms, in general, can develop resistance (Larsson et al., 2022). "Superbugs" are bacteria resistant to many drugs or utterly resistant to all drugs. Bacterial resistance can develop naturally due to genetic mutation or as a result of one species acquiring resistance from another (Nweze et al., 2020).

On the other hand, extended usage of antimicrobials appears to increase mutation selection, rendering antimicrobials useless. Millions of people die each year due to infections caused by antibiotic resistance (Ventola, 2015). Resistant microorganism infections are more challenging to treat, necessitating greater antibiotic doses or alternative treatments that may be more harmful. These methods may potentially be more costly. Multidrug-resistant bacteria are resistant to various antimicrobials (Ayukekbong et al., 2017).

The most common bacterial pathogens associated with gonorrhoea and various sequelae is *Neisseria gonorrhoeae* (Unemo et al., 2019). *Neisseria gonorrhoeae* is a bacterial pathogen that causes symptoms when an asymptomatic infection spreads across the vaginal tract or to distant tissues (Yakobi et al., 2022). Increased transmission and antimicrobial resistance in *Neisseria*

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gonorrhoeae is a global health concern, with alarming trends in decreased susceptibility to the last-line extended-spectrum antibiotic ceftriaxone (Adamson et al., 2020). Increased transmission and antimicrobial resistance in *Neisseria gonorrhoeae* is a global health concern with alarming trends in decreased susceptibility to the last-line extended-spectrum antibiotic ceftriaxone (Al-Maslamani et al., 2022). Albert Neisser isolated *Neisseria gonorrhoeae*, also known as gonococcus (singular) or gonococci (plural), as a Gram-negative diplococci bacteria in 1879. It causes gonorrhoea, a sexually transmitted genitourinary infection, as well as disseminated gonococemia, septic arthritis, and gonococcal ophthalmia neonatorum, among other forms of the gonococcal disease (Humbert and Christodoulides, 2020). The genitals, throat, and eyes can all be infected with *N. gonorrhoeae*. Both males and females are susceptible to asymptomatic infection. Untreated gonorrhoea infection can spread throughout the body, especially to the joints (disseminated gonorrhoea infection) (septic arthritis). In women, untreated infection can lead to pelvic inflammatory disease and, as a result of scarring, infertility (Walker and Sweet, 2011).

To combat the public health threat posed by antimicrobial resistance, new drugs with novel chemistry and modes of action are badly needed worldwide. The pharmaceutical industry's research of new antibiotics, which had previously been successful in battling antibiotic-resistant germs, had come to a halt due to economic and regulatory barriers (Cui et al., 2021). As a result, judicious investment in innovative therapeutic alternatives to combat resistant bacteria is critical to meet unmet patient needs while balancing the exponentially expanding financial load on global health systems (Dadgostar, 2019). Molecular docking is a well-established structure-based *in silico* approach commonly utilized in drug discovery. Without knowing the chemical structure of other target modulators, docking allows for identifying novel therapeutic compounds the prediction of ligand-target interactions at the molecular level, and the delineation of structure-activity connections (Qifeng et al., 2018). It is one of the most widely used virtual screening approaches, mainly when the target protein's 3D structure is available. This technique was able to estimate the ligand-protein binding affinity and the design of the protein-ligand complex, which is valuable information for lead optimization (Zhang et al., 2022). Several natural compounds were deliberated against *Neisseria gonorrhoeae* mutated enzyme, which was responsible for the bacterial resistance, to predict a novel compound for inhibiting it through *in silico* docking analysis.

2. Material and methods

2.1. Biological data

The biological interactions, functions, and enzymes of the resistant *Neisseria gonorrhoeae* bacteria were determined from the UniProtKB database (<https://www.uniprot.org/uniprot/P08149>). The enzyme sequence was obtained from the NCBI database (WP_003703066). Because our target was the mutated enzyme, the resistant domain was utilized from the PDB database (ID: 6hzj) and a sequence of 581 amino acids (Fig. 1). This enzyme's function is to catalyze the cross-linking of the peptidoglycan cell wall at the division septum. Also, this protein participates in the peptidoglycan manufacturing pathway, a part of cell wall biogenesis (Aliashkevich and Cava, 2021).

2.2. Ligand assemblage and modifications

Ten new compounds were aggregated from the MarinLit database (MarinLit - A database of the marine natural products litera-

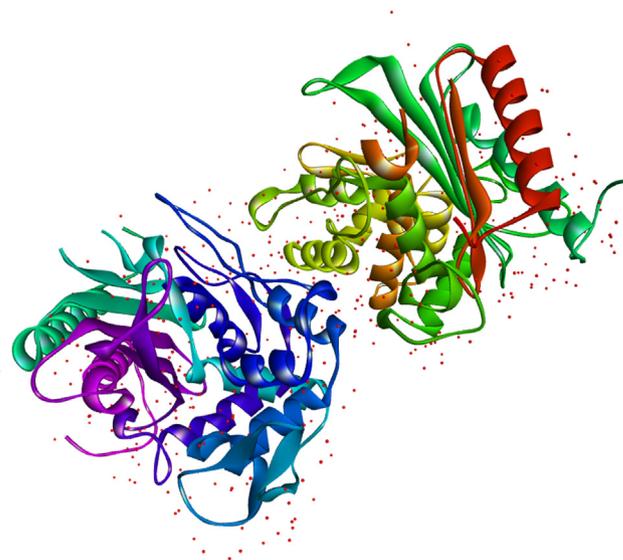


Fig. 1. The 6hzj domain with the two identical chains A and B (Bellini et al., 2019).

ture (rsc.org) (Fig. 2). Energy minimization was performed for them by SPDBV software, and the formats were converted from PDB to pdbqt by Open babel software. SWISS-ADME examined the toxicity of each compound after converting the standard InChI format to the smile format by Open babel software (Rifaioğlu et al., 2019).

2.3. *In-silico* docking

We first changed the protein structure using the Discovery Studio software by eliminating chain B and water molecules, adding protein and ligand hydrogen atoms, adding hydrogen, and treating metals (version 2019) (Aallaei et al., 2022). The PDBQT format was used to save the structure file. The random setting was used for rudimentary placement, orientation, and torsions of the ligand. The grid box was then created using AutoDock Vina. Affinity grid maps with 50 60 50 XYZ points and 1.00 spacing were created using the AutoGrid software. The van der Waals were ranked using AutoDock. Chemical interactions, H-bonds, and hydrophobic areas were implicated in the results, which included binding energy and inhibition constant. PyMOL was used to visualize the interaction surface (Sébastien et al., 2018). The intricate interactions of each ligand - 6hzj domain were investigated.

3. Results

3.1. Drug-likeness

Some of the drawbacks of *in vitro* bioassays, particularly the absence of integration of toxicokinetics, can be partially mitigated by combining them with computer (*in-silico*) modeling based on structure-activity connections (Mark et al., 2022). The chemical structure and other Physico-chemical features of a material can be utilized to predict its toxicokinetic once they are understood. The anticipated threshold of toxicological concern can be assigned to the chemical based on available toxicity databases, which can then be used to create a provisional drinking water guideline value (Canady et al., 2013). As exhibited in Table 1, all the chosen hits had acceptable lipophobicity, water Solubility, pharmacokinetics, and drug-likeness.

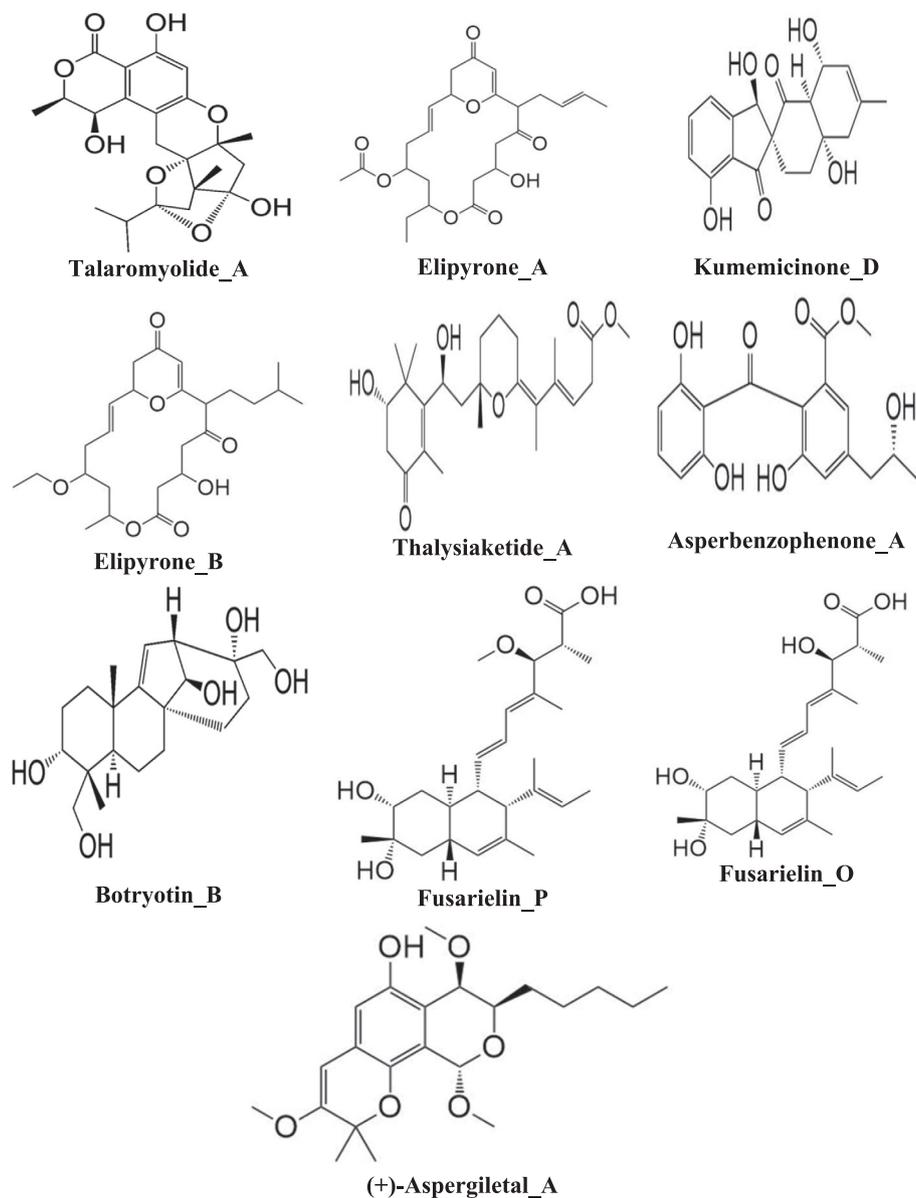


Fig. 2. The chemical structure of the compound (Diallo et al., 2021).

Table 1

The results of investigating the ten compounds through the SWISS-ADME database. Lipophilicity, water-solubility, pharmacokinetics, and Druglikeness parameters were examined.

Compound name	Lipophilicity (Log Po/w (iLOGP))	Water Solubility	Pharmacokinetics (GI absorption)	Druglikeness
Talaromyolide_A	3.07	Soluble	High	Yes
Elipyronone_A	2.99	Soluble	High	Yes
Kumemycinone_D	2.08	Soluble	High	Yes
Elipyronone_B	3.74	Soluble	High	Yes
Thalysiaketide_A	2.18	Soluble	High	Yes
Asperbenzophenone_A	1.87	Soluble	High	Yes
Botryotin_B	2.17	Soluble	High	Yes
Fusarielin_P	3.84	Soluble	High	Yes
Fusarielin_O	3.87	Soluble	High	Yes
(+)-Aspergiletal_A	4.34	Moderately soluble	High	Yes

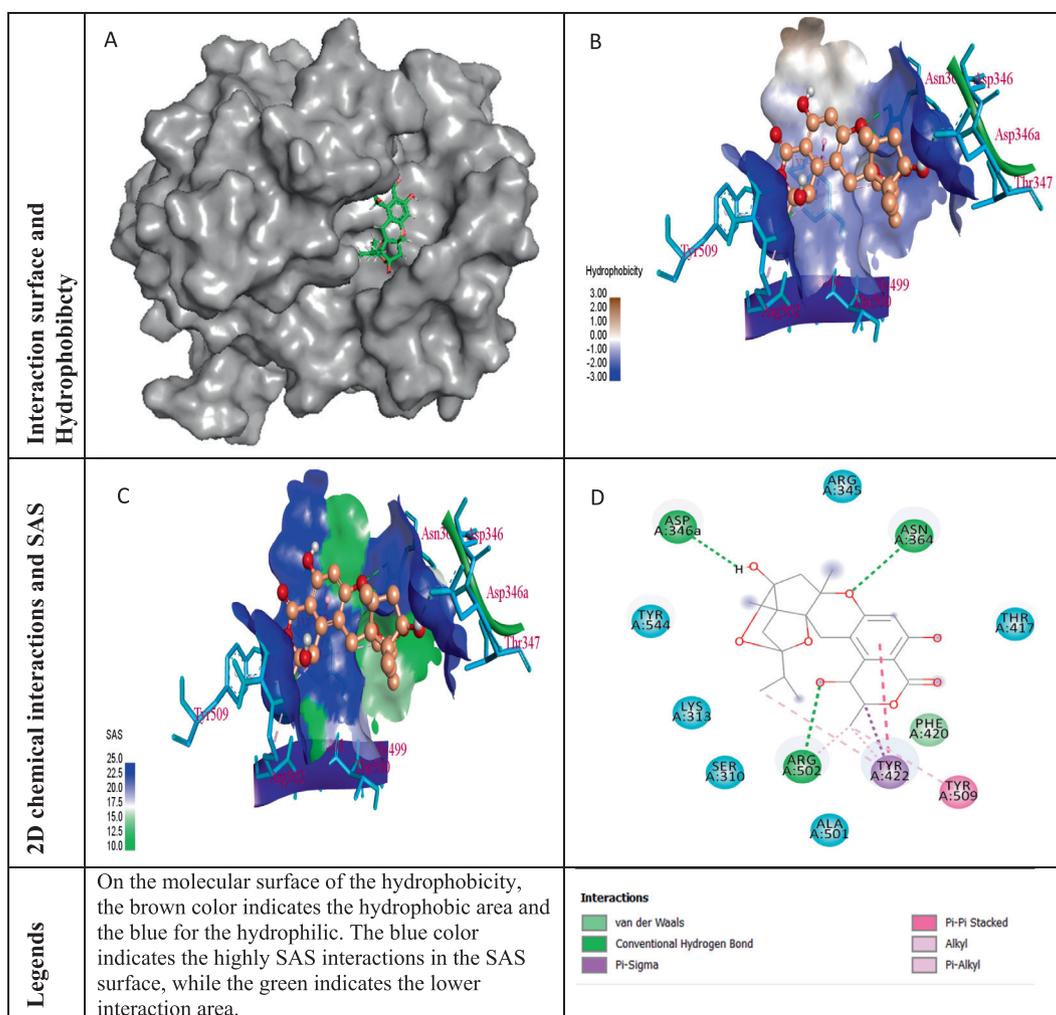


Fig. 3. the molecular interaction results from the docked 6hzj-talaromyolide_A complex, investigated the interaction surface (A), hydrophobicity (B), SAS (C), and the 2D chemical interactions (D).

3.2. Binding affinity

It is feasible to develop compounds that inhibit resistance proteins, restoring the effectiveness of failing antimicrobials, by studying the biology of antimicrobial resistance, specifically the structures and activities of the proteins involved (Khameneh et al., 2019). As a result, in-silico drug design criteria like “Docking” are highly helpful in discovering potent inhibitors for aflatoxin, particularly for polyketide synthase protein. The changed product template 3D domain (6hzj) was populated in the discovery studio.

Table 2

The binding affinity results of the docked compounds.

Compound name	Binding energy (kcal/mol)
Talaromyolide_A	-9.2
Elipyron_A	-8.5
Kumemicinone_D	-8.3
Elipyron_B	-8
Thalysiaketide_A	-7.9
Asperbenzophenone_A	-7.6
Botryotin_B	-7.3
Fusarielin_P	-7.2
Fusarielin_O	-7.2
(+)-Aspergiletal_A	-7.2

The domain's active site was discovered and docked with a list of compounds (Punjabi et al., 2018). The interaction of the 6hzj domain with each chemical was investigated. Based on the binding energy ratings, ten molecules were found to have strong interactions with the 6hzj domain's plurality (Table 2). The total interaction energy was calculated using AutoDock using a semiempirical free energy calculation assignment that contains two factors (intramolecular and intermolecular energy) and is proportional to the number of interactions between the ligand and receptor (Santos-Martins et al., 2021).

3.3. Docking analysis

Docking analysis was investigated for each complex at first by using pymol software to illustrate the interaction surface (Kwon et al., 2022). The interaction of these natural chemicals with the altered enzyme can be studied using molecular docking analysis. The structure-activity relationship can also be leveraged to create novel natural derivatives with more potent inhibition (Altaf et al., 2022). Fig. 3., Fig. 4., Fig. 5., Fig. 6., Fig. 7., S1, S2, S3, S4, and S5 illustrated the interaction surface, the hydrophobicity, solvent accessible surface (SAS), and the 2D chemical interactions for Talaromyolide_A, Elipyron_A, Kumemicinone_D, Elipyron_B,

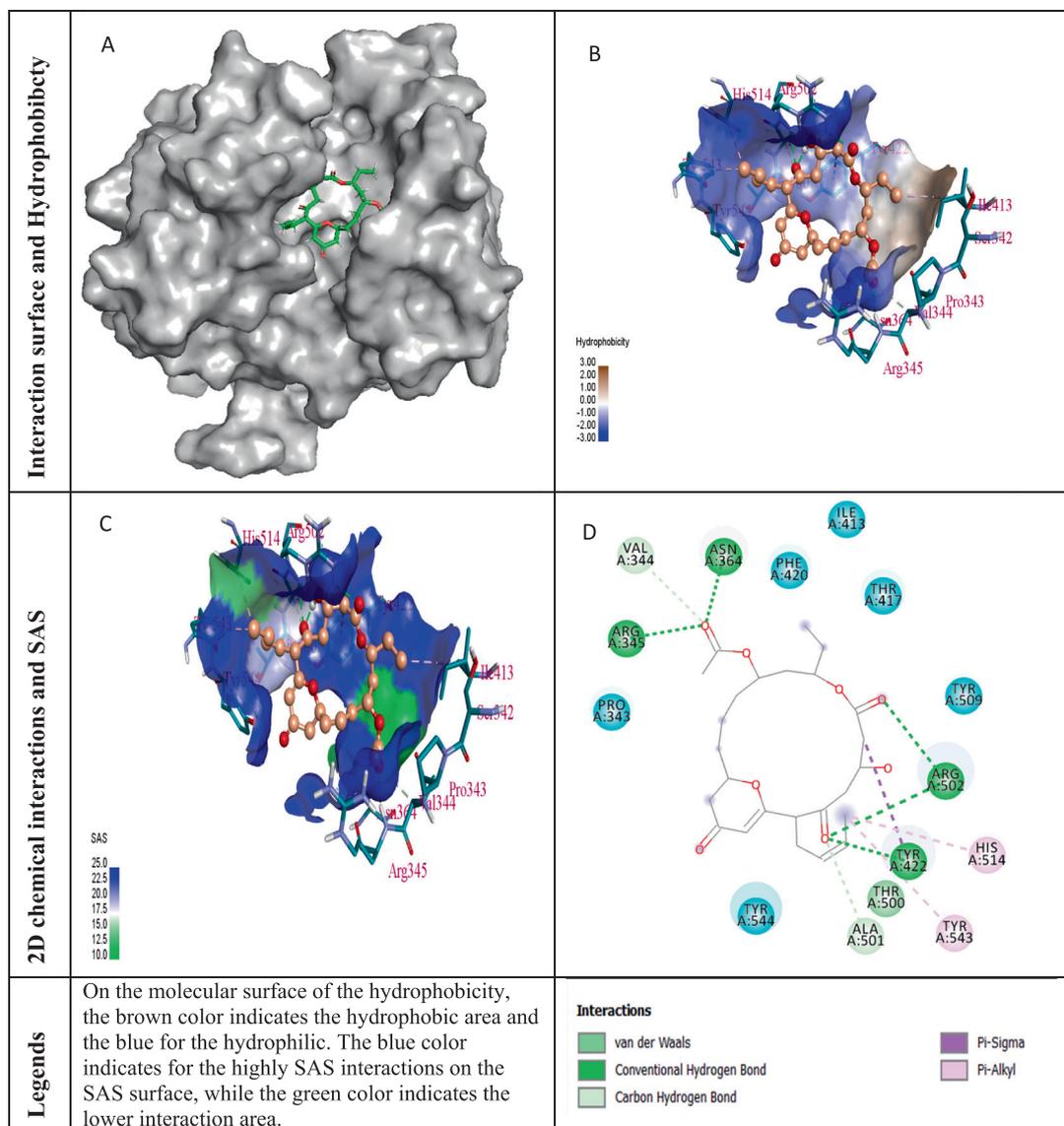


Fig. 4. the molecular interaction results from the docked 6hzj- elipyrone_A complex, investigated the interaction surface (A), hydrophobicity (B), SAS (C), and the 2D chemical interactions (D).

Thalysiaкетide_A, Asperbenzophenone_A, Botryotin_B, Fusarielin_P, Fusarielin_O, and (+)-Aspergiletal_A complexes respectively.

4. Discussion

Molecular docking had been an essential method in drug discovery and development. The lowest binding energy between the domain and the ligand, the best chemical interaction with the domain, and the best drug-likeness are all well-known criteria for selecting the best inhibitor (Mathpal et al., 2022). As illustrated in Table 1, all the ten compounds appeared with non-expected toxic effects through the examination by the SWISS-ADME database. Among the investigation of the binding affinity of each complex, the 6hzj-Talaromyolide_A complex was the best, with the binding energy of -9.2 . In contrast, 6hzj-(+)-Aspergiletal_A was the lowest binding energy of -7.2 . Hydrophobicity is one of the inhibiting parameters during docking scores. Using substituent

constants and regression analysis, the function of hydrophobic interactions in blocking the relatively specific enzymatic activities of an enzyme system by a series of congeneric medicines has been demonstrated. Surface Area that can be reached SAS is the location of the solvent molecule's center as it rolls over the protein's van der Waals surface (Lu et al., 2022). Among all the examined compounds, asperbenzophenone_A was higher in the hydrophobic areas in the brown color. In contrast, talaromyolide_A, kumemycinone_D, and botryotin_B were the lowest with the blue color. On the other hand, all the investigated compounds illustrated huge SAS interaction areas in blue. The 2D chemical interactions always had been a critical parameter in analyzing the best inhibition process. The vigorous interactions, such as conventional hydrogen bond, carbon-hydrogen bond, and pi-donor hydrogen bond, ensured the stability of the docked complex (Chen et al., 2016). All the docked ligands showed several vigorous chemical interactions. Beginning with the 6hzj-talaromyolide_A complex, three

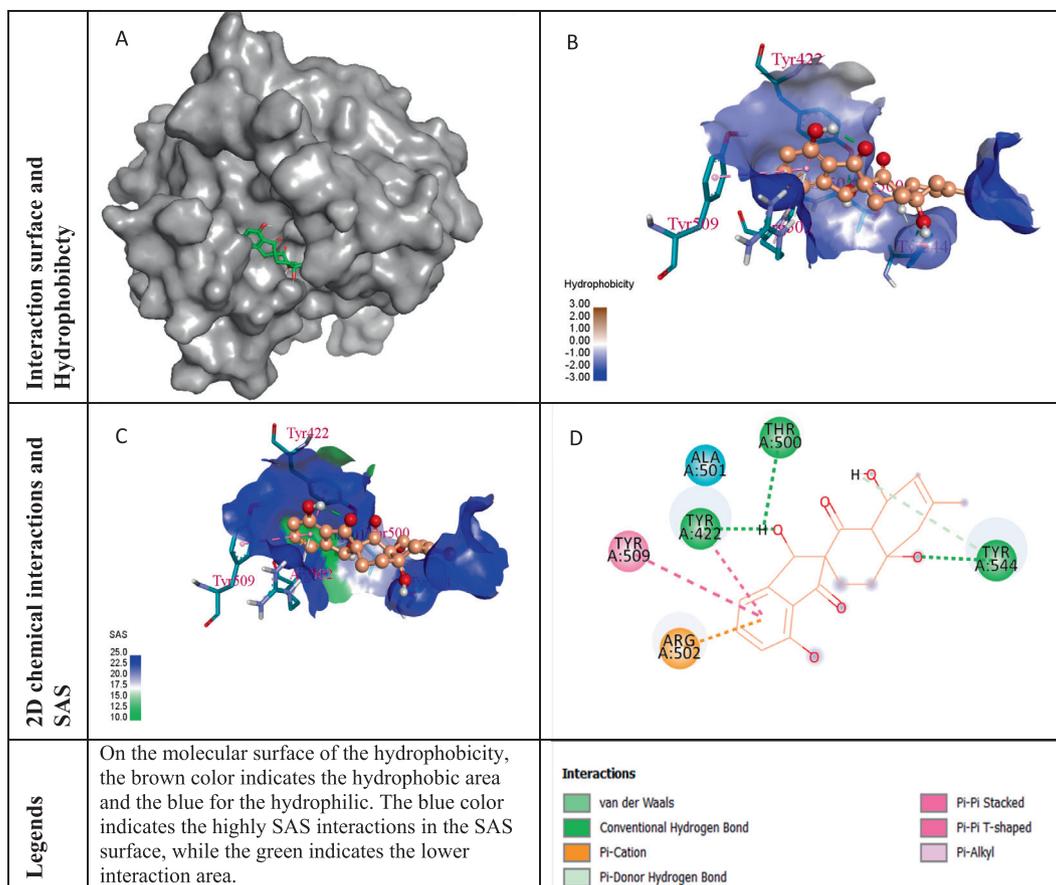


Fig. 5. the molecular interaction results from the docked 6hzj- kumemicinone_D complex, investigated the interaction surface (A), hydrophobicity (B), SAS (C), and the 2D chemical interactions (D).

conventional hydrogen bonds were detected in the amino acids ASN A: 364, ASP A: 346, and ARG A:502. Next, in the 6hzj-elipyrone_A docked complex, five conventional hydrogen bonds were detected in the amino acids ASN A: 364, ARG A: 345, two in ARG A: 502, and TYR A: 422, beside one carbon-hydrogen bond in the amino acid VAL A: 344. After that, in the 6hzj - kumemicinone_D docked complex, three conventional hydrogen bonds were detected in the amino acids THR A: 500, TYR A: 422, and TYR A: 544, beside one pi-donor hydrogen bond in the amino acid TYR A: 544. Also, in the 6hzj-elipyrone_B docked complex, three conventional hydrogen bonds were detected in the amino acids TYR A: 422, THR A: 417, and ASN A: 364. Besides, in the 6hzj- thalysiaketide_A docked complex, one conventional hydrogen bond was detected in the amino acid PRO A: 343 and one carbon-hydrogen bond in the amino acid SER A: 342. On the other

hand, in the 6hzj- asperbenzophenone_A docked complex, four conventional hydrogen bonds were detected in the amino acids ASP A: 304, THR A: 264, LEU A: 504, and LYS A: 503, beside one carbon-hydrogen bond in the amino acid GLN A: 425. In the 6hzj-botryotin_B docked complex, five conventional hydrogen bonds were detected in the amino acids TYR A: 544, LYS A: 313, two with ARG A: 502, and one carbon-hydrogen bond in the amino acid THR A: 500. In the 6hzj-fusarielin_P docked complex, three conventional hydrogen bonds were detected in the amino acids TYR A: 422, THR A: 347, and THR A: 500. In the 6hzj-fusarielin_O docked complex, one conventional hydrogen bond was detected in the amino acid TYR A: 422. Finally, in the 6hzj-(+)-aspergileta_A docked complex, three conventional hydrogen bonds were seen in the amino acids GLN A: 425, MET A: 305, and LYS A: 503.

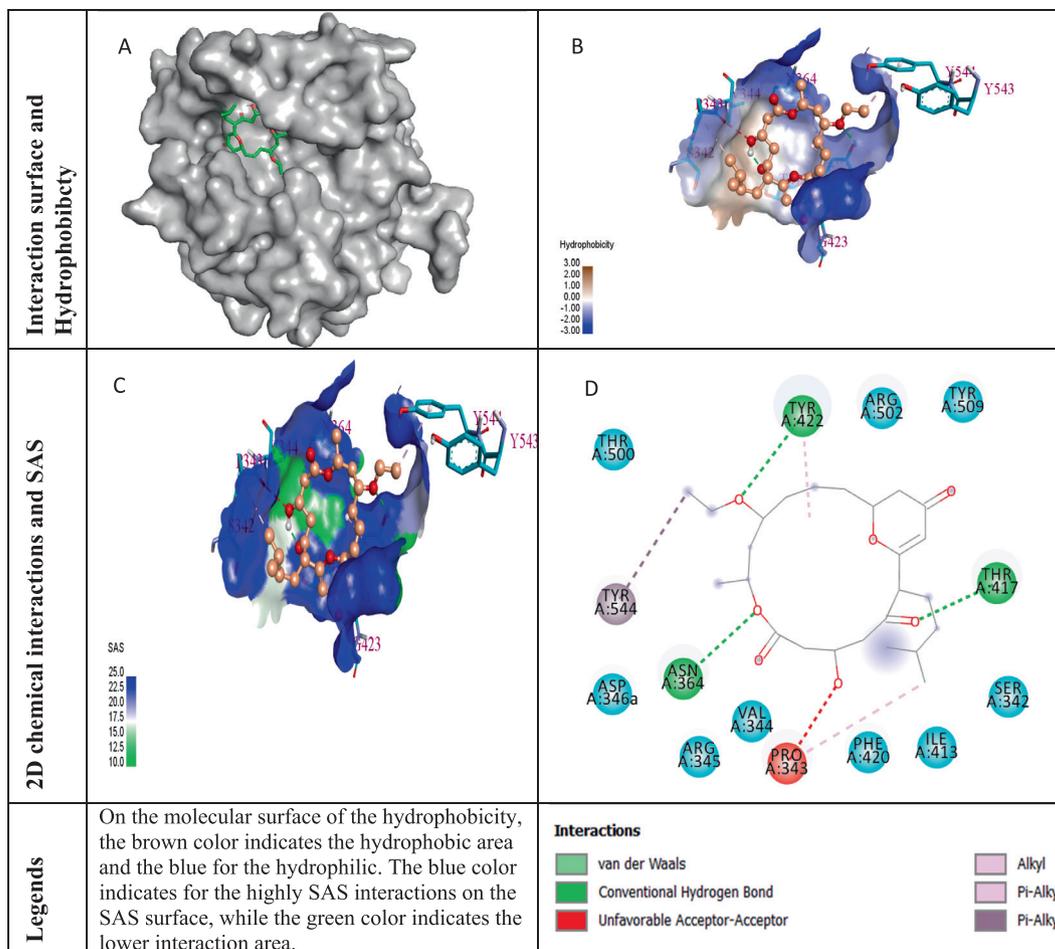


Fig. 6. the molecular interaction results from the docked 6hzj- elipyrone_B complex, investigated the interaction surface (A), hydrophobicity (B), SAS (C), and the 2D chemical interactions (D).

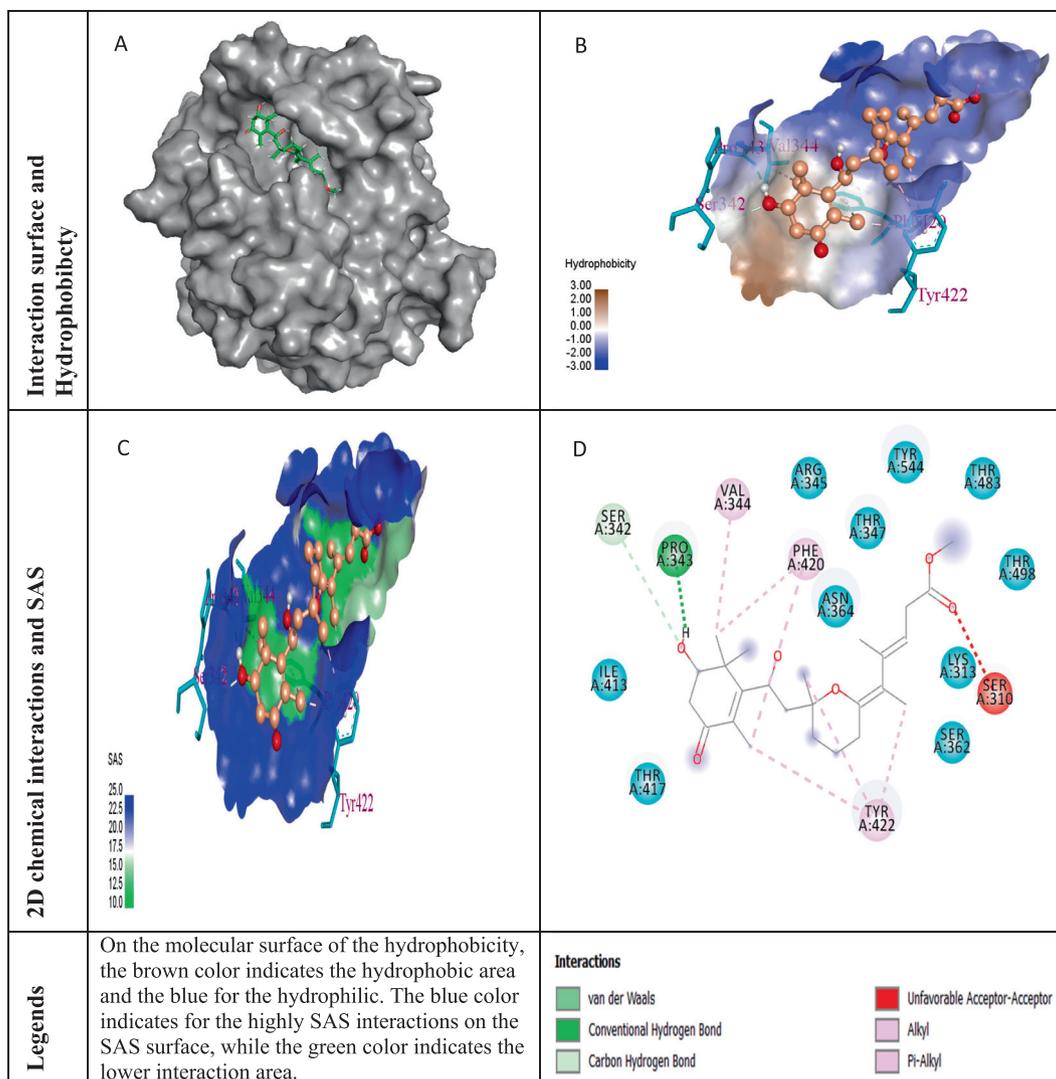


Fig. 7. the molecular interaction results from the docked 6hzi- thalysiaetide_A complex, investigated the interaction surface (A), hydrophobicity (B), SAS (C), and the 2D chemical interactions (D).

5. Conclusion

The most potent compound which was able to inhibit the 6hzi domain with the best chemical interactions, binding energy, and hydrophobicity was elipyrone_A.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2022.103424>.

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