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In-Silico characterisation of antiviral activities of marine fungal derivatives: Focusing on TMPRSS2 as a therapeutic target

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ABSTRACT

Marine fungi represent an underexplored source of bioactive metabolites with significant antiviral potential. The enzyme TMPRSS2, which primes the SARS-CoV-2 spike protein, is a crucial target for preventing viral entry into cells. Natural TMPRSS2 inhibitors derived from marine fungi offer promising therapeutic possibilities. The study evaluated the TMPRSS2-inhibiting potential of 10 antiviral marine fungal derivatives using molecular docking with the Schrödinger Glide software against the co-crystal structure of TMPRSS2 (PDB ID: 7Y0F). Drug-likeness was assessed after docking using QikProp. Molecular dynamics simulations were performed over 120 ns using Desmond, analysing stability parameters such as RMSD, RMSF, solvent-accessible surface area, binding free energy, and radius of gyration. The compounds Norlichexanthone (-6.618 kcal/mol) and Alterporriol Q (-6.516 kcal/mol) demonstrated strong binding to the TMPRSS2 catalytic residues. Norlichexanthone formed stable hydrogen bonds with Ser436, Ser441, and Ser460, while Alterporriol Q engaged through hydrogen bonds and a salt bridge involving His296, Ser441, Ser436, and Lys342. MD simulations indicated stable binding and structural stability, with Norlichexanthone showing advantageous compactness and a binding energy of -120.72 kcal/mol. Alterporriol Q stabilised after initial fluctuations and maintained a significant binding energy of -127.66 kcal/mol. Predictions of drug-likeness favoured Norlichexanthone for oral bioavailability, whereas Alterporriol Q's high molecular weight and lower absorption suggested the need for optimisation. Norlichexanthone and Alterporriol Q could serve as promising natural scaffolds for TMPRSS2 inhibition and broad-spectrum antiviral development, with Norlichexanthone being especially notable for its drug-like properties.

Background

Marine fungi inhabiting ocean sediments, mangroves, and swamps have emerged as essential candidates for medical drug discovery due to their unique metabolites, diverse ecological roles, and promising therapeutic properties [1]. These fungi produce a wide array of secondary metabolites with often distinct chemical structures [2,3]. Marine fungal metabolites are recognised for their specificity and potency, enabling targeted action against pathogens such as viruses and multidrug-resistant bacteria [1]. Recent studies highlight marine fungi as a valuable source for antiviral drug leads. Their metabolites can act through diverse mechanisms—blocking viral entry, inhibiting replication, and disrupting host-virus interactions—making them vital for preventing emerging viral diseases and meeting unmet medical needs. Marine fungi represent a largely untapped resource for drug discovery; they are less studied than terrestrial fungi due to challenges in sampling and cultivation, as well as issues with gene expression, such as biosynthetic gene clusters (BGCs), and concerns over sustainability [4,5].

Marine fungi have been shown to produce compounds with significant antiviral activities against a wide range of viruses [3]. In this context, we aimed to understand the antiviral impact of selected marine fungi derivatives with noted antiviral properties. These marine fungi derivatives were selected based on their strong antiviral properties, especially against HIV-1 integrase, influenza (H1N1) inhibition, anti-RSV effects, SARS-CoV-2 inhibition (in-silico), and their general broad-spectrum antiviral activity [6–8]. The selected compounds are

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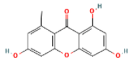
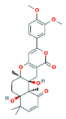
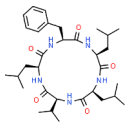
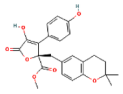
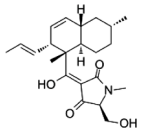
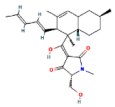
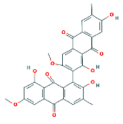
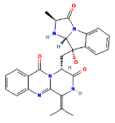
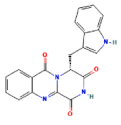
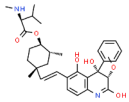
supported by prior reports of antiviral and bioactive properties, and represent diverse, well-characterized structural classes with proven affinity for protein and enzyme targets, especially viral host proteins. Moreover, these compounds could exhibit favorable drug-like molecular complexity with functional groups well-suited for reliable molecular docking and molecular dynamics simulations [7,8]. The in-silico study aimed to identify the most effective marine fungi derivative (s) from the chosen list against Transmembrane Serine Proteases 2 [TMPRSS2]. The protein TMPRSS2 is a host cell enzyme with a central role in SARS-CoV-2 infection. TMPRSS2 primes the viral spike protein for membrane fusion, enabling the virus to enter human cells by facilitating fusion with the cell membrane after binding to the ACE2 receptor [9,10]. Small-molecule TMPRSS2 inhibitors could block viral entry and serve as an attractive antiviral strategy [10,11]. Therefore, TMPRSS2 is a key host factor in COVID-19, mediating viral entry, influencing disease outcomes, and representing a promising broad-spectrum antiviral target [10,12].

COVID-19 remains a globally monitored infectious disease, with increased cases in some regions but an overall reduced threat due to vaccination and improved treatments. Recent data published by the World Health Organisation (WHO) show stable circulation of SARS-CoV-2 worldwide, with higher activity observed in parts of Central America, tropical South America, South, West, North, and Eastern Europe, Western North Africa, and Eastern, Southern, and East Asia [13]. Current treatment options include antiviral drugs, immunotherapies, and supportive care, yet many have limited efficacy or adverse effects [14–18]. Considering this scenario, we conducted a computational analysis to identify the most promising derivatives among the selected ones with inhibitory potential against TMPRSS2. We chose the Nafamostat binding site of TMPRSS2 as a reference for comparative analysis. Nafamostat is highly valued for its ability to bind to the TMPRSS2 catalytic centre and for its preventive effect against the S protein's

Table 1
List of marine-fungi derivatives.

S.No	Compound Name	PubChem ID	Molecular weight (g/mol)	Chemical Structure	Marine Fungi (Species Name)
1	Norlichexanthone	5281657	258.23		Mould Fungi
2	Arisugacin A	10255275	496.549		Penicillium
3	Sansalvamide A	10348337	585.778		Fusarium
4	Aspernolide A	25265784	424.443		Aspergillus terreus
5	Equisetin	54684703	373.486		Fusarium heterosporum and Phoma
6	Phomasetin	54693801	413.55		Fusarium heterosporum and Phoma
7	Alterporriol Q	57332381	566.503		Alternaria Stemphylium
8	Norquinadoline A	71725622	471.508		Cladosporium
9	Oxoglyantrypine	71725700	358.35		Cladosporium
10	22-O-(N-Me-L-valyl)-21-epi-aflaquinolone B	101893728	550.686		Aspergillus

proteolytic process [10].

Methods

Selected ligands from the marine sources

Table 1 lists ten antiviral drugs derived from various marine fungi sources. These drugs are recognised for their effective antiviral properties against several viruses such as Herpes Simplex Viruses (HSVs) (Compounds 1-4), Human Immunodeficiency Virus (HIV) (Compounds 5 and 6), Influenza virus (IFV) (Compound 7), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (Compounds 8 and 9), and Molluscum Contagiosum Virus (MCV) (Compound 10) [19].

Molecular docking QikProp module of Schrödinger Suite

Molecular docking studies were performed to evaluate the binding affinity and interaction modes between TMPRSS2 and fungal metabolites using Schrödinger Suite (version 2025-2). The crystal structure of TMPRSS2 in complex with the ligand, UK-371804 (PDB ID: 7Y0F), was obtained from the Protein Data Bank. Protein preparation was performed using Schrödinger's Protein Preparation Wizard, in which water molecules within 5 Å of the ligand were removed, and missing hydrogens and loops were added. The structure was then subjected to restrained energy minimisation using the OPLS3 force field. A receptor grid was generated based on the energy-minimised structure, with the grid box centred on the co-crystallised ligand. The grid box dimensions were set to 12 × 12 × 12 $Å^3$. The 3D structures of the ligands were sourced from the PubChem database and optimised at a near-neutral pH (7 ± 1). Tautomeric and stereoisomeric variants were generated, and suitable protonation states were assigned. Ligand preparation, including structural refinement and energy minimisation, was carried out using the LigPrep module with the OPLS3 force field. Finally, the binding affinities of the prepared ligands for TMPRSS2 were evaluated using the Glide docking module within Schrödinger Suite. The drug-likeness properties of the ligands were assessed through the Qikprop module of Schrödinger Suite [20,21].

Molecular dynamics

To assess the stability of the binding interactions between the selected compounds and TMPRSS2 in an explicit solvent environment, molecular dynamics (MD) simulations were conducted using the Desmond module in Schrödinger. The docked protein–ligand complexes served as input structures and were prepared with Desmond's system setup utility. The systems were solvated with the TIP3P water model and neutralised by adding Na^+ and Cl^- ions. After setting up the system, energy minimisation and equilibration were performed under NPT conditions using Desmond's default relaxation protocol. MD simulations were then performed under periodic boundary conditions in the NPT ensemble, using the OPLS3 force field. The temperature was maintained at 300 K with the Nose–Hoover thermostat, while the pressure was stabilised at 1 atm through isotropic pressure scaling. A 120 ns production run was subsequently carried out. The production MD simulations were performed using a 2-fs integration timestep under periodic boundary conditions, with all bonds involving hydrogen atoms constrained. A RESPA multiple time-step scheme was utilised, with long-range electrostatics evaluated every 6 fs [22–24].

Results

Molecular Docking Analysis

The advanced docking method developed by Schrödinger revealed potential binding mechanisms and affinities of compounds with TMPRSS2. Among the ten analysed marine metabolites, several showed significant binding affinities (Fig. 1). The standard inhibitor, Nafamostat, exhibited a binding score of -8.735 kcal/mol and formed hydrogen bonds with SER 436 (bond length - 1.66 Å), GLY 646 (bond length - 1.92 Å), ASP 435 (bond length - 2.16 Å), and two hydrogen bonds with GLU 299 (bond lengths - 2.6 Å and 1.52 Å) (Fig. 2A). The high negative value indicates a thermodynamically stable complex, with hydrogen bonds forming between core amino acids central to enzyme activity and substrate recognition, which explains the compound's strong inhibitory effect. Among the studied compounds, Norlichexanthone and Alterporriol Q demonstrated high binding affinity with the protein. Norlichexanthone's binding score was estimated at -6.618 kcal/mol, with hydrogen bonds to SER 436 (bond length - 1.5 Å), SER 460 (bond length - 2.08 Å), and SER 441 (bond length - 2.19 Å) (Fig. 2B). Norlichexanthone forms hydrogen bonds with key residues at the C-terminal serine protease (SP) domain, which is responsible for catalytic activity, suggesting that the compound can inhibit TMPRSS2's proteolytic function. Alterporriol Q also interacted strongly with the protein, with a binding score of -6.516 kcal/mol, forming hydrogen bonds with SER 436 (bond length - 1.6 Å), HIS 296 (bond length - 2.7 Å), and SER 441 (bond length - 2.09 Å). Additionally, it made a salt bridge with LYS 342 across a bond length of 4.9 Å (Fig. 2C). Binding with these residues facilitates peptide bond cleavage, and the interaction of Alterporriol Q with these residues suggests that the compound may block TMPRSS2's proteolytic activity, which is related to viral protein priming. The QikProp module of the Schrödinger Suite was used to assess the drug-likeness properties of the ligands. QikProp evaluates the reactive functional groups of a compound and compares its physicochemical properties with those of established drugs. Key parameters, including molecular weight, hydrogen bond donors, number of rotatable bonds, acceptors, human oral absorption, and polar surface area, were calculated. The predicted values—including rotatable bond count, molecular weight, hydrogen bond acceptors and donors, octanol/water partition coefficient (log P), qualitative human oral absorption, and polar surface area—for Norlichexanthone and Alterporriol Q were within or close to the recommended ranges. These computed properties and their respective standard ranges are presented in Table 2. In Table 2 - A) represents number of rotatable bonds (recommended range: 0-15), B) represents molecular weight (recommended range: 130-725), C) represents number of hydrogen-bond donors (recommended range: 0-6), D) represents number of hydrogen-bond acceptors (recommended range: 2-20), E) represents predicted octanol/water partition coefficient (recommended range: 2-6.5), F) represents predicted qualitative human oral absorption: 1, 2, or 3 for low, medium, or high, G) represents polar surface area (recommended range: 7-200).

Molecular dynamics analysis

The molecular dynamics (MD) simulations were performed for 120 ns on two different protein-ligand complexes: Norlichexanthone-TMPRSS2 and Alterporriol Q-TMPRSS2 (ligands chosen based on their Glide scores). The MD simulation results were analysed to understand the trajectories of the ligand and protein during the simulation and to identify any constraints on the protein. The study included RMSD, RMSF, SASA, Potential Energy, and Ligand Gyration. The interpretation of each component of the MD simulation is provided below.

Root mean square deviation (RMSD)

RMSD measures conformational changes in both the protein and ligands during simulations. Generally, for globular proteins, RMSD values within 3–4 Å are considered highly acceptable, while larger deviations indicate significant conformational shifts and potential instability in the protein-ligand complex. The Norlichexanthone - TMPRSS2 complex showed high stability throughout the simulation, with protein RMSD remaining below 2.5 Å and ligand RMSD staying under 4 Å (Fig. 3A). Key hydrogen bonds with amino acid residues such as ASP435, SER436,

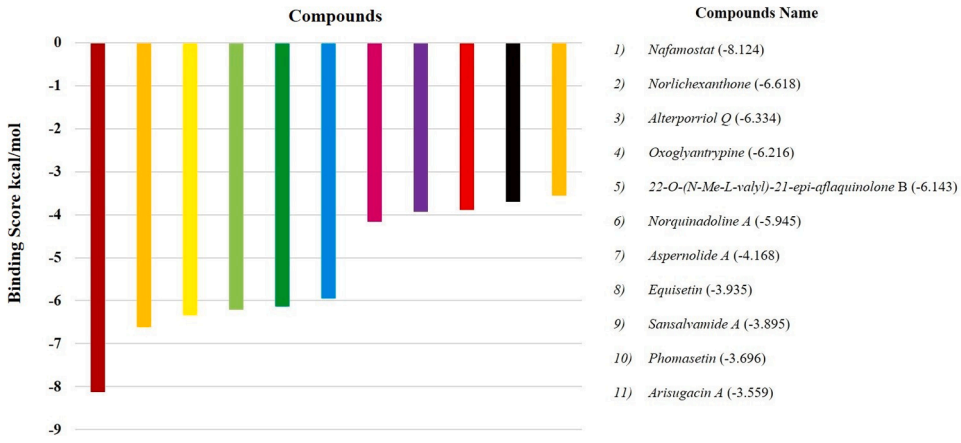


Fig. 1. Docking Score Result.

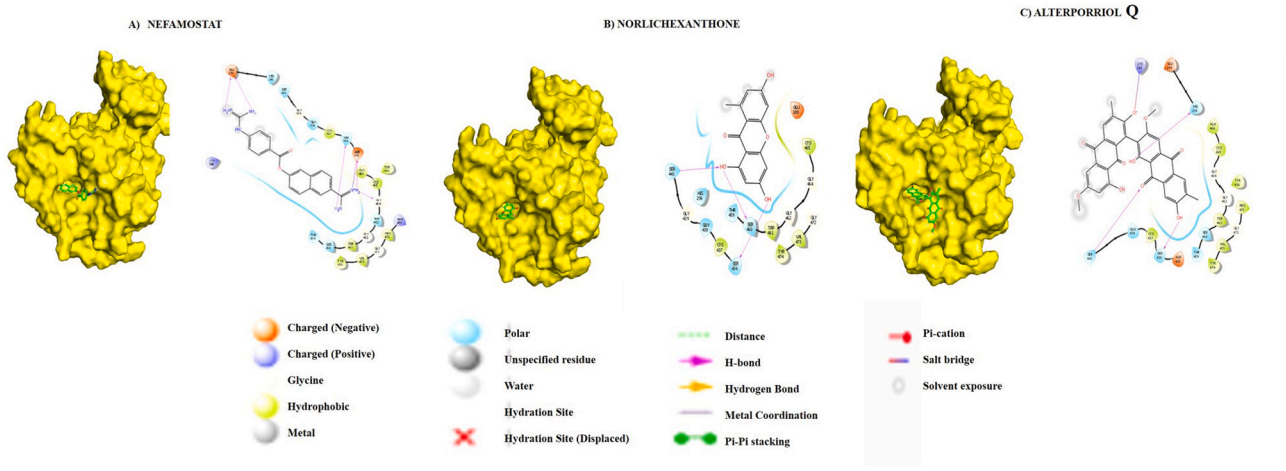


Fig. 2. Protein structure and docking analysis.

Table 2
Prediction of drug likeness properties.

Compound Name	A rotor[a]	B mol_MW[b]	C Donor HB[c]	D Accpt HB[d]	E QPlogPo/w[e]	F Human Oral Absorption[f]	G PSA[g]
Norlichexanthone	3	258.66	2	3	1.374	3	203.545
Alterporriol Q	7	566.56	2	10	2.822	1	208.208
Nafamostat	7	347.376	7	6	0.954	2	152.159

and GLY464 remained stable during the simulation (Fig. 3B). The data indicate that the complex maintained high structural stability during MD, with minimal conformational changes and reliable complex formation. Stable hydrogen bonds throughout the simulation are vital for the long-term retention of Norlichexanthone in the TMPRSS2 active site. The Alterporriol Q - TMPRSS2 complex exhibited slightly higher structural fluctuations, with the protein RMSD reaching up to 3.3 Å. Despite initial fluctuations, the complex stabilised in the latter part of the simulation, and the essential hydrogen bonds involving LYS390, GLY391, and SER436 were consistently maintained (Fig. 4A). The data suggest that initially, the compound displayed moderate dynamic binding. After structural adjustments in TMPRSS2, the ligand adapts to Alterporriol Q, allowing the protein-ligand complex to reach equilibrium in later stages. The consistent hydrogen bonds with key amino acids throughout the MD simulation emphasise that these residues secure Alterporriol Q in the binding site (Fig. 4B). Overall, the RMSD analysis strongly suggests that the marine metabolites Norlichexanthone and Alterporriol Q can inhibit the functional activity of TMPRSS2 more effectively than other marine-derived compounds studied.

Root mean square fluctuation (RMSF)

The RMSF measures the flexibility of individual residues in a protein, indicating the extent to which each residue fluctuates during an MD simulation. The data provide a broad view of the protein's dynamic behaviour and structural flexibility. According to the simulation, higher RMSF values for specific residues or regions suggest increased mobility, which may be vital for protein function — such as facilitating molecular interactions or enabling conformational changes. In the case of the TMPRSS-Norlichexanthone complex, the key binding site residues, including Asp250, Ser215, Pro140, and Ser122, showed relatively higher RMSF values than other residues, indicating increased flexibility in these areas. The data indicate that the complex exhibits exceptionally high structural stability throughout the simulation, suggesting minimal

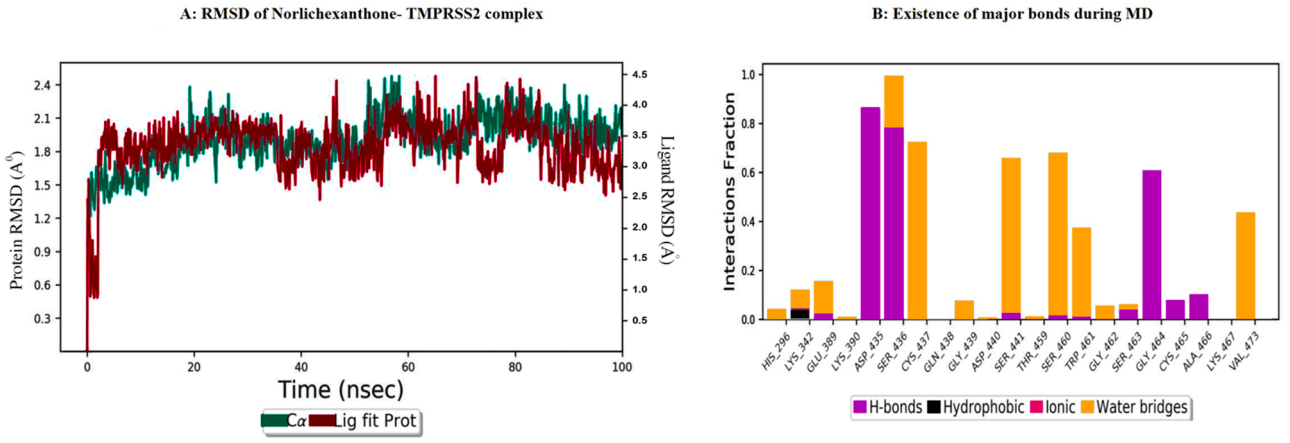


Fig. 3. RMSD and major bonds - Norlichexanthone.

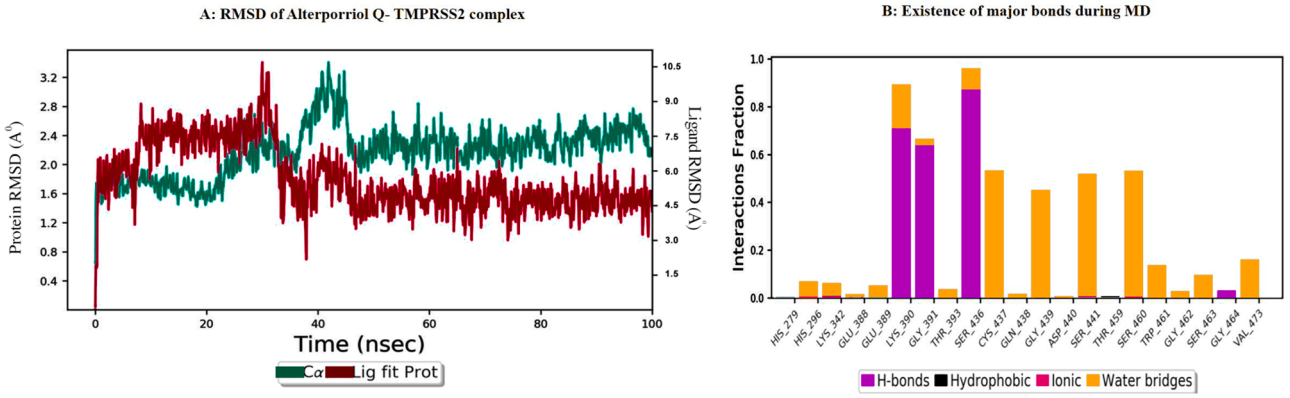


Fig. 4. RMSD and major bonds - Alterporriol Q.

conformational changes and reliable complex formation. Stable hydrogen bond formation throughout the simulation is crucial for the long-term retention of Norlichexanthone in the TMPRSS2 active site. Such flexibility at residues in the key binding area can be critical for a protein to adapt its conformation and maintain productive ligand interactions, thereby potentially enhancing its binding affinity or specificity. Similarly, in the TMPRSS-Alterporriol Q complex, amino acid residues such as Ser122, Ile200, Ser215, Val219, Asp250, and Gly428 exhibited greater fluctuations, indicating dynamic behaviour during the simulation. Table 3 and Fig.s 5A and 5B illustrate the RMSF variations observed throughout the MD simulation period. The results suggest that these residues, situated in the surface-exposed regions near the binding site, exhibit conformational fluctuations that enable the protein to adapt to the presence of Alterporriol Q. This kind of dynamic behaviour is typical in proteins for fitting binding sites, where the protein adjusts its conformation to better engage with the ligand, thereby enhancing binding affinity and specificity. Thus, the data emphasise that the selected compounds interact strongly with the protein, potentially restricting its structure despite changes in amino acid residues.

Table 3
RMSF changes of Compounds.

Compound	Residues	RMSF Å
Norlichexanthone	Asp 250	4.05
	Ser 215	3.46
	Pro 140	2.77
	Ser 122	3.42
Alterporriol Q	Ser 122	4.54
	Ile 200	5.01
	Ser 215	3.8
	Val 219	3.04
	Asp 250	3.07
	Gly 428	3.04

Solvent accessible surface area (SASA)

SASA is a key parameter in molecular dynamics simulations, as it measures the surface area of a molecule accessible to solvent molecules. Data obtained from SASA can provide valuable insights into molecular interactions and the compound's conformational changes during the simulation. Over time, significant fluctuations in SASA indicate conformational shifts or structural flexibility, while lower values highlight the depth of ligand binding within the protein's binding site, potentially leading to a more stable complex with stronger interactions. For Norlichexanthone, the initial SASA was calculated to be 461.827 Å^2. The SASA values consistently decreased below 160 Å^2 during the simulation, indicating stable binding within the TMPRSS binding pocket. A high initial value suggests that Norlichexanthone is exposed to the solvent upon initial binding. The reduced SASA below 160 Å^2 consistently implies that the ligand is buried in the binding pocket and forms a stable complex with less surface exposed to solvent molecules (Fig. 6A). Similarly, Alterporriol Q had an initial SASA of 847.61 Å^2, which decreased to below 500 Å^2 during the simulation, indicating a stable interaction with TMPRSS2. It began with extensive solvent exposure, typically before or at the start of binding (Fig. 6B). A sustained reduction below 500 Å^2 indicates that the protein covers the ligand and is deeply embedded in the binding site. Therefore, the SASA data support the antiviral potential of both marine-fungus-derived TMPRSS2 inhibitors.

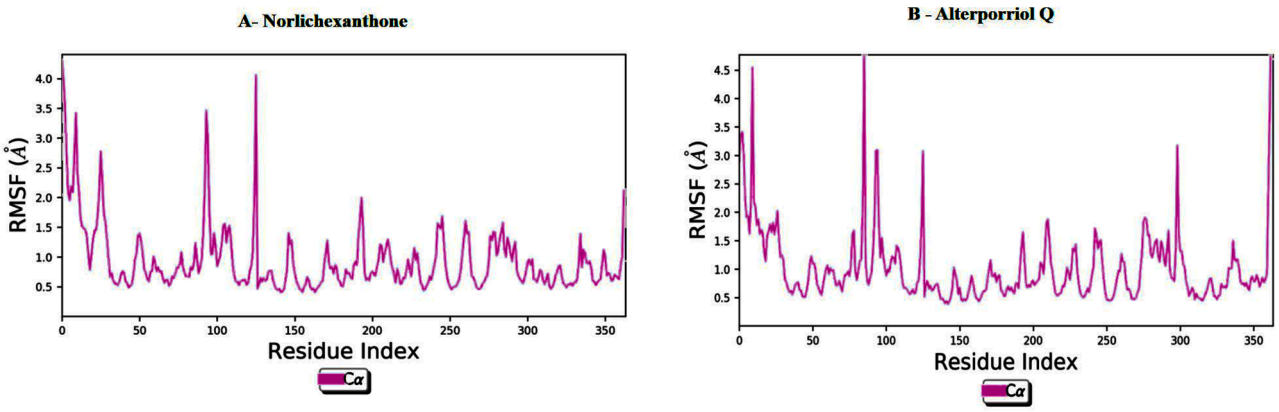


Fig. 5. RMSF changes.

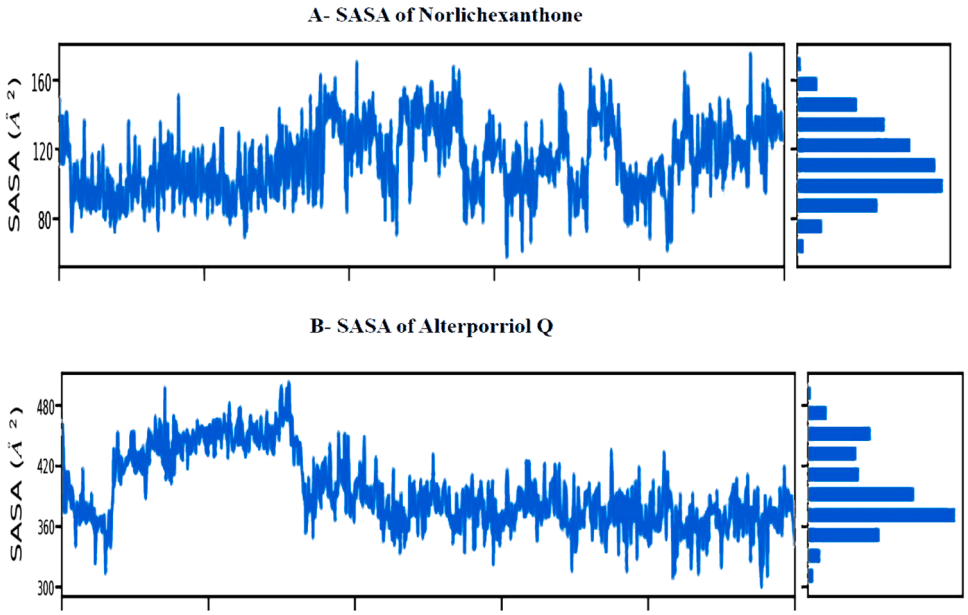


Fig. 6. Solvent Accessible Surface Area.

Binding free energy

The binding free energy was calculated by selecting every 100th frame from a 1000-frame molecular dynamics trajectory, resulting in 10 frames for analysis. Norlichexanthone exhibited an average binding energy of -120.72 ± 4.56 kcal/mol and formed the most stable complex during the MD simulation. The other derivative, Alterporriol Q, also showed a favourable binding energy profile, with an average of -127.66 ± 10.75 kcal/mol. Thus, both complexes display favourable binding energy landscapes and indicate strong binding potential with TMPRSS2. Such stability is desirable for potential inhibitors, as it supports their persistent enzyme inhibition and reliable pharmacological effects.

Radius of gyration

The Rg is an important parameter indicating the overall size and compactness of a molecule during molecular dynamics (MD) simulations. It is recorded over time to monitor structural changes, providing insights into the system's stability and conformational behaviour. Rg is especially useful in assessing how ligand binding affects the structural integrity of a protein. In this study, the compound Norlichexanthone maintained a relatively low Rg value of below 3.30 Å, indicating that the complex does not undergo significant unfolding or destabilisation during the simulation, thereby preserving its structural integrity (Fig. 7A). The compound Alterporriol Q showed an Rg value below 5.25 Å throughout the simulation. Similarly, a lower and stable Rg value for the Alterporriol Q-TMPRSS2 complex suggests minimal expansion or unfolding of the protein complex (Fig. 7B). The data indicate that both compounds can maintain stable structural integrity and preserve compactness during the MD simulation.

Discussion

Compared with synthetic terrestrial drugs, chemical derivatives extracted from marine fungi may offer fewer side effects and greater efficacy in treating infections and chronic diseases [4]. They demonstrate potent specificity and strength, enabling targeted action against pathogens, including viruses and multidrug-resistant bacteria [1, 25–30]. In this study, we used Schrödinger Suite (Version 2025-2) for molecular docking analysis and the Desmond module within Schrödinger for molecular dynamics simulation. Among the selected marine fungal metabolites, Norlichexanthone (-6.618 kcal/mol) and Alterporriol Q (-6.516 kcal/mol) demonstrate higher binding scores, indicating relatively strong binding affinity with TMPRSS2. Additionally, the reference compound Nafamostat shows a higher binding score of -8.735 kcal/mol.

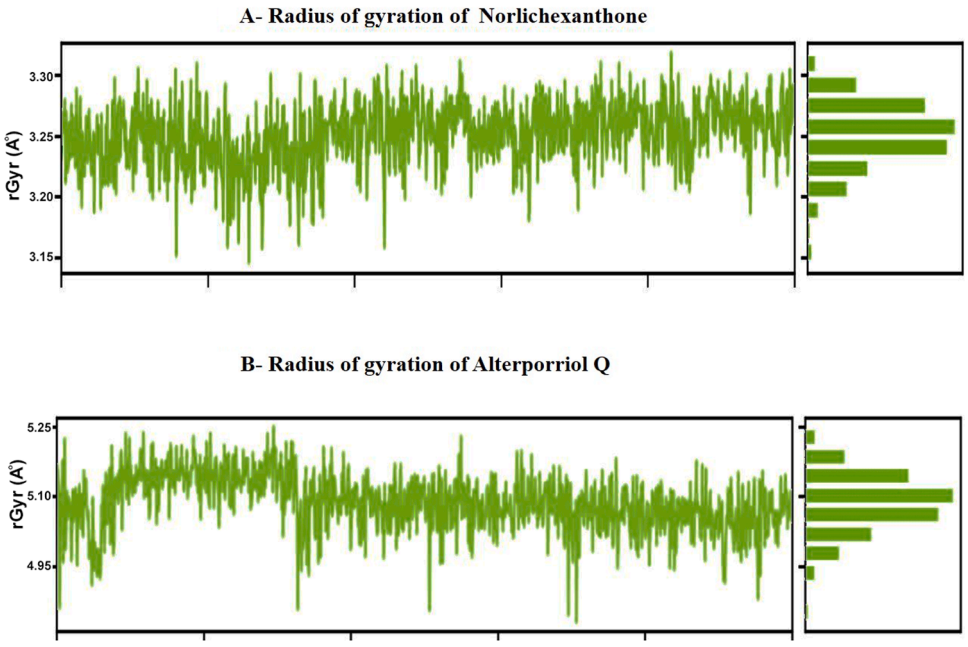


Fig. 7. Radius of Gyration.

The metabolite Norlichexanthone is a demethylated lichexanthone derived from fungi and lichens. It exhibits various activities, including antioxidant and antibacterial effects [31,32]. It has significant pharmacological potential, particularly against the bacterium Staphylococcus aureus, including antibiotic-resistant strains such as MRSA [31, 33–35]. There is no direct or specific evidence or studies supporting the compound's inhibitory potential against TMPRSS2. This report is the first in silico data identifying the antiviral properties, particularly the inhibitory potential of Norlichexanthone, on TMPRSS2. Our molecular data reveal that Norlichexanthone forms hydrogen bonds with key residues, including Ser436, Ser460, and Ser441, within the C-terminal serine protease (SP) domain, which is responsible for catalytic activity. The amino acid residue near the active site cleft (Ser436) contributes to substrate-binding specificity and often interacts with inhibitors or substrates via hydrogen bonding. Meanwhile, Ser460, found within the hydrophobic pocket near the active site, also contributes to protein conformation and substrate interaction through flanking amino acid cleavage sites. The residue Ser441, part of the catalytic triad, is directly involved in serine protease activity (peptide bond cleavage) and aids viral entry into host cells [36]. Thus, the binding of Norlichexanthone to these key, highly conserved serine residues within the serine protease domain suggests that the compound can inhibit TMPRSS2's proteolytic activity by affecting substrate recognition, binding, and catalysis — all critical steps in activating viral proteins. Such inhibition may reduce or prevent viral infection. Therefore, Norlichexanthone shows promise as an antiviral agent because its binding can impair TMPRSS2's enzymatic functions by directly inhibiting its serine protease activity, potentially blocking viral entry and acting as an effective antiviral mechanism. The drug-likeness data of Norlichexanthone also support the notion that the compound exhibits favourable characteristics for oral drug-likeness, including moderate flexibility, a low molecular weight, moderate lipophilicity, and high predicted oral absorption.

The marine fungi compound Alterporriol Q is a hydroxyanthraquinone primarily isolated from marine fungi such as Alternaria and Stemphylium. This anthraquinone is recognised for its wide range of pharmacological activities, including antiviral, anticancer, antibacterial, and anti-inflammatory effects [37–39]. Computational studies identify Alterporriol Q as a potent natural inhibitor of the SARS-CoV-2 main protease, suggesting it may theoretically block viral replication and transcription. Data indicate that Alterporriol Q binds near the catalytic site residues His41 and Cys145 through various non-covalent interactions, including hydrogen bonds and hydrophobic contacts. These residues constitute the active site, a critical functional region for TMPRSS2. Specifically, His41 helps deprotonate the thiol group of Cys145, enabling Cys145 to act as a nucleophile in the attack on peptide bonds during substrate cleavage [38]. Molecular data emphasise that Alterporriol Q could form hydrogen bonds with key active residues in the serine protease (SP) domain of TMPRSS2, such as Ser436, His296, and Ser441, as well as a salt bridge with Lys342. These residues facilitate peptide bond cleavage, and the interaction of Alterporriol Q with these sites suggests that it may inhibit the proteolytic activity of TMPRSS2, which is involved in priming viral proteins for entry into cells. The salt bridge involving residues such as Lys342, located in the S2 substrate-binding subsite, further supports the idea that the compound inhibits TMPRSS2 function primarily through salt bridge formation. Consequently, the docking data verify that the compound is positioned to act as a competitive inhibitor by blocking the proteolytic function essential for SARS-CoV-2 entry. Previous potent inhibitors, such as Nafamostat and Camostat, also demonstrate stronger binding scores and covalent/catalytic triad engagement with Ser441, Asp345, and His296 [40]. Although Alterporriol Q is non-covalent, it can form multi-residue interactions, like those observed in known inhibitors, supporting its stable interaction, albeit at slightly lower affinity, which remains highly relevant pharmacologically. Our findings align with earlier docking and MD studies, which identified the compound as having strong ligand stability and inhibitory potential with TMPRSS2 [38]. Although direct experimental data on the interaction of Alterporriol Q with TMPRSS2 are not yet available, the similar interaction profile and energies suggest that Alterporriol Q could serve as a promising lead for developing natural antiviral agents targeting host proteases. Furthermore, the drug-likeness profile indicates that, despite its high molecular weight and numerous hydrogen-bond acceptors, the compound exhibits low predicted oral absorption, suggesting reduced bioavailability.

The RMSD results strongly support the idea that Norlichexanthone can stably occupy the TMPRSS2 active site through hydrogen bonds and maintain the structural integrity of the complex. This suggests that the molecules are robust as functional inhibitors, indicating the establishment of a potent TMPRSS2 inhibitor. This data aligns with earlier reports on Nafamostat and Camostat, in which long-lasting hydrogen bonds to comparable active-site residues demonstrate greater stability

and thus potential antiviral activity [41]. Conversely, the RMSD of the Alterporriol Q-TMPRSS2 complex shows that although the complex initially exhibited moderate structural fluctuations, with a protein RMSD reaching up to 3.3 Å, its stabilisation in later simulations suggests it could effectively inhibit enzyme function, with strong and sustained inhibitory activity. In the case of Norlichexanthone, the higher RMSF values for Asp250, Ser215, Pro140, and Ser122 indicate that these amino acid residues actively participate in the binding-site dynamics in conjunction with TMPRSS2, which are crucial for molecular interactions and conformational changes. This type of strategic residue mobility can help expose catalytic or critical interaction sites further, locking the ligand in place or enabling water-mediated contacts that are favourable for drugability. Similarly, the higher RMSF values for Ser122, Ile200, Ser215, Val219, Asp250, and Gly428 in the TMPRSS2-Alterporriol Q complex emphasise the compound's dynamic role in achieving and maintaining protein-ligand binding, thereby facilitating the necessary conformational changes for robust and specific molecular maturation. This further supports the pivotal role of Alterporriol Q as a biological inhibitor of viral activity.

The observed SASA shows that, although both Norlichexanthone and Alterporriol Q become less accessible to solvent initially, as the MD simulation progresses, both compounds demonstrate stable, persistent binding within the TMPRSS2 binding pocket. This suggests the potential of these fungal derivatives as antiviral inhibitors targeting the core function of TMPRSS2. The binding free energy of Norlichexanthone (-120.72 kcal/mol) indicates that it forms the most stable complex with TMPRSS2 in the simulations, making it a strong candidate for future inhibitor optimisation and development. Compared to other classical TMPRSS2 inhibitors, Norlichexanthone's more negative binding energy suggests it can form a more robust and extensive interaction with TMPRSS2′s active site [42–46]. The highly negative average binding energy of Alterporriol Q (-127.66 kcal/mol) suggests that it can also form a highly stable complex with TMPRSS2. This demonstrates a strong and stable interaction, supporting its potential as an antiviral inhibitor candidate and potentially matching or exceeding the efficacy of known inhibitors in silico [26].

The low and stable Rg value (3.30 Å) of Norlichexanthone indicates that the TMPRSS2-ligand complex remains compact and stable, reinforcing the ligand's potential effectiveness as a TMPRSS2 inhibitor. The Rg value of Alterporriol Q, which is below 5.25 Å, suggests that the compound could maintain the structural integrity of TMPRSS2 during simulation and supports a stable and effective inhibitory potential of the respective derivative. This compactness directly correlates with sustained inhibitory efficacy, as it prevents large-scale structural fluctuations and therefore reduces binding or enzymatic inhibition. This compact state, together with other parameters such as SASA and binding energy, underpins the conclusion that both marine fungi derivatives, including Norlichexanthone and Alterporriol Q, form a robust and stable interaction with TMPRSS2.

Conclusion

Due to their unique adaptations and metabolic capabilities, derivatives from marine fungi remain a rich and abundant source of drugs with diverse applications in human health research. Marine fungi-derived drugs are highly desired for their capacity to address issues such as drug resistance and toxicity, which are common in synthetic antivirals. Marine fungal metabolites, such as Norlichexanthone and Alterporriol Q, show significant potential as natural TMPRSS2 inhibitors with antiviral properties. MD results revealed strong binding affinity for both compounds and interactions with key catalytic residues of TMPRSS2. Molecular dynamics simulations confirmed the stability of both ligand-protein complexes, with Norlichexanthone exhibiting highly stable conformations and demonstrating effective inhibitory potential. Alterporriol Q showed initial fluctuations but stabilised during the simulation, indicating a robust interaction with TMPRSS2. Although Alterporriol Q is limited by low oral absorption and high molecular weight, it's strong in silico inhibition profile makes it a promising lead for further optimisation. Conversely, Norlichexanthone combines potent inhibitory ability with favourable drug-likeness parameters, supporting its potential as an orally active antiviral agent. Notably, our investigation reveals that marine fungal metabolites can serve as novel scaffolds for inhibiting TMPRSS2. This is the first in silico study of its kind, further emphasising the need for advanced research, such as in vitro and in vivo studies, to facilitate practical application in antiviral therapeutics.

Abbreviation

TMPRSS2- Transmembrane serine protease 2, MD- Molecular Dynamics, MS- Molecular Simulation, RMSD- Root Mean Square Deviation, RMSF- Root Mean Square Fluctuations, PE- Potential Energy, SASA-Solvent Accessible Surface Area.

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CRediT authorship contribution statement

Reji Manjunathan: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simi Sam:** Writing – review & editing, Visualization, Validation. **Vijeesh Vayyattil:** Formal analysis, Data curation. **Arun Kumar:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The author declares that there are no competing interests.

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Data availability

Data will be made available on request.

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