Secondary metabolites from *Caulerpa cylindracea* (Sonder) could be alternative natural antiviral compounds for COVID-19: A further *in silico* proof

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Abstract

SARS-CoV-2 has been exhibiting extremely spreading property all around the world since its existence from Wuhan-China in December-2019. Although it has caused a death toll of over than 1.3 M people, no validated vaccine has been proposed yet. On the other hand, very dense studies on the vaccine development have been carrying out in some countries such as the US, Germany, UK, China and Russia. Due to side effects of current antiviral agents used in the therapy of COVID-19, there is a great need for the development of alternative compounds for this disease. Caulerpin (CPN) and caulerpenyne (CYN), predominant natural secondary metabolites from invasive marine green algae *Caulerpa cylindracea*, are proposed to neutralize the virus from two targets: spike protein (5XLR) and main protease (6YB7) in this study. The results show that the binding energies related to CPN-6YB7 and CYN-6YB7 interactions are found to be -8.02 kcal/mol and -6.83 kcal/mol, respectively. The binding energies were -9.68 kcal/mol and -7.53 kcal/mol, respectively, for CPN-5XLR and CYN-5XLR. In the molecular dynamics results, RMSD values show that CPN and CYN can form stable complexes with the proteins where CYN is more stable with 6YB7 and CPN interacts better with 5XLR. These differences seem to be based on the type of interactions of the complexes. In conclusion, caulerpin and caulerpenyne can further be investigated experimentally for their anti-SARS-CoV-2 efficiency.

Keyword: blind docking, COVID-19, in-silico, molecular dynamics, SARS-CoV-2, structural bioinformatics.

Introduction

An outbreak in the seafood bazaar of Wuhan-China was reported in December 2019 and then the outbreak transformed into a pandemic very fast. It was found that the new strand of coronavirus named SARS-CoV-2 was responsible for this pandemic [1-3]. The disease caused by SARS-CoV-2 was named as COVID-19 by WHO [4]. Corona viruses can be classified under four class: alpha-CoV, beta-CoV, gamma-CoV, and delta-CoV. The new strain (SARS-CoV-2) is clustered under beta-CoV [5]. The severe symptoms of SARS-CoV-2 infection are pulmonary edema and pneumonia [6]. With these symptoms the SARS-CoV-2 virus has spread widely around the world, making it one of the well-known species in the history of virology. The main protease enzyme of SARS-CoV-2 (Mpro) has attracted the attention of researchers to eliminate the virus and produce new drugs. Mpro is the enzyme that allows the virus to fabricate itself inside the host cell, thereby creating new copies that are capable of infecting other healthy cells [7]. Spike protein of SARS-CoV-2 (S-Protein) is also an important target for SARS-CoV-2 since it is responsible to infuse the cell via human angiotensin-converting enzyme 2 (hACE-2). hACE-2 is expressed mostly in lungs. SARS-CoV-2 uses hACE-2 as an entrance gate to human cells. Therefore, Mpro and S-Protein are critical targets to neutralize the virus. Although current and known antiviral agents are currently used in the therapy of COVID-19, they are not effective and they are not SARS-CoV-2 specific. Therefore, the virus has been spreading and is still responsible for the death of over than 1.3 M people (According to the date of 22.11.2020). Therefore, there is a great need for the development of drugs to eradicate this virus [8-9]. For the development of drugs, in-silico tools are of great importance since they decrease the time required for discovery and they are cost-effective. Therefore, these tools can be used to discover and develop new valuable drugs. Although antiviral drugs such as tipranavir, raltegravir, remdesivir, ribavirin,

chloroquine, and favipiravir have shown promising results in the treatment of SARS-CoV-2, new agents with lower side effects must be discovered [10-11]. Some in-silico studies have shown that some natural bioactive compounds including caulerpin (CPN) have inhibitory properties for the Mpro [12]. Same research group published a subsequent report to show the inhibitory properties of CPN and its derivatives on the Mpro and S-Protein of SARS-CoV-2 [13]. In this study, we wanted to study further by using Blind docking technology developed by UCAM-HPC and also propose another important secondary metabolite caulerpenyne (CYN) of *C.cylindracea*. Other important dimension of the present study is to reveal the importance of an alien seaweed in the Mediterranean Sea. *Caulerpa cylindracea* (Sonder) (hereafter *C.cylindracea*) is an invasive seaweed in the Mediterranean Sea. It was first observed in the Sousse harbor of Tunisia. Starting from 1991, it has shown the invasive properties by affecting indigenous species in the Mediterranean Sea [14]. Since there has been no proposed or approved eradication method on this alien seaweed, alternative evaluation methods are needed. Many papers have so far been reported for the use of biomass this species. The reports are generally focused on CYN and CPN. They are sesquiterpene and bisindole based structures, respectively. They have so far been recommended for many biotechnology-based processes including enzyme inhibitions [14]. Ahmed et al (2020) proposed caulerpin and its 20 different derivatives as the inhibitors of Mpro and S-Protein of SARS-CoV-2. They also found high binding affinity to these receptors. On the other hand, they did not study the interactions of the ligands outside of the active site of these proteins [12-13]. In our investigation, the interactions outside of the active site are also reported by using the blind docking methodology available online at Blind Docking (BD) Server. This software was used to improve the knowledge about molecular pathways of auto inflammatory diseases [15], also this technique was utilized in infectious diseases including the discovery of new possible treatments against virus like HIV-1 or SARS-CoV-2 [16-17]. More interaction

points reported by the present study may contribute further support to the idea of development of *C.cylindracea*'s secondary metabolite-based SARS-CoV-2 neutralizing agents predicted by BD technique [18].

Materials and Methods

Protein and Ligands

The sdf files of CPN (CID:5326018) and CYN (CID:5311436) were retrieved from https://pubchem.ncbi.nlm.nih.gov/ [19]. The files of Mpro (6YB7) and S-Protein (5XLR) of SARS-CoV-2 were retrieved from https://www.rcsb.org/ in the pdb format [20].

Molecular Docking

Blind docking calculations were carried out through Blind Docking Server, available at: http://bio-hpc.eu/software/blind-docking-server/ [18]. Before the docking, water molecules were removed, Kollman charges and polar hydrogens were added to Mpro (6YB7) and S-Protein (5XLR) of SARS-CoV-2 using AutoDock Tool version 1.5.6 [21-22]. In order to examine the interactions between ligands (CYN and CPN) and receptors (Mpro and S-Protein), Maestro (Maestro version 9.4 Schrödinger, LLC) was used.

Molecular Dynamics

The complexes obtained in the docking results were used for the Molecular Dynamics (MD) simulations. These complexes were carried out with MD engine Desmond (Desmond, Schrödinger, LLC, NY, USA) which is proceed by Maestro (Maestro version 9.4 Schrödinger,

LLC). Also this software was used to analyzed the score of MM/GBSA values of all complexes (Data now shown). All complexes were immersed into a box filled with water molecules with the simple point charge (SPC). The box was the following dimensions: x=10 Å, y=10 Å and z=10 Å. Counter ions (6 Na^+) were added to neutralize charges. Energy minimization was made by 2000 steps using the steepest descent method with a threshold of 1.0 kcal/mol/Å. The NPT simulations were realized at 300 K with the Nosé-Hoover algorithm [23-24] and the pressure was maintained at 1 bar with the Martyna-Tobias-Klein barostat [25]. The simulation length was 100 ns. Periodic boundary conditions were used. The cutoff of 9 A was established to van der Waals interactions and the Particle Mesh Ewald (PME) method with a tolerance of 10^{-9} was used in the electrostatic part. The force field used in all runs was OPLS3e [26].

Prediction of drug-likeness descriptors

Lipinski's parameters, relations with P-glycoprotein, gastrointestinal absorption and blood-brain barrier transportation information were obtained via using the SwissADME tool [27]. The SwissADME tool accepts SMILES format as input data. The SMILES formulas of ligands were retrieved from https://pubchem.ncbi.nlm.nih.gov/.

Results and Discussion

The binding affinities of CPN and CYN on the S-Protein and Mpro were obtained by using Blind Docking Server. While best docking score values for 6YB7-CPN and 6YB7-CYN were found to be -8.02 kcal/mol and -6.83 kcal/mol (Table 1), the best scores of 5XLR-CPN and 5XLR-CYN were obtained as -9.68 kcal/mol and -7.53 kcal/mol (Table 2). The highest 6YB7-CPN, 6YB7-CYN, 5XLR-CPN and 5XLR-CYN binding energies, interactions, interacted

amino acids and distances are given in Table 3-4. The binding sites and interacted residues of each protein-ligand docking were presented in Figure 1-4. Lipinski's rules are evaluated to filter compounds that do not accomplish ideal pharmacological properties and it is aimed to be filtered at the beginning of drug development stages. In this study, Lipinski's parameters were calculated by using the SwissADME tool. The SMILES formulas of ligands were obtained in Table 5. The molecules that follow Lipinski's rule of five (RO5) must meet the rules of molecular mass < 500; Hydrogen-bond donors (HBD) < 5; Hydrogen-bond acceptors (HBA) < 10; and Log P < 5. According to our analysis, CPN and CYN were complied with these rules. All data about Lipinski's RO5 analysis can be found in Table 6. Gastrointestinal absorption and access to the brain are two pharmacokinetic models that should be predicted at various stages of drug discovery processes. In order to get this information, the BOILED-Egg graphs were created using the SwissADME tool. According to BOILED-Egg graphs, CPN and CYN were found not to be effluated from the central nervous system by the P-glycoprotein (PGP-) and passively absorbed by the gastrointestinal tract (Figure 5-6).

In order to study the dynamic stability of the ligand-protein complex, MD simulations were analyzed. The evolution of the root-mean-square-deviation (RMSD) on all residues was useful to observe the stability of the complex. Regarding the complex made by CYN and Mpro (6YB7), Figure 7 showed that conformation of Mpro changed slowly. One of the evidences of this affirmation is that the value of RMSD at the end of the simulations was higher than 4 Å. Also RMSD values of CYN showed that the ligand changed slightly its positions in the beginning of the simulation. The CYN-Mpro interactions were shown in Figure 8. In the complex, CYN had hydrophobic interactions with three residues (Tyr118, Tyr126 and Phe140) and hydrophilic interactions with two (Ser139 and Phe140). Figure 9 showed that acetyl groups of CYN were the functional groups that more contact with the Mpro. Whereas the complex made by CPN and the Mpro, the Figure 10 showed that Mpro

changed its conformation and in the end of the simulation RMSD reached a value of 4Å. RMSD values of CPN showed that the ligand changed its positions along the simulation. As shown Figure 11, the analysis of the contact between ligand and protein shown that the ligand had hydrophilic interactions with three threonines (198,199 and 239), Lys236 and Asn238, and hydrophobic interactions with two residues (Met235 and Lys236). The interaction with Lys236 was a π-cation interaction (Figure 12). However, the contacts most important were hydrogen-bonds between atoms of the ligand.

Regarding complex made by CYN and the S-Protein (5XLR) the Figure 13 shows that the conformation of the spike protein was in unstable status until the end of the simulation. The evidence of this lack of stability; it's the fact that the value of RMSD was rising until the end of the simulation (final value was 4 Å). The CYN-S-Protein interactions were shown in Figure 14. In the complex the CYN had hydrophobic interactions with three residues (Phe69, Ile234 and Ala250) and hydrophilic interactions with Ala78. Figure 15 shown that the three acetyl groups had interactions with hydrophilic residues of S-protein. Concerning the CPN-S-Protein, the Figure 16 showed that the conformation of the protein is more stable than in the CYN-S- protein, because the value of RMSD at the end of simulation was 2.7 Å. The CPN-S-Protein interactions were shown in Figure 17. In the complex, the CPN had hydrophobic interaction with three residues (Lys291, Phe815 and Lys946) and hydrophilic interactions with Thr943 and Lys946. Figure 18 showed that one of the acetyl groups interacts with Thr943 and the other with Lys946. Also two lysines (291 and 946) had interactions with the rings of the CPN.

Whilst CYN seemed to be a better candidate could be the better candidate to inhibit Mpro, CPN was the best candidate to inhibit S-Protein. Both affirmations were based on the fact that the CYN-S-Protein complex and CPN-S-Protein complex were the most stable complexes. One of the main reasons for these differences could be the type of interaction. While

hydrophobic interactions seemed to stabilize the proteins, the hydrophilic interactions increased the RMSD values, that is to say, the complexes with this type of interactions had less stability.

Abdelraheem et al (2020) investigated ten different bioactive compounds including CPN. Crystal structures of 3CLpro (6LU7), Mpro (2GTB and 3TNT) were used as receptors. According to their analysis, CPN was found as the best binding ligand to the receptors. Therefore, they examined CPN and its derivatives in their recent study [13]. They used 6LU7 (Mpro) and 6VYB (S-Protein) as receptors for CPN derivatives. Binding energy, ligand-receptor interactions and active site residues are taken into consideration by Ahmed et al (2020) in their calculations. The detailed comparison of CPN and CYN interactions with 6YB7 and 5XLR are shown in Table 7-11. According to our analysis, CPN interacts with Asn95 and Thr98 residuals of 6YB7 through polar interactions, with Pro96 and Pro99 through hydrophobic interactions, with Lys12,Lys97 and Lys100 through charged (positive) interactions and with Asp33 and Asp155 through charged (positive) interactions as shown in Figure 1 and Table 3. CYN interacts with Ala7, Phe8, Pro9 and Phe305 residuals of 6YB7 through hydrophobics interactions, with Arg298 through charged (positive) interaction and with Thr304 through polar interaction as shown in Figure 2 and Table 3. CPN interacts with Thr51,Gln818 and Asn942 residuals of 5XLR through polar interactions, with Leu52, Tyr53 and Val555 through hydrophobic interactions and with Lys291 and Lys946 through charged (positive) interactions as shown in Figure 3 and Table 4. CYN interacts with Gly77 residuals of 5XLR through glycine type interaction, with Val97, Val98, Ile234, Phe238, Ala249, Ala250 and Tyr252 through hydrophobic interactions and with Thr236 through polar interactions as shown in Figure 4 and Table 4 . Since the stability of the 6YB7 and 5XLR are very high, development of new drugs will be very difficult. Current drugs may not inhibit the activity or function of these proteins completely [13-28-29]. CPN can easily be obtained by single step

isolation from invasive *C.cylindracea* distributed in the Mediterranean Sea. Since it has been used as a drug in traditional medicine in far east countries, CPN-based consumption can also be proposed to people to prevent the disease. *Caulerpa lentillifera* that is known as green caviar in the far east is commonly consumed as salad [30]. Since CPN also existed in the tissues *C.lentillifera* [31], aquaculture of this species or *Caulerpa* genus members can be exploited. CPN based functional foods can also be prepared since it has no side effect reported so far on this molecule. The present paper reports the interaction of CPN with SARS-CoV-2 targets (6YB7 and 5XLR) outside the functional side of these proteins. Interaction of CPN or CYN with these receptors outside their functional site may also stop their functions vie conformational changes. CYN is also an important secondary metabolite for *Caulerpa* members. Since they are siphonous algae, any damage in their tissues can result in the leakage of genetic materials [14]. In order to protect this kind of external damage, members of the *Caulerpa* genus have developed excellent wound-closure in the evolutionary process [32]. Even if CYN is reported as a secondary metabolite in some papers [14-34], main function of the CYN is also associated with wound closure to prevent the leakage of genetic material [32-33]. Practically, after a damage, an acetyl group of CYN is removed by esterase based mechanisms. After enzymatic transformation, CYN is transformed into oxytocin which is more reactive compared to CYN [33]. Oxytocin plays like a cross linked agent in the damaged tissues by interacting side chains of specific amino acids within damaged tissues [33-34]. Similar mechanisms can also be created to fight with SARS-CoV-2. CYN and esterase can be co-encapsulated within hACE-2 including membranes. When SARS-CoV-2 is attached to the CYN-esterase including membrane via hACE-2, the RNA and related enzymes of SARS-CoV-2 can be destroyed by degradation of CYN with esterase. Alternatively, semi-synthetic CPN or CYN based compounds can be synthesized in the laboratory for a sustainable production since the isolation of these compounds can be problematics because of

many parameters such as sun light, sea water temperature or the physicochemical parameters of the sea water [34].

In conclusion, since *Caulerpa* genus members, especially invasive ones, reveal important biological activities including antiviral effects, they could be further tested in vitro and in vivo studies. This study provides further confirmation for not only for caulerpin but also caulerpenyne.

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