

Research Article

Evaluation of Natural Peptides to Prevent and Reduce the Novel SARS-CoV-2 Infection

María Paredes-Ramos ^{1,2}, Enma Conde Piñeiro ³, Horacio Pérez-Sánchez ⁴,
and José M. López-Vilariño ²

¹METMED Research Group, Physical Chemistry Department, Universidade da Coruña (UDC), Campus da Zapateira, A Coruña, Spain

²Hijos de Rivera S.A.U., C/José María Rivera Corral 6, A Coruña, Spain

³GLECEX (Global and Ecofriendly Natural Extracts S.L.), Edificio CITI, Parque Tecnológico de Galicia, San Cibrao das Viñas, Ourense, Spain

⁴Structural Bioinformatics and High-Performance Computing Research Group (BIO-HPC), Computer Engineering Department, Universidad Católica San Antonio de Murcia (UCAM), Campus de los Jerónimos s/n, Murcia, Spain

Correspondence should be addressed to María Paredes-Ramos; mparedes@estrellagalicia.es

Received 22 December 2021; Revised 27 January 2022; Accepted 2 February 2022; Published 10 March 2022

Academic Editor: Muhammad H. Alu'datt

Copyright © 2022 María Paredes-Ramos et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In a preventive context, natural peptides can play a major role against SARS-CoV-2, so their character of GRAS (generally recognized as safe) means they would not need innocuity analyses to be employed. This study analyses the potential of pea peptides, LSDRFS and SDRFSY, and amaranth peptides, GGV, IGV, IVG, VGV, and VIKP, against the SARS-CoV-2 hosts, ACE2 (angiotensin-converting enzyme 2), ACE (angiotensin-converting enzyme), and CD26 (cluster of differentiation 26), and SARS-CoV-2 enzymes, spike glycoprotein and 3CLpro (3-chymotrypsin-like protease). Also, currently used drugs were analysed to contrast drug and peptide behaviour. Employing docking, virtual screening, and molecular dynamics assays, SDRFSY, LSDRFS, and VIKP were detected as potential bioactive peptides by blocking ACE2 and CD26 or reducing the inflammation associated with COVID-19. Enzyme inhibition analyses were also performed, proving the ability of SDRFSY and LSDRFS as ACE2-blocking agents against the spike glycoprotein with inhibition capacities above 80%.

1. Introduction

The high transmission (R_0 1.5–6.8) [1, 2] and mortality rates (1–3%) [3] of SARS-CoV-2 had caused a global epidemic. Although several vaccines were developed in a record time, they are not yet available in many parts of the world, so another strategy is required not only to treat COVID-19 patients, but also to prevent the infection of healthy individuals and the reinfection of recovered patients or, at least, to mitigate the harmful effects of the infection.

Great efforts were done by the scientific community to reposition several approved antiviral and anti-inflammatory drugs [4–7]. In this regard, it is well known that some hydrolysed peptides show very high bioactivity when

interacting with different human proteins, generating functional benefits in some cases and food allergies in others [8]. Therefore, these peptides could be a good option to take a step forward against SARS-CoV-2 infection. They are directly extracted from natural sources, with no modification of their molecules, so they are not structurally optimized to exert a certain effect and they possess less efficacy than drugs. Nevertheless, the absence of toxic effects related to their consumption makes them excellent candidates to be used as a preventive treatment or even as a treatment for those patients with minor symptoms, acting similarly as cough syrups for mild colds.

To fight this novel coronavirus, two different strategies can be applied: the direct treatment of the virus or the

treatment of the host. Several studies were performed to identify the main receptors of both, and among them, spike glycoprotein, 3-chymotrypsin-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), and papain-like protease (PLpro) are signalled as the key proteins of the virus. The spike glycoprotein is located on the outer envelope of the virion and contributes to the cell receptor binding, and the 3CLpro enzyme is indispensable to the viral replication and infection process, thereby making them ideal targets for antiviral therapy [9]. Among the host receptors, angiotensin-converting enzyme 2 (ACE2) and dipeptidyl peptidase, known as cluster of differentiation 26 (CD26), are identified as the most important human cell proteins employed as hosts by SARS-CoV-2 [5, 10–13].

Also, critical patients of COVID-19 show extremely high inflammatory parameters, including creatine-reactive protein (CRP) and proinflammatory cytokines (e.g., IL-6 and IL-8) [14, 15]. These proinflammatory cytokines are triggered by the nuclear factor kappa B (NF- κ B), and thus, NF- κ B can be also an appropriate target to overcome not the SARS-CoV-2 itself but one of their acutest side effects [16]. One of the cell enzymes that mediate the NF- κ B pathway is angiotensin-converting enzyme (ACE), so its inhibition would reduce the production of the proinflammatory cytokines already mentioned [17–20]. Hence, ACE is also considered a promising host target.

To find inhibitors for these receptors, computer-aided methods are required. Since the 1990s, huge efforts have been made to test an almost unlimited number of drug-like compounds in an automatic way [21]. Hence, computer-aided drug design (CADD) software is nowadays commonly employed for discovering the bioactive molecules, which can then be employed for the pharmaceutical industry as drugs or for the food industry as nutraceutical supplements or functional food/beverages.

Accordingly, a computational approach rooted in molecular docking-based virtual screening is employed to examine the receptors, namely, 3CLpro (PDB code: 6lu7), ACE2 (PDB code: 6m18), spike glycoprotein (PDB code: 6vsb), CD26 (PDB code: 6l8q), and ACE (PDB code: 1o8a). As inhibitors, a library of pea and amaranth peptides was studied, including LSDRFS, SDRFSY, GGV, IGV, IVG, VIKP, and VGVV. Their computed binding affinities and docking poses were compared to FDA-approved drugs. Then, *in vitro* assays were performed to test the capacity of these peptides to inhibit the spike glycoprotein-ACE2 interaction.

2. Materials and Methods

2.1. Resources and Programs. UniProt Knowledgebase (UniProtKB), Swiss Protein (SwissProt) database, ChemSpider database, and Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) were searched.

Openbabel GUI 2.4.1 [22], Acypype [23], Gromacs 2018 [24–26], Chimera UCSF 1.13.1 [27], AutoDock Tools 4.2 [28], AutoDock Vina 2.0 [29], PyMOL 2.3 (The PyMOL Molecular Graphics System, version 2.3 Schrödinger, LLC), Python 2.7.6

(Python Software Foundation), PoseView 1.1.2 (ZBH University of Hamburg, BioSolveIT GmbH), Omega 2.5.1.4 (OpenEye Scientific Software) [30], PLIP 1.3.2 [31], Maestro suite 2019.4, Schrödinger LLC, and the Shuttlemol suite of HPC scripts for virtual screening (<https://bio-hpc.eu>) were used.

2.2. Materials and Reagents. Synthetic GGV, VIKP, LSDFRS, and SDRFSY peptides were purchased from APeptide Co. Ltd. (Shanghai, China) and presented a purity degree above 95%. The Spike glycoprotein-ACE2 binding assay kit was purchased from RayBiotech® (Atlanta, United States) and the ACE2 inhibitor screening kit from AssayGenie Ltd. (London, United Kingdom).

2.3. Molecular Modelling

2.3.1. Selection of Ligands. Several amaranth and pea peptides were identified as bioactive peptides in numerous studies. Some amaranth peptides are well known for their 3-hydroxy-3-methylglutaryl reductase (HMGCR) and angiotensin-converting enzyme (ACE) inhibition capacities, thus reducing cholesterol production and hypertension [32–37]. Several pea peptides are proved to be ACE inhibitors but also ACE2 enhancers, boosting Ang 1–7 and Ang 1–9 production and consequently acting as vasodilators [38, 39].

Accordingly, GGV, IGV, IVG, VIKP, and VGVV from amaranth and LSDFRS and SDRFSY from pea are selected as ligands.

2.3.2. Selection of Receptors. There are two different target groups that can be employed to overcome the COVID-19 infection: (1) receptors of the virus and (2) receptors of the host [5, 10–13].

The spike glycoprotein (protein S) is mainly responsible for the SARS-CoV-2 entry at the host receptor, so it fuses the virus and the host membranes [40, 41]. Accordingly, this receptor is selected for docking studies (PDB code: 6vsb). Also, there are several virus proteins (3CLpro, RdRp, and PLpro) that are considered important factors for the infection [5, 42]. Among them, the 3CLpro is considered the key for virus replication, so it is selected as a virus receptor for docking analysis (PDB code: 6lu7).

As host enzymes, angiotensin-converting enzyme 2 (ACE2) [40, 43], dipeptidyl peptidase (CD26) [13, 44, 45], and angiotensin-converting enzyme (ACE) [19] are identified as the most important human cell enzymes, so PDB codes 6m18, 6l8q, and 1o8a corresponding to these receptors were downloaded from RCSB.

2.3.3. Molecular File Preparation. 3CLpro (PDB code: 6lu7), ACE2 (PDB code: 6m18), spike glycoprotein (PDB code: 6vsb), CD26 (PDB code: 6l8q), and ACE (PDB code: 1o8a) were downloaded from RCSB.

Ligands were removed from pdb files employing PyMOL 2.3. Then, AutoDock Tools 4.2 was used to remove water molecules, add hydrogens, assign AD4 type to all atoms,

compute Gasteiger charges, and save protein files both in pdb and pdbqt formats.

Ligand molecules were downloaded from ChemSpider in mol format and converted to sdf format using the Openbabel GUI 2.4.1. Then they were converted to mol2 format with the Molconvert script from ChemAxon and minimized in the Merck Molecular Force Field (mmff94). AutoDock Tools 4.2 was employed to convert files to pdbqt format.

2.3.4. Blind Docking Analysis. A blind docking (BD) study was performed to detect the regions of interaction between ligands and the aforementioned receptors: ACE, ACE2, spike glycoprotein, CD16, and 3CLpro [46].

Series of single docking simulations were performed in each alpha carbon of the protein, detecting the most favourable binding pockets in terms of bond energy but also the spatial conformation of the ligand and its bound residues. Affinities of ligand-protein interactions are determined with AutoDock 4.2 and individual energies of each bonded residue are calculated with PoseView 1.1.2, representing global bond energy and key residues bond energy, respectively.

2.3.5. Virtual Screening Analysis. To delve deeper into the binding pockets of each ligand, virtual screening (VS) studies were performed, allowing ligands to rotate and move within a box of 30 Å side and increasing the exhaustiveness of the blind docking analysis. The coordinates employed in this step had been obtained from the previous blind docking analyses.

2.3.6. Molecular Dynamics Simulation. Molecular dynamics (MD) is an approximation of the behaviour of the system against real conditions and allows to test the stability of the ligand-protein contacts, which were detected during the BD and VS analyses, during a period of time.

MD simulations were carried out using the GPU version of Desmond included with Maestro suite 2019.4 (Schrödinger LLC) on a workstation with a NVIDIA QUADRO 5000. The system conformed by the ligand and protein of interest was solvated in an aqueous environment, in a cubic box with a minimal distance of 10 Å between the biomolecule and the box boundary (for periodic boundary conditions). Next, systems were neutralized and maintained in 0.15 M NaCl. The OPLS3 force field and the TIP3P-TIP4P water model were employed. Initially, the systems were simply energy-minimized for 2000 time steps. Next, systems were allowed to execute free dynamics in the NPT ensemble; pressure was controlled using the Martyna–Tobias–Klein methodology, and the Nose–Hoover thermostat was employed to maintain the system near 310K. Production-grade MD trajectories were extended to a total duration of 20 ns per system.

MD trajectories were characterized in terms of the root mean square deviation (RMSD) of fluctuations of ligand and receptor, particularly in terms of the main interactions with the top interacting residues. The trajectories were also used

to assess the stabilities of the protein secondary structures (in complex with potential inhibitor) by plotting RMSDs.

2.4. In Vitro Assay

2.4.1. Spike-ACE2 Binding Assay. The *in vitro* enzyme-linked immunosorbent assay is a sensitive method to characterize the binding of the spike-ACE2 complex in the presence of potential inhibitors. The assay uses a 96-well plate coated with recombinantly expressed receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. The testing reagent-of-choice is then added to the wells in the presence of recombinant human ACE2 protein. Unbound ACE2 is removed by washing, and a goat anti-ACE2 antibody that binds to the spike-ACE2 complex is added.

HRP-conjugated anti-goat IgG is then applied to the wells in the presence of 3,3', 5,5'-tetramethylbenzidine (TMB) substrate. The HRP-conjugated anti-goat IgG binds to the ACE2 antibody and reacts with the TMB solution, producing a blue colour that is proportional to the amount of bound ACE. The HRP-TMB reaction is halted with the addition of the stop solution, resulting in a blue-to-yellow colour change. The intensity of the yellow colour is then measured at 450 nm. The percentage of inhibition of the formation of the spike-ACE2 complex was calculated as follows:

$$\text{Spike - ACE2 binding inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \cdot 100, \quad (1)$$

where A_{control} is the absorbance without inhibitor (considered as 100% Spike-ACE2 binding) and A_{sample} is the absorbance of sample with inhibitor.

2.4.2. ACE2 Inhibitor Screening Assay. The ACE2 inhibitor screening kit was used to detect potent inhibitors of ACE2 activity. It utilizes the ability of an active ACE2 to cleave a synthetic MCA-based peptide substrate to release a fluorescent fluorophore. The released MCA is easily quantified using a fluorescence microplate reader (excitation wavelength 320 nm and emission wavelength 420 nm). In the presence of an ACE2-specific inhibitor, the enzyme loses its peptidase activity which results in the decrease of fluorescence intensity. The ACE2 percentage inhibition was calculated as follows:

$$\text{ACE2 inhibition (\%)} = \frac{F_{\text{control}} - F_{\text{sample}}}{F_{\text{control}}} \cdot 100, \quad (2)$$

where F_{control} is the fluorescence with ACE2 and without inhibitor (considered as 100% enzyme activity) and F_{sample} is the fluorescence of sample with ACE2 and inhibitor.

3. Results and Discussion

3.1. Molecular Docking and Virtual Screening. Angiotensin-converting enzyme 2 is known to be one of the essential host receptors for SARS coronaviruses, and it is

also proved to be the major cellular receptor for the novel SARS-CoV-2. ACE2 is a membrane protein expressed in the lungs, kidneys, heart, and intestine, and it is responsible for vasoconstriction and blood pressure control, being its decreased expression associated with cardiovascular diseases.

COVID-19 disease causes ACE2 downregulation, affecting the tissues where it is expressed, but with major implications for lung and heart tissues, causing both pulmonary and cardiovascular complications [47, 48]. There are several studies that identify the region of interaction between the virus and this receptor, proving that the $\alpha 1$ and $\alpha 2$ helices are the most important areas of interaction [10–12]. Hence, the development of effective treatment should consider the blockage of this area to avoid SARS-CoV-2 binding, but the capacity for ACE2 expression enhancement should be also kept in mind.

ACE2 infection is caused due to the fusion between this enzyme and the spike glycoprotein of SARS-CoV-2. The S1 and S2 subunits of the spike are known to be responsible for the receptor binding (S1) and for the membrane fusion among the viral subunit and ACE2 (S2) [49].

The crystal structure between ACE2 and the spike glycoprotein is elucidated, and the residues which take part in the binding process are identified. To avoid virus infection, the blockage of the spike glycoprotein or the host cell receptors should be considered.

Similar to ACE2, dipeptidyl peptidase is considered an important entry receptor for SARS coronaviruses, including MERS-CoV, SARS-CoV, and SARS-CoV-2 [44, 50, 51].

CD26 has a key role in immune regulation as a T-cell activation molecule and in immune-mediated disorder, thus acting as a key immunoregulatory factor in viral infections. The S1 loop is considered to be the area of interaction of this receptor and SARS-CoV-2, being its key residues clearly identified, so therapies to overcome the COVID-19 can be developed by targeting this region [13].

As a SARS-CoV-2 host receptor, angiotensin-converting enzyme was also identified as an important target. ACE is the precursor of angiotensin II, which is a strong inducer of vascular smooth muscle cells (VSMCs) by activating the AT1 receptor. The VSMCs are essential to maintain vascular function and homeostasis, being its upregulation responsible for the overexpression of proinflammatory cytokines and growth factors as IL-6, IL-8, and NF- κ B, among others [14].

Lungs are one of the most affected organs when the overexpression of the inflammatory cascade takes place. Accordingly, high inflammation levels are associated with major complications of COVID-19, so ACE inhibition may be a suitable strategy to avoid or mitigate this process [19, 20]. The ACE residue Glu384 is considered the key of the hot spot, so its regulation can be achieved by triggering this amino acid.

A different strategy to fight the COVID-19 infection can be the direct treatment of the virus. The viral protease 3CLpro has been proved to be essential for coronavirus replication and is thus considered the most potent drug target among the virus receptors [5, 9]. The dyad His41-

Cys145 conforms the hot spot of this receptor, so an effective SARS-CoV-2 disabling can be developed by targeting these key residues.

To target these five receptors, the pea peptides, LSDRFS and SDRFSY, and the amaranth peptides, GGV, IGV, IVG, VGVL, and VIKP, are proposed. Its binding capacities are analysed in a docking study which detects, first, the regions of interaction of each peptide among the whole target receptor. This will indicate whether the peptide reaches the hot spot or remains trapped in ineffective areas of the protein.

Thereafter, these effective interactions are characterized in terms of affinity energy. The different types of interaction (hydrogen bond, hydrophobic interaction, π - π interaction, and salt bridge) between ligand and receptor will determine its magnitude giving an associated energy value or score. Hence, those ligands which reach the hot spot area with sufficient energy are susceptible of being effectively bound to the receptor, enhancing or inhibiting its associated biological activity.

3.1.1. Angiotensin-Converting Enzyme 2 (ACE2).

Angiotensin-converting enzyme 2 (ACE2) is considered one of the major host receptors of β -coronaviruses, including SARS-CoV, MERS-CoV, and SARS-CoV-2. Regarding the SARS-CoV-2, its interaction takes place among the $\alpha 1$ and $\alpha 2$ helices of ACE2, where residues Gln24, Asp30, His34, Tyr41, Gln42, Met82, Lys353, and Arg357 play a major role in reinforcing virus interaction [11, 12].

There is some controversy about the regulation of ACE2. Some authors indicate that ACE2 expression reduction could be beneficial, so the main host receptor for SARS-CoV-2 would be diminished, involving a less acute infection [52]. Nevertheless, this would cause an increase of the ratio Ang II:Ang 1–7, exacerbating the pulmonary tissue damage initially provoked by SARS-CoV-2 [47, 48, 53]. It is true that cardiovascular tissues or cells that express ACE2 are potentially at risk for SARS-CoV-2 infection; however, ACE2 expression reduction would be also detrimental.

The moderate enhancing production of ACE2 with a blocking agent, which helps to protect the area of interaction between ACE2 and SARS-CoV-2, may be a good approach to prevent COVID-19 disease while an adequate ratio Ang II:Ang 1-7 is maintained to avoid pulmonary tissue damage.

Several pea peptides had been proved to be bioactive against ACE2, so ligands as LSDRFS and SDRFSY are known to interact in the hot spot of this receptor [39]. Besides, it is well known that when a ligand shows bioactivity against one receptor, it would bind its hot spot but also other nonactive areas of the target. Thus, considering the key area of interaction of SARS-CoV-2 in ACE2, a blind docking analysis was performed to detect both the region of interaction and its magnitude for the pea peptides, LSDRFS and SDRFSY, and the amaranth peptides, GGV, IGV, IVG, VGVL, and VIKP.

Table 1 shows the global affinity values, calculated with AutoDock 4.2, and the affinities related to the key residues for virus interaction, calculated with PoseView 1.1.2.

TABLE 1: Angiotensin-converting enzyme 2 (ACE2) docking analysis.

Drug/peptide	E bond _{global} (kcal/mol)	E bond _{key residues} (kcal/mol)		Drug/peptide	E bond _{global} (kcal/mol)	E bond _{key residues} (kcal/mol)		
Ritonavir (347980)	-7,98	>-2		IGV	-5,05	-2,557		
	-5,86	-2,274	-2,080		-4,7	>-2		
	-5,79	-2,279	-2,055	-2,313	IVG	-5,25	-3,559	
Lopinavir (83706)	-5,88	-2,557		VGVL	-4,88	-2,106		
	-5,3	-2,903			-5,14	>-2		
	-4,85	>-2			-4,93	-4,000		
Emtricitabine (54859)	-4,94	-5,184		VIKP	-4,28	-5,587		
	-4,83	-4,453			-5,18	-2,049		
Hydroxychloroquine (3526)	-5,06	>-2		LSDRFS	-4,69	>-2		
	-4,71	>-2			-4,66	-2,543		
	-4,46	-3,965			-5,93	-4,115	-8,610	
Chloroquine (2618)	-4,8	>-1		SDRFSY	-5,69	-5,649	-2,366	-2,557
	-4,39	>-2			-5,51	-2,351		
	-4,18	>-2			-4,69	-11,390	-2,191	
GGV	-5,78	-4,866		SDRFSY	-7,56	-5,494		
	-4,76	-2,557			-6,32	-2,759	-2,352	-4,389
	-4,32	-3,576			-6,05	-6,452		

LSDRFS and SDRFSY show the best performance against ACE2 (Table 1), even compared to drugs that were studied for drug repositioning such as ritonavir, lopinavir, emtricitabine, hydroxychloroquine, or chloroquine [54–56]. Regarding the global interaction energy, SDRFSY has a better affinity value. Nevertheless, the ACE2 key residues that are fundamental for virus infection (Figure 1, yellow colour) are more strongly bound to LSDRFS than to SDRFSY. This means that these positions would be involved in the LSDRFS interaction, blocking the access of the SARS-CoV-2 to this area (Figure 1).

Comparing the area of interaction of these two peptides, LSDRFS covers a more extensive area of the hot spot region than SDRFSY. There are four positions of interaction that are involved with key residues (Figure 1(a)), and SDRFSY peptides let exposed the key residues Tyr41 and Lys353, located at the left side of $\alpha 2$ helix, and Gln24 and Met82, located at the right side of both helices. Thus, due to the stronger interactions and larger blocked area, LSDRFS is expected to be more effective than SDRFSY to prevent ACE2 infection.

VGVL and VIKP also show good global affinity values, but only two positions of the key binding area are effectively blocked (Supplementary Figures 29 and 33), within key residue energies lower than -2.00 kcal/mol (Supplementary Figures 30 and 36), so this would probably cause poor blocking capacity against SARS-CoV-2 infection.

GGV also shows a good performance, regarding the global and key residue energies, but the small size of this amaranth peptide does not allow to cover the whole area where the virus interacts (Figure 1(c)). However, the combination of GGV and LSDRFS can be a promising match. Figure 1(d) shows that there are no competitive interactions between these ligands, so they interact along the whole $\alpha 1$ and $\alpha 2$ helices covering the main area of virus interaction and matching their strong binding capacities. This LSDRFS-GGV combination may be the most promising strategy to protect ACE2 from SARS-CoV-2 infection.

3.1.2. Spike Glycoprotein. The spike glycoprotein or S protein of coronaviruses, including SARS-CoV-2, is one of their most important structural proteins. It recovers the external capsid of the virion, giving the appearance of a crown. Conformed by three major sections, ectodomain, single-pass transmembrane, and intracellular tail, the most important one is the ectodomain. This section is divided in two different subunits: the S1, responsible for the receptor binding to the host cells, and the S2, the membrane fusion unit [40, 49, 50].

The main host receptor for SARS-CoV-2 and thus spike glycoprotein is ACE2. Several studies had identified the residues that take part in this interaction, revealing the importance of Tyr449, Tyr453, Leu455, Phe456, Phe486, Asn487, Tyr489, Gln493, Gly496, Gln498, Thr500, Asn501, Gly502, and Tyr505 [41, 51].

Blind docking and virtual screening analyses were performed to detect the possible interactions between spike glycoprotein and the amaranth peptides, GGV, IGV, IVG, VGVL, and VIKP, and the pea peptides, LSDRFS and SDRFSY (Supplementary Table 1, Supplementary Figures 44–64). Regarding the affinity scores (Supplementary Table 1), it seems that these ligands are not suitable to shield the crown of the SARS-CoV-2 and prevent an infection (Supplementary Figures 44–64). Nevertheless, this can be considered as a beneficial behaviour. It is well known that a certain molecule can act as a protein-protein stabilizer, enhancing the interaction between two (or more) different proteins [57]. This ineffective interaction with the protein S proves that there is no possibility for these peptides to enhance the ACE2 infection, so these results reinforce the promising behaviour of LSDRFS, SDRFSY, and the combination LSDRFS-GGV against ACE2 host receptor.

3.1.3. Cluster of Differentiation 26 (CD26). The cluster of differentiation 26 (CD26), also known as dipeptidyl peptidase, has been recognized as an important host receptor of

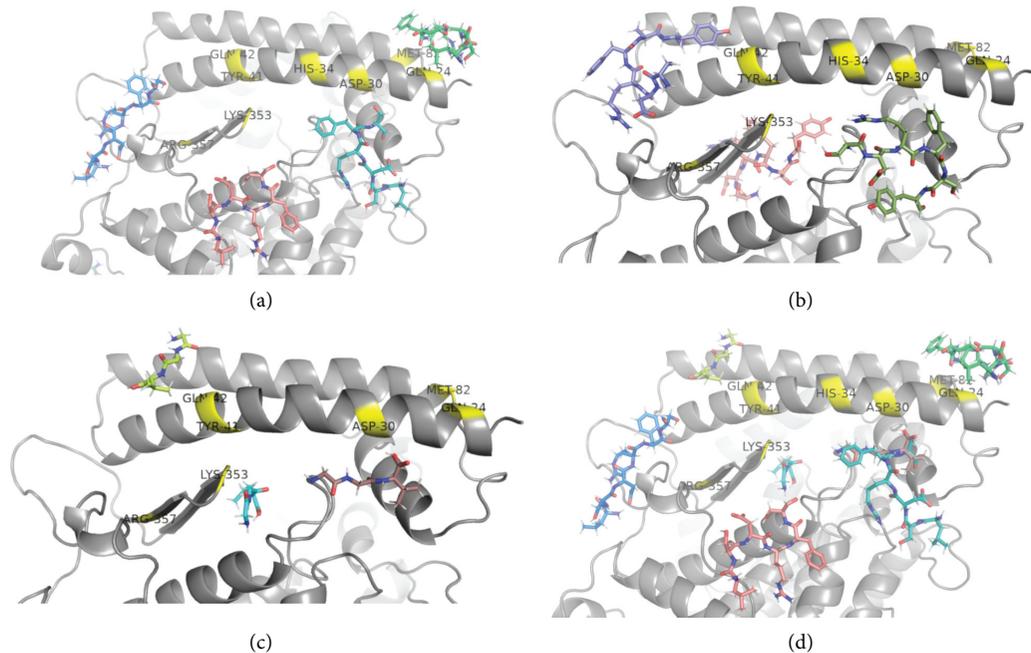


FIGURE 1: Angiotensin-converting enzyme 2 blind docking analysis. (a) LSDRFS, (b) SDRFSY, (c) GGV, and (d) LSDRFS and GGV. Yellow = hot spot area/key residues. ACE2 PBD: 6m18.

SARS-CoV-2. Residues Lys267, Gln286, Ile287, Thr288, Ala289, Ala291, Leu294, Ile295, Arg317, Tyr322, Arg336, and Asn338 from S1 loop were identified as key residues in virus interaction [13].

A blind docking analysis was performed to detect the possible interactions between this receptor and the proposed ligands LSDRFS, SDRFSY, GGV, IGV, IVG, VGVL, and VIKP. Also, the approved drugs chloroquine, hydroxychloroquine, and ritonavir, studied for drug repurposing, were tested following the same procedure. Accordingly, peptide and drug results can be compared (Supplementary Table 2, Supplementary Figures 65–74), showing similar responses for SDRFSY and hydroxychloroquine, with high-affinity values against CD26.

The hydroxychloroquine exerts a good performance against CD26, with great binding energies and blocking Val341, Asn338, Arg336, Gln286, and Lys287 from virus interaction (Supplementary Figure 66, Supplementary Table 2). Nevertheless, the central area conformed by residues Ala291, Tyr322, Leu294, Ile295, and Arg317 is uncovered, exposing important residues from the hot spot and allowing the virus binding. SDRFSY performance can be compared with the hydroxychloroquine (Supplementary Figure 74, Supplementary Table 2), so it has also good energy values for global and key residues interaction. Due to the size of this pea peptide, a broad area of CD26 is expected to be inhibited, so Supplementary Figure 74 shows almost a total binding of the hot spot, being only Tyr322 and Arg336 unbound. This would help to protect the receptor against virus entry. Regarding the other studied drugs and peptides, low binding capacities are detected, so they are not expected to be effective virus blockers (Supplementary Table 2, Supplementary Figures 65–74).

3.1.4. Angiotensin-Converting Enzyme (ACE). Angiotensin-converting enzyme was selected as target because of its connexion to the inflammatory process mediated by NF- κ B transcription factor, which is overexpressed in patients with major COVID-19 complications.

Several pea and amaranth peptides are proved to have ACE inhibitory capacity, so LSDRFS, SDRFSY, GGV, IGV, IVG, VGVL, and VIKP ligands are studied against ACE by means of blind docking and virtual screening analyses. Also, the approved drug captopril was tested following the same procedure to contrast drug and peptide results (Supplementary Table 3, Supplementary Figures 75–90).

Glu384 is considered by UniProtKB database as the key residue to inhibit the hypertension effect caused by ACE. Supplementary Table 3 summarizes the global and key residue binding energies of the proposed drugs and peptides, showing that LSDRFS and VIKP are expected to behave as effective hypertension reduction agents, thus also helping to reduce the inflammation levels of the organism. IGV and VGVL show also great inhibitory potentials, being only GGV results below captopril ones (Supplementary Figures 75–90). This is an indication of the angiotensin reduction capacity of these peptides, which may be suitable to inhibit the NF- κ B transcription factor overexpression while helping to block the area of interaction of ACE2 and SARS-CoV-2. Hence, peptides as LSDRFS, with favourable binding capacities against ACE2 and ACE, are expected to exert a double action against COVID-19.

3.1.5. Chymotrypsin-Like Protease (3CLpro). The chymotrypsin-like protease enzyme (3CLpro) is indispensable to the viral replication and infection process, so it is considered

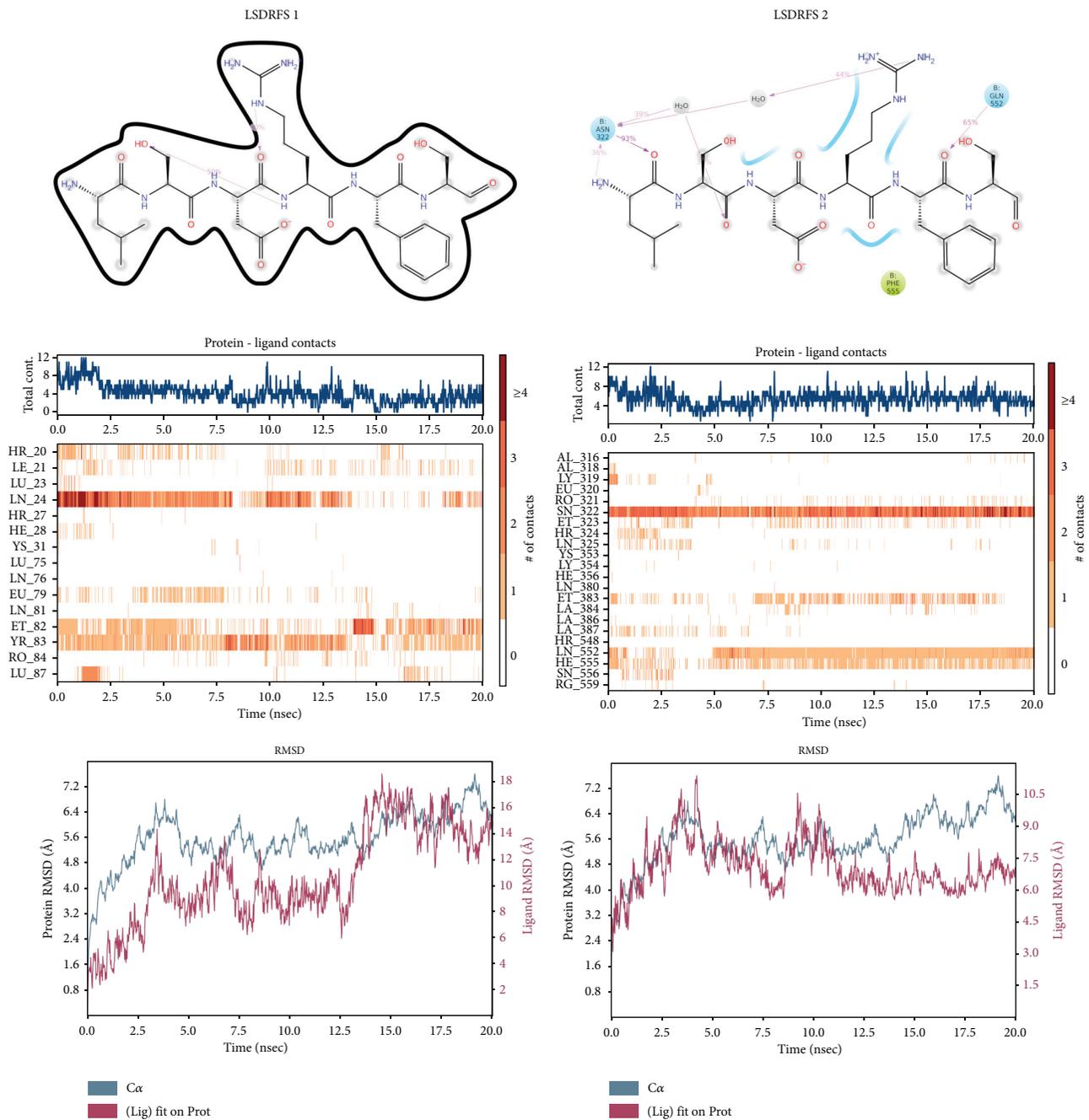


FIGURE 2: Molecular dynamics simulation LSDRFS-ACE2. Analysis of positions 1 and 2: interactions and RMSD fluctuation.

nowadays the most important target to develop an antiviral therapy [9]. Several approved drugs are being studied for drug repositioning by targeting this receptor, such as demeclocycline, doxycycline, oxytetracycline, lymecycline, nicardipine, telmisartan, and conivaptan [5].

It is unlikely that the proposed peptides would bind and inhibit 3CLpro, but due to the favourable binding of some of these ligands with ACE and ACE2, it is important to test their possible interferences with the virus itself. The hot spot of the 3CLpro receptor is the catalytic dyad His41-Cys145 [5, 9]. Thus, blind docking and virtual screening analyses were performed for 3CLpro and peptides LSDRFS, SDRFSY, GGV,

IGV, IVG, VGV, and VIKP regarding this binding site. Also, demeclocycline, doxycycline, oxytetracycline, lymecycline, nicardipine, telmisartan, and conivaptan are studied following the same procedure to contrast drug and peptide results (Supplementary Table 4, Supplementary Figures 91–103).

The poor bond energies of this analysis suggest, as it was expected, an ineffective interaction between these ligands and the 3CLpro virus receptor results (Supplementary Table 4, Supplementary Figures 91–103). However, this noninteraction between peptides and the main virus receptor can be favourable because it proves that there is no possibility of these peptides to act as a protein–protein stabilizer, enhancing the interaction

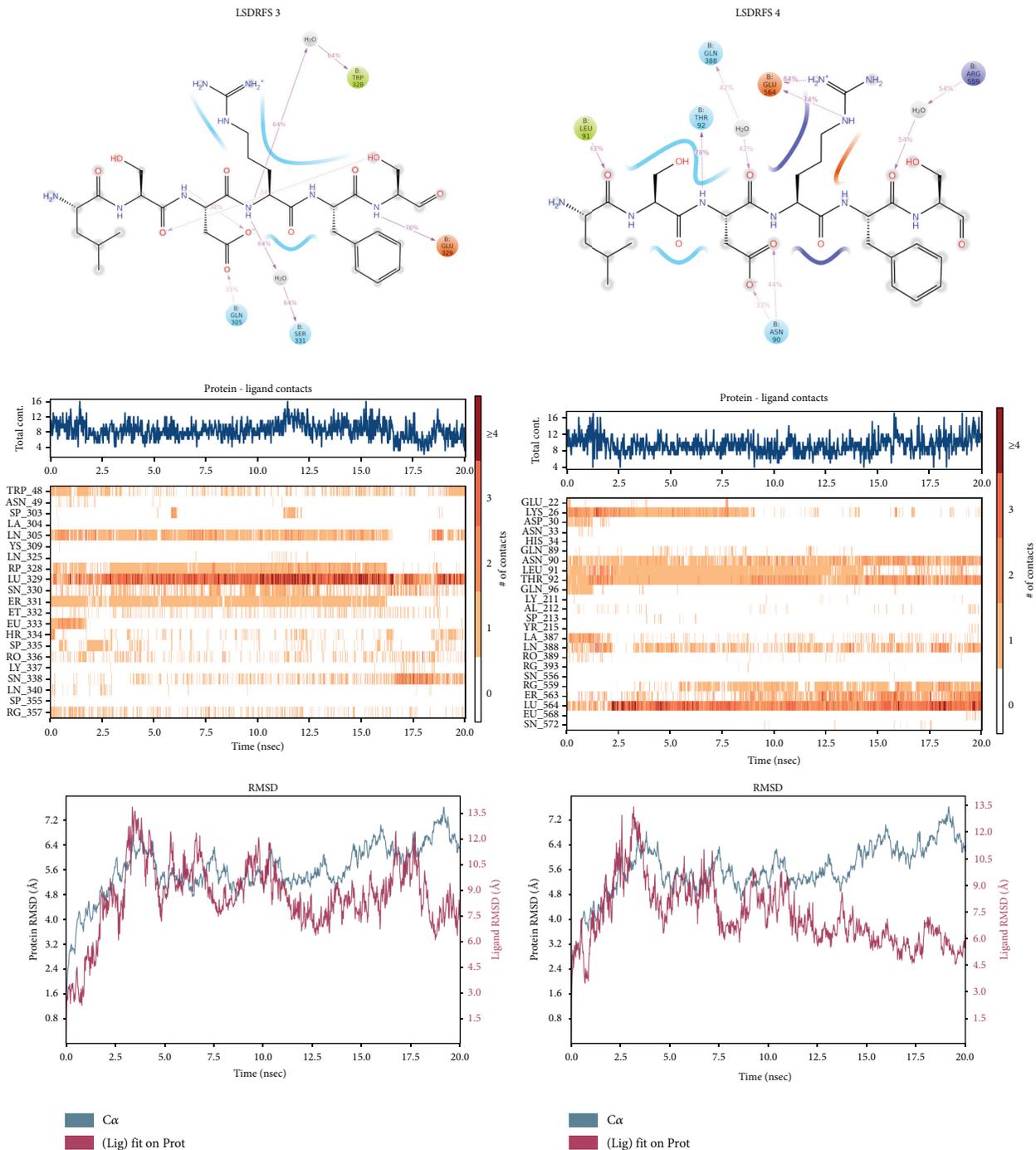


FIGURE 3: Molecular dynamics simulation LSDRFS-ACE2. Analysis of positions 3 and 4: interactions and RMSD fluctuation.

between two (or more) different proteins [57]. Hence, these peptides are well-suited to inhibit the regions of interaction of ACE2 (LSDRFS and SDRFSY peptides) and mitigate the inflammation process by triggering ACE (LSDRFS and VIKP peptides) without exerting an attraction effect against the 3CLpro nor the spike protein SARS-CoV-2 receptors.

3.2. Molecular Dynamics Simulation. Molecular dynamics simulation study was performed to test the stability of the interactions detected during the molecular blind docking

and virtual screening analyses. The LSDRFS, SDRFSY, and VIKP-ACE2 systems were studied following this protocol. Also, hydroxychloroquine was submitted to the same process to contrast peptide and drug performances [54]. Each ligand interaction with ACE2 was analysed, reporting the main protein-interacting residues during the simulation period and its correspondent RMSD plots (Figures 2–5, Supplementary Figures 104 and 105).

The first docking position of LSDRFS (Figure 2) shows a persistent interaction with Gln24 during the first 10 ns, but it becomes unstable after that, and a more intermittent but

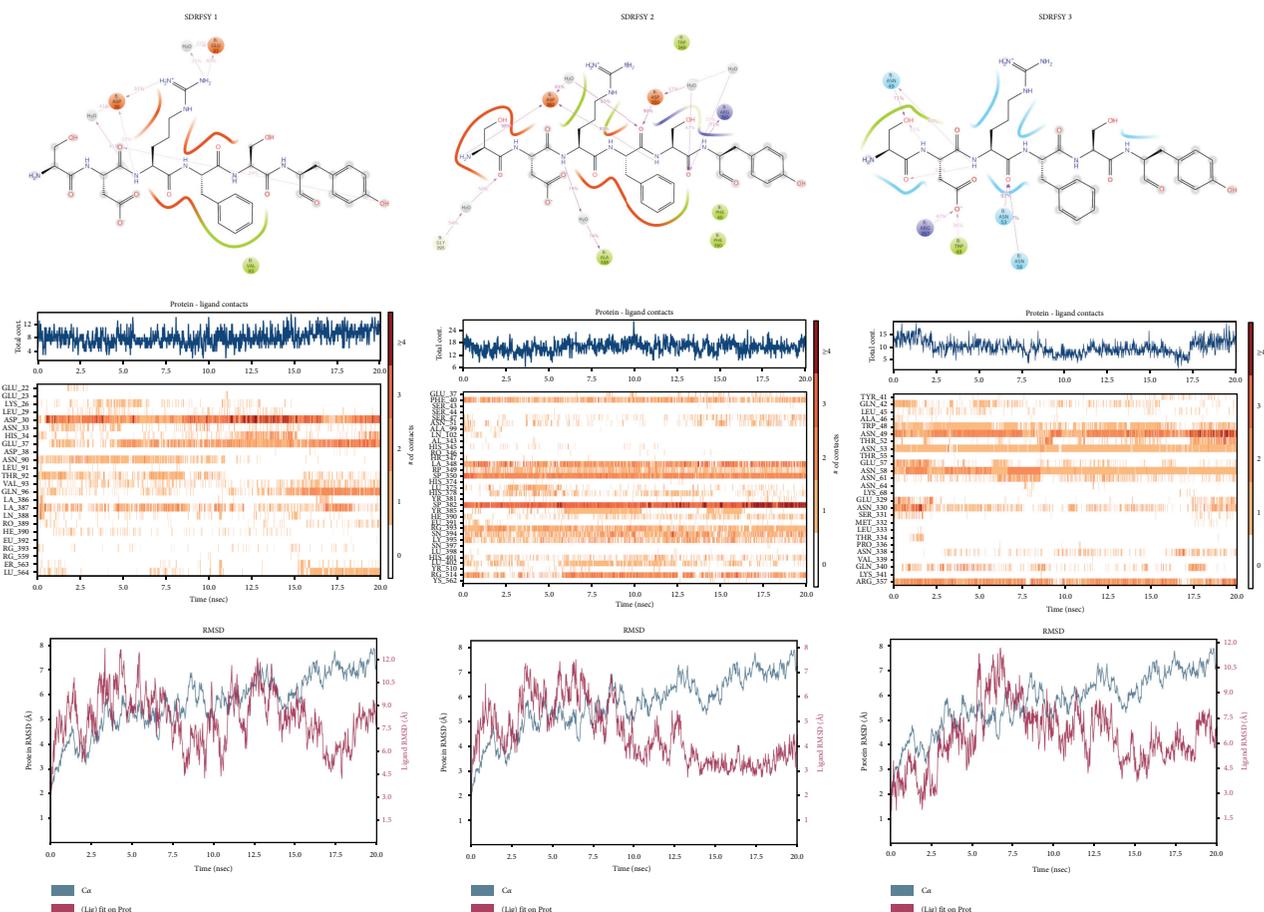


FIGURE 4: Molecular dynamics simulation SDRFSY-ACE2. Analysis of positions 1, 2, and 3: interactions and RMSD fluctuation.

stable interaction with Met82. There are no persistent contacts during the whole simulation, so this is translated to the RMSD values, which show a high value around 18 Å. The second docking position (Figure 2) interacts persistently with Asn322 and Gln552, and more intermittently but stably with Phe555. This position is maintained during the whole simulation, causing lower and stable RMSD values (around 7 Å). Furthermore, the peptide head and tail are involved in these interactions, fixing the whole position of this peptide and thus blocking a wide area which otherwise would be available for spike glycoprotein reception.

A quite similar behaviour is present at the third docking position (Figure 3), which has stable interactions with Gln305, Trp328, and Ser331 and a persistent interaction with Glu329. Accordingly, the RMSD values are moderate and stable around 8 Å. The fourth position analysis (Figure 3) reveals persistent interactions with Asn90, Thr92, and Glu564 and high stable interactions with Leu91, Gln388, Arg559, and Ser563. This is translated to the RMSD value, which is stable and low (around 6 Å). The whole structure of this peptide (head, centre, and tail) is well fixed to the protein, causing a high stable interaction and allowing to block a big area where the spike glycoprotein interacts.

LSDRFs shows stable interactions with ACE2, specially the second and fourth positions, followed by the third one (Figures 2 and 3). This is expected to efficiently block the

virus entry by covering an important area where the spike glycoprotein causes the ACE2 infection (Figure 1(a)).

The first docking position of SDRFSY shows a persistent interaction with Asp30 and high stable interactions both with Glu37 and Ala387. Also, it has more intermittent but stable interactions with Val93 and Gln96 (Figure 4). The peptide is fixed to the protein in its central zone, leaving the head and tail amino acids free to rotate. This makes the RMSD values slightly high (around 8.5 Å), but still covers a large area of the protein in a stable way. The second position (Figure 4) shows persistent interactions with Phe40, Ala348, Trp349, Asp350, Asp382, Arg393, Asn394, and Gly395 and also stable but slightly more intermittent interactions with Phe390, His401, and Arg514. This high number of contacts makes the peptide interaction very stable, with RMSD values around 4 Å. In addition, the whole peptide structure is bound to the protein, covering a large area of the protein in a very stable way, so a high capacity to prevent virus binding is expected. The third position (Figure 4) has persistent interactions with Trp48, Asn49, Asn53, Asn59, and Arg357. It also interacts slightly more intermittently with Asn61 and Asn330. The head amino acids are specially well fixed to the protein (Figure 4), leaving the tail free to rotate, which causes mid-low RMSD values around 7 Å.

SDRFsY shows three interactions along the area of interaction of ACE2 and spike glycoprotein (Figure 1(b)).

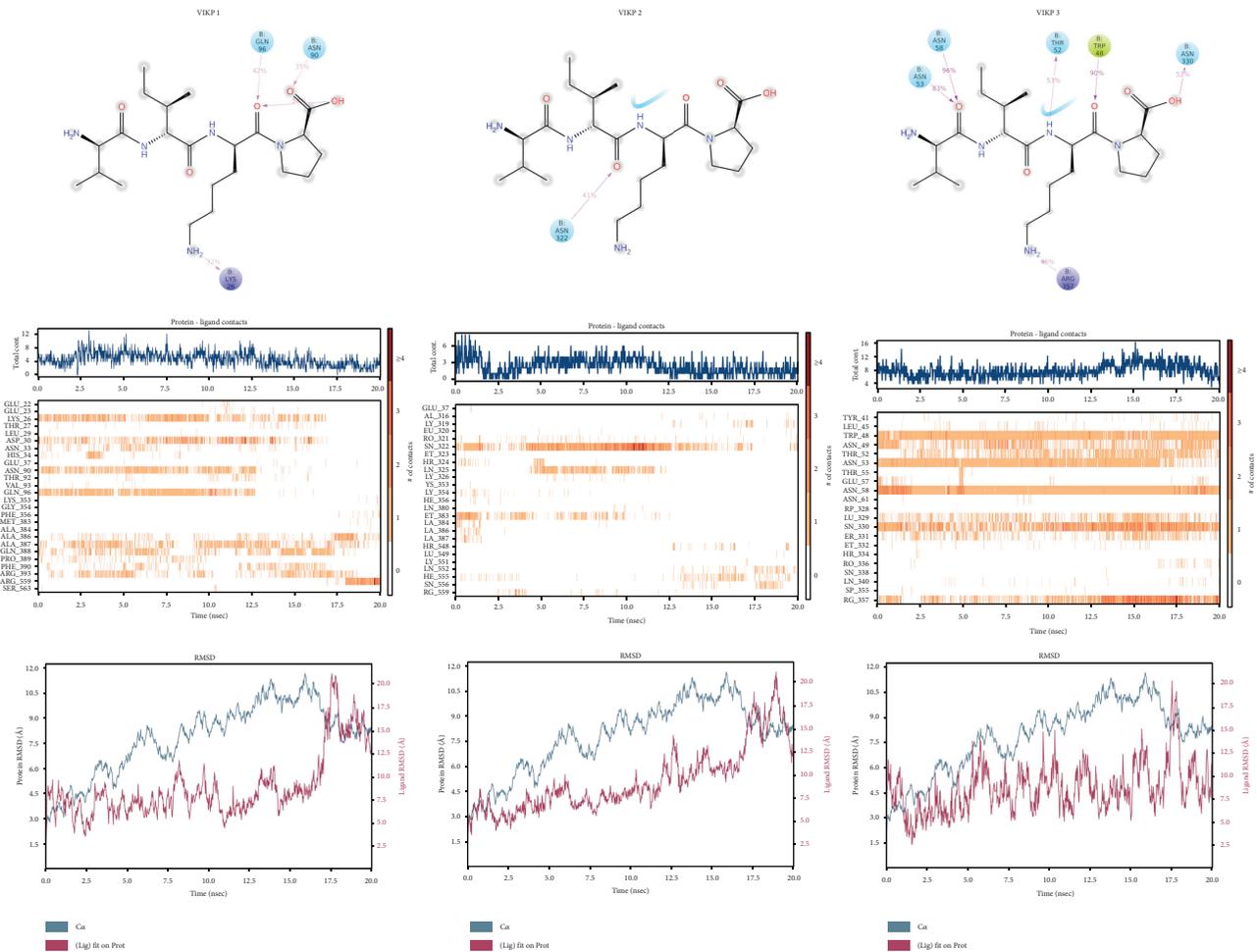


FIGURE 5: Molecular dynamics simulation VIKP-ACE2. Analysis of positions 1, 2, and 3: interactions and RMSD fluctuation.

These three docking positions have a high number of persistent contacts with ACE2 (Figure 4), making SDRFSY interactions very stable and thus preventing virus binding by covering its main area of interaction.

VIKP shows intermittent and unstable interactions for the first and second docking positions (Figure 5), with high RMSD values around 12 Å. Only the third position is considered stable, so it has persistent interactions with Trp48, Asn53, and Asn58 and low RMSD values around 4 Å (Figure 5).

Blind docking and virtual screening analyses reveal VIKP as a weaker candidate for ACE2 binding. This was also proved by molecular dynamics analysis (Figure 5), which show intermittent and unstable interactions specially for the first and second docking positions. Despite the stable interaction of the third position, the small size of this peptide does not allow to cover a large area of ACE2, so it is not expected to act as spike glycoprotein blocker.

The high number of persistent contacts and low RMSD values of SDRFSY show the high stability of SDRFSY-ACE2 interactions, so this peptide is expected to behave as a good inhibitor for spike glycoprotein binding, followed by LSDRFS, with slightly higher instability but also strong interactions with several ACE2 key residues.

These molecular dynamics results were compared with hydroxychloroquine, showing a better performance to prevent the virus infection than this drug which was used for COVID-19 treatment (Supplementary Figures 104 and 105), so SDRFSY and LSDRFS peptides are potential candidates to act preventively against mid SARS-CoV-2 infection.

3.3. In Vitro Assays. The *in vitro* analyses were carried out following the fluorimetric procedure explained before. The percentages of inhibition of peptides LSDRFS, SDRFSY, VIKP, and GGV for spike glycoprotein-ACE2 interaction and ACE2 activity are shown in Table 2.

SDRFSY and LSDRFS show a higher inhibitory potential at spike-ACE2 and also ACE2 analysis. This is an indicative of the strong interaction of these peptides within the ACE2 receptor, which would prevent virus (spike glycoprotein) and host (ACE2) from forming an effective interaction. This is in accordance with the results obtained by the computational method, which predicted a good performance for SDRFSY and LSDRFS (Table 1, Figures 1-4).

VIKP shows lower inhibition values, as it was also reported by the docking and molecular dynamics analyses (Table 1, Figure 5). However, results for the combination of

TABLE 2: Inhibitory effects of peptides on the interaction between SARS-CoV-2 spike glycoprotein and the human ACE2 receptor and on the ACE2 activity. a = 15 g/L, b = 7.5 g/L, c = 15 g/L (1 : 1), and d = 15 g/L (2 : 1), n.d. = not detected.

Peptides	Spike-ACE2			ACE2	
	PI (%) ^a	PI (%) ^c	PI (%) ^d	PI (%) ^a	PI (%) ^b
LSDRFS	79.56	—	—	95.50	52.17
SDRFSY	84.65	—	—	95.81	71.33
VIKP	62.03	—	—	94.37	16.4
GGV	34.99	—	—	6.07	n.d
LSDRFS + GGV	—	4.08	38.29	—	—

LSDRFS and GGV widely differ from the molecular modelling ones, which foresaw a synergetic effect for the combination of these peptides (Figure 1(d)). This is probably due to the incorrect positioning of the peptides in the interaction zone of ACE2 and spike glycoprotein. Although after the BD analysis these ligands were located at compatible poses and positions, avoiding binding competition between them, regarding the obtained results, GGV probably presents a higher instability that interferes with LSDRFS interaction positions, causing poor inhibition capacities.

4. Conclusions

β -Coronaviruses have crossed the animal–human barrier, causing several outbreaks during recent years, but none of them with the dimensions of SARS-CoV-2, which has been spread for over the world [58], so both treatment and preventive approaches are urgently required. In this scenario, natural peptides play a major role offering a preventive strategy or a treatment for non-acute patients due to its GRAS (generally recognised as safe) property.

In this study, several pea and amaranth peptides were analysed against the major SARS-CoV-2 receptors—both host and virus. Molecular docking and dynamics simulation results show that SDRFSY and LSDRFS have strong and stable interactions with ACE2, so these peptides can be suitable to shield the area of ACE2 where the spike glycoprotein of the virus exerts its interaction (Table 1, Figures 1(a) and 1(b)). It was also proved that none of the studied peptides interacts with the spike glycoprotein, so their presence does not cause an increase in the interactions between ACE2 and the spike glycoprotein, which verifies that these peptides do not have the potential to act as protein–protein interaction (PPI) stabilizers (Supplementary Table 2). These theoretical results were confirmed by *in vitro* analyses, proving that SDRFSY and LSDRFS prevent the spike glycoprotein–ACE2 interaction with inhibition percentages above 80% at 15 g/L (Table 2).

Also, computational results show that CD26 can be similarly protected with SDRFSY and VIKP (Supplementary Table 2), and ACE, which is inextricably linked to the cytokine cascade that causes major complications in acute COVID-19 patients, can be inhibited by LSDRFS and VIKP (Supplementary Table 3).

Hence, it can be assessed that the pea peptides, SDRFSY and LSDRFS, and the amaranth peptide, VIKP, are susceptible of being useful not to overcome but to mitigate and prevent the effects of the SARS-CoV-2 in an effective and quick manner.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this publication.

Acknowledgments

This work was financed by the Hi-Bio 4.0 Project (IN854A 2019/13) supported by the Axencia Galega de Innovación–Xunta de Galicia and the European Regional Development Fund. Supercomputing resources in this work have been supported by the infrastructures of Poznan Supercomputing Center, the e-infrastructure program of The Research Council of Norway, and the supercomputer center of UiT—the Arctic University of Norway.

Supplementary Materials

Blind docking and virtual screening analyses of spike glycoprotein, cluster of differentiation 26 (CD26), angiotensin-converting enzyme (ACE), and 3-chymotrypsin-like protease enzyme (3CLpro). (*Supplementary Materials*)

References

- [1] G. Viceconte and N. Petrosillo, “COVID-19 R0: magic number or conundrum?” *Infectious Disease Reports*, vol. 12, pp. 12–13, 2020.
- [2] Y. Liu, A. A. Gayle, A. Wilder-Smith, and J. Rocklöv, “The reproductive number of COVID-19 is higher compared to SARS coronavirus,” *Journal of Travel Medicine*, vol. 27, no. 2, pp. 1–4, 2020.
- [3] K. F. Amanat Fatima, “SARS-CoV-2 vaccines: status report,” *Cell*, vol. 52, no. 4, pp. 1–7, 2020.
- [4] M. Wang, R. Cao, L. Zhang et al., “Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro,” *Cell Research*, vol. 30, no. 3, pp. 269–271, 2020.
- [5] C. Wu, Y. Liu, Y. Yang et al., “Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods,” *Acta Pharmaceutica Sinica B*, vol. 10, 2020.
- [6] Y. Wang, D. Zhang, P. G. Du et al., “Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial,” *Lancet*, vol. 6736, no. 20, pp. 1–10, 2020.
- [7] M. Koromina, M.-T. Pandi, and G. P. Patrinos, “Rethinking drug repositioning and development with artificial intelligence, machine learning, and omics,” *OMICS: A Journal of Integrative Biology*, vol. 23, no. 11, pp. 539–548, 2019.

- [8] K. H. Roux, S. S. Teuber, and S. K. Sathe, "Tree nut allergens," *International Archives of Allergy and Immunology*, vol. 131, no. 4, pp. 234–244, 2003.
- [9] T. Pillaiyar, M. Manickam, V. Namasivayam, Y. Hayashi, and S.-H. Jung, "An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy," *Journal of Medicinal Chemistry*, vol. 59, no. 14, pp. 6595–6628, 2016.
- [10] Y. Chen, Y. Guo, Y. Pan, and Z. J. Zhao, "Structure analysis of the receptor binding of 2019-nCoV," *Biochemical and Biophysical Research Communications*, vol. 525, no. 1, pp. 135–140, 2020.
- [11] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, and Q. Zhou, "Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2," *Science*, vol. 2762, pp. 1–10, 2020.
- [12] J. S. Morse, T. Lalonde, S. Xu, and W. R. Liu, "Learning from the past: possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV," *ChemBioChem*, vol. 21, no. 5, pp. 730–738, 2020.
- [13] N. Vankadari and J. A. Wilce, "Emerging COVID-19 coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26," *Emerging Microbes & Infections*, vol. 9, no. 1, pp. 601–604, 2020.
- [14] M. Suski, A. Gębska, R. Olszanecki et al., "Influence of atorvastatin on angiotensin I metabolism in resting and TNF- α -activated rat vascular smooth muscle cells," *Journal of the Renin-Angiotensin-Aldosterone System*, vol. 15, no. 4, pp. 378–383, 2014.
- [15] W. Zhang, Y. Zhao, F. Zhang et al., "The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the experience of clinical immunologists from China," *Clinical Immunology*, vol. 214, Article ID 108393, 2020.
- [16] T. Liu, L. Zhang, D. Joo, and S. C. Sun, "NF- κ B signaling in inflammation," *Signal Transduction and Targeted Therapy*, vol. 2, 2017.
- [17] T. Skurk, V. Van Harmelen, and H. Hauner, "Angiotensin II stimulates the release of interleukin-6 and interleukin-8 from cultured human adipocytes by activation of NF- κ B," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 7, pp. 1199–1203, 2004.
- [18] D. Fliser, K. Buchholz, and H. Haller, "Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation," *Circulation*, vol. 110, no. 9, pp. 1103–1107, 2004.
- [19] U. Kostakoglu, A. Topcu, M. Atak, L. Tumkaya, T. Mercantepe, and H. A. Uydu, "The protective effects of angiotensin-converting enzyme inhibitor against cecal ligation and puncture-induced sepsis via oxidative stress and inflammation," *Life Science*, vol. 241, Article ID 117051, 2020.
- [20] R. Kranzhöfer, J. Schmidt, C. A. Pfeiffer, S. Hagl, P. Libby, and W. Kübler, "Angiotensin induces inflammatory activation of human vascular smooth muscle cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 7, pp. 1623–1629, 1999.
- [21] S. Puertas-Martín, A. J. Banegas-Luna, M. Paredes-Ramos et al., "Is high performance computing a requirement for novel drug discovery and how will this impact academic efforts?" *Expert Opinion on Drug Discovery*, vol. 15, no. 9, pp. 981–986, 2020.
- [22] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, "Open babel: an open chemical toolbox," *Journal of Cheminformatics*, vol. 3, no. 10, pp. 1–14, 2011.
- [23] A. W. Sousa Da Silva and W. F. Vranken, "AcPyPe—AnteChamber PYthon parser interFAce," *BMC Research Notes*, vol. 5, pp. 1–8, 2012.
- [24] S. Pronk, S. Páll, R. Schulz et al., "Gromacs 4.5: a high-throughput and highly parallel open source molecular simulation toolkit," *Bioinformatics*, vol. 29, no. 7, pp. 845–854, 2013.
- [25] H. J. C. Berendsen, D. van der Spoel, and R. van Drunen, "GROMACS: a message-passing parallel molecular dynamics implementation," *Computer Physics Communications*, vol. 91, no. 1–3, pp. 43–56, 1995.
- [26] C. Kutzner, S. Páll, M. Fechner, A. Esztermann, B. L. Groot, and H. Grubmüller, "More bang for your buck: improved use of GPU nodes for GROMACS 2018," *Journal of Computational Chemistry*, vol. 40, no. 27, pp. 2418–2431, 2019.
- [27] E. F. Pettersen, T. D. Goddard, C. C. Huang et al., "UCSF chimera? a visualization system for exploratory research and analysis," *Journal of Computational Chemistry*, vol. 25, no. 13, pp. 1605–1612, 2004.
- [28] G. M. Morris, R. Huey, W. Lindstrom et al., "AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, no. 16, pp. 2785–2791, 2009.
- [29] O. Trott and A. Olson, "Autodock vina: improving the speed and accuracy of docking," *Journal of Computational Chemistry*, vol. 31, no. 2, pp. 455–461, 2010.
- [30] P. C. D. Hawkins, A. G. Skillman, G. L. Warren, B. A. Ellingson, and M. T. Stahl, "Conformer generation with OMEGA: algorithm and validation using high quality structures from the protein databank and cambridge structural database," *Journal of Chemical Information and Modeling*, vol. 50, no. 4, pp. 572–584, 2010.
- [31] S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, and M. Schroeder, "PLIP: fully automated protein-ligand interaction profiler," *Nucleic Acids Research*, vol. 43, no. W1, pp. W443–W447, 2015.
- [32] A. Arnoldi, L. Carmen, and G. Aiello, "Cholesterol-reducing foods: proteins and peptides," *Encyclopedia of Food Chemistry*, vol. 3, pp. 323–329, 2018.
- [33] R. Soares, S. Mendonça, L. Í. de Castro, A. Menezes, and J. Arêas, "Major peptides from Amaranth (*Amaranthus cruentus*) protein inhibit HMG-CoA reductase activity," *International Journal of Molecular Sciences*, vol. 16, no. 2, pp. 4150–4160, 2015.
- [34] A. V. Quiroga, P. Aphalo, A. E. Nardo, and M. C. Añón, "In vitro modulation of renin-angiotensin system enzymes by amaranth (*amaranthus hypochondriacus*) protein-derived peptides: alternative mechanisms different from ACE inhibition," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 34, pp. 7415–7423, 2017.
- [35] A. Ayala-Niño, G. M. Rodríguez-Serrano, R. Jiménez-Alvarado et al., "Bioactivity of peptides released during lactic fermentation of amaranth proteins with potential cardiovascular protective effect: an in vitro study," *Journal of Medicinal Food*, vol. 22, pp. 1–6, 2019.
- [36] A. Montoya-Rodríguez, M. A. Gómez-Favela, C. Reyes-Moreno, J. Milán-Carrillo, and E. González de Mejía, "Identification of bioactive peptide sequences from amaranth (*Amaranthus hypochondriacus*) seed proteins and their potential role in the prevention of chronic diseases," *Comprehensive Reviews in Food Science and Food Safety*, vol. 14, no. 2, pp. 139–158, 2015.

- [37] B. Vecchi and M. C. Añón, "ACE inhibitory tetrapeptides from *Amaranthus hypochondriacus* 11S globulin," *Phytochemistry*, vol. 70, no. 7, pp. 864–870, 2009.
- [38] G. Boschini, G. M. Scigliuolo, D. Resta, and A. Arnoldi, "ACE-inhibitory activity of enzymatic protein hydrolysates from lupin and other legumes," *Food Chemistry*, vol. 145, pp. 34–40, 2014.
- [39] W. Liao, H. Fan, P. Liu, and J. Wu, "Identification of angiotensin converting enzyme 2 (ACE2) up-regulating peptides from pea protein hydrolysate," *Journal of Functional Foods*, vol. 60, Article ID 103395, 2019.
- [40] I. Astuti and Ysrafil, "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): an overview of viral structure and host response," *Diabetes & Metabolic Syndrome: Clinical Research Reviews*, vol. 14, no. 4, pp. 407–412, 2020.
- [41] D. Wrapp, N. Wang, K. S. Corbett et al., "Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation," *Science*, vol. 367, no. 6483, pp. 1260–1263, 2020.
- [42] V. Kumar, K.-P. Tan, Y.-M. Wang, S.-W. Lin, and P.-H. Liang, "Identification, synthesis and evaluation of SARS-CoV and MERS-CoV 3C-like protease inhibitors," *Bioorganic & Medicinal Chemistry*, vol. 24, no. 13, pp. 3035–3042, 2016.
- [43] M. Letko, A. Marzi, and V. Munster, "Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses," *Nature Microbiology*, vol. 5, 2020.
- [44] G. Lu, Y. Hu, Q. Wang et al., "Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26," *Nature*, vol. 500, no. 7461, pp. 227–231, 2013.
- [45] R. Strollo and P. Pozzilli, "DPP4 inhibition: preventing SARS-CoV-2 infection and/or progression of COVID-19?" *Diabetes/Metabolism Research and Reviews*, vol. 36, no. 1, 2020.
- [46] A. Tapia-Abellán, D. Angosto-Bazarra, H. Martínez-Banachocha et al., "MCC950 closes the active conformation of NLRP3 to an inactive state," *Nature Chemical Biology*, vol. 15, no. 6, pp. 560–564, 2019.
- [47] Y. Li, W. Zhou, L. Yang, and R. You, "Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor," *Pharmacological Research*, vol. 157, Article ID 104833, 2020.
- [48] Y.-J. Geng, Z.-Y. Wei, H.-Y. Qian, J. Huang, R. Lodato, and R. J. Castriotta, "Pathophysiological characteristics and therapeutic approaches for pulmonary injury and cardiovascular complications of coronavirus disease 2019," *Cardiovascular Pathology*, vol. 47, Article ID 107228, 2020.
- [49] B. Vellingiri, K. Jayaramayya, M. Iyer et al., "COVID-19: a promising cure for the global panic," *The Science of the Total Environment*, vol. 725, Article ID 138277, 2020.
- [50] F. Li, W. Li, M. Farzan, S. C. Harrison et al., "Structure of SARS coronavirus spike receptor-binding domain complexed with receptor," *Science*, vol. 309, no. 5742, pp. 1864–1868, 2005.
- [51] J. Lan, J. Ge, J. Yu et al., "Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor," *Nature*, vol. 581, no. 7807, pp. 215–220, 2020.
- [52] J. J. Mourdad and B. I. Levy, "Interaction between RAAS inhibitors and ACE2 in the context of COVID-19," *Nature Reviews Cardiology*, vol. 17, p. 313, 2020.
- [53] A. M. South, D. I. Diz, and M. C. Chappell, "COVID-19, ACE2, and the cardiovascular consequences," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 318, no. 5, pp. H1084–H1090, 2020.
- [54] J. Liu, R. Cao, M. Xu et al., "Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro," *Cell Discovery*, vol. 6, no. 1, pp. 16–19, 2020.
- [55] S. Hraiech, J. Bourenne, K. Kuteifan et al., "Lack of viral clearance by the combination of hydroxychloroquine and azithromycin or lopinavir and ritonavir in SARS-CoV-2-related acute respiratory distress syndrome," *Annals of Intensive Care*, vol. 10, no. 1, pp. 63–66, 2020.
- [56] N. J. White, J. A. Watson, R. M. Hoglund, X. H. S. Chan, P. Y. Cheah, and J. Tarning, "COVID-19 prevention and treatment: a critical analysis of chloroquine and hydroxychloroquine clinical pharmacology," *PLoS Medicine*, vol. 17, no. 9, Article ID e1003252, 2020.
- [57] S.-M. Lin, S.-C. Lin, J.-N. Hsu et al., "Structure-based stabilization of non-native protein-protein interactions of coronavirus nucleocapsid proteins in antiviral drug design," *Journal of Medicinal Chemistry*, vol. 63, no. 6, pp. 3131–3141, 2020.
- [58] S. M. Lin, S. C. Lin, J. N. Hsu et al., "A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2," *Cell Host & Microbe*, vol. 27, pp. 671–e2, 2020.