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Inhibition of SARS-CoV-2 pathogenesis by potent peptides designed by the mutation of ACE2 binding region

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ABSTRACT

The outbreak of COVID-19 has resulted in millions of deaths. Despite all attempts that have been made to combat the pandemic, the re-emergence of new variants complicated SARS-CoV-2 eradication. The ongoing global spread of COVID-19 demands the incessant development of novel agents in vaccination, diagnosis, and therapeutics. Targeting receptor-binding domain (RBD) of spike protein by which the virus identifies host receptor, angiotensin-converting enzyme (ACE2), is a promising strategy for curbing viral infection. This study aims to discover novel peptide inhibitors against SARS-CoV-2 entry using computational approaches. The RBD binding domain of ACE2 was extracted and docked against the RBD. MMPBSA calculations revealed the binding energies of each residue in the template. The residues with unfavorable binding energies were considered as mutation spots by OSPREY. Binding energies of the residues in RBD-ACE2 interface was determined by molecular docking. Peptide inhibitors were designed by the mutation of RBD residues in the virus-receptors complex which had unfavorable energies. Peptide tendency for RBD binding, safety, and allergenicity were the criteria based on which the final hits were screened among the initial library. Molecular dynamics simulations also provided information on the mechanisms of inhibitory action in peptides. The results were finally validated by molecular docking simulations to make sure the peptides are capable of hindering virus-host interaction. Our results introduce three peptides P7 (RAWTFLDKFNHEAEDLRYQSSLASWN), P13 (RASTFLDKFNHEAEDLRYQSSLASWN), and P19 (RADTFLDKFNHEAEDLRYQSSLASWN) as potential effective inhibitors of SARS-CoV-2 entry which could be considered in drug development for COVID-19 treatment.

1. Introduction

Peptides with therapeutic potential have been considered during the last decades and the number of FDA-approved peptide drugs has increased [1]. They offer several advantages like ease of synthesis, high specificity, and limited accumulative behavior. These benefits have made peptides favorable agents in the development of diagnostic approaches, vaccines, and drugs against highly infectious viruses including influenza, acquired immunodeficiency syndrome, chronic hepatitis B, dengue virus, and coronavirus disease 2019 [2,3]. Moreover, antiviral peptides (AVPs) have the potential to block a virus’ cycle at different levels from viral attachment to the host cell to its replication. Some AVPs have natural origins [4–7] while others are discovered or rationally designed by bioinformatics techniques machine learning [8–11]. Since the outbreak of COVID-19, which is caused by the SARS-CoV-2 virus, peptide inhibitors have also been among the promising anti-covid agents from various resources [12]. While some studies seek to find peptide anti-COVID-19 agents from natural resources [13], others tried to rationally design novel peptides [12].

Due to the importance of the entrance step in the pathogenesis of viruses as obligate intracellular parasites, it has been the main target for anti-viral development. This is also the case about SARS-CoV-2 whose pathogenesis depends on angiotensin-converting enzyme II as a receptor [14–16] to either directly fuse its genetic materials into the cell or enter
in endosome-based endocytosis [17]. Host receptor recognition is mediated by the SARS-CoV-2 spike S1 subunit which is expressed on the viral envelope. Four main domains make up S, one of which is located in the direct interaction with ACE2, named receptor-binding domain (RBD) [15,18]. On the receptor side, the N-terminal helix of ACE2 is recognized by RBD and interacts with the virus [16,19].

Peptide inhibitors against SARS-CoV-2 entry have been proposed using three main strategies. Firstly, many peptides focused on virus fusion. This process is made possible by the catalytic cleavage of spike protein by the host proteases TMPRSS2 (transmembrane protease serine 2), furin, and cathepsin-L [20–22], is shown that can be blocked by peptides [23–27]. The second strategy is approached by receptor antagonists. The peptides in this category are designed or shown to block virus entry by occupying the host N-terminal helix of ACE2 [28,29]. While fusion inhibitors and receptor antagonists must approach the host cell to perform their anti-COVID-19 activity, in the last category, virus inactivators, peptides can prevent the viral infection before the virus achieve ACE2 N-helix in blood. These inhibitors mostly interact with the SARS-CoV-2 RBD domain and neutralize its binding affinity for ACE2 [30–32].

As the computational methods provide the cost and time effective manner for investigating the biomolecular interactions in detailed state [33,34], in this study, we applied computational approaches with the mutation-based rational design of peptides to prevent host cell recognition mediated by the SARS-CoV-2 RBD domain, thereby discovering peptides with potential competitive affinity for blocking viral pathogenesis.

2. Materials and methods

The overall diagram of the methods is illustrated in Fig. 1.

2.1. Interface analysis and template extraction

ACE2 residues that embrace viral spike RBD were considered as an inspiration for inhibitor design due to their ability to form a stable complex with the virus. Therefore, the peptides which can mimic the binding behavior of the virus binding region may interfere with the viral attachments and subsequently, may prevent viral fusion and pathogenesis.

The PDB structure of SARS-CoV2 RBD complexed with ACE2 (PDB ID: 6m0j) was obtained from the RCSB protein data bank (https://www.rcsb.org/) and the interactions in the interface of the virus and the receptor were identified and visualized by LigPlot+ v. 2.2.4 [35].

2.2. Molecular docking and molecular dynamics simulations

Molecular dockings were carried out applying the fully-automated ClusPro server (https://cluspro.org/) [36,37] to estimate spike-peptides binding modes. This server needs a simple provision of two molecule files being introduced as a receptor or peptide. ClusPro considers both molecules as rigid and automatically docks them using a fixed grid box for the receptor and a movable grid for the peptide. The docking score is calculated based on the following equation in ClusPro:

\[
E = w_1 E_{rep} + w_2 E_{attr} + w_3 E_{des} + w_4 E_{DARS}
\]

Here, \( E_{rep} \), \( E_{attr} \), and \( E_{DARS} \) represent the repulsive and attractive contribution to the van der Waals interaction and electrostatic energy, respectively. \( E_{DARS} \) denotes the Decoys as Reference State (DARS) approach which is measured by the amount of free energy change following the removal of water molecules from the protein interface. This parameter takes the desolvation contribution into account. W1-4 coefficients are weighted which is calculated for different types of docking problems [36].

After the separate introduction of the receptor’s and peptides’ files to the server, each run was performed for the receptor and a peptide with default parameters. Among the results, the conformations with the lowest docking scores were selected for further analysis.

In molecular dynamics (MD) simulations, the candidate peptides-ACE2 complexes were put into a 100-ns simulation using GROMACS package v. 2020 [38], Gromos96 54a7 [39], and SPCE water model. Gromos 96 was selected since it has a favorable agreement with the NMR data and the X-ray crystal structure of the protein [40]. Each complex was centered in a cubic box with a minimum distance of 1.0 nm from the edges. The energy of all systems was minimized for 50,000 steps followed by a thermal equilibrium step using Berendsen thermostat at 310 K. The pressure was equilibrated for 1 ns to achieve the pressure of 1 bar using Berendsen barostat, LINCS algorithm [41] for bond constrain, and PME mesh [42] for the calculation of long-range electrostatic interactions. Moreover, the Fourier grid spacing and Coulomb radius were set at 0.16 and 1.2 nm, respectively, and the van der Waals interactions were limited to 1.2 nm. Finally, the production states were performed under the leapfrog algorithm for 100 ns.

The resulted trajectories were then analyzed by built-in GROMACS utilities e.g. root mean squared deviation (RMSD), root mean squared fluctuation (RMSF), radii of gyration(Rg), principle component analysis (PCA) and solvent accessible surface area (SASA).

2.3. Binding energy calculation

To identify how important is the contribution of each residue in spike binding, the resides of decoy peptide was analyzed using g.mmpbsa program [43], the definitions of which can be applied to our system as

\[
\Delta G_{bind} = \Delta G_{Spike-template} - (\Delta G_{Spike} + \Delta G_{Template})
\]

Where \( \Delta G_{Spike-template} \), \( \Delta G_{Spike} \), and \( \Delta G_{Template} \) describe the total energy of spike-template peptide complex, solution free energy of spike, and solution free energy of free template peptide, respectively.

2.4. Library construction

Peptide library was built by OSPREY v. 3.0 (Open-Source Protein Redesign for You) [44] python-based script in which the input pdb files of the template was introduced as a strand. The mutation hotspots were then defined by strand. Flexibility and the probable residues of Ile, Trp, Ser, Thr, and Asp were determined by setLibraryRotamers scripts, respectively. The defined strand was used to make a conf space and the osprey forcefield parameters were selected. The remained scripts were performed as with default parameters according to the OSPREY documentation.
2.5. Validation of toxicity and allergenicity

The toxicity and allergenicity properties of screened peptides were predicted by ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/protein.php) [45] and AllerTop v. 2.0 (http://www.ddg-pharmfac.net/AllerTOP/) servers [46]. Both servers offer fully automated predictions which need peptide sequence as input. In ToxinPred, fragment length of 10 residues and other default parameters were set.

3. Results and discussion

It is well known that the SARS-COV-2 virus is equipped with a glycoprotein anchor, Spike (S), which guarantees host cell recognition and viral entry by interacting with angiotensin-converting enzyme 2 (ACE2) which is expressed on the surface of human cells [1,2]. Spike protein has been the initial main target for drug design. It is formed by S1 and S2 subunits, both of which are comprised of various domains. While the S1 subunit is known to be responsible for cell recognition, the S2 subunit plays a vital role in membrane fusion, following which the viral entry occurs. However, it is following the direct establishment of

![Fig. 2. The illustration of (A) ACE2-RBD complex and (B) the interactions made by ACE2 and SARS-Cov-2 RBD. The viral residues with hydrogen bonds and hydrophobic forces are indicated by pink and blue fonts and the green and black fonts indicate ACE2 residues with hydrophobic and hydrogen bonds, respectively. The atoms with H-bonds are connected with green dashed lines.](image-url)

| Table 1 | The mmpbsa energy analysis of the template residues revealed by a 100-ns MD simulation. |
|---------|------------------------------------------------------------------------------------------|
| Template Residue | ΔG binding (kJ/mol) |
| 24GLN | 64.533 |
| 25ALA | 5.905 |
| 26LYS | 77.717 |
| 27THR | −20.011 |
| 28PHE | −21.064 |
| 29LEU | −5.489 |
| 30ASP | −74.37 |
| 31LYS | 52.018 |
| 32PHE | −3.903 |
| 33ASN | −8.814 |
| 34HIS | −0.822 |
| 35GLU | −80.445 |
| 36ALA | −0.604 |
| 37GLU | −116.525 |
| 38ASP | −107.831 |
| 39LEU | −5.742 |
| 40PHE | 0.095 |
| 41TYR | −13.531 |
| 42GLN | −3.414 |
| 43SER | −5.384 |
| 44SER | −4.1 |
| 45LEU | −16.746 |
| 46ALA | −7.268 |
| 47SER | −6.086 |
| 48TRP | −14.855 |
| 49ASN | −111.155 |

| Table 2 | The inhibitory peptides with the highest OSPREY scores and the assessment of their allergenicity. |
|---------|------------------------------------------------------------------------------------------|
| ID | Peptide Inhibitor | OSPREY Score | Allergenicity |
| 1 | RARTFLDKFNAEEDLRYQSLSWSW | −90.6 | PROBABLE ALLERGEN |
| 109 | DARTFLDKFNAEEDLRYQSLSWSW | −79.6 | PROBABLE ALLERGEN |
| 19 | RADTFDLKNNIENANRWSW | −77.7 | Non |
| 37 | WARTFLDKFNAEEDLRYQSLSWSW | −74.7 | PROBABLE ALLERGEN |
| 4 | RARTFLDKFNAEEDLRYQSLSASW | −73.4 | PROBABLE ALLERGEN |
| 181 | TARTFLDKFNAEEDLRYQSLSASW | −72.6 | Non |
| 7 | RAWTDLFKFNHEADLRWYQSLSASW | −72.6 | Non |
| 73 | SARTFLDFNHEEDLRQYSSOLSW | −72.4 | PROBABLE ALLERGEN |
| 31 | RARTFLDKFNAEEDLRYQSLSWSW | −71.9 | PROBABLE ALLERGEN |
| 13 | RASTFLDKFNAEEDLRQYSSOLSW | −71.8 | Non |
| 2 | RARTFLDKFNAEEDLRWYQSLSWSW | −71.7 | PROBABLE ALLERGEN |
interaction between the S1 and ACE2 peptide domain that the spike undergoes proteolytic cleavage, and the viral infection initiates [47]. S1 includes a domain called receptor-binding domain (RBD) (residues 333–526) with two main subdomains: five anti-parallel β-sheets (e.g., β1, β2, β3, β4, and β7) and connecting loops, α4, β5, β6, and α5 form a region named receptor-binding motif (RBM) and it includes most of the residues responsible for receptor binding [16,19]. A variety of inhibitors have been proposed for hindering ACE2-RBD interaction small inhibitors [48], antibodies [49], phytochemicals [50], and FDA-approved drugs [51,52].

Whether the SARS-CoV-2’s genetic material enters the cytoplasm directly or the whole virus makes use of endosomes for cell penetration, both mechanisms require ACE2 recognition by its RBD domain. Therefore, RBD binders may inhibit viral attachment to the receptor and subsequently perform as a virus inactivator. In the present study, ACE2-derived peptides were computationally designed based on the RBD-binding residues of ACE2 and their potential propensities were predicted using computer-aided approaches.

To construct a peptide inhibitor library, the interactions that bind SARS-CoV-2 to the host ACE2 were raised using the experimentally approved PDB structure of their complex. Among the residues shown in Fig. 2, ACE2 residues which are targeted by the virus were Gln24, Thr27, Phe28, Asp30, Lys31, His34, Glu37, Asp38, Tyr41, Gln42, Leu79, Met82, Tyr83, Asn330, Lys353, Gly354, Asp355, and Arg357.

We chose the ACE2 chain A residues placed in 24–49 positions as a template peptide for inhibitor design. The selection of this region is supported by alanine scanning results that showed the critical role of residues 22–57 for S1 attachment [53]. The template was then docked against spike protein to validate its binding potential. The docking result showed that it chose the RBM motif for binding, as the source PDB, with the binding score of −700.6. The designed peptide, therefore, must have greater negative energy than the template and a higher affinity for RBD than the receptor to effectively prohibit the virus-receptor interaction.

The template-receptor complex was put into 100ns of MD simulation to reveal the spots where their interactions are relatively weak and can be substituted by other amino acids in sequence. MMPBSA calculation indicated that the residues 24, 26, 31, and 40 had unfavorable binding energies suggesting their negative effect on binding the spike to ACE2 (Table 1). This offers an opportunity to design peptide inhibitors with stronger binding affinity.

Following the mutation-based peptide design, a peptide library of 216 unique sequences was constructed using OSPREY (Table S1). Several studies introduced peptide inhibitors against RBD. However, they used different approaches to design peptides [54–60], and therefore, their results may seem incomparable to the present study. In the next step, 11 peptides having the greatest OSPREY scores were chosen and their probable allergenicity and toxicity were evaluated. As shown in Table 2, peptides 19, 181, 7, and 13 were allergically safe. Having no toxicity, these four peptides were considered as final candidates for docking simulations using the Chus pro server.

The results showed that except for peptide P181 (~673.1), other designed peptides had greater binding scores compared to template peptides (~761.6, ~726.6, and ~761.6 for P7, P13, and P19) (Table 3). This suggests that peptides P7, P13, and P19 had a higher affinity for spike protein, with peptide P7 having the highest affinity.

The higher binding potential of P7 may be explained by its highest number of hydrogen bonds (five) and the highest number of spike residues involved in its binding (Table 3). Among the viral residues, Arg403 was only connected with P7 while others formed interactions with at least two peptides (Fig. 3). Moreover, it is evident from Fig. 3 that three peptides attached the RBM motif so that the number of hydrogen bonds (five) and the highest number of spike residues involved in its binding (Table 3). Among the viral residues, Arg403 was only connected with P7 while others formed interactions with at least two peptides (Fig. 3). Moreover, it is evident from Fig. 3 that three peptides attached the RBM motif so that the number of hydrogen bonds (five) and the highest number of spike residues involved in its binding (Table 3). Among the viral residues, Arg403 was only connected with P7 while others formed interactions with at least two peptides (Fig. 3).

### Table 3

The comparison of sequence, binding affinities (docking scores), and interactions of the template and top hits of computationally improved peptides binding. The residues involved in H-binding are indicated in bold.

| ID   | Sequence                      | Chus Pro Docking Score | Residues                  | Spike RBD                  | Peptide                  |
|------|-------------------------------|------------------------|---------------------------|---------------------------|--------------------------|
| P7   | QAKTLFDKFNHEADELFYQSSLASWN    | −700.6                 | Lys417, Gly446, Tyr449,   | Tyr453, Leu455, Phe456,   | Gly24, Thr27, phe28,     |
|      |                               |                        | Tyr457, Ala475, Asn475,   | Ala487, Tyr489, Gly493,   | Asp30, Lys31, His34,     |
|      |                               |                        | Gly496, Thr500, Met501,   | Gly498, Thr500, Tyr500,   | Glu37, Asp38, Tyr41,     |
|      |                               |                        | Gly498, Thr500, Tyr500,   | Tyr505                     | Glu42                     |
| P13  | RASTLFKDFNHEADELFYQSSLASWN   | −761.6                 | Arg403, Lys417, Gly446,   | Arg403, Lys417, Gly446,   | Ala25, Trp26, Phe28,     |
|      |                               |                        | Gly447, Tyr449, Tyr453,   | Tyr453, Leu455, Thr473,   | Leu29, Asp30, Lys31,     |
|      |                               |                        | Gly493, Thr493, Gly495,   | Thr493, Arg546, Gly493,   | His34, Glu37, Asp38,     |
|      |                               |                        | Thr495, Gly496, Phe497    | Thr495, Arg546, Gly493,   | Tyr41, Glu42              |
| P19  | RASTLFKDFNHEADELFYQSSLASWN   | −730.1                 | Lys417, Gly446, Gly447,   | Lys417, Gly446, Gly447,   | Thr27, Phe28, Lys31,     |
|      |                               |                        | Asn448, Tyr449, Asn450,   | Asn448, Gly447, Tyr449,   | Asp38, His34, Glu42,      |
|      |                               |                        | Tyr451, Thr453, Leu455,   | Tyr451, Thr453, Leu455,   | Thr27, Phe28, Asp30,     |
|      |                               |                        | Phe456, Arg546, Arg547,   | Phe456, Arg546, Arg547,   | Lys31, His34, Glu37,     |
|      |                               |                        | Tyr473, Ala475, Thr473,   | Tyr473, Ala475, Thr473,   | Asp38, His34, Glu42      |
|      |                               |                        | Tyr489, Gly493, Glu493,   | Tyr489, Gly493, Glu493,   | Thr27, Phe28, Asp30,     |
|      |                               |                        | Leu492, Gly493, Glu498    | Leu492, Gly493, Glu498    | Lys31, His34, Glu37,     |
| P181 | TARTLFKDFNHEADELFYQSSLASWN   | −673.1                 | Lys417, Lys444, Val445,   | Lys417, Lys444, Val445,   | Thr27, Asp30, Lys31,     |
|      |                               |                        | Gly447, Asn448, Tyr449,   | Gly447, Asn448, Tyr449,   | His34, Glu37, Asp38,     |
|      |                               |                        | Tyr453, Leu455, Phe456,   | Tyr453, Leu455, Phe456,   | Tyr41, Glu42, Leu45,     |
|      |                               |                        | Gly493, Ser494, Gly496,   | Gly493, Ser494, Gly496,   | Trp48                     |
|      |                               |                        | Ser494, Gly496, Gly498    | Ser494, Gly496, Gly498    |                           |
|      |                               |                        | Tyr505                     |                           |                           |

SASa analysis also gained information about the volatility of each residue in all studied systems (Fig. 4). This suggests that the conformational changes in RBD induced by P7 and P19 were more severe than those of other system (Fig. 4).

Rg analysis which is an indicator of protein compactness is interpreted as a characteristic of protein stability [62]. The results showed that the interaction of all peptides with RBD caused a little compactness in protein structure. There are also significant fluctuations in the Rg diagram of the P7 complexed RBD which show there is recurrent open/close in protein structure indicating the binding of peptide to protein lead to instability in its structure (Fig. 4B).

Solvent accessible surface area (SASA) of proteins is a surface around a protein determined by a hypothetical center of a solvent sphere which has van der Waals contact surface with the protein. The results obtained in SASA analysis are shown in Fig. 4C and as can be seen there is no significant changes in the final values of SASA in all studied systems (Fig. 4C). This is a sign for the systems that did not undergo high values of opening or compactness which is in agreement with the results of Rg. SASA analysis also gained information about the volatility of each RBD residue during the simulation. It is evident from Fig. 4D that the greatest amino acid fluctuation is seen in the RBD when it is complexed with P7. This in line with the results obtained from other MD analysis which indicated that this peptide forces the protein to possess higher mobility which can lead to instability in its structure. Definition Secondary Structure of Protein (DSSP) gives information about the frequency of each secondary structure in protein’s conformation. DSSP
analysis also revealed no significant changes in various types of secondary structures over the span of simulation time (Table 4). Principal Component Analysis (PCA) provides the main components of protein motion during the simulation [63]. The 2D diagram of the RBD movements for different systems was obtained using the projection of the first two principal components. As can be seen in Fig. 5, the P7-RBD pattern in more propagated over the diagram plane indicating the higher protein flexibility and movements in this system which is in good agreement with other results.

The number of hydrogen bonds was also investigated since they are the main interactions that stabilize a complex. The number of H-bonds during 100 ns simulations varied for each peptide. In comparison with other two peptides the results indicated that P7 formed more stable hydrogen bonds with the RBD (Fig. 6).

Furthermore, the free non-bonded binding energies (e.g., van der Waals, electrostatic, polar solvation, and SASA energy) of the final systems were calculated using MM/PBSA method. The binding energies between spike RBD domain and peptides during the whole simulation revealed that P19 had a stronger interaction with the spike however other binding energies are also potent for complex formation (Table 5). These results are also in good agreement with that of docking results.

Finally the resulted RBD-peptide complexes were docked against host receptor to validate their inhibitory activity. As shown in Fig. 7, the RBD domain proved divergence from the position it must take to infect
the host cell. As it can be seen, binding the peptides P7 and P13 to the protein resulted in non-proper binding of the spike RBD with the ACE2. The amino acids involve in RBD-receptor binding are listed in Table 6 for all complexes. In previous studies the vital role of RBD Lys417 is approved in the virus binding affinity, transmission, and immune escape by mutation analysis [64]. Accordingly, P7 and P13 might be preferable since they successfully limited this residue. Moreover, the energetic assessment of residues lying in the RBD-ACE2 complex indicated the stabilizing impact of Tyr449, Leu455, Phe456, Ala475, Phe486, Glu493, Gly496, Gln498, Thr500, Asn501, Gly502, and Tyr505 in the virus-host complex formation [65]. Also it can be seen that P7 let only 3 RBD residues (Phe456, Asn501, and Tyr505) to access the receptor. However, this number increased to 4 and 6 residues for P13 (Leu455, Phe456, Gln493, and Gly496) and P19 (Tyr449, Leu455, Phe456, Ala475, Gln493, and Gln498). Regarding ACE2, X-ray diffraction experiments demonstrated residues Gln24, Lys31, Tyr41, Gln42, Leu79, Met82,
Tyr83, and Lys353 as essential elements for viral recognition [16]. In control docking, it is evident that ACE2 could involve its vital residues the most when bound to P7 with Lys31, Tyr41, Gln42, Tyr83, and Lys353 but fewer host residues were involved due to the inhibition of P13 (ACE2 Lys31, and Gln42) and P19 (ACE2 Lys31). These data suggest that although P19 and P13 let several important pathogenic RBD residues free, they hindered RBD to accommodate the ACE2 recognition region. In contrast, P7 stifled the RBD-ACE2 complex formation by preventing most of RBD determinant residues.

4. Conclusion

In the present study, a library of 216 peptides was designed to investigate their inhibitory potential in complex formation of RBD domain of SARS-CoV-2 and its main receptor the ACE2. At first using the pre docking and MD simulation analysis the interaction of ACE2 derived peptide and the RBD was evaluated. Among all the obtained peptides, based on the binding scores and biological factors such as immunogenicity and stability, number of 3 peptides was selected for the rest of calculations. The results of molecular dynamic simulation indicated that the P3 peptide causes more instability in protein dynamic. This is extracted from the higher values of RMSD and residue RMSF during the simulation. Also the more severe fluctuation in the Rg and more distribution in 2D PCA diagrams of the RBD confirmed the noted conclusion. Also the results illustrated that in all systems the h-bonding interactions are a part of complex stabilization forces between the RBD and designed peptide. The results of non-bonded interactions showed that the P19 peptide performed the more strong interaction with the RBD however others also possess a tight binding to the protein. Finally the docking results confirmed that among the three studied peptides both the P7 and P13 have more adverse effects on binding of the RBD to

Fig. 6. Estimation of hydrogen bonds of candidate peptides P7 (A), P13 (B), and P19(C) with RBD domain of SARS-CoV-2 spike over the span of 100 ns.

| ID | Van der Waal Energy (kJ/mol) | Electrostatic Energy (kJ/mol) | Polar Solvation Energy (kJ/mol) | SASA Energy (kJ/mol) | Binding Energy (kJ/mol) |
|----|-------------------------------|-------------------------------|-------------------------------|---------------------|-----------------------|
| Template | -263.9 | -782.2 | 795.9 | -35.9 | -286.2 |
| P7 | -244.9 | -631.2 | 660.6 | -32.7 | -248.2 |
| P13 | -222.6 | -479.9 | 563.2 | -29.6 | -169.0 |
| P19 | -252.6 | -739.7 | 726.0 | -34.8 | -301.2 |

Fig. 7. The representations of the best docking pose of top three peptides (A) P7, (B) P13, and (C) P19) bound to the RBD (gray) domain of the SARS-CoV-2 with the best docking scores. The surface regions indicate RBD position in receptor recognition.

Table 5

| ID | Residues | Spike RBD | ACE2 |
|----|----------|-----------|------|
| P7 | Gly406, Arg408, Asp420, Phe456, Tyr473, Gly476, Ser477, Asn501, Val503, Tyr505 | Thr27, Lys31, Tyr41, Gln42, Lys68, Gly75, Tyr83, Gly326, Asn330, Lys353, Asp355 | Thr27, Phe28, Lys31, His34, Asp38, Gln42 |
| P13 | Tyr453, Leu455, Phe456, Tyr473, Tyr489, Gln493, Tyr495, Gly496, Phe497 | Thr27, Phe28, Lys31, His34, Gly64 | |
| P19 | Lys417, Gly446, Gly447, Asn448, Tyr449, Asn450, Tyr451, Tyr453, Leu455, Phe456, Arg457, Tyr473, Ala475, Tyr489, Leu492, Gln493, Gln498 | Thr27, Phe28, Asp30, Lys31, His34, Gly35 | |
its receptor. From all the results obtained in this study it can be concluded that the as designed P7 peptide is capable of being promising blocker of SARS-CoV-2 host cell recognition with high affinity.

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Appendix A. Supplementary data

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