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Design Novel Peptide Derived from Protein-Protein Interfaces of 3CL-Pro SARS/CoV-2 and ACE-II Enzyme Complexes for Inhibition of COVID-19 Virus Replications by Computational Approach

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ABSTRACT

The novel coronavirus disease 2019 (COVID-19 or SARS/CoV-2) is rapidly expanding infection around the globe. This infection causes many deaths on a daily basis all over the world. Therapeutic options are currently limited. There is an emergent need of finding potential therapeutic agents for COVID-19. As a key enzyme in the life-cycle of corona-virus, the 3C-like main protease (3CL-Pro or M^{-}-Pro) is the most attractive for antiviral drug design. Therefore, current work are focused on RNA interference silences gene expression in COVID-19 through short interfering of SARS/CoV-2-RNA segments that guide mRNA degradation in a sequence-specific fashion at blocking the 3CL-Pro-SARS/CoV-2 active site further to block the proteins accession on infected human host cells leads to inhibit the virus replications. Thus, we developed the Structure-based drug design, based on a recently solved structure 3CL-Pro-SARS/CoV-2 (PDB ID: 6LU7 its 99% identical of COVID-19 reported and 6VW1 is a SARS/CoV-2 3CL-Pro complexed with ACE-II), for the intention of generating novel peptides has been derived from protein-protein interfaces of SARS-CoV-2-3CL-Pro and ACE-II (it's an entry receptor for COVID-19) as, Yu-Chuan Chang et.al., 2020 complexes, based on the structure-based optimization, we obtained the peptide-3 as shown a near perfect dock in the overlap region of the protein pocket, the active sites inferred from three selected target proteins of 3CL-Pro/SARS-CoV-2/ACE-II are compatible with the docking score. Hence, these peptide-3 can be used as potential candidate's leads for the inhibition of protein-protein interactions of SARS/CoV-2 and ACE-II with against the COVID-19 through binding and activation.

Keywords: SARS/CoV-2, 3CL-Proteases, COVID-19, Peptides, Protein-protein interfaces, Docking

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19 or SARS/CoV-2) is a rapidly expanding infection around the globe. The COVID-19 compared to previous outbreaks of severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS) is more contagious. The World Health Organization (WHO) in March 2020 announced the Coronavirus pandemic. This infection causes many deaths on a daily basis all over the world. Therapeutic options are currently limited. There is an emergent need of finding potential therapeutic agents for COVID-19[1,2].

The SARS/CoV-2 Coronaviruses are single-stranded positive-sense RNA viruses that possess large viral RNAgenomes. Recent studies showed that SARS-CoV-2 has a similar genomic organization to other beta-coronaviruses,

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consisting of a 5'-untranslated region (UTR), a replicase complex (orf1ab) encoding non-structural proteins (nsps), a spike protein (S) gene, envelope protein (E) gene, a membrane protein (M) gene, a nucleocapsid protein (N) gene, 3'-UTR, and several unidentified non-structural open reading frames. Although SARS-CoV-2 is classified into the beta-coronaviruses group, it is diverse from MERS-CoV and SARS-CoV. Recent studies highlighted that SARS-CoV-2 genes share <80% nucleotide identity and 89.10% nucleotide similarity with SARS-CoV genes. Usually, beta-coronaviruses produce a ~800 kDa polypeptide upon transcription of the genome. This polypeptide is proteolytically cleaved to generate various proteins. The proteolytic processing is mediated by papain-like protease (Plpro) and 3-chymotrypsin-like protease (3Clpro or $3CL^{Pro}$ also known as M^{Pro}). The 3Clpro cleaves the polyprotein at 11 distinct sites to generate various non-structural proteins that are important for viral replication. 3Clpro play a critical role in the replication of virus particles and unlike structural/accessory protein-encoding genes, it is located at the 3' end which exhibits excessive variability. Therefore, it is a potential target for anti-coronaviruses espeasiallyCOVID-19 inhibitors and vaccine screening. In addition, the alveolar epithelial cells have abundant expression of angiotensin-converting enzyme 2 (ACE2), which is targeted by the virus. The recognition of ACE2 by the S protein of the virus enables the invasion of the coronavirus into the human circulation system [1,2]. Recent study demonstrates that ACE2 is the SARS-CoV-2 receptor, which is required for cell entry [3]. Single-strand RNA (ssRNA) viruses such as the coronavirus (SARS/CoV-2) family replicate the virus genomes by taking advantage of host cells. For example, after coronavirus approaches the ribosome of the epithelial cells or other host cells, it uses the ribosome of the host cell to replicate polyproteins. The replication and subsequent processes of precursor polyproteins can occur in the epithelial cells [3]. After the coronavirus' polyproteins are expressed, two enzymes specifically, coronavirus main proteinase (3Clpro) and the papain-like protease (Plpro) are thought to be involved in cleaving the polyproteins into smaller products used for replicating new viruses [3]. In order to generate the daughter RNA genome, the coronavirus expresses an RNA-dependent RNA polymerase (RdRp), which is a crucial replicase that catalyzes the synthesis of a complementary RNA strand using the virus RNA templates [1,2].

Therefore, current work are focused on RNA interference silences gene expression in COVID-19 through short interfering of SARS/CoV-2-RNA segments that guide mRNA degradation in a sequence-specific fashion at blocking the 3CL-Pro-SARS/CoV-2 active site further to block the proteins accession on infected human host cells leads to inhibit the virus replications. Thus, we developed the Structure-based drug design, based on a recently solved structure 3CL-Pro-SARS/CoV-2 (PDB ID: 6LU7 its 99% identical of COVID-19 reported as., Yu-Chuan Chang et.al. [1] and 6VW1 is a SARS/CoV-2 3CL-Pro complexed with ACE-II), for the intention of generating novel peptides has been derived from protein-protein interfaces of SARS-CoV-2-3CL-Pro and ACE-II (it's a entry receptor for COVID-19) complexes, based on the structure-based optimization, we obtained as peptide-3 shows a near perfect dock in the overlap region of the protein pocket, the active sites inferred from three selected target proteins of 3CL-Pro/SARS-CoV-2/ACE-II are compatible with the docking score. Hence, these peptide-3 can be used as potential candidate's leads for the inhibition of protein-protein interactions of SARS/CoV-2 and ACE-II with against the COVID-19 through binding and activation.

Although, Tin-Yun Ho et al. [2], Antiviral Research 69 (2006) 70–76 reported SARS-Cov inhibitors of small peptides derived from S protein on the binding of S protein to ACE2 and on the S-protein-pseudotyped retrovirus infectivity. SP-4 (residues 192–203), SP-8 (residues 483–494), and SP-10 (residues 668–679) significantly blocked the interaction between S protein and ACE2 by biotinylated enzyme-linked immunosorbent assay, with IC 50 values of 4.30 ± 2.18, 6.99 ± 0.71, and 1.88 ± 0.52 nmol, respectively [2,3]. Based on that, we developed peptides derived from the protein-protein interfaces regions of 3-CL protease-SARS-CoV-2 and ACE-II complexes with the aim of blocking the 3CL-Pro cleavage in SARS/CoV-2 interacting cellular receptors especially inhibit the ACE-2 receptor entry.

MATERIALS &METHODS

The Proteins/Macromolecules of COVID-19, the SARS/CoV-2 3clpro/MproPDB ID: 6LU7[Ref] structures were obtained from PDB (https://www.rcsb.org/), in the file format of dot.pdb format. The PDB is an archive for the crystal structures of biological macromolecules from worldwide [4,5]. The 6LU7 protein which contains two chains, A and C, which form a homodimer. Chain A was used for macromolecule preparation. The native ligand for 6LU7is n-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide and the PDB ID: 6VW1 is a crystal structure of $3CL^{Pro}$-SARS/CoV-2 complexes with ACE-II [6-7] were also used for this studies.

Protein-protein docking

In order to mimic the $3CL^{Pro}$-SARS/CoV-2 – ACE-II complex, the 3D crystal structures of proteins: $3CL^{Pro}$-SARS/CoV-2 (PDB ID: 6LU7) and ACE-II (PDB ID: 6VW1_A, B) were retrieved from the Protein Data Bank. These, Protein-Protein complex prediction was mimicked by using Patch Dock. First, the $3CL^{Pro}$-SARS/CoV-2 was docked with the ACE-II complex, finally to obtain a mimicked structure.

Protein-protein interfaces and peptide designing

The obtained 3D complex of 3CLPro-SARS/CoV-2-ACE-II was introduced to the online meta-PPISP server for the prediction of protein-protein interface region **(Table 1)**. The interface region was interpreted and analyzed by SwissPDB-Viewer. The possible three peptide structures that mimic the interface region structures were obtained and drawn by using ChemDraw. ChemDraw has advanced chemical editor for drawing chemical structures and it has a rich list of editing features, further the obtained peptides were saved as dot.MOLformar **(Tables 1-3).**

***In silico*-high throughput structure-based screening**

The three peptides of protein-protein interfaces were designed based on drug-like properties of their ability is due to the ligand to interact and inhibit the native ligand catalytic site of 3-CL Pro SARS/CoV-2 and ACE-II protein. A lead compounds or scaffolds can be identified from diversified compound pool and accelerated screening, a screened pool is focused for bio-targets to inhibit the diseases. Hence, we used structural based screening, through molecular docking [8,9] by using binding and activation with the GOLD drug discovery software.

GOLD (Genetic Optimization for Ligand Docking) is a genetic algorithm for docking flexible ligands into protein binding sites. GOLD offers a choice of scoring functions of GoldScore/ChemScore. The GOLD fitness function is made up of four components: 1st protein-ligand hydrogen bond energy (external H-bond). 2nd the protein-ligand van der Waals (vdw) energy (external vdw). Thired, ligand internal vdw energy (internal vdw) and 4th the ligand torsional strain energy (internal torsion). The Input Parameters of Protein: Allows specification of the protein input file and ligand: Thus, Added file or Update selected file button or Add all files in directory button to add the chosen ligand file or directory to Set atom types: Controls whether atom types are set manually or automatically for (a) the ligand(s) and (b) the protein for Defining the Binding Site we should specify the approximate centre and extent of the binding site. This can be done in several ways: such as from a point, from a protein atom, from a file containing a list of atoms, from a protein residue, from a file containing a list of residues and from a reference ligand and we can use cavity detection to confine the calculation to regions enclosed within concave parts of the binding site surface cavity volume, as determined by the cavity detection algorithm, can also be output and define active site from: Allows specification of the position of the binding site with respect to a point, a protein atom close to the centre of the site, a set of protein atoms lining the site, or a reference ligand and active site radius: Allows specification of the radius of the binding site and Covalent: Allows specification of a protein-ligand covalent bond.

The Fitness and Search Options used to control: the Ligand flexibility during docking, including whether ligand ring conformations are varied, whether torsion angles around ligand amide bonds and bonds to trigonal nitrogen are allowed to vary during docking, whether intramolecular hydrogen bonds are permitted between ligand atoms, and whether protonated carboxylic acids are permitted to rotate or flip and the parameters testing is based on Edit Constraints: Allows specification of distance constraints, hydrogen bond constraints, regional (hydrophobic) constraints, and binding mode similarity constraints and Van der Waals: Only available if Gold Score selected. Allows specification of the van der Waals annealing parameter and Hydrogen Bonding: Only available if Gold Score selected. Allows specification of the hydrogen-bonding annealing parameter and the Genetic Algorithm Parameters were selected GA Presets and Automatic Settings: Allows specification of speed and accuracy of docking runs to the Population Size: Allows specification of the population size and Selection Pressure: Allows specification of the selection pressure and number of Operations: Allows specification of the total number of operations to be performed in a genetic algorithm run and further number of Islands: Allows the genetic algorithm to be split over n islands, where n is user specified and Niche Size: Allows specification of the niche size to be used and Migrate/Mutate/Crossover: Controls the relative frequencies with which the three types of genetic operations occur. Thus, the complete strategy followed in this study was reversed to ensure that the identified potential leads really fit the generated models and active sites of both targets. All the parameters required for molecular docking were set as used in actual process.

RESULTS & DISCUSSION

The three-protein complex of 3CL-Pro-SARS/CoV-2-ACE-II obtained by Patch Dock. The complex is 1394 amino-acids long and the interfaces were predicted as by the online server meta-PPISP. The positive interface regions were analyzed using Swiss PDB-Viewer tools. The Protein-protein interface mimics were used for predicting peptides similar to the interface region. There were three positive interface regions predicted. **Table 1** shows the results of the positive Interface regions from the protein complex. The Interface Region-1 contained the amino-acids sequence of >6LU7; FDVVRQCSGVTF while The Interface Region-2 contained the amino acids of >6LU7; ELLQNGMNGRTILGSA and the interface region-3 containing the sequences of >6LU7; ELLQNGMNGRTILGSA. All the interface regions were present in the A-chain of SARS/CoV-2. Based on the interface regions-1,2 and 3 mimicked as Peptide-1, 2 and 3 respectively **(Tables 2 and 3)**. Those structures were considered as novel peptides for the inhibition of COVID-19 virus replication by Insilco high through put structure-based screening.

Column 1: AA (Amino Acid code)

Column 2: Ch (Chain ID)
Column 3: AA# (Amino Acid number)
Column 4-7: Prediction scores of cons-PPISP, PINUP, Promate, and meta-PPISP
Note: PINUP and Promate scores are scaled by 100
Column 8: Prediction of whether the residue is in an interface
(P = Positive; N = Negative; - = Burried and not predicted)
(Note: P corresponds to a score > 0.34 in 7th column; user may set a different threshold for positive prediction.)

According to study of RNA interference silences gene expression in COVID-19 through short interfering of SARS/CoV-2-RNA segments that guide mRNA degradation in a sequence-specific fashion at blocking the $3CL^{Pro}$-SARS/CoV-2 active site further to block the proteins accession on infected human host cells leads to inhibit the virus replication. Hence, we developed the Structure-based drug design, based on a recently solved structure COVID-19 (PDB ID: 6LU7 its 97% identical of COVID-19 reported by Yu-Chuan Chang etal. [1] and 6VW1 is a SARS/CoV-2 $3CL^{Pro}$ complexed with ACE-II), for the intervention of generating novel peptides, has been derived from protein-protein interfaces of SARS-CoV-2-$3CL^{pro}$ and ACE-II (it's an entry receptor for COVID-19) complexes, with the aim of inhibition of the virus reproduction by targeting the mRNAs for either blocking of the $3CL^{pro}$ cleavage in SARS/CoV-2 and ACE-II interacting cellular receptors. Based on the structure-based optimization of 3-CL Pro SARS/CoV-2-protein RNAs signaling pathway in viral network, there are various sites present in 3-CL Pro SARS/CoV-2-protein.

Thus, in this study, we chose three target one is 3-chymotrypsin-like protease (3CL-protease), the main protease used to cleave polyproteins into replication-related proteins, and RdRp, the main protein for RNA replication, as the target receptors. The 3CL-protease structure of COVID-19 (PDB ID: 6LU7) was obtained from the RCSB Protein Data Bank, which was recently released on February 5th, 2020. Hence, we can give us a strong structural insight about its mechanism of action and also enable us to trace important target residues for designing drug inhibitors against the 3-CL Pro SARS/CoV-2-protein signaling pathway. The minimized free energy was calculated with electrostatics and van der Waals interactions between residues of complex, the active site and binding site amino acid can be labeled **(Figure 1)**.

The second target was focused on SARS/CoV-2 PDB ID: 6VW1_A, these active site amino acid residues can be labeled **(Figure 2)**. The final target was focused on angiotensine converting enzyme-II, these protein active site amino acid resedues can be also labeled **(Figure 3)** which is an entry receptor for COVID-19, based on the structure based optimization, the newly designed three peptides **(Tables 2 and 3)** separately docked with above selected three targets with the aim of blocking these three target mechanism for against COVID-19.

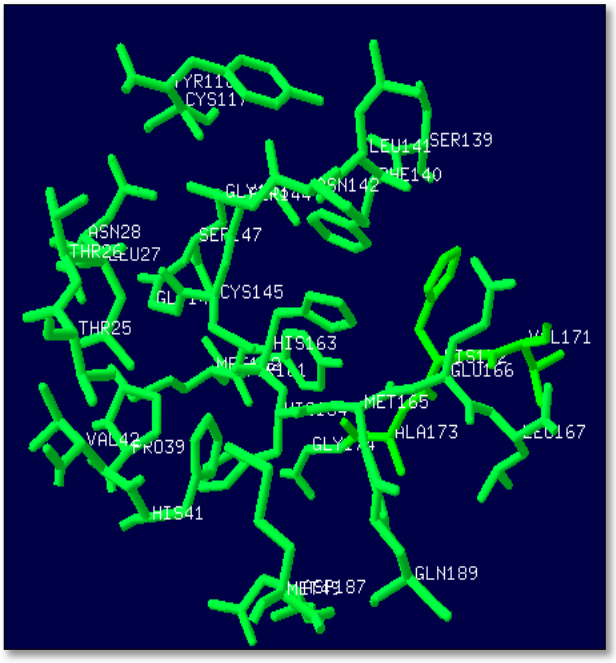


Figure 1. Representation of Ligand binding pockets amino acid residues of COVID-19-Main protease (3CL-Pro PDB: 6LU7) it's an enzyme, which is responsible for cleaves the RNA polymerase such as RdRb for entry into the ACE-II receptor to further activate the virus replication and disease. The represented binding amino acids are, Thr-25, Thr-26,Leu-27,Asn-28,Pro-39,His-41,Val-42,Met-49,Ser-139,Phe-140,Leu-141,Asn-142,Gly-143,Ser-144,Cys-145,Gly-146,Ser-147,Tyr-161,Met-162,His-164,164,Met-165,Glu-166,Leu-167,Val-171,His-172,Ala-173,Gly-174,Asp-187,Gln-189.

A molecular docking study by GOLD drug discovery software was performed to calculate of binding energy and H-bond interaction, H-bond donor and acceptors, hydrophobicity and lipophobicity at all three focused peptides with targets. In order to gain functional and structural insight into the mechanism of most active lead compounds, molecular docking simulation was performed by the aid of GOLD software. Docking simulation is a popular approach for the preliminary screening in structure-based drug design. By performing docking simulation, information on feasible conformations of the ligand within the protein binding site can be obtained. This information can also reflect the nature and quality of the interaction. In our study, we examined three target separately docked with the ligands (peptides) the grid box for docking simulation was built with enough size to enable probing into the binding with first 3CL Proteases and second SARS/CoV-2 and thired ACE-II is the first human protein whose three dimensional X-ray crystal structure is broadly used in molecular docking studies to expose the binding properties of COVID-19 inhibitors.

Table1. Prediction by meta-PPISP: met method for Protein-Protein Interaction Site Prediction**.**

S A 1 0.980 0.370 0.376 0.404 P	
G A 2 0.491 0.370 0.401 0.372 P	
F A 3 0.019 0.000 0.416 0.220 N	
R A 4 0.277 0.370 0.399 0.355 P	
K A 5 0.015 0.490 0.401 0.369 P	
M A 6 0.881 0.870 0.430 0.568 P	
A A 7 0.046 0.990 0.427 0.580 P	
F A 8 0.000 0.990 0.435 0.000 -	
P A 9 0.975 0.990 0.405 0.651 P	
S A 10 0.131 0.870 0.398 0.493 P	
G A 11 0.221 0.870 0.336 0.448 P	
K A 12 0.057 0.620 0.332 0.330 N	
V A 13 0.000 0.000 0.000 0.000 -	
E A 14 0.032 0.370 0.393 0.407 P	
G A 15 0.014 0.240 0.330 0.209 N	
C A 16 0.000 0.000 0.323 0.000 -	
M A 17 0.000 0.000 0.401 0.000 -	
V A 18 0.000 0.000 0.353 0.000 -	
Q A 19 0.951 0.000 0.324 0.197 N	
V A 20 0.000 0.000 0.000 0.000 -	
T A 21 0.982 0.000 0.347 0.189 N	
C A 22 0.000 0.000 0.387 0.000 -	
G A 23 0.799 0.000 0.400 0.182 N	
T A 24 0.080 0.000 0.389 0.070 N	
T A 25 0.936 0.000 0.394 0.177 N	
T A 26 0.214 0.000 0.384 0.220 N	
L A 27 0.000 0.000 0.395 0.000 -	
N A 28 0.000 0.000 0.000 0.000 -	
G A 29 0.000 0.000 0.000 0.000 -	
L A 30 0.000 0.000 0.000 0.000 -	
W A 31 0.000 0.000 0.323 0.000 -	
L A 32 0.000 0.000 0.429 0.000 -	
D A 33 0.034 0.000 0.408 0.047 N	
D A 34 0.187 0.000 0.374 0.061 N	
V A 35 0.007 0.000 0.393 0.057 N	
V A 36 0.000 0.000 0.000 0.000 -	
Y A 37 0.000 0.000 0.443 0.000 -	
C A 38 0.000 0.000 0.000 0.000 -	

P A 39 0.000 0.000 0.000 0.000 -	
R A 40 0.046 0.000 0.433 0.062 N	
H A 41 0.000 0.000 0.408 0.000 -	
V A 42 0.000 0.000 0.396 0.000 -	
I A 43 0.000 0.000 0.373 0.000 -	
C A 44 0.000 0.000 0.361 0.000 -	
T A 45 0.079 0.000 0.371 0.113 N	
S A 46 0.043 0.000 0.345 0.113 N	
E A 47 0.007 0.000 0.321 0.082 N	
D A 48 0.109 0.000 0.335 0.115 N	
M A 49 0.014 0.000 0.312 0.054 N	
L A 50 0.217 0.000 0.221 0.020 N	
N A 51 0.034 0.000 0.271 0.029 N	
P A 52 0.000 0.000 0.309 0.000 -	
N A 53 0.418 0.000 0.350 0.137 N	
Y A 54 0.000 0.000 0.370 0.000 -	
E A 55 0.047 0.000 0.356 0.032 N	
D A 56 0.447 0.000 0.319 0.105 N	
L A 57 0.000 0.000 0.338 0.000 -	
L A 58 0.000 0.000 0.383 0.000 -	
I A 59 0.022 0.000 0.309 0.072 N	
R A 60 0.522 0.000 0.322 0.097 N	
K A 61 0.163 0.000 0.360 0.081 N	
S A 62 0.317 0.000 0.370 0.077 N	
N A 63 0.014 0.000 0.386 0.090 N	
H A 64 0.205 0.000 0.377 0.139 N	
N A 65 0.453 0.000 0.373 0.162 N	
F A 66 0.000 0.000 0.333 0.000 -	
L A 67 0.795 0.000 0.315 0.171 N	
V A 68 0.000 0.000 0.300 0.000 -	
Q A 69 0.219 0.000 0.294 0.135 N	
A A 70 0.431 0.000 0.296 0.172 N	
G A 71 0.397 0.000 0.279 0.176 N	
N A 72 0.476 0.000 0.259 0.173 N	
V A 73 0.483 0.000 0.265 0.156 N	
Q A 74 0.220 0.000 0.282 0.120 N	
L A 75 0.000 0.000 0.304 0.000 -	
R A 76 0.317 0.000 0.350 0.083 N	
V A 77 0.000 0.000 0.373 0.000 -	
I A 78 0.008 0.000 0.354 0.050 N	

G A 79 0.015 0.000 0.354 0.065 N	
H A 80 0.251 0.000 0.353 0.072 N	
S A 81 0.013 0.000 0.372 0.056 N	
M A 82 0.451 0.000 0.367 0.084 N	
Q A 83 0.010 0.000 0.397 0.101 N	
N A 84 0.008 0.000 0.405 0.062 N	
C A 85 0.006 0.000 0.423 0.073 N	
V A 86 0.000 0.000 0.000 0.000 -	
L A 87 0.000 0.000 0.000 0.000 -	
K A 88 0.010 0.000 0.373 0.086 N	
L A 89 0.000 0.000 0.000 0.000 -	
K A 90 0.024 0.000 0.350 0.066 N	
V A 91 0.000 0.000 0.367 0.000 -	
D A 92 0.564 0.000 0.360 0.112 N	
T A 93 0.414 0.000 0.332 0.105 N	
A A 94 0.346 0.000 0.356 0.059 N	
N A 95 0.000 0.000 0.333 0.000 -	
P A 96 0.010 0.000 0.322 0.025 N	
K A 97 0.082 0.000 0.312 0.159 N	
T A 98 0.085 0.000 0.339 0.107 N	
P A 99 0.014 0.240 0.357 0.194 N	
K A 100 0.176 0.370 0.372 0.338 N	
Y A 101 0.254 0.240 0.442 0.151 N	
K A 102 0.108 0.370 0.453 0.309 N	
F A 103 0.470 0.240 0.465 0.186 N	
V A 104 0.975 0.240 0.466 0.300 N	
R A 105 0.265 0.000 0.476 0.113 N	
I A 106 0.398 0.120 0.436 0.278 N	
Q A 107 0.267 0.000 0.429 0.213 N	
P A 108 0.281 0.000 0.369 0.075 N	
G A 109 0.000 0.000 0.319 0.000 -	
Q A 110 0.097 0.000 0.349 0.103 N	
T A 111 0.000 0.490 0.376 0.000 -	
F A 112 0.000 0.000 0.000 0.000 -	
S A 113 0.000 0.000 0.000 0.000 -	
V A 114 0.000 0.000 0.598 0.000 -	
L A 115 0.000 0.000 0.449 0.000 -	
A A 116 0.000 0.240 0.583 0.000 -	
C A 117 0.000 0.000 0.000 0.000 -	
Y A 118 0.989 0.240 0.500 0.325 N	

N A 119 0.201 0.120 0.393 0.206 N	
G A 120 0.979 0.240 0.394 0.305 N	
S A 121 0.884 0.240 0.430 0.321 N	
P A 122 0.381 0.370 0.427 0.269 N	
S A 123 0.980 0.240 0.532 0.377 P	
G A 124 0.124 0.490 0.521 0.384 P	
V A 125 0.319 0.620 0.467 0.469 P	
Y A 126 0.732 0.620 0.513 0.452 P	
Q A 127 0.000 0.620 0.420 0.000 -	
C A 128 0.000 0.000 0.439 0.000 -	
A A 129 0.000 0.000 0.384 0.000 -	
M A 130 0.000 0.000 0.514 0.000 -	
R A 131 0.000 0.000 0.297 0.000 -	
P A 132 0.334 0.000 0.318 0.067 N	
N A 133 0.012 0.000 0.369 0.043 N	
F A 134 0.435 0.000 0.474 0.137 N	
T A 135 0.000 0.000 0.428 0.000 -	
I A 136 0.000 0.000 0.493 0.000 -	
K A 137 0.011 0.000 0.376 0.152 N	
G A 138 0.661 0.120 0.495 0.279 N	
S A 139 0.052 0.240 0.576 0.252 N	
F A 140 0.000 0.000 0.572 0.000 –	
L A 141 0.934 0.240 0.551 0.415 P	
N A 142 0.877 0.120 0.453 0.256 N	
G A 143 0.896 0.120 0.435 0.291 N	
S A 144 0.000 0.000 0.541 0.000 -	
C A 145 0.000 0.120 0.478 0.000 -	
G A 146 0.000 0.000 0.000 0.000 -	
S A 147 0.000 0.000 0.665 0.000 -	
V A 148 0.000 0.000 0.659 0.000 -	Peptide Linkages
G A 149 0.000 0.000 0.000 0.000 -	
F A 150 0.000 0.000 0.000 0.000 -	
N A 151 0.930 0.740 0.430 0.377 P	
I A 152 0.944 0.740 0.462 0.616 P	
D A 153 0.981 0.740 0.462 0.581 P	
Y A 154 0.237 0.740 0.469 0.493 P	
D A 155 0.062 0.620 0.397 0.399 P	
C A 156 0.000 0.490 0.416 0.000 -	
V A 157 0.000 0.000 0.000 0.000 -	
S A 158 0.000 0.490 0.479 0.000 -	

F A 159 0.000 0.000 0.000 0.000 -	
C A 160 0.000 0.000 0.000 0.000 -	
Y A 161 0.000 0.000 0.671 0.000 -	
M A 162 0.000 0.000 0.000 0.000 -	
H A 163 0.000 0.000 0.570 0.000 -	
H A 164 0.000 0.000 0.453 0.000 -	
M A 165 0.000 0.000 0.532 0.000 -	
E A 166 0.399 0.000 0.445 0.215 N	
L A 167 0.000 0.000 0.316 0.000 -	
P A 168 0.011 0.000 0.277 0.023 N	
T A 169 0.455 0.000 0.297 0.057 N	
G A 170 0.061 0.000 0.371 0.038 N	
V A 171 0.229 0.000 0.350 0.109 N	
H A 172 0.000 0.000 0.503 0.000 -	
A A 173 0.000 0.000 0.558 0.000 -	
G A 174 0.000 0.000 0.604 0.000 -	
T A 175 0.000 0.000 0.000 0.000 -	
D A 176 0.000 0.000 0.473 0.000 -	
L A 177 0.000 0.000 0.436 0.000 -	
E A 178 0.456 0.000 0.434 0.155 N	
G A 179 0.000 0.000 0.434 0.000 -	
N A 180 0.515 0.000 0.441 0.141 N	
F A 181 0.042 0.000 0.466 0.065 N	
Y A 182 0.000 0.000 0.528 0.000 -	
G A 183 0.019 0.000 0.467 0.105 N	
P A 184 0.083 0.000 0.413 0.074 N	
F A 185 0.000 0.000 0.409 0.000 -	
V A 186 0.012 0.000 0.329 0.064 N	
D A 187 0.000 0.000 0.366 0.000 -	
R A 188 0.008 0.000 0.288 0.066 N	
Q A 189 0.201 0.000 0.234 0.048 N	
T A 190 0.007 0.000 0.209 0.034 N	
A A 191 0.024 0.000 0.228 0.029 N	
Q A 192 0.012 0.000 0.270 0.032 N	
A A 193 0.128 0.000 0.312 0.064 N	
A A 194 0.025 0.000 0.330 0.075 N	
G A 195 0.114 0.000 0.351 0.086 N	
T A 196 0.012 0.000 0.322 0.087 N	
D A 197 0.131 0.000 0.305 0.059 N	
T A 198 0.477 0.000 0.298 0.052 N	

T A 199 0.256 0.000 0.310 0.140 N	
I A 200 0.000 0.000 0.271 0.000 -	
T A 201 0.000 0.000 0.303 0.000 -	
V A 202 0.000 0.000 0.299 0.000 -	
N A 203 0.000 0.000 0.298 0.000 -	
V A 204 0.000 0.000 0.000 0.000 -	
L A 205 0.000 0.000 0.000 0.000 -	
A A 206 0.000 0.000 0.000 0.000 -	
W A 207 0.000 0.000 0.403 0.000 -	
L A 208 0.000 0.000 0.000 0.000 -	
Y A 209 0.000 0.000 0.000 0.000 -	
A A 210 0.000 0.000 0.000 0.000 -	
A A 211 0.000 0.000 0.000 0.000 -	
V A 212 0.000 0.000 0.428 0.000 -	
I A 213 0.000 0.000 0.405 0.000 -	
N A 214 0.463 0.120 0.412 0.177 N	
G A 215 0.126 0.240 0.447 0.262 N	
D A 216 0.019 0.120 0.467 0.106 N	
R A 217 0.007 0.000 0.461 0.132 N	
W A 218 0.527 0.000 0.479 0.199 N	
F A 219 0.000 0.000 0.495 0.000 -	
L A 220 0.208 0.000 0.485 0.099 N	
N A 221 0.080 0.000 0.445 0.060 N	
R A 222 0.004 0.000 0.430 0.083 N	
F A 223 0.006 0.000 0.436 0.017 N	
T A 224 0.004 0.000 0.387 0.042 N	
T A 225 0.006 0.000 0.393 0.051 N	
T A 226 0.053 0.000 0.368 0.044 N	
L A 227 0.062 0.000 0.335 0.040 N	
N A 228 0.053 0.000 0.355 0.082 N	
D A 229 0.021 0.000 0.361 0.030 N	
F A 230 0.000 0.000 0.447 0.000 -	
N A 231 0.014 0.000 0.353 0.089 N	
L A 232 0.309 0.000 0.366 0.120 N	
V A 233 0.842 0.000 0.381 0.182 N	
A A 234 0.000 0.000 0.383 0.000 -	
M A 235 0.025 0.000 0.353 0.030 N	
K A 236 0.459 0.000 0.379 0.194 N	
Y A 237 0.961 0.000 0.376 0.236 N	
N A 238 0.917 0.000 0.326 0.196 N	

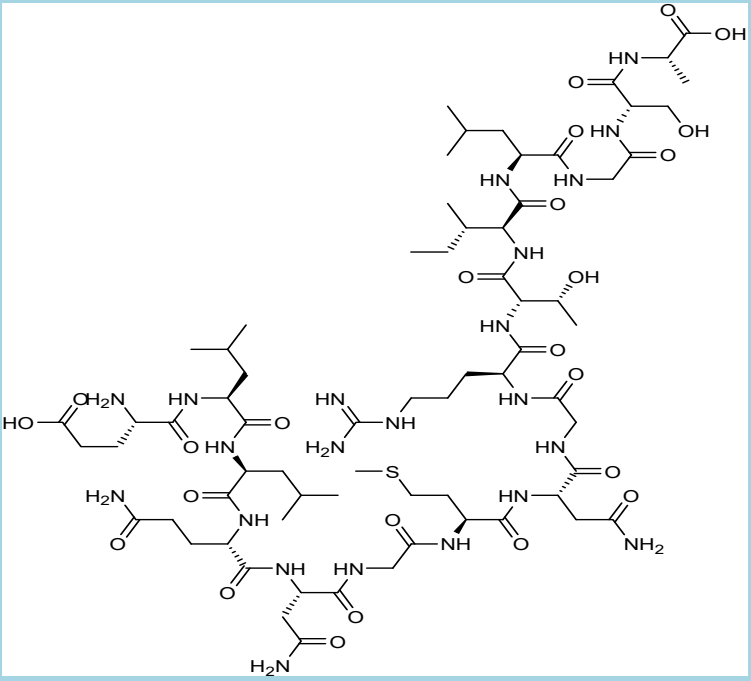
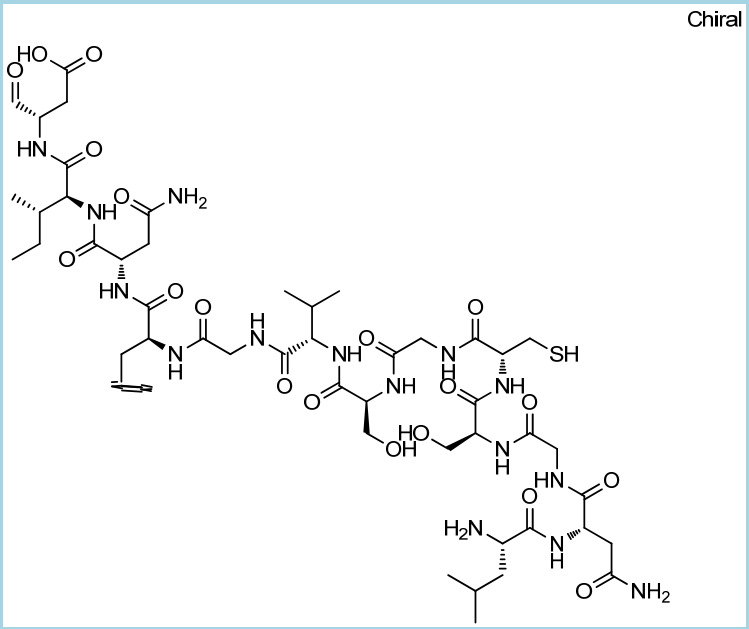
Y A 239 0.000 0.000 0.377 0.000 -	
E A 240 0.136 0.000 0.315 0.028 N	
P A 241 0.023 0.000 0.323 0.074 N	
L A 242 0.000 0.000 0.318 0.000 -	
T A 243 0.020 0.000 0.309 0.036 N	
Q A 244 0.005 0.000 0.301 0.025 N	
D A 245 0.004 0.000 0.269 0.047 N	
H A 246 0.031 0.000 0.274 0.043 N	
V A 247 0.325 0.000 0.295 0.076 N	
D A 248 0.011 0.000 0.284 0.095 N	
I A 249 0.006 0.000 0.302 0.148 N	
L A 250 0.000 0.000 0.000 0.000 -	
G A 251 0.008 0.120 0.278 0.110 N	
P A 252 0.898 0.240 0.335 0.244 N	
L A 253 0.000 0.000 0.383 0.000 -	
S A 254 0.006 0.000 0.284 0.058 N	
A A 255 0.034 0.120 0.294 0.098 N	
Q A 256 0.277 0.120 0.358 0.189 N	
T A 257 0.007 0.000 0.362 0.156 N	
G A 258 0.005 0.120 0.330 0.123 N	
I A 259 0.000 0.000 0.370 0.000 -	
A A 260 0.023 0.000 0.356 0.062 N	
V A 261 0.000 0.000 0.315 0.000 -	
L A 262 0.012 0.000 0.345 0.047 N	
D A 263 0.057 0.000 0.407 0.059 N	
M A 264 0.000 0.000 0.000 0.000 -	
C A 265 0.000 0.000 0.000 0.000 -	
A A 266 0.000 0.000 0.456 0.000 -	
S A 267 0.000 0.000 0.470 0.000 -	
L A 268 0.000 0.000 0.000 0.000 -	
K A 269 0.014 0.000 0.395 0.091 N	
E A 270 0.938 0.000 0.418 0.173 N	
L A 271 0.000 0.000 0.444 0.000 -	
L A 272 0.916 0.000 0.399 0.186 N	
Q A 273 0.981 0.000 0.406 0.176 N	
N A 274 0.950 0.000 0.405 0.180 N	
G A 275 0.087 0.000 0.421 0.129 N	
M A 276 0.967 0.000 0.426 0.150 N	
N A 277 0.948 0.000 0.413 0.181 N	
G A 278 0.041 0.000 0.413 0.196 N	Peptide Linkages

R A 279 0.464 0.000 0.436 0.171 N	
T A 280 0.015 0.000 0.412 0.148 N	
I A 281 0.000 0.000 0.000 0.000 -	
L A 282 0.041 0.120 0.433 0.134 N	
G A 283 0.409 0.120 0.398 0.117 N	
S A 284 0.945 0.000 0.387 0.230 N	
A A 285 0.970 0.000 0.394 0.144 N	
L A 286 0.134 0.000 0.397 0.063 N	
L A 287 0.144 0.000 0.403 0.159 N	
E A 288 0.018 0.000 0.373 0.171 N	
D A 289 0.020 0.000 0.309 0.034 N	
E A 290 0.010 0.120 0.361 0.111 N	
F A 291 0.000 0.120 0.419 0.000 -	
T A 292 0.000 0.120 0.393 0.000 -	
P A 293 0.000 0.240 0.348 0.000 -	
F A 294 0.912 0.620 0.458 0.492 P	
D A 295 0.000 0.740 0.427 0.000 -	
V A 296 0.000 0.000 0.000 0.000 -	
V A 297 0.986 0.370 0.424 0.363 P	
R A 298 0.054 0.740 0.458 0.577 P	
Q A 299 0.913 0.370 0.425 0.426 P	
C A 300 0.000 0.000 0.419 0.000 -	Peptide Linkages
S A 301 0.353 0.370 0.396 0.332 N	
G A 302 0.453 0.370 0.359 0.348 P	
V A 303 0.971 0.370 0.363 0.387 P	
T A 304 0.894 0.240 0.366 0.302 N	
F A 305 0.955 0.240 0.391 0.270 N	
Q A 306 0.398 0.240 0.380 0.180 N	
A C 2 0.006 0.000 0.251 0.040 N	
V C 3 0.024 0.000 0.356 0.048 N	
L C 4 0.090 0.000 0.000 -0.001 N	

The peptide-3, which displayed superior COVID-19 inhibitory activity in all selected target mechanism such as 3CL Proteases/SARS/CoV-2/ACE-II enzymes, which possessed highest docking activity **(Figure 4-6 and Table 4-6)** among the rest of selected and designed compounds. This simulation may assist to reveal binding orientation and interaction of these molecules with amino acid residues composing active site gorge in these 3CL Proteases/SARS/CoV-2/ACE-II enzymes.

The ligand-protein interactions indicated in pep-3 with docked Confirmation of COVID-19 main protease (3CL-Pro) PDB ID: 6LU7; explaining the Ligand-Protein interactions and score having the Fitness is -136.14, S(hb_ext) is 4.52, S(vdw_ext) is 42.34, S(hb_int) is 0.00 and S(vdw_int) is -198.89 and number of hydrogen bond presented in 10, indicated that hydrogen bonding, hydrophobic and mild polar interactions are the three major interactions incorporating the attachment of this ligand to 3CL Proteases enzymes.

Table 2. Table 2. 2D Structure of Novel Peptide-1 to Peptide-3.

Peptide Name	2D-Chemical Structure
PEP-1	
Peptide Name	**2D-Chemical Structure**
Pep-2	

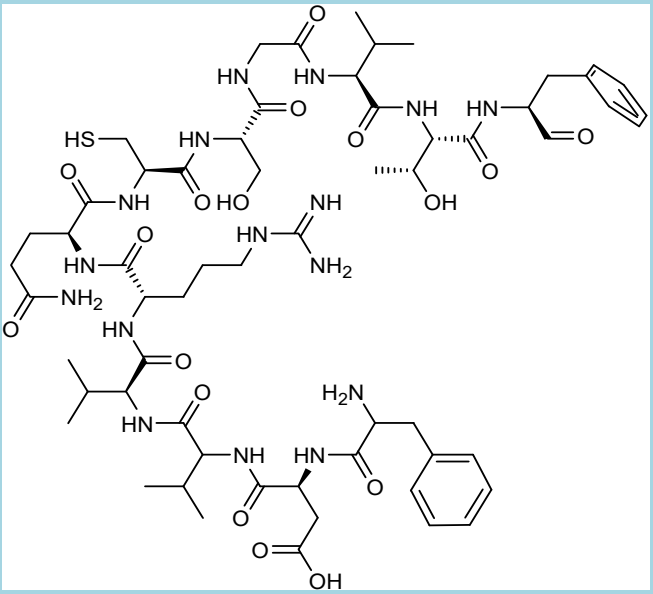
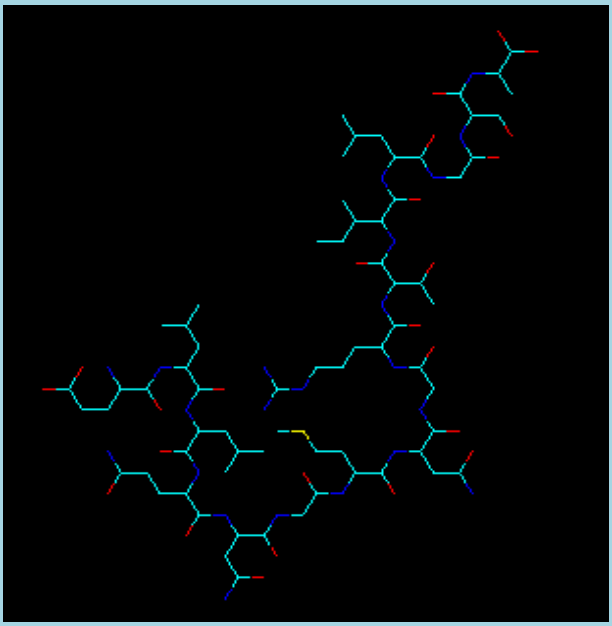
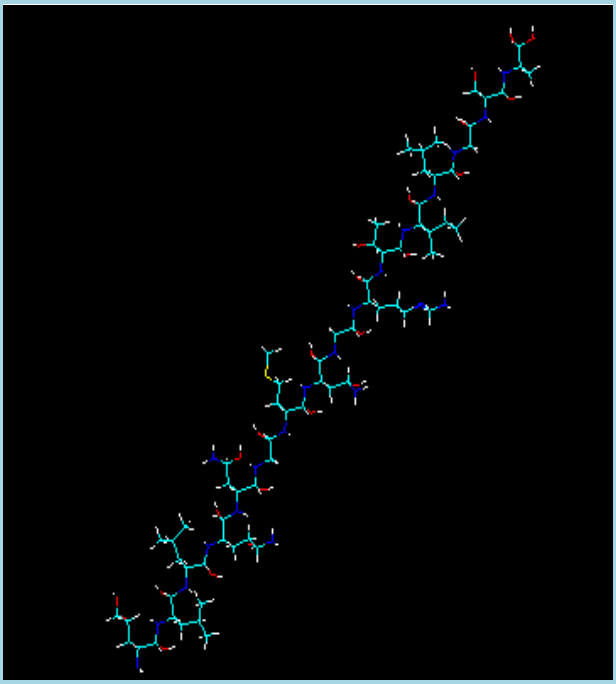
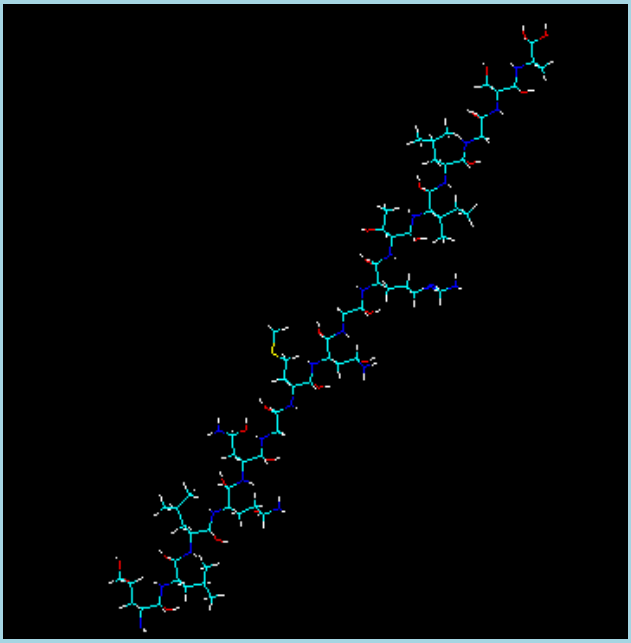
Peptide Name	Chemical structure
PEP-3	

Table 3. Primary Sequence and 3D-Ligand Confirmation of Peptide-1 to Peptide-3

Peptide Name	Sequence (FASTA Format)	3D-Ligand Confirmation (PDB Format)
Pep-1	>6LU7 FDVVRQCSGVTF	

Pep-2	>6LU7 ELLQNGMNGRTILGSA	
Pep-3	>6LU7 LNGSCGSVGFNID	

In second, **Pep-3** with docked confirmation of SARS-COV-2 Chimeric Receptor-Binding Domain Chain-E PDB_6VW1_E with the potential lead Pep-3; Explaining the orientation of the blocking the SARS/CoV-2 interactions in the entry receptors through binding and activation of certain parameters such as shown Fitness is -238.78, S(hb_ext) is 26.43, S(vdw_ext) is 34.36, S(hb_int) is 0.00 and S(vdw_int) is -312.45 and number of hydrogen bond presented in 8.

In finally the pep-3 Docked confirmation of human ACE2 angiotensin-converting enzyme-Chain-A PDB: 6VW1_A: Explaining the blocking of SARS/CoV-2 interactions in entry into the ACE-II receptor through binding and activation, having the scores of Fitness is –110.02, S(hb_ext) is 10.47, S(vdw_ext) is 32.48, S(hb_int) is 0.00 and S(int) is -165.14 and number of hydrogen bond presented in 10. The preliminary results suggested that the

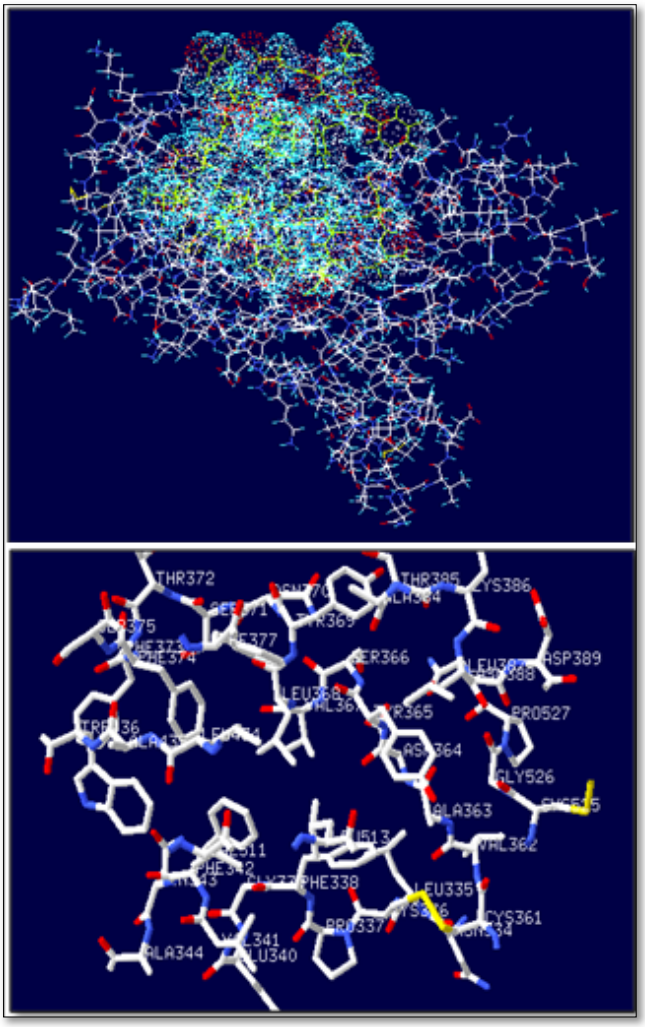


Figure 2. SARS-COV-2 Chimeric Receptor-Binding Domain Chain-E PDB: 6VW1_E: The blue coloured spheres indicating the ligand binding cavity of SARS/CoV-2, Below shown amino acids residues.

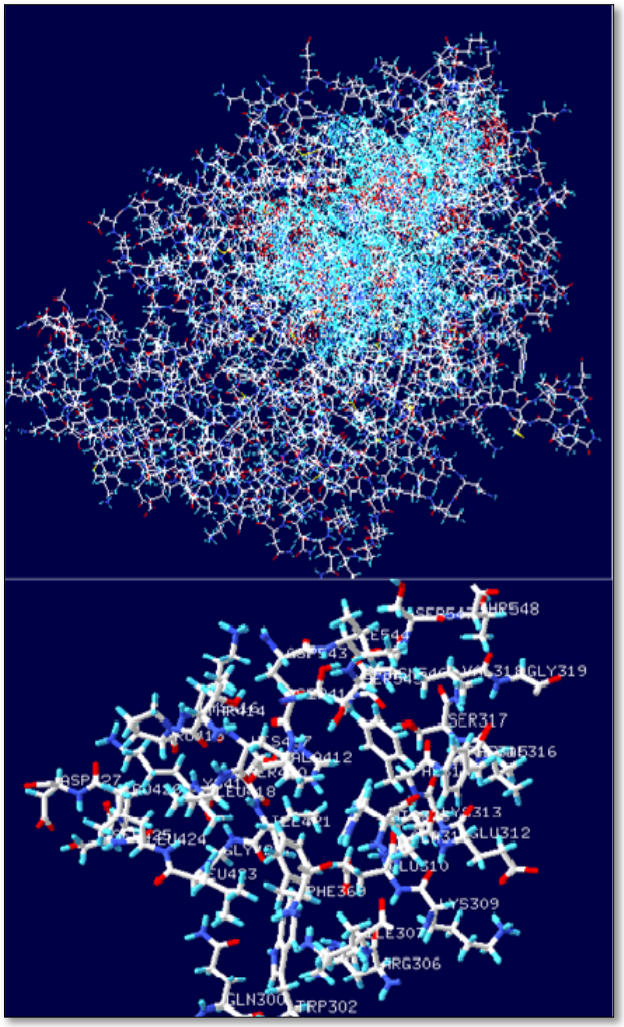


Figure 3. Shown human ACE2 Angiotensin-Converting Enzyme Ligand binding site PDB:6VW1_A representing the binding cavity surface and below figure indicating amino acid resedues presents in the binding cavity surface.

best docking scores and the comparison of the docking sites of these Pep-3 ligands shows a near perfect dock in the overlap region of the protein pocket, the active sites inferred from three selected target proteins of 3CL-Pro/SARS-CoV-2/ACE-II are compatible with the docking score. After analyzing them on the basis of their interactions and docking values were compared to the Tin-Yun Ho, et.al, [2]. Antiviral Research 69 (2006) 70–76 reported peptides derived from spike protein of SARS/Cov and retrovirus, has been efficiently blocked the binding of S protein to ACE2, in contrast, our targeted PEP-3 has been derived from the COVID-19 of 3CL-Pro-SARS/CoV-2 and ACE-II

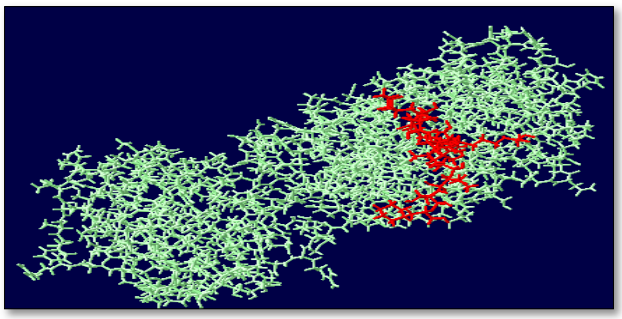


Figure 4. Docked Confirmation of COVID-19 main protease (3CL-Pro) PDB ID: 6LU7 with potential lead PEP-3; explaining the Ligand-Protein interactions and score having the Fitness is -136.14, S(hb_ext) is 4.52, S(vdw_ext) is 42.34, S(hb_int) is 0.00 and S(vdw_int) is -198.89 and number of hydrogen bond presented in 10.

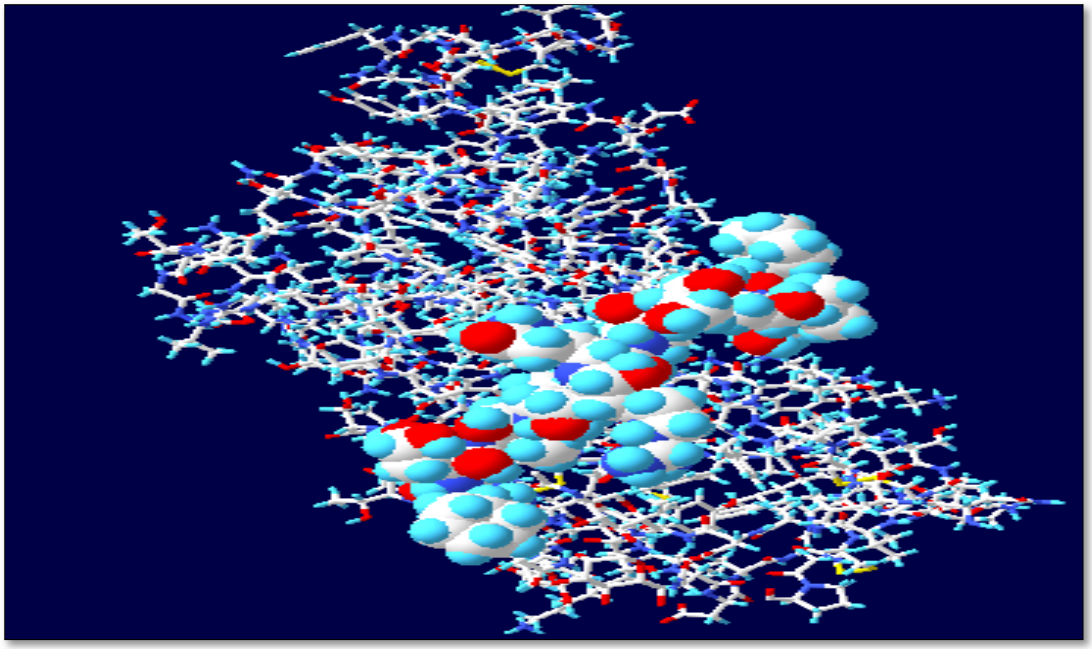


Figure 5. Docking confirmation of SARS-COV-2 chimeric receptor-binding domain chain-E PDB_6VW1_E with the potential lead Pep-3; Explaining the orientation of the blocking the SARS/CoV-2 interactions in the entry receptors through binding and activation of certain parameters such as shown Fitness is -238.78, S(hb_ext) is 26.43, S(vdw_ext) is 34.36, S(hb_int) is 0.00 and S(vdw_int) is -312.45 and number of hydrogen bond presented in 8.

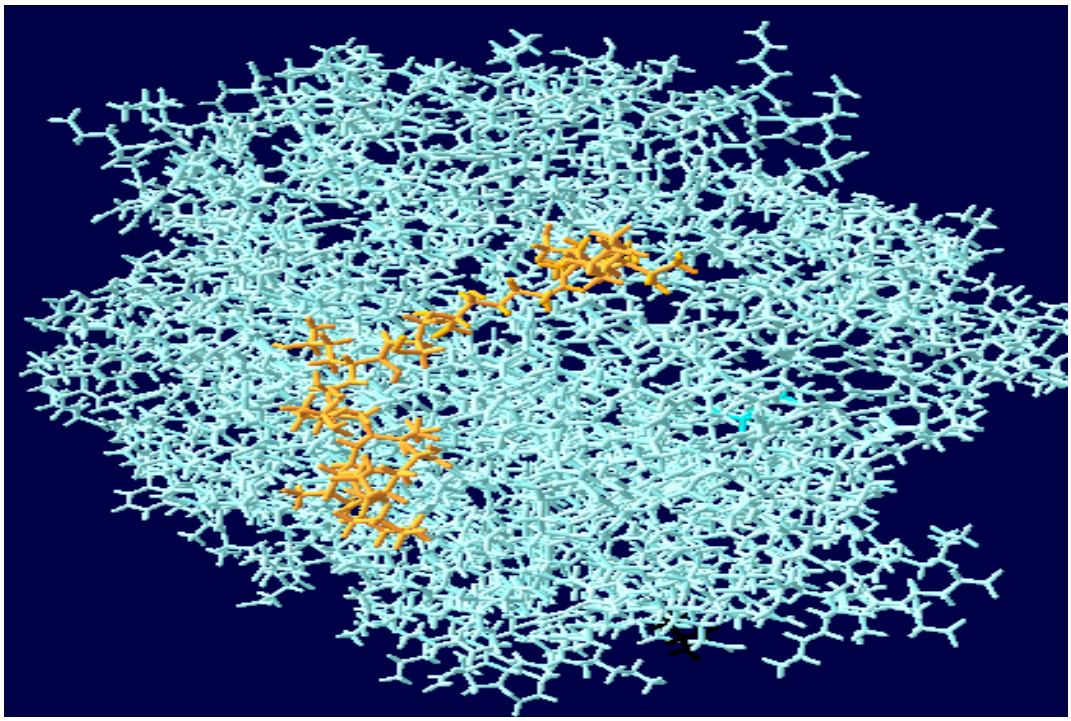


Figure 6. Docked confirmation of human ACE2 Angiotensin-Converting Enzyme-Chain-A PDB: 6VW1_A with PEP-3; Explaining the blocking of SARS/CoV-2 interactions in entry into the ACE-II receptor through binding and activation, having the scores of Fitness is -110.02, S(hb_ext) is 10.47, S(vdw_ext) is 32.48, S(hb_int) is 0.00 and S(int) is -165.14 and number of hydrogen bond presented in 10.

cellular receptors especially inhibit the ACE-2 receptor entry through binding and activation.

Hence, the Pep-3 was identified as potential lead candidate drugs for the inhibitory activity of 3CL Proteases/SARS-CoV-2/ACE-II for against COVID-19, which may give to the concern in its safety and efficacy for future development of the pre-clinical pharmacological therapeutics for COVID-19.

Table 4. Docking result of COVID-19 main protease (3CL Pro) PDB: 6LU7_A and Peptides.

Peptide name	No of H bonds	H bond energy	Gold score
PEP-1	8	1.16	-352.86
PEP-2	2	0.14	-299.18
PEP-3	**10**	**4.56**	-136.14

Note: As per above docking interaction PEP-3 is much better then (hydrogen bonds, H bond energy and docking score) rest of the PEP- 1 and PEP- 2
Highest binding score (lowest in negative) = -136.14

Table 5. Docking results of PDB ID; 6VW1 Chain-E (SARS-COV-2 Chimeric Receptor-Binding Domain Chain-E) and peptides.

Peptide name	No of H bonds	H bond energy	Gold score
PEP-1	7	10.30	-389.29
PEP-2	7	11.60	-384.58
PEP-3	**8**	**26.43**	-238.78

Note: As per above docking interaction PEP-3 is much better then (hydrogen bonds, H bond energy and docking score) rest of the PEP- 1 and PEP-2

Table6. Docking results of PDB ID; 6VW1 Chain-A (Human ACE2 angiotensin-converting enzyme) with peptides.

Peptide name	No of H bonds	H bond energy	Gold score
PEP-1	7	7.25	-322.18
PEP-2	7	0.67	-109.72
PEP-3	**10**	**10.47**	-110.02

Note: As per above docking interaction PEP-3 is much better then (hydrogen bonds, H bond energy and docking score) rest of the PEP- 1 and PEP- 2

CONCLUSION

Demonstrated the utility of in-silico structure-based drug design, we were competent to achieve the novel PEP-3 has been derived from protein-protein interfaces of SARS-CoV-2-3CL^{pro} and ACE-II (it's a entry receptor for COVID-19) complexes, with indicated the inhibition of the virus reproduction by targeting the mRNAs for either blocking of the 3CL^{pro} cleavage in SARS/CoV-2 and ACE-II interacting cellular receptors. Hence the pep-3shown potential anti-viral-COVID-19 activity through binding and activation.

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