Epitope-based peptide vaccine design and elucidation of novel compounds against 3C like protein of SARS-CoV-2

Muhammad Sajid\(^1\), Saigha Marriam\(^2\), Hamid Mukhtar\(^3\), Summar Sohail\(^4\), Muhammad Sajid\(^1\)*, Sheikh Arslan Sehgal\(^5\)*

\(^1\) Department of Biotechnology, University of Okara, Okara, Pakistan, \(^2\) Department of Microbiology and Molecular Genetics, University of Okara, Okara, Pakistan, \(^3\) School of Law, University of Okara, Okara, Pakistan, \(^4\) Department of Forestry, Kohsar University Murree, Murree, Pakistan, \(^5\) Department of Bioinformatics, University of Okara, Okara, Pakistan

* sajid@uo.edu.pk (MS); arslansehgal@yahoo.com (SAS)

Abstract

Coronaviruses (CoVs) are positive-stranded RNA viruses with short clubs on their edges. CoVs are pathogenic viruses that infect several animals and plant organisms, as well as humans (lethal respiratory dysfunctions). A novel strain of CoV has been reported and named as SARS-CoV-2. Numerous COVID-19 cases were being reported all over the World. COVID-19 and has a high mortality rate. In the present study, immunoinformatics techniques were utilized to predict the antigenic epitopes against 3C like protein. B-cell epitopes and Cytotoxic T-lymphocyte (CTL) were designed computationally against SARS-CoV-2. Multiple Sequence Alignment (MSA) of seven complete strains (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2) was performed to elucidate the binding domain and interacting residues. MHC-I binding epitopes were evaluated by analyzing the binding affinity of the top-ranked peptides having HLA molecule. By utilizing the docked complexes of CTL epitopes with antigenic sites, the binding relationship and affinity of top-ranked predicted peptides with the MHC-I HLA protein were investigated. The molecular docking analyses were conducted on the ZINC database library and twelve compounds having least binding energy were scrutinized. In conclusion, twelve CTL epitopes (GTDLEGNFY, TVNVLAWLY, GSVGFNIDY, SEDMLNPNY, LSQTGIAV, VLDMCASLK, LTQDHVDIL, TTDNDFNLV, CTSEDMLNP, TTITVNVLA, YNGSPSGVY, and SMQNCVLKL) were identified against SARS-CoV-2.

1. Introduction

The viral parentage is used to inhibit a range of diseases and even serves as a tool for the study for previously unknown pathogens. Numerous novel viruses possessing cytotoxic properties have been discovered, including those that do not replicate in cell culture and those that induce cytotoxicity (CPE) \[1\]. Coronaviridae viruses are RNA-encapsulated viruses belongs to the
Coronavirus (CoV) family. RNA is of 27 to 32 kb in length [2]. CoV are respiratory viruses found in rats, bats, turkeys, humans, and other animals [3].

On January 19, 2020, the Chinese Center for Disease Surveillance released a novel CoV, published with the consent of the Centers for Disease Control and Prevention [4]. SARS-CoV is the fourth type of coronavirus. SARS-CoV-2 is considered as the third pandemic of this century and was discovered in a significant number of patients at the Seafood market of Hunan, China on December 31st, 2019 [4, 5]. SARS-CoV-2 symptoms include fever, cough, and trouble breathing similar to other coronaviruses including the acute respiratory syndrome coronavirus and the Middle East respiratory coronavirus [3].

11th of January, 2020, several Chinese institutions submitted SARS-CoV-2 sequences to GSAID database. Thousands of people have died as a consequence of SARS-CoV-2 [6]. The signs and symptoms were severe including dry cough, fever, leukopenia, and shortness of breath [7]. The old age patients are less likely to contract SARS-CoV infection and the mortality rate is about 10% [8, 9].

SARS-CoV-2 was first transmitted via direct human contact [10]. Research of human serology found a link between bat coronavirus proteins and SARS-CoV-2. About 89% of the genome was shared with two SARS-CoV-2 variants (C45 and Z21). 75% of the amino acids in the SARS-CoV-2 spike protein are similar to SARS-CoV proteins. Infections with SARS-CoV-2 were found in 7.3% of medical and non-medical workers, implying a 7.0% absolute risk (95% confidence interval for risk difference). The nursing staff was the most infected (WHO) individuals [7].

Our present research aims to utilize immunoinformatics approaches to anticipate and identify the possible B and T cell epitopes for vaccine development against SARS-CoV-2. Furthermore, to identify particular peptides from CoV proteome that may bind to the major histocompatibility complex (MHC), which is one of the most important phases in the vaccine development process.

2. Research methodology

2.1 Sequence retrieval

The amino acid sequence of SARS-CoV-2 protease inhibitor (PDB 6M2N) was retrieved from Protein Data Bank (PDB) [11, 12] and X-ray crystallographic structure of a selected protein (306 residues) was retrieved having resolution of 2.20 Å. ProtParam was used to evaluate the biochemical properties of the target protein [13].

2.2 Alignment of multiple sequences

Seven different genomes from CoV family were retrieved through NCBI [14]. Multiple Sequence Alignment (MSA) was performed by utilizing Clustal Omega [15–17]. MSA was carried out for the whole genomes of selected CoV genomes such as SARS-CoV-2 = NC_045512.2, SARS-CoV = NC_004718.3, MERS-CoV = NC_019843.3, HCoV-NL63 = NC_005831.2, HCoV-229E = NC_002645.1, HCoV-OC43 = NC_006213.1, HCoV-HKU1 = NC_006577.2 [18–21]. WebLogo3 was used to evaluate the conserved residues among the selected genomes and target protein [16, 17].

2.3 Conformational and linear B-cell epitopes prediction

B-lymphocytes are divided into two cell types as a result of interactions between the B-cell epitope and anti-B-lymphocyte as memory cells and plasma antibody secretors.
The surface accessibility and hydrophilic nature of B-cell epitopes are significant for B-cell epitopes [22] by accessing the Immune Epitope Database and Analyses Resource (IEDB), as stated by Parker Hydrophilicity Prediction (PHP), Karplus and Schulz Flexibility Prediction [23], Kolaskar and Tongaonkar antigenicity scale, and Emini surface accessibility prediction [24]. Three different methods were used in conjunction with the Ellipro to predict IEDB analysis resource conformational B-cell epitopes, including nearby clustering of residues based on the protrusion index (PI), approximating the protein shape, and the prediction of IEDB analysis resource conformational B-cell epitopes [24], the criteria of the least possible score of 0.5 and maximum distance of 6 were followed [25].

2.4 Prediction of cytotoxic effects of epitopes on T-Lymphocytes (CTL)

To evaluate CTL epitope predictions, the NetCTL.1.2 server was utilized [4]. The features of NetCTL.1.2 was set as super type A1 including C-terminal cleavage weight 0.15, TAP transport efficiency weight 0.05, and epitope prediction thresholds. The C-terminal cleavage weight was 0.15, the TAP transport efficiency weight was 0.05, and the epitope prediction threshold was 0.75. MHC molecules are antigens, and CTLs are activated by the surface of these molecules. The server was used to combine the antigen processing (TAP) transport efficiency transporter, proteasome C-terminal cleavage, and Class I prediction into one analytics system. FASTA sequences from the selected species were utilized to analyze the human leucocyte antigen (HLA) alleles and the length of the polypeptide sequence. TAP transportation efficiency was assessed by using a weight matrix and T-epitome forecasts, and an artificial neural network was utilized to forecast proteasome C-terminal division and MHC Class-I binding [4].

2.5 Coverage of world population

The IEDB server was utilized to perform worldwide population coverage analyses. Ten different epitopes and the area-country-ethnicity combination were employed for population coverage analyses in the global population inquiry. CTL epitopes were employed against particular allele sets to cover the selected populations. Japan, China, Italy, Iran, and other countries with high COVID-19 mortality rates were selected for global population coverage analyses [26].

2.6 Molecular docking analyses of MHC protein complex peptide

SARS-CoV-2 predicted epitopes were analyzed by utilizing molecular docking analyses for target proteins that include antigens residues. The 3D structures of the proposed peptides were predicted by using PEP-FOLD 3 [27] and 100 simulation runs were conducted in various conformations changes. sOPEP energy ratings were used to assess compliance models clustered with PEP-FOLD3 [28]. The peptides having highest values throughout the screening procedure were subsequently submitted for docking analyses with MHC class I binding molecules by PatchDock [29]. All the unnecessary receptor atoms from all the docked complexes were removed for reliable results and categorized all the remaining complexes by using geometric complementary. FireDock was employed to further refine the selected docked complexes [30]. The docked complexes were set to decrease the number of scoring errors while simultaneously increase the flexibility of the docking experiments [31]. To determine and evaluate the binding affinity and hydrogen bonding interactions of the docked complexes, PyMOL, Discovery Studio, and UCSF Chimera 1.14 were used for interactional analyses [32].
2.7 Molecular docking analyses

For simulated scanning and molecular docking analyses, the library of FDA-approved compounds was selected for virtual screening. ZINC database was used to retrieve the 2122 FDA approved compounds and was minimized by utilizing CHemDraw and UCSF Chimera [33]. A nonstructural coronavirus protein (PDB 6M2N) was retrieved that plays significant role for replication of SARS-CoV-2. PyRx [33], AutoDock, and AutoDock Vina [34] were used to perform molecular docking analyses [34]. Root-mean-square deviation (RMSD) values were used to scrutinize the suitable docked complexes. The drug-like physical and chemical properties were assessed by using admetSAR and ADMETlab. UCSF Chimera and Discovery Studio were used to investigate and visualize the interacting residues [35, 36].

3. Results and discussion

The genome of SARS CoV-2 was organized into 14 ORF (Open Read Frames) encoding 27 proteins and a projected RNA molecule of approximately 29,900 nucleotides with a positive-stranded RNA (Fig 1). The majority of the genome contains ORF1a and ORF1b coding for sixteen distinct non-structural proteins within the replica complex, however few have alternative and essential function models (nsp1-nsp16) [37]. The remaining one-third genome has four ORFs (spike S, envelope E, membrane M, nucleocapsid N) and ten extra proteins (ORF7a, ORF7b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b, ORF9b, ORF9c, ORF10). Some ORFs overlap and also present in larger ORF (Fig 1). At each end of the genome, the 5′ UTR and 3′ UTR (non-coding or unconventional regions) were present. The UTRs are approximately of 230 bases long and play essential regulatory roles [38]. The viruses cause cold in humans including CoVs and CoVs are also responsible for high mortality rate. SARS-CoV and MERS-CoV were identified in animal sources [39]. 31st December 2019, a novel strain of CoV was identified named as SARS-CoV-2. The conclusive reason of pandemic and consequences remain unclear due to the constantly change of environment around the disease [40].

To construct a epitope vaccine, immunoinformatics approaches were used to anticipate the relevant antigen epitopes of the target protein [40]. The goal of current effort was to predict peptide vaccines by using immunoinformatics approaches to recognize CTL epitopes [41]. Immunoinformatics analyses can identify several vaccine candidates with promising preclinical results using computational techniques [42].

CTL epitopes have been discovered to develop a peptide vaccine against HLA-B protein. The epitope-based vaccines were being used to target SARS-CoV-2 structural proteins, and CTL epitopes of the target proteins were anticipated to boost the immune response of host [39]. A non-structural protein (PDB 6M2N) was selected for epitope based vaccine design as it has significant role in the replication of SARS-CoV-2. Vaxijen and Allergen F.P. were used to evaluate the antigenicity and allergenicity of CTL epitope.

In China, population coverage estimations of predicted epitopes showed 16.08 MHC class I coverage with an average hit of 0.48. Numerous epitopes were predicted and top ranked twelve epitopes were selected for further experiments (Tables 1 and 2). The molecular docking analyses were performed against all the selected top ranked 12 peptides to evaluate the effective binding site (Tables S1 and 2).

3.1 Analyses of SARS-CoV-2 surface accessibility

The surface accessibility of the predicted peptides were observed >1.0 dipepts that the selected peptides were located on the surface. The predicted peptides were observed on the basis of y-
axis, and the most likely predicted peptides of SARS-CoV-2 were selected for surface probability (y-axis) and sequence position (x-axis) analyses. The highest score of the predicted peptides were observed 0.911, ranges from 301 to 306 amino acids with the sequence SGVTFQ, while the lowermost value was observed 0.508, ranges from 1 to 5 amino acids with the sequence SGFRK (S1 Fig).

https://doi.org/10.1371/journal.pone.0264700.g001

**Fig 1. Decoding of coronavirus genome.** The CoV genome consists of a 5’ end, a leading sequence, replicase protein, spikes, envelope, membrane, nucleocapsid, and 3’ UTR Poly-A-tail end.
3.2 Surface flexibility of SARS-CoV-2 protein

The atomic vibrational motions in the protein structure determined by B-factor and temperature were calculated and analyzed by using Karplus and Schulz flexibility method. The stability and organization of the structure were determined by the B-factor values [23]. The B-factor values determine the quality of the model however a lower B-factor value suggests a reliable model and higher B-factor value depicts less organized and poorly ordered structures [24]. With the heptapeptide sequences of MESLVPG and SSDVLVN, the lowest and highest flexibility scores were observed as 0.999 and 1.0 respectively [24].

3.3 Prediction of SARS-CoV-2 Parker Hydrophilicity

Parker hydrophilicity scale method was used to evaluate the hydrophilicity of the predicted peptides by using reversed-phase HPLC on a C18 column to estimate the peptide retention durations. The association between antigenic sites and hydrophilic regions was calculated through immunological analyses. The hydrophilicity of each predicted peptide was observed through a hydrophilicity graph, with y-axis measuring hydrophilicity and x-axis showed the residual positions, to assess the hydrophilicity of SARS-CoV-2-predicted peptides (Fig 2). Parker’s hydrophilicity forecast has a maximum hydrophilicity score.
3.4 Antigenicity prediction for SARS-CoV-2 using Kolaskar and Tongaonkar

At locations 85 and 91 of the protein sequence 85 CVLKLKV 91 was determined 1.22 and showed highest antigenicity, while NGMNGRT had 0.84 as lowest antigenicity at positions 274 to 280.

![Fig 2. The probability of a residue surface influenced by accessibility to surfaces, surface flexibility, Parker’s hydrophilicity, and antigenicity predictions of non-structural protein (PDB 6M2N) determined by IEDB Parker hydrophilicity prediction has a maximum score of 5.329, ranging from 92 to 98, with the heptapeptide sequence 92 DTANPKT 48, and a minimum value of -4.257, ranging from 204 to 210, with the VLAWLYA sequence of the peptide.](https://doi.org/10.1371/journal.pone.0264700.g002)
Table 3. Summary of designed peptides MHC class I HLA-B interactions against SARS-CoV-2.

| Sr. No. | Peptide | Global energy (kcal/mol) | Attractive energy (kcal/mol) | H-bond energy (kcal/mol) | Peptidase-MHC pair | Bond distance (Å) | Conserved residues |
|---------|---------|--------------------------|------------------------------|--------------------------|-------------------|------------------|-------------------|
| 1       | GTDLEGNFY | -36.48                  | -24.10                       | -0.24                    | LEU 4 N MET 49.A CE ASP 3 OD2 SER 46.A OG GLU 5 N MET 49.A CG GLU 5 CG LEU 50.A CG GLU 5 O2 LEU 50.A CD1 GLU 5 N MET 49.A CB LEU 4 C MET 49.A CB | 3.145 3.788 4.041 3.642 3.873 3.883 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 2       | TVNVLAWLY | -67.09                  | -28.10                       | -2.77                    | VAL 2 C GLN 189.A O VAL 2 O GLN 189.A CB VAL 4 CG1 GLU 166.A CA VAL 2 CG2 ALA 191.A CA THR 1 CB GLN 192.A O LEU 8 CD2 HIS 164.A O THR 1 OG1 ALA 191.A CA | 3.387 3.659 4.127 4.131 3.672 3.674 3.726 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 3       | GSVGFNIDY | -40.56                  | -23.81                       | -2.75                    | VAL 3 CG1 LEU 141.A C PHE 5 CE1 ASN 142.A ND2 ASP 8 CG ASP 187.A CG VAL 3 CG1 ASN 142.A ND2 VAL 3 CG1 GLU 166.A OE1 ASN 6 CA CYS 44.A O ILE 7 CD ASN 142.A OD1 | 3.695 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 4       | QTFSVLACY | -45.92                  | -31.22                       | -2.17                    | SER 4 CB PRO 293.A CA ALA 7 CA THR 111.A O SER 4 CB PHE 294.A CG TYR 9 CZ SER 158.A OG GLN 1 CD PRO 252.A CD CYS 8 N ASN 151.A OD1 TYR 9 CE1 ASP 153.A O | 4.127 4.127 3.871 3.453 3.882 3.456 3.580 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 5       | DYDCVSFCY | -56.19                  | -27.17                       | -1.73                    | TYR 9 OZ ILE 249.A O PHE 7 CD1 PHE 294.A CZ ASP 3 OD2 SER 158.A N PHE 7 CZ PHE 294.A CE1 CYS 4 SG VAL 104.A CB ASP 1 O SER 158.A OG PHE 7 CA PHE 294.A CD1 | 3.204 3.894 3.441 3.901 4.031 3.264 4.028 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 6       | TANPKTPKY | -43.59                  | -28.20                       | -4.13                    | THR 6 CG2 ILE 249.A CG2 LYS 3 NZ ILE 249.A CD1 PRO 7 CB ASN 151.A ND2 PRO 7 CG PHE 294.A CD1 ALA 2 N VAL 104.A CG1 PRO 4 N GLN 110.A NE2 TYR 9 N PHE 294.A CD2 | 4.105 3.882 3.886 4.011 3.904 3.676 3.800 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |

(Continued)
| Sr. No. | Peptide     | Global energy (kcal/mol) | Attractive energy (kcal/mol) | H-bond energy (kcal/mol) | Peptidase-MHC pair Bond distance (Å) | Conserved residues |
|--------|-------------|--------------------------|------------------------------|--------------------------|--------------------------------------|--------------------|
| 7      | SEDMLNPY    | -34.88                   | -25.96                       | -2.40                    | 3.978 3.600 3.577 3.417 4.120 3.871 3.698 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 8      | GTDTITV     | -27.73                   | -23.12                       | -2.60                    | 4.013 4.015 3.726 4.147 3.727 3.690 3.885 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 9      | LSAQTGIAV   | -39.43                   | -18.89                       | -3.80                    | 3.675 3.677 3.598 3.906 4.147 3.230 3.644 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 10     | VLDMCASLK   | -49.11                   | -23.03                       | -0.91                    | 3.755 4.124 3.867 3.441 3.873 3.913 3.884 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 11     | GTTTLNGLW   | -44.31                   | -20.50                       | -0.64                    | 4.112 4.112 3.670 3.631 3.688 3.614 3.888 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 12     | TTTLNLWL    | -57.54                   | -26.76                       | -1.84                    | 3.655 4.079 3.979 3.450 3.725 4.150 4.153 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |

(Continued)
| Sr. No. | Peptide     | Global energy (kcal/mol) | Attractive energy (kcal/mol) | H-bond energy (kcal/mol) | Peptidase-MHC pair Bond distance (Å) | Conserved residues |
|---------|-------------|--------------------------|------------------------------|--------------------------|---------------------------------------|-------------------|
| 13      | FTTTLNDFN   | -43.52                   | -23.56                       | -1.63                    | 3.648 3.323 3.405 3.978 4.117 3.861 4.154 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 14      | QTGIAVLDM   | -44.24                   | -20.49                       | -2.81                    | 3.655 4.093 4.102 4.118 3.653 3.896 3.912 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 15      | ILEDEFTPF   | -41.71                   | -23.91                       | -2.83                    | 4.127 3.670 3.866 3.219 4.145 3.730 4.153 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 16      | HVDILGPLS   | -40.51                   | -16.69                       | -1.31                    | 4.052 3.939 3.855 3.996 4.122 4.129 3.911 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 17      | LTQDHVIDL   | -40.26                   | -25.73                       | -1.13                    | 3.697 3.610 4.128 3.893 3.405 3.692 3.809 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 18      | TTLNDFNLV   | -54.90                   | -25.46                       | -1.23                    | 3.880 3.662 3.431 4.016 3.220 3.871 3.872 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |

(Continued)
| Sr. No. | Peptide       | Global energy (kcal/mol) | Attractive energy (kcal/mol) | H-bond energy (kcal/mol) | Peptidase-MHC pair                                                                 | Bond distance (Å)                   | Conserved residues |
|--------|---------------|--------------------------|------------------------------|--------------------------|----------------------------------------------------------------------------------|-------------------------------------|-------------------|
| 19     | FVDRQAQA      | -43.60                   | -24.43                       | -4.09                    | THR 6 N GLN 110.A OE1 GLN 5 CA GLN 110.A CD ALA 9 O2 HIS 246.A NE2 ALA 9 C PRO 108.A O ALA 9 CB GLY 109.A N GLN 5 CB GLN 110.A CB GLN 5 NE2 PHE 294.A CD1 | 3.424 3.855 3.432 3.403 3.907 4.149 3.798 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 20     | NLVAMKNY      | -42.71                   | -21.84                       | -3.68                    | TYR 9 OH HIS 246.A CD2 TYR 9 CA ASP 245.A CG TYR 9 CE2 ASP 245.A CA TYR 9 O2 ILE 249.A CB TYR 9 CA ASP 248.A CB LYS 6 O ASP 245.A OD2 TYR 9 CB ILE 249.A CG1 | 3.520 4.063 3.952 3.644 4.106 3.190 4.138 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 21     | CTSEDMLNP     | -43.85                   | -32.50                       | -6.39                    | THR 2 CA GLU 166.A O THR 2 CA GLU 166.A CB ASN 8 ND2 HIS 41.A CG GLU 4 CD MET 165.A CA LEU 7 CD2 THR 26.A O CYS 1 CA MET 165.A CB MET 6 CG ASN 142.A CG | 3.667 4.134 3.625 4.136 3.690 4.154 3.887 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 22     | TTITVNLA      | -42.29                   | -20.11                       | -2.27                    | ALA 9 O2 ASN 142.A CG ALA 9 O1 ASN 142.A ND2 VAL 5 CG2 SER 46.A CA ALA 9 O2 ASN 142.A CA VAL 5 CB SER 46.A OG ALA 9 CB ASN 142.A CG VAL 5 CG1 MET 49.A CG | 3.026 3.088 3.807 3.558 3.630 3.814 4.130 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 23     | YNGPSGVY      | -57.72                   | -25.02                       | -2.35                    | SER 6 C GLU 166.A O TYR 1 OH MET 49.A CB GLY 7 N GLU 166.A CG TYR 1 OH MET 49.A CA GLY 7 CA GLU 166.A N ASN 2 CB ASN 142.A CG SER 6 C GLU 166.A OE1 | 3.380 3.698 3.879 3.703 3.884 3.868 3.426 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |
| 24     | VSFCYMHMM     | -54.36                   | -30.86                       | -4.07                    | SER 2 OG VAL 202.A CG1 HIS 8 CB GLN 110.A CD SER 2 OG ILE 249.A CG2 TYR 5 CB GLN 110.A NE2 TYR 5 OH PHE 294.A CD1 PHE 3 CZ ASP 245.A CG VAL 1 C HIS 246.A CD2 | 3.721 3.871 3.722 3.903 3.604 4.031 3.764 | TYR9 LEU5 ILE7 HIS41 MET49 ASN142 HIS164 ARG188 GLN189 |

(Continued)
3.5 SARS-CoV-2 structure-based epitope prediction

Antigenicity, epitope prediction, accessibility, and flexibility in the 3D structure were also evaluated to overcome the errors [43]. The protein-antibody interactions were also investigated for all the selected epitopes and top ranked 3 SARS-CoV-2 conformational epitopes having >0.7 score were further evaluated. The proportion of atoms across the molecular substance and the antibody binding was determined for the target protein by using pI (isoelectric point value) score [44] and 5.95 score after titration was observed. The score range between 0.685 and 0.714 was observed along with names, lengths, and positions of the residues, as well as the scores of the three-member conformational epitope prediction panel.

3.6 Molecular docking analyses

Top ranked 25 CTL epitopes were identified and comparative molecular docking analyses were conducted. The binding affinity and global energy ranges from -23.45 to -32.5 kcal/mol and -27.73 to -67.09 kcal/mol respectively were observed against all the selected CTL epitopes (Table 3). Interestingly, it was observed that all the 25 CTL epitopes were docked at similar binding residues. Top ranked eight docked complexes were evaluated (Fig 3), and similar residues (TYR9, LEU5, ILE7, HIS41, MET49, ASN142, HIS164, ARG188, and GLN189) were observed.

3.7 Population coverage analyses

Epitopes linked with particular HLA alleles were evaluated to scrutinize MHC class I and MHC class II epitopes. MHC class I and MHC class II epitopes were found to be related to 58.73% and 18.06% of the world’s population, respectively. MHC class I epitope coverage was observed higher in the Italian and Chinese populations as 100% (S1 File).

3.8 Multiple Sequence Alignment (MSA)

The genomes of seven coronaviruses were retrieved for MSA alignment to elucidate the conserved region among all. It was observed that the binding domain has conserved region in all of the selected coronavirus strains (S2 and S3 Files).

3.9 Virtual screening and comparative docking

The selected peptides have a substantial anti-SARS-CoV-2 value as per followed methodology [4]. Virtual screening was performed against non structural protein of SARS-CoV-2 (PDB 6M2N) and FDA approved library from ZINC database was utilized for virtual screening purpose. Comparative molecular docking anayses were performed against the complete FDA approved library. All the docked complexes were evaluated on the basis of least binding energy,
Fig 3. A: Peptide-MHC class I HLA-B binding interacting residues of four top-ranked peptides and their sequences presented in different colors.

B: Peptide-MHC class I HLA-B binding interacting residues of eight top-ranked peptides and their sequences presented in different colors. Conserved residues are already shown in Table 3.

https://doi.org/10.1371/journal.pone.0264700.g003
contacts, pharmacological characteristics and efficient binding affinity and top ranked 100 compounds were further evaluated. All the docked complexes bound at the similar binding residues and top ranked twelve compounds (ZINC3816514, ZINC3932831, ZINC72318121, ZINC43207238, ZINC1548097, ZINC3995811, ZINC3830405, ZINC43207238, ZINC100016058, ZINC3920266, ZINC16052277 and ZINC84441937) (Table 4) showed the most promising

| Sr No. | Ligands   | Binding Affinity (Kcal/mol) | RMSD Value | Molecular Weight (g/mol) | H-bond acceptor | H-Bond Donor | Rotable Bond | Interacting Residues | Density | A logp Value | Acute oral toxicity(Kg/mol) | Pfizer | Lipinski Rule | CSK  |
|-------|-----------|-----------------------------|------------|--------------------------|----------------|-------------|--------------|----------------------|---------|---------------|-----------------------------|---------|--------------|------|
| 1     | ZINC3816514 | -8.9                        | 1.738      | 500.48                   | 3              | 2           | 5            | HIS41 CYS44 MET49 TYR54 CY5145 GLU166 ASP187 ARG188 GLN192 | 1.077    | 5.73          | 3.288                      | NO      | Yes          | NO  |
| 2     | ZINC3932831 | -8.3                        | 0.487      | 528.54                   | 2              | 2           | 2            | THR24 THR25 HIS41 ASN142 GLY143 CYS145 MET165 THR190 ALA191 | 1.072    | 6.58          | 3.743                      | NO      | NO           | NO  |
| 3     | ZINC72318121 | -7.9                      | 1.836      | 506.61                   | 8              | 1           | 7            | HIS41 CYS44 MET49 PRO52 GLN189 | 1       | 4.94          | 2.927                      | Yes     | Yes          | No  |
| 4     | ZINC43207238 | -7.8                      | 0.912      | 444.52                   | 6              | 4           | 5            | HIS41 CYS44 MET49 ASN142 MET165 PRO168 TH190 ALA191 GLN192 | 1.017    | 2.97          | 2.901                      | Yes     | Yes          | Yes |
| 5     | ZINC1548097 | -7.7                        | 1.784      | 426.49                   | 6              | 0           | 8            | HIS41 MET49 MET165 PRO168 GLN192 | 0.988    | 4.83          | 2.492                      | No      | Yes          | Rejected No |
| 6     | ZINC3995811 | -7.5                        | 0.575      | 606.78                   | 3              | 2           | 14           | MET165 PRO168 THR190 GLN192 | 1.019    | 8.68          | 2.964                      | No      | No           | Rejected No |
| 7     | ZINC3830405 | -7.5                        | 1.591      | 454.52                   | 12             | 2           | 7            | MET49 TYR54 GLU166 LEU167 PRO168 | 1.214    | -0.64         | 1.589                      | No      | Yes          | Rejected No |
| 8     | ZINC3816292 | -7.5                        | 1.781      | 426.86                   | 5              | 3           | 6            | THR25 HIS41 SER46 MET49 MET165 ARG188 | 1.051    | 4.07          | 2.287                      | Yes     | Yes          | Rejected No |
| 9     | ZINC100016058 | -7.4                    | 1.02       | 602.68                   | 6              | 2           | 11           | HIS41 CYS44 MET49 ASN142 MET165 GLU166 ARG188 GLN192 | 1.031    | 7.33          | 3.286                      | No      | Yes          | Rejected No |
| 10    | ZINC3920266 | -7.3                        | 1.371      | 497.50                   | 10             | 5           | 3            | HIS41 MET49 TYR54 ASN142 MET165 GLU166 ASP187 | 1.032    | 1.02          | 3.704                      | Yes     | Yes          | Rejected No |
| 11    | ZINC16052277 | -7.2                       | 1.345      | 444.44                   | 9              | 7           | 2            | HIS41 CYS44 MET49 CYS145 HIS164 MET165 GLU166 | 1.042    | 0.70          | 2.191                      | Yes     | Yes          | Rejected No |
| 12    | ZINC84441937 | -7.1                       | 1.568      | 444.44                   | 9              | 6           | 2            | HIS41 MET49 TYR54 ASN142 HIS164 MET165 ASP187 | 1.042    | -0.21         | 2.537                      | Yes     | Yes          | Rejected No |

https://doi.org/10.1371/journal.pone.0264700.t004
results among all (Figs 4 and 5). All the docked complexes showed interactions at similar site and HIS41, CYS44, MET49, ASN142, MET165, GLU166, ARG188 and GLN192 residues were conserved (S2 and S3 Tables).
The selected protein plays a significant role in the replication of SARS-CoV-2. The scrutinized compounds have the ability to inhibit the target protein based on extensive \textit{in silico} analyses. The scrutinized compounds followed the lipinsky’s rule of five and better oral bioavailability. The scrutinized compounds showed the solubility in water at 25°C. The binding sites and maximum binding affinity of all selected compounds have promising results (S2 and S3 Tables).

There is an urgent need of an effective cure for coronaviruses. SARS-CoV-2 pandemic became a medical emergency in all over the globe [45]. Vaccine development is of significant interest to peptide inhibitors [46]. The peptide targets include lower toxicity, lower side effects and faster action than traditional medicinal products based on ligands. Immunoinformatics methodologies help scientists to minimise the laboratory load, less expedient and cost-effective [47]. There have been major advances in \textit{in silico} drug design over the last decade [48–51]. A large number of biological difficulties were tackled by the use of different bioinformatics approaches [52–54]. The epitopes of non structural protein (PDB 6M2N) were designed and CTL epitopes were also predicted against SARS-CoV-2. The binding affinities for the predicted peptides for MHC-I were further evaluated through comparative molecular docking analyses. Eight peptides showed effective MHC-I (HLA-B) interactions. Based on the global energy value, twelve peptides have been selected with the greatest antigenicity and binding affinities (S2 Table).

4. Conclusion

The goal of current effort was to elucidate the efficient peptide based inhibitors against SARS-CoV-2 non-structural protein. The predicted epitopes were designed followed by comparative molecular docking studies against MHC-I. Moreover, the interactional studies of the scrutinized docked complexes were analyzed. In conclusion, 12 epitopes (GTDLEGNFY,
TVNLAWLY, GSVGNIDY, SEDMLNPNY, LSAQTGIAV, VLDMCASLK, LTQDHVDIL, TTLNDFNLV, CTSEDMLNP, TTITTVNLVA, YNGSPSGVY, and SMQNCVLKL), were predicted having potential targets as peptide vaccine against SARS-CoV-2.

Supporting information

S1 Fig. The probability of a residue surface influenced by accessibility to surfaces. (DOCX)

S1 Table. Predicted CTL epitopes and predicted amino acid residues from the SARS-CoV-2, Table 2. Top-ranked selected discontinuous epitopes, interacting residues, and scores predicted discontinuous epitopes. (DOCX)

S2 Table. Fig 5. Drug like properties and comparative molecular docking analyses of selected compounds scrutinize through virtual screening. (XLSX)

S3 Table. Drug like properties and comparative molecular docking analyses of selected compounds scrutinize through virtual screening. (CSV)

S1 File. Population coverage. (PDF)

S2 File. Multiple sequence alignment. (DOCX)

S3 File. Phylogenetic tree. (PDF)

Author Contributions

Conceptualization: Hamid Mukhtar, Muhammad Sajid, Sheikh Arslan Sehgal.

Data curation: Hamid Mukhtar.

Formal analysis: Muhammad Sajid, Saigha Marriam, Summar Sohail.

Methodology: Muhammad Sajid, Summar Sohail, Sheikh Arslan Sehgal.

Project administration: Sheikh Arslan Sehgal.

Software: Muhammad Sajid, Muhammad Sajid, Sheikh Arslan Sehgal.

Supervision: Sheikh Arslan Sehgal.

Validation: Muhammad Sajid, Muhammad Sajid, Sheikh Arslan Sehgal.

Visualization: Muhammad Sajid, Saigha Marriam, Muhammad Sajid, Sheikh Arslan Sehgal.

Writing – original draft: Muhammad Sajid, Saigha Marriam, Muhammad Sajid, Sheikh Arslan Sehgal.

Writing – review & editing: Muhammad Sajid, Saigha Marriam, Hamid Mukhtar, Summar Sohail, Muhammad Sajid, Sheikh Arslan Sehgal.

References

1. Stohlman SA, Hinton DR, et al. Viral induced demyelination. Brain pathology. 2001; 11(1):92–106. https://doi.org/10.1111/j.1750-3639.2001.tb00384.x PMID: 11145206
2. Guy JS, Breslin JJ, Breuhaus B, Vivrette S, Smith LG. et al. Characterization of a coronavirus isolated from a diarrheic foal. Journal of Clinical Microbiology. 2000; 38(12):4523–6. https://doi.org/10.1128/JCM.38.12.4523-4526.2000 PMID: 11101590

3. Riva L, Yuan S, Yin X, Martín-Sancho L, Matsunaga N, Pache L. et al. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. Nature. 2020; 586(7827):113–9. https://doi.org/10.1038/s41586-020-2577-1 PMID: 3270573

4. Waqas M, Haider A, Rehman A, Qasim M, Umar A, Sufyan M. et al. Immunoinformatics and Molecular Docking Studies Predicted Potential Multiprotein-Based Vaccine Candidate and Novel Compounds against Novel SARS-CoV-2 through Virtual Screening. BioMed research international. 2021;2021. https://doi.org/10.1155/2021/1596834 PMID: 33728324

5. Ji W, Wang W, Zhao X, Zai J, Li X. et al. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. Journal of medical virology. 2020.

6. Tahir RA, Mughal S, Nazir A, Sufyan M, Siraj S, Sehgal SA. et al. Determine the potential epitope based peptide vaccine against novel SARS-CoV-2 targeting structural proteins using immunoinformatics approaches. Combinatorial Chemistry & High Throughput Screening. 2021.

7. Wu P, Hao X, Lau EH, Wong JY, Wu JT, et al. Real-time tentative assessment of the epidemiological characteristics of novel coronavirus infections in Wuhan, China, as at 22 January 2020. Eurosurveillance. 2020; 25(3):2000044. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000044 PMID: 31992388

8. Robertson D. et al. nCoV’s relationship to bat coronaviruses & recombination signals (no snakes)-no evidence the 2019-nCoV lineage is recombinant. Virological org. 2020.

9. Tahir RA, Mughal S, Nazir A, Sufyan M, Siraj S, Sehgal SA. et al. Determine the potential epitope based peptide vaccine against novel SARS-CoV-2 targeting structural proteins using immunoinformatics approaches. Combinatorial chemistry & high throughput screening. 2021.

10. Waqas M, Rehman A, Sufyan M, Siraj S, Sehgal SA. et al. Determine the potential epitope based peptide vaccine against novel SARS-CoV-2 targeting structural proteins using immunoinformatics approaches. Combinatorial chemistry & high throughput screening. 2021.

11. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Di Costanzo L, et al. et al. RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic acids research. 2019; 47(D1):D464–D74. https://doi.org/10.1093/nar/gky1004 PMID: 30357411

12. Noor A JF, Noor F. et al. Possible strategies and treatments of SARS-CoV-2 outbreak. Biomedical Letters 2020; 6 (2):112–7.

13. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. et al. et al. Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook. 2005:S71–67.

14. Sherry ST, Ward M-H, Kholodenko M, Bi C, Chen L, Di Costanzo L, et al. et al. dbSNP: the NCBI database of genetic variation. Nucleic acids research. 2001; 29(1):308–11. https://doi.org/10.1093/nar/29.1.308 PMID: 11125122

15. Sajid M SM, Murtaza A, Mukhtar H, Sohail S, Shahid A, Sabir I. et al. Adaptive evolutionary analyses of the COVID-19 pandemic in twelve most affected countries. Biomedical Letters 2021; 7(1):72–9.

16. Sievers F, Higgins DG. et al. Clustal omega. Current protocols in bioinformatics. 2014; 48(1):3.13. 1–3.

17. Sievers F, Higgins DG. et al. Clustal Omega for making accurate alignments of many protein sequences. Protein Science. 2018; 27(1):135–45. https://doi.org/10.1002/pro.3290 PMID: 28884485

18. Desforges M, Le Coupance A, Stodola JK, Meessen-Pinard M, Talbot PJ. et al. Human coronaviruses: viral and cellular factors involved in neuroinvasiveness and neuropathogenesis. Virus research. 2014; 194:145–58. https://doi.org/10.1016/j.virusres.2014.09.011 PMID: 25281913

19. Dijkman R, Van Der Hoek L. et al. Human coronaviruses 229E and NL63: close yet still so far. Journal of the Formosan Medical Association. 2009; 108(4):270–9. https://doi.org/10.1016/S0929-6646(09)60066-8 PMID: 19369173

20. Lim YX, Ng YL, Tam JP, Liu DX. et al. Human coronaviruses: a review of virus–host interactions. Diseases. 2016; 4(3):26. https://doi.org/10.3390/diseases4030026 PMID: 28933406

21. Van Den Brand JM, Smits SL, Haagmans BL. et al. Pathogenesis of Middle East respiratory syndrome coronavirus. The Journal of pathology. 2015; 235(2):175–84. https://doi.org/10.1002/path.4458 PMID: 25294366

22. Nair DT, Singh K, Siddiqui Z, Nayak BP, Rao KV, Salunke DM. et al. Epitope recognition by diverse antibodies suggests conformational convergence in an antibody response. The Journal of Immunology. 2002; 168(5):2371–82. https://doi.org/10.4049/jimmunol.168.5.2371 PMID: 11859128
23. Parker J, Guo D, Hodges R, et al. New hydrophilicity scale derived from high-performance liquid chromatography peptide data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry. 1986; 25(19):5425–32. https://doi.org/10.1021/bi00367a013 PMID: 2430611

24. Wang Y, Wang G, Zhang D, Yin H, Wang M, et al. Identification of novel B cell epitopes within Toxoplasma gondii GRA1. Experimental parasitology. 2013; 135(3):606–10. https://doi.org/10.1016/j.exppara.2013.09.019 PMID: 24090568

25. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004; 25(13):1605–12. https://doi.org/10.1002/jcc.20084 PMID: 15264254

26. Vita R, Mahajan S, Onerton JA, Dhanda SK, Martin S, Cantrell JR, et al. The immune epitope database (IEDB): 2018 update. Nucleic acids research. 2019; 47(D1):D339–D43. https://doi.org/10.1093/nar/gky1006 PMID: 30357391

27. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, Tuffery P, et al. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. Nucleic acids research. 2016; 44(W1):W449–W54. https://doi.org/10.1093/nar/gkw329 PMID: 27131374

28. Maupetit J, Tuffery P, Derreumaux P, et al. A coarse-grained protein force field for folding and structure prediction. Proteins: Structure, Function, and Bioinformatics. 2007; 69(2):394–408. https://doi.org/10.1002/prot.21505 PMID: 17600832

29. Mashiach E, Schniedman-Duhovny D, Andrusier N, Nussinov R, Wolfson HJ, et al. FireDock: a web server for fast interaction refinement in molecular docking. Nucleic acids research. 2008; 36(suppl_2):W229–W32. https://doi.org/10.1093/nar/gkn186 PMID: 18424796

30. Andrusier N, Nussinov R, Wolfson HJ, et al. FireDock: fast interaction refinement in molecular docking. Proteins: Structure, Function, and Bioinformatics. 2007; 69(1):139–59. https://doi.org/10.1002/prot.21495 PMID: 17598144

31. Kingsford CL, Chazelle B, Singh M, et al. Solving and analyzing side-chain positioning problems using linear and integer programming. Bioinformatics. 2005; 21(7):1028–39. https://doi.org/10.1093/bioinformatics/bti144 PMID: 15546935

32. Hornbeck PV, Kornhauser JM, Tkachev S, Zhang B, Skrzypek E, Murray B, et al. PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. Nucleic acids research. 2012; 40(D1):D261–D70. https://doi.org/10.1093/nar/gkr1122 PMID: 22135298

33. Vilar S, Cozza G, Moro S, et al. Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. Current topics in medicinal chemistry. 2008; 8(18):1555–69. https://doi.org/10.1080/156802608786786624 PMID: 19075767

34. Daliaikyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Chemical biology: Drug discovery. 2005; 8(15):1555–69. https://doi.org/10.1016/j.cub.2005.03.017 PMID: 16802908786786624

35. Ayati A, Falahati M, Irannejad H, Emami S, et al. Synthesis, in vitro antifungal evaluation and in silico study of 3-azolyl-4-chromanone phenylhydrazones. DARU Journal of Pharmaceutical Sciences. 2012; 20(1):1–7. https://doi.org/10.1186/1560-8115-20-1 PMID: 23226110

36. Wallace AC, Laskowski RA, Thornton JM, et al. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein engineering, design and selection. 2020; 8(2):127–34. https://doi.org/10.1093/biometas/abz020-P003-8 PMID: 32015508

37. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579(7798):265–9. https://doi.org/10.1038/s41586-020-2420-w PMID: 32015508

38. Yang D, Leibowitz JL, et al. The structure and functions of coronavirus genomic 3' and 5' ends. Virus research. 2015; 206:120–33. https://doi.org/10.1016/j.virusres.2015.02.025 PMID: 25736566

39. Ji W, Wang W, Zhao X, Zai J, Li X, et al. Cross-species transmission of the newly identified coronavirus 2019-nCoV. J Med Virol. 2020; 92(4):433–40. https://doi.org/10.1002/jmv.25682 PMID: 31967321

40. De Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ, et al. Host factors in coronavirus replication. Roles of host gene and non-coding RNA expression in virus infection. 2017; 1–42. https://doi.org/10.1093/nar/gkw1006 PMID: 30357391

41. Irwin JJ, Shoichet BK, et al. ZINC—A free database of commercially available compounds for virtual screening. Journal of chemical information and modeling. 2005; 45(1):177–82. https://doi.org/10.1021/ci049714+ PMID: 15667143

42. Sehgal SA, Mirza AH, Tahir RA, Mir A. Quick guideline for computational drug design: Bentham Science Publishers; 2018.

43. Martina BE, Haagmans BL, Kuiken T, Fouchier RA, Rimmelzaan GF, Van Amerongen G, et al. SARS virus infection of cats and ferrets. Nature. 2003; 425(6961):915. https://doi.org/10.1038/425915a PMID: 14586458
44. Ponomarenko J, Bui H-H, Li W, Fusseder N, Bourne PE, Sette A. et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC bioinformatics. 2008; 9(1):1–8. https://doi.org/10.1186/1471-2105-9-514 PMID: 19055730

45. McClain CS. et al. A new look at an old disease: smallpox and biotechnology. Perspectives in biology and medicine. 1995; 38(4):624–39. https://doi.org/10.1353/pbm.1995.0000 PMID: 7659492

46. Chew M-F, Poh K-S, Poh C-L. et al. Peptides as therapeutic agents for dengue virus. International journal of medical sciences. 2017; 14(13):1342. https://doi.org/10.7150/ijms.21875 PMID: 29200948

47. Mirza MU, Rafique S, Ali A, Munir M, Ikram N, Manan A. et al. Towards peptide vaccines against Zika virus: Immunoinformatics combined with molecular dynamics simulations to predict antigenic epitopes of Zika viral proteins. Scientific reports. 2016; 6(1):1–17. https://doi.org/10.1038/s41598-016-0001-8 PMID: 28442746

48. Cavasotto CN, Di Filippo JI. et al. In Silico drug repurposing for COVID-19: Targeting SARS-CoV-2 proteins through docking and consensus ranking. Molecular informatics. 2021; 40(1):2000115. https://doi.org/10.1002/minf.202000115 PMID: 32722864

49. Chandra A, Gurjar V, Ahmed MZ, Alqahtani AS, Qamar I, Singh N. et al. Exploring potential inhibitor of SARS-CoV2 replacem ent from FDA approved drugs using insilico drug discovery methods. Journal of Biomolecular Structure and Dynamics. 2021;1:1–8. https://doi.org/10.1080/07391102.2020.1871416 PMID: 33491573

50. Ibrahim MA, Abdelrahman AH, Hegazy M-EF. et al. In-silico drug repurposing and molecular dynamics puzzled out potential SARS-CoV-2 main protease inhibitors. Journal of biomolecular Structure and Dynamics. 2021; 39(15):5756–67. https://doi.org/10.1080/07391102.2020.1791958 PMID: 32684114

51. Sharma S, Bhatia V. et al. Drug Design of GLP-1 Receptor Agonists: Importance of In Silico Methods. Current Pharmaceutical Design. 2021; 27(8):1015–24. https://doi.org/10.2174/1381612826666201118094502 PMID: 33213316

52. Krivov GG, Shapovalov MV, Dunbrack RL Jr. et al. Improved prediction of protein side-chain conformations with SCWRL4. Proteins: Structure, Function, and Bioinformatics. 2009; 77(4):778–95. https://doi.org/10.1002/prot.22488 PMID: 19603484

53. Lefèvre F, Rémy M-H, Masson J-M. et al. Alanine-stretch scanning mutagenesis: a simple and efficient method to probe protein structure and function. Nucleic acids research. 1997; 25(2):447–8. https://doi.org/10.1093/nar/25.2.447 PMID: 9016577

54. Liu W, Zhu H-M, Niu G-J, Shi E-Z, Chen J, Sun B, et al. et al. Synthesis, modification and docking studies of 5-sulfon yl isatin derivatives as SARS-CoV 3C-like protease inhibitors. Bioorganic & medicinal chemistry. 2014; 22(1):292–302.