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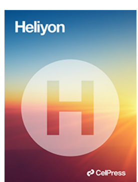
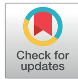


Research article

Variations of SARS-CoV-2 in the Iranian population and candidate putative drug-like compounds to inhibit the mutated proteins



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A R T I C L E I N F O

Keywords:
SARS-CoV-2
COVID-19
Nonstructural proteins (NSPs)
Open reading frame (ORF)
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Candidate putative drug-like compounds

A B S T R A C T

The first cases of the novel coronavirus, SARS-CoV-2, were detected in December 2019 in Wuhan, China. Nucleotide substitutions and mutations in the SARS-CoV-2 sequence can result in the evolution of the virus and its rapid spread across the world. Therefore, understanding genetic variants of SARS-CoV-2 and targeting the conserved elements responsible for viral replication have great benefits for detecting its infection sources and diagnosing and treating COVID-19. In this study, we used the SARS-CoV-2 sequence isolated from a 59-year-old man in Ardabil, Iran, in April 2020 and sequenced using Oxford Nanopore technology. A meta-analysis comparing the sequence under study with other sequences from Iran indicated long nucleotide insertions/deletions (indels) that code for NSP15, the NSP14-NSP10 complex, open reading frame ORF9b, and ORF1ab polyproteins. In addition, replicating the NSP8 protein in the study sequence is another topic that can affect viral replication. Then using the DNA structure of NSP8, NSP15, NSP14-NSP10 complex, and ORF1ab as a genetic target can help find drug-like compounds for COVID-19. Potential drug-like compounds reported in this study for their mechanism of action and interactions with SARS-CoV-2 genes using drug repurposing are resveratrol, erythromycin, chloramphenicol, indomethacin, ciclesonide, and PDE4 inhibitor. Ciclesonide appears to show the best results when docked with chosen viral proteins. Therefore, different proteins isolated from nucleotide mutations in the virus sequence can indicate distinct inducers for antibodies and are important in vaccine design.

1. Introduction

COVID-19 is a disease caused by the new coronavirus, SARS-CoV-2, which emerged in Wuhan, Hubei Province, China. It is now distributed worldwide, proving the need for biological research to discover such kinds of viruses in human life (Benvenuto et al., 2020). Fatigue, fever, and dry cough are the main symptoms of COVID-19. According to the World Health Organization (WHO), COVID-19 is classified as an infectious disease that has spread to more than 200 countries worldwide, and the number of cases is still increasing (Xu et al., 2020). Important challenges about COVID-19 diagnosis have been covered in one recent study reviewing molecular and antigen tests (Dinnes et al., 2021).

Infection in the human respiratory tract is the main effect of SARS-CoV-2, which invades host cells through receptors on the cell surface; namely, the S protein on the coronavirus surface binds to the receptor, and then the virus goes through clathrin-mediated endocytosis to invade the host cell. Viral genetic studies can help in virus early detection and the development of better antiviral drugs optimized to be effective against emerging viral mutants using drug repurposing. For example, the viral evolution of SARS-CoV-2 in nature and its rapid spread may result from nucleotide substitution and mutations (Chatterjee et al., 2020). Therefore, understanding genetic variations in SARS-CoV-2 can help win the battle against COVID-19 (Ceraolo and Giorgi, 2020).

Different studies have reported SARS-CoV-2 sequences in different countries. For example, previous studies reported SARS-CoV-2 sequences in India, Nepal, and China (Yadav et al., 2020; Wang et al., 2020; Sah et al., 2020). SARS-CoV-2 genomes have been studied in 56 countries (Safari et al., 2020), and 1969 nucleotide variants were identified to assess virus evolution. Moreover, the full genome of SARS-CoV-2 has been analyzed, and 44 nucleotide mutations with 26 protein sequence mutations were identified from 20 patients in Iran (Salehi et al., 2020). Nucleotide mutations can be used for diagnosis to reduce errors in qPCR-based diagnosis tests. The key stage of the SARS-CoV-2 infection cycle is the replication of its viral genome inside the infected cell. The

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transcription of structural genes in viral replication is catalyzed by an RNA-dependent RNA polymerase complex or viral replication complex (Romano et al., 2020). The development of therapeutic drugs or preventive vaccines for COVID-19 is a research process called COVID-19 drug development. Greater attention must be paid to the replicase proteins to identify new therapeutic targets for the replication machinery of SARS-CoV-2.

The slow process of drug discovery and substantial costs in COVID-19 research motivated scientists to investigate and repurpose existing drugs. This process is called drug repurposing or drug repositioning. Identifying genes involved in diseases and their interactions with other genes or known drugs is one approach to identifying repurposing candidates because it is likely that drugs interacting with the human genome are more effective than others. Different drug dosages are required to treat novel diseases using drug repurposing compared to the original target disease (Wishart et al., 2018; Yoo et al., 2015). This study aims to detect genetic mutations in SARS-CoV-2 sequences and their protein structures in an Iranian population that can be utilized for further studies to provide new vaccines and therapeutic options.

Recently, the outbreak of COVID-19 creates challenges to select an appropriate treatment option considering little time for drug discovery. For that reason, as a rapid and low-cost approach that can directly enter clinical trials, drug repurposing has been used as an effective strategy for finding therapeutic agents. Because of insufficient data to propose therapy for COVID-19, some studies concentrated on drug-repurposing and identified potential pharmacological agents for SARS-CoV-2 and proposed drugs like Remdesivir, Favipiravir, Ribarvirin, Ebselen, etc. that can be tested at the clinical level (Singh et al., 2020; Kifle et al., 2021). However, there are just a few pieces of research concerning drug repurposing for COVID-19. This is because the methods are based on the knowledge of SARS-CoV-2, which only started less than a decade ago. Therefore, it is necessary to grow indispensable knowledge and develop research in the field that can be applied to biomedicine (Dotolo et al., 2021). As an example of drug repurposing analysis using the result of genetic analysis, a study by Mortezaei and Khosravi (2021) have detected genetic variants in association with cancer, and then drug-repurposing analysis has been used to detect some drug-like compounds that can be used potentially for cancer targeted therapies.

The results of drug repurposing can be validated using molecular docking. Docking determines the best binding modes of a ligand molecule to a defined protein structure and the affinities of the respective. However, the widely used Autodock Vina algorithm is not error free, and false positives and false-negatives may arise in the results. So, we used another, independent algorithm, implemented in LeDock package to verify the docking results. The Autodock algorithm has been enhanced in precision and accuracy and packaged as Autodock Vina. modes. Autodock is the most widely used docking program. Subsequently, qVina accelerated the speed of the Vina algorithm. In a recent study, it was found that qVina outperforms Vina in docking. Hence, we used qVina in the current study. In the recent study, LeDock has been found to have still slightly higher accuracy than qVina, and hence was used here to validate qVina results (Cinaroglu and Timucin, 2019).

2. Methods

2.1. Protein detection

Detection of SARS-CoV-2 nucleic acid is possible using quantitative reverse transcriptase-polymerase chain reaction (PCR) after isolation of the virus and its genomic sequence. In this study, throat swabs were collected from a 59-year-old COVID-19 male patient in a hospital in Tehran, Iran, in April 2020 who was positive for the SARS-CoV-2 virus. Genomic RNA was extracted and confirmed, and sequenced using Oxford Nanopore technology and Sanger dideoxy sequencing at Kawsar Biotech Company, Tehran, Iran. Sequence coverage or total sequence of nucleotides used to create the complete consensus paired-end sequence of the SARS-CoV-2 genome in this study is 900. The sequence under study for SARS-CoV-2 (Supplementary 1: SARS-CoV-2 DNA sequence) was initially compared with submitted sequences in the NCBI database (Sayers et al., 2018) using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (McGinnis and Madden, 2004; Kircher and Kelso, 2010).

In addition, a meta-analysis was performed using other submitted SARS-CoV-2 sequences in NCBI from Iranian patients with accession numbers MT646036.1, MT447177.1, MT825091.1, MT459928.1, MT320891.2, and MT281530.2 for comparison with the sequence under study (Supplementary 1: SARS-CoV-2 DNA sequence). Differences between those sequences at the nucleotide level were observed and then used to construct their amino acid sequences using the "translate" resource of the ExPASY database (Gasteiger et al., 2003) and to construct their protein structures using the Swiss-Model database (Waterhouse et al., 2018). Unsolved protein structures may be modelled through different approaches including ab initio, threading and homology modelling (Xu et al., 2000). Homology modeling of proteins with solved structures usually gives the superior results. Among several approaches to homology modelling, the approach introduced by Andrej Sally (Eswar et al., 2008) and implemented in MODELLER is widely used (Webb and Sali, 2016). The SWISSMODEL website is an automated pipeline based on MODELLER that can model proteins having similar solved structures using their sequence.

After that, the models created by the Swiss Model Server were validated using BLASTP to compare protein sequences of modeled sequences with existing sequences in NCBI. After identifying proteins with differences between the sequence under study and other sequences from Iranian patients, proteins were studied using the Gene Ontology (GO) database (The Gene Ontology Consortium, 2019), MEME-Suite (http://meme-suite.org/) (Bailey et al., 2009) and the EBI database (ebi.ac.uk) (Madeira et al., 2019) to detect their function and how they can affect virus replication. The modeled proteins were verified using SAVES server.

2.2. Drug-like compounds for COVID-19

When studying genetic mutations and protein structures in the SARS-CoV-2 sequence analyzed in this study for the Iranian population, their genetic drug targets were studied using drug repurposing and considering gene-drug interactions in the DrugBank (Ganter et al., 2005), DSigDB (Kirchdoerfer and Ward, 2019) and DrugMatrix (Ong et al., 2020) databases to determine candidate drug-like compounds for the treatment of COVID-19. Then, the mechanism of action for selected drug-like compounds was studied to predict more effective drug-like compounds for COVID-19. SARS-CoV-2 genetic mutations considered in this study and the structure and function of their modeled protein can be used for molecular docking to detect the best drug-like compound for COVID-19.

The two-dimensional (2D) structures of the four ligands were downloaded from Drugbank (https://go.drugbank.com/releases/latest), a free cheminformatic database containing data on drugs, their interactions and their targets. Ten Different three dimensional (3D) conformations were created for each ligand, using the Balloon software (Puranen et al., 2010). The potential energy of the obtained conformers where then minimized using universal force field via openbabel to obtain realistic form of the ligands (O'Boyle et al., 2011) and converted to the desired format using Marvin Suite (MarvinSketch, 2014). The formats are pdbqt and sdf, required by the docking programs described below.

Two different docking programs were used. QuickVina-W (Hassan et al., 2017) was initially used to obtain the binding modes and interaction energies of ligands to each protein. The results were further verified using LeDock (Wang et al., 2016). Raw PDB files obtained from RCSB.org database or SWISSMODEL homology modelling pipeline cannot be directly used by docking programs. To add hydrogens and charges to proteins for docking, Pyrx (Dallakyan and Olson, 2015) and lepro (Liu and Xu, 2019) were respectively used before

Table 1. Long nucleotide substitution and indel mutations. Mutations were observed when comparing the sequence under study with the MT281530.2, MT459928.1 and MT646036.1 sequences. In this sequence comparison, sequence 1 is the sequence under study, and sequence 2 is the other sequence.

	Accession	Genomic position	Mutant sequence
1	MT281530.2	933-1107 of genomic sequence under study	seq1 AGAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAAATTTGACACCTTCA 982 seq2 AGAGCTATGANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1020 ********** seq1 ATGGGGAATGTCCAAATTTTGTATTTCCCTTAAATTCCATAATCAAGACTATTCAACCAA 1042 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1080 seq1 GGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTG 1102 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 1140 seq1 CGTCACCAAATGAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTG 1162 seq2 NNNNNCCAAATGAATGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTG 1200
2	MT281530.2	6168-6247 of genomic sequence under study	seq1 ATAAACCTATTGTTTGGCATGTTAACAATGCAACTAATAAAGCCACGTATAAACCAAATA 6202 seq2 ATAAACCTATTGTTTGGCATGTTAACANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 6240 seq1 CCTGGTGTATACGTTGTCTTTGGAGCACAAAACCAGTTGAAACATCAAATTCGTTTGATG 6262 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCAAATTCGTTTGATG 6300
3	MT281530.2	6435-6490 of genomic sequence under study	seq1 AAACTACCGAAGTTGTAGGAGACATTATACTTAAACCAGCAAATAATAGTTTAAAAATTA 6442 seq2 AAACTACCGAAGTTGTAGGAGACATTATACTTAAACCAGCAAATAATAGTTTNNNNNNNN 6480 seq1 CAGAAGAGGTTGGCCACACAGATCTAATGGCTGCTTATGTAGACAATTCTAGTCTTACTA 6502 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTAGTCTTACTA 6540
4	MT281530.2	7830-8194 of genomic sequence under study	seq1 CAAAATGTGAAGAATCATCTGCAAAATCAGCGTCTGTTTACTACAGTCAGCTTATGTGTC 7882 seq2 CAAAATGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 7920 ******* seq1 AACCTATACTGTTACTAGATCAGGCATTAGTGTCTGATGTTGGTGATAGTGCGGAAGTTG 7942 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 7980 seq1 CAGTTAAAATGTTTGATGCTTACGTTAATACGTTTTCATCAACTTTTAACGTACCAATGG 8002 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 8040 seq1 AAAAACTCAAAACACTAGTTGCAACTGCAGAAGCTGAACTTGCAAAGAATGTGTCCTTAG 8062 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 8100 seq1 ACAATGTCTTATCTACTTTTATTTCAGCAGCTCGGCAAGGGTTTGTTGATTCAGATGTAG 8122 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 8160 seq1 AAACTAAAGATGTTGTTGAATGTCTTAAATTGTCACATCAATCTGACATAGAAGTTACTG 8182 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 8220 seq1 GCGATAGTTGTAATAACTATATGCTCACCTATAACAAAGTTGAAAACATGACACCCCGTG 8242 seq2 NNNNNNNNNNNNATAACTATATGCTCACCTATAACAAAGTTGAAAACATGACACCCCGTG 8280
5	MT281530.2	27725-28372 of genomic sequence under study	seq1 CTTTCATTAATTGACTTCTATTTGTGCTTTTTAGCCTTTCTGCTATTCCTTGTTTTAATT 27742 seq2 CTTTCATTAATTGACTTCTATTTGTGCTTTTTAGCCTTTCTGNNNNNNNNNNNNNNNNNN 27780 seq1 ATGCTTATTATCTTTTGGTTCTCACTTGAACTGCAAGATCATAATGAAACTTGTCACGCC 27802 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 27840 seq1 TAAACGAACATGAAATTTCTTGTTTTCTTAGGAATCATCACAACTGTAGCTGCATTTCAC 27862 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 27900 seq1 CAAGAATGTAGTTTACAGTCATGTACTCAACATCAACCATATGTAGTTGATGACCCGTGT 27922 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 27960 seq1 CCTATTCACTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTTA 27982 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28020 seq1 ATTGAATTGTGCGTGGATGAGGCTGGTTCTAAATCACCCATTCAGTACATCGATATCGGT 28042 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28080 seq1 AATTATACAGTTTCCTGTTTACCTTTTACAATTAATTGCCAGGAACCTAAATTGGGTAGT 28102 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28140 seq1 CTTGTAGTGCGTTGTTCGTTCTATGAAGACTTTTTAGAGTATCATGACGTTCGTGTTGTT 28162 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28200 seq1 TTAGATTTCATCTAAACGAACAAACTAAAATGTCTGATAATGGACCCCAAAATCAGCGAA 28222 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28260 seq1 ATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAG 28282 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28320 seq1 AACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGT 28342 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 28380 seq1 CTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAG 28402 seq2 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAGGAAGACCTTAAATTCCCTCGAGGACAAG 28440
6	MT459928.1	19183-20118 of genomic sequence under study	seq1 TATCCTGCTAATTCCATTGTTTGTAGATTTGACACTAGAGTGCTATCTAACCTTAACTTG 19190 seq2 TATCCTGCTAATTCCATTGTTTGTAGATTTGACACTAGAGTGCTATCTAACC——— 19192 seq1 CCTGGTTGTGATGGTGGCAGTTTGTATGTAAATAAACATGCATTCCACACACCAGCTTTT 19250 seq2 ———————————————————————— 19192 seq1 GATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTCTATTACTCTGACAGTCCA 19310 seq2 ———————————————————————— 19192 seq1 TGTGAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGTACCACTAAAGTCTGCT 19370 seq2 ———————————————————————— 19192 seq1 ACGTGTATAACACGTTGCAATTTAGGTGGTGCTGTCTGTAGACATCATGCTAATGAGTAC 19430 seq2 ———————————————————————— 19192 seq1 AGATTGTATCTCGATGCTTATAACATGATGATCTCAGCTGGCTTTAGCTTGTGGGTTTAC 19490 seq2 ———————————————————————— 19192 seq1 AAACAATTTGATACTTATAACCTCTGGAACACTTTTACAAGACTTCAGAGTTTAGAAAAT 19550 seq2 ———————————————————————— 19192 seq1 GTGGCTTTTAATGTTGTAAATAAGGGACACTTTGATGGACAACAGGGTGAAGTACCAGTT 19610 seq2 ———————————————————————— 19192

(*continued on next page*)

Table 1 (*continued*)

	Accession	Genomic position	Mutant sequence
			seq1 TCTATCATTAATAACACTGTTTACACAAAAGTTGATGGTGTTGATGTAGAATTGTTTGAA 19670 seq2 ———————————————————————————— 19192 seq1 AATAAAACAACATTACCTGTTAATGTAGCATTTGAGCTTTGGGCTAAGCGCAACATTAAA 19730 seq2 ———————————————————————————— 19192 seq1 CCAGTACCAGAGGTGAAAATACTCAATAATTTGGGTGTGGACATTGCTGCTAATACTGTG 19790 seq2 ———————————————————————————— 19192 seq1 ATCTGGGACTACAAAAGAGATGCTCCAGCACATATATCTACTATTGGTGTTTGTTCTATG 19850 seq2 ———————————————————————————— 19192 seq1 ACTGACATAGCCAAGAAACCAACTGAAACGATTTGTGCACCACTCACTGTCTTTTTTGAT 19910 seq2 ———————————————————————————— 19192 seq1 GGTAGAGTTGATGGTCAAGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTACA 19970 seq2 ———————————————————————————— 19192 seq1 GAAGGTAGTGTTAAAGGTTTACAACCATCTGTAGGTCCCAAACAAGCTAGTCTTAATGGA 20030 seq2 ———————————————————————————— 19192 seq1 GTCACATTAATTGGAGAAGCCGTAAAAACACAGTTCAATTATTATAAGAAAGTTGATGGT 20090 seq2 ———————————————————————————— 19192 seq1 GTTGTCCAACAATTACCTGAAACTTACTTTA——————————————— 20121 seq2 ——————————————TTANNNNNNNNNNNNNNNNNNNNNNNNNNNNN 19224
7	MT646036.1	19183-20030 of genomic sequence under study	seq1 TATCCTGCTAATTCCATTGTTTGTAGATTTGACACTAGAGTGCTATCTAACCTTAACTTG 19190 seq2 TATCCTGCTAATTCCATTGTTTGTAGATTTGACACTAGAGTGCTATCTAACC———— 19192 seq1 CCTGGTTGTGATGGTGGCAGTTTGTATGTAAATAAACATGCATTCCACACACCAGCTTTT 19250 seq2 ———————————————————————————— 19192 seq1 GATAAAAGTGCTTTTGTTAATTTAAAACAATTACCATTTTTCTATTACTCTGACAGTCCA 19310 seq2 ———————————————————————————— 19192 seq1 TGTGAGTCTCATGGAAAACAAGTAGTGTCAGATATAGATTATGTACCACTAAAGTCTGCT 19370 seq2 ———————————————————————————— 19192 seq1 ACGTGTATAACACGTTGCAATTTAGGTGGTGCTGTCTGTAGACATCATGCTAATGAGTAC 19430 seq2 ———————————————————————————— 19192 seq1 AGATTGTATCTCGATGCTTATAACATGATGATCTCAGCTGGCTTTAGCTTGTGGGTTTAC 19490 seq2 ———————————————————————————— 19192 seq1 AAACAATTTGATACTTATAACCTCTGGAACACTTTTACAAGACTTCAGAGTTTAGAAAAT 19550 seq2 ———————————————————————————— 19192 seq1 GTGGCTTTTAATGTTGTAAATAAGGGACACTTTGATGGACAACAGGGTGAAGTACCAGTT 19610 seq2 ———————————————————————————— 19192 seq1 TCTATCATTAATAACACTGTTTACACAAAAGTTGATGGTGTTGATGTAGAATTGTTTGAA 19670 seq2 ———————————————————————————— 19192 seq1 AATAAAACAACATTACCTGTTAATGTAGCATTTGAGCTTTGGGCTAAGCGCAACATTAAA 19730 seq2 ———————————————————————————— 19192 seq1 CCAGTACCAGAGGTGAAAATACTCAATAATTTGGGTGTGGACATTGCTGCTAATACTGTG 19790 seq2 ———————————————————————————— 19192 seq1 ATCTGGGACTACAAAAGAGATGCTCCAGCACATATATCTACTATTGGTGTTTGTTCTATG 19850 seq2 ———————————————————————————— 19192 seq1 ACTGACATAGCCAAGAAACCAACTGAAACGATTTGTGCACCACTCACTGTCTTTTTTGAT 19910 seq2 ———————————————————————————— 19192 seq1 GGTAGAGTTGATGGTCAAGTAGACTTATTTAGAAATGCCCGTAATGGTGTTCTTATTACA 19970 seq2 ———————————————————————————— 19192 seq1 GAAGGTAGTGTTAAAGGTTTACAACCATCTGTAGGTCCCAAACAAGCTAGTCTTAATGGA 20030 seq2 ———————————————————————————— 19192

QuickVina-W(Handoko et al., 2012) and LeDock (Nafisa et al., 2017). As the proteins did not contain any bound ligands, we considered all protein volume as the ligand-binding box for our docking studies. The same box was used for blind docking using QuickVina-W (Handoko et al., 2012) and LeDock. (Liu and Xu, 2019). For the docking performed by QuickVina-W, the exhaustiveness of the algorithm, the parameter adjusting the amount of sampling performed by qVina, was increased according to the size of the box.

3. Results

3.1. Mutations

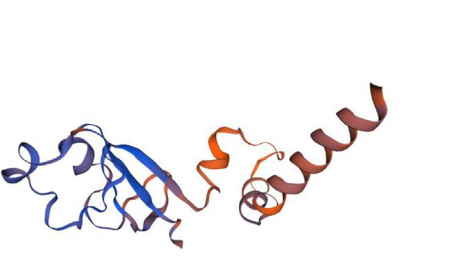
The consensus sequence of SARS-CoV-2 under study contains 29,776 bps and is included in Supplementary 1 (SARS-CoV-2 DNA sequence). Genomic comparison of that sequence with the existing sequences of SARS-CoV-2 in NCBI, including sequences with accession numbers MT114414.1, MT641746.1, LC553269.1, MT184909.2, and MT184907.2, shows that the sequence under study has approximately 99.9% similarity with other SARS-CoV-2 submitted sequences. On the other hand, a meta-analysis comparison of the sequence under study with other submitted SARS-CoV-2 sequences from Iran with the accession numbers MT646036.1, MT447177.1, MT825091.1, MT459928.1, MT320891.2, and MT281530.2 showed a similarity of 97.3%, 99.97%, 99.2%, 99.96%, 99.98%, and 95.5%, respectively. Long nucleotide insertions/deletions (indels) observed in the sequence under study compared with MT281530.2, MT459928.1, and MT646036.1 are reported in Table 1. Comparing the sequence under study with other submitted sequences from Iran did not show any additional long nucleotide insertions/deletions (indels).

Having detected mutations in the DNA sequence of SARS-CoV-2 in this study, amino acid sequences were deduced for the mutant DNA and used to model the tertiary structure. When we modeled those amino acid sequences using a Swiss-model database (Waterhouse et al., 2018), we observed some DNA mutations of SARS-CoV-2 presented in the third column of Table 1, encode proteins with structures similar to ORF9b, ORF1ab, ORF8, NSP15, and the NSP14-NSP10 complex, as reported in Table 2. To prove those similarities, the results of BLASTp for created models indicated that the modeled proteins have 100% similarity with the existing ones reported previously in NCBI. To verify the structure of the modeled proteins, we used SAVES server. The overall quality factor of the modelled proteins was as follows: NSP14: 79.3814, NSP15: 94.8768, ORF-1: 85.7143, ORF-8: 91.0714, ORF-9: 92.6471. As explained in the next paragraphs, when studying the roles of these proteins in viral

Table 2. Tertiary modeled structures of large mutations in the sequence under study when compared with other sequences from Iran.

Name	Amino-acid sequence	Modeled structure of mutant part
ORF9b (X-ray Crystallographic Structure of Orf9b from SARS-CoV-2)	MDPKISEMHPALRLVDPQ IQLAVTRMENAVGRDQNNVGPKVYPI ILRLGSPLSLNMA	
ORF8	LFLVLIMLIIFWFSLELQDHNETCHA-TNMKFLVFLGIITTVAAFHQECSLQS CTQHQPYVVDDPCPIHFYSKWYIRVGA RKSAPLIELCVDEAGSKSPIQYIDIGN YTVSCLPFTINCQEPKLGSLVVRCSFY EDFLEYHDVRVVLDFI-TNKLKCLIMDPKISEMHPALRLVDPQI QLAVTRMENAVGRDQNNVGPKVYPIIL RLGSPLSLNMA	
ORF1ab	KELLVYAADPAMHAASGNLLLDKRTT CFSVAALT	
NSP15	SLENVAFNVVNKGHFDGQQGE VPVSIINNTVYTKVDGVDVELFENKTTL PVNVAFELWAKRNIKPVPEVKILNNLGV DIAANTVIWDYKRDAPAHISTIGVCSMTD IAKKPTETICAPLTVFFDGRVDGQVDL FRNARNGVLITEGSVKGLQPSVGPKQ ASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPE TYFTQSRNL	
nsp14-nsp10 complex with functional ligand SAM	VLSNLNLPGCDGGSLYVNKHAFHTPAFD KSAFVNLKQLPFFYYSDSPCESHGKQVVS DIDYVPLKSATCITRCNLGGAVCRHHANEY RLYLDAYNMMISAGFSLWVYKQFDTYNLW NTFTR	

a. Before replication (original protein)



b. After replication (protein under study)

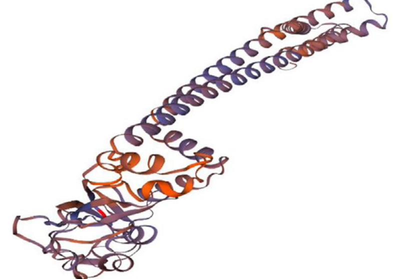


Figure 1. Tertiary structure of the original NSP8 protein obtained from the NCBI and the mutant protein created from the sequence under study for SARS-CoV-2.

Table 3. Drug-like compounds with interactions with specific reported genes involved in SARS-CoV-2.

Protein	Drugs
ORF9b	GSK10071028, BX-912, TAE684, GSK260, Staurosporine, Cyclosporin
NSP8	Pyridine, 24-Diaminophenol, Piracetam, Erythromycin, (+)-pulegone, colchicine, streptozotocin, Cyproheptadine, Pentamidine, Diethylstilbestrol, Chloramphenicol, 6-Mercaptopurine, Nortriptyline, Streptomycin, Rapamycin, 44-Methylenedianiline, Beta-Estradiol, Propylene, Arsenenous acid, O-phospho-L-tyrosine, estradiol, L-alanine, Imatinib mesyate, Chembl 460515, phenylarsine oxide, Garcinol, N-Phenyl-1H-Pyrazole-3-carboxamide, Silica, Sulfuguanidine, Cicloheximide HL60, Iatamoxef, Ouabain, dihydroergotamine HL60, (-)-isoprenaline, pergolide, prestwick, quinpirole, 15-delta prostaglandin J2, Zaradaverine, Focalin, dihydroergotamine, Aflatoxin, Tetradioxin, Zinc, Resveratrol, Quercetin
NSP10	Chlortetracycline, Methyl Salicylate, Amitriptyline, 3-Methylcholanthrene, Streptozotocin, Mecaptopurine-5′, Rapamycin, Hydrazine, Enoxacin, Indomethacin, Nicotine, Azathioprine, Indomethacin, Amoxapine, Oxcabazepine, Raloxifene, Diethanolamine, Griseofulvin, Carboplatin, 7646-79-9, quercitrin, Honokiol, o-Cresolphthalein, IB-ME-CA CTD 00003042, equilin, acetaminophen, Tetradioxin, 0175029-0000, Quercetin, Copper Sulfate, Copper Boss, Carganathin, Pyrazole, diltiazem, Enkephalin, Hexadecand, MethySergide meleate, estradiol, Methyl methanesulfonate, Aflatoxin
NSP14	Rofecoxib, Levosulpiride, Prednisolone, Zidovudine, Doxorubicin, Cimetidine, Epinephrine, Hydroxyurea, Resveratrol, 483-60-3, AC1NRCGS, Pindolol, Suramin, Fucose
NSP15	Ciclesonide, Mitoxantrone, ellipticine, Tamibarotene, Berberine, Cytochalasin, N-formylmethionylieu, dioxidanide, 1-butand

Table 4. Novel candidate drug-like compounds for COVID-19 using drug repurposing.

Drug	Mechanism of action
Resveratrol	In Herpes simplex virus (HSV) infected cells, Resveratrol suppresses NF-kappaB (NF-kappaB) activation. During infection which is one of an essential step in virus replication scheme, NF-kappaB can be activated by HSV [41].
Erythromycin	Erythromycin can bind to the 23S ribosomal RNA molecule and inhibit the protein synthesis. By inhibition of protein synthesis in transpeptidation step, it can stop bacterial protein synthesis. Various bacterial infections can be controlled in this way [43].
Chloramphenicol	chloramphenicol can diffuse trough the membrane and then bind to 16 protein of the 50S subunit in bacterial ribosomes that can prevent growing peptide chains by transferring amino acids. This can result in inhibition of subsequent protein synthesis and peptide bond formation [44].
Indomethacin	This is a non-steroidal anti-inflammatory drug that also has antipyretic and analgesic properties. This is also a reversible and nonspecific inhibitor of the cyclo-oxygenase enzyme with two isoforms that either involve in prostaglandis, thromboxane A2 synthesis or express in response to inflammation or injury [46].
Ciclesonide	Ciclesonide is a kind of Glucocorticoids that can act as humoral immune response suppressor, leukocyte infiltration inhibition, and inflammatory response mediation. Ciclestonide limits vascular structure permeability and dilatation capillary that can result in vasoactive kinins release reduction. Ciclesonide can also reduce prostaglandis formation by inhibition of arachidonic acid from phospholipids. Glucocorticoid response elements cab bind to the complex translocated corticoreptor-ligand that leads to interaction of transcription factors with DNS bound receptor and result in changing the expression level of specific target genes [47].

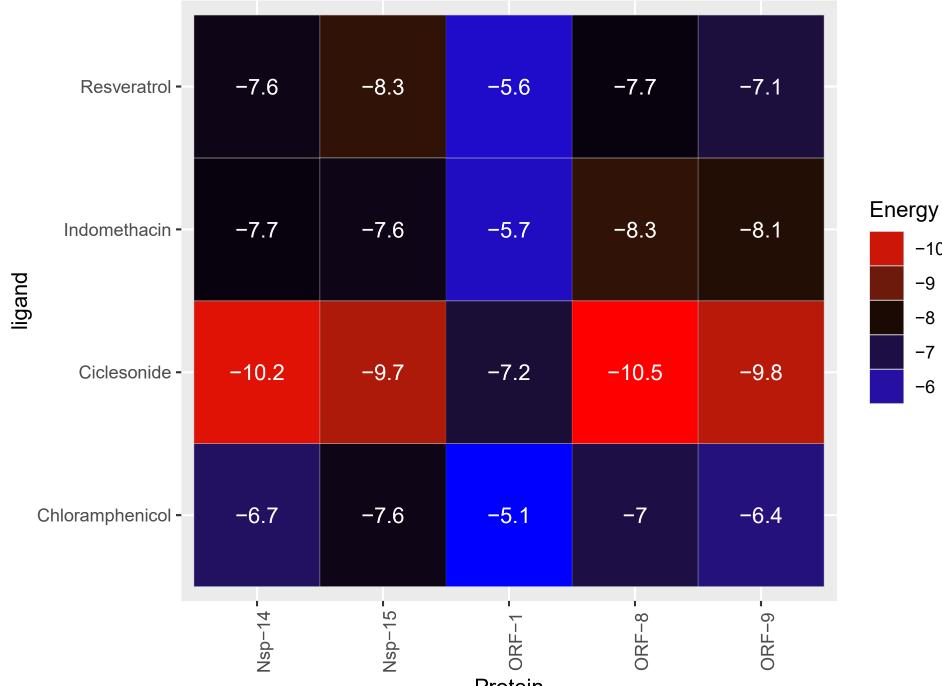


Figure 2. Heatmap of the docking results. Colors represents the binding affinity (Kcal/mol) of the ligands to proteins reported by qVina. A docking score of ≥7 is considered to represent an excellent binding of the ligand to the protein.

replication, we observed that the new study sequences had inserted portions with important roles in viral replication. The amino acid sequences of related mutations were reported in the second column of Table 2, and the third column shows the tertiary structure of related modeled proteins for those mutant sequences. As explained in the next paragraphs, compared to the roles of these proteins in viral replication, the new study sequence had inserted portions with important roles in viral replication. The amino acid sequences of related mutations are reported in the second column of Table 2, and the third column indicates the tertiary structure of related modeled proteins for those mutant sequences.

NSP8 and NSP7 proteins form a cylinder-like structure in RNA-dependent RNA polymerase (RdRp) complex (Tvarogova et al., 2019). RdRp in SARS-CoV-2, which is necessary for viral replication, has NSP8 and NSP7 as two accessory subunits and NSP12 as a catalytic subunit that mediates the activity of RdRp. The efficiency of RNA replication can be enhanced through the important roles of NSP8. RdRp activities were previously discovered as important in coronavirus replication; NSP8 is one of the RdRp (Te Velthuis et al., 2012). In addition, for virus genome replication, RdRp, NSP8, and NSP7 form a super-complex for a transcription/replication machinery. NSP8 mutations may contribute to transcription stabilization (Reshamwala et al., 2021). It has also been discovered that NSP8 has a direct association with NSP12 and greatly stimulates its polymerase activity. The direct interaction of NSP8 and NSP12 suggests their important roles in viral replication. Biochemical studies have identified that NSP12 requires the presence of NSP7 and NSP8 to perform sufficient RNA synthesis and for viral replication, which is one of the important events in the positive regulation of NSP12 (Kirchdoerfer and Ward, 2019; Hillen et al., 2020). In addition, NSP8 can negatively regulate antiviral immune responses when localized and largely expressed in host cells and favoring viral replication and infection (Yang et al., 2020). In addition, ORFs mutations have previously been shown to enhance host-genome similarity, which can delay host innate immunity. Those mutations can help the virus evade a range of host-immune system pressures and therefore support virus replication and spread (Sun, 2020).

For NSP8 in the sequence under study, a duplicated protein was observed when their duplicate parts were bound to the other, which can

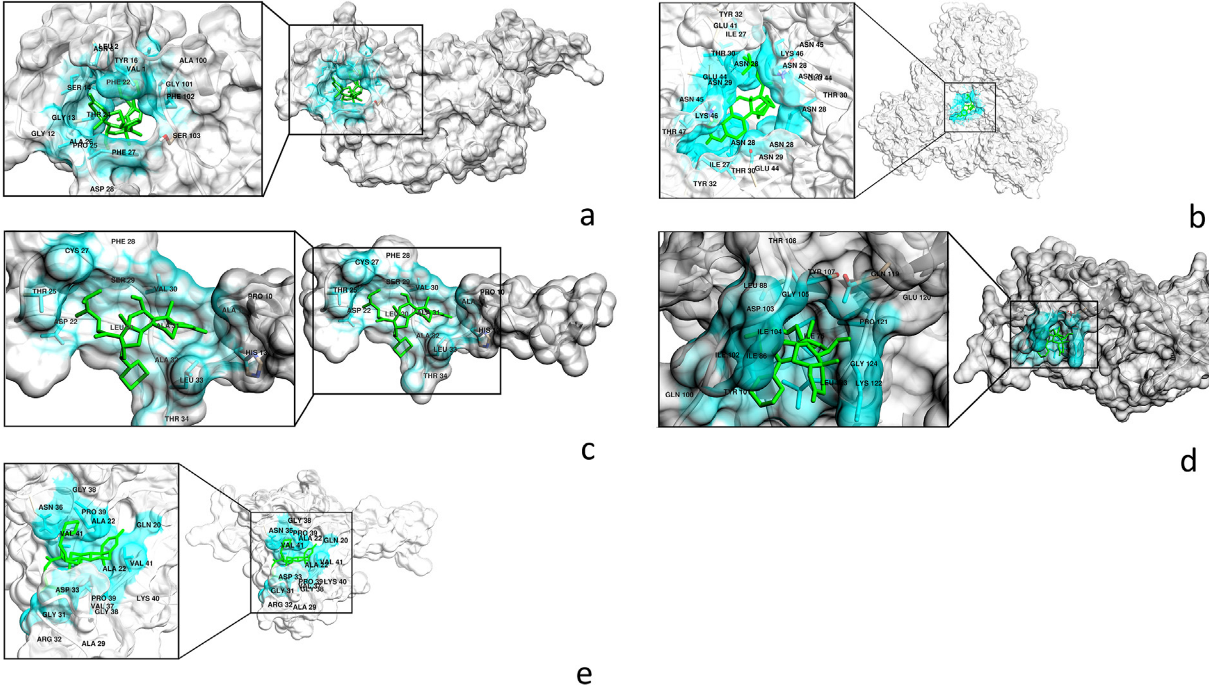


Figure 3. The structure oif ciclesonide in complex with NSP 14 (a), NSP 15 (b), ORF 1 (c), ORF8 (d) and ORF9 (e.).

have an effect on viral replication. Heterotetrameric has been shown in previous studies as a SARS-CoV-2 replication complex consisting of one NSP7 chain, one NSP12 chain, and two NSP8 chains. In that complex, the NSP8 cofactor has an essential role in stimulating NSP12's polymerase activity, and the NSP12-NSP8 complex has an important viral replication role in SARS-CoV-2 (Mutlu et al., 2022). Therefore, for inhibition of the virus replication and making control over virus infection, it is required to obstruct those NSPs interactions (Sarma et al., 2022). The structure of replicated NSP8 in the sequence under study is reported in Figure 1 (b), and its original single protein structure obtained from NCBI and observed without any replication is shown in Figure 1 (a). NSP8 has been found to have highly protective antigenicity and should be evaluated as a vaccine candidate to stimulate protective immunity (Romano et al., 2020; Matsuyama et al., 2020; Han et al., 2020). The endonuclease activity of NSP15 can affect RNA processing and coronavirus infection and replication. NSP15 is a conserved protein among coronaviruses and is essential in virulence and the coronavirus life cycle. It can participate in viral replication either directly or by interfering with the host's immune response (Smith et al., 2013).

One of the key factors in viral replication and transcription of the SARS-CoV-2 is highly conserved NSP14. Proofreading activity of an exoribonuclease domain (EXoN) enzyme in NSP14 protects viruses from mutagenesis. The deleterious effects of mutagens on SARS-CoV-2 can be prevented by EXoN proofreading. In RNA viruses, the EXoN has been reported as the first proofreading protein (Ma et al., 2015). Therefore, targeting an EXoN is an ideal method to develop new sensitizers and inhibitors for coronaviruses. Interaction of NSP10 with EXoN of NSP14 can stimulate and fully unleash its activity. The complex structure of NSP14 with NSP10 can provide uniquely valuable information for developing potential antiviral drugs (Denison et al., 2011). A decrease in virus replication fidelity can result from a disturbance in NSP14-NSP10 interaction. In addition, the NSP14-NSP10 complex can function as an RNA viral proofreading useful in understanding RNA virus evolution. It has been demonstrated that a lack of RNA viral proofreading activities can cause errors in the biology of viruses, which can result in virus replication (Smith, 2017; Narayanan and Nair, 2020).

On the other hand, vesicular stomatitis virus (VSV) replication can be facilitated by SARS-CoV-2 open reading frame (OFR)9b overexpression. Therefore, SARS-CoV-2 ORF9b can facilitate virus replication by negatively regulating antiviral immunity. In addition, other viral proteins have shown a wide range of interactions with ORF9b (Ogando et al., 2020).

3.2. Candidate putative drug-like compounds

The genetic mutations in the study SARS-CoV-2 sequence can be used for drug repositioning to a candidate some drug-like compounds for COVID-19. The drugs that interacted with mutant genes using drug repositioning and reported in this study are shown in Table 3. Among them, 4,4′-methylenedianiline, doxorubicin, bromocriptine, harmine, methanesulfonate, bromocriptine, isoprenaline, streptozocin, estradiol, dihydroergotamine, resveratrol, quercetin, and ellipticine interact with more than one related gene. Among them, resveratrol has some mechanism of action that has been reported previously as a potential drug-like compound for COVID-19 (Taylor et al., 2015; Mittra et al., 2020; Liao et al., 2021).

Among the rest of the potential drug-like compounds that interact with only one related gene, bepridil, primaquine, pimozide, camptothecin, piracetam, erythromycin, colchicine, cyproheptadine, pentamidine, diethylstilbestrol, chloramphenicol, nortriptyline, streptomycin, alanine, ouabain, dexmethylphenidate, amitriptyline, enoxacin, indomethacin, azathioprine, amoxapine, oxcarbazepine, carboplatin, diltiazem, methysergide, rofecoxib, prednisolone, zidovudine, cimetidine, pindolol, BX-912, cyclosporin and ciclesonide can have effects on the treatment of COVID-19 based on their mechanism of action (Ganter et al., 2005; Kirchdoerfer and Ward, 2019).

In addition, a PDE4 inhibitor (PDE4i), with its mechanism of action, has been found to modulate immunity as a drug-like compound for severe diseases such as COVID-19 by ameliorating lung inflammation. Additionally, the severe cytokine storm in COVID-19 can be attenuated by PDE4i, and SARS-CoV-2 infection can be potentially treated using the inhibitor (Wu et al., 2020; Bridgewood et al., 2020). As reported in Table 4, resveratrol is a candidate drug-like compound for COVID-19 treatment since it suppresses NF-kappa B (NF-kappa B) activation. Camptothecin binds to the topoisomerase, and erythromycin binds to the 23S ribosomal RNA molecule and inhibits protein synthesis.

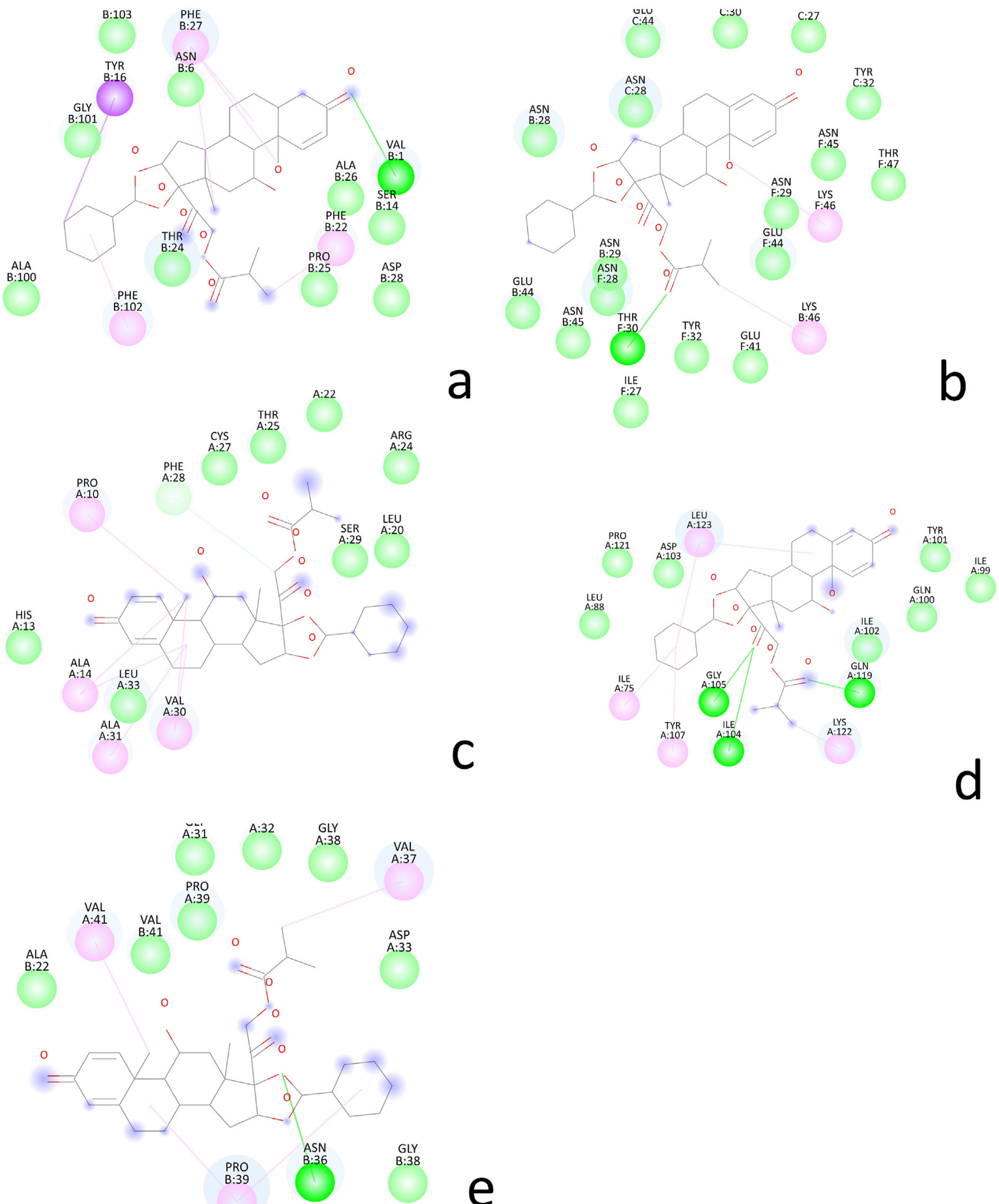


Figure 4. Interaction of cicleosonide with different modelled proteins. NSP 14 (a), NSP 15 (b), ORF 1 (c), ORF8 (d) and ORF9 (e).

Chloramphenicol diffuses through the bacterial cell membrane and then binds to 16 proteins of the 50S subunit in bacterial ribosomes, preventing peptide chains from growing by transferring amino acids that can help to treat viral infection. Indomethacin has reversible and nonspecific properties as an inhibitor of the cyclo-oxygenase enzyme, and ciclesonide binds to the complex translocated corticoreptor ligand, which leads to the interaction of transcription factors with DNS-bound receptors and results in changes in the expression levels of specific target genes (Pushpakom et al., 2018; King et al., 2019; Merwe and Bjornsti, 2007).

Previous studies have revealed that viral replication can be decreased by the inhibition of NSP15. Therefore, NSP15 has been identified as a potential SARS-CoV-2 chemotherapeutic target. Ciclesonide is known as a safe drug-like compound that can be used to reduce lung inflammation in the host and viral replication. Ciclesonide can be used to block SARS-CoV-2 replication by targeting viral NSP15 endonuclease (Neto et al., 2020). Previous studies have also reported drugs as candidates for COVID-19; for example, a study by Mitra et al. also reported on resveratrol (Pringle et al., 2007), Alakwaa studied camptothecin (Lucas, 2016),

Pani et al. studied azithromycin (Dvorak and Pavek, 2010), Marinella studied indomethacin (Kimura et al., 2020), and Iwabuchi et al. and Yamasaki et al. studied ciclesonide (Mittra et al., 2020; Alakwaa, 2020).

Furthermore, the attenuating effect of NSP14 on the function of drugs that terminate viral genome replication can be targeted for COVID-19 drug development (Pani et al., 2020; Dinos, 2017). For example, Middle East respiratory syndrome coronavirus (MERS-CoV) NSP14 has been suggested as a key replicative enzyme that can be targeted for antiviral drug development (Muhammed, 2020). In addition, ORF7a is a transmembrane coronavirus protein that can cause loss of its antiviral function (Marinella, 2020). Therefore, ORF7a is a potential target for antiviral drug discovery. This target protein shows a high binding affinity to a series of clinical drugs with anti-inflammatory effects (Wabuch et al., 2020).

The result of molecular docking for potential drugs candidate in this study, indicated that all ligands and proteins were prepared without error. The raw output from the docking programs were analyzed and the top scoring (most negative binding energy) ligand conformers were selected. Both qVina and leDock results showed the best binding energy for Ciclesonide for the five proteins. Results from qVina are depicted in Figure 2. The best ligand pose along with the respective protein was visualized using Chimera software (Figure 3). Subsequently, the interacting residues for each protein with the Ciclessonide was obtained using Discovery Studio software (Figure 4).

Some of the existing experimental or observational clinical trials can be used to validate our findings as potential antiviral drugs to treat COVID-19 inferred from this study. For example, in an experimental study, patients in Mumbai with severe COVID-19 received resveratrol and copper, and the results indicated a nearly 2-fold reduction in mortality rate (Mittra et al., 2020). Also, the antiviral activity of resveratrol was observed in African monkey kidney cells. In those cases, virus progeny production was reduced by adding resveratrol. That result indicated an antiviral activity of resveratrol for inhibiting SARS-CoV-2 infection (ter Ellen et al., 2021). In addition, one previous study showed that erythromycin was recommended for COVID-19 management in 10 African countries (Adebisi et al., 2021). Another study used tetracyclines to treat COVID-19 in 38 adults in Rome, and as a result, the symptoms of all COVID-19 patients were resolved (Gironi et al., 2020). According to another study, experimental cell studies for indomethacin showed an increase in SAR-CoV-2 inhibition (Kiani et al., 2021). Another case study examined indomethacin on 210 COVID-19 patients, indicating some symptomatic relief. Such a study indicated that indomethacin could be used as a safe drug for COVID-19 patients (Ravichandran et al., 2021). On the other hand, another study used 90 COVID-19 patients' data from Japan and showed that Ciclesonide in cell culture could suppress viral replication and potentially be used for COVID-19 treatment (Terada-Hirashima et al., 2020). In addition, one other study used experimental epithelial cells to indicate the Ciclesonide suppressive effects on SARS-CoC-2 viral replication (Matsuyama et al., 2020). A case study in Japan indicated that using the antiviral activity of Ciclesonide for COVID-19 treatment was successful (Iwabuchi et al., 2020).

4. Conclusions

We have observed the emergence of SARS-CoV-2, which can mutate and recombine rapidly to infect human. We have investigated nucleotide genetic mutations in the SARS-CoV-2 sequence in Iran and identified their related proteins. Proteins with roles in viral replication have been further studied. Phylogenetic analysis of SARS-COV-2 indicated that the MT320891 sequence with the most similarity with the study sequence is related to the newer version of SARS-CoV-2 (Li et al., 2020). This result could lead us to conclude that comparing the SARS-CoV-2 sequences in this study help detect virus replication in a newer version of the virus, which can complete the evolution of SARS-CoV-2. It will be a long fight against COVID-19, but the process can be facilitated by considering genetic variations of the virus. For example, treatment of COVID-19 and reducing its burden required strategies to target the conserved elements of the viral life cycle, such as those responsible for the transcription and replication of viral RNA genomes, such as NSPs or ORFs. By looking at the effect of emerging mutations on essential viral replication protein, new potential interactions with existent drug-like compounds can be inferred by in silico methods to derive further investigation on more effective chemotherapeutic agents against COVID-19. Protein structure information can be used for immunogen design, in which crystal structures of antigens are the lounging point, and distinct inducers for those antibodies are important to design a vaccine.

Our focus in this study was introducing genes in association with COVID-19; therefore, based on the genetic studies of COVID-19, only some drug-like compounds were introduced for some follow-up studies to interpret new treatment approaches. We should clarify that experimental or observational clinical trials are not within the scope of our manuscript. Our manuscript focuses on bioinformatic techniques, and performing experimental studies is worth enough for independent research. However, based on pharmacological studies, the follow-up studies can be pharmacophore analysis, toxicological analysis, molecular dynamic, cell-like and then animal test analysis, and finally clinical trial analysis. As COVID-19 targeted therapy, some toxicological and pharmacological tests can be used to test for the effectiveness of the proposed drugs for the suitable cell line. We used a bottom-up procedure, initially performed bioinformatic analysis, and accepted our genetic findings; then, we searched for potential drug-like compounds for COVID-19. Based on our findings, we are not claiming a new treatment for COVID-19 because it required some follow-up studies like MD simulation, pharmacodynamic analysis, mode of activity analysis, experimental analysis, animal tests, and clinical trials. Therefore, our results may help detect potential drug-like compounds that might be used for COVID-19 treatment.

Declarations

Author contribution statement

Zahra Mortezaei: Conceived and designed the analysis; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ali Mohammadian: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mahmood Tavallaei: Conceived and designed the analysis; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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