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Virtual Development of COVID-19 Drugs Targeting Nsp16-Nsp10 Complex

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ABSTRACT

The search for pharmaceutical treatment for COVID-19 has been progressive. Currently approved medicines include therapeutic antibodies and antiviral drugs. However, the effectiveness of current antiviral drugs is far from satisfactory, which suggests the necessity for improvement. In this research, we selected the non-structural protein complex Nsp16-Nsp10, which is responsible for methylating SARS-CoV-2's RNA, as a drug target. With computer-aided drug development technology, we screened two potential drugs either by inhibiting the SAM catalytic pocket on the complex or inhibiting the binding between Nsp16 and Nsp10 from preventing the Nsp16-Nsp10 complex helping SARS-CoV-2 duplicate, and that will work in COVID-19 treatment.

Introduction

The coronavirus disease of 2019 (COVID-19) has been a global pandemic caused by SARS-CoV-2, lasting over three years.

SARS-CoV-2 mainly consists of a lipid bilayer membrane, viral RNA, and proteins. During entry, the viral membrane merges with that of hosts, releasing the viral genome into the cytoplasm. The viral genome is a positive-strand RNA (+ssRNA), which encodes proteins necessary for the virus to enter the cell and replicate itself. Proteins of SARS-CoV-2 can be classified as an accessory, structural and non-structural (Gorkhali et al., 2021). Structural proteins are part of the complete virus, mainly consisting of the spike, envelope, and nucleocapsid proteins. Non-structural proteins (Nsp) are not present in the virus; instead, they function together to replicate the virus inside the host. Non-structural proteins are named in order of the viral RNA reading frame.

The duplication process of SARS-CoV-2 begins when the viral RNA-dependent RNA polymerase (RdRp/Nsp12) synthesizes its RNA. According to Gorkhali *et al.*, the newly synthesized RNA strands will be capped with a methylation mark, GMP, and a methyl group at the end, which is done with the viral methyltransferase, the Nsp16-Nsp10 complex (Gorkhali et al., 2021). The capped viral RNA mimics specific mRNA segments of the host, such that it will not be detected by the host's pathogen identifier nor modified by host proteins, which secures the newly synthesized SARS-CoV-2 RNA from being degraded by host cells' immune factors. It will be extremely inefficient or impossible for the virus to duplicate without capping by the Nsp16-Nsp10 complex; Hence, the Nsp16-Nsp10 complex is chosen to be the target for our drug inhibition.

Methods

The research revolves around two parts to develop a COVID-19 drug: 1) Screen a drug target protein or protein complex that plays key roles in SARS-CoV-2's life cycle and pilot approach to disrupt the functional domain of the target protein or protein complex; 2) Employ computer-aided drug development (CADD) technology for the virtual screening of potential drugs with high-affinity potency to the sites on the target protein.

Nsp16 and Nsp10 Complex

Nsp16-Nsp10 caps and methylates viral RNA with GMP and methyl groups, respectively, similar to the role of cap-specific mRNA transferase (CMTr1) of the host (Chen et al., 2011). Nsp16 has a negatively charged pocket that an S-Adenosyl methionine (SAM) molecule binds to. SAM is a messenger molecule that delivers methyl groups to Nsp16, which is then transferred onto the viral RNA. Four active sites in Nsp16-SAM binding pocket are responsible for transferring the methyl group, two of which directly bind with the SAM molecule. Therefore, inhibiting one or more of the four active sites may prevent the methylation of viral RNA, rendering Nsp16-Nsp10 unfunctional. The inhibition could be done with a drug molecule similar in shape to SAM that can competitively bind against SAM to the four active sites of Nsp16.

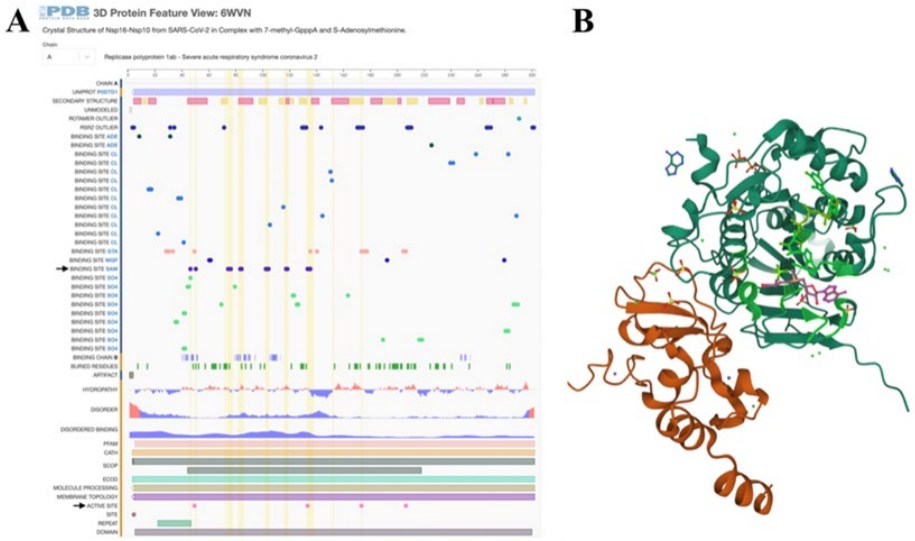

Figure 1. Four active sites in Nsp16-SAM binding pocket. A) The protein sequence of Nsp-16, marking the binding sites of SAM and the active sites, B) The 3D model of SARS-CoV-2 Nsp16-Nsp10 complex, with SAM highlighted in pink and adjacent molecules or residues highlighted in green.

Nsp16 and Nsp10 are both monopeptide proteins, separately translated before they combine. According to Gorkhali, Nsp10 has a hydrophobic, positively charged projection that matches the shape of a hydrophobic, negatively charged pocket on Nsp16 (Gorkhali et al., 2021). After Nsp16 and Nsp10 are synthesized and come close to each other in the right orientation, the Nsp10 projection will be attracted to the Nsp16 pocket, causing them to combine. As shown, several hydrogen bonds form between the residues, locking them together.

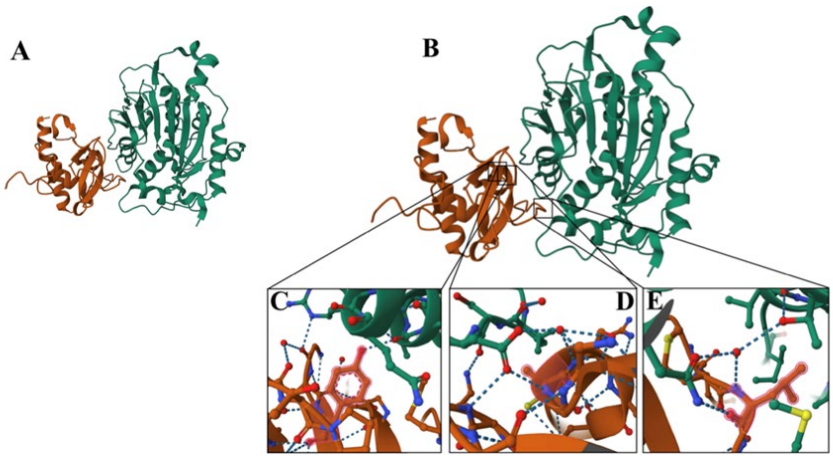

Figure 2. A) A complete view of the Nsp16-Nsp10 complex, B) The complex with magnified binding sites on Nsp10: C) Leucine 48, D) Alanine 74, E) Tyrosine 99.

So far, it has been reported that Nsp16 and Nsp10 bind together to function properly. A probable explanation is that the attractive charges between the two proteins can stabilize SAM binding (Gorkhali et al., 2021). In other words, it can be deduced that Nsp16 depends on Nsp10 to activate its functionality. Therefore, another strategy could also be used for drug development of preventing or destabilizing the binding between Nsp16 and Nsp10 to inhibit their function.

Virtual Screening of Potential Drugs

The 6WVN protein structure file in PDB was chosen because it provides detailed insight into the molecular structure of the Nsp16-Nsp10 complex, including the location of its active sites. Also, the protein has great structural purity and a clear resolution of around 2.00 Å (Minasov et al., 2021). All this information is beneficial in investigating the properties of the protein, which can lead to the development of effective drugs.

A virtual molecular docking and screening algorithm that predicts interaction patterns between proteins and small molecules to evaluate the binding between two molecules is used to select potential drugs. The algorithm screened candidate drugs with high binding coefficients to target proteins' functional domains. The four amino acids on the active sites and the structural information of the Nsp16-Nsp10 complex are input into Cheminformatic Tools and Databases for Pharmacology screen potential Nsp16-Nsp10 binding drugs in the SciFinder/CAS database. The results provide the binding affinity or percentage score between the Nsp16-Nsp10 complex and matched molecules and the properties of potential drugs.

Results

Potential Drug *Levalbuterol*

For the first approach, the drug *Levalbuterol* is selected after screening drug candidates combining the four active sites on of Nsp16 in Nsp16-SAM binding pocket (ASP133, GLU206, LYS49, and LYS173) for its relatively high binding affinity (72.88%) and original use.

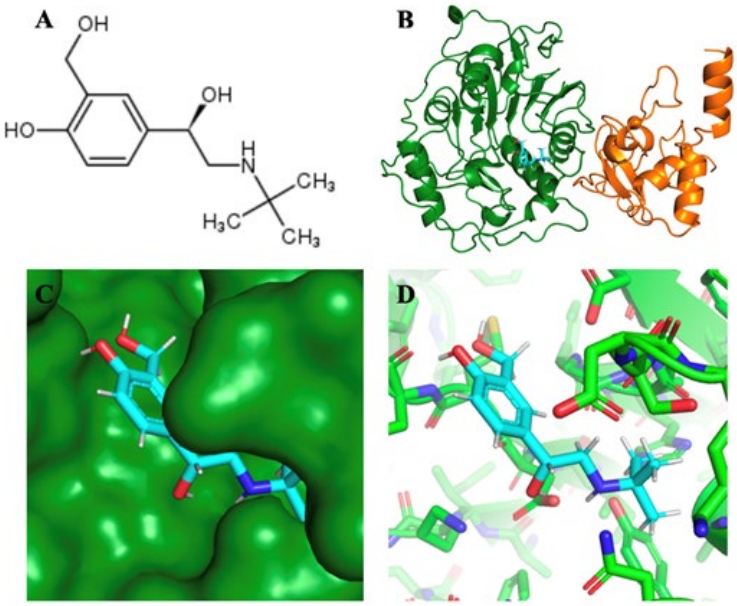

Figure 3. Simulated use of the potential drug *Levalbuterol*. A) The structural formula of *Levalbuterol*, B) Complete view of Nsp16-Nsp10 complex with *Levalbuterol* embedded, C) Close-up view of *Levalbuterol* with the gaussian surface of Nsp16, D) Close-up view of *Levalbuterol* with residues of Nsp16.

Potential Drug *Latanoprostene Bunod*

For the second approach, after screening drug candidates combining the three binding sites between Nsp16 and Nsp10 on Nsp10 (ALA74, LEU48, and TYR99), the drug *Latanoprostene Bunod* (binding to site ASP133 of Nsp16) is selected for its high binding affinity (85.62%) and original use.

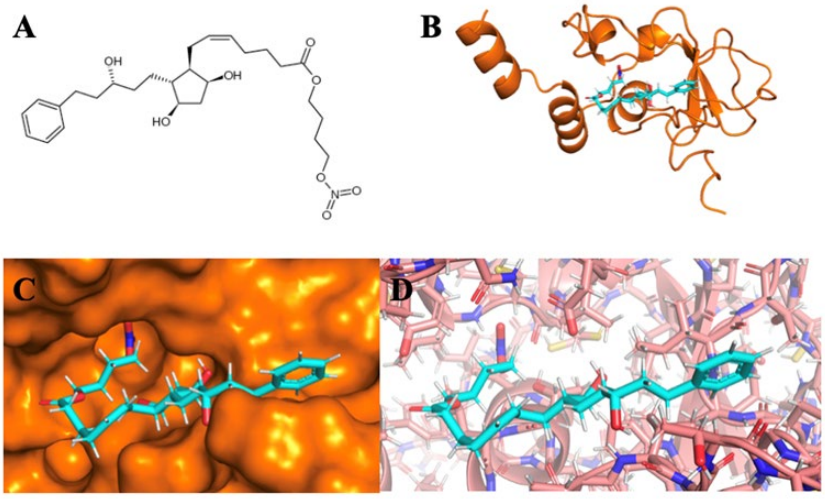

Figure 4. Simulated use of the potential drug *Latanoprostene Bunod*. A) the structural formula of *Latanoprostene Bunod*, B) A complete view of Nsp16-Nsp10 complex with *Latanoprostene Bunod* embedded, C) A close-up view of *Latanoprostene Bunod* (binding to site LEU48 of Nsp10) with the gaussian surface of Nsp16, D) Close-up view of *Latanoprostene Bunod* with residues of Nsp16.

Discussion

The Drug *Levalbuterol* Selected by the First Approach

Levalbuterol is classified as an inhaled corticosteroid drug. Specifically, it's used as an adrenergic bronchodilator to prevent or relieve shortness of breath, coughing, and chest tightness caused by lung disease. Evidence shows that inhaled corticosteroids have been used to treat COVID-19. Because it disrupts the methylation of viral RNA of COVID-19 and can treat symptoms that coincide to some extent with those of COVID-19. Their anti-inflammatory effects can potentially reduce the risk of severe illness caused by excessive inflammation in COVID-19 (Griesel, 2022). Hence, *Levalbuterol* could be a very valuable drug to treat COVID-19.

The Drug *Latanoprostene Bunod* Selected by the Second Approach

Latanoprostene Bunod is a prostaglandin analogue. Although studies on the use of prostaglandin analogues in the treatment of acute respiratory distress syndrome are very rich, based on this literature study and drug properties, *Latanoprostene Bunod* can be developed into a drug for the treatment of COVID-19 in the future. It is helpful in the treatment of covid-19 associated acute respiratory distress syndrome (ARDS), including (1) Improving oxygenation; (2) Alleviating the coagulation dysfunction directly related to SARS-Cov-2; (3) Increasing the production of nitric oxide; (4) Possible anti-inflammatory effects (Mulia et al., 2021). Since acute respiratory distress syndrome (ARDS) associated with covid-19 is one of the most important conditions in the treatment of SARS-Cov-2, *Latanoprostene Bunod* is essential for the treatment of SARS-Cov-2.

Considering the uncommon and unstable nature of the Nsp16-Nsp10 assembly process, future research should be conducted on the evolution of the Nsp16-Nsp10 complex, as well as more potential drugs that inhibit the proper binding between the two proteins.

Conclusion

In this research, we developed a widely feasible drug research process, and used it to select two potential drugs to treat COVID-19. We first overviewed SARS-CoV-2's structure, life cycle and important proteins functioning in its reproduction. Then, we identified that the Nsp16-Nsp10 complex as a critical factor in methylating viral RNA to avoid immune attack from host cells. The fact that Nsp16 have prominent pockets to cap the viral RNA and its dependence on Nsp-10 are vulnerabilities for inhibition; thus, drug molecules can bind to the residues of the catalytic and Nsp10-binding pockets and prevent either SAM or Nsp10 from binding with Nsp16, which disables the complex.

For the first approach, we identified four residues in the Nsp16 catalytic pocket that bind with the SAM molecule. For the second approach, we identified three residues on either Nsp16 or Nsp10 that bind the Nsp16-Nsp10 complex. We screened the FDA drug database according to each of the residues in the two approaches. Then, we evaluate the drugs based on their match score as well as how their original pharmaceutical purpose relates to the treatment of COVID-19 and selected two optimal drugs — *Levalbuterol* and *Latanoprostene Bunod* — as potential drugs to treat COVID-19 infection.

Limitation

The virtual screening of COVID-19 drugs can provide a broad overview of potential drug candidates. However, a limitation associated with virtual screening results is that the list of drug candidates is ranked by binding

affinity. This means that the results are limited by the accuracy of the screening algorithm. This screening process does not account for the real situation, such as the potential interaction of drugs with other medication conditions. Furthermore, current research does not provide a conclusive answer to the functionality and significance of Nsp10 to Nsp16, affecting the reliability of the second approach mentioned above.

Acknowledgments

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