Article title: Investigation of N Terminal Domain of SARS CoV 2 Nucleocapsid Protein with Antiviral Compounds Based on Molecular Modeling Approach

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Keywords: SARS-CoV-2, COVID-19, molecular docking, MD simulations

Investigation of the N-Terminal Domain of SARS-CoV-2 Nucleocapsid Protein with Antiviral Compounds Based on Molecular Modeling Approach: Molecular Docking and Molecular Dynamic Simulation

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Abstract

The recent outbreak of coronavirus disease (COVID-19) in China caused by SARS-CoV-2 virus continually lead to worldwide human infections and deaths. It is currently no specific viral protein targeted therapeutics yet. The nucleocapsid (N) protein of coronaviruses (CoVs) is a multifunctional RNA-binding protein necessary for viral RNA replication and transcription. Therefore, it is a potential antiviral drug target, serving multiple critical functions during the viral life cycle. Herein, we focus here on the potential to repurpose antiviral compounds approved or in development for treating infections caused by human CoVs. For this purpose, we used the docking methodology to better understand the inhibition mechanism of SARS-CoV-2 N protein with this existing 34 antiviral compounds. The results of this analysis were showed that Nafamostat, Rapamycin, Saracatinib, Imatinib and Camostat are the top hit compounds with binding energy (-10.24 kcal/mol, -9.88 kcal/mol, -9.66 kcal/mol, -9.23 kcal/mol, -9.07 kcal/mol) and K_i (0.0313 μ M, 0.05736 μ M, 0.08304 μ M, 0.17224 μ M, 0.22413 μ M). In addition, this analysis also showed that the most common residues that interact with the compounds are *Lys65*, *Phe66*, *Arg 68*, *Glu69*, *Tyr123*, *Gly124*, *Lys127*, *Ile 130*, *Val133* and *Ala134*. These results suggest that these residues are potential drug targeting sites for the SARS-CoV-2 N protein. Subsequently, protein-ligand complex stability was examined with Molecular Dynamics (MD) simulations for the **Nafamostat** compound, which showed the best binding affinity. According to the results of this study, the interaction between the compound and the crucial residues of the target were maintained. Based on this information, we propose guidelines to develop novel N protein-based antiviral agents that target CoVs.

Keywords: SARS-CoV-2, COVID-19, CoVs, N protein, molecular docking, MD simulations

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1. Introduction:

Coronaviruses (CoVs), which are the subject of the basic research of this study, are an envelope and single-stranded of RNA viruses (positive-sense) [1–6]. The classification of CoVs has been based on genomic organization, similarities in genomic sequence, antigenic properties of viral proteins, replication strategies, and structural characteristics of virions, pathogenic, cytopathogenic and physicochemical properties [6]. Until 2019, only six CoVs were known to cause disease in humans: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [7,8]. In late 2019 and early 2020, when a novel coronavirus was discovered to be the cause of a large and rapidly spreading outbreak of respiratory disease, including potentially fatal pneumonia, in Wuhan, China.

The newly discovered Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was characterized as a betacoronavirus and recognized as the seventh discrete coronavirus species capable of causing human disease [9]. The disease caused by the virus is officially named Coronavirus Disease 2019 (Covid-19) by World Health Organization (WHO). The emerged global epidemic spread rapidly with 823,626 confirmed cases and 40,598 deaths across 204 countries (COVID-19 situation Report WHO, 1 April 2020). Despite remarkable efforts on containing spread of the virus, there is no specific targeted therapeutic currently.

The SARS-CoV-2 as a betacoronavirus with approximately 80% similarity in genetic sequence to SARS-CoV [9,10] overall, and more than 90% sequence identity with respect to various essential enzymes [11]. The nucleocapsid (N) protein of coronaviruses (CoVs) plays an essential role in the virus structure, the replication and transcription of CoVs via interactions with the large positive-strand RNA viral genome. The pivotal roles of N protein are binding to viral RNA genome forming a ribonucleoprotein (RNP) complex which is critical for maintaining an ordered RNA conformation suitable for replicating and transcribing the viral genome [12]. Other studies indicate the N protein in the regulation of cellular processes, such as actin reorganization, host cell cycle progression, and apoptosis [13,14]. Besides, it is shown to induce protective immune responses against CoV and is a significant antigen to develop a sensitive diagnostic assay [15].

The common domain architecture of coronavirus N protein contains two structurally independent domains which are called as N-terminal domain (NTD) and C-terminal (CTD) dimerization domain linked by a charged linker region which is rich in Ser/Arg (SR-rich linker). Previous studies have revealed that the NTD are responsible for RNA binding, CTD for oligomerization, an SR-rich linker for primary phosphorylation, respectively [16–19]. Many studies indicated that several critical residues have been identified for RNA binding and virus infectivity in the NTD of CoVs N proteins [20–22]. Therefore, The NTD domain of SARS-CoV-2 N protein is one of the most attractive targets for the development of new drugs against CoVs due to its important role in the replication and transcription of the virus.

Given the serious problems of the 2019-nCoV outbreaks, there is an urgent need for a new drug against COVID-19. Therefore, drug repurposing studies for coronavirus infections may be an alternative approach that can help discover potential antiviral molecules relatively quickly. Since the molecules considered in these studies go through

several stages and have well-defined profiles, they will be excellent candidates in case of disease emergencies or outbreaks, without the need for long-term preclinical studies [23,24].

In this study, molecular modelling approach is applied for repurpose of antiviral agents, which is approved or under development to treat infections caused by human CoVs, against SARS-CoV-2 N protein. This study has identified drug molecules, which can be directly tested for *in vitro* and *in vivo* studies, to combat a global threat of COVID-19.

2. Materials and methods

2.1 Structure Preparation and Molecular docking

The crystal structure SARS-CoV-2 N protein was downloaded from protein data bank web site (<http://www.rcsb.org/pdb>) (PDB ID: 6VYO, Resolution 1.7 Å). This structure includes the RNA binding domain (amino acids range 50-173) of the N protein. Small antiviral compounds used in docking studies were obtained from PubChem as SDF form and were drawn in the Hyperchem software [25] then subjected to conformational search with geometric optimization. Possible docking modes between small compounds and the SARS-CoV-2 N protein were studied using the Autodock 4.2 [26] and Lamarckian genetic algorithm was employed for blind docking simulations. A grid box dimensions of 126, 126, and 126 points in x, y, and z directions was set with a grid spacing of 0.375 Å. The program was run for a total number of 100 Genetic algorithm runs. The default settings were applied for all other parameters. The visualization of results was performed with the help of the BIOVIA Discovery Studio 2018 [27].

2.2 Molecular Dynamics Simulations

MD simulations were performed for the enzyme with compound using GROMACS 5.0.7 package [28]. Topology parameters were prepared with CGenFF Server [29] for the ligands and with CHARMM36 all-atom forcefield [30] for the protein. Protein-ligand complexes were placed in a dodecahedral unit cell shape with explicit TIP3P water models. A short energy minimization (1000 steps) was carried out with Steepest Descent method. Following equilibration for 100 ps in NVT and 100 ps in NPT then production for 10 ns in NPT were performed for each complex at constant temperature (300 K) and pressure (1 atm). During the production run, the timesteps were set to 2 fs respectively. The root mean square deviation (RMSD), root mean square fluctuation (RMSF) and radius of gyration (Rg) were examined during the complete MD simulation.

3. Results and Discussions

3.1 Identification of SARS-CoV-N protein binding antiviral compounds with molecular docking

Previous analyzes indicate that the catalytic regions of SARS-CoV-2 enzymes are highly conserved and share a high sequence similarity with the corresponding SARS-CoV and MERS-CoV protein [31]. Accordingly, it is likely that important drug-binding pockets in viral proteins are protected in human CoVs. It is, therefore, reasonable to consider repurposing existing human CoVs antiviral agents for SARS-CoV-2.

In light of this information, we focus here on the potential to repurpose antiviral agents used to treat infections caused by human CoVs [32] against SARS-CoV-2 with molecular docking. Based on this study result, we analyzed the 34 antiviral compounds (see in Supplementary Material Table S1) tested and found that Nafamostat, Rapamycin, Saracatinib, Imatinib and Camostat was the highly binding affinity against the SARS-CoV-2 with low micromolar K_i values among the evaluated compounds (see in Table 1).

Experimental studies showed that, the Nafamostat was the most potent inhibitor (IC_{50} : 0.1 μ M) to ability of it to block MERS-CoV infection [33] and inhibited the SARS-CoV-2 at a low-micromolar concentration (EC_{50} = 2.12 μ M; CC_{50} > 35.53 μ M; SI > 16.76) [34] *in vitro*. Our *in silico* study showed that, Nafamostat has the best binding affinity to the SARS-CoV-2 N protein (Binding energy: -10.24 kcal/mol, K_i 0.0313 μ M) such as experimental study. This compound has been observed to be bound the residues *Phe66*, *Ala125*, *Lys127*, *Ile130*, *Ala134* and *Trp132* of SARS-CoV-2 N protein with a hydrogen bond (see in Figure 1). It could be suggested that these residues can contribute to enhancing ligand affinity for SARS-CoV-2 N protein.

In addition, Nafamostat, Rapamycin, Saracatinib, Imatinib and Camostat compounds, which show a high affinity for SARS-CoV-2, interacted with the stronger H-bond the residues *Ala134*, *Trp132* and *Lys127* in the SARS-CoV-2 RNA-binding site domain (See in Figure 2). It could be these residues key roles inhibition mechanisms. Furthermore, these antiviral agents have frequent interactions with *Lys65*, *Phe66*, *Arg 68*, *Glu69*, *Tyr123*, *Gly124*, *Lys127*, *Ile 130*, *Val133* and *Ala134* in SARS-CoV-2 N protein (see in Supplementary Material Table S1). In line with this study, we can suggest that these residues are potential drug targeting sites for the SARS-CoV-2 N protein.

3.2 Molecular Dynamics Simulation analysis of SARS-CoV-2 N with most effective compound

The molecular dynamics simulation was carried out on these protein-ligand complexes to probe the stabilities of ligand binding modes for most effective inhibitor Nafamostat. This simulation system was conducted in dodecahedron simulation boxes solvated with TIP3P water and neutralized with Na^+ ion and then, 10ns-MD simulation was performed. These results indicated that Nafamostat is kept to maintain interactions with *Lys65*, *Gly69*, *Gln70*, *Pro67*, *Phe66*, *Lys123*, *Trp132* and *Ala134* of SARS-CoV-2, especially the

hydrogen bond interaction between the compound and the *Ala134* was stable during the 10-ns MD simulation time (see in Figure 1).

Likewise, the range of 0.1-0.3 nm, 0.05-0.2 nm and 0.5-0.8 nm were observed with RMSD between conformation of these protein, ligands and protein-ligand complex in the MD simulations for a period of 10 ns. These results suggest that the stability of the interactions in the complex structure is reliable during the simulation (see in Figure 3A). The average RMSF of the SARS-CoV-2 is 0.09 nm and 0.4 nm in the complexes and the maintain interaction residues (*Lys65*, *Gly69*, *Gln70*, *Pro67*, *Phe66*, *Lys123*, *Trp132* and *Ala134*) lowest fluctuations (rigid behavior) (see in Figure 3B).

Besides, we examined radius of gyration (calculated as the root mean square distance of the objects from each atom of protein to their center of gravity or a given axes) for proteins in the MD simulation data. The Rg of a protein is a measure of its compactness. It is regarding how regular secondary structures can be compactly packed into 3D structure of the protein. This analysis shows that, Rg values (1.5-1.55 nm) of the proteins remain stable, in its compact (folded) form over the course of 10 ns at 300 K (see in Figure 3C). Finally; the interaction total energy of the system was calculated for these complex structures. This energy average value is -386.647 kcal/mol for SARS-CoV-2 & Nafamostat protein-ligand complex (see in Figure 3D).

4. Conclusions

Many studies demonstrated that N proteins will be a good drug-targeting candidate in other CoVs since they process several critical functions, such as RNA genomic packing, viral transcription and assembly, in the infectious cell [14,16,17,19,22,35–37]. However, the molecular mechanism basis for newly emerged novel SARS-CoV-2 N protein with inhibitors remain largely unknown. Understanding these aspects should facilitate the discovery of agents that specifically inhibits CoV genome replication.

Here, we analyzed the interaction mechanism of therapeutic antiviral compounds against to SARS-CoV-2 N RNA binding domain at the molecular level with the help of structure-based computational methods. Structure-based drug discovery has been known to be an advance approach for the discovery and refinement of therapeutic agents [38,39]. Because it is quite difficult and costly to examine interaction of many compounds with enzymes based on the molecular level in the experimental conditions. Thus, these computational methods are providing a significant advantage in the hit identification phase of the costly drug discovery process.

These study results indicated that Nafamostat, Rapamycin, Saracatinib, Imatinib and Camostat compounds are effectively inhibits the SARS-CoV-2 N protein. These compounds can be clinically tested and used for the treatment of COVID-19. Furthermore, *Lys65*, *Phe66*, *Arg 68*, *Glu69*, *Tyr123*, *Gly124*, *Lys127*, *Ile 130*, *Val133* and *Ala134* are potential drug targeting sites for the SARS-CoV-2 N protein. Based on this information, we propose guidelines to develop novel antiviral agents that target SARS-CoV-2.

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Appendix A. Supplementary data

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Name	Binding Energy	Ki (uM)	2D-Structure
Nafamostat	-10.24	0.0313
Rapamycin	-9.88	0.05736
Saracatinib	-9.66	0.08304
Imatinib	-9.23	0.17224
Camostat	-9.07	0.22413

Table 1. Two-dimensional (2D) structures, binding energy and inhibition constant (Ki) values of the top 5 five compounds from each docking simulation.

Figure 1: The two-dimension (2D) interaction analysis of SARS-CoV-2 with Nafamostat before and after MD simulation.

Figure 2: The two-dimension interaction analysis (2D) of SARS-CoV-2 with the top 5-compounds from each docking simulation.

Figure 3: (A) The RMSD trajectory of protein-ligand complex, protein and ligand during the MD simulation, (B) RMSF profile of SARS-CoV N protein in the protein-ligand complex, (C) The radius of gyration analysis of SARS-CoV N protein in the protein-ligand complex, (D) The potential and total energy interaction trajectory of protein-ligand complex during the MD simulation.