

RESEARCH ARTICLE

Computational drug repurposing study of the RNA binding domain of SARS-CoV-2 nucleocapsid protein with antiviral agents

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Abstract

The recent outbreak of coronavirus disease (COVID-19) in China caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to worldwide human infections and deaths. 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. This study addresses the potential to repurpose antiviral compounds approved or in development for treating human CoV induced infections against SARS-CoV-2 N. For this purpose, we used the docking methodology to better understand the inhibitory mechanism of this protein with the existing 34 antiviral compounds. The results of this analysis indicate that rapamycin, saracatinib, camostat, trametinib, and nafamostat were the top hit compounds with binding energy (−11.87, −10.40, −9.85, −9.45, −9.35 kcal/mol, respectively). This analysis also showed that the most common residues that interact with the compounds are *Phe66*, *Arg68*, *Gly69*, *Tyr123*, *Ile131*, *Trp132*, *Val133*, and *Ala134*. Subsequently, protein-ligand complex stability was examined with molecular dynamics simulations for these five compounds, which showed the best binding affinity. According to the results of this study, the interaction between these compounds and crucial residues of the target protein were maintained. These results suggest that these residues are potential drug targeting sites for the SARS-CoV-2 N protein. This study information will contribute to the development of novel compounds for further in vitro and in vivo studies of SARS-CoV-2, as well as possible new drug repurposing strategies to treat COVID-19 disease.

KEYWORDS

COVID-19, CoVs, drug repurposing, MD simulations, molecular docking, N protein, RNA binding domain, SARS-CoV-2

1 | INTRODUCTION

Coronaviruses (CoVs) that are the subject of the main research of this study are large, enveloped and single-stranded (positive-sense) RNA viruses.^{1–6} Until 2019, only six CoVs have been observed to cause disease in humans: (a) HCoV-229E,

(b) HCoV-OC43, (c) HCoV-NL63, (d) HCoV-HKU1, (e) Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and (f) Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV).^{7,8} In late 2019 and early 2020 in Wuhan, China, a novel coronavirus was discovered to be the cause of a rapidly spreading outbreak of the respiratory disease.

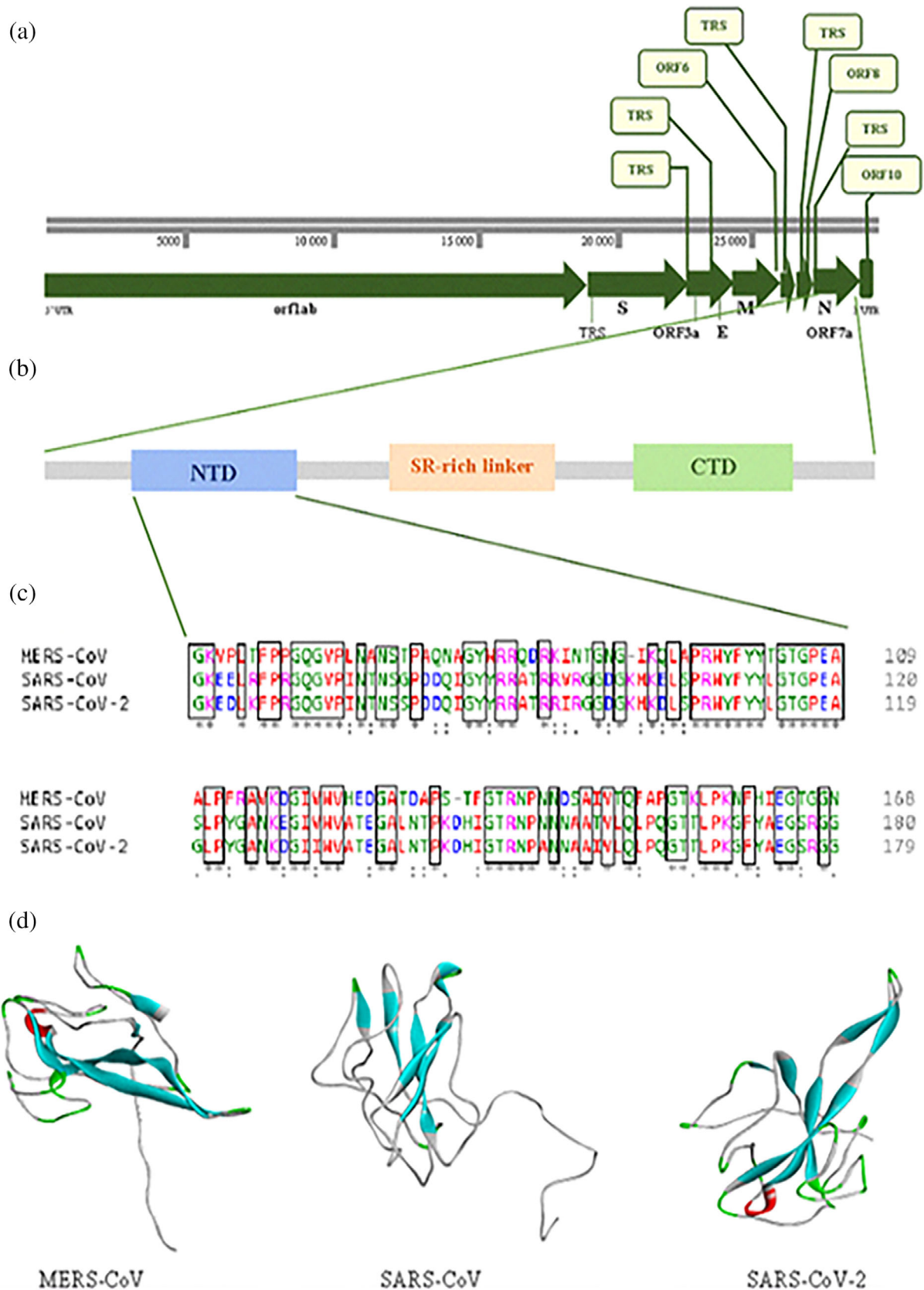


FIGURE 1 Sequence features and structures of CoVs nucleocapsid protein. (a) Complete genome of SARS-CoV-2. (b) Domain architectures of coronavirus nucleocapsid protein. (c) Multiple sequence alignment of CoVs N-NTD analysis with Clustal Omega Service (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). (d) 3D structures of CoVs N-NTD (MERS-CoV: PDB code: 4UD1, SARS-CoV: PDB code: 1SSK, SARS-CoV-2: PDB code: 6VYO)

The newly discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was characterized as a betacoronavirus and considered the seventh discrete coronavirus species that can cause a human disease.⁹ The disease caused by the virus was officially named Coronavirus Disease 2019 (COVID-19) by the World Health Organization (WHO). The emerging global epidemic spread rapidly with 42,512,186 confirmed cases and 1,147,301 deaths across 218 countries (COVID-19 situation Report WHO, October 25, 2020). Given the serious nature of the 2019-nCoV outbreaks, an urgent need for a new drug against COVID-19 has arisen.

The amino acid sequence of the SARS-CoV-2 shows a high similarity to both SARS-CoV and MERS-CoV. The sequence has approximately 82% similarity in its amino acid sequence to SARS-CoV^{9,10} overall, and more than 90% sequence identity with respect to various essential enzymes and structural proteins.¹¹ Especially, the nucleocapsid protein of SARS-CoV-2 has a 90.52% sequence identity compared to SARS-CoV.

In this case, medical chemistry efforts for new therapeutic options for human CoVs can be very helpful to identify potential treatments for SARS-CoV-2.^{12,13} Therefore, drug repurposing studies for coronavirus infections are used as an alternative approach that can help discover potential antiviral molecules quickly and reliably. Since the molecules considered in these studies proceed through several stages

and have well-defined profiles, they would be excellent candidates in case of disease emergencies or outbreaks, without the need for long-term preclinical studies.^{14,15}

Coronaviruses contain a very large RNA genome of about 30 kb in length, and a unique replication strategy. These viruses encode two overlapping open-reading frames (ORF) that transform into two polyproteins, (a) pp1a and (b) pp1ab. These polyproteins are also processed to generate four main structural proteins (a) Spike (S), (b) Membrane (M), (c) Envelope glycoproteins (E), and (d) Nucleocapsid (N) and 16 non-structural proteins (nsps) as shown as in Figure 1.¹⁶

The N protein of coronaviruses (CoVs) plays a pivotal role in the viral structure in addition to, the replication and transcription of CoVs via interactions with the large positive-strand RNA viral genomes.¹⁷ RNA chaperones are nonspecific nucleic acid binding proteins that facilitate folding of RNA and protein substrates into their correct functional structures.¹⁸ The main function of the N protein of CoVs is to bind to the viral RNA genome to promote the correct folding of the hammerhead ribozyme avoiding unproductive RNA conformations and make them into a helical capsid structure or ribonucleoprotein (RNP) complex, whose packaging is critical to viability.^{19,20} Because of this function, it is suggested that the CoV's N protein is an RNA chaperone and conducts a special step in viral genomic RNA replication.²¹

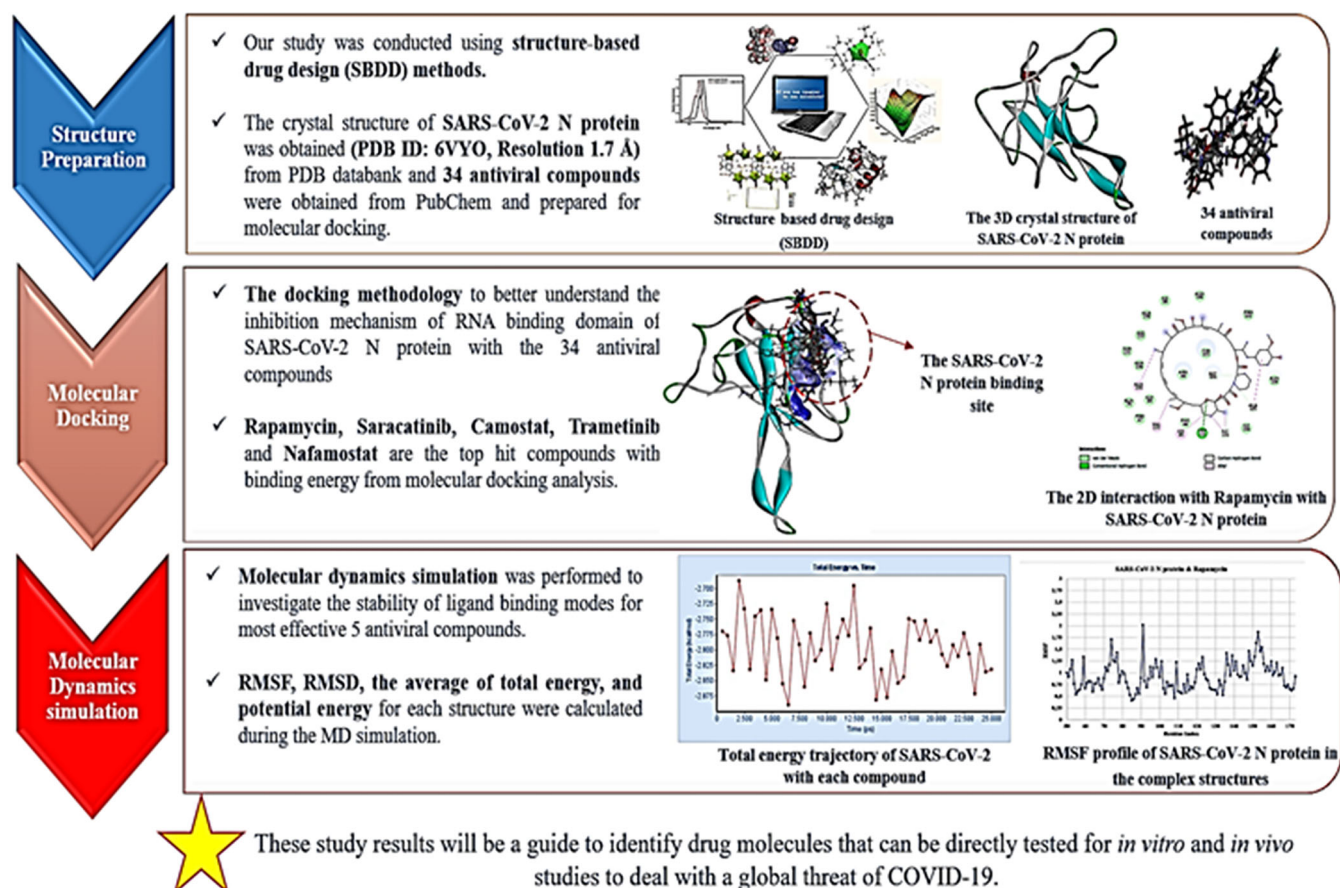


FIGURE 2 Flowchart showing the steps to screen antiviral compounds for the SARS-CoV-2 N protein

Furthermore, *in vitro* studies showed that the N protein promotes the binding of DNA and viral transcriptional regulatory sequence (TRS) RNAs, confirming its role as an RNA chaperon in a coronavirus associated system.¹⁸ The N protein also plays a major role in increasing the yield of transcription and assembly during virion assembly by interacting with the M protein.^{22,23} In addition, the N protein is involved in the regulation of cellular processes, such as actin reorganization, host cell cycle progression, and apoptosis.^{24,25} Besides, the N protein has been shown to induce protective immune responses against CoV and is a significant antigen to develop a sensitive diagnostic assay.²⁶ Thus, it is one of the potential antiviral drug targets, serving critical functions throughout the viral life cycle.

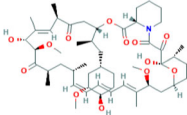
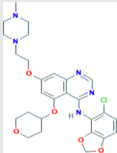
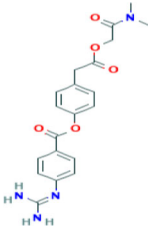
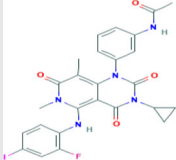
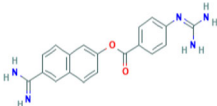
The CoVs N protein has two main domains: (a) the RNA binding domain (N-terminal domain [NTD]) and the (b) C-terminal dimerization domain (CTD). The NTD domain is known to play an important role in CoV replication and transcription by binding to the 3' end of the viral RNA genome due to electrostatic interactions of positive amino acids.^{19,27} Some *in vitro*, *in vivo*, and *in silico* studies have revealed that several critical amino acids play a role in RNA binding and virus

infectivity via the NTD of CoV N proteins. Specifically, they indicated that several conserved aromatic residue groups in the SARS-CoV N-NTD were identified for RNA binding and virus infectivity.^{27–30} Therefore, SARS-CoV N-NTD is considered an important drug target, as processes involving virus viability, such as viral replication, transcription, and assembly begin with the binding of NTD to RNA.^{22,31}

In recent times, three-dimensional (3D) crystal structures of CoVs N-NTD solved in isolation or complex with other molecules are available in the protein database (PDB) as shown in Figure 1. However, these important roles in their biological mechanisms are largely unknown at the molecular level. Understanding these aspects will make it easier to develop agents that specifically inhibit CoV genome replication, transcription, and viral assembly.

In light of this information, the current study aimed to identify the structural mechanism of SARS-CoV-2 N protein with approved drugs and antiviral agents and elucidated the residues of this enzyme that play an important role in the inhibition of CoV genome replication at the molecular level. Accordingly, a computational molecular

TABLE 1 Two-dimensional (2D) structures, interaction residues, indication, lowest binding energy and inhibition constant (K_i) values of the top five compounds from each docking simulation. Bold colors indicate common interactions in the binding site of SARS-CoV-2 N-NTD domain

Ligand name	Binding energy (kcal/mol)	Inhibition Constant (μ M)	SARS-CoV-2 N residues interacting with ligands	Compound structure	Indication
Rapamycin	−11.87	0.001	Lys65, Phe66 , Pro67, Arg68 , Gly69 , Gln70, Ile84, Pro122, Tyr123 , Gly124, Ala125, Asn126, Ile130, Ile131 , Trp132 , Val133 , Ala134 , Thr135, Glu136, Gly137, Ala138, Asn140		Antiviral drug used for the treatment of MERS-CoV. ³⁵
Saracatinib	−10.40	0.023	Lys65, Phe66 , Pro67, Arg68 , Gly69 , Gln70, Tyr123 , Gly124, Ile131 , Trp132 , Val133 , Ala134 , Thr135, Glu136, Gly137, Ala138		Antiviral drug used for the treatment of MERS-CoV. ³⁶
Camostat	−9.85	0.060	Lys65, Phe66 , Arg68 , Gly69 , Tyr123 , Gly124, Ala125, Asn126, Ile130, Ile131 , Trp132 , Val133 , Ala134 , Thr135, Glu136, Gly137, Ala138		Antiviral drug used for the treatment of SARS-CoV, MERS-CoV, HCoV-229E ³⁷
Trametinib	−9.45	0.118	Phe66 , Pro67, Arg68 , Gly69 , Gln70, Tyr123 , Ile131 , Trp132 , Val133 , Ala134		Antiviral drug used for the treatment of SARS-CoV, MERS-CoV. ³⁵
Nafamostat	−9.35	0.140	Phe66 , Pro67, Arg68 , Gly69 , Gln70, Gly71, Tyr123 , Gly124, Ala125, Asn126, Lys127, Asp128, Gly129, Ile130, Ile131 , Trp132 , Val133 , Ala134		Antiviral drug used for the treatment of 2019-nCoV, MERS-CoV ^{38,39}

modeling approach was applied for the repurpose of these agents, which is approved or under development to treat infections caused by human CoVs, against SARS-CoV-2 N protein. Computational approaches such as structure-based virtual screening provide significant savings in experimental cost and time in drug discovery. These study results will be a guide for identifying drug molecules that can be directly tested for in vitro and in vivo studies to deal with a global threat of COVID-19.

2 | MATERIALS AND METHODS

Our study was conducted using structure-based drug design (SBDD) methods. In the first stage, structural analyses of the SARS-CoV-2 N protein (target protein) and antiviral compounds (ligand compounds) were performed. Next, molecular docking was applied to study the mechanism of interaction between target protein and ligand compounds. In the last stage, the stability analysis of the important contact interaction between these compounds and the target enzyme was investigated by molecular dynamic (MD) simulations (see in Figure 2). The visualization of the results was carried out with the help of BIOVIA Discovery Studio.

2.1 | Structure preparation of protein and ligands

The crystal structure of the NTD of the SARS-CoV-2 N protein was obtained from the PDB web site at <http://www.rcsb.org/pdb> (PDB ID: 6VYO, Resolution 1.7 Å). This structure contains a range of 50–173 amino acids. Water and ion molecules were removed from this crystal structure. The structure was then prepared for continuous electrostatic calculations by reconstructing the missing atoms in the crystal structure with the APBS-PDB2PQR³² that added hydrogens, assigned atomic charges and radii from specified force fields. Antiviral compounds for molecular docking studies were obtained from PubChem as SDF form and the 3D versions of these compounds were drawn in Marvin Sketch (Marvin 18.12, ChemAxon (<https://www.chemaxon.com>)). To make sure that all values of the heavy atoms were satisfied, all of the hydrogen atoms were added these structures, geometrically cleaned, and then converted to .pdb format with BIOVIA Discovery Studio.³³

2.2 | Molecular docking

Blind docking is used in cases in which the ligand binding site of the protein is not known. It can be used to predict the possible ligand binding sites on the whole protein target, and it is anticipated that each ligand will bind to the site with the lowest calculated docking energy. In this study, the ligand binding site of the NTD domain of the target SARS-CoV-N protein is not known yet. Therefore, blind docking studies were performed to estimate the binding energies of antiviral compounds to the therapeutic protein targets of SARS-CoV-2 N

TABLE 2 The non-covalent interaction analysis of SARS-CoV-2 N protein with top five compounds initial and final stage in MD simulation. The stable interactions are indicated in bold color

Complexes	Initial stage		Final stage	
	H-bond interaction	H-bond length	H-bond interaction	H-bond length
SARS-CoV-2 N-NTD & Rapamycin	Trp132, Ile131 , Gln124 , Ala125	2.81, 1.76, 2.73, 2.68	Lys65 , Ala125 , Ile131 , Ala134 , Val133	1.94, 2.36, 2.95, 2.41, 2.97
SARS-CoV-2 N-NTD & Saracatinib	Ala134 , Lys65 , Gln124 , Gln70 , Ala138 , Glu136 , Gly69	2.02, 2.19, 2.18, 2.88, 2.96, 2.87, 2.81	Arg68 , Val133 , Tyr123 , Ala134 , Ile131 , Phe66 , Lys65	2.73, 2.92, 2.89, 2.58, 2.71
SARS-CoV-2 N-NTD & Camostat	Glu136 , Phe66 , Gln69 , Ala134 , Ala138 , Lys65 , Arg68 , Ile131 , Ala125	2.36, 2.11, 1.85, 2.20, 2.15, 2.86, 2.94, 1.85, 3.66	Trp132 , Arg68 , Ala134	2.18, 2.38, 2.28, 1.89, 1.94, 2.44, 2.78, 2.33
SARS-CoV-2 N-NTD & Trametinib	Gln69 , Ala134 , Arg68 , Trp132	2.16, 1.90, 2.67, 2.81	Lys65 , Phe66 , Gln69 , Ala134 , Lys65 , Val133	2.28, 2.50, 2.13, 2.51, 2.98, 2.71
SARS-CoV-2 N-NTD & Nafamostat	Ala134 , Gln70 , Pro67 , Asn126 , Ile130 , Gln124 , Trp132	2.09, 1.93, 1.88, 2.02, 2.61, 2.69, 1.84	Asp63 , Trp132 , Thr135 , Asp128 , Asn126	2.13, 2.43, 1.95, 1.85, 2.14
				Hydrophobic interaction
				Lys65 , Ala134 , Ile131 , Arg68 , Ile84
				Tyr123 , Arg68 , Ala134 , Trp132 , Ile131
				Val133 , Ile131 , Phe66 , Ala134
				Arg68 , Val133 , Ile131

protein using the AutoDock 4.2.³⁴ The PDB files of the protein and ligands prepared in the previous step were converted to the PDBQT format. All molecular docking studies were performed with a total number of 200 runs with the Lamarckian Genetic algorithm and grid box dimensions of 126, 126, and 126 points in x, y, and z directions, respectively, which were set with grid spacing of 0.375 Å between them. The default settings were applied for all other parameters.

2.3 | Molecular dynamic simulations

MD simulations were performed for structural stability, conformational changes, and other critical aspects of protein function analysis of the protein-ligand complex. This analysis was carried out using the NAMD module of the BIOVIA Discovery Studio³³ for SARS-CoV-2 with the top five compounds selected as a result of molecular docking. First, energy minimization (1,000 steps) was carried out with Steepest Descent algorithm, and then with the Conjugate Gradient algorithm (1,000 numsteps), provided that a low-energy starting point is provided to

subsequent dynamic stages. Heating and equilibration were performed distribute the energy in the system in accordance with all degrees of freedom and to ensure that the system reached thermal equilibrium at the target temperature of 300 K. The production run was carried out at 25 ns with both a constant temperature and volume canonical ensemble (NVT) for each complex. During this run, the timesteps were set to 2 fs. The root mean square deviation and root mean square fluctuation (RMSD and RMSF, respectively) were examined using MD trajectories during the complete MD simulation.

3 | RESULTS AND DISCUSSIONS

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

In this study, we used molecular docking to focus on 34 approved antiviral agents used to treat human CoVs infections against the SARS-

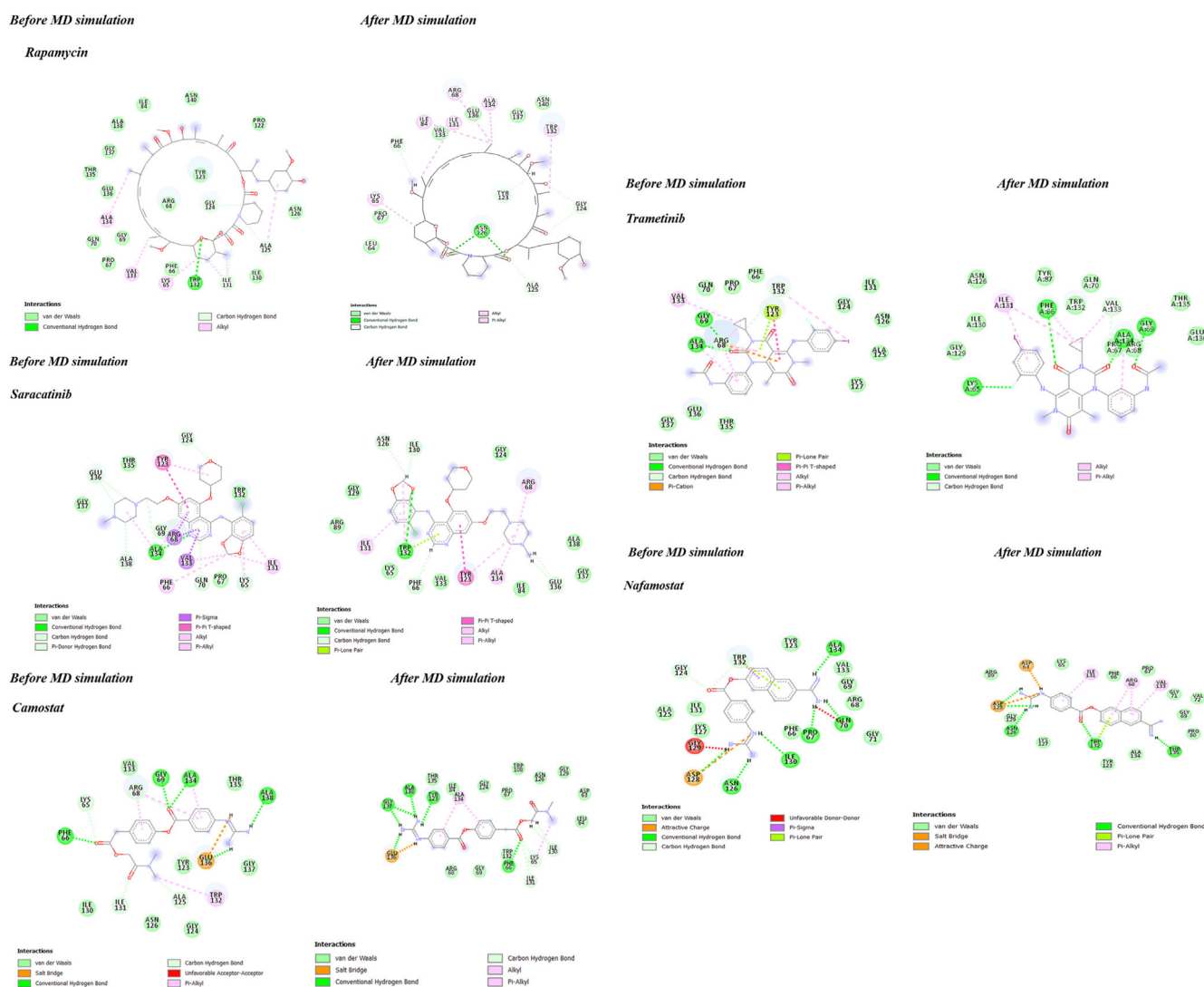


FIGURE 3 Two-dimensional (2D) analysis of the lowest energy binding conformations of SARS-CoV-2-N-NTD and the top five compounds is given

CoV-2 N protein (Table S1). Based on results, we found that rapamycin has the best binding affinity to the SARS-CoV-2 N protein (Binding energy: -11.87 kcal/mol with low micromolar K_i values (K_i : <0.001 μM) among the 34 compounds (see Table 1, Table S1). Besides, saracatinib (-10.40 kcal/mol), camostat (-9.85 kcal/mol), trametinib (-9.45 kcal/mol), and nafamostat (-9.35 kcal/mol) also exhibited significant binding affinity.

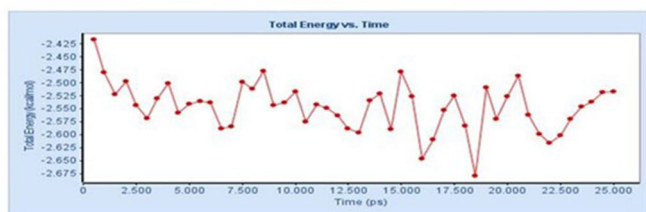
Previous studies have indicated that the catalytic regions of SARS-CoV-2 proteins are highly conserved, sharing more than 90% sequence similarity with the corresponding SARS-CoV and MERS-CoV proteins.⁴⁰ Accordingly, sequence identities between the sub-families of the receptor and, target complexes are expected to share the same binding mode (drug binding pockets) and exhibit similar activities. The high efficiency of these drug molecules against various viruses, especially MERS-CoV and SARS-CoV has already been proven by many experimental studies. For instance, rapamycin has been shown to be a key factor in regulating the replication of various viruses, including Andes orthohantavirus and CoVs. Rapamycin has also been reported to effectively block viral protein expression and virion release.⁴¹ Also, in an in vitro study, it was reported that rapamycin at a 10 μM inhibited MERS-CoV infection by 61% and at the lowest concentration tested (0.1 μM) by 24%. The other clinical trial reported that saracatinib, which is an inhibitor of the Src/abl family of kinases, exhibited significant antiviral activity against MERS-CoV with an $\text{EC}_{50} = 2.9$ μM and $\text{CC}_{50} > 50$ μM , resulting in selectivity (SI) of about >17 . Moreover, Shin et al also indicated that saracatinib showed broad antiviral activity against hCoV-OC43 with an $\text{EC}_{50} = 5.1$ μM .³⁶ Furthermore, nafamostat was the most potent

inhibitor (IC_{50} : 0.1 μM) based on its capability of blocking MERS-CoV infection⁴² and inhibition of SARS-CoV-2 at a low-micromolar drug concentration in vitro with an $\text{EC}_{50} = 2.12$ μM ; $\text{CC}_{50} > 35.53$ μM , and $\text{SI} > 16.76$.³⁸ Likewise, trametinib and camostat demonstrated inhibitory activity against MERS-CoV and SARS-CoV infection at low concentrations.^{35,43} As a result, our in silico study results are compatible with available experimental data.

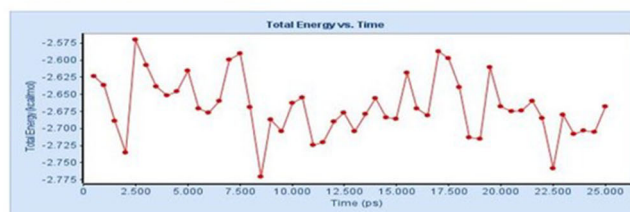
In addition, in this study, the energetically favorable binding mechanism of the compounds was predicted and a better understanding of this mechanism at the molecular level was provided. Accordingly, rapamycin, the compound with the best binding affinity, was observed to bind to the residues *Gly124*, *Ala125*, *Ile131*, and *Trp132* of SARS-CoV-2 N protein with a hydrogen bond and *Lys65*, *Ala125*, *Ala134*, and *Val133* with an alkyl bond (see Table 2 and Figure 3). Besides, other significantly effective compounds mainly common interacted with *Phe66*, *Arg68*, *Gly69*, *Tyr123*, *Ile131*, *Trp132*, *Val133*, and *Ala134* (see Table 1), which are conserved amino acids in the MERS-CoV, SARS-CoV, and SARS-CoV-2 N-NTD domains (see Figure 1c). The results of this study showed that these compounds bind with high affinity to conserved sites in human CoVs, such as MERS-CoV and SARS-CoV. The conserved sites in the N-NTD is responsible for RNA binding and virus infectivity.

Thus, it can be suggested that conserved amino acids involved in the non-covalent interactions may contribute to increase ligand binding affinity for human CoV proteins, facilitating the binding of drug molecules to the NTD-N-protein, thereby reducing the efficacy of the N protein to prevent coronavirus infections.

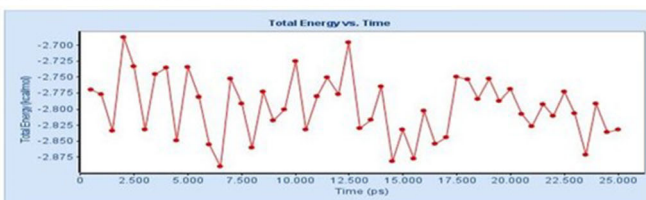
SARS-CoV-2 N-NTD & Rapamycin



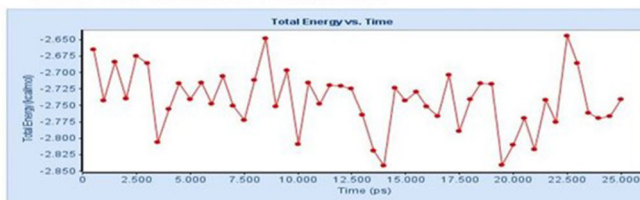
SARS-CoV-2 N-NTD & Saracatinib



SARS-CoV-2 N-NTD & Camostat



SARS-CoV-2 N-NTD & Trametinib



SARS-CoV-2 N-NTD & Nafamostat

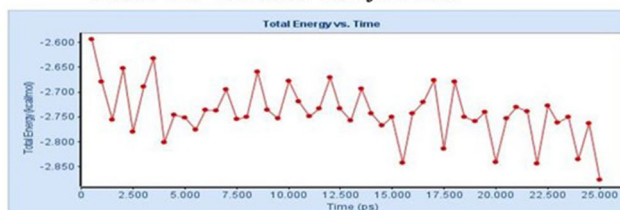


FIGURE 4 The total energy trajectory of SARS-CoV-2 N-NTD and top 5 compounds at the phase (25 ns) in the simulation

3.2 | Molecular dynamics simulation analysis of SARS-CoV-2 N with most effective compounds

The molecular dynamic simulations were carried out on these protein-ligand complex to probe the stabilities of ligand binding modes for most effective compounds. Accordingly, the non-covalent interaction analysis, RMSF, RMSD, the average of total energy, and potential energy for each structure were calculated during the MD simulation (see Table 2 and Figures 3 and 4, Table S2).

Non-covalent interactions such as hydrogen bonding and hydrophobic interactions, play a key role in stabilizing energetically favored ligands at the active site of a protein structure, and help improve binding affinity and drug efficacy. Thus, we also analyzed and compared the stabilization of these interactions in the dynamic process at the initial and final stages to understand that the protein-ligand complex system was stably preserved during the MD simulation analysis. Our analyses indicated that rapamycin, which was most efficient according to molecular docking analysis, continued to maintain interactions with *Lys65*, *Gly124*, *Ala125*, *Ile131*, and *Ala134* of SARS-CoV-2 during the 25-ns MD simulation period. Especially, the hydrogen bond interaction with *Gly124* and *Ala125* was stable and stronger in the SARS-CoV-2 N-rapamycin complex structure (bond length: 2.68 to 2.36 and, 3.34 to 2.95) after the MD simulation. Likewise, the hydrogen bond interaction with *Gly136* in SARS-CoV-2 N-saracatinib and camostat, *Gln69* and *Ala134* in SARS-CoV-2 N-trametinib, *Asn126*, and *Trp132* in SARS-CoV-2 N-nafamostat continued to interact steadily during the MD simulation. The results of this analysis showed that these important contacts were preserved in the SARS-CoV-2 N-potent compounds (see in Figure 3 and Table 2). In addition, occupancy values were calculated for SARS-CoV-2 residues and hydrogen bond interactions that were below 5.0 Å between compounds. Higher occupancy values were obtained for shorter and, therefore, more stable interactions. According to the results of this analysis, these values were found to be high in important amino acids interacting in the binding site (see in Table S3).

Furthermore, the RMSF values of *Phe66*, *Arg68*, *Gly69*, *Tyr123*, *Ile131*, *Trp132*, *Ile131*, *Trp132*, *Val133*, and *Ala134*, which play an important role in protein-ligand binding affinity, were between 0.42 and 1.32 nm. In addition, the RMSD values of backbone C α atoms were calculated to be in the range of 1.87 to 2.70 Å between the initial and the final simulated structures (Table S2 and Figure S1). The results of this analysis show that the stability of the complex structure is reliable and that the interactions that play an important role in the binding affinity of all potent molecules with the protein remain stable throughout the simulation.

4 | CONCLUSIONS

Many studies have reported that N proteins will be important candidates for drug-targeting in other CoVs as they process various critical functions, such as RNA genomic packing, viral replication and transcription, and assembly in infectious cells.^{21,22,25,29,30,44-48} Few in silico studies have attempted to design a new drug targeting to inhibit interactions between the SARS-CoV-2 N and RNA at the molecular

level. For instance, Sarma et al showed that theophylline and pyrimidine drug groups,²¹ while Amin et al⁴⁹ showed that chloroquine and hydroxychloroquine drug groups are effective against SARS-CoV-2 N-NTD domain. However, the basis of the molecular mechanism of these functions of the newly emerging new SARS-CoV-2 N protein is largely unknown. Knowledge of these aspects will contribute significantly to the development of agents that specifically inhibit CoVs replication, transcription, and viral assembly.

In this study, we investigated the interaction mechanism of therapeutic antiviral compounds against SARS-CoV-2 N RNA binding domain at the molecular level using structure-based computational methods. SBDD has been shown as an advanced approach for the discovery and refinement of therapeutic agents^{21,47-54} because it is quite difficult and costly to examine the interaction of many compounds with enzymes at the molecular level under 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 rapamycin, saracatinib, camostat, trametinib, and nafamostat compounds effectively inhibited the SARS-CoV-2 N protein. Rapamycin (Sirolimus) is an agent that can inhibit the mammalian target of rapamycin (mTOR) via the phosphoinositide-3-kinase/protein kinase B/mTOR (PI3K/AKT/mTOR) pathway. This pathway plays an important role in CoVs infection and is a new drug target for therapeutic intervention strategies. In experimental studies, it has been reported that rapamycin can inhibit the expansion of Th17 cells in COVID-19 patients, as in patients with Systemic Lupus Erythematosus.^{55,56} Likewise, the approved drugs (saracatinib, camostat, trametinib, and nafamostat) are effective in treating HCoV-229E, MERS-CoV, SARS-CoV, SARS-CoV-2 infections.^{2,21,28,29,34}

Understanding non-covalent interactions in protein-ligand complexes contributes significantly to modern biochemistry and the discovery of new drugs. The affinity of a ligand is related to the binding constant and corresponds to a free energy determined by enthalpic entropic effects. The binding enthalpy is generally treated as the changes in energy resulting from the formations of non-covalent interactions at the binding interface.⁵⁷ Therefore, in this study, we investigated the effects of non-covalent interactions on binding affinity between protein-ligand complexes through dynamic processes at the molecular level. In light of this information, our study indicated that energetically favored ligands based on molecular docking analysis, formed hydrogen and hydrophobic interactions with *Phe66*, *Arg68*, *Gly69*, *Tyr123*, *Ile131*, *Trp132*, *Val133*, and *Ala134* in the SARS-CoV-2 N-NTD domain. These interactions continued to persist during the MD simulation period. Amino acid residues that play a role in these interactions may be the most important contribution to binding affinity in protein-ligand complexes.

In conclusion, this study offers a novel testable hypothesis with systematic drug repositioning for rapid and low-cost identification of approved drugs using computational approaches for the potential treatment of 2019-nCoV. It also provides better understanding of potential drug targeting sites for the SARS-CoV-2 N protein at the molecular level, thus opening new avenues for in vitro validations.

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AUTHOR CONTRIBUTIONS

Gizem Tatar: Conceptualization; Methodology; resources. **Ezgi Ozyurt:** Methodology; resources. **Kemal Turhan:** Project administration; supervision.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/btpr.3110>.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article and if further data are needed, they are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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