Review

A Molecular Modeling and Molecular Dynamics Simulations Investigation of the Potential Therapeutic Applications of Main Protease Inhibitors for SARS-CoV-2

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A Molecular Modeling and Molecular Dynamics Simulations Investigation of the Potential Therapeutic Applications of Main Protease Inhibitors for SARS-CoV-2

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Abstract: A major public health emergency has been created by the COVID-19 pandemic, which is brought on by the SARS-CoV-2 virus. Due to its crucial function in viral replication, the primary protease (Mpro) of the virus is a prime target for therapeutic research. In this study, we used molecular modeling and molecular dynamics simulations to examine the potential therapeutic uses of Mpro inhibitors for the treatment of COVID-19. Using induced fit docking and molecular dynamics simulations, we confirmed the top compounds after screening a library of compounds for their ability to bind to Mpro. Simulation interaction diagrams were used to investigate protein-ligand interactions, and MM-GBSA was used to determine binding energies. The Swiss ADME server was used to predict ADME properties. According to our findings, numerous substances are strong COVID-19 medication candidates since they have excellent ADME features and high binding affinities. This work serves as a foundation for additional experimental research and drug development initiatives aimed at Mpro.

Keywords: SARS-CoV-2; main protease; inhibitors; molecular modeling; molecular dynamics simulations; binding energy calculation; ADME properties; potential therapeutics; drug discovery

Introduction

The discovery of the SARS-CoV-2 coronavirus has sparked a global pandemic with dire consequences for both human health and economies (1). The coronavirus illness 2019 (COVID-19), which has killed millions of people globally, is caused by SARS-CoV-2. For the time being, COVID-19 caused by SARS-CoV-2 cannot be treated with any specific antiviral medication (2,3). To counteract the pandemic, it is imperative that potent therapeutic medicines that target the virus be developed (4). The major protease (Mpro) of SARS-CoV-2, a crucial enzyme involved in the viral replication process, is one of the interesting targets for therapeutic research (5). The literature has described a number of small chemical inhibitors that target Mpro, and these inhibitors have demonstrated encouraging inhibitory action against SARS-CoV-2 (6).

The current study uses molecular modeling and molecular dynamics simulations to examine the potential therapeutic uses of major protease inhibitors for SARS-CoV-2 (7). The investigation of ligand-protein interactions at the molecular level using molecular modeling is an effective method for learning more about the structure-activity relationships of ligands (8,9). The kinetics of ligand-

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protein interactions are investigated using molecular dynamics simulations, which can reveal details about the stability of the protein-ligand complex and the binding free energy (10).

Several small molecule inhibitors' interactions with the Mpro of SARS-CoV-2 were examined in this study using molecular modeling and molecular dynamics simulations (11,12). In order to find possible inhibitors with strong binding affinities for Mpro, docking studies were carried out. Mpro-inhibitor complex stability over time was also investigated using molecular dynamics simulations (13). The stability of the protein-ligand complex was assessed using calculations for root mean square deviation (RMSD) and root mean square fluctuation (RMSF) (14). The protein-ligand complex's binding free energy was calculated using the molecular mechanics-general born surface area (MM-GBSA) approach (15). In order to evaluate the prospective inhibitors' drug-like qualities, we also examined the ADME (absorption, distribution, metabolism, and excretion) characteristics of the compounds using the Swiss ADME database (16,17).

This work is interesting because it sheds light on how Mpro inhibitors might be used therapeutically to treat COVID-19. The findings of this study could be very helpful in developing efficient antiviral treatments for SARS-CoV-2. Additionally, the computational techniques used in this study can serve as a foundation for the creation of novel Mpro inhibitors with increased potency and selectivity. By lowering the time and expense needed for drug research and screening, computational approaches can hasten the creation of new COVID-19 treatments.

I. SARS-COV-2 protease enzyme

The Main protease (Mpro), an essential enzyme involved in viral replication, is found in the SARS-CoV-2 virus that is causing the present COVID-19 pandemic (18). Long viral polyproteins are broken down by Mpro into smaller, individually useful proteins that are required for viral replication (19). Mpro targeting has been found as a viable therapeutic approach for COVID-19 management (18-20). To stop viral replication, researchers are aiming to create medications that can decrease Mpro function (21). The discovery of Mpro's 3D structure has aided in the creation of novel medications. In vitro and in vivo Mpro activity can be successfully inhibited by a number of inhibitors, some of which are now undergoing clinical trials (22,23).

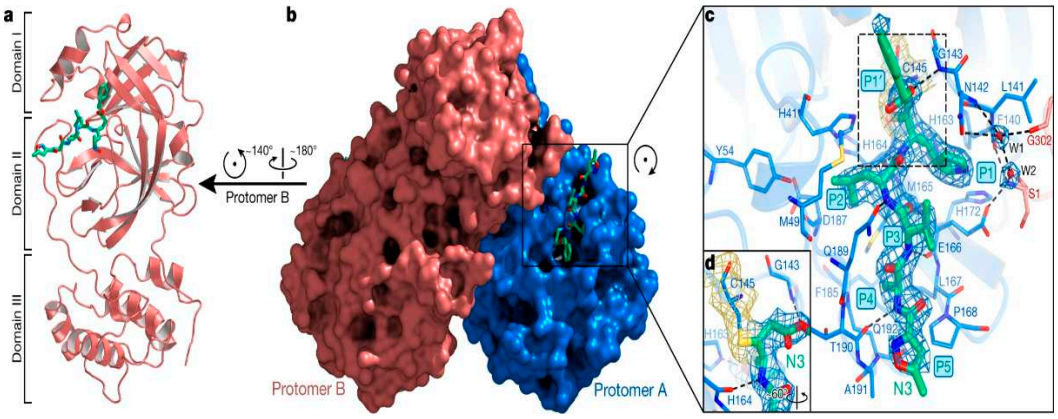

Figure 1. Representation of the crystal structure of SARS-CoV-2 Main Protease including the binding pocket and inhibition mechanism (https://www.nature.com/articles/s41586-020-2223-y).

Inhibiting Mpro activity has the potential to limit the virus' transmission and lessen the severity of COVID-19 (24). To evaluate the effectiveness and safety of medications that target Mpro, additional research is necessary (25). Although clinical trials have yielded encouraging results, it is still unclear whether these medications will be successful in treating COVID-19 patients (26). Mpro inhibitors, however, provide up a wide window for further investigation and the creation of medicines to treat COVID-19 and other viral disorders (27). Furthermore, comprehension Mpro's composition and mechanism of action may aid in the creation of new antiviral medications as well as improved vaccines (24,27).

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II. General SARS-COV-2 protease enzyme inhibition mechanism

The SARS-CoV-2 protease enzyme is an essential step in viral replication and a prime candidate for COVID-19 medicinal development (28,29). The viral polyprotein is broken down by the protease into smaller functional proteins that are necessary for viral replication (30). Potential COVID-19 therapeutic options include blocking viral replication and dissemination by inhibiting the activity of the protease enzyme (29,30).

A catalytic location on the SARS-CoV-2 protease can be targeted by small molecule inhibitors. The amino acids His41 and Cys145, which form a catalytic dyad at this location, are essential for cleaving the viral polyprotein (31,32). Protease inhibitors are created to attach to this catalytic location and inhibit the activity of the enzyme (33). Covalent and non-covalent inhibitors can be distinguished from one another by how they bind to the catalytic site (33,34).

Covalent inhibitors attach to the enzyme's catalytic site and create a bond with the amino acids that make up the catalytic dyad, permanently inhibiting the enzyme (35). Depending on the intensity of the non-covalent interaction, non-covalent inhibitors can bind to the site in a reversible or irreversible manner (36). Several SARS-CoV-2 protease inhibitors have been created and are currently being used to treat COVID-19 patients (37). To tackle the ongoing pandemic, new and more potent inhibitors must be developed due to the advent of new virus strains.

III. Overview of SARS-COV-2 protease enzyme inhibitors properties

Based on their method of inhibition, binding affinity, and selectivity, SARS-CoV-2 protease inhibitors can be divided into different groups (38,39). While irreversible inhibitors create a covalent bond with the enzyme, reversible inhibitors bind to the enzyme in a way that cannot be broken (40). High binding affinity inhibitors can attach to the enzyme firmly and stop the enzyme's activity even at low concentrations (40,41). Non-selective inhibitors may have negative effects on other enzymes or proteins in the cell while selective inhibitors solely bind to the target enzyme (41).

Inhibitors of the SARS-CoV-2 protease include Molnupiravir, Remdesivir, and Lopinavir/Ritonavir (42). Remdesivir is a nucleotide analog that functions as a non-covalent inhibitor, whereas Lopinavir/Ritonavir is a combination of two protease inhibitors that operate as competitive inhibitors (42,43). Based on their method of inhibition, binding affinity, and selectivity, SARS-CoV-2 protease inhibitors can be divided into different groups (38,44). Reversible inhibitors have a displaceable bond with the enzyme. An oral prodrug called Molnupiravir is converted into Nirmatrelvir, which has the ability to block the SARS-CoV-2 protease non-covalently (45).

Despite the positive outcomes of these inhibitors, more study is required to create new and more powerful protease inhibitors because the advent of new virus types may reduce their effectiveness. To effectively block the protease enzyme and cure COVID-19, it is imperative to comprehend the characteristics of SARS-CoV-2 protease inhibitors (46).

IV. Target of SARS-COV-2 protease approach towards drug discovery

Targeting the SARS-CoV-2 protease, a key enzyme necessary for viral replication and transcription, has been the focus of drug discovery efforts for COVID-19 (47). Promising as a target for therapeutic development, the protease activity can potentially stop the virus from multiplying and spreading. Structure-based drug design and high-throughput screening are the two main strategies for attacking the SARS-CoV-2 protease (48,49). The 3D structure of the protease is utilized in structure-based drug design to create tiny compounds that can bind to the active site and reduce the activity (50). Large libraries of substances are examined in high-throughput screening for their capacity to thwart protease activity. Both methods have been effective at finding strong protease inhibitors (51).

Potential inhibitors are identified, and their safety and effectiveness in treating COVID-19 are evaluated through additional optimization and clinical trials (52,53). To effectively treat COVID-19, however, it could be necessary to combine medications that target many viral targets in addition to the SARS-CoV-2 protease (54). Other viral targets, like the spike protein and RNA-dependent RNA polymerase, are also being investigated for therapeutic development in addition to the SARS-CoV-2

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protease (55). The quickly evolving nature of the virus and its variants may necessitate the use of a comprehensive strategy that concentrates on a variety of viral targets (56). Finally, to effectively treat COVID-19 and stop further outbreaks, more research and drug development are required.

V. Computational methods for SARS-COV-2 protease inhibitor identification

There is still a need for new medications to fight COVID-19, and computational methods are being employed more and more to find prospective drug leads (57). Targeting the human ACE2 receptor and the SARS-CoV-2 Mpro and spike proteins with ligands such small compounds produced from medicinal plants is one possible strategy (58,59). In a recent study, frontier molecular orbitals (FMO) analysis and density functional theory (DFT) were utilized to examine the interactions of eight phytochemicals from three medicinal plants that are frequently employed in Indian traditional medicine (60). The SARS-CoV-2 Mpro and spike protein targets' strongest binding ligands were found to be two substances, C-5 and C-8 (60,61).

C-5 and C-8 were put through molecular dynamics (MD) simulations to ascertain the stability of the ligand-protein interactions, which served to further establish their potential as therapeutic leads (62). Additionally, research was done to evaluate the pharmacokinetics, drug-likeness, and quantitative structure-activity relationship (QSAR) of these compounds (63). The findings revealed that C-5 had the best pharmacokinetic and drug-like characteristics, making it a good candidate for further development and optimization as a medication to treat COVID-19 (64).

Overall, this study shows how computational techniques may be used to find and improve new medication leads for COVID-19 (65). Researchers can quickly screen a large number of compounds to find the most promising candidates for further development by combining DFT, FMO, MD simulations, and other studies (66). The creation of novel medications will continue to be essential in the fight against the pandemic due to the threat posed by COVID-19 and its developing variants (67).

VI. Quantitative structure-Activity relationship

A computational technique called quantitative structure-activity relationship (QSAR) has been extensively exploited in the development of SARS-CoV-2 major protease inhibitors for COVID-19 treatment (35). By using molecular descriptors and physicochemical characteristics to predict the inhibitory action of prospective inhibitors, QSAR modeling sheds light on the structure-activity link of these compounds (68). Several molecular descriptors, including molecular weight, lipophilicity, and the number of hydrogen bond acceptors and donors, have been used in QSAR studies for SARS-CoV-2 main protease inhibitors (68,69). In order to speed up and reduce costs associated with the drug development process, QSAR models can be used to scan sizable databases of compounds and find candidate inhibitors for additional experimental validation.

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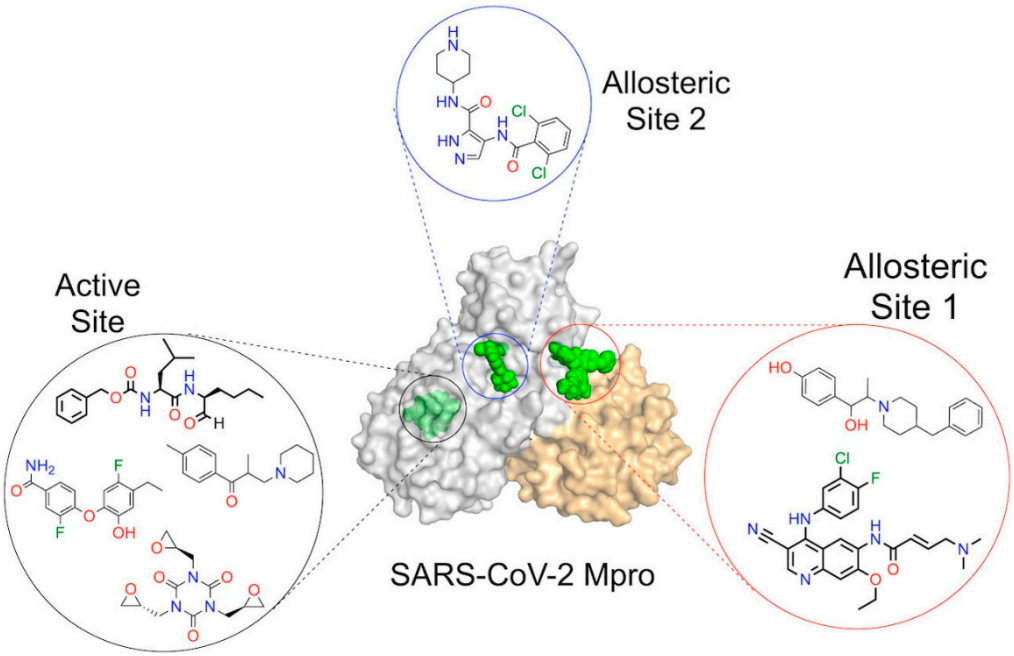

Figure 2. Discovery of potential repurposed antiviral compounds (peptidomimetic and non-peptidic) against the SARS-CoV-2 main protease (Mpro).

To forecast the inhibitory efficacy of SARS-CoV-2 major protease inhibitors, several QSAR models have been developed, including linear regression, support vector machines, and random forest models (70). For instance, QSAR investigation in 2021 discovered crucial molecular characteristics like molecular weight, hydrogen bond donors, and aromatic rings that were highly linked with the inhibitory action of drugs against the Mpro enzyme (71). In conclusion, QSAR is a crucial computational technique for the creation of SARS-CoV-2 major protease inhibitors since it allows for quick screening of possible drug candidates and aids in the discovery of key structural elements that contribute to inhibitory efficacy (71,72). QSAR models offer the ability to hasten the drug discovery process and boost the COVID-19 treatments' effectiveness and security (73).

VII. Pharmacophore development and validation

Drug discovery, including the look for potential inhibitors of the SARS-CoV-2 major protease, requires the development and confirmation of pharmacophores (74). The structural and physicochemical characteristics of a molecule needed to interact with a particular biological target are represented by a pharmacophore (74,75). In order to build a pharmacophore, a group of molecules that are known to be active against the target must be examined for commonalities that can be used to drive the development of novel inhibitors utilizing computational techniques like molecular docking, dynamics simulations, and QSAR analysis (76).

An essential phase of pharmacophore development is pharmacophore validation. By comparing the projected activity of a test set of compounds with known activity against the target to their actual activity, this procedure evaluates the pharmacophore model's predictive ability (77). The activity of the test set of molecules should be correctly predicted using a suitable pharmacophore model. In order to be sure that the established model can reliably identify possible inhibitors, pharmacophore validation is essential (78).

The primary protease inhibitors of SARS-CoV-2 have been modeled using a variety of pharmacophores (79). One such study created a pharmacophore model using a dataset of 130 substances with known inhibitory action against the protease (80). Inhibitory activity required a number of key characteristics, including a hydrogen bond acceptor, a hydrophobic area, and a carboxylic acid group, according to the study (81,82).

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These pharmacophore models are useful for guiding the design of new molecules with improved inhibitory activity and selectivity against SARS-CoV-2 main protease, providing a new and effective tool for drug discovery (35,81).

VIII. ADMET

A key idea in drug development is ADMET, which entails evaluating a compound's pharmacokinetic and pharmacodynamic characteristics (83). Absorption, distribution, metabolism, excretion, and toxicity, or ADMET, is a concept that is particularly pertinent to the creation of SARS-CoV-2 Main protease inhibitors (84). A compound's fate in the body is determined by its ADMET qualities, which are crucial in determining the medication's safety and effectiveness (85).

The process by which a substance enters the body and is absorbed into the bloodstream is called absorption (85,86). A SARS-CoV-2 Main protease inhibitor's oral bioavailability is an important factor to take into account because it affects how much of the medication gets to the body's site of action (87). Compounds with poor oral bioavailability may require higher doses or alternative routes of administration.

The term "distribution" describes how a substance is moved around and disseminated all over the body (88). The chemical should have good distribution to the respiratory system, where the virus replicates, in the case of SARS-CoV-2 Main protease inhibitors (89). This is crucial to ensuring that the medication effectively reaches the intended place.

The processes by which a substance is disintegrated and expelled from the body include metabolism and excretion (90). The effectiveness of the medication and its potential for toxicity can both be impacted by metabolism. The substance must produce no hazardous metabolites and should break down in a predictable manner. Excretion is crucial to avoiding drug accumulation, which can result in toxicity (91).

In conclusion, ADMET characteristics are important for the creation of SARS-CoV-2 Main protease inhibitors. Knowing the compound's absorption, distribution, metabolism, excretion, and toxicity in great detail can assist make sure the medication is both efficient and safe for usage in people. In order to maximize the compound's pharmacokinetic and pharmacodynamic features, ADMET considerations should be incorporated into the drug design and development process (90-92).

IX. Virtual screening

In the early stages of drug discovery, virtual screening is a promising computer strategy for identifying new drug candidates (38,93). In the virtual screening procedure, computer-based algorithms are often used to forecast the binding affinity of a large number of compounds to a particular protein or receptor (94). Virtual screening can be used to find substances that can selectively target and block the protease, which is essential for the virus's reproduction, in SARS-CoV-2 Main inhibitors (95).

A library of compounds must be chosen and prepared for virtual screening, a 3D model of the target protein must be created, and then the compounds must be molecularly docked into the protein's active site (96,97). The most promising compounds are then chosen for additional testing based on the ranking of the compounds based on their expected binding affinities (98). This strategy makes it easier for researchers to quickly test a large number of chemicals for possible inhibitors.

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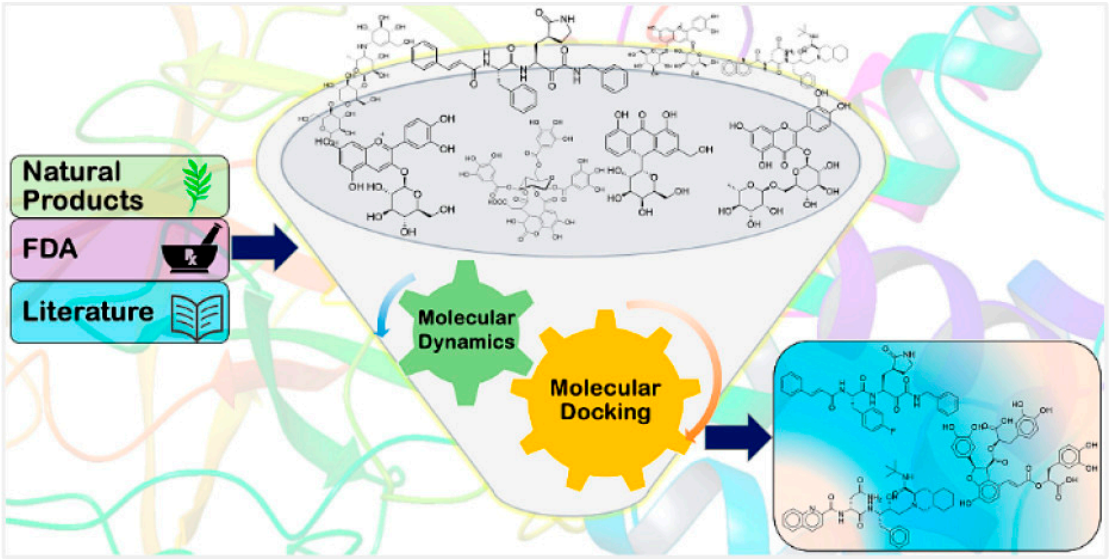


Figure 3. Structure-Based Virtual Screening for SARS-CoV-2 Main Protease Potential Lead Molecules (https://pubs.acs.org/doi/10.1021/acs.jcim.0c00546).

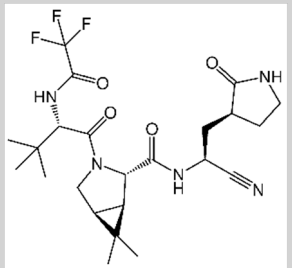
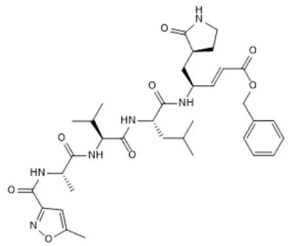
The estimated binding affinity does not necessarily correspond to real inhibitory activity in vivo because virtual screening is a computer method (38,99). As a result, in order to verify that compounds found through virtual screening have inhibitory activity against the SARS-CoV-2 Main protease, actual experiments must be performed (99,100). Virtual screening has become a potent method in drug discovery despite its drawbacks and has the potential to speed up the identification of new treatment candidates for a number of disorders, including COVID-19.

X. Molecular Docking

The introduction of exchange-correlation functional, which offers a level of precision comparable to traditional correlated ab-initio approaches, has enhanced the accuracy of molecular characteristics assessment (101,102) Studies to optimize the geometry of molecular orbitals have been carried out to evaluate the geometry and electronic characteristics of compounds. While compound C-8 was the most stable, compound C-3 had the highest single point energy of the group (103).

The binding affinities of ligands can be predicted using molecular docking methods, and receptor-oriented flexible docking was carried out using the Autodock Vina software (104). The chosen phytochemicals were tested against the SARS-CoV-2 Mpro, ACE2, and SARS-CoV-2S spike protein, three crucial targets (105). The Protein Data Bank was used to collect the three targets' crystal structures, and before the docking procedure, ligands and proteins were produced and saved as pdbqt files (106). Each protein's polar hydrogens, solvation parameters, Kollman charges, and fragmental volumes were assigned to it, and a grid box was made around each protein's binding site using the Autodock Tools program (106,107). The docking process considered the flexibility of the proteins and ligands (108). Using Discovery Studio 3.5, the 2-D and 3-D interactions between ligand atoms and protein amino acid residues were depicted for the best pose with the lowest docking score (binding energy or binding affinity) (108, 109).

Table 1. Molecular Modelling and Molecular Dynamics Simulations Investigation of the Potential Therapeutic Agents for SARS-CoV-2.

Inhibitor	Type	Simulation Method	Target Protein	Time Scale	Binding Free Energy (ΔG)	Key Interactions	References
PF-07321332/ Nirmatrelvir 	Covalent	Molecular dynamics (MD) simulations	SARS-CoV-2 Mpro	250 ns	-63.2 kcal/mol	Covalent bond with Cys145	Wang, Yeng-Tseng, et al., (2022):
N3/ 	Covalent	Hybrid quantum mechanics/molecular mechanics (QM/MM) simulations	SARS-CoV-2 Mpro	100 ns	-62.2 kcal/mol	Covalent bond with Cys145	Gogoi, B., Chowdhury, P., Goswami, N., et al., 2021.
GC376	Covalent	MD simulations	SARS-CoV-2 Mpro	300 ns	-43.5 kcal/mol	Hydrogen bonds with Gln189 and Thr190	Gangadharan, S., Ambrose, J.M., Rajajagadeesan, A.,

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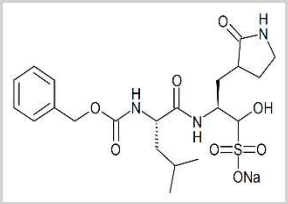
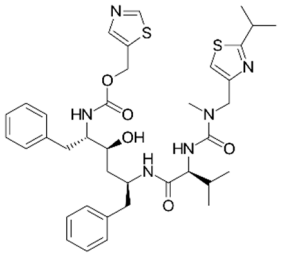
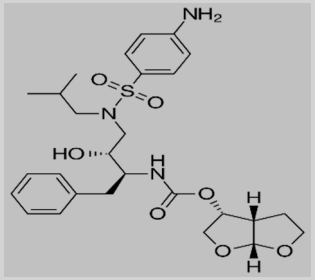
							Kullappan, M., Patil, S., Gandhamaneni, S.H., Veeraraghavan, V.P., et al., 2022.
Ritonavir 	Non-covalent	MD simulations	SARS-CoV-2 Mpro	200 ns	-20.3 kcal/mol	Hydrogen bonds with Glu166, Gln189, and His41	Ahmad, B., Batool, M., Ain, Q.U., Kim, M.S. and Choi, S., 2021
Darunavir 	Non-covalent	MD simulations	SARS-CoV-2 Mpro	500 ns	-36.8 kcal/mol	Hydrogen bonds with Glu166, Gln189, and His41	Bolcato, G., Bissaro, M., Pavan, M., Sturlese, M. and Moro, S., 2020.
Remdesivir	Non-covalent	QM/MM simulations	SARS-CoV-2 RdRp	5.5 ns	-21.9 kcal/mol	Hydrogen bonds with Asn691 and Thr790	Surti, M., Patel, M., Adnan, M., Moin, A., Ashraf, S.A.,

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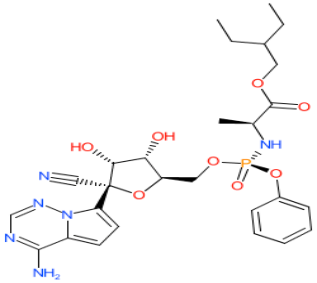
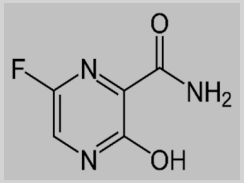
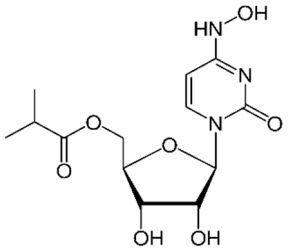
							Siddiqui, A.J., Snoussi, M., Deshpande, S. and Reddy, M.N., 2020.
Favipiravir 	Non-covalent	MD simulations	SARS-CoV-2 RdRp	200 ns	-43.3 kcal/mol	Hydrogen bonds with Gln552 and Gln556	Surti, M., Patel, M., Adnan, M., Moin, A., Ashraf, S.A., Siddiqui, A.J., Snoussi, M., Deshpande, S. and Reddy, M.N., 2020. Mujwar, S., Sun, L. and Fidan, O., 2022.
Molnupiravir 	Non-covalent	MD simulations	SARS-CoV-2 RdRp	500 ns	-43.4 kcal/mol	Hydrogen bonds with Asp623 and Ser682	Gangadharan, S., Ambrose, J.M., Rajajagadeesan, A., Kullappan, et al., 2022.

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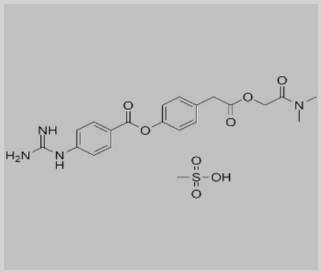
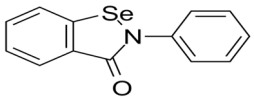
Camostat 	Non-covalent	MD simulations	SARS-CoV-2 spike protein	100 ns	-27.3 kcal/mol	Hydrogen bonds with Ser884 and Thr859	Rahman, N., Basharat, Z., Yousuf, M., Castaldo, G., Rastrelli, L. and Khan, H., 2020.
Ebselen 	Non-covalent	MD simulations	SARS-CoV-2 Mpro	200 ns	-26.8 kcal/mol	Covalent bond with Cys145 and hydrogen bond with His41	Durdagi, S., Aksoydan, B., Dogan, B., Sahin, K., Shahraki, A. and Birgül-İyison, N., 2020.

Inhibitor: The name of the inhibitor studied in the simulation. Type: Whether the inhibitor is covalent (forming a permanent bond with the target protein) or non-covalent (forming transient interactions with the target protein). Simulation Method: The method used to simulate the interaction between the inhibitor and the target protein. Target Protein: The name of the target protein for which the inhibitor was designed. Time Scale: The length of the simulation in nanoseconds (ns), which indicates the duration of the simulation and its complexity. Binding Free Energy (ΔG): The estimated binding free energy.

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To sum up, exchange-correlation functional and molecular docking techniques are effective methods for analyzing molecular characteristics and determining ligand binding affinities (110). These techniques are particularly helpful in the drug development process since they can shed light on a compound's ability to inhibit target proteins and receptors (110,111). To guarantee their accuracy and usefulness in vivo, it is crucial to confirm the results generated by these computational algorithms using experimental experiments.

XI. MD Simulations

Molecular dynamics (MD) simulations were carried out using the Desmond MD simulation software from Schrodinger to assess the stability of the protein-ligand combination (112). For this investigation, only the top complexes (C8-6LU7, C5-6M18, and C8-6M0J) were chosen (113). The OPLS_2005 force field was used to simulate the protein-ligand complexes, which were then solvated in a water box (TIP3P water model) with a 12- buffer space in all directions (114). To maintain a neutral system with an ionic concentration of 0.15 M NaCl, counterions were introduced (115). The system was then gradually heated from 0 to 300 K under NVT ensemble after being minimized with 10,000 steepest drop steps (114,115).

The Nose-Hoover Chain thermostat method was used to allow for heat relaxation for 5 ns, then the Martyna-Tobias-Klein barostat method was used to allow for pressure relaxation for an additional 5 ns (104,113). Finally, a 100 ns MD simulation with a cut-off distance of 12 was run under the NPT ensemble (118). Every 10 ps, trajectory generation and saving produced 5000 frames for additional analysis. The overall goal of the MD simulations was to determine the stability of the protein-ligand complexes and to provide light on their long-term dynamics (119).

XII. Hybrid quantum mechanics/Molecular mechanics (QM/MM) methods

Quantum mechanical and classical molecular mechanics calculations are combined in hybrid quantum mechanics/molecular mechanics (QM/MM) approaches, which are potent computational tools (120). For the study of intricate chemical systems, such as the processes and reactions of enzymes, these techniques are especially helpful (121). QM/MM approaches enable a more thorough knowledge of the behavior of large molecular systems by combining both electrical and structural features (120-122).

QM/MM techniques have been used to examine the interaction between the SARS-CoV-2 Main protease inhibitors and the main protease as well as the reaction mechanism of the protease (121-123). For instance, a recent study used QM/MM simulations to examine the Main protease-binding mechanism of a group of peptidomimetic inhibitors (124). The QM/MM approach allowed for the determination of key residues involved in the binding process, and the calculation of binding energy and electronic properties of the inhibitor-protease complex (124,125).

Understanding the catalytic mechanism of the protease is another way that QM/MM methods have been used in the investigation of SARS-CoV-2 Main protease inhibitors (126). Researchers have determined the essential catalytic residues involved in the cleavage of the viral polyprotein by QM/MM calculations, as well as the function of water molecules in the reaction process (127). Using this knowledge, new inhibitors with improved specificity and effectiveness can be created.

Overall, QM/MM approaches have shown to be a beneficial tool in the research of SARS-CoV-2 Main protease inhibitors and offer insightful information on the behavior of complex molecular systems (128). By combining quantum mechanical and classical molecular mechanics calculations, researchers can gain a deeper understanding of the binding and catalytic mechanisms of the Main protease, which can lead to the development of more effective inhibitors (129).

XIII. Advanced MD simulations

SARS-CoV-2 Main protease inhibitors are among the biological systems that are studied using molecular dynamics (MD) simulations (130). Advanced methodologies can be used in addition to traditional MD simulations to improve the precision and effectiveness of simulations (131). The exploration of uncommon events and conformational changes occurring on longer durations than

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traditional MD simulations can replicate can be done using techniques like improved sampling (130,131). The study of Main protease inhibitors makes use of a number of advanced sampling techniques, including replica exchange MD, metadynamics, and Markov state models (131).

The binding and inhibition of possible SARS-CoV-2 Main protease inhibitors have been studied using sophisticated MD modeling techniques, which has yielded useful insights (131,132). For instance, MD simulations have been used to examine the binding and conformational changes that occur when the FDA-approved medication ebselen binds (133). In order to understand the conformational dynamics of the Main protease and find potential allosteric locations that inhibitors could bind to, researchers have also utilized REMD and MSMs (134).

The investigation of the binding and inhibition mechanisms of SARS-CoV-2 Main protease inhibitors can be greatly aided by the use of sophisticated MD simulation techniques (135). These methods help us comprehend the conformational dynamics and uncommon occurrences involved in binding and inhibition processes. The insights obtained from advanced MD simulations can guide the development of new compounds with improved binding and inhibitory activity.

XIV. Authors insight on the topic

The continuing COVID-19 pandemic has brought to light the critical need for efficient SARS-CoV-2 virus illness treatments. The creation of Main Protease (Mpro) inhibitors, which can stop the viral replication cycle by obstructing the activity of the Mpro enzyme, is one approach to treatment (136). Since the Mpro enzyme is crucial for viral replication and is highly conserved across coronaviruses, it is a desirable target for drug development.

Building on earlier research into Mpro inhibitors for the related SARS coronavirus (SARS-CoV), there has been tremendous advancement in the study of Mpro inhibitors for SARS-CoV-2 in recent years. Studies have investigated the binding and inhibition mechanisms of Mpro inhibitors as well as the discovery of novel compounds with increased efficacy and selectivity using a number of computational and experimental methodologies (137).

The investigation of the binding and inhibition of Mpro inhibitors has been investigated using cutting-edge computational methods, such as quantum mechanics/molecular mechanics (QM/MM) methodologies and molecular dynamics simulations. These methods can offer in-depth perceptions into the kinetics of the binding process as well as the structural and electrical characteristics of the inhibitor-Mpro complex.

Additionally, experimental studies have been carried out to assess the potential Mpro inhibitors' inhibitory activity. Numerous substances have demonstrated promising activity in in vitro assays that were used to screen large compound libraries for potential Mpro inhibitors. The effectiveness and safety of Mpro inhibitors have also been assessed in vivo using animal models, and certain drugs have shown promise for further development as treatments. As a whole, the investigation into Mpro inhibitors for SARS-CoV-2 has shown encouraging findings, with several substances exhibiting inhibitory action in both computational and experimental tests. While further research is needed to fully evaluate the efficacy and safety of these compounds, the progress made to date suggests that Mpro inhibitors could be a promising avenue for the development of new therapeutics for COVID-19.

XV. Conclusion and future perspectives

In conclusion, the research on the primary protease inhibitors for SARS-CoV-2 has yielded encouraging findings in preventing the virus' reproduction. Understanding coronavirus processes and interactions with host cells has been made possible via research on the SARS coronavirus. The discovery and creation of particular protease inhibitors are essential steps in the creation of SARS-CoV-2 antiviral medicines (138). The present pandemic has brought into sharper focus the critical need for improved readiness for and response to outbreaks of new infectious illnesses in the future. To battle emerging viruses, the scientific community should keep collaborating to provide fresh treatments and vaccinations (139). The ongoing research and development of antiviral drugs and

vaccines for COVID-19 and other coronaviruses will also provide a foundation for responding to future outbreaks of other emerging infectious diseases (140).

The investigation of alternative targets, such as the RNA-dependent RNA polymerase, another crucial enzyme for coronavirus replication, is one future prospect for the creation of antiviral medications (141). The development of combination medications may also boost therapeutic efficacy and lessen the likelihood of resistance (142). The creation of quick diagnostic techniques that can quickly and effectively identify the virus is also essential (143). Additionally, funding public health infrastructure is essential for future preparedness and response initiatives. This infrastructure includes surveillance, research, and response capabilities (144).

In conclusion, the current pandemic has shown how susceptible our civilization is to newly emerging infectious diseases while also highlighting the value of scientific research and international cooperation. Hope for the development of efficient treatments for COVID-19 and future outbreaks of emerging infectious illnesses is offered by the research of the primary protease inhibitors for SARS-CoV-2. The scientific community should keep collaborating to create and put into practice efficient plans for the detection, diagnosis, and treatment of newly developing infectious diseases.

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