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Research Article

Exploration of SARS-CoV-2 M^{pro} inhibitors through molecular docking studies

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A B S T R A C T

The SARS-CoV-2 main protease (M^{pro}) is indispensable for viral replication and thus represents a highly attractive therapeutic target. In this study, molecular docking was employed to explore natural bioactive compounds as potential inhibitors of M^{pro}. The crystal structure of SARS-CoV-2 M^{pro} (PDB ID: 6LU7) was retrieved from the RCSB Protein Data Bank, and a ligand library of 38 phytochemicals derived from *Azadirachta indica*, *Curcuma longa*, *Zingiber officinale*, *Ocimum basilicum*, and *Panax ginseng* was compiled from PubChem. Docking simulations were carried out using AutoDock 4.2 with the Lamarckian Genetic Algorithm to evaluate binding affinities and interaction patterns within the protease's substrate-binding pockets. Visualization and interaction analyses were performed with LigPlot^{+} and UCSF Chimera to validate hydrogen bonding, hydrophobic contacts, and potential allosteric effects. Several compounds, particularly flavonoids such as casticin, exhibited strong binding energies and favorable interactions with both the catalytic dyad and allosteric regions of M^{pro}, suggesting dual mechanisms of inhibition. The integration of phytochemical screening with structure-based docking highlights promising leads for the development of safe, plant-derived antivirals. Collectively, these results provide a computational framework for identifying novel SARS-CoV-2 M^{pro} inhibitors and contribute to the broader effort of advancing effective therapeutic strategies against COVID-19.

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1. Introduction

Coronaviruses (CoVs) are enveloped, positive-sense RNA viruses with large genomes and a distinctive crown-like surface morphology formed by spike proteins. They infect a wide range of hosts, causing respiratory and enteric diseases in animals and, in humans, illnesses ranging from mild colds to life-threatening pneumonia [1].

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 led to the COVID-19 pandemic, which has had an unparalleled global impact [2, 3]. While four human coronaviruses (229E, NL63, OC43, and HKU1) are endemic and generally associated with mild infections, the appearance of SARS-CoV, MERS-CoV, and SARS-CoV-2 over the past two decades highlights the pandemic potential of this viral family [4]. Phylogenetic studies indicate that SARS-CoV-2 is closely related to bat coronaviruses identified in 2015 and 2017, suggesting a shared evolutionary lineage [5].

The most frequently observed symptoms at the onset of the illness include cough, fever, and muscle pain or fatigue, while less frequently reported symptoms are headache, production of sputum, coughing up blood, and diarrhea [6]. In severe instances, patients may experience organ failure such as acute respiratory distress syndrome (ARDS), shock, acute heart injury, or even death [7]. Additionally, SARS-CoV-2 has been found to interfere with the body's typical immune responses, triggering an excessive inflammatory reaction in serious cases. Consequently, individuals with severe forms of the disease often show reduced lymphocyte counts (lymphopenia), abnormal lymphocyte activation and function, irregularities in granulocytes and monocytes, as well as elevated levels of immunoglobulin G (IgG) [8].

Efforts to control COVID-19 have benefited from antiviral agents such as remdesivir and the protease inhibitor nirmatrelvir, the latter marketed with ritonavir as Paxlovid. Among viral proteins, the main protease (M^{pro}, also called 3CLpro) is of particular interest because of its essential role in processing viral polyproteins and its high degree of conservation across coronaviruses. These properties make M^{pro} an attractive target for drug discovery [9]. Recent computational studies have further reinforced the

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therapeutic potential of M^{pro} inhibitors. Bharadwaj et al. [2] conducted a virtual screening of 653 natural compounds and identified four candidates with strong binding affinities to the catalytic dyad His41 and Cys145. These compounds were validated through ADMET profiling and quantum mechanical simulations, highlighting their drug-like properties and potential efficacy against SARS-CoV-2 M^{pro}.

Similarly, Narkhede et al. [10] evaluated the binding interactions of existing antiviral and antimalarial drugs, including ritonavir, remdesivir, and favipiravir, with M^{pro} and the spike glycoprotein-ACE2 complex. Their results suggest that these repurposed drugs may offer therapeutic benefits by targeting key viral proteins. Ghasemlou et al. [11] explored the mutational landscape of M^{pro} and its implications for drug design. Through comparative docking analyses and ligand optimization, they identified several potent inhibitors, including Nelfinavir-derived compounds (NE1, NE2, NE3), which demonstrated high binding affinity across multiple M^{pro} variants. These studies collectively underscore the value of both natural and synthetic compounds in protease-targeted antiviral strategies. Additionally, Das et al. [12] performed blind docking of 33 compounds including natural products and approved antivirals, and found rutin to exhibit the strongest affinity near the catalytic residues His41 and Cys145, further supporting its potential as an M^{pro} inhibitor.

Several small molecules, including boceprevir, carmofur, nirmatrelvir, and ensitrelvir, have shown inhibitory activity against M^{pro}, underscoring the feasibility of protease-targeted therapies [13]. However, most strategies focus on competitive inhibition at the catalytic site, which often requires high binding affinity and sustained drug concentrations. Allosteric inhibition, by contrast, offers an alternative mechanism that could complement conventional approaches [14].

In this context, the present study explores phytochemical compounds from medicinal plants as potential allosteric inhibitors of SARS-CoV-2 M^{pro}. Using molecular docking and structure-based screening, we assess their binding interactions with both active and non-catalytic sites, aiming to identify natural products with favorable safety profiles and therapeutic promise.

This study introduces a targeted high-throughput in silico screening strategy to identify natural inhibitors of the SARS-CoV-2 main protease (M^{pro}). Building upon structural insights into the protease's active site, particularly the S1, S2, P1, and P3 pockets crucial for substrate recognition, the work integrates ligand-based similarity analysis with molecular docking to rationally prioritize candidates. Unlike previous approaches dominated by synthetic compounds, this research focuses on plant-derived phytochemicals with favorable safety profiles, aiming to deliver antiviral leads that are not only potent but also biocompatible. The novelty lies in merging natural product chemistry with structure-based drug design to exploit the dimeric nature and catalytic architecture of M^{pro}, offering an efficient, cost-effective, and goal-oriented pathway for antiviral drug discovery.

2. Materials and method

2.1. Protein/macromolecule

The study focused on the main protease of SARS-CoV-2. We obtained its three-dimensional structure in PDB format from the RCSB Protein Data Bank. The protease is known by the PDB ID **6LU7** and is a homodimer, made up of two identical **A** chains, each containing 306 amino acids. The **N3** molecule acts as an inhibitor for this protease [15].

2.2. Ligands

A total of 38 bioactive compounds, sourced from five plants, *Azadirachta indica, Curcuma longa, Zingiber officinale, Ocimum basilicum, and Panax ginseng*, were selected as potential ligands. We pulled their chemical structures from PubChem in .sdf format. For docking, the compounds were converted to .pdb format using Biovia Discovery Studio Visualizer [15].

2.3. Molecular docking studies

For the docking evaluations, both protein and ligands were imported into AutoDock Tools (ADT) 1.5.6. Gasteiger partial charges were assigned after merging non-polar hydrogen, and rotatable bonds were designated, which allowed the ligands to flex during docking. The docking evaluations were carried out using the protein model with polar hydrogen, kollman charges, and solvation parameters implemented from ADT. AutoDock 4.2 provides three distinct search algorithms, each varying in search efficiency for evaluating binding sites. For this study, we employed the Lamarckian Genetic Algorithm due to its robust performance in docking simulations. The grid box was configured to cover the entire binding site of the target proteins, giving ligands enough space to translate and rotate. The docking runs differed in number, each containing 30 independent runs with up to 27,000 genetic algorithm operations on a population of 150. We also used the maximum of default operator settings with a crossover rate of 0.80, a mutation of 0.02, and an elitism of 1. In order to visualize and analyze the results we used LigPlot+ (v.1.4.5) and UCSF Chimera (v.1.10.2) to examine and plot the interactions involved in the protein–ligand complexes [14].

3. Results and Discussion

Fig.1 illustrates a rational approach to designing potent inhibitors targeting the main protease (M^{pro}) of SARS-CoV-2 and elucidates their mechanistic impact on the viral replication cycle. In the top portion of the diagram, the structural evolution of protease inhibitors is depicted, beginning with the lead compound 11r. Through targeted modifications at specific substrate-binding pockets (notably S1, S2, P1, and P3), compounds such as 13a, 13b, and 14b were generated. The structural features interacting with each pocket are highlighted, demonstrating a structure-guided optimization strategy to enhance inhibition efficacy and binding specificity toward M^{pro}. The bottom section outlines the SARS-CoV-2 replication lifecycle, emphasizing the critical role of M^{pro} in proteolytic cleavage of viral polyproteins (pp1a/pp1ab) into functional nonstructural proteins (Nsps) required for viral RNA replication and assembly. The diagram demonstrates that administration of M^{pro} inhibitors effectively disrupts this process. By binding to the active site of M^{pro}, these inhibitors block its proteolytic function, thereby halting the maturation of viral proteins and preventing the assembly and release of new viral particles.

Collectively, these results show the centrality of M^{pro} as an antiviral target and underscore the efficacy of rational structure-based drug design. The inhibitors impede SARS-CoV-2 replication at multiple stages by interfering directly with a pivotal enzymatic step in the viral life cycle. This approach not only inhibits viral propagation but also provides a framework for developing therapeutics against current and future coronavirus threats. The schematic effectively bridges the gap between medicinal chemistry design and virological mechanism, supporting the potential of M^{pro} inhibitors as a promising class of antiviral agents.

Fig. 2 highlights the structural biology behind the inhibition of the SARS-CoV-2 main protease (M^{pro}), a critical enzyme involved in viral replication. The upper segment of the image depicts the architecture of M^{pro} as a homodimer, with both monomers contributing structurally and functionally to the active site. This dimeric arrangement is essential for the protease's enzymatic cleavage activity. The lower part illustrates the strategic design of an M^{pro} inhibitor, focused on occupying key substrate-binding pockets, namely S1, S1', S2, and S3. By delineating these pockets, the image demonstrates the rational targeting of M^{pro}'s active site. Occupancy of these sites by tailored inhibitors can effectively block proteolytic processing of viral polyproteins, thereby halting the production of essential nonstructural proteins required for viral replication. This structural insight not only underscores the value of M^{pro} as a pharmacological target but also guides the molecular design of next-generation antiviral agents based on precise, pocket-specific interactions. The schematic thus provides a clear mechanistic rationale for inhibitor development and supports ongoing efforts in structure-based drug discovery against SARS-CoV-2.

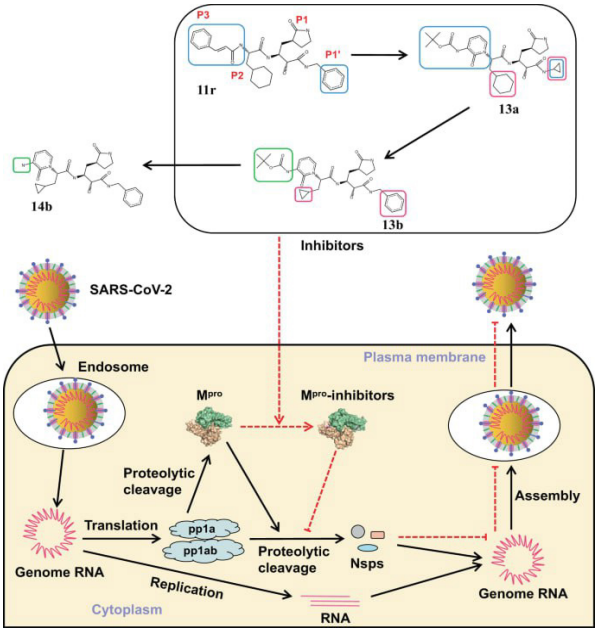

Fig. 1. Stepwise design and functional overview of SARS-CoV-2 main protease (M^{pro}) inhibitors. The upper panel illustrates the chemical transformation of lead compound 11r into potent inhibitors 13a, 13b, and 14b, highlighting key molecular interactions within the substrate-binding pockets (S1, S2, P1, P3) of M^{pro}. The lower panel depicts the SARS-CoV-2 viral life cycle, emphasizing the role of M^{pro} in cleaving viral polyproteins (pp1a/pp1ab) to generate nonstructural proteins (Nsps) necessary for viral RNA replication and assembly. The inhibitors effectively block M^{pro} activity, thereby preventing viral replication and assembly.

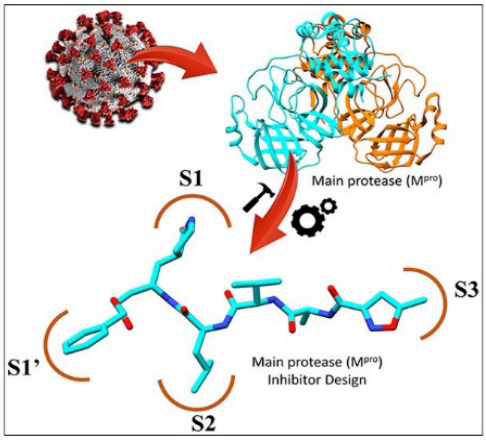

Fig. 2. Structural basis of inhibitor binding to SARS-CoV-2 M^{pro}. The dimeric M^{pro} structure with key substrate-binding pockets (S1, S2, S1', S3) is shown, highlighting a modeled inhibitor and its essential interactions for protease inhibition.

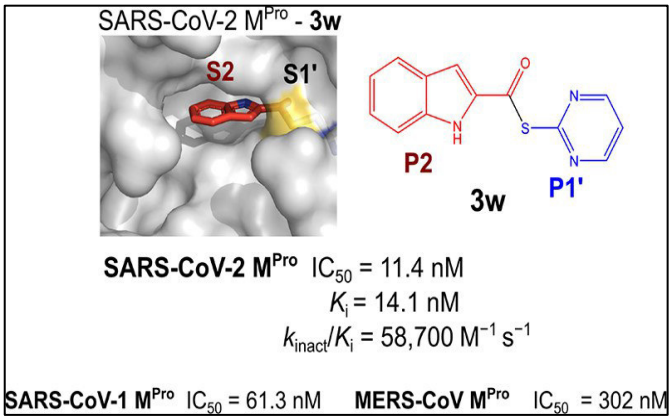

Fig. 3. Molecular interaction and inhibitory potency of compound 3w against SARS-CoV-2 M^{pro}. a) Surface representation of SARS-CoV-2 M^{pro} with compound 3w bound in the S2 and S1' substrate-binding pockets, highlighting its spatial fit. b) Chemical structure of 3w indicating the P2 (red) and P1' (blue) moieties. c) The enzymatic inhibition parameters: IC_{50} and K_i values against SARS-CoV-2 M^{pro}, along with the k_{inact}/K_i rate constant, demonstrating high affinity and rapid enzyme inactivation. Comparative IC_{50} values against SARS-CoV-1 and MERS-CoV M^{pro} reveal enhanced selectivity and potency of 3w for SARS-CoV-2.

Fig. 3 presents the structural and biochemical characterization of the SARS-CoV-2 M^{pro} inhibitor 3w. The three-dimensional model (top left) illustrates compound 3w occupying the critical S2 and S1' substrate-binding pockets of M^{pro}, demonstrating a precise fit that likely underpins its high inhibitory potency. The chemical structure further clarifies the functional groups engaging with these pockets, specifically the quinolinone (P2) and pyridine (P1') moieties. Kinetic data provided on the right reveal that compound 3w exhibits an IC_{50} of 11.4 nM and a K_i of 14.1 nM against SARS-CoV-2 M^{pro}, values indicative of strong binding affinity and efficient inhibition. Moreover, the k_{inact}/K_i ratio of 58,700 M^{-1} s^{-1} highlights the compound's rapid and effective inactivation of the protease. Notably, when compared with M^{pro} from related coronaviruses, 3w demonstrates substantially higher potency for SARS-CoV-2 (IC_{50}= 11.4 nM) over SARS-CoV-1 (IC_{50}= 61.3 nM) and MERS-CoV (IC_{50}= 302 nM), underscoring both the selectivity and therapeutic promise of this inhibitor scaffold. These results collectively validate the structure-guided optimization strategy and point toward the clinical potential of 3w and related molecules in targeting SARS-CoV-2 replication.

4. Conclusion

This study presents a comprehensive investigation into the design and efficacy of M^{pro} inhibitors through molecular docking and structure-based approaches. By elucidating the structural features of M^{pro} and its critical substrate-binding pockets, including S1, S2, and S1', key inhibitor interactions were identified and optimized to enhance binding affinity and enzymatic inhibition. The development of compounds such as 3w demonstrated potent inhibition of SARS-CoV-2 M^{pro}, with superior selectivity and activity compared to related coronaviral proteases. These inhibitors effectively disrupt the protease's function, thereby impeding viral polyprotein processing and halting viral replication. The integration of molecular docking with biochemical evaluation underscores the utility of structure-guided drug design in identifying promising antiviral agents. Collectively, these findings provide valuable insights for the rational development of therapeutic

inhibitors targeting SARS-CoV-2 and present a foundational framework for advancing effective antiviral strategies against COVID-19.

Author Contributions

Michael Askari: Conceptualization, Methodology, Validation, Data Curation Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data is available.

Ethical issues

The authors confirm full adherence to all ethical guidelines, including the prevention of plagiarism, data fabrication, and double publication.

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