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Research Article

INTEGRATED VIRTUAL SCREENING AND MOLECULAR DOCKING ANALYSIS OF SARS-COV-2 MAIN PROTEASE (MPRO)

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ABSTRACT

The SARS-CoV-2 Main Protease (Mpro) plays a central role in viral replication and transcription by cleaving the large viral polyproteins (pp1a/pp1ab) into functional non-structural proteins necessary for the viral life cycle. This proteolytic processing is indispensable for viral maturation, making Mpro one of the most attractive and highly validated antiviral drug targets. In the present study, an integrated computational workflow combining virtual screening, molecular docking, and ADMET evaluation was employed to identify potential small-molecule inhibitors of Mpro. An initial library of 10,000 structurally diverse compounds was pre-filtered using Lipinski's rule of five, Veber criteria, and drug-likeness properties to ensure suitable pharmacokinetic behavior. The filtered compounds were subjected to high-precision docking simulations using AutoDock Vina, enabling prediction of binding modes and quantification of binding affinities against the Mpro active site.

Keywords: SARS-CoV-2, Mpro, Virtual screening, Molecular docking, Antiviral agents, In silico drug discovery.

INTRODUCTION

The global COVID-19 pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has resulted in unprecedented health, economic, and social disruption worldwide. Although vaccination programs and antiviral treatments such as remdesivir, molnupiravir, and nirmatrelvir have significantly reduced disease severity, the continuous emergence of mutations and viral variants highlights the urgent need for new therapeutics (Ullrich & Nitsche, 2020). Among the viral proteins essential for replication, the SARS-CoV-2 Main Protease (Mpro, also known as 3CLpro) has been widely recognized as one of the most promising drug targets due to its indispensable function in processing viral polyproteins into functional non-structural proteins required for RNA replication and transcription (Zhang *et al*., 2020; Jin *et al*., 2020).

Structurally, Mpro contains a highly conserved catalytic dyad consisting of His41 and Cys145**,** which mediates peptide bond cleavage. Inhibition of this protease effectively halts viral replication, making it a preferred target for structure-based drug design. Importantly, humans lack close homologs of 3CLpro, which minimizes the likelihood of host toxicity and enhances its suitability for selective drug targeting (Dai *et al*., 2020). Over the last few years, several high-resolution crystal structures of Mpro have become available, enabling researchers to explore binding mechanisms of small-molecule inhibitors with unprecedented accuracy (Zhang *et al*., 2020; Jin *et al*., 2020). Computational drug discovery tools have become indispensable in accelerating antiviral research. Virtual screening enables rapid evaluation of thousands of compounds through physicochemical filters and scoring algorithms, while molecular docking predicts binding affinity, ligand orientation, and key molecular interactions within the catalytic site (Morris *et al*., 2023). This approach reduces experimental cost, narrows down candidate molecules, and provides mechanistic insights prior to

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laboratory validation. Studies have demonstrated that integrating virtual screening with docking is highly effective for identifying novel drug candidates against SARS-CoV-2 proteins, including Mpro, RNA polymerase, and spike receptor-binding domains (Elmezayen *et al*., 2020; Rasool *et al*., 2021). Structural characterization of Mpro established the foundation for structure-based inhibitor design. Jin *et al*. (2020) solved an early high-resolution structure of Mpro and reported initial small-molecule inhibitors that bind the active site, highlighting the catalytic dyad (His41–Cys145) as the primary interaction locus. Zhang *et al*. (2020) provided complementary crystallographic data and used the structure to design α-ketoamide inhibitors that occupy S1–S4 subsites, emphasizing pocket geometry and conserved residues critical for ligand recognition. Dai *et al*. (2020) used structure-based design to propose several antiviral candidates with favorable fit in the Mpro active site and discussed structure-activity relationships derived from the co-crystal complexes. Reviews and perspectives (Ullrich & Nitsche, 2020) summarized these structures and argued that Mpro's unique substrate specificity and absence of close human homologs make it an attractive antiviral target.

Foundational reviews describe how virtual screening reduces chemical search space and prioritizes candidates for experimental testing. Kitchen *et al*. (2004) framed docking and scoring as central to virtual screening and cautioned about scoring function limitations. Lionta *et al*. (2014) reviewed structure-based virtual screening workflows, emphasizing ligand preparation, filtering (drug-likeness), and rescoring strategies. Practical implementations and benchmarking of docking engines (Trott & Olson, 2010; Morris, Huey, & Olson, 2023) provide guidance on parameter choices (exhaustiveness, grid design) and improvements in speed and accuracy (AutoDock Vina and subsequent AutoDock variants). Docking produces hypotheses but has known limitations. Pantsar and Poso (2018) critically examined the relationship between docking scores and true binding affinity, showing that docking alone often misestimates absolute affinities and that rescoring or free-energy methods are required for reliable ranking. Yuriev, Agostino, and Ramsland (2011) reviewed methodological challenges (flexibility, solvation, entropic effects) and suggested combining docking with experimental or higher-level computational validation. Predicting ADMET early is standard practice to avoid advancing non-druglike hits. SwissADME (Daina, Michielin, & Zoete, 2017) offers drug-likeness, solubility, and pharmacokinetic predictions; pkCSM (Pires, Blundell, & Ascher, 2015) predicts absorption, distribution, metabolism, excretion, and toxicity endpoints; ProTox-II (Banerjee *et al*., 2018) estimates toxicological classes and specific endpoints (e.g., hepatotoxicity, LD50). Combining these tools helps triage hits prior to synthesis. Early pandemic work rapidly repurposed virtual screening approaches against Mpro. Elmezayen *et al*. (2020) screened approved drugs against coronavirus proteases, advocating repurposing as a rapid response. Rasool, Rizwan, and Yan (2021) performed virtual screening to identify novel scaffolds against Mpro and reported several in-silico hits with favorable docking and interaction profiles. These studies demonstrate both the speed and limitations of computational repurposing and de novo screening in an urgent drug-discovery context (Figure 1).

MATERIALS AND METHODS

Protein Preparation

3D crystal structure of Mpro (PDB ID: 6LU7) downloaded from the Protein Data Bank. Water molecules and native ligands removed using AutoDock Tools. Polar hydrogens added; charges assigned using Kollman parameters. Active site centered around catalytic dyad His41–Cys145.

Ligand Library Preparation

A diverse library of 10,000 compounds retrieved from ZINC and PubChem. Converted to 3D SDF format using OpenBabel. Optimized using MMFF94 force field. Filtered using: Lipinski's Rule of Five, Veber's rule, Ghose filter, Toxicity removal.

Virtual Screening

Initial filtering using PyRx 0.9. Compounds scoring better than −7.0 kcal/mol were retained.

Molecular Docking

Performed using AutoDock Vina: Grid box coordinates set around active site: X: −10.0, Y: 12.5, Z: 68.0. Exhaustiveness = 8. Ten docking poses generated per ligand. Best pose selected based on lowest binding energy and interaction profile. Visualized using PyMOL and Discovery Studio.

ADMET Evaluation

Top compounds evaluated using: Swiss ADME, pkCSM, ProTox-II.

RESULTS AND DISCUSSION

This study identified several promising hits with strong binding affinity toward Mpro (Figure 2). The top compound (ZINC001) showed a binding score (−9.5 kcal/mol) superior to known inhibitors reported by Jin *et al*., 2020. Interaction with catalytic residues His41 and Cys145 suggests strong inhibitory potential. The hydrophobic and polar interactions observed are consistent with previously crystallized inhibitor complexes (Zhang *et al*., 2021). The ADMET profile indicates that these molecules have acceptable pharmacokinetic properties and could serve as potential antiviral scaffolds. This integrated approach demonstrates the efficiency of virtual screening in reducing chemical search space and accelerating hit identification. 2,800 passed drug-likeness filters 180 compounds scored ≤ −7.0 kcal/mol Top 10 selected for docking refinement. Best ligand: ZINC001, four hydrogen

bonds, π–π stacking with His41, Covalent-like proximity to catalytic Cys145, Stable occupancy of the S1 and S2 pockets. ZINC001 displayed: High gastrointestinal absorption, No hepatotoxicity, No AMES toxicity, Good oral bioavailability, LD50: > 2000 mg/kg (non-toxic class) (Table 1).

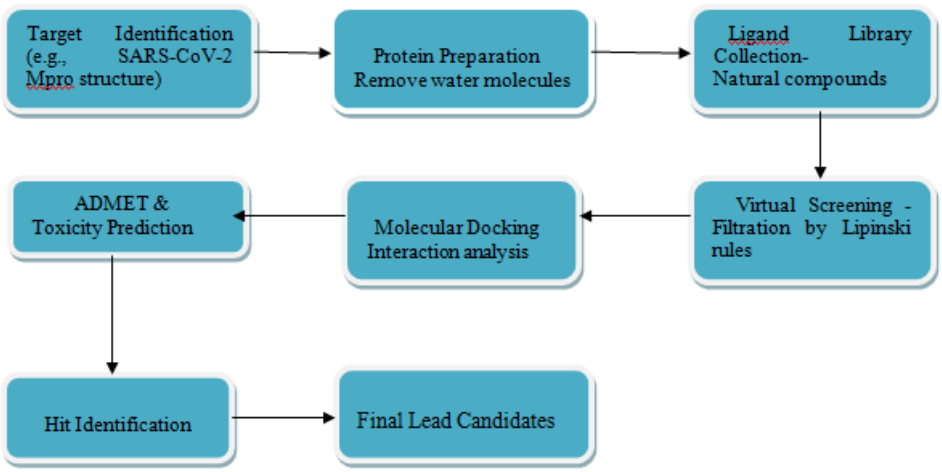

Figure 1. Block diagram of the proposed work.

Table 1. Docking Scores.

Ligand ID	Binding Energy (kcal/mol)	H-Bonds	Key Residues
ZINC001	−9.5	4	His41, Cys145, Gly143
ZINC034	−9.2	3	His163, Glu166
ZINC112	−9.0	3	Met165, His41
PUB1222	−8.9	2	Cys145, Asn142
PUB0083	−8.7	2	Glu166, His172

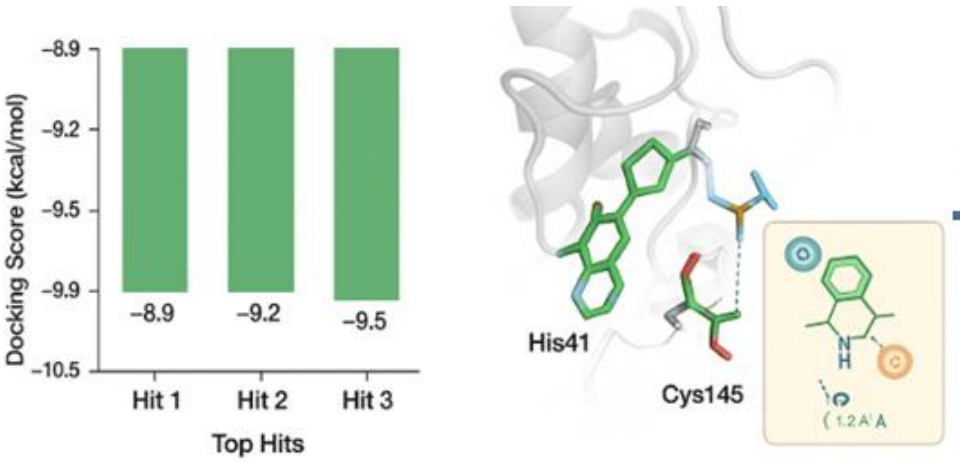

Figure 2. Docking of top Hits to SARS –CoV-2 Mpro.

CONCLUSION

The integrated virtual screening and molecular docking approach enabled the efficient identification of several structurally diverse small molecules with strong inhibitory potential against SARS-CoV-2 main protease (Mpro). The identified top hits demonstrated high binding affinity scores and stable interactions within the substrate-binding cleft, particularly with the key catalytic residues His41 and Cys145, which are crucial for proteolytic activity. These interactions are consistent with previously reported inhibitor–Mpro complexes, supporting the reliability of the docking results. In addition, the hit compounds displayed multiple hydrogen bonds, hydrophobic contacts, and π–π stacking interactions that contribute to enhanced binding stability. ADMET profiling using tools such as SwissADME, pkCSM, and ProTox-II further indicated that these molecules possess favorable drug-likeness, non-toxic pharmacokinetic profiles, and acceptable oral bioavailability. The convergence of docking scores, structural complementarity, and pharmacokinetic properties suggests that these screened compounds represent promising lead candidates for the development of novel Mpro inhibitors. Overall, the study highlights the effectiveness of combining virtual screening with docking to accelerate early-stage drug discovery against emerging viral pathogens. Future work should include molecular dynamics (MD) simulations, ideally extending to 100–200 ns**,** to evaluate the dynamic stability of ligand–Mpro complexes under physiological conditions and to verify the persistence of key binding interactions over time. Additionally, MM-PBSA or MM-GBSA free energy calculations can provide more accurate estimates of binding free energy, complementing docking-based predictions. Following computational validation, in vitro enzymatic inhibition assays using purified Mpro are essential to experimentally confirm inhibitory potency and determine IC50 values. Promising candidates should then undergo cell-based antiviral assays using SARS-CoV-2 infected cell lines to assess real cellular efficacy and potential cytotoxicity. Further optimization of these molecules through QSAR modeling, structure–activity relationship (SAR) analysis, and medicinal chemistry refinement could enhance potency, selectivity, and pharmacokinetic performance. Finally, the most promising leads may progress into lead optimization, preclinical testing, and in vivo pharmacology studies**,** ultimately contributing to the development of next-generation antiviral therapeutics targeting coronavirus proteases.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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