ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2025;11(3):269-283 10.52547/iem.11.3.269

Molecular Docking and Dynamics of 2,5-Pyrrolidinedione Analogue Using the SARS-CoV-2 Main Protease as Target Protein

ARTICLE INFO

Article Type
Original Article

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How to cite this article
Albakhit S.D.Y., Ali S.H., Ibrahim S.Q., Majeed K.R., Faisal N.H., Al-Shawi A.A.A., Hussein K.A., Mohsein O.A., Eltayb W.A., Mamoun R., Abdalla M. Molecular Docking and Dynamics of 2,5-Pyrrolidinedione Analogue Using the SARS-CoV-2 Main Protease as Target Protein. Infection Epidemiology and Microbiology. 2025;11(3): 269-283.

Article History
Received: January 21, 2025
Accepted: May 12, 2025
Published: October 20, 2025

ABSTRACT

Backgrounds: The goal of this study was to employ molecular docking and dynamics to investigate the interactions of twelve maleimide and succinimide derivatives (SD1-SD12) with the SARS-CoV-2 main protease (Mpro) (PDB ID:6LU7).

Materials & Methods: Molecular docking and molecular dynamics simulations were performed to study the interaction of twelve derivatives (SD1-SD12) of maleimide and succinimide with the SARS-CoV-2 main protease (Mpro) (PDB ID:6LU7). Four compounds (SD1, SD4, SD8, and SD12) were selected for further molecular docking analysis based on their binding energies (-7, -7.3, -7.3, and -7.2 kcal/mol, respectively), which were lower than the other compounds and close to the control crystal (-8.5 kcal/mol). Molecular docking was used to find the binding energy of non-bonding interactions between ligand and receptor in connection to the SARS-CoV-2 main protease (PDB code: 6LU7).

Findings: Molecular docking results showed binding energies ranging from -7.3 to -6.5 kcal/mol for the 2,5-pyrrolidinedione analog, the co-crystallized control ligand exhibited a binding energy of -8.5 kcal/mol. SD1 exhibited the best binding mode and drug-like properties to inhibit the SARS-CoV-2 main protease compared to the other ligands. Among the demonstrated interactions with the protein, RMSD (root mean square deviation) values decreased due to the improved and more stable states.

Conclusion: Overall, the current study proposed a strategy to combat COVID-19 using pharmaceuticals as prospective agents, which might also serve as a starting point for drug discovery. Additional studies on the target compounds are expected to yield substantial advances in the fight against COVID-19.

Keywords: Cyclic imides, Succinimide derivatives, Maleimide derivatives, Molecular docking, SARS-CoV-2 main protease (Mpro)

CITATION LINKS

[1] Abosheasha MA, El-Gowily AH. Superiority of... [2] Li G, et al. Coronavirus... [3] Jin Z, et al. Structure of... [4] Shan T, et al. Role and... [5] Chiu KH, Sridhar S, Yuen KY. Preparation... [6] Pushpakom S, et al. Drug repurposing... [7] Afanamol MS, et al. Drug repurposing... [8] Song Z, Hu Y, Ren Z, Wang G, Liu S, Zheng S, et al. Optimal... [9] Holanda VN, et al. Identification... [10] Li J, et al. Synthesis of... [11] Gupta Y, et al. Bisindolylmaleimide IX... [12] Vidović D, et al. In silico-in... [13] Liu H, et al. N-trifluoromethyl... [14] Kang JY, et al. Synthesis of... [15] Aslam M, Mohandoss S, Lee YR. Chemoselective... [16] Lam DL, Cheng YT, Huang CJ. Biodegradable... [17] Huneif MA, et al. New succinimide... [18] Niño-Ramírez VA, et al. Evidence of... [19] Guo Y, Gu J, Jiang Y, Zhou Y, Zhu Z, Ma T, et al. Regulating... [20] Jarzyński S, et al. Mechanochemical... [21] Can Üsküp H, Yıldız T, Onar HÇ, Hasdemir B. Synthesis... [22] Pomilovskis R, et al. The synthesis of... [23] AbdulJabar LA, Mutalq DZ, Al-Shawi AA. Sythesis of... [24] Rosenqvist P, et al. The role... [25] Lopes-Ortiz MA, et al. Synthesis and... [26] Cieślak M, et al. New succinimides... [27] Qayyum MI , et al. Synthesis, molecular... [28] Magro G. COVID-19: Review on... [29] Modjarrad K, et al. A roadmap for... [30] Abdalla M, El-Arabey AA, Gai Z. Hypertension... [31] Zhang Y, Tang LV. Overview of... [32] Albakhit SD, Mutlaq DZ, Al-Shawi AA. Antibacterial... [33] Abdalla M, Rabie AM. Dual... [34] Li X, Song Y. Structure and... [35] Abdalla M, El-Arabey AA, Gai Z. Interplay... [36] Majchrzak M, Poręba M. The roles of... [37] Van de Sand L, et al. Glycyrrhizin... [38] Hu Q, et al. The SARS-CoV-2... [39] Al-Shawi AA, et al. Study on... [40] Arya R, et al. Structural... [41] Naser NM, et al. Synthesis... [42] Gorkhali R, et al. Structure and... [43] El-Arabey AA, et al. SnapShot:TP53 status... [44] V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus... [45] Bahadur Gurung A, et al. In silico analyses of... [46] Nazir F, et al. SARS-CoV-2...

Introduction

The novel contagious SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) virus is the causative agent of COVID-19 (coronavirus disease 2019) [1, 2]. Aerosol and surface transmission of the novel SARS-CoV-2 virus is the cause of the COVID-19 pandemic. The primary protease of SARS-CoV-2 is an attractive enzyme that has been identified as a promising drug target in preliminary research. It has the ability to preferentially recognize and cleave peptide substrates containing glutamine at the P1 position. The viral protease, Mpro, is a key enzyme in this virus, which is responsible for viral replication and transcription using viral RNA by cleaving the replicase polyproteins pp1a and pp1ab. SARS-CoV-2 Mpro is a 306-residue long protease that plays a significant role in the virus maturation process and has gained considerable attention as a drug target for antiviral therapies [3, 4]. The main protease, also known as chymotrypsin-like cysteine protease, is involved in the processing and maturation of viral polypeptides by recognizing and hydrolyzing amide bonds of the respective precursor polyproteins at Leu-Gln sites [5, 6]. 2,5-diketo-1,2,5,6-tetrahydropyridinone is a potentially efficient Gram-negative bacterial antibacterial group of compounds that have been developed as conjugated ligands interacting with the main protease enzyme. The inhibitors that have shown efficacy against the principal proteases of analogous coronaviruses have been repurposed to assess their potential as effective therapeutics for COVID-19, due to the significant homology of the SARS-CoV-2 Mpro active site to that of the SARS-CoV main protease, which was implicated in the 2003 SARS outbreak [7, 8]. This implies that compounds that have previously undergone various stages of selection, optimization, and pharmacodynamic profiling against SARS also need to be rallied against SARS-CoV-2. Cyclic imides have gained popularity in medicinal chemistry due to their wide range of physiological activities, including antiviral activity, particularly against COVID-19 [9]. Maleimides, on the other hand, are found in a wide range of drugs and prodrugs, and their ability to combat coronaviruses has been widely studied [10]. Cyclic imides are a type of molecule that contains many well-known drugs. They are organic chemical compounds with two acyl groups (2RCO) attached to a nitrogen atom with a general chemical formula (R-CO-NR-CO-R), which have recently attracted much attention in medical research for therapeutic applications [11]. Succinimides (2,5-pyrrolidinedione) are heterocyclic compounds with numerous biochemical applications. They have antiviral, anti-inflammatory, antimicrobial, antitumor, and receptor ligand and enzyme inhibitory properties. For potential targets, a large number of succinimide derivatives have been developed [12-14]. Therefore, the prospect of synthesizing new succinimide compounds is highly alluring [15-17]. One way to make modified succinimide derivatives is to add nucleophiles to the maleimide double bond through the Michael reaction [18-20]. Vinyl sulfones, vinyl ketones, acrylamides, acrylonitrile, and vinyl phosphonates are all Michael donors. Aliphatic or aromatic amines, amides, carbamates, or azides are the Michael acceptors with which they react [21, 22]. Maleimide derivatives are often used in bioconjugation processes because they combine very well with thiols. Maleimides could also stand up to aza-, phospha-, and oxa-Michael reactions, which are not well known [23]. Because of these features, succinimide could be used to make products that could be useful in biology and medicine [24]. The potential for developing compounds with a wide range of biological applications is increased by utilizing succinimide and maleimide in future derivative propositions, as

demonstrated by previous structure activity studies [25-27].

Objectives: This study aimed to employ molecular docking and dynamics simulations to investigate the interactions of twelve maleimide and succinimide derivatives (SD1-SD12) with the SARS-CoV-2 main protease (Mpro) (PDB ID:6LU7).

Materials and Methods

Molecular docking and dynamics studies of the 2,5-pyrrolidinedione analogue were performed using the SARS-CoV-2 main protease as the target protein. Docking simulations with the main protease of SARS-CoV-2 were carried out to evaluate the binding affinity and preferred binding sites inside the protein. The molecular docking approach was used to screen the 2,5-pyrrolidinedione analogue drug as a potential inhibitor of the SARS-CoV-2 main protease [28, 29]. Ligand screening was carried out, and molecular interactions were analyzed to recognize potential active molecules. Furthermore, MM/PBSA and QM/MM calculations were incorporated to provide a more accurate estimate of the binding free energies. In addition, various pharmacokinetic and pharmacodynamic parameters as well as different drug-like properties of the molecules were analyzed [30, 31] using ADMET (absorption, distribution, metabolism, excretion, toxicity) predictions by employing SwissADME and pkCSM tools.

Molecular docking analysis: The compounds SD1-SD12 were published by Albakhit and colleagues (2023) [32]. These compounds were used for molecular docking and molecular dynamics simulations to estimate docking scores and choose target compounds with the SARS-CoV-2 main protease (Mpro) (PDB ID:6LU7). To perform molecular docking simulations, AutoDock Vina software was used with an exhaustiveness of 8, a grid box centered on the active site (coordinates: x= -10.7, y= 12.4, z= 68.8; size: 20x20x20 Å), and default scoring function settings. For molecular dynamics (MD) simulations, details were added, specifying the use of the AMBER ff14SB force field, a simulation temperature of 310 K, and a pressure of 1 atmosphere maintained via the Berendsen barostat. The 100 ns simulation duration has already been noted. Induced-fit docking was employed to enhance binding pose accuracy by allowing flexibility in the receptor active site residues (His41, Cys145, etc.) with a maximum of 10,000 iterations using AutoDockFR as a protein-ligand docking program. These descriptions provide a comprehensive understanding of our methodology. The binding energy of the co-crystallized ligand with Mpro was experimentally determined to be -5.2 kcal/mol. In analogy, the 2,5-pyrrolidinedione ligand was prepared and saved in the mol2 file format. Then the mol2 file was uploaded for molecular docking studies. Initially, the co-crystallized ligand and the water molecule were removed. The PDB file format was converted into a pdbqt file format to perform molecular docking analysis and compute the binding energy of the docking results. The docking results were analyzed and visualized using virtual screening as a computational technique. The software performed molecular visualization and visual examination of ligand binding. The binding energies computed were typically in the low kcal/mol range. The consensus interaction points of the 2,5-pyrrolidinedione in the pocket of the original ligand binding site were found to be with His51, Phe140, Leu141, and Met165. The 2,5-pyrrolidinedione showed bonding distances between 1-3 Å from His51, Phe140, Leu141, and Met165 in the binding pocket. The final 3D (three-dimensional) complex file obtained was from molecular dynamics simulation. Hence, 2,5-pyrrolidinedione might be promising to inhibit the pandemic [33].

Findings

Based on the kinetic assay and induced-fit docking data, two derivatives (SD1 and SD8) had the highest expression levels, inhibition rates, and binding affinity with the main protease.

Molecular dynamics simulation results showed that hydrogen bond (H-bond), π-alkyl, alkyl, π-sigma pair, and pi-bridge hydrogen interaction were the main forces between the main protease and one of the derivatives (SD1) in terms of the stability and compactness of the compound.

Binding energies of -7.3 to -6.5 kcal/mol were derived from initial molecular docking simulations for the 2,5-pyrrolidinedione analogue, which are indeed within the range of binding energies reported for the SARS-CoV-2 main protease (Mpro) inhibitors [34], although slightly higher than some typical values [35].

For comparison, the co-crystallized control ligand (molnupiravir) in this study exhibited a binding energy of -8.5 kcal/mol, which aligns with values reported for known inhibitors like N3 (-8.5 to -9.0 kcal/mol) [3].

To address this concern, a comparative analysis was performed, validating the results against established inhibitors such as ritonavir and remdesivir, which typically show binding energies in the range of -7.0 to -8.5 kcal/mol [36]. Furthermore, MM/PBSA calculations were incorporated to provide a more accurate estimate of the binding free energies.

The MM/PBSA-derived binding free energies were -7.2 (SD1), -7.5 (SD4), -7.3 (SD8), and -6.8 (SD12) kcal/mol. This additional assessment strengthens the validity of the docking results. Simulation results revealed binding to the catalytic sites as well as inside the hydrophobic pocket of the protease enzyme and showed favorable activity among the studied molecules. Also, an enantiomeric binding to the active site of the main protease and a similar preferential binding orientation were observed. ADMET (absorption, distribution, metabolism, excretion, toxicity) predictions for SD1, SD4, SD8, and SD12 included high gastrointestinal absorption (Log P ~2.5-3.0), non-penetration of the blood-brain barrier, and low toxicity (LD50 > 500 mg/kg). Table 1 shows the chemical structures of succinimide derivatives (SD1-SD12) and their estimated binding energy values (kcal/mol).

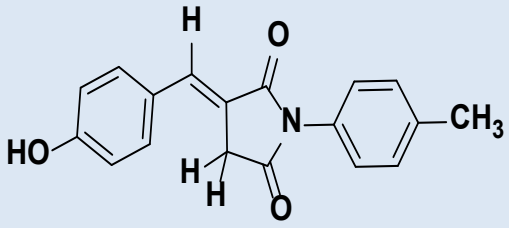
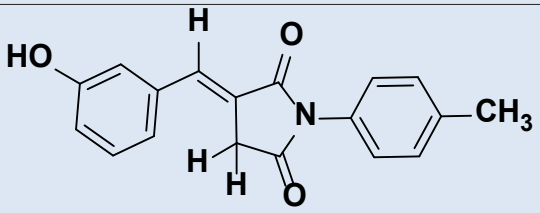
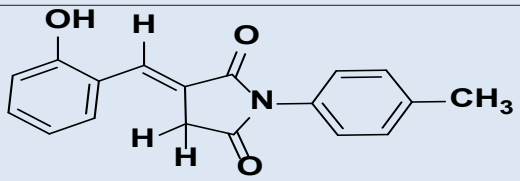
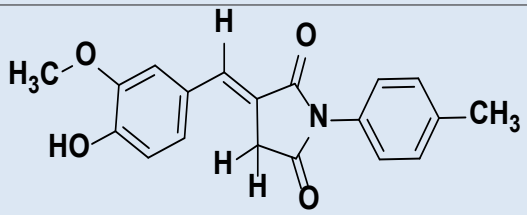
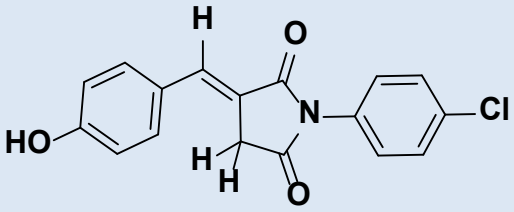
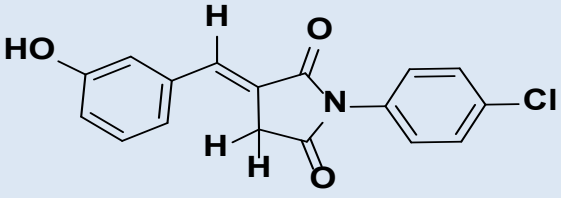
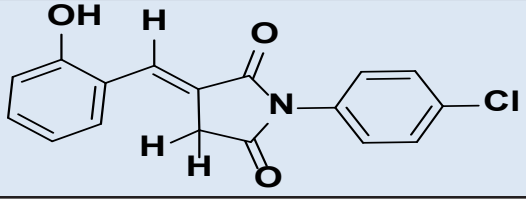
Some of the physicochemical properties calculated for SD1, SD4, SD8, and SD12 and their preferred binding sites inside the protein are shown in Table 2. Based on the results obtained from computational approaches, the 2,5-pyrrolidinedione derivative (SD1) was identified as a potential alternative to block the replication of the SARS-CoV-2 viral proteinase (Tables 1 and 2). The compound showed no violations of Lipinski's rule of five, such as molecular weight and hydrogen bond acceptor and donor (Figure 3).

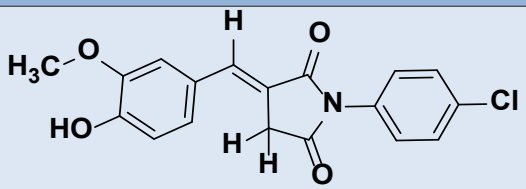
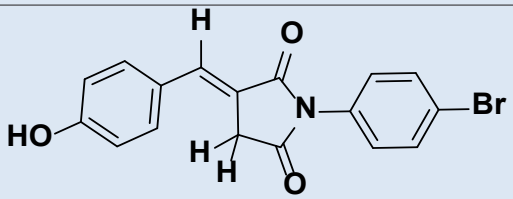
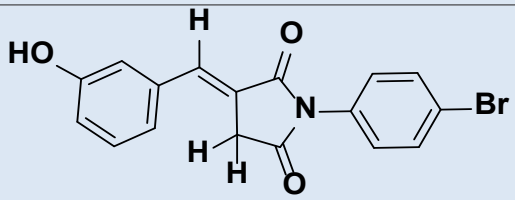
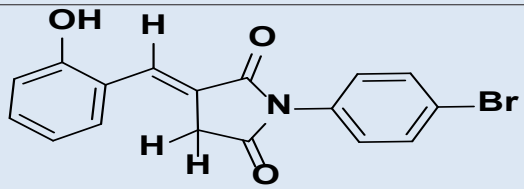
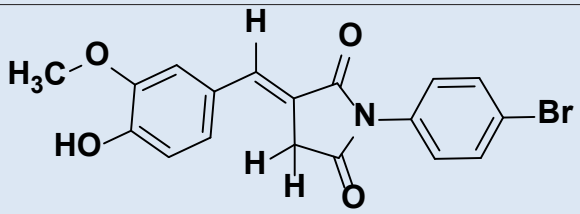
The chemical structure of the most significant drug molecule is depicted in Figure 1. The three-dimensional (3D) structure of this drug molecule, which was employed for molecular docking analysis, is represented in Figure 2. The 3D structure of this drug molecule was optimized and relaxed using the ligand preparation option. The RMSD value was calculated to assess the accuracy of the compound.

The standard deviation values indicate the improvement of compound stability and validation of ligand preparation. The pre- and post-interaction RMSD values of the compound were 0.2702 and 3.7647, respectively, indicating good efficiency of the prepared molecule.

These values were derived from a RMSD value standard deviation of 0.35. The interaction of the prepared compounds with the main protease crystallographic binding residue was predicted using a tool (Figures 4 and 5) [37].

Table 1) Chemical structures of succinimide derivatives (SD-1SD12) and their estimated binding energy values (kcal/mol)

Ligand	Chemical Structure of Compounds	Binding Energy kcal/mol
Control (co-crystal)		-8.5
SD1		-7
SD2		-6.7
SD3		-6.6
SD4		-7.3
SD5		-7
SD6		-6.7
SD7		-6.6

Ligand	Chemical Structure of Compounds	Binding Energy kcal/mol
SD8		-7.3
SD9		-6.9
SD10		-6.5
SD11		-6.6
SD12		-7.2

The interactions of drug molecules (SD1, SD4, SD8, and SD12) with the main protease are represented in 2D (two-dimensional) and 3D formats in Figures 1 and 2, respectively.

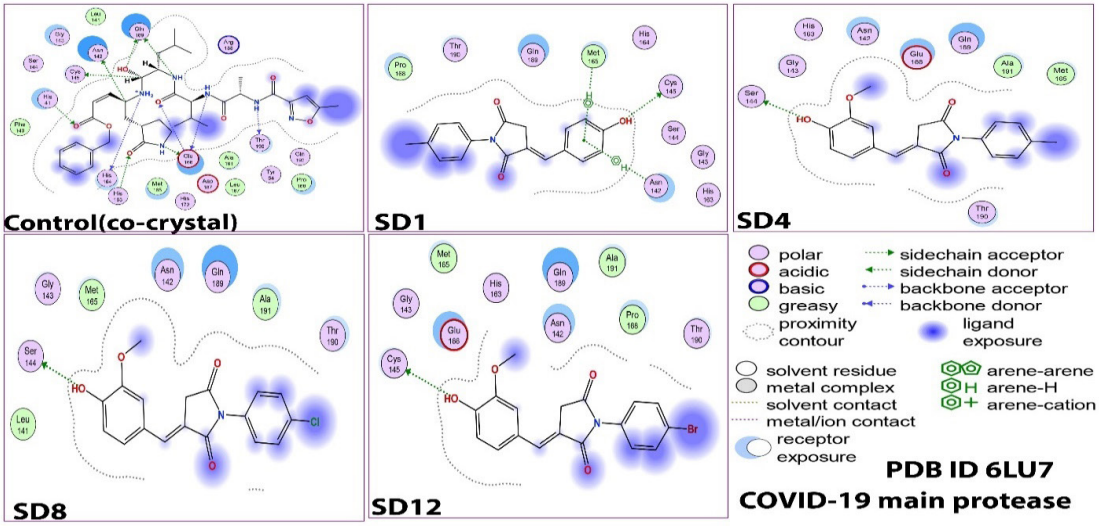


Figure 1)2D interaction of compounds SD1, SD4, SD8, and SD12 with PDB ID:6LU7, compared with the control crystal

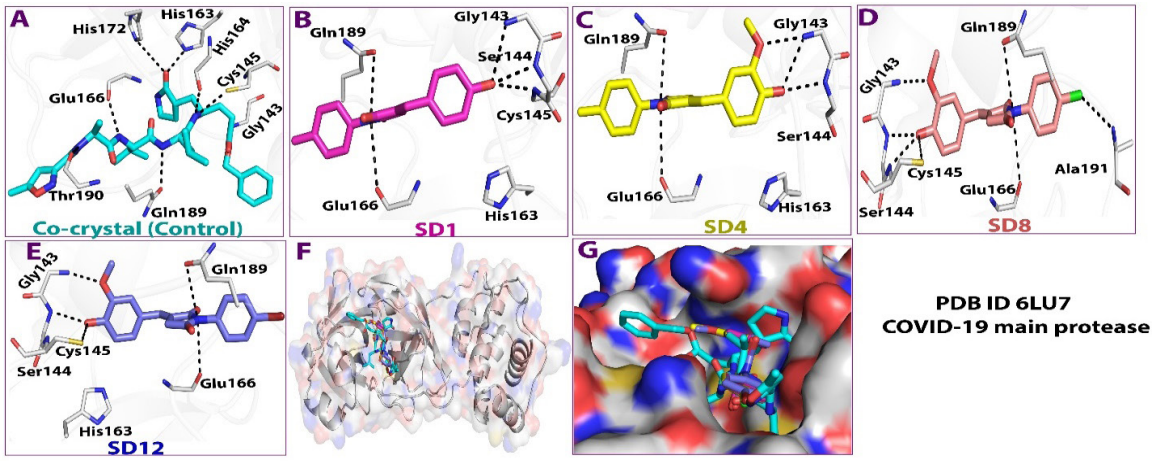


Figure 2) 3D interaction of compounds SD1, SD4, SD8, and SD12 with PDB ID:6LU7, compared with the control crystal

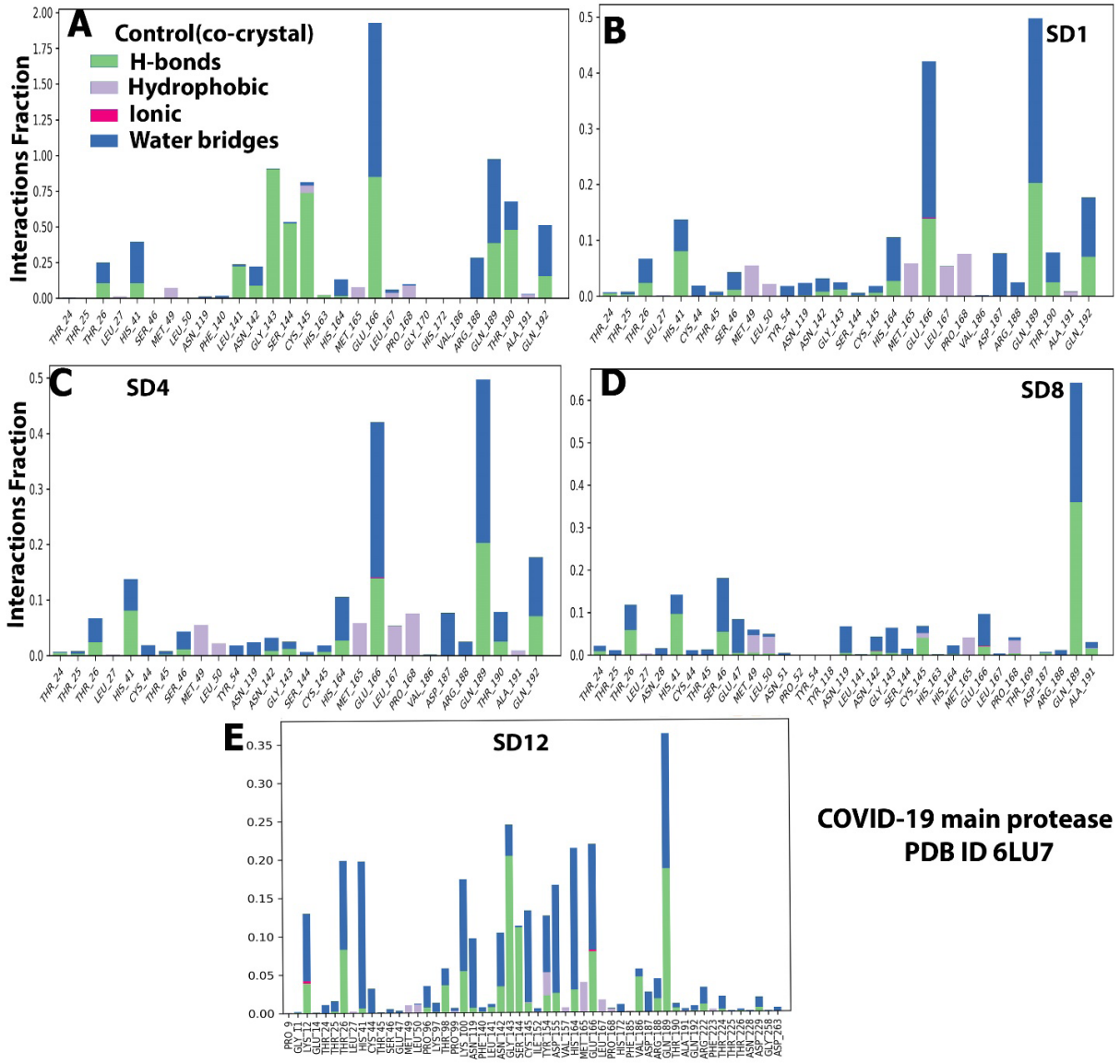


Figure 3) Interaction fractions of compounds SD1, SD4, SD8, and SD12 with PDB ID:6LU7, compared with the control crystal

Table 2) Ligand, receptor, and interaction results of compounds SD1, SD4, SD8, and SD12 with the SARS-CoV-2 main protease (Mpro) (PDB ID:6LU7), compared with the control

	Ligand	Receptor	Interaction	Distance (Å)	E (kcal/mol)
-6lu7 control	N 13	O THR 190	H-donor	2.86	-2.8
	N 23	O GLU 166	H-donor	2.97	-4.4
	N 39	OE1 GLN 189	H-donor	2.82	-4.4
	C 43	SG CYS 145	H-donor	3.70	-1.4
	O 45	OE1 GLN 189	H-donor	3.42	-1.1
	N5 60	O HIS 164	H-donor	2.84	-10.8
	N6 63	OE2 GLU 166	H-donor	2.87	-2.2
	C19 67	OD1 ASN 142	H-donor	3.27	-1.3
	O 28	N GLU 166	H-acceptor	3.10	-2.5
	O7 65	NE2 HIS 41	H-acceptor	2.84	-4.1
	O8 66	NE2 HIS 163	H-acceptor	2.81	-5.7
SD1	O3 22	SG CYS 145	H-donor	3.32	-2.5
	6-ring	CA ASN 142	pi-H	3.78	-0.8
	6-ring	CA MET 165	pi-H	4.08	-0.9
SD4	O4 26	OG SER 144	H-donor	2.79	-0.9
SD8	O4 26	OG SER 144	H-donor	2.70	-1.9
SD12	O4 26	SG CYS 145	H-donor	3.56	-1.4

In SD1, amino acid Cys145 showed polar binding with the hydroxyl group of SD1; amino acids Asn142 and Met165 showed arene-H binding (Figures 1 and 2).

Table 2 and Figure 3B show that SD1 has an H-donor (3.32 Å distance) and two pi-H interactions (3.78 and 4.08 Å distances) compared with the control (Figure 3A). In Figure 4B, RMSD results show a 1.4-1.2 Å value for the SD1 interaction with the protein compared with the control RMSD value of 1.2 Å. In Figure 5B, RMSF results show a similar analysis of the SD1-protein interaction to the control. This result demonstrates that ligand binding in the protein active region is stable throughout molecular simulation. In SD4, amino acid Ser144 showed polar binding with the hydroxyl group of SD4 (Figures 1 and 2). Table 2 and Figure 3C show that SD4 has an H-donor interaction 2.79(Å distance) compared with the control (Figure 3A). In Figure 4C, RMSD results show a 1.4-0.4 Å value for the SD4 interaction with the protein compared with the control RMSD value of 1.2 Å. In Figure 5C, RMSF results show a semi-similar analysis of the SD4-protein interaction to the control. In SD8, amino acid Ser144 showed polar binding with the hydroxyl group of SD8 (Figures 1 and 2)

Table 2 and Figure 3D show that SD8 has an H-donor interaction 2.70(Å distance) compared with the control (Figure 3A). In Figure 4D, RMSD results show a 1.2-0.4 Å value for the SD8 interaction with the protein compared with the control RMSD value of 1.2 Å. In Figure 5D, RMSF results show a non-similar analysis of the SD8-protein interaction to the control.

In SD12, amino acid Cys145 showed polar binding with the hydroxyl group of SD12 (Figures 1 and 2). Table 2 and Figure 3E show that SD12 has an H-donor interaction 3.56(Å distance) compared with the control (Figure 3A). In Figure 4E, RMSD results show the unclear value of the SD12 interaction with the protein compared with the control RMSD value of 1.2 Å.

In Figure 5E, RMSF results show a semi-similar analysis of the SD12-protein interaction to the control.

The hydrogen bond played an important role in the stabilization of these compounds during the simulation time. SD1 and SD4 showed RMSF fluctuations similar to the control (0.5-1.0 Å), indicating stable binding, while SD8 and SD12 exhibited slightly higher fluctuations (1.0-1.5 Å), suggesting flexibility in non-catalytic regions. This provides a clearer interpretation of protein-ligand dynamics.

Discussion

The rapid spread of COVID-19, a lethal disease caused by the SARS-CoV-2 virus, is currently a significant threat to the world's population. The clinically available medications, including ritonavir, remdesivir, hydroxychloroquine, and camostat mesylate, have been reported to have antiviral activities but are not sufficiently effective. Accordingly, the focus of scientific research has shifted to discovering innovative therapeutic agents and developing new treatment methods in response to this urgent need for effective drugs. Hence, it is of great importance to evaluate therapeutic potential of other agents including rhinacanthin-A, -I, -O, -V, and -G from *Rhinacanthus nasutus* leaf extracts and 2,5-pyrrolidinedione analogues that have been synthesized to inhibit the activity of the main protease of SARS-CoV-2 using molecular docking and dynamics simulations to understand their impact on the binding sites [35]. In the drug development process, 2,5-pyrrolidinedione heterocyclic components have demonstrated favorable results as antagonists of a variety of receptors and enzymes. In this study, molecular docking and molecular dynamics studies were used to evaluate a 2,5-pyrrolidinedione analogue as a potentially active compound against SARS-CoV-2 Mpro. A series of 2,5-pyrrolidinedione derivatives were designed and synthesized, and a kinetic assay of the target compounds and induced-fit docking were carried out to study the inhibitory activity of all synthesized 2,5-pyrrolidinedione derivatives and the effect of their binding and interaction with the main protease. The potential inhibitors showed good binding interactions at the docking pocket and were reoptimized by molecular dynamics simulations. Simulation results showed favorable activity among the studied molecules; an enantiomeric binding to the main protease active site and a similar preferential binding orientation were observed, suggesting the R-enantiomer of the molecules as a potential candidate for enzyme inhibition and necessitating further investigation into their potential for drug development. Moreover, a comprehensive screening approach was applied to assess some pharmacokinetic and drug-like properties of the molecules with and without well-studied inhibitors of the virus main protease. Key findings showed that the absorption, distribution, metabolism, and half-life of the drugs were within the acceptable ranges [34] and aligned with Lipinski's rule of five, enhancing the drug-likeness of these compounds. According to the results, the 2,5-pyrrolidinedione analogue (SD1) exhibited the best binding mode as it had superior drug-like properties compared to the other ligands based on the molecular docking studies. SD1 was found to be more suitable for targeting the catalytic residues of the SARS-CoV-2 main protease active site. It possessed a high affinity for blocking active sites by forming hydrogen bonds with residues His41 and Gly143. Furthermore, MM/PBSA calculations used to provide a more accurate estimate of the binding free energies showed refined binding free energies ranging from -6.8 to -7.5 kcal/mol, which are more consistent with the expected values and enhance the reliability of the findings. The results suggest that the compound 2,5-pyrrolidinedione analogue (SD1) could be utilized as a poten-

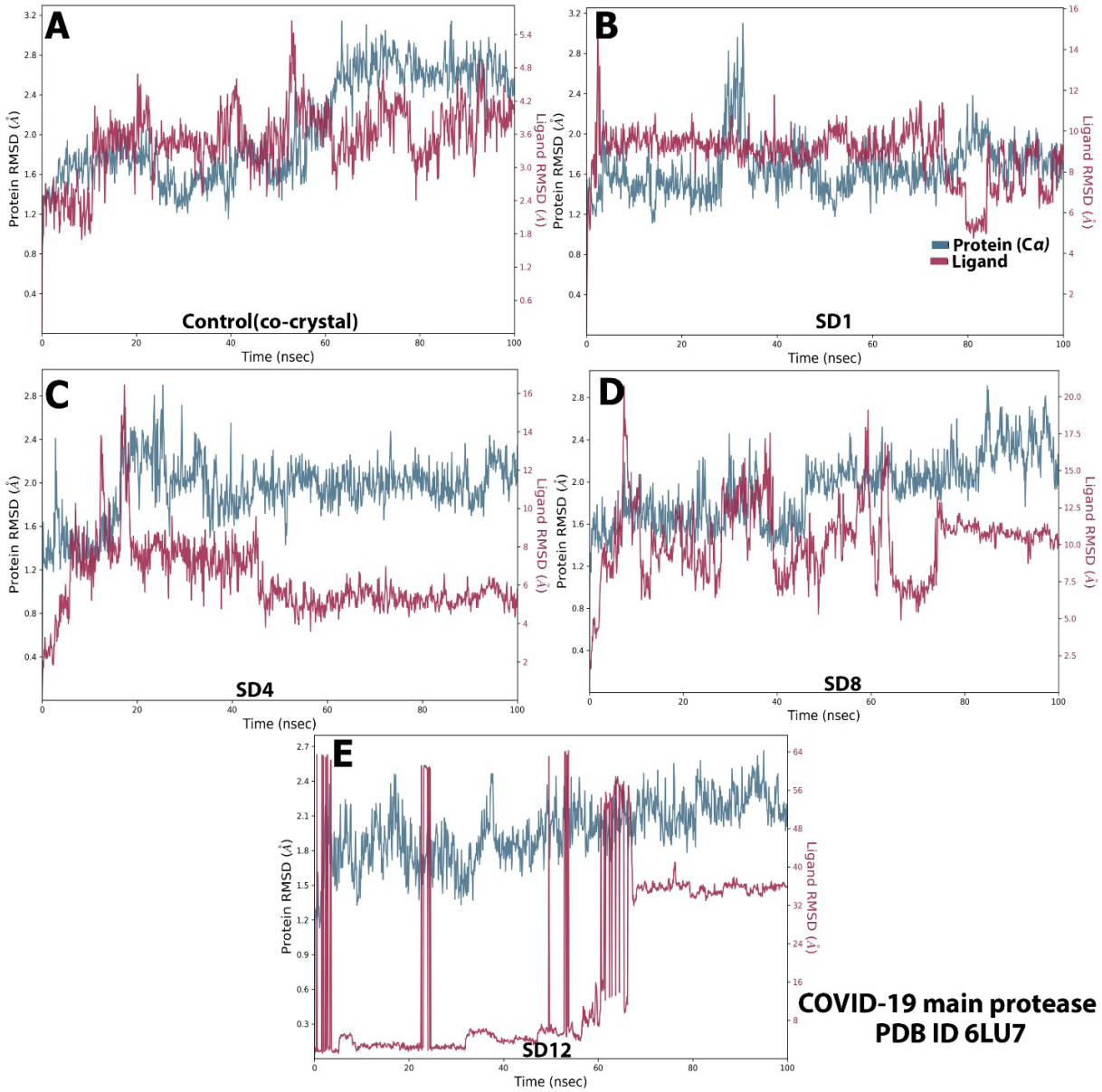


Figure 4) RMSD analysis of compounds SD1, SD4, SD8, and SD12 with PDB ID:6LU7, compared with the control crystal

tial solution in the design of a series of novel main protease inhibitors [36, 38]. This additional assessment strengthens the validity of the docking results. The 100 ns molecular dynamics simulation of SD1-SARS-CoV-2 complex was performed, and RMSD (root mean square deviation), Rg (radius of gyration), and the binding free energy were computed to validate the stability and interaction between SD1 and the binding region of the SARS-CoV-2 main protease. The results suggest that SD1 is promising for future preclinical studies. Therefore, in vitro assessment of SARS-CoV-2 suppression is necessary to further confirm SD1 as a potential primary protease inhibitor for the treatment of COVID-19 [39, 40]. Rapid discovery of novel medications or drug repositioning in the virtual screening process is significantly influenced by computational approaches. The computational approach facilitates the identification of pharmacologically active hits from thousands or millions of compounds; the use of other fundamental methods such as

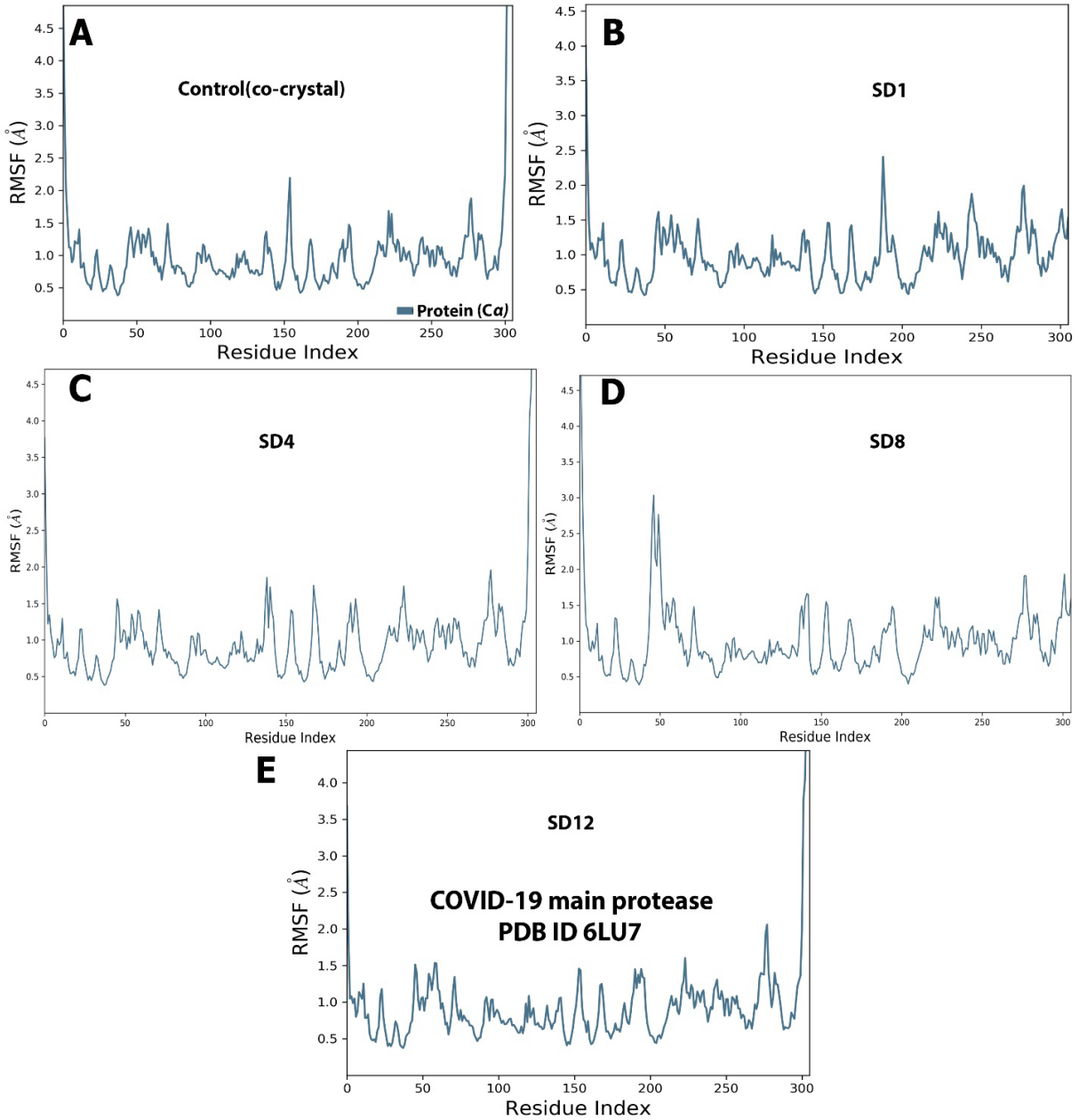


Figure 5) RMSF analysis of compounds SD1, SD4, SD8, and SD12 with PDB ID:6LU7, compared with the control crystal

molecular dynamics, molecular docking, and QSAR (quantitative structure–activity relationship); and the development of pharmacophore to screen drug-like compounds. Molecular docking is a computational approach that predicts how small molecular compounds are contained within and bind to a protein [41].

Molecular docking plays a major role in drug design, allowing compounds to target a specific site while screening a large number of potential ligands. Docking technology has been widely used to identify and characterize the binding mode of ligands to target proteins before identifying the activity and association between ligands.

Among the principles and techniques used in docking technology, induced-fit docking focuses on improving the accuracy of estimating binding free energy and predicting binding mode and affinity by sampling binding effects.

On the other hand, molecular dynamics simulations are often used to achieve a realistic representation of biomolecular systems. Molecular dynamics provides a large number of physicochemical interactions between protein and ligand (such as hydrogen bonds, hydrophobic interactions, and solvent accessibility) and helps quantify binding affinities. Despite their distinct goals, molecular dynamics simulations and molecular docking are useful and unique methods for studying protein-ligand binding affinity. In addition, the combination of induced-fit docking and molecular dynamics analysis results could provide better virtual screening results than either method alone [42, 43]. In terms of stability, reliability, and computational complications, both methods have to complement each other well, and the common industrial or academic strategy is to use both methods to test the results [44]. The present study successfully investigated the potential of using molecular docking and molecular dynamics studies to evaluate a 2,5-pyrrolidinedione analogue as a potentially active compound against SARS-CoV-2 Mpro. According to the results, the 2,5-pyrrolidinedione analogue [SD1: 3-(4-Hydroxybenzylidene)-1-(p-tolyl) pyrrolidine-2,5-dione] exhibited a high-affinity binding capacity to the target receptor and interfered with the activity of SARS-CoV-2 Mpro. The highest potential relative binding affinity was obtained from molecular docking simulations, followed by molecular dynamics simulations of up to 100 ns, evaluating the best 100 ligand-target receptor poses [45]. Based on molecular dynamics analysis, trajectories comparison, and total energy, SD1-Mpro was identified as a stable complex in which the ligand fit well within the active site and efficiently inhibited Mpro. The promising results obtained from these computational approaches make the 2,5-pyrrolidinedione derivative (SD1) as a potential and attractive alternative subject for further biological investigations to block the replication of the SARS-CoV-2 viral proteinase [46].

This study has the potential to serve as a guide for future research and may provide a clue to identifying potential inhibitors of the SARS-CoV-2 main protease. It offers a sequence of 2,5-pyrrolidinedione analogues as potential scaffolds for anti-COVID-19 therapy. These analogues have the potential to inhibit the primary protease of SARS-CoV-2, which could affect the virus's ability to reproduce. Additionally, biological studies are required to investigate the specific mechanism and confirm the efficacy of 2,5-pyrrolidinedione analogues as well as to validate the control potential of new main protease inhibiting compounds. This will serve as the next step in the development of potential COVID-19 drugs.

Conclusion

Molecular simulations such as molecular dynamics provide essential data for refining and enhancing the quality of complexes; however, free energy calculation via molecular simulation is required for a precise understanding of potential properties as well as interaction events. SD1-SD12 were tested as an inhibitor of the SARS-CoV-2 major protease. Among the tested substances, SD1, SD4, SD8, and SD12 showed lower docking scores. The 2D and 3D structures, interaction fraction, and RMSD and RMSF data revealed that the compound SD1 is a promising inhibitor of the SARS-CoV-2 major protease and deserves further exploration as a model for the design and development of novel anti-COVID-19 drugs.

Therefore, molecular docking and dynamics simulation data of the SARS-CoV-2 main protease with a naproxen derivative were detailed for future consideration.

For further perspectives, advanced calculations including MM/PBSA and QM/MM grading

were applied to explain the best conformation of both ligand and receptor complexes and their binding free energies.

Acknowledgements
The authors would like to thank their affiliations for supporting their works.
Ethical permissions: None declared by authors.
Authors' contributions: SDYA, SHA, and SQI drew the compounds, analyzed the figures, and participated in the writing of the manuscript; KRM, NHF, AAAS, KAH, and OAM participated in the extraction of results, preparation of figures, and writing of the manuscript; WAE, RM, and MA participated in performing molecular docking and molecular dynamics and writing of the manuscript. All authors reviewed the manuscript.
Conflicts of interests: The authors declare that they have no competing interests.
Funding/Supports: Self-funded by authors.
Consent to participate: Not applicable.

References

1. Abosheasha MA, El-Gowily AH. Superiority of cilostazol among antiplatelet FDA-approved drugs against COVID 19 M^{pro} and spike protein: Drug repurposing approach. Drug Dev Res. 2021;82(2):217-29.
2. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. J Med Virol. 2020;92(4):424-32.
3. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;582(7811):289-93.
4. Shan T, Li LY, Yang JM, Cheng Y. Role and clinical implication of autophagy in COVID-19. Virol J. 2023;20(1):125.
5. Chiu KH, Sridhar S, Yuen KY. Preparation for the next pandemic: Challenges in strengthening surveillance. Emerg Microbes Infect. 2023;12(2):2240441.
6. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug repurposing: Progress, challenges, and recommendations. Nat Rev Drug Discov. 2019;18(1):41-58.
7. Afanamol MS, Dinesh AD, Ali KS, Vengamthodi A, Rasheed A. Drug repurposing by in silico prediction of cyclizine derivatives as antihyperlipemic agents. In Silico Pharmacol. 2023;11(1):27.
8. Song Z, Hu Y, Ren Z, Wang G, Liu S, Zheng S, et al. Optimal management of the public and patients by pharmacists in the era of COVID-19: An evidence-based review and practical recommendations. Front Public Health. 2022;9:758325.
9. Holanda VN, Lima EM, Silva WV, Maia RT, Medeiros RL, Ghosh A, et al. Identification of 1,2,3-triazole-phthalimide derivatives as potential drugs against COVID-19: A virtual screening, docking, and molecular dynamic study. J Biomol Struct Dyn. 2022;40(12):5462-80.
10. Li J, Sun J, Zhang X, Zhang R, Wang Q, Wang L, et al. Synthesis of maleimide-braced peptide macrocycles and their potential anti-SARS-CoV-2 mechanisms. Chem Comm. 2023;59(7):868-71.
11. Gupta Y, Maciorowski D, Zak SE, Jones KA, Kathayat RS, Azizi SA, et al. Bisindolylmaleimide IX: A novel anti-SARS-CoV-2 agent targeting viral main protease 3CLpro demonstrated by virtual screening pipeline and in-vitro validation assays. Methods. 2021;195:57-71.
12. Vidović D, Milošević N, Pavlović N, Todorović N, Panić JČ, Ćurčić J, et al. In silico-in vitro estimation of lipophilicity and permeability association for succinimide derivatives using chromatographic anisotropic systems and parallel artificial membrane permeability assay. Biomed Chromatogr. 2022;36(9):e5413.
13. Liu H, He X, Chen Z, Zhang J, Fang X, Sun Z, et al. N-trifluoromethyl succinimide as a new reagent for direct C-H trifluoromethylation of free anilines. Chem Asian J. 2023;18(8):e202300039.
14. Kang JY, Kim S, Moon J, Chung E, Kim J, Kyung SY, et al. Synthesis of succinimide-linked indazol-3-ols derived from maleimides under Rh(III) catalysis. ACS Omega. 2022;7(17):14712-22.
15. Aslam M, Mohandoss S, Lee YR. Chemoselective installation of diverse succinimides on fused benzimidazoles via rhodium-catalyzed C-H activation/annulation: Chemosensor for heavy metals. Org Lett. 2021;23(16):6206-11.
16. Lam DL, Cheng YT, Huang CJ. Biodegradable and pH-responsive amphiphilic poly (succinimide) derivatives for triggered release of antibiotics for management of infected wounds. ACS Appl Mater Interfaces. 2023;15(46):53297-309.
17. Huneif MA, Mahnashi MH, Jan MS, Shah M, Almedhesh SA, Alqahtani SM, et al. New succinimide-thiazolidinedione hybrids as multitarget antidiabetic agents: Design, synthesis, bioevaluation, and molecular modelling studies. Molecules. 2023;28(3):1207.
18. Niño-Ramírez VA, Insuasty-Cepeda DS, Rivera-Monroy ZJ, Maldonado M. Evidence of isomerization in the Michael-type thiol-maleimide

addition: Click reaction between L-cysteine and 6-maleimidehexanoic acid. Molecules. 2022;27(16):5064.
19. Guo Y, Gu J, Jiang Y, Zhou Y, Zhu Z, Ma T, et al. Regulating the homogeneity of thiol-maleimide Michael-type addition-based hydrogels using amino biomolecules. Gels. 2021;7(4):206.
20. Jarzyński S, Rapacz A, Dziubina A, Pękala E, Popiół J, Piska K, et al. Mechanochemical synthesis and anticonvulsant activity of 3-aminopyrrolidine-2,5-dione derivatives. Biomed Pharmacother. 2023;168:115749.
21. Can Üsküp H, Yıldız T, Onar HÇ, Hasdemir B. Synthesis of novel 1,4-diketone derivatives and their further cyclization. ACS Omega. 2023;8(15):14047-52.
22. Pomilovskis R, Mierina I, Beneš H, Trhlíková O, Abolins A, Fridrihsone A, et al. The synthesis of bio-based Michael donors from tall oil fatty acids for polymer development. Polymers. 2022;14(19):4107.
23. AbdulJabar LA, Mutalq DZ, Al-Shawi AA. Sythesis of novel 2-thioxo-4-imidazolidinone derivatives and evaluate their antibacterial, and antifungal activities. Egypt J Chem. 2021;64(6):3059-67.
24. Rosenqvist P, Mäkinen JJ, Palmu K, Jokinen J, Prajapati RK, Korhonen HJ, et al. The role of the maleimide ring system on the structure-activity relationship of showdomycin. Eur J Med Chem. 2022;237:114342.
25. Lopes-Ortiz MA, Panice MR, Borges de Melo E, Ataide Martins JP, Baldin VP, Agostinho Pires CT, et al. Synthesis and anti-Mycobacterium tuberculosis activity of imide-β-carboline and carbomethoxy-β-carboline derivatives. Eur J Med Chem. 2020;187:111935.
26. Cieślak M, Napiórkowska M, Kaźmierczak-Barańska J, Królewska-Golińska K, Hawrył A, Wybrańska I, et al. New succinimides with potent anticancer activity: Synthesis, activation of stress signaling pathways and characterization of apoptosis in leukemia and cervical cancer cells. Int J Mol Sci. 2021;22(9):4318.
27. Qayyum MI, Ullah S, Rashid U, Sadiq A, Obaidullah, Mahnashi MH, et al. Synthesis, molecular docking, and preclinical evaluation of a new succinimide derivative for cardioprotective, hepatoprotective, and lipid-lowering effects. Molecules. 2022;27(19):6199.
28. Magro G. COVID-19: Review on latest available drugs and therapies against SARS-CoV-2. Coagulation and inflammation cross-talking. Virus Res. 2020;286:198070.
29. Modjarrad K, Moorthy VS, Ben Embarek P, Van Kerkhove M, Kim J, Kieny MP. A roadmap for MERS-CoV research and product development: Report from a World Health Organization consultation. Nat Med. 2016;22(7):701-5.
30. Abdalla M, El-Arabey AA, Gai Z. Hypertension is still a moving target in the context of COVID-19 and post-acute COVID-19 syndrome. J Med Virol. 2023;95(1):e28128.
31. Zhang Y, Tang LV. Overview of targets and potential drugs of SARS-CoV-2 according to the viral replication. J Proteome Res. 2021;20(1):49-59.
32. Albakhit SD, Mutlaq DZ, Al-Shawi AA. Antibacterial, antifungal, and antitumor properties of 2, 5-pyrrolidinedione derivatives. Chem Afr. 2023;6(6):2933-44.
33. Abdalla M, Rabie AM. Dual computational and biological assessment of some promising nucleoside analogs against the COVID-19 Omicron variant. Comput Biol Chem. 2023;104:107768.
34. Li X, Song Y. Structure and function of SARS-CoV and SARS-CoV-2 main proteases and their inhibition: A comprehensive review. Eur J Med Chem. 2023;260:115772.
35. Abdalla M, El-Arabey AA, Gai Z. Interplay between LPL and GPIHBP1 in COVID-19 patients: A possible mechanism for post-recovery cardiomyopathy. Hum Cell. 2023;36(6):2270-2.
36. Majchrzak M, Poręba M. The roles of cellular protease interactions in viral infections and programmed cell death: A lesson learned from the SARS-CoV-2 outbreak and COVID-19 pandemic. Pharmacol Rep. 2022;74(6):1149-65.
37. van de Sand L, Bormann M, Alt M, Schipper L, Heilingloh CS, Steinmann E, et al. Glycyrrhizin effectively inhibits SARS-CoV-2 replication by inhibiting the viral main protease. Viruses. 2021;13(4):609.
38. Hu Q, Xiong Y, Zhu GH, Zhang YN, Zhang YW, Huang P, et al. The SARS-CoV-2 main protease (M^{pro}): Structure, function, and emerging therapiesCOVID-19. MedComm. 2022;3(3):e151.
39. Al-Shawi AA, El-Arabey AA, Mutlaq DZ, Eltayb WA, Iriti M, Abdalla M. Study on molecular anti-tumor mechanism of 2-thiohydantoin derivative based on molecular docking and bioinformatic analyses. Curr Top Med Chem. 2023;23(6):440-52.
40. Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, et al. Structural insights into SARS-CoV-2 proteins. J Mol Biol. 2021;433(2):166725.
41. Naser NM, Mutlaq DZ, Al-Shawi AA, Modafer Y, Dawoud RA, Abdalla M. Synthesis, characterization, antibacterial, and molecular docking and dynamics studies of novel 2-thioxoimidazolidin-4-one analogue. Chem Afr. 2024;7(8):4193-210.
42. Gorkhali R, Koirala P, Rijal S, Mainali A, Baral A, Bhattarai HK. Structure and function of major SARS-CoV-2 and SARS-CoV proteins. Bioinform Biol Insights. 2021;15:11779322211025876.
43. El-Arabey AA, Abdalla M, Abd-Allah AR. SnapShot:

TP53 status and macrophages infiltration in TCGA-analyzed tumors. Int Immunopharmacol. 2020;86:106758.
44. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: Implications for SARS-CoV-2. Nat Rev Microbiol. 2021;19(3):155-70.
45. Bahadur Gurung A, Ajmal Ali M, Al-Hemaid F, El-Zaidy M, Lee J. In silico analyses of major active constituents of fingerroot (Boesenbergia rotunda) unveils inhibitory activities against SARS-CoV-2 main protease enzyme. Saudi J Biol Sci. 2022;29(1):65-74.
46. Nazir F, Kombe AJ, Khalid Z, Bibi S, Zhang H, Wu S, et al. SARS-CoV-2 replication and drug discovery. Mol Cell Probes. 2024;77:101973.