ORIGINAL RESEARCH ARTICLES

IN SILICO BASED SCREENING EMPLOYED TO ASSESS THE ACTIVITY OF PHYTOCHEMICAL, SYNTHETIC AND MARINE COMPOUNDS ON THE SARS-COV-2 MAIN PROTEASE

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ABSTRACT

Our study focuses on screening ligands against the target 6Y2E using the iGemDock docking program, encompassing phytochemical, synthetic and marine sources, suggesting suitability for oral use against SARS-CoV-2 Main protease. Docking process involves iGemDock program for assessment, Argus Labs, for binding energy determination, Swiss ADME for evaluating pharmacological properties, and Chimera for visualizing interactions. Docking score gauges how effectively a ligand inhibits SARS-CoV-2 M^{pro}, with compounds ranked based on their docking scores. Rosmarinic acid, a phytochemical compound, achieved a docking score of -117.629 and energy of -11.051. Remdesivir, a synthetic compound, attained a docking score of -118.091 and energy of -9.31522. Spongouridine, a marine compound, secured a docking score of -83.1317 and energy of -6.38, making them the top-ranked molecules in the docking process.

Keywords: Binding efficacy**,** SARS-CoV-2, Rosmarinic acid, remdesivir, spongouridine, drug design

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a significant loss of lives. Overall, 13,595,721,080 vaccine doses have been administered, as reported by WHO[1,19]. COVID-19 pandemic, attributed to SARS-CoV-2, has prompted extensive research on the virus's Main protease (M^{pro}), a pivotal enzyme crucial for the viral life cycle, as it breaks down essential polyprotein necessary for replication[2]. Inhibiting M^{pro} is seen as a promising strategy for the development of antiviral drugs and treatments for COVID-19[3]. Researchers, using the crystal structure of the free enzyme (PDB ID 6Y2E) obtained through X-ray diffraction with a resolution of 1.75 Å, sought to enhance understanding and potentially intervene in the virus's replication process. Recent advanced approaches made by computational method in structural biology and drug design, predicting the ideal alignment and binding strength between two molecules, typically a smaller ligand and a larger target protein, are used to create a stable complex[4]. The molecules for this process are chosen, and their sequence files are retrieved from Protein Data Bank and PubChem databases in PDB (Protein Data Bank) and SDF (Structure Data File) formats. Subsequently, the protein-ligand docking commences, and the outcomes are assessed. iGemDock is specifically employed for docking purposes[5]. The PDB file for protein 6Y2E is obtained from the Protein Data Bank. PubChem, managed by the National Center for Biotechnology Information and acts as a repository for chemical compounds and their interactions in biological assays[6]. Open Babel serves as a versatile chemical toolkit, facilitating communication in various chemical data languages for molecular modeling, chemistry, solid-state materials, biochemistry and related fields[7]. iGemDock streamlines the entire process, from preparing target proteins and ligand libraries to post-screening analysis and inferring pharmacological interactions. It is a valuable tool for understanding ligand binding mechanisms and discovering potential lead compounds[8]. Argus Labs, designed for Windows, is a molecular modeling and drug design program used to determine the binding energy of target proteins and

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ligands[9]. Swiss ADME, developed for drug discovery and medicinal chemistry, balances precision and efficiency in managing a high volume of molecules[10]. UCSF Chimera is a versatile software application for interactive exploration and examination of molecular structures. It covers density maps, supramolecular assemblies, sequence alignments, docking outcomes, molecular trajectories and conformational ensembles[11]. Therefore, our study put in a way to screen the binding efficacy on docking based virtual screening of phytochemical, synthetic and marine compound's activity towards the active sites of SARS-CoV-2 main protease using iGemDock software.

MATERIALS AND METHODS

Protein information

Crystal structure of the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease PDB DOI: https://doi.org/10.2210/pdb6Y2E/pdb

Organism(s): Severe acute respiratory syndrome coronavirus

2 Mutation(s): No

Method: X-RAY DIFFRACTION

Resolution: 1.75 Å

Deposition Author(s): Zhang, L., Sun, X., Hilgenfeld, R.

Ligand preparation

As previously mentioned, the selected protein is 6Y2E, the Corona Virus M^{pro}. The chosen ligands consist of phytochemical drugs, synthetic drugs and marine compounds known for their anti-viral properties. These ligands were explored to identify a compound exhibiting anti- viral characteristics, potentially acting as an inhibitor for Covid-19 M^{pro}. A total of 45 compounds were selected, comprising 20 each of phytochemical and synthetic drugs, and 5 marine compounds. Noteworthy drugs from the pandemic period, such as remdesivir, hydroxychloroquine, favipiravir, and dexamethasone, were included in the experiment for docking, aligning with their usage in Covid-19 treatment.

Docking and visualization

Employing iGemDock for docking, the target proteins were assessed with various ligands. To ascertain binding energy, Argus Labs software was utilized. Swiss ADME aids in evaluating pharmacological properties, including absorption, distribution, metabolism, excretion and toxicity. Chimera is employed for visualizing bonds, ligand poses, and the attachment of the drug to the protein[12]. Furthermore, the selected ligands adhere to the Lipinski Rule of Five. This rule serves as a guideline for researchers and pharmaceutical companies, aiding them in prioritizing and directing their efforts toward compounds that are more likely to succeed in terms of oral bioavailability and their potential as viable drugs[13].

RESULTS AND DISCUSSION

Phytochemical compounds, synthetic compounds and marine compounds chosen in this experiment were used as ligands. Their docking scores were checked through iGemDock and Argus Lab (Table I, II & III).

From the phytochemical compounds, 18 compounds chosen for this experiment were used as ligands. Their docking score was checked through iGemDock and Argus Lab and the energy values, and docking scores are shown in Table I.

From the synthetic compounds, 19 compounds chosen for this experiment were used as Ligands. Their docking score and energy values were checked through iGemDock and Argus Lab respectively as shown in Table II.

Only 3 Marine derived compounds were chosen in this experiment and were used as Ligands. Their docking score and energy values were checked through iGemDock and Argus Lab, respectively, the results are listed in the Table III.

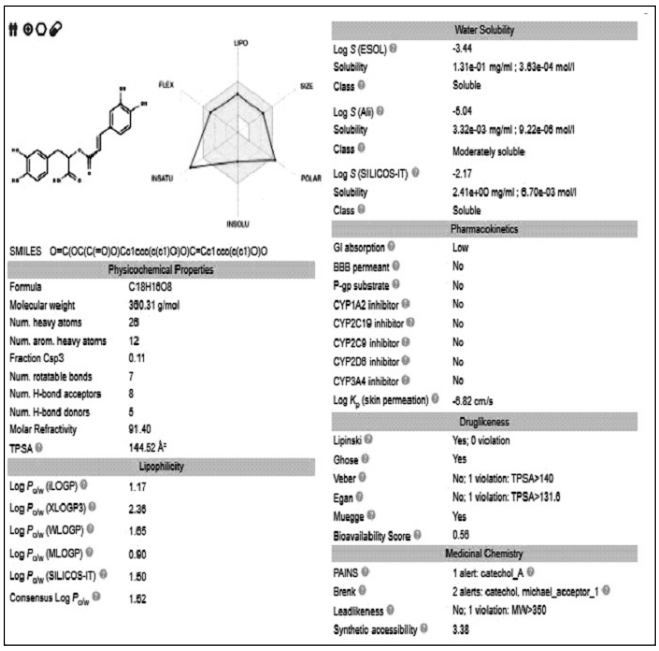
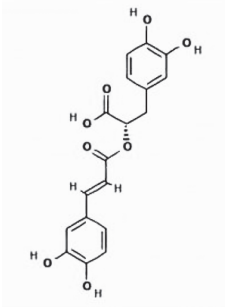
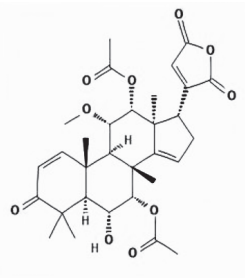
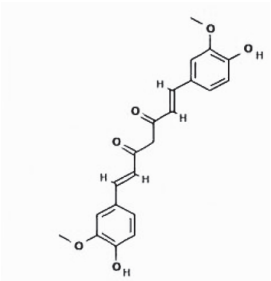
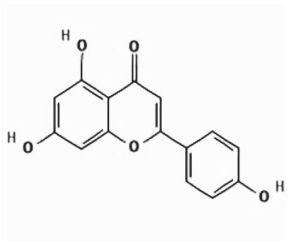
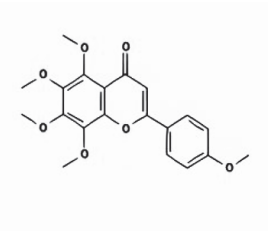
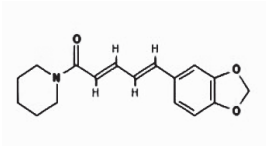
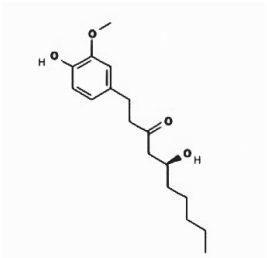
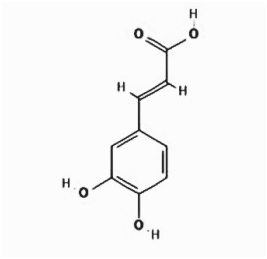
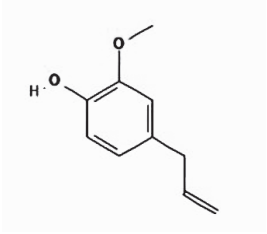
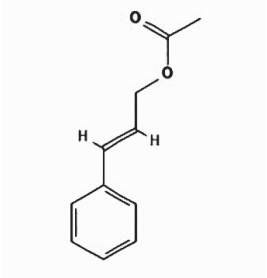
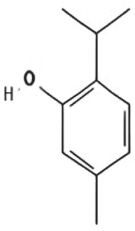


Fig. 1: Rosmarinic acid ADME analysis

Table I: Docking results of protein 6y2e and phytochemical compounds

S. No.	COMPOUND	ENERGY (ARGUS LAB)	DOCKING SCORE (IGEMDOCK)	STRUCTURE
1.	ROSMARINATE *(S)*-ROSMARINIC ACID	-11.051	-117.629	
2.	MELIACIN ANHYDRIDE	-9.9398	-113.315	
3.	CURCUMIN	-11.3512	-94.3145	
4.	APIGENIN	-9.0499	-88.6656	
5.	TANGERETIN	-7.65133	-84.662	

6.	PIPERINE	-9.43996	-84.0049	
7.	GINGEROL	-9.2454	-81.2314	
8.	CAFFEIC ACID	-9.14004	-80.8456	
9.	EUGENOL	-8.44547	-70.2146	
10.	CINNAMYL ACETATE	-9.08467	-69.1472	
11.	THYMOL	-8.94441	-66.3466	

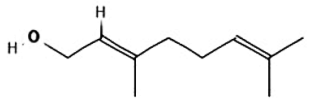
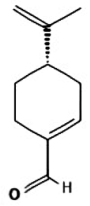
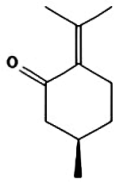
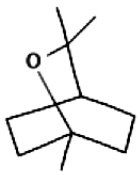
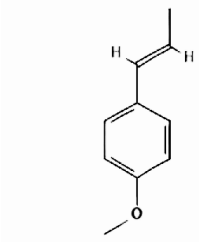
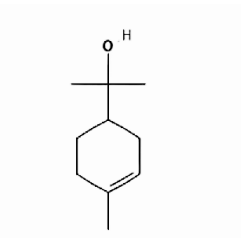
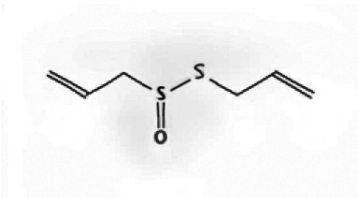
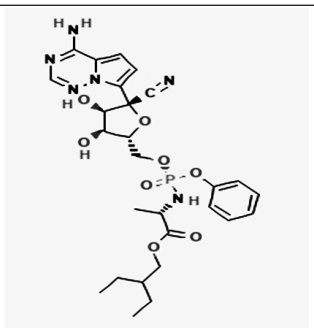
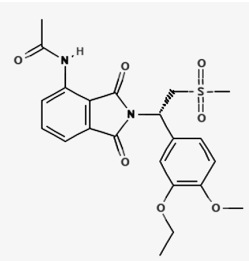
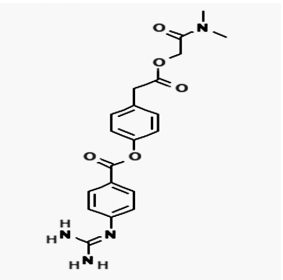
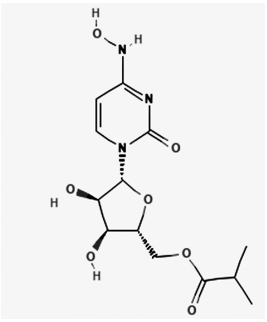
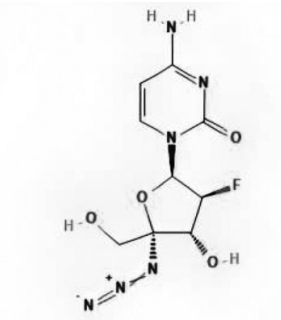
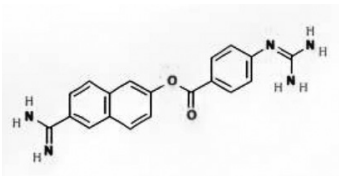
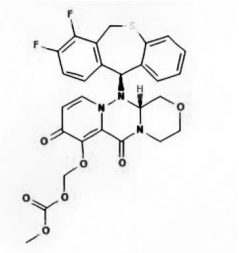
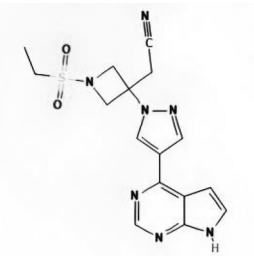
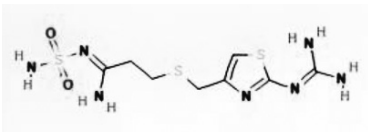
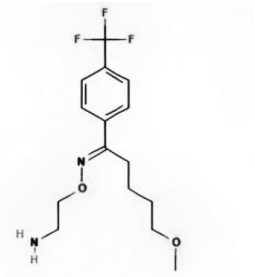
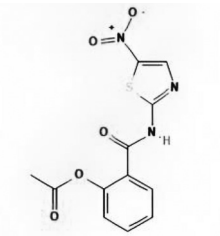
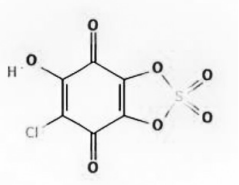
12.	GERANIOL	-9.24542	-59.111	
13.	PERILLALDEHYDE	-8.90205	-56.5403	
14.	PULEGONE	-8.77632	-55.8814	
15.	1,8 CINEOLE	-6.93044	-55.0702	
16.	ANETHOLE	-8.47362	-53.8517	
17.	ALPHA TERPINEOL	-9.03036	-52.8523	
18.	ALLICIN	-7.50241	-46.0436	

Table II: Docking results of protein 6y2e and synthetic compounds

S. No.	COMPOUND	ENERGY (ARGUS LAB)	DOCKING SCORE (IGEMDOCK)	STRUCTURE
1.	REMDESIVIR	-9.31522	-118.091	
2.	APREMILAST	-8.12335	-111.153	
3.	CAMOSTAT	-10.5078	-108.435	
4.	MOLNUPIRAVIR	-7.93192	-107.879	
5.	AZVUDINE	-6.59227	-103.479	

6.	NAFAMOSTAT	-8.44019	-102.59	
7.	BALOXAVIR MARBOXIL	-2.96115	-102.411	
8.	BARICITINIB	-7.78724	-101.247	
9.	PEPCID	-8.63656	-98.0682	
10.	FLUVOXAMINE	-9.22373	-90.4405	
11.	NITAZOXANIDE	-8.44019	-89.0023	
12.	HYDROXYCHLORO-QUINONE	-8.62378	-88.6533	

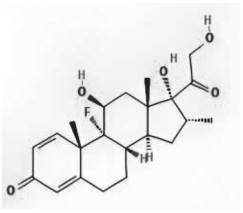
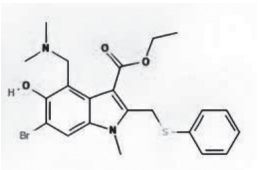
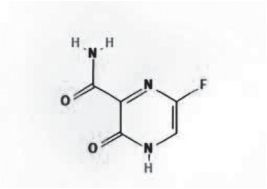
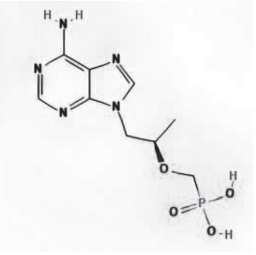
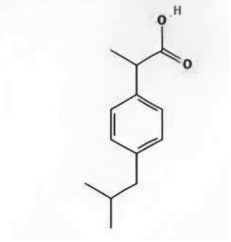
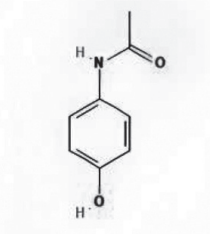
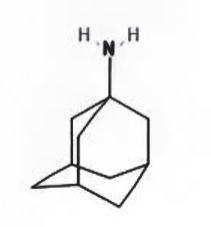
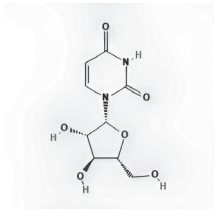
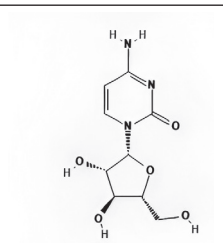
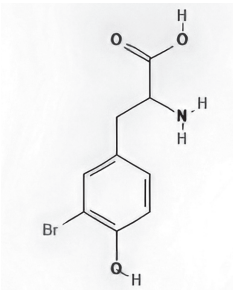
13.	DEXAMETHASONE	-10.2407	-87.6556	
14.	ARBIDOL	-9.73946	-87.962	
15.	FAVIPIRAVIR	-5.88838	-77.8227	
16.	TENOFOVIR	-6.24264	-77.4262	
17.	IBUPROFEN	-8.39304	-72.3116	
18.	PARACETAMOL	-7.7047	-64.0383	
19.	AMANTADINE	-8.00728	-60.6343	

Table III: Docking results of protein 6y2e and marine compounds

S. No.	COMPOUND	ENERGY (ARGUS LAB)	DOCKING SCORE (IGEMDOCK)	STRUCTURE
1.	SPONGOURIDINE	-6.38	-83.1317	
2.	CYTARABINE	-6.26664	-79.5596	
3.	BROMOTYROSINE	-8.27482	-78.164	

SWISS ADME Analysis of rosmarinic acid

Comprehending ADME characteristics is imperative for drug developers and researchers. It enables them to anticipate a drug's conduct, bioavailability, potential interactions and safety within the body, all of which are pivotal in evaluating a drug's efficacy and suitability for clinical application. This is shown in Fig. 1 and Visualization of protein and rosmarinic acid binding using Chimera is shown in Fig. 2.

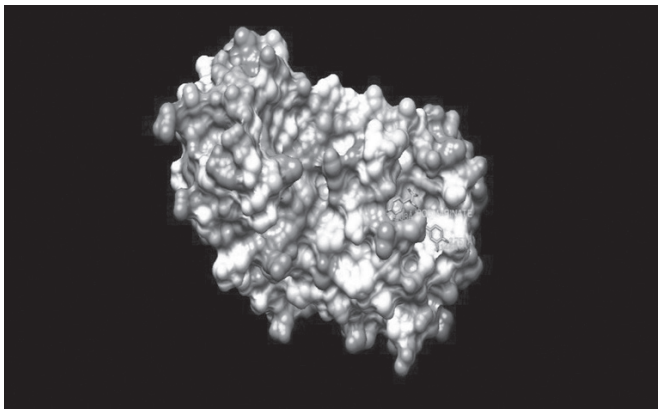


Fig. 2: Visualization of protein and rosmarinic acid binding using Chimera

Table IV: Bonding interactions between ligand and receptor

LIGAND	VAN DER WAALS FORCE (VDW)	HYDROGEN BONDS (H Bond)
Rosmarinic acid	-70. 79	-13. 75
Remdesivir	-106. 59	-18. 18
Spongouridine	-46. 96	-36. 59

In the docking process, we have selected the structure code 6Y2E, obtained through X-ray diffraction with a high resolution of 1.75 Å. This viral protein structure was chosen due to its exceptional resolution compared to other available crystallographic structures, and its particular interest lies in containing the compound within its active site[14]. The docking score gauges how effectively a ligand inhibits SARS-CoV-2 M^{pro}, with compounds ranked based on their docking scores. Rosmarinic acid, a phytochemical compound, achieved a docking score of -117.629, and energy of -11.051. Remdesivir, a synthetic compound, attained a docking score of -118.091 and

energy of -9.31522. Spongouridine, a marine compound, secured a docking score of -83.1317 and energy of -6.38, making them the top-ranked molecules in the docking process. Binding energy, expressed in energy units like kilocalories per mole (kcal mol^{-1}) or kilojoules per mole (kJ mol^{-1}), represents the thermodynamic energy associated with establishing a ligand-receptor complex. Lower values indicate more potent interaction reflecting the formation of a more stable complex. Molecular docking software anticipates binding score and binding energy for various ligands interacting with a specific receptor, offering valuable insights. However, these computational predictions require validation through experimental investigations such as binding assay to confirm actual binding affinity and functional activity[15]. Argus Lab provides binding energy values for compounds and the target protein, where lower energy indicates greater stability.

The interaction between ligands and receptors is crucial in pharmacology as it can trigger or hinder receptor activity and regulate the opening or closing of ion channels within the cellular membrane.

Despite their seemingly weak nature, van der Waals forces serve as a vital element in ensuring the stability of the drug-receptor interaction. The impact of these weak intermolecular forces on the binding affinity of ligand-protein complexes is significant in anchoring a ligand to the protein structure interface. Hydrogen bonds are particularly noteworthy in biological contexts, being widespread and pivotal in processes such as protein folding, protein-ligand interactions, and catalysis[20]. The results showed that rosmarinic acid has -70.79 VWF with the hydrogen bonding of -13.75Å, similarly for remdesivir it was found to be -106.59 VWF, for hydrogen bonding of -18.18 Å and for spongouridine it was found to be -46.96 VWF, for hydrogen bonding is of -36.59 Å as shown in Table IV.

ADME properties offer insights into a drug's influence on the body, determining safety and potential toxicity. Evaluating pharmacological characteristics related to Absorption, Distribution, Metabolism, and Excretion (ADME) is crucial in the initial selection of potential chemical leads, establishing standards for compounds developed during lead optimization. Lead optimization aims to enhance ADME properties while maintaining efficacy and selectivity, recognizing that more effective compounds may exhibit improved ADME properties[16]. Early pharmacological evaluation is crucial in the drug discovery process, incorporating a multifaceted approach. The Lipinski rule assesses a compound's drug-likeness and suitability for consumption. All compounds in this experiment adhere to at least one of the Lipinski Rule of Five conditions, suggesting their potential as drugs against SARS-CoV-2 Main protease[17]. "Optimal ligand orientation" refers to the ligand's predicted position and alignment, crucial for effective binding affinity and stable interactions with the receptor. This information aids researchers in selecting potential drug candidates that can modulate the target protein effectively. To visualize the best binding orientation, the software tool Chimera is employed. This process helps to identify the most effective ligands for inhibiting SARS-CoV-2 M^{pro}[18]. Chimera finds applications in drug targeting, controlled release, tracking drugs through fluorescent probes, investigating target engagement and elucidating mechanisms of action.

CONCLUSION

In our current research, we introduced and assessed various compounds from phytochemical, synthetic, and marine sources against the target 6Y2E using the iGemDock docking program. Phytoconstituents, synthetic constituents and marine constituents suitable for oral use, and their analogues, undergo docking into the active site of the Covid M^{pro} using iGemDock and Argus Lab. Protein-ligand interaction is made visible through Chimera and Swiss ADME and the data used to explore the molecular aspects of interaction, stability, binding affinity, and other physiochemical properties. Our results suggest that the top three compounds rosmarinic acid (a phytochemical compound), remdesivir (a synthetic drug) and spongouridine (a marine-derived compound) exhibit orientations near the active site. These compounds show promise for further pharmaceutical development as potential drugs for the treatment of COVID-19.

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