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Rilpivirine inhibits SARS-CoV-2 protein targets: A potential multi-target drug

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ABSTRACT

Background: COVID-19 disease caused by SARS-CoV-2 is lacking efficient medication although certain medications are used to relief its symptoms.
Objectives: We tested an FDA-approved antiviral medication namely rilpivirine to find a drug against SARS-CoV-2.
Methods: The inhibition of rilpivirine against multiple SARS-CoV-2 therapeutic targets was studied using *in silico* method. The binding attraction of the protein-ligand complexes were calculated using molecular docking analysis.
Results: Docking rilpivirine with main protease (Mpro), papin like protease (PLpro), sprike protein (Spro), human angiotensin converting enzyme-2 (ACE2), and RNA dependent-RNA polymerase (RdRp) yielded binding energies of −8.07, −8.40, −7.55, −9.11, and −8.69 kcal/mol, respectively. The electrostatic interaction is the key force in stabilizing the RdRp-rilpivirine complex, while van der Waals interaction dominates in the ACE2 rilpivirine case. Our findings suggest that rilpivirine can inhibit SARS-CoV-2 replication by targeting not only ACE2, but also RdRp and other targets, and therefore, it can be used to invoke altered mechanisms at the pre-entry and post-entry phases.
Conclusion: As a result of our in silico molecular docking study, we suggest that rilpivirine is a compound that could act as a powerful inhibitor against SARS-CoV-2 targets. Although *in vitro* and *in vivo* experiments are needed to verify this prediction we believe that this antiviral drug may be used in preclinical trials to fight against SARS coronavirus.

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Introduction

WHO (The World Health Organization) declared the pandemic of COVID-19 caused by SARS-coronavirus-2 (SARS-CoV-2) in March 2020. SARS-CoV-2 infection had been identified in 171,782,908 cases around the world until June 2021 including 3,698,621 deaths [1]. The disease incidence has been high in Saudi Arabia, and therefore, all actions are needed to prevent the disease spreading [2]. COVID-19 causes fatigue, fever, muscle aches, dry cough, and shortness of breath, leading to pneumonia or acute respiratory distress syndrome, the last resulting oxygen deprivation and death.

Extensive research on responsive and active anti-viral mediators of SARS-CoV-2 have taken place throughout the world. Since it takes a long time for new drug to be licensed, several trials have been performed to assess the effectiveness of previously permitted medicines to treat COVID-19 disease. Various medicines have been shown to possess anti-viral activity against SARS-CoV-2. Old anti-malarials (hydroxychloroquine, chloroquine phosphate and chloroquine) [3] an anthelminthic drug (ivermectin) [4], viral RNA polymerase (RdRp) inhibitors (favipiravir and remdesivir) [5], viral protease inhibitor [6] and several other antiviral drugs have shown their potential as COVID-19 drugs [7]. However, efficient treatments that could be used throughout the world are still needed.

SARS-CoV-2 is an RNA virus with 4 structural proteins (envelope protein, membrane protein, spike protein, and nucleo-capsid protein) and 16 non-structural proteins that are accountable for viral replication and other infection-related functions. Since RNA viruses have high mutation frequency, and thus, high evolution frequency [8], multi-targeting drugs are useful by reducing the viral resistance to a single protein [9,10]. Among the most important targets

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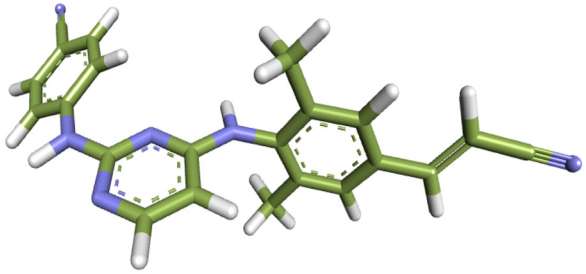

Fig. 1. 3D structure of rilpivirine (CID: 6451164).

are Chymo-trypsin Like Protease-3CLpro, also identified as main protease-Mpro, and papain like protease-PLpro aiding virus replication, Spike protein-Spro stimulating virus entry into a human tissue or cell, Angiotensin Converting Enzyme-2 (ACE2) aiding virus entry, and RNA-dependent RNA-polymerase (RdRp) aiding viral replication [9,11,12].

Rilpivirine is a Tibotec-developed prescription drug to treat HIV infection [13]. It's a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI). New NNRTIs are more active and have a longer half-life and less side-effects than older NNRTIs such as efavirenz [14,15]. However, the antiviral effects of SARS-CoV-2 proteins on patients are unknown. In their virtual drug screening using VINI *in silico* model of cancer, HIV drugs appeared to be the most promising drugs. To the best of our knowledge, nor further studies on rilpivirine against SARS-CoV have been carried out. The aim of this study was to use molecular docking to evaluate the binding affinity of different viral proteins to rilpivirine, and thus show the antiviral properties of rilpivirine against SARS-CoV-2.

Material and methods

Ligand preparation

The ligand was prepared at Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal, Telangana State, India and Molecular lab 2b 60, Department of Botany & Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. Firstly, rilpivirine's three-dimensional SDF structure (CID: 6451164) from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to apply BIOVIA Discovery Studio 3.0 [16], to convert the 3D structure of the ligand file format from SDF to PDB (Fig. 1). UCSF Chimera tools were used to prepare the ligand from a PDB file (energy minimization, hydrogen atoms added, and charges added where needed) [17]. Lopinavir, ritonavir, chloroquine phosphate, emodin, and remdesivir drugs were antagonist of SARS-CoV-2 PLpro, Mpro, ACE2, Spoke glycoprotein and RdRp respectively were chosen as positive control for comparative study [18].

Retrieval of target proteins

RCSB Protein Data Bank (https://www.rcsb.org/) [19] was used to obtain high-resolution 3D structures of the newly described SARS-CoV-2 enzymes, including the PLpro (PDB ID = 4OVZ), Mpro (PDB ID = 6LU7), ACE2 (PDB ID = 6CS2), Spike Glycoprotein (PDB ID = 6CS2), and RdRp (PDB ID = 6NUR) (Fig. 2).

Preparation of the target proteins

Molecular docking method cannot be performed on the raw PDB protein structure. Just heavy atoms, liquids, cofactors, and metal ions make up the PDB structure, which can be multimeric [20]. Topologies, bond orders and proper atomic charges are not present in these structures. Since X-ray structure investigation cannot differentiate between NH_2 and oxygen ionisation and tautomeric situations are also unassigned, the terminal amide groups may be misaligned. As a result, the raw PDB structure must be primed for docking in an appropriate manner.

All target proteins were refined, and energy was optimized before moving into docking research. The protein was processed and prepared using the UCSF Chimera software's Protein Preparation Wizard that guides through the process of properly preparing a protein macromolecule for docking. This method will transform a PDB structure into completely prepared all-atom protein simulations [21]. All target proteins' X-ray crystal structures were prepared by removing all water molecules from the structure. The implicit H_2 atoms were applied to the atoms to saturate their necessary valences, and ligands in the protein structure were omitted because the raw data did not contain any hydrogen. The bond orders, bond angles, and topology of the structure were then assigned, and the structure was optimized. For the amino acid residues, the formal atomic charges were set, and minimization of energy was performed.

In the process of molecular docking, based on predicted active sites, the grid box was set to 60 Å × 60 Å × 60 Å centred at 225.616, 226.490, 225.337 (XYZ coordinates) for spike protein (6CS2), grid box 26 Å × 26 Å × 26 Å centered at −10.712, 12.411, 68.8312 (XYZ coordinates) for 3CLpro (6LU7), grid box 60 Å × 60 Å × 60 Å centered at −8.611, 38.916, −41.012 (XYZ coordinates) for PLpro (4OVZ), grid box 60 Å × 60 Å × 60 Å centered at 138.751, 163.504, 136.636 (XYZ coordinates) for RdRp (6NUR) and grid box 60 Å × 80 Å × 60 Å centered at 190.404, 101.754, −0.753 (XYZ coordinates) for ACE2 (6CS2).

Molecular docking

Following the preparation of the ligand and proteins for docking, Auto Dock Tools 1.5.6 was applied to conduct the docking procedure by taking the ligand and target proteins together [22]. Each target protein was docked with the ligand molecule separately. In this docking process, the various conformations for the ligand were produced, and the ultimate energy modification of the ligand posture happened. Each docking phase had a total of ten runs. Furthermore, the maximum iterations were 2000, with a 100 kcal/mol energy threshold. The lowest docked binding energy was used to choose the best conformations for each docking operation. The docked conformations remained saved in PDB format and then visualized using Discovery studio 4.0 to assess docking site recognition.

Results and discussion

The binding interactions of drug and target proteins in the screening was scored using a knowledge-based approach. According to the docking, selected rilpivirine can form traditional hydrogen bonds with different residues and interact successfully with the selected a five-target protein. It also binds to all protein targets due to van der Waals interaction. These interactions are low-energy, confirming their presence in comparison to target enzymes.

SARS-COV-2 target protein inhibition occurred with binding attractions ranging from −7.55 kcal/mol to −9.11 kcal/mol, suggesting substantial interactions at the binding pocket active site (Table 1 and Fig. 3). Hydrogen bonds, electrostatic interactions, and hydrophobic interactions all helped to stabilize these interactions. Pi-interactions, such as Pi–Alkyl interaction, Pi–Pi interaction, Pi–Sigma, Pi–S interaction, and Pi–Pi stacking, were also observed with all target proteins and the interactions involved the transfer

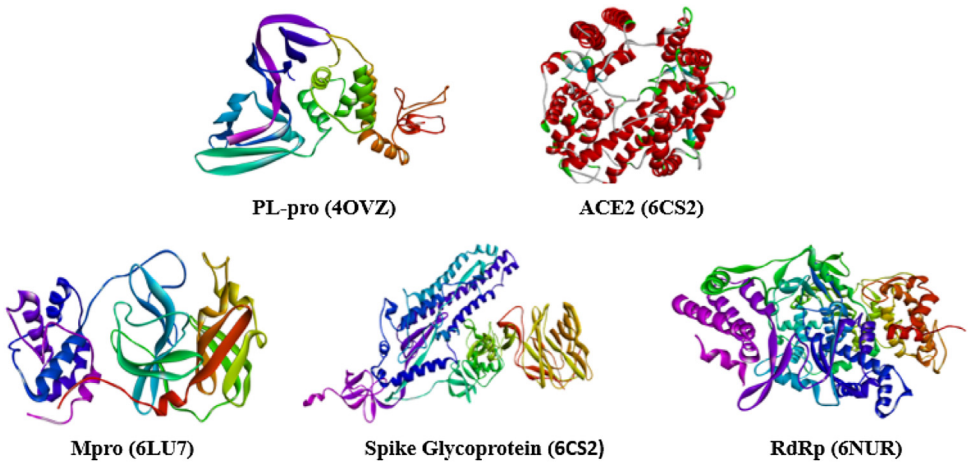

Fig. 2. 3D structures of SARS-CoV-2 therapeutic target proteins.

Table 1
Molecular docking score and interactive amino acid residues with rilpivirine docked against multiple targets of SARS-CoV-2.

Protein target (PDB ID)	Positive control drugs (Binding energy)	Binding energy (kcal/mol)	Interactive residues	No. of H bonds	Interaction of residues forming H_2 bonds
ACE2 (6CS2—D chain)	Chloroquine phosphate (−7.10)	−9.11	PHE:40, 356, 390, ARG:393, GLU:37, 375, GLY:352, 354, LYS:353, SER:44, ASN:394, ALA:386, 348, HIS:378, 401, TYR:385,	1	ASP:350
RdRp (6NUR)	Remdesivir (−7.60)	−8.69	LEU:270, 271, 329, ARG:331, TYR:273, THR:344, 324, HIS:355, SER;325, PHE:324, 326, MET:666, VAL:675, 398,330, PRO:328, GLY:327	2	LEU:270 ARG:331
PLpro (4OVZ)	Lopinavir (−8.01)	−8.40	LEU:81, 59, 76, ARG:66, PHE:70, 80, THR:75, ASP:63,77, SER:67, GLU:71, 78, PRO:60, ALA:69.	1	LEU:81
Mpro (6LU7)	Ritonavir (−7.38)	−8.07	GLY:143, CYS:145, SER:144, HIS:41,163,164,172, TYR:54, ASP:187, ARG:188, MET:165, GLN:189, GLU:166, ASN:142, LEU:141, PHE:140	5	GLY:143 CYS:145 SER:144 HIS:41 TYR:54
Spike Glyco- Protein (6CS2, A-chain)	Emodin (−6.1)	−7.55	CYS:576, 524, PRO:575, SER:574, ALA:577, 609, PHE:578, ASP:600, 605, VAL:537, 601, 307, ASN:522, GLN:523, 614, 599,	2	CYS:576 PRO:575

of charges. The ligands were imbedded in the target protein-active/binding site for these Pi interactions.

Binding interactions of rilpivirine with ACE2 receptor

ACE2, a homolog of angiotensin-converting enzyme (ACE) found in a number of human organs and tissues, has a wide range of biological activities, including the ability to reverse the detrimental effects of the renin-angiotensin system (RAS) in a variety of diseases [13]. ACE2 is an entry receptor of SARS-CoV-2 that can bind to the viral spike protein [13]. We can stop virus replication while inhibition of ACE2 catalytic pocket by small molecules could change the conformation of ACE2 in such a way that it could block SARS-CoV-2 entry inside host cells through ACE2 [14]. As a result, ACE2 may be used to prevent virus replication.

According to the docking results, rilpivirine took a negative binding attraction of −9.11 kcal/mol whereas, positive control drug chloroquine phosphate binding energy is −7.10 kcal/mol. This states that, rilpivirine binding affinity is high when compared with positive control drug. Rilpivirine formed one traditional hydrogen bond with ASP350 (Table 1 and Fig. 4). The stable binding interaction may also be related to the Pi–Sigma interaction of the rilpivirine ring with TYR385 and the Pi–Alkyl interaction of the ACE2 binding site with ALA386. The stability of binding is sustained by the hydrophobic interactions with ACE2 amino acids PHE:40, 356, 390, ARG:393, GLU:37, 375, GLY:352, 354, LYS:353, SER:44, ASN:394, ALA:348, HIS:378, 401, PHE:40, 356, 390, ARG:393, GLU:37, 375, GLY:352, 354, LYS:353, SER:44 (Fig. 4).

Binding interactions of rilpivirine with RdRp

RdRp plays an important part in the life cycle of the virus. Since the dynamic site of RdRp is the most preserved and nearby region, inhibiting viral replication by targeting this region may be a successful therapeutic strategy [24].

Rilpivirine demonstrated stable binding with a binding attraction of −8.69 kcal mol^{-1} in docking studies against the RdRp enzyme of the SARS-CoV-2 virus (Table 1). The binding energy of rilpivirine is low when compared to positive control drug remde-

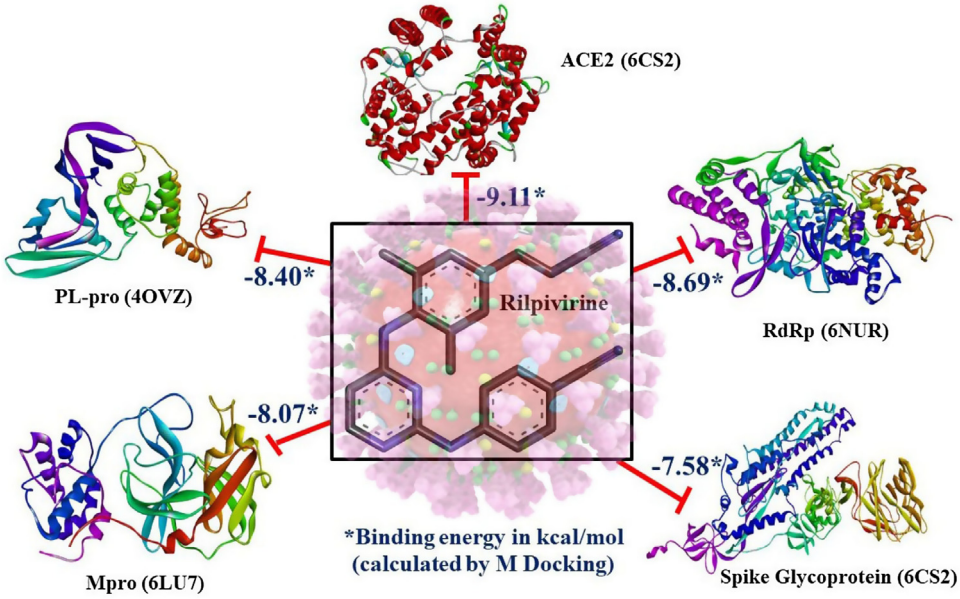

Fig. 3. Illustration of rilpivirine inhibition against the SARS-CoV-2 target proteins: ACE2, RdRp, Spike Glycoprotein, Mpro and PL-pro.

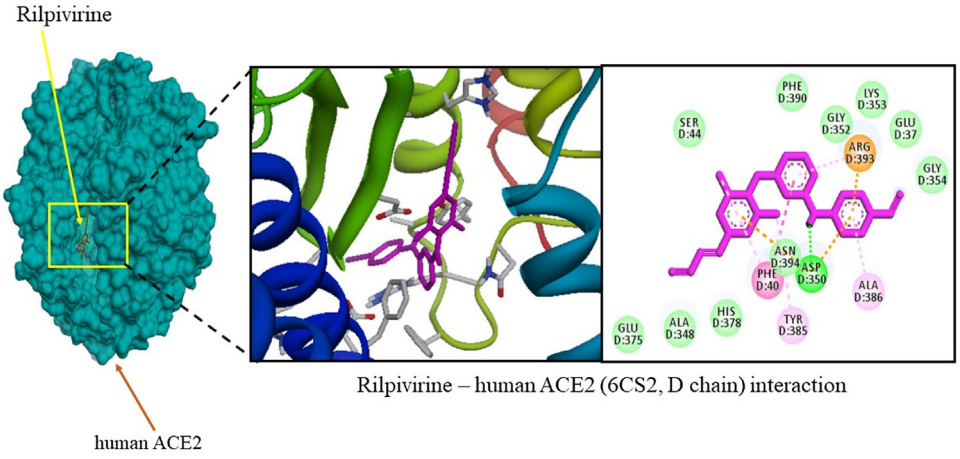

Fig. 4. Molecular interactions between ACE2 receptor protein (PDB ID: 6CS2, D Chain) and rilpivirine.

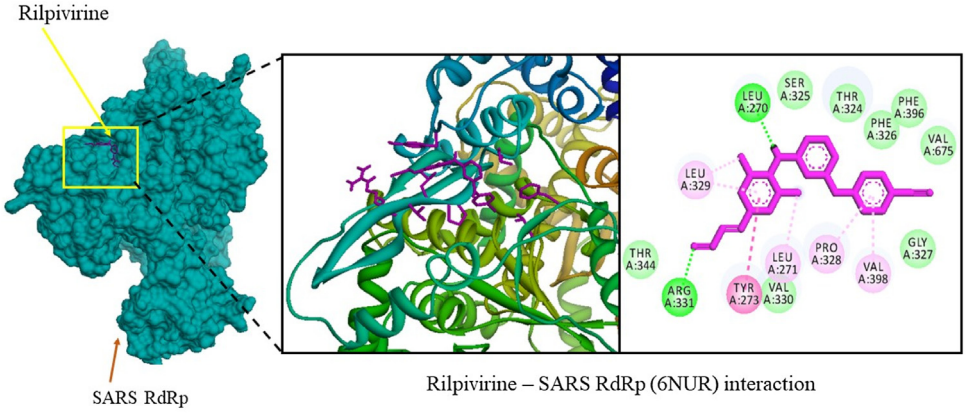

Fig. 5. Molecular interactions between SARS-CoV-2 RdRp (PDB ID: 6NUR) and rilpivirine.

sivir (−7.60 kcal/mol). This states that, rilpivirine is more potential than remdesivir. This is due to its two traditional hydrogen bonds with LEU270 and ARG331, as well as hydrophobic interactions with LEU:270, 271, 329, ARG:331, TYR:273, THR:344, 324, HIS:355, SER:325, PHE:324, 326, MET:666, VAL:675, 398, 330, PRO:328, GLY:327 (Fig. 5). Based on these findings, rilpivirine is a possible RdRp inhibitor to be used in the fight against SARS-CoV-2 infection.

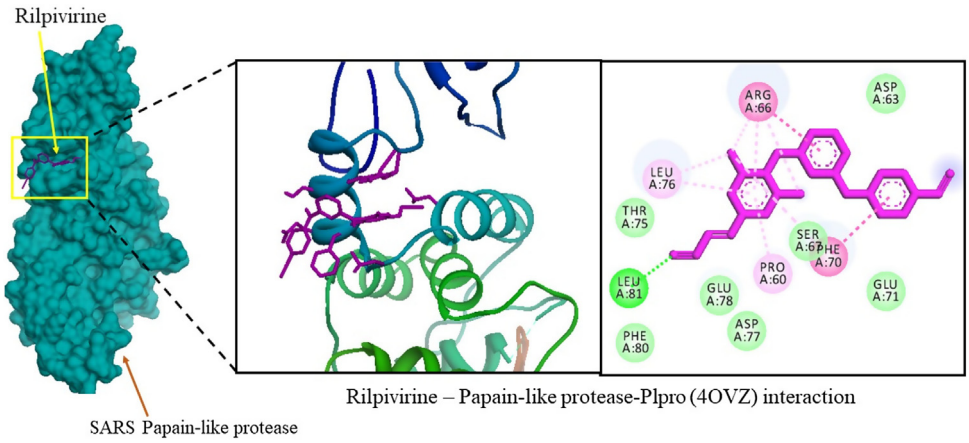

Fig. 6. Molecular interactions between SARS-CoV-2 PLpro (PDB ID: 4OVZ) and rilpivirine.

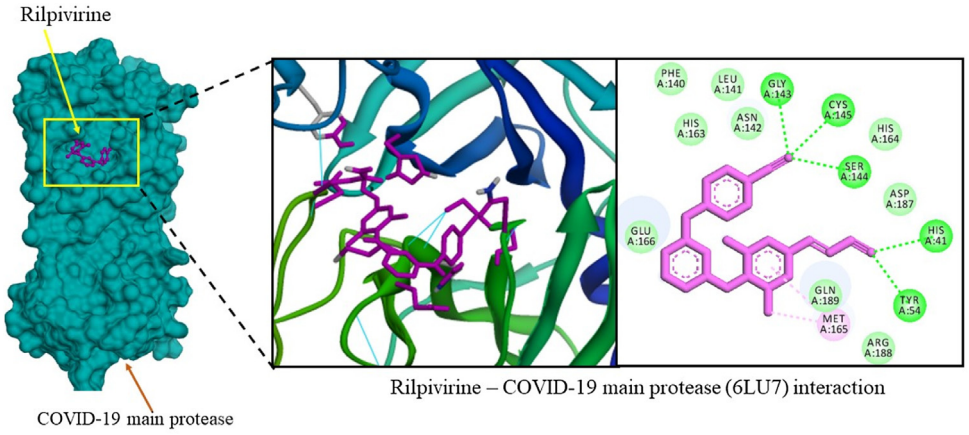

Fig. 7. Molecular interactions between SARS-CoV-2 Mpro (PDB ID: 6LU7) and rilpivirine.

Binding interactions of rilpivirine with SARS-CoV-2 PLpro

Papain like SARS-CoV-2 protease (PLpro), has 83% sequence similarity to SARS-CoV but is not related to MERS-CoV PLpro [7]. PLpro is a multifunctional cysteine protease that converts viral polyproteins into an efficient replicase composite that promotes viral replication [11]. Deubiquitination, which obstructs essential signaling pathways instigating viral attack of the inherent immune response by the appearance of type I interferon, is also a feature of PLpro [11]. This evidence strongly indicates that inhibiting PLpro activity will stop viral replication, making it an important target for antiviral drugs. The binding energy of rilpivirine with the PLpro of SARS coronavirus was found to be −8.01 kcal/mol, whereas, the positive control drug lopinavir binding energy is −8.01 kcal/mol in a molecular docking analysis.

The rilpivirine forms one hydrogen bond with LEU:81 and thirteen hydrophobic bonds with LEU:59, 76, ARG:66, PHE:70, 80, THR:75, ASP:63, 77, SER:67, GLU:71, 78, PRO:60, ALA:69 resulting in a binding potential energy of −8.40 kcal/mol (Table 1 and Fig. 6). Based on these findings, rilpivirine may be used as a drug candidate to inhibit PLpro of SARS-CoV-2.

Binding interactions of rilpivirine with SARS-CoV-2 Mpro

Mpro of the SARS coronavirus, also known as 3C-like proteins, is a cysteine protease with 3-domains (domains I–III) and a 33.8 kDa size [25]. Mpro is involved in polyprotein cleavage at 11 conserved sites, resulting in established and inter-mediate non-structural proteins [26]. Mpro has no canonical dyad between domains I, II of Cys145–His41, which is connected through a loop with domain III [19]. The amino acids Cys145 and His41 are essential for substrate recognition [26]. For computer-generated screening of the selected rilpivirine with the Mpro of SARS-CoV-2, a docking grid was created about these residues (6LU7).

The docking results show that rilpivirine has the highest binding affinity against Mpro, with a binding energy of −8.07 kcal/mol as compared to the control drug ritonavir (−7.38 kcal/mol). Rilpivirine interacts with five H_2 bonds with Mpro's GLY:143, CYS:145, SER:144, HIS:41, and TYR54 (Table 1). Rilpivirine interacts hydrophobically with the amino acids HIS:163, 164, 172, ASP:187, ARG:188, MET:165, GLN:189, GLU:166, ASN:142, LEU:141, PHE:140 (Fig. 7). Rilpivirine inhibits the activity of Mpro, conferring to the docking results of our report.

Binding interactions of rilpivirine with SARS-CoV-2 spike protein (Spro)

Digital screening aided molecular docking with anti-HIV drug rilpivirine on the active pocket of spike proteins was used to examine possible anti-viral drugs against SARS-CoV-2 spike protein. Table 1 shows the docking scores of rilpivirine, which were chosen for the study of SARS-CoV-2. Spro inhibition.

Molecular docking using AutoDock, for the Rilpivirine was analyzed by Chimera. Fig. 8 shows that rilpivirine has a binding affinity of −7.55 kcal/mol as compared to the control drug emodin (−6.10 kcal/mol) and rilpivirine forms two H_2 bonds with the spike protein

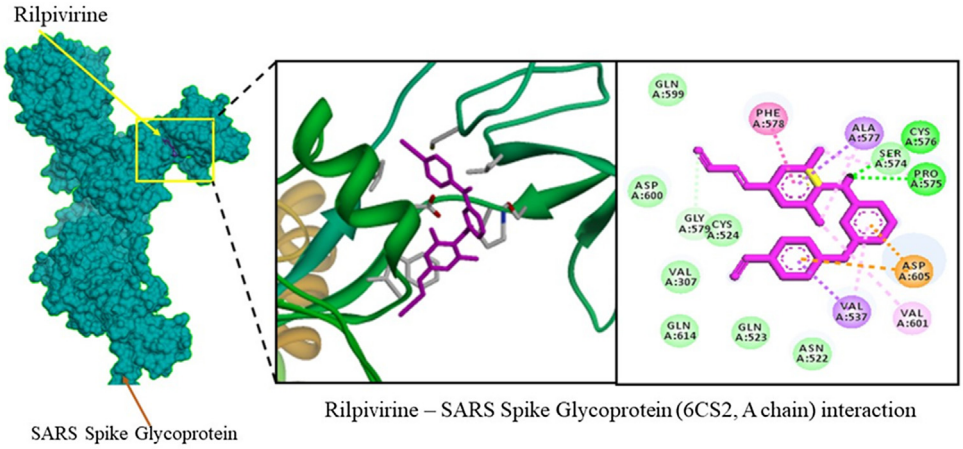

Fig. 8. Molecular interactions between SARS-CoV-2 Spro (PDB ID: 6CS2, A Chain) and rilpivirine.

(Fig. 7). Hydrophobic interactions were also observed, with hotspot residues CYS:524, SER:574, ALA:577, 609, PHE:578, ASP:600, 605, VAL:537, 601, 307, ASN:522, GLN:523, 614, and 599 clearly demonstrating their ability to bind and block interactions with active site residues. When these findings are analyzed, it can be shown that rilpivirine has low binding energy with SARS-CoV-2 Spro, indicating that *in vitro* and *in vivo* research is required before they can be considered as potential COVID-19 drugs.

Conclusion

Rilpivirine can be considered as potential medication in COVID-19 therapy. Since rilpivirine binds to both the Spike Glycoprotein and ACE-2, the receptors of the human cell, it may be tangled in preventing the virus from infecting the host tissue or cell. It also binds to PLpro and Mpro of SARS coronavirus, suggesting that it may play a role in stopping the viral polyprotein post-translational mechanism. The efficient binding of rilpivirine with RdRp suggests that the rilpivirine plays a role in inhibiting the viral replication and assembly. As a result of our *in silico* molecular docking study, we suggest that rilpivirine is a compound that could act as a powerful inhibitor against SARS-CoV-2 targets. Although *in vitro* and *in vivo* experiments are needed to verify this prediction we believe that this antiviral drug may be used in preclinical trials to fight against SARS coronavirus.

Limitation of the study

- Study design: an *in silico* study design was used this study to test rilpivirine against inhibition of SARS-CoV-2 multiple targets. This study applied molecular docking in the drug design field to simulate ligand-receptor interactions. However, some defects still exist; the accuracy and speed of docking calculation is a challenge to explore and these methods can be enhanced as a solution to docking problem.
- Approached technique: we used molecular dynamic simulations in this study. The binding attraction of the protein-ligand complexes were calculated using molecular docking analysis. *In vitro* and *in vivo* experiments are needed to verify the docking and simulation prediction.

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Competing interests

None declared.

Ethical approval

Not required.

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