Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Rilpivirine inhibits SARS-CoV-2 protein targets: A potential multi-target drug

Fuad Ameen a,∗, Estari Mamidala b, Rakesh Davella b, Shravan Vallala c

a Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
b Infectious Diseases Research Lab, Department of Zoology, Kakatiya University, Warangal 506 009 TS, India
c Texas Tech University Health Sciences Center, El Paso, TX, USA

A R T I C L E   I N F O

Article history:
Received 16 June 2021
Received in revised form 13 July 2021
Accepted 15 July 2021

Keywords:
Rilpivirine
SARS-CoV-2
Docking
In silico
ACE2
RdRp

A B S T R A C T

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), papain like protease (PLpro), spike 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.

© 2021 The Authors. Published by Elsevier Ltd on behalf of King Saud Bin Abdulaziz University for Health Sciences. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 anthelmintic 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

https://doi.org/10.1016/j.jiph.2021.07.012
1876-0341/© 2021 The Authors. Published by Elsevier Ltd on behalf of King Saud Bin Abdulaziz University for Health Sciences. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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 VIN 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 = 4OZV), 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 NH₂ 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 H₂ 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 A × 60 A × 60 Å at 225,616, 226,490, 225,337 (XYZ coordinates) for spike protein (6CS2), grid box 26 A × 26 A × 26 A centered at −10.712, 12.411, 68.8312 (XYZ coordinates) for 3CLpro (6LU7), grid box 60 A × 60 A × 60 Å centered at −8.611, 38.916, −41.012 (XYZ coordinates) for Plpro (4OZV), grid box 60 A × 60 A × 60 Å centered at 138.751, 163.504, 136.636 (XYZ coordinates) for RdRp (6NUR) and grid box 60 A × 80 A × 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
of charges. The ligands were imbedded in the target protein-active/binding site for these Pi interactions.

**Binding interactions of rilpirivine 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, rilpirivine 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, rilpirivine binding affinity is high when compared with positive control drug. Rilpirivine 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 rilpirivine 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, LYS:353, SER:44, ASN:394, ALA:348, HIS:378, 401, PHE:40, 356, 390, ARG:393, GLU:37, 375, LYS:353, SER:44 (Fig. 4).

**Binding interactions of rilpirivine 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].

Rilpirivine 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 rilpirivine 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.

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.
**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 replicate 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 $-8.01\text{ kcal/mol}$, whereas, the positive control drug lopinavir binding energy is $-8.01\text{ kcal/mol}$ 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 $-8.40\text{ kcal/mol}$ (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 $-8.07\text{ kcal/mol}$ as compared to the control drug ritonavir ($-7.38\text{ kcal/mol}$). 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 $-7.55\text{ kcal/mol}$ as compared to the control drug emodin ($-6.10\text{ kcal/mol}$) and rilpivirine forms two H$_2$ bonds with the spike protein.
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.

**Funding**

This research was funded by Researchers Supporting Project number (RSP-2021/364), King Saud University, Riyadh, Saudi Arabia.

**Competing interests**

None declared.

**Ethical approval**

Not required.

**Acknowledgments**

This research was funded by Researchers Supporting Project number (RSP-2021/364), King Saud University, Riyadh, Saudi Arabia.

**References**

[1] Organization WH, et al. COVID-19 weekly epidemiological update, 25 May 2021; 2021.
[2] Ameer F, Anna T, Alghamdi AAA, AlKahtani MDF, Alyahya SA. Covid-19 pandemic outbreak in Saudi Arabia: a glimpse. Saudi J Biol Sci 2020;27:3547.
[3] Noureddine O, Issaoui N, Al-Dossary O. DFT and molecular docking study of chloroquine derivatives as antiviral to coronavirus COVID-19. J King Saud Univ 2021;33:101248.
[4] Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug remdesivir inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res 2020;178:104787.
[5] National Health Commission of the PRC (NHCPRC); 2020. n.d. [http://en.nhc.gov.cn/]
[6] Mugisha CS, Vuong HB, Puray-Chavez M, Kariuay SB. A facile Q-RT-PCR assay for monitoring SARS-CoV-2 growth in cell culture. BioRxiv 2020, [http://dx.doi.org/10.1101/2020.06.26.174698].
[7] Gurung AB, Ali MA, Lee J, Farah MA, Al-Anazi KM. Structure-based virtual screening of phytochemicals and repurposing of FDA approved antiviral drugs unravels lead molecules as potential inhibitors of coronavirus 3C-like protease enzyme. J King Saud Univ 2020;32:2845–53.
[8] Bruning AHL, Aatola H, Toivola H, Ikonen N, Savolainen-Kopra C, Blomqvist S, et al. Rapid detection and monitoring of human coronavirus infections. New Microbes New Infect 2018;24:52–5.
[9] Dyall J, Gross R, Kindrachuk J, Johnson RF, Olinger GC, Hensley LE, et al. Middle East respiratory syndrome and severe acute respiratory syndrome: current therapeutic options and potential targets for novel therapies. Drugs 2017;77:1935–66.
[10] Mammadala E, Davella R, Gurrapu S. An in silico approach for identification of inhibitors as a potential therapeutics targeting SARS-CoV-2 protease. Asian J Pharm Res Health Care 2020;12:3–9.
[11] Mammadala E, Davella R, Gurrapu S, Shivakrishna P. In silico identification of clinically approved medicines against the main protease of SARS-CoV-2, causative agent of covid-19. ArXiv Prepr ArXiv200410555 2020;11:107–22.
[12] Arul MN, Kumar S, Jeyakanthan J, Srivastav V. Searching for target-specific and multi-targeting organics for Covid-19 in the Drugbank database with a double scoring approach. Sci Rep 2020;10, [http://dx.doi.org/10.1038/s41598-020-75762-7].
[13] Stellbrink H-J. Antiviral drugs in the treatment of AIDS: what is in the pipeline? Eur J Med Res 2007;12:483–95.
Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoetelmans R, et al. Short-term antiviral activity of TMC278—a novel NNRTI—in treatment-naive HIV-1-infected subjects. Aids 2006;20:1721–6.

Pozniak A, Morales-Ramirez J, Mohapi L, Santoscoy M, Chetchotisakd P, Hereygers M, et al. 48-week primary analysis of trial TMC278-C204: TMC278 demonstrates potent and sustained efficacy in ART-naive patients. 14th Conference on Retroviruses and Opportunistic Infections 2007:25–8.

Tomic D, Davidovic D, Szasz AM, Rezeli M, Petrik J, et al. The screening and evaluation of potential clinically significant HIV drug combinations against the SARS-CoV-2 virus. Inf Med Unlocked 2021:100529.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 2009;30:2785–91.

Wang K, Gheblawi M, Oudit GY. Angiotensin converting enzyme 2: a double-edged sword. Circulation 2020;142:426–8.

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020;581:215–20.

Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Loefgren SM, Okafor EC, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. N Engl J Med 2020;383:517–25.

Jia H, Gong P. A structure-function diversity survey of the RNA-dependent RNA polymerases from the positive-strand RNA viruses. Front Microbiol 2019;10:1945.

Shin D, Mukherjee R, Grew D, Bojkova D, Baek K, Bhattacharya A, et al. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. Nature 2020;587:657–62.

Báez-Santos YM, John SES, Mesecar AD. The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antiviral Res 2015;115:21–38.

Anand K, Palm GI, Mesters JR, Siddell SG, Ziebuhr J, Hilgenfeld R. Structure of coronavirus main protease reveals combination of a chymotrypsin fold with an extra α-helical domain. EMBO J 2002;21:3213–24.

Mirza MU, Froeyen M. Structural elucidation of SARS-CoV-2 viral proteins: computational methods reveal potential drug candidates against main protease, Nsp12 polymerase and Nsp13 helicase. J Pharm Anal 2020;10:320–8.