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Dual targeting of 3CL^{pro} and PL^{pro} of SARS-CoV-2: A novel structure-based design approach to treat COVID-19

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ABSTRACT

With the rapid growth of the COVID-19 (coronavirus disease 2019) pandemic across the globe, therapeutic attention must be directed to fight the novel severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). However, developing new antiviral drugs and vaccines is time-consuming, so one of the best solutions to tackle this virus at present is to repurpose ready-to-use drugs. This paper proposes the repurposing of the Food and Drug Administration (FDA)-approved, purchasable, and naturally occurring drugs for preventive and therapeutic use. We propose to design a dual-inhibitor for the SARS-CoV-2 cysteine proteases—3 Chemotrypsin-like protease or main protease (3CL^{pro} or M^{pro}) and Papain-like protease (PL^{pro}) responsible for processing the translated poly-protein chain from the viral RNA yielding functional viral proteins. For virtual screening, an unbiased blind docking was performed from which the top nine dual-targeting inhibitors for 3CL^{pro} and PL^{pro} were selected. The nine repurposed drugs, block the catalytic dyad (His41 and Cys145) of 3CL^{pro} as well as the catalytic triad (Cys111, His272, and Asp286) of PL^{pro}. Repurposing known drugs will not only pave the way for rapid in-vitro and in-vivo studies to battle the SARS-CoV-2 but will also expedite the quest for a potent anti-coronaviral drug.

1. Introduction

In late December 2019, there was an outbreak of the new strain of the coronavirus termed as SARS-CoV-2, spawning the coronavirus disease-2019 (COVID-19) pandemic. Since there are no specific drugs or -vaccines to tackle the virus, the disease has taken hundreds of thousands of human lives (Acter et al., 2020; Guo et al., 2020; Chatterjee et al., 2020). Presently, several strategies, including the use of broad-spectrum antiviral drugs, anti-malarial drugs, anti-HIV infection drugs, alongside the target-specific molecular screening of these existing drugs, are being used to counter the virus (Li et al., 2020; Vincent et al., 2005; Wang et al., 2020a, 2020b; Arabi et al., 2020; Chu et al., 2004; Lim et al., 2020). The lack of site-specific target inhibition by these broad-spectrum drugs renders them therapeutically less specific and, consequently, less effective against the virus. Moreover, the possibilities of undetermined side effects to patients treated with these drugs cannot be ruled out. However, the strategy of high-throughput screening of these drugs opens up the possibility to identify and repurpose the purchasable drugs that would help treat COVID-19-affected cases effectively (Issa et al., 2013; Wang, 2020; Jain et al., 2020; Senanayake, 2020). The computational screening, interaction, and physiochemical analysis of these identified drugs, with the objective of repurposing, constitutes one of the fastest ways to determine their efficacy in vitro and *in vivo* before they are approved for clinical use for the treatment of the patients. This repurposing reduces further efforts and medicine costs, almost bypassing the initial trials, which is a prerequisite step to test new drugs to determine any unknown side effects and dose administration. In addition, the already available formulation and distribution system of such medicines in the pharmaceutical market make them to fulfill the requirement in less time. Around 80 clinical trials are going on to test the treatment for COVID-19, where many of them include repurposing of drugs (Maxmen, 2020). A recently concluded study identified 24 clinical trials for COVID-19 treatment, in which 19 medicines are in phase 1, 2, and 3 of the clinical phase, such as human immunoglobulin, interferons, chloroquine, hydroxychloroquine, arbidol, remdesivir, favipiravir, lopinavir, ritonavir, oseltamivir, methylprednisolone, bevacizumab, and traditional Chinese medicines (TCM) (Rosa and Santos, 2020). Each listed drugs differently target the machinery of SARS-CoV-2. Interestingly, we have attempted to come up with virtually-screened, FDA-approved, naturally occurring purchasable drugs as a dual-inhibitor for 3CL^{pro} and PL^{pro}, with therapeutic potential to act simultaneously on the activity of both proteases. For a long time, bioactive and natural products from plants,

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microbes, and animal sources have been used to treat infectious and non-infectious diseases (Kim et al., 2016, 2018), and they play a vital role in innovative drug discovery in the face of this 21st-century pandemic (Thomford et al., 2018). According to records, one-third of all FDA-approved drugs between 1981 and 2019 were either natural products or their derivatives (Newman and Cragg, 2016, 2020). Meanwhile, in recent times, natural products have been significantly contributing to the computer-aided discovery of molecular targets, drug development, and repurposing for diseases (Thomford et al., 2018; Li et al., 2019).

The SARS-CoV-2 shares the highest genome identity, of around 80%, with SARS-CoV, the other human coronavirus that exhibits the structural and functional similarities of the encoded proteins (Wu et al., 2020a; Ul Qamar et al., 2020; Chen and Zhong, 2020). The non-structural proteins $3CL^{pro}$ and PL^{pro} are the two major important cysteine proteases of SARS-CoV-2, quite conserved with SARS-CoV (Wu et al., 2020b; Baez-Santos et al., 2015; Yoshimoto, 2020; Xue et al., 2008), which process the polyproteins translated from the viral RNA-genome to yield the active functional proteins necessary for viral replication and, hence, for more infections (Wu et al., 2020b; Zhang et al., 2020; Rut et al., 2020; Luigi, 2020). Thus, inhibition of these proteases may significantly hamper the viral machinery and overall viral infection rate. Notably, the similar mechanism of infection in human host by SARS-CoV and SARS-COV-2 have helped much in the context of therapeutics (Tu et al., 2020). Such comparative analysis has also been studied between the other human coronavirus, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) and effective drugs against it was expected to target SARS-CoV-2 and treat COVID-19 (Tu et al., 2020; Baron et al., 2020). Previously, major attempts have been made to target the SARS-CoV-2 spike protein, RdRp (RNA dependent RNA polymerase), and $3CL^{pro}$ with less focus on other important protease PL^{pro} almost equally responsible for the processing of several polypeptides of SARS-CoV-2 (Wu et al., 2020b; Alexpandi et al., 2020; Sternberg et al., 2020). Also the catalytic similarities between these two proteases suggest a common therapeutic target. This study, therefore, aims to identify the virtually screened drugs capable of inhibiting the activity of these proteases and, further, establishing these drugs as a single drug with the potential to block both the proteases simultaneously. In agreement with previous studies for other viral diseases (Modhiran et al., 2019; Xiao et al., 2019; Raghavendra et al., 2018), we strongly suggest that such a treatment regimen of dual-target for COVID-19 may prove more beneficial and may largely reduce the combinatorial drug application as well as multi-drug dose burden in host system.

2. Material and methods

Structural data retrieval and preparation- For molecular docking studies, the experimentally solved low-resolution crystal structure of both the $3CL^{pro}$ and PL^{pro}available at the PDB (Protein Data Bank) database was retrieved and prepared for docking studies. The pdb file format of the apo-enzyme structure of $3CL^{pro}$ and PL^{pro} (PDB ID 6M2Q and 6W9C, respectively) was downloaded from the PDB database (https://www.rcsb.org/). Before docking study, the water molecules were deleted and polar hydrogens were added to the structures. The Chain A of homo-trimeric 6W9C (PL^{pro}) was only used for docking studies, deleting the other two chains from 6W9C pdb file in Discovery Studio (Systèmes, 2019). Further, both the structures were energetically minimized in UCSF Chimera (Pettersen et al., 2004). For virtual screening, drug library was selected from ZINC15 drug database (Sterling and Irwin, 2015). The filter was applied to specifically select only the FDA approved, in-stock naturally occurring purchasable drugs and a total of 291 drugs (http://zinc.docking.org/substances/subsets/fda+for-sale+standard-ok+biogenic/?q=natural-products) matched to the set filter were downloaded. The downloaded compounds in SDF (Structure-data file-) format were converted into PDBQT and mol2 file format using open babel version 3.0 (O'Boyle et al., 2011) for docking and screening against the above prepared protease structures.

In-silico Molecular docking, Virtual screening and Molecular Dynamics (MD) simulation studies-For virtual screening of the 291 FDA approved compounds, the AutoDock Vina (Trott et al., 2010) and Swiss Dock (Bitencourt-Ferreira and de Azevedo, 2019; Grosdidier et al., 2011) protein-ligand docking platforms were used. The 291 compounds were docked with $3CL^{pro}$ (6M2Q) and PL^{pro} (6W9C) separately, and top compounds interacting specifically with all the catalytic residues of $3CL^{pro}$ and PL^{pro} were only selected and later the compounds common for interacting with catalytic sites of both the protein structures were finally identified and used for further analysis. The docked result was analysed using UCSF Chimera (Pettersen et al., 2004) and BIOVIA Discovery Studio Visualizer (Systèmes, 2019). All protein-ligand interaction images were prepared in BIOVIA Discovery Studio Visualizer. The top 09 drug candidates for repurposing as a dual-inhibitor can be identified with their name and ZINC15 ID. The docked complexes of top three compounds with $3CL^{pro}$ and PL^{pro} were subjected to MD Simulation studies in GROMACS 4.5.5 for analysing the stability in terms of RMSD (Root Mean Square Deviation) and a total period of 50ns simulation was performed for each complex. The files were prepared for MD simulation in MDWeb server. The GROMOS 53a6 force field was applied and the whole system was solvated, equilibrated and simulation was performed at constant temperature of 300 K. The stability of these protein-ligand complexes were analysed using the RMSD of backbone atoms plotted against the total simulation time. The coordinates of the structure were stored and examined using the analytical tool in the GROMACS 4.5.5 package (Pronk et al., 2013).

Prediction of activity spectra for substances (PASS) - The software program can evaluate the general biological potential of an organic drug-like candidate (http://www.pharmaexpert.ru/PASSonline/predict.php). It provides simultaneous predictions of a wide range of biological activity based on the structure of a compound. Thus, PASS can be used to estimate the biological activity profiles for molecules prior to their chemical synthesis and biological testing. This tool provided quantitative structure-activity relationships based on the decomposition of chemical structures using 2D and/or 3D descriptors, followed by the generation of models obtained from bioactive ligands (Khurana et al., 2011). The activity is estimated in terms of Pa (probable activity) and Pi (probable inactivity). Structures with Pa greater than Pi were the only compounds considered for a particular pharmacological activity (Filimonov et al., 2018; Lagunin et al., 2011).

In silico physicochemical and pharmacokinetic analysis–For physiochemical and pharmacokinetic analysis, components of lipinski's Rule of 5 (RO5) and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the final 09 compounds were predicted using MedChem Designer (Simulations Plus Inc. Lancaster, CA) (https://www.simulations-plus.com/) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/), (Pires et al., 2015) respectively.

3. Results and discussion

Repurposing of drugs against $3CL^{pro}$and PL^{pro}(SARS-CoV-2)–Like the SARS-CoV, the SARS-CoV-2 main protease $3CL^{pro}$ and PL^{pro} have similarly conserved catalytic residues His41 and Cys145 as well as Cys111, His272 and Asp286 along with oxyanion hole-stabilizing Trp106 respectively in the active region which is responsible for its enzymatic activity to process the newly synthesized polypeptide chain of the virus for functional non-structural proteins (Wu et al., 2020b; Baez-Santos et al., 2015; Pillaiyar et al., 2016). Inhibition of these proteases will not only stop the production of functional polypeptides of SARS-CoV-2 but also help to reduce the viral replication and its load on the host. Considering the fact, the $3CL^{pro}$ and PL^{pro} were targeted to virtually identify the potential drug specifically blocking its catalytic dyad and triad, respectively. Virtual screening of drugs were performed on the crystal structure of the targets. The unmutated crystal structure 6M2Q for $3CL^{pro}$ and 6W9C for PL^{pro} from the PDB database was selected (Fig. 1). The chains of 6W9C were processed in Discovery studio by removing

duplicate chain B and C, keeping only Chain A for the docking study. Further, both the structures were prepared by removing the water molecules, hetro-atoms and any co-crystlaized ligands followed by adding polar hydrogen atoms in Discovery Studio (Systèmes, 2019) and was finally energetically minimized in UCSF Chimera (Pettersen et al., 2004). The unbiased, blind molecular docking of the 291 FDA approved naturally occurring drugs from the ZINC library was performed with the prepared 6M2Q and 6W9C apo-enzyme crystal structure in AutoDock Vina (Trott et al., 2010) to screen out the inhibitory drug's binding specifically in the pocket of the enzymes active site. In the output result, the binding pose of all docked compounds were analysed in Discovery Studio and UCSF Chimera (Pettersen et al., 2004), which results in 45 and 65 drugs binding either to any of the catalytic residues or with all of the catalytic residues along with other interacting residues in the enzymes active region. The selective screening of the drugs was performed based on the parameter of their interacting residues, ligand efficiency, and binding energy. The compounds, interacting with all the catalytic residues along with their binding energy value above −5 kcal/mol were shortlisted. Similar to SARS-CoV PL^{pro} Trp107 in the active site, the Trp106 in SARS-CoV-2 PL^{pro} also plays a significant role in stabilization to the catalytic triad throughout the catalysis by its indole ring nitrogen present in the oxyanion hole (Baez-Santos et al., 2015; Ratia et al., 2006). Therefore, the Trp106 was also considered as the significant residue and a parameter for compounds selection interacting with it. Interestingly, we found a total of 18 and 13 drugs for $3CL^{pro}$ and PL^{pro}, respectively, whose solutions specifically fit into the active site and interacts with all the above said residues along with others. To further validate this result, another docking platform Swiss Dock (Grosdidier et al., 2011) was used for screening again in the blind docking mode with the same $3CL^{pro}$ and PL^{pro}prepared crystal structure. The Swiss dock virtual screening of 45 and 65 drugs confirmed the result in agreement with the selected drugs obtained from Autodock Vina result. This also confirms the binding specificity of the repurposed drugs toward the catalytic site of these enzymes. The selected drugs were involved both in conventional and carbon Hydrogen bonding along with other electrostatic interaction Van Der Waal forces as well as pi-Alkyl bonding as analysed in the BIOVIA Discovery Studio Visualizer protein-ligand interaction tool (Systèmes, 2019). Finally, the selected drugs were assessed for its repurposing role as the dual-inhibitor of $3CL^{pro}$ and PL^{pro}.

Common repurposed drugs targeting both $3CL^{pro}$ and PL^{pro}(-SARS-CoV-2)– The assessment of all the 18 and 13 drugs for protease enzyme $3CL^{pro}$ and PL^{pro} was performed in the curiosity to find these naturally occurring drugs as the dual-inhibitor of both the enzymes activity. Due to their similarity as the cysteine proteases and enzymatic function in virus, we were curious to analyze the final selected drugs to repurpose them as a dual inhibitor of SARS-CoV-2 proteases $3CL^{pro}$ and PL^{pro}. Interestingly, the 11 drugs out of the 18 and 13 shortlisted drugs were common for both the enzymes. Several previous virtual screening studies on either $3CL^{pro}$ or PL^{pro} have identified the individual inhibitor compounds, most of which are either druggable compound or lead molecules. The novel finding of our study suggests the repurposing of already marketed approved naturally occurring drugs as the dual-inhibitor of the $3CL^{pro}$ and PL^{pro} which shows potential binding to simultaneously inhibit both the enzymes of SARS-CoV-2. Such a simultaneous inhibition may not only reduce the SARS-CoV-2 infection at large but will also provide the major solution to the unknown side effects to the host because of the use of combinatorial drugs. Each 11 drugs were individually studied for their known pharmacological action and route of administration to categorize them for a better understanding of their chemical nature as well as possible mechanisms being utilized for in-vitro and in-vivo study to establish it as potent coronaviral drugs. Therefore, drugs for human use with an oral and injectable route of administration, and not the topical, were only selected. This led us to remove 02 drugs (ZINC 1554 and ZINC 1011) majorly for topical use only. All the other top 09 drugs, with their ZINC ID, a common name with known pharmacological actions, and their binding energies, as confirmed from both Autodock Vina (Trott et al., 2010) and Swiss Dock (Grosdidier et al., 2011) are given in Table 1.

The fitting and interaction of these drugs in the active pocket of enzymes were individually analysed in BIOVIA Discovery Studio Visualizer protein-ligand interaction tool (Systèmes, 2019). The interaction pattern of all the drugs, as shown individually in Fig. 2, demonstrates the type of bonding between each ligand and protein structure. Besides the other residues interaction in active site vicinity, the catalytic residues His41 and Cys145 in $3CL^{pro}$ and Trp106, Cys111, His272, and Asp286 in PL^{pro} have shown the stable conventional and carbon-hydrogen bonding, Van

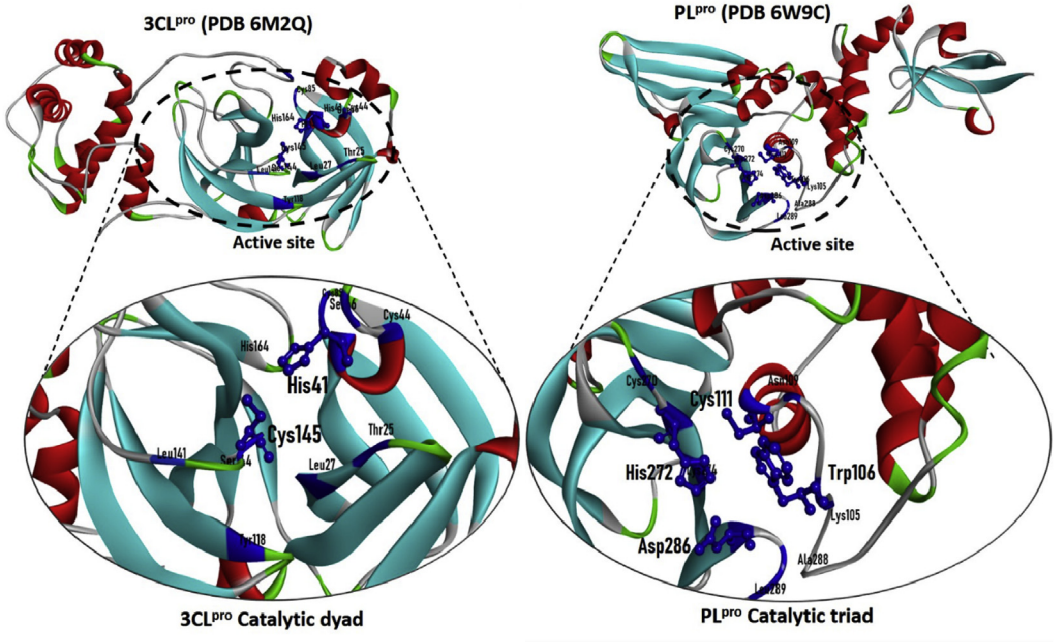
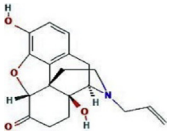
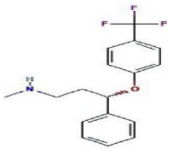
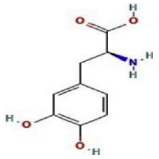
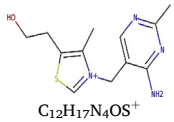
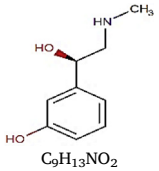
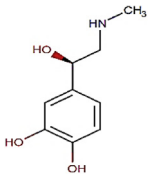
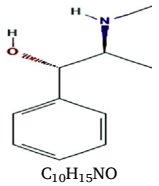


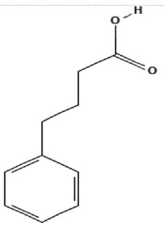
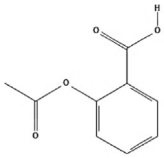
Fig. 1. Representation of SARS-CoV-2 proteases active sites and catalytic residues. (A) Catalytic dyad His41 and Cys145 of $3CL^{pro}$, and (B) catalytic triad Cys 111, His 272, and Asp 286, respectively. SARS-CoV-2- severe acute respiratory syndrome-coronavirus-2, $3CL^{pro}$ – 3Chemotrypisn-like protease, and PL^{pro}-papain-like protease.

Table 1
List of virtually screened naturally occurring drugs identified as dual-inhibitor of SARS-CoV-2 cysteine proteases $3CL^{pro}$ and PL^{pro}. All the listed drugs are FDA approved, whose know pharmacological action, chemical structure, formula, and common name is provided along with their ZINC ID. The binding energy of these drugs obtained both by AutoDock Vina, and Swiss Dock docking studies for $3CL^{pro}$ and PL^{pro} are given in right.

No.	ZINC ID	Structure & Molecular Formula	Drug Name/ Commercial Name	Pharmacological action	Route of Adminis-tration	3CLpro (6M2Q) Binding energy (kcal/mol)		PLpro (6W9C) Binding energy (kcal/mol)	
						Vina	Swiss Dock (Full Fitness)	Vina	Swiss Dock (Full Fitness)
Anti-psychotic Drugs									
1.	ZINC 389747	 $C_{19}H_{21}NO_4$	Naloxone/ Narcan	Block opioids effect by binding opioid receptors in Central Nervous system	Intravenous/ Intramuscular/ Subcutaneous	-6.8	-3168.67	-7.8	-3098.25
2.	ZINC 1530637	 $C_{17}H_{18}F_3NO$	Fluoxetine/ Prozac	Selective serotonin reuptake inhibitor (SSRI) antidepressant	Oral	-6.5	-3188.24	-7.5	-3074.37
3.	ZINC 895199	 $C_9H_{11}NO_4$	L-Dopa/ Levodopa	A prodrug crosses blood brain barrier and metabolized into dopamine	Oral	-5.8	-3164.75	-7.3	-3032.75
Vitamin-Based Drug									
4.	ZINC 49153	 $C_{12}H_{17}N_4OS^+$	Thiamine	To treat or prevent Vitamin B1 deficiency and role in Intracellular glucose metabolism	Intramuscular/ Intravenous	-6.6	-3178.34	-7.8	-3048.24
Hormonal Drugs-									
5.	ZINC 113355	 $C_9H_{13}NO_2$	Phenylephrine/ Metasympatol	alpha-adrenergic agonist, a cardiotonic drug, a mydriatic agent, a protective agent, a vasoconstrictor agent, a sympathomimetic agent and a nasal decongestant	Oral	-5.6	-2646.89	-6.8	-2247.25
6.	ZINC 39089	 $C_9H_{13}NO_3$	Epinephrine/ Adrenaline	used in asthma and cardiac failure, causes systemic vasoconstriction and gastrointestinal relaxation, stimulates the heart, and dilates bronchi and cerebral vessels	Intravenous/ Intramuscular/ Subcutaneous/ Inhalation	-5.5	-3045.94	-6.1	-2456.15
7.	ZINC 20259	 $C_{10}H_{15}NO$	Pseudoephedrine/ Isoephedrine/Sudafed	a sympathomimetic agent, an anti-asthmatic drug, a bronchodilator agent, a vasoconstrictor agent, a central nervous system drug, a nasal decongestant	Oral	-5.5	-3104.24	-5.5	-2948.14

(*continued on next page*)

Table 1 (*continued*)

No.	ZINC ID	Structure & Molecular Formula	Drug Name/ Commercial Name	Pharmacological action	Route of Administration	3CLpro (6M2Q) Binding energy (kcal/mol)		PLpro (6W9C) Binding energy (kcal/mol)	
						Vina	Swiss Dock (Full Fitness)	Vina	Swiss Dock (Full Fitness)
Natural Acid-BasedDrugs-									
8.	ZINC 56568	 $C_{10}H_{12}O_2$	Benzenebutyric acid/ Phenylbutanoic acid	histone deacetylase inhibitor role as anticancer activity, inhibits cell proliferation, invasion and migration and induces apoptosis in glioma cells	Oral	-5.4	-2759.12	-6.3	-3024.25
9.	ZINC 53	 $C_9H_8O_4$	Acetylsalicylic acid/ 2-Acetoxybenzoic acid/ Aspirin	an analgesic, antipyretic and anti-inflammatory, prevent heart attacks and various cancer	Oral	-5.4	-2604.34	-5.3	-2147.25

$3CL^{pro}$ – 3Chemotrypisn-like protease and PL^{pro}-papain-like protease.

Der Waals interactions (VdW), Alkyl and pi-Alkyl interactions. To our surprise, most of the listed drugs as the hormonal drugs, natural acid drugs, in addition to our identified $3CL^{pro}$ and PL^{pro} dual-inhibitor, are also symptoms reliever or risk reducer for cardiovascular, a bronchodilator in respiratory condition, anti-inflammatory, analgesic (pain relief), antipyretic (fever) and decongestant for running nose and notably these all are associated with SARS-CoV-2 infection and severity too. This may also suggest that the use of such drugs in particular pre-medical conditions along with the SARS-CoV-2 infection can have a synergistic effect and proved to be more beneficial to curb the disease. The repurposing of these drugs as a dual-inhibitor candidate, however, requires experimental validation before its recommendation for clinical use.

4. Results of MD simulations

The MD simulation was performed in GROMACS 4.5.5 package (Pronk et al., 2013) where the stability of three ligands (ZINC 389747, ZINC 1530637, and ZINC 49153) with $3CL^{pro}$ and PL^{pro} complexes were analysed using the RMSD of backbone atoms. As represented in Fig. 3, the RMSD (root mean square deviation) graph of both proteins $3CL^{pro}$ and PL^{pro} complexed with ZINC 389747, ZINC 1530637, and ZINC 49153 shows an average root mean square deviation of 0.25–0.5 nm, 0.3–0.4 nm, and 0.4–0.45 nm as well as 0.35–0.4 nm, 0.5–0.55 nm and 0.35–0.4 nm, respectively (Fig. 3). Interestingly, at the end of the 50 ns of time scale simulation, all the protein-ligand complexes attained their stable conformations as also evident from their convergence and lower RMSD value confirming the stability.

PASS activity prediction- The shortlisted repurposed drugs (of ZINC 389747, ZINC 895199, ZINC 113355, ZINC 39089, ZINC 20259, ZINC 56568, and ZINC 53) were further subjected to determine the broad biological activity spectra by the online PASS program. The predictions were interpreted and used in a flexible manner to determine the possible biological activity of repurposed drugs. The antiviral activities of the screened drugs were predicted as PASS predicted probable antiviral activity (Pa) (supplementary table 1). Based on the PASS prediction and docking studies, we expect the identified repurposed drug would have antiviral activity by blocking the PL^{pro} and $3CL^{pro}$.

Physiochemical and pharmacokinetic properties of identified repurposed dual-inhibitors of SARS-CoV-2 $3CL^{pro}$ and PL^{pro}-The obtained physiochemical and ADMET score is displayed in Table 2. In physicochemical properties analysis by MedChem designer (https://www.simulations-plus.com/), the final compounds satisfy all the components (hydrogen bond donor should be less than 5 and acceptor less than 10, molecular mass less than 500 Da and octanol-water partition coefficient (logP) should not exceed 5) of Lipinski's rule of 5 (RuleOf5 or RO5) (Lipinski et al., 2001). The MlogP scores are revealing the lesser lipophilicity and higher soluble nature of these compounds naturally. Even though the lipophilicity of the second drug (ZINC 1530637) is a little higher (RO5 value 1, Table 2), this compound has exhibited remarkable *in silico* functional values such as second lowest binding energy and an affinity for the proteases. The Hydrogen bond donor and acceptor (HBDA) are also less than 5 and 10, respectively. The pharmacokinetic properties of the compounds were obtained through the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction) (Pires et al., 2015) submitting the SMILES files of each compound for prediction. Mainly total clearance (TC) and Fraction unbound (human) value for each compound was recorded, as shown in Table 2.

5. Conclusion

The pandemic of COVID-19 caused by SARS-CoV-2 has dangerously emerged as the worst deadly virus of the century after the pandemic of Influenza Virus (H1N1) (Johnson and Mueller, 2002; Taubenberger and Morens, 2006; −19 Situation Re, 2020). The outbreak of human infecting coronavirus various strains SARS-CoV, MERS-CoV, and now this novel SARS-CoV-2 in the last two decades have evolved more and more lethal and reminded the absence of significant therapeutic measure against it. The further variant strains of SARS-CoV-2 may pose a serious threat of recurrence in the near future, and one more failure in early medical preparation will put humankind far behind both in terms of better global health and economy. The current outbreak and statistics of the spike in COVID-19 related deaths worldwide have already put the global health system in an emergency and demands an immediate effective therapeutic approach to curb this outbreak now and forever. Interestingly, the

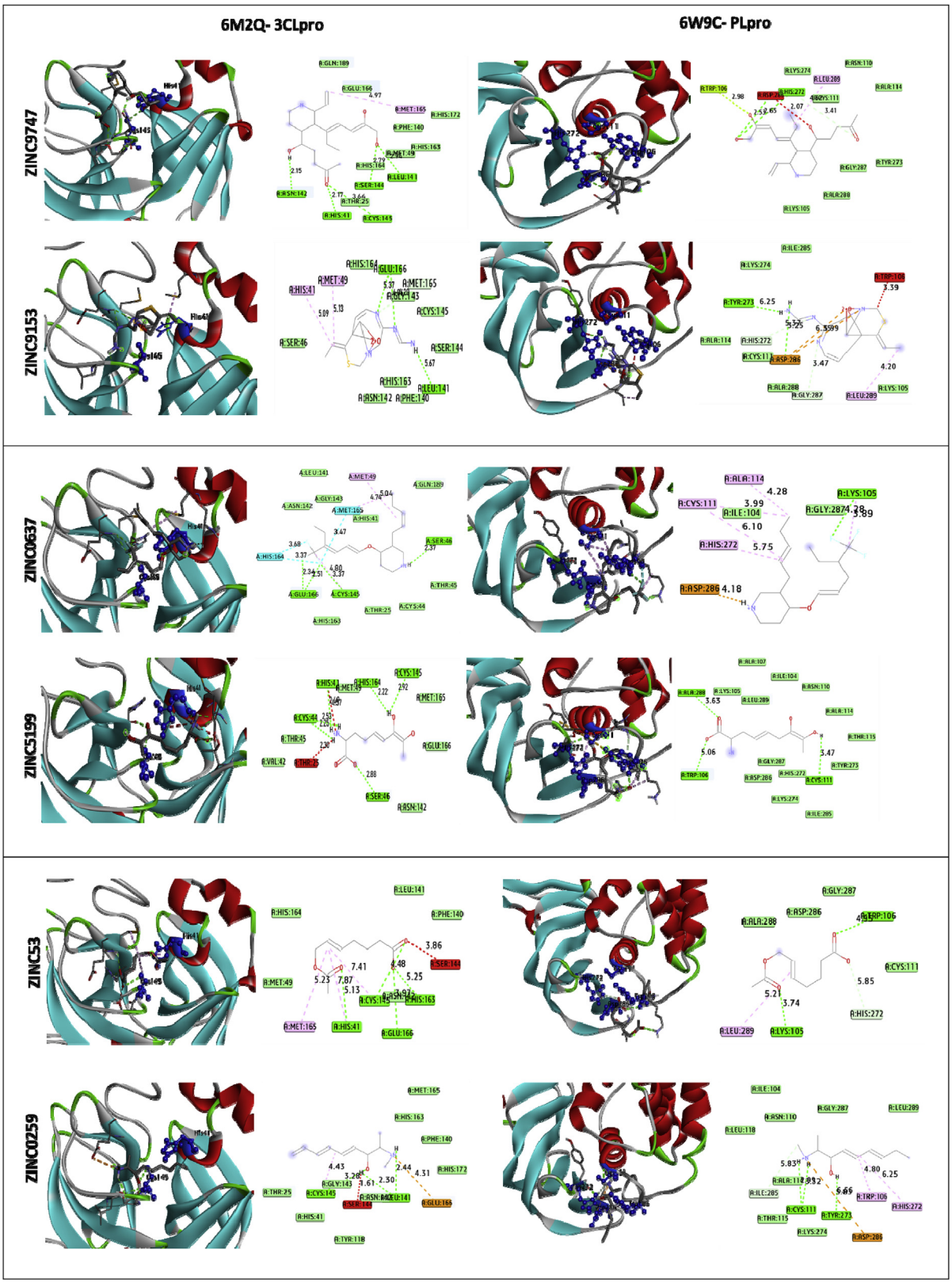


Fig. 2. The protease-drug interaction pattern. The crystal structure of $3CL^{pro}$ (PDB 6M2Q) and PL^{pro} (PDB 6W9C) of SARS-CoV-2 docking complex with virtually screened FDA approved naturally occurring drugs from the ZINC15 library. The interaction of each drug molecule is presented in the 3D and 2D patterns. The Catalytic residues in $3CL^{pro}$ (His41 and Cys145) and PL^{pro} (Trp106, Cys111, His272, and Asp286) are highlighted with blue color in a ball and stick format in the 3D image while the bonding types with residues in 2D formats are highlighted with individual color as given in the legend.

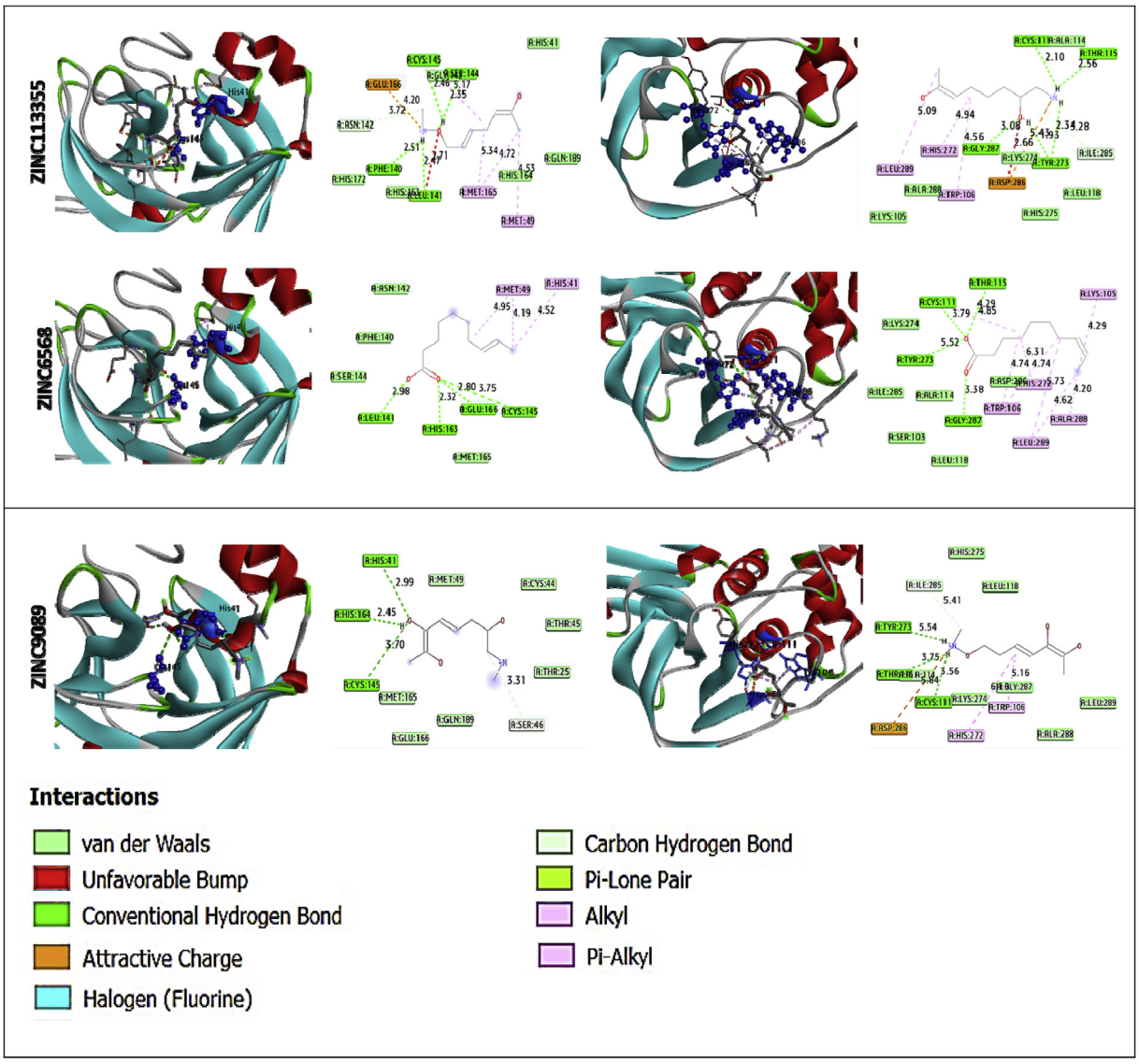


Fig. 2. (*continued*).

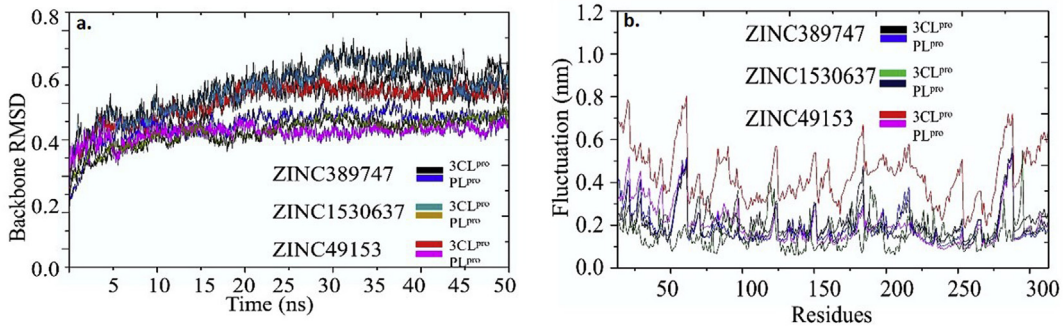


Fig. 3. Plots of Backbone RMSD of (a) three compounds, ZINC389747, ZINC1530637 and ZINC49153 complexed with $3CL^{pro}$ and PL^{pro} of SARS-COV-2 and, (b) its residue fluctuation. *RMSD- Root Mean Square Deviation.*

genetic identity of SARS-CoV-2 with other coronavirus strains, especially with humans infecting SARS-CoV, the similarity in host cell interaction, infection, and replication machinery, and the presence of conserved region in these major potential therapeutic targets such as Spike (S) glycoprotein receptor-binding domain (RBD), non-structural proteins (NSPs) cysteine proteases $3CL^{pro}$ (NSP5) and PL^{pro} (NSP3), RNA dependent RNA polymerase (RdRp) and others have supported at the great extent to use the range of therapeutic drugs being employed previously

Table 2
ADMET properties of screened compounds.

Sr No	Name	Diff Coef	MlogP	S + logP	S + logD	Mol mass	N&O	T_PSA	HBDA	RuleOf5	RuleOf5_Code	TC	FU (human)
1.	ZINC389747	0.75	2.038	1.199	0.947	327.38	5	70	2	0	None	1.28	0.57
2.	ZINC1530637	0.746	4.153	4.388	2.025	309.33	2	21.26	1	1	LP	0.69	0.05
3.	ZINC895199	1.144	1.165	0.753	0.747	152.15	3	57.53	2	0	None	0.62	0.72
4.	ZINC49153	0.804	0.317	−1.841	−1.842	265.36	5	75.91	3	0	None	1.02	0.69
5.	ZINC113355	1.014	0.793	−0.026	−1.63	167.21	3	52.49	3	0	None	0.92	0.58
6.	ZINC39089	0.992	0.764	−0.621	−1.922	183.21	4	72.72	4	0	None	0.94	0.51
7.	ZINC20259	0.972	1.664	1.173	−0.722	165.24	2	32.26	2	0	None	0.99	0.49
8.	ZINC56568	1.014	1.98	2.282	−0.264	164.21	2	37.3	1	0	None	0.36	0.33
9.	ZINC53	1.061	1.4	1.74	−1.307	180.16	4	63.6	1	0	None	0.72	0.48

DiffCoef– Differential co-efficient; MlogP - Moriguchi estimation of logP; S + logP – Simulated logP; RuleOf5 (RO5) - Lipinski's Rule of Five: a score indicating the number of potential problems a structure might have with passive oral absorption; RuleOf5_Code - Lipinski's Rule of Five codes: LP = logP; Mol masst = molecular mass(Daltons); HBDA – Hydrogen bond Donor and Acceptor; T_PSA – Topological polar surface area; N&O – Number of N (Nitrogen) and O (Oxygen) atoms, TC- Total clearance (log ml/min/kg), FU- Fraction unbound (Fu).

for other strains as well as to study for new or repurposed drugs and vaccines (Li et al., 2020; Wang et al., 2020a; Chen and Zhong, 2020; Wu et al., 2020b; Khailany et al., 2020; Lu et al., 2020). It is a serious matter of concern that since the 2003 SARS-CoV outbreak, we don't have any clinically approved new drugs. However, the repurposing of available approved drugs is one of the best options for rapid analysis and approval for clinical use against SARS-CoV-2 as an effective measure (Wang, 2020; Senanayake, 2020).

The broad-spectrum anti-HIV drugs like lopinavir and ritonavir are known for HIV protease inhibition, and Chloroquine phosphate as anti-malarial drugs used in emergencies for SARS-CoV previously without clinical trials are being used again as a combination drug to counter SARS-CoV-2 (Arabi et al., 2020; Chu et al., 2004; Lim et al., 2020; Simsek Yavuz and Unal, 2020). However, its speculation to block SARS-CoV-2 proteases and other targets, respectively, have shown a poor effect in terms of binding specificity to these targets, and having toxic side effects demand the requirement of target-specific drugs with better therapeutic efficacy against SARS-CoV-2. In comparison to these, other structural studies have shown better and improved drug candidates than the above-mentioned broad-spectrum drugs individually acting on various targets of SARS-CoV-2 (Wu et al., 2020b; Chen et al., 2020). Further to this, both to largely reduce the burden of combinatorial drugs administration and its increased side effects, the attempt of our study was to combinedly target the main cysteine protease $3CL^{pro}$ and the other PL^{pro} of SARS-CoV-2 with a single drug to provide a platform for an effective counter of COVID-19. The similarity in the functional and catalytic nature of these two proteases inspires us to pursue the search for dual-inhibitors. We prepared the available crystal structure of these proteases for docking and assessment of drugs filtered from the ZINC15 library. The finding of our structural study focused on the repurposing of FDA approved naturally occurring drugs, which can act as a dual inhibitor for SARS-COV-2 proteases. The 291 drugs obtained from the ZINC15 library were virtually screened by unbiased, blind docking studies against each of the crystal structures of $3CL^{pro}$ and PL^{pro}. The drugs binding specifically with all the catalytic residues His41 and Cys145 in $3CL^{pro}$ and Trp106, Cys111, His272, and Asp286 were only considered as positive (Fig. 3). Furthermore, the assessment of such positive drugs was done to find the type of interactions and list of common drugs being positive for both proteases.

Finally, the one parameter in the last step selection of such drugs, already available in the market, was based on their oral or injectable route of administration, excluding topical drugs from our study to provide rapid relief post-approval for clinical use after in vitro and invivo experimental analysis. We expect to perform these experimental studies of the listed top 09 naturally occurring drugs shortly to finalize the promising dual-inhibitor drugs repurposing for SARS-CoV-2 cysteine proteases $3CL^{pro}$ and $PL^{pro,}$ and hence these virtually screened drugs provide us with the fastest way toward its further validation for repurposing as dual-inhibitor. Most of the drugs screened in this study have a pharmacological action for the symptoms of cardiovascular conditions, respiratory conditions, as a decongestant, anti-inflammatory, antipyretic, and analgesic. Notably, the clinical studies of SARS-CoV-2 infection report that COVID-19 patients either show such symptoms like running nose, fever, sore throat, or those already with such pre-medical conditions have shown an increase in the disease severity. Therefore, we may suggest that the repurposing of these drugs as a dual-inhibitor of SARS-CoV-2 proteases may also help as a risk-reducer in the case of patients with a particular pre-medical condition like respiratory, cardiovascular, or psychotic problems. Henceforth, if approved, the synergistic effect of these drugs, along with dual inhibition of SARS-CoV-2 proteases, can extensively improve the patient treatment.

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Contributions

M.S.B. conceived and designed the research. M.A. and S.R. executed, compiled, and analyzed the data. M.S.B. have written, reviewed and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crstbi.2020.12.001.

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