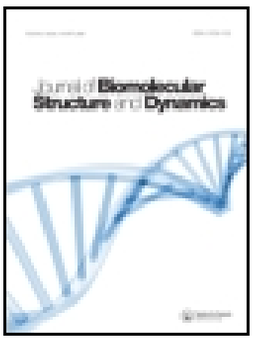

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Possibility of HIV-1 protease inhibitors-clinical trial drugs as repurposed drugs for SARS-CoV-2 main protease: a molecular docking, molecular dynamics and binding free energy simulation study

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ABSTRACT

Initially, the SARS-CoV-2 virus was emerged from Wuhan, China and rapidly spreading across the world and urges the scientific community to develop antiviral therapeutic agents. Among several strategies, drug repurposing will help to react immediately to overcome the COVID-19 pandemic. In the present study, we have chosen two clinical trial drugs against HIV-1 protease namely, TMB607 and TMC310911 to use as the inhibitors of SARS-CoV-2 main protease (M^{pro}) enzyme. To make use of these two inhibitors as the repurposed drugs for COVID-19, it is essential to know the molecular basis of the binding mechanism of these two molecules with the SARS-CoV-2 M^{pro}. To understand the binding mechanism, we have performed molecular docking, molecular dynamics (MD) simulations, and binding free energy calculations against the SARS-CoV-2 M^{pro}. The docking results indicate that both molecules form intermolecular interactions with the active site amino acids of M^{pro} enzyme. However, during the MD simulations, TMB607 forms strong interaction with the key amino acids of M^{pro}, and remains intact. The RMSD and RMSF values of both complexes were stable throughout the MD simulations. The MM-GBSA binding free energy values of both complexes are −43.7 and −34.9 kcal/mol, respectively. This *in silico* study proves that the TMB607 molecule binds strongly with the SARS-CoV-2 M^{pro} enzyme and it may be suitable for the drug repurposing of COVID-19 and further drug designing.

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KEYWORDS
SARS-COV-2M^{pro}; HIV-1 protease clinical trial drugs; drug repurposing; molecular docking; molecular dynamics; binding free energy

1. Introduction

World health organization (WHO) declared global public health emergency due to the outbreak of Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) from the city of Wuhan, China in December 2019; subsequently, so far 192 countries were affected over the world (Minah et al., 2020). Coronaviruses (CoV) belongs to the family of enveloped positive-strand RNA viruses, in which SARS-CoV-2 are the β-coronavirus and has severe infectivity and transmissibility than SARS and MERS-CoVs (Su et al., 2016; Zhu et al., 2020; Tang et al., 2020). Generally, the coronaviruses are the enveloped viruses, consist of structural proteins such as spike (S), membrane (M), envelope (E) and nucleocapsid (N) (Hossam et al., 2020; Boopathi et al., 2020). The S-spike protein of SARS-CoV-2 binds with the angiotensin-converting enzyme 2 (ACE2) with 10 fold more rapidly than SARS-CoV to facilitate further infection (Li-Sheng et al., 2020). Further, viral mRNA translates the viral polyproteins (Elfiky, 2020) and they are cleaved into functional polypeptides by SARS-CoV-2 main protease (M^{pro}) which plays an important role in the development of new viruses. Hence, targeting M^{pro} and developing the potential inhibitor will help to control the disease progression. The SARS-CoV-2 M^{pro} acts as an attractive target and the structure of viral M^{pro} contains 306 amino acids with three major domains (I-III) and 11 cleavage sites for single polyprotein 1ab. The domains I and II form a deep cleft where the substrate binding site occurs and the catalytic dyad (His41 and Cys145) present in the centre of the site (Xiaoyu et al., 2008; Linlin et al., 2020). In addition, the substrate-binding site M^{pro} has conserved amino acids that are critical for pocket formation and they are common in all other CoVs including Leu27, His41, Tyr53, Phe139, Gly142, His163, Glu166, Leu167, His172, Asp187 and Gln192 (Fenghua et al., 2016). Hence, to inhibit this viral protease, drugs should form strong interactions with the catalytic dyad and these conserved amino acids. To solve the drug crisis, the drug repurposing strategy is being adopted and it is functional in this global health emergency; the present work provides strong evidence for the drug activity and possible for drug repurposing. Currently, the anti-viral drugs lopinavir, ritonavir (HIV protease inhibition) and oseltamivir (influenza virus) are being used to treat the severe infected patients of COVID-19 (https://www.the-scientist.com/news-opinion/flu-and-anti-hiv-drugs-show efficacyagainstcoronavirus-67052; Muralidharan et al., 2020; Elmezayen et al., 2020; Khan et al., 2020; Adeoye et al., 2020). In this pandemic scenario, computational based identification of potential drug candidates against SARS-CoV-2 M^{pro} is contributing an essential role in drug designing in a short period of time (Al-Khafaji et al., 2020). Hence, several studies including synthetic (Pant et al., 2020; Joshi et al., 2020; Das et al., 2020;

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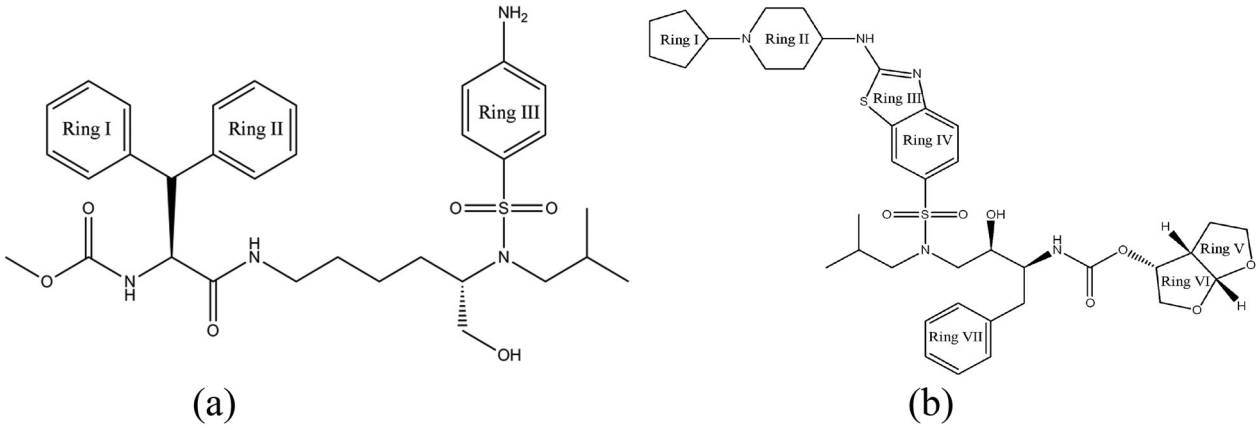

Scheme 1. Chemical structure of (a) TMB607 and (b) TMC310911 molecules.

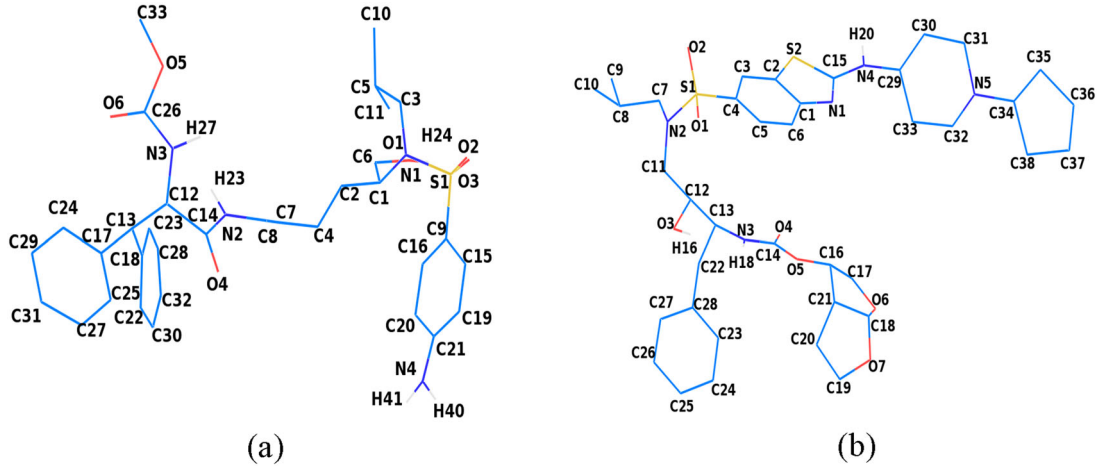

Figure 1. Molecular structure and the atom labelling of (a) TMB607 and (b) TMC310911.

Lobo-Galo et al., 2020) and plant derived (Aanouz et al., 2020; Enmozhi et al., 2020; Islam et al., 2020; Wahedi et al., 2020; Umesh et al., 2020; Kumar et al., 2020; Gyebi et al., 2020) molecules are showing better inhibition against SARS-CoV-2 M^{pro}. Also *in silico* and *in vitro* studies on HIV protease inhibitor nelfinavir shows that it exhibits remarkable inhibition activity against SARS-CoV and SARS-CoV-2 (Yamamoto et al., 2004; Bolcato et al., 2020; Yamamoto et al., 2020). The molecules lopinavir, ritonavir and nelfinavir form strong interactions with the active site amino acids including Asp25 and Asp25′of HIV-1 protease enzyme (Lv et al., 2015). Similarly, these drugs also form strong interaction with the catalytic dyad His41, Cys145 and other active site residues of the SARS-CoV-2 (Bolcato et al., 2020). Furthermore, studies on the inhibition mechanism of several inhibitors of the SARS-CoV M^{pro} (Bacha et al., 2004; Chen et al., 2005; Zhou et al., 2006) have been reported to understand the binding mechanism of inhibitors. Several research groups actively involved in searching a potential drug from the approved/clinical trial drugs to use as the repurposed drugs and are in progress. In the present study, we have chosen two clinical trial drugs (inhibitors) of HIV-1 protease (Scheme 1) to be used as the repurposed drug from the *in silico* study.

(i) un-boosted TMB607 (https://clinicaltrials.gov/ct2/show/NCT03110549) and (ii) TMC310911 boosted with ritonavir (https://clinicaltrials.gov/ct2/show/NCT00838162) shown in Figure 1.

Reportedly, these two drugs were found to be safe and well-tolerated among the healthy volunteers with no-dose limiting toxicity (Stellbrink et al., 2014; Jinzi et al., 2006). The molecular binding mechanism of these two drugs with SARS-CoV-2 M^{pro} enzyme is not yet known; to understand the same, here we have carried out the molecular docking, molecular dynamics (MD) simulations and binding free energy calculations. The detailed information about the stability, intermolecular interactions and binding affinity of these two drug molecules in the active site of the SARS-CoV-2 M^{pro} enzyme are provided from the molecular docking and MD simulations. These results are useful to evaluate the two clinical trial drugs to consider as the repurposed drugs for treatment of the devastating COVID-19 disease after *in vitro* and clinical studies. Furthermore, we also interested to understand the intermolecular interactions of TMB607 and TMC310911 with the catalytic dyad and conserved amino acids among seven coronaviruses reported in Fenghua et al. (2016), which will be helpful to identify the potential inhibitor.

2. Materials and methods

2.1. Ligand preparation and molecular docking

The geometry of molecules TMC310911 and TMB607 were optimized with B3LYP/6-311G** level of density functional

Table 1. Intermolecular interaction distances of TMB607···SARS-CoV-2 M^{pro} complex.

	Distance (Å)	
TMB607··· SARS-CoV-2 M^{pro}	Dock	MD
Hydrogen bonding interactions		
C24···NE2/His41	4.0	2.8
C33···O/Leu141	3.2	–
O6···HD21/HA/Asn142	2.4, 2.6	–
H27···SG/Cys145	3.0	–
O6···HB2/Cys145	7.6	2.5
H23···HN/Glu166	3.4	–
O5···HN/Glu166	–	2.7
O1···HE21/Gln189	2.1	–
O4···HE22/Gln189	2.7	1.9
H24, C6···O/Thr190	2.5, -	-, 3.4
O1···HN/Gln192	–	1.9
Hydrophobic interactions		
Ring II···His41 (π-π-stacked)	4.2	–
Ring I···SD/Met49 (π-orbital···Sulfur)	4.9	5.3
Ring I···SG/Cys145(π-orbital···Sulfur)	5.0	5.3
C33···His163 (alkyl···π-orbital)	4.8	4.8
C33···His172 (alkyl···π-orbital)	5.1	–

Table 2. Intermolecular interaction distances of TMC310911···SARS-CoV-2 M^{pro} complex.

	Distance (Å)	
TMC310911···SARS-CoV-2 M^{pro}	Dock	MD
Hydrogen bonding interactions		
S2···O/Phe140	2.4	–
H40···O/His164	3.5	–
H15···OE1/Glu166	2.2	–
H20···O/Glu166	–	2.0
S···OE2/Glu166	3.5	–
C17···O/Leu167	3.4	–
H45···O/Arg188	–	2.7
C32···OE1/Gln189	3.7	–
Electrostatic interaction		
O6···OE2/Glu166 (Anion···π-orbital)	3.5	–
Hydrophobic interactions		
Ring I···His41 (alkyl···π-orbital)	4.9	–
Ring I···Met49 (alkyl···π-orbital)	–	5.0
C38···Met165 (alkyl···alkyl)	4.7	5.5
C16···Pro168 (alkyl···alkyl)	4.3	4.2

theory (DFT) (Parr & Yang, 1989) using *Gaussian03* software (Frisch et al., 2004). The X-ray crystal structure of the SARS-CoV-2 M^{pro} (PDB: 6LU7) was retrieved from Protein Data Bank (PDB). The structure was optimized with OPLS_2005 force field using *protein preparation wizard* and the selected two molecules were prepared and optimized by the *Ligprep* routine of *Maestro* application incorporated in the *Schrödinger program* suit LLC (Jacobson et al., 2004). Further, an induced fit docking (IFD) was carried out for these two compounds with the SARS-CoV-2 M^{pro}. In the IFD, the standard protocol was chosen; the gird box was centred on the catalytic site residues. The van der Waals scaling of both ligands and protein was fixed at 0.5. Finally, the *extra precision* (XP) mode of IFD was performed. The intermolecular interactions and electrostatic potential map of both ligand-M^{pro} complexes were analyzed using *PyMol* (Delano, 2002) and *Discovery studio visualizer* softwares (Dassault Systems BIOVIA, 2017).

2.2. MD Simulations and binding free energy

The MD simulations of SARS-CoV-2 M^{pro}, TMC310911-SARS-CoV-2 M^{pro} and TMB607-SARS-CoV-2 M^{pro} complexes were carried out using *Sander* routine of *AMBERTOOLS14* package (Case et al., 2014) to understand the stability and the binding affinity of the two molecules in the substrate binding site of SARS-CoV-2 M^{pro} enzyme. The orthorhombic shell of TIP3P water box was generated with a minimum solute-wall at 8 Å distance and to neutralize the charges of the complex system, 4 Na^+ ions were added. Further, both complexes were minimized for the removal of steric clashes present in the complexes and annealed from 0 to 300 K for 500ps time period and equilibrated at 300 K for 500ps with the maintenance of canonical ensemble (NVT) (Glenn et al., 1999). The MD production phase was initiated and continued to 50 ns in 2fs time step at constant temperature (300 K) and pressure (1 bar) using *Langevin thermostat* and *Berendsen barostat* as in the heating process (Andrew & Ben, 2011; Berendsen et al., 1984). *VMD* (Humphrey et al., 1996) and *CPPTRAJ* software (Daniel & Cheatham, 2013) were used to analyze the MD trajectory. MM-GBSA calculation was carried out for both complexes based on the GB model (Onufriev et al., 2000).

3. Results and discussion

3.1. Molecular docking and intermolecular interactions

The glide energy of TMB607-SARS-CoV-2 M^{pro} and TMC310911-SARS-CoV-2 M^{pro} complexes obtained from the molecular docking simulation is −10.3 and −7.1 kcal/mol, respectively. The intermolecular interactions of TMB607 and TMC310911 molecules with the SARS-CoV-2 M^{pro} enzyme were identified and presented in Tables 1 and 2. The H27 atom of molecule TMB607 forms hydrogen bonding interactions with one of the catalytic dyad amino acids Cys145 with a distance of 3.0 Å. In addition, the molecule also forms π···π stacked type of hydrophobic interaction with the amino acid His41 at the distance of 4.2 Å. And TMB607 also forms hydrogen bonding interaction with Glu166 at the distance of 3.4 Å and alkyl···π-orbital type of hydrophobic interaction with His163 of catalytic dyad at the distance of 4.8 Å, the conserved amino acids of M^{pro} enzyme. In contrast to TMB607, the molecule TMC310911 only forms alkyl···π-orbital type of interaction with the amino acid His41 at the distance of 4.9 Å and hydrogen bonding interaction with the conserved amino acids Glu166 and Leu167 at the distance of 2.7 and 2.3 Å, respectively. Further, to understand the conformation, stability of these intermolecular interactions and binding affinity of both drug molecules with the M^{pro} enzyme, the MD simulation has been performed.

3.2. Evaluation of MD simulation and intermolecular interactions

The MD simulations for the SARS-CoV-2 M^{pro} (apo-enzyme), TMB607-SARS-CoV-2 M^{pro} and TMC310911-SARS-CoV-2 M^{pro} complexes have been carried out for 50 ns time period. Figure 2 shows the RMSD and RMSF values of both complexes and are found to be stable over the 50 ns simulations;

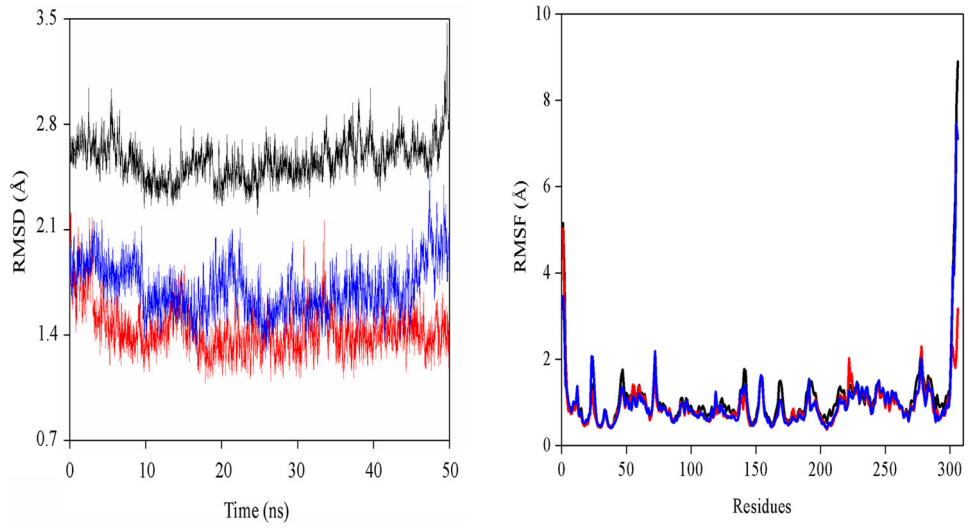

Figure 2. (a) RMSD and (b) RMSF plots of apo-protein, TMB607 and TMC310911-SARS-CoV-2 M^{pro} complexes. [Black: SARS-CoV-2 M^{pro}; Red: TMB607-SARS-CoV-2 M^{pro}; Blue: TMC310911-SARS-CoV-2 M^{pro} complexes.].

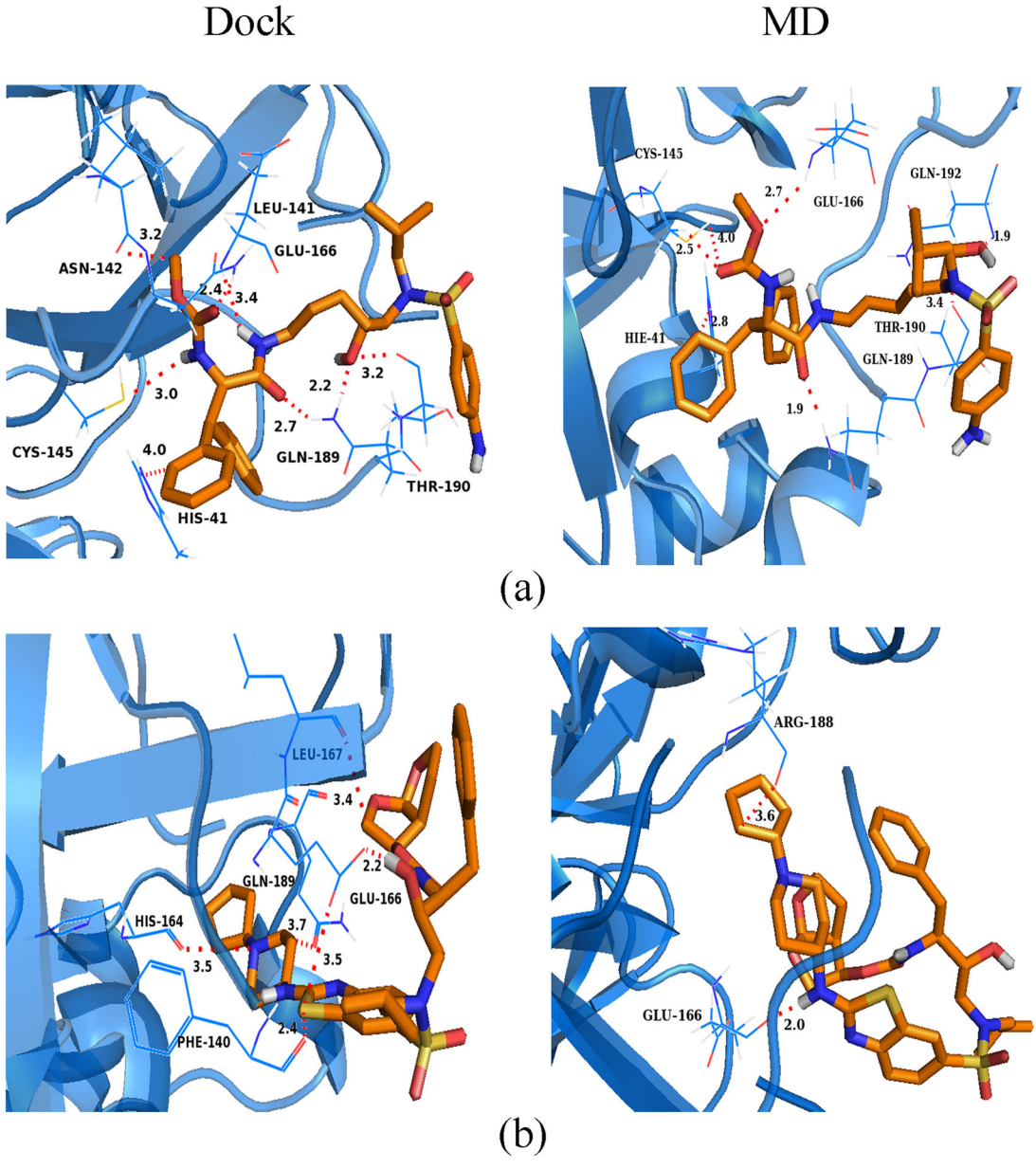

Figure 3. Intermolecular interactions of (a) TMB607-SARS-CoV-2 M^{pro} and (b) TMC310911-SARS-CoV-2 M^{pro} complexes obtained from docking and the MD simulations.

Table 3. List of catalytic dyad and conserved amino acids of SARS-CoV and SARS-CoV-2 M^{pro} interacting with various inhibitors.

	SARS-CoV-2 M^{pro}					SARS-CoV M^{pro}		
						Isatin derivatives		
Catalytic dyad & Conserved amino acids of M^{pro}	TMB607	TMC 310911	Lopinavir (Bolcato et al., 2020)	Ritonavir (Bolcato et al., 2020)	Nelfinavir (Bolcato et al., 2020)	Compound 5f (Zhou et al., 2006)	Compound 4k (Chen et al., 2005)	Compound 4o (Chen et al., 2005)
His41	His41	–	His41	His41	His41	His41	His41	His41
Cys145	Cys145	–	–	Cys145	Cys145	Cys145	Cys145	Cys145
Leu27	–	–	–	–	–	–	–	–
Tyr53	–	–	–	–	–	–	–	–
Phe139	–	–	–	–	–	–	–	–
Gly142	–	–	–	–	–	–	–	–
His163	His163	–	–	–	–	His163	–	–
Glu166	Glu166	Glu166	Glu166	Glu166	Glu166	Glu166	–	–
Leu167	Leu167	–	Leu167	–	–	–	–	–
His172	His172	–	–	–	–	His172	–	–
Asp187	–	–	Asp187	–	–	–	–	–
Gln192	Gln192	–	–	–	–	–	–	–

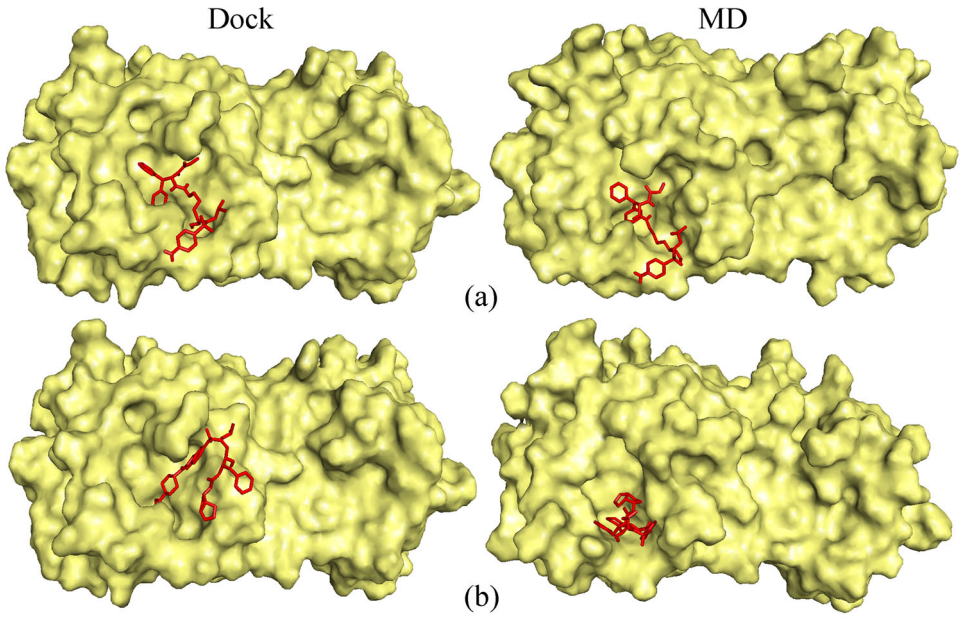

Figure 4. Connolly representation of (a) TMB607-SARS-CoV-2 M^{pro} and (b) TMC310911-SARS-CoV-2 M^{pro} complexes obtained from docking and MD simulations.

in which the RMSD values of the TMB607-SARS-CoV-2 M^{pro} complex are less (∼1.4 Å) than the TMC310911-SARS-CoV-2 M^{pro} complex (∼1.8 Å) and SARS-CoV-2 M^{pro} (∼2.4 Å) over the entire simulations; this confirms the high stability of TMB607-SARS-CoV-2 M^{pro} complex. Further, the RMSF values (Figure 2b) illustrate that the fluctuations of active site residues are found to be low for both complexes, in which notably, the RMSF values of TMB607-SARS-CoV-2 complex is lower than TMC310911-SARS-CoV-2 M^{pro} complex and SARS-CoV-2 M^{pro} (Figure 2). This difference reveals the strong interactions between TMB607 and the active site residues of SARS-CoV-2-M^{pro}.

The intermolecular interactions of the molecules TMC310911 and TMB607 with the neighbouring amino acids present in the active site of SARS-CoV-2 M^{pro} during the MD simulation are listed in Tables 1 and 2 respectively. The hydrogen bonding interactions of the complexes obtained from the docking and MD simulations are shown in Figure 3(a and b).

It is found that at the 50 ns MD simulation, the molecule TMB-607 forms strong interactions with the amino acids His41 and Cys145 of catalytic dyad, which is stronger than the same found in docked complex and the corresponding hydrogen bonding interaction distances are 2.8 and 2.5 Å, respectively. Further, the molecule also forms strong hydrogen bonding interaction with the conserved amino acids including Glu166, Gln189 and Gln192 at the distance of 2.7, 1.9 and 1.9 Å, and alkyl···π-orbital type of hydrophobic interaction with the residue His163 at the distance of 4.8 Å, respectively. Interestingly, these interactions are in correlation with the recently reported inhibitor α-ketomide (Linlin et al., 2020). The intermolecular interaction of TMB607 molecule with the M^{pro} enzyme are compared with some of the reported SARS-CoV M^{pro} enzyme inhibitors (Bacha et al., 2004; Chen et al., 2005; Zhou et al., 2006) and HIV protease as well as SARS-CoV-2 M^{pro} inhibitors including lopinavir, ritonavir and nelfinavir (Lv et al., 2015; Bolcato et al., 2020). The corresponding interactions are compared in Table 3 and the results indicate that how the molecule TMB607 is potentially interact with SARS-CoV-2 M^{pro} enzyme.

Intermolecular interactions of TMC310911-SARS-CoV-2 M^{pro} complex is shown in Figure 3. In this complex, it is found that except with Glu166, the molecule does not form

any strong interaction with the conserved amino acids present in the active site of SARS-CoV-2 M^{pro}. Furthermore, the Connolly surface representation and intermolecular interactions (Figure 3b) indicate that this molecule was unable to cover the substrate-binding site during the MD simulation; hence, the molecule is found to be less stable in the active site. These modifications can be well understood from the Connolly surface representation, which displays the position of the ligands in the active site (Figure 4).

3.3. Binding free energy

The MM-GBSA, MM-PBSA and decomposition free energy values of TMB607-SARS-CoV-2 M^{pro} and TMC310911-SARS-CoV-2 M^{pro} complexes were calculated from the MD trajectories. The MM-GBSA free energy values of both complexes are −43.7 and −34.9 kcal/mol, respectively. Contributions of various energy components to the binding free energy for TMB607 and TMC310911 with SARS-CoV-2 M^{pro} of both complexes are listed in Table 4.

The decomposition free energy values of these two complexes are plotted in Figure 5. In concurrence with the results of intermolecular interactions, the TMB607 molecule contributes low decomposition free energy with the catalytic dyad amino acids His41 (−1.4 kcal/mol) and Cys145 (−1.9 kcal/mol) and conserved residues including Leu167 (−1.3 kcal/mol), Gln189 (−4.4 kcal/mol) and Gln192 (−1.6 kcal/mol), respectively. The decomposition free energy contribution of the molecule TMC310911 with the conserved residues is quite higher than TMB607. The molecule has low binding energy contribution with the conserved amino acids Leu167 (−1.9 kcal/mol) and Gln189 (−0.97 kcal/mol). Hence, the interaction between TMB607 molecule and the active site residues (His41, Met49, Cys145, Glu189, Ala191 and Gln192) contribute for the low binding energy of TMB607-SARS-CoV-2 M^{pro} complex. Whereas in TMC310911-SARS-CoV-2 M^{pro} complex, only four amino acids (Met165, Leu167, Pro168 and Ala191) are contributing, the binding energy is higher than TMB607.

Table 4. Contributions of various energy components to the binding free energy (kcal/mol) for TMB607-SARS-CoV-2 M^{pro} and TMC310911-SARS-CoV-2 M^{pro} complexes.

Energy components	TMB607	TMC310911
ΔE_{vdw}	−60.4	−46.6
$\Delta E_{electrostatic}$	−31.1	−12.1
$\Delta G_{PB/GB}$	51.8	−28.9
ΔE_{SA}	−7.7	−5.1
ΔE_{gas}	−87.8	−58.7
ΔG_{sol}	44.1	23.8
ΔG_{Total}	−43.7	−34.9

4. Conclusion

Currently, the HIV-1 protease inhibitor combinations are being used to treat severely infected COVID-19 patients successfully. Besides this, several laboratories are involved in the preparation of repurposed drugs from the approved/clinical trial drugs. In the present study, we have chosen two clinical trial HIV-1 protease inhibitors TMB607 and TMC310911 and performed molecular docking, MD simulation and binding free energy calculations. The docking analysis shows that the molecule TMB607 forms strong interactions with the catalytic dyad residues His41 and Cys145. Further, the RMSD and RMSF values of TMB607-SARS-CoV-2 M^{pro} complex are found to be low on comparing with TMC310911-SARS-CoV-2 M^{pro}. During the MD simulation, the molecule TMB607 maintains the strong intermolecular interactions with the catalytic dyad residues His41, Cys145 and the conserved amino acid residues including Glu166, Gln189 and Gln192, which plays a crucial role in proteolytic process. Whereas, the molecule TMC310911 forms strong interaction with the conserved residue Glu166 alone and this molecule also exhibits less stability in the active site of SARS-CoV-2 M^{pro}. The MM-GBSA and decomposition binding free energy analysis also confirm that the molecule TMB607 strongly binds with the SARS-CoV-2 M^{pro}. Hence, from the present *in silico* study, we conclude that the molecule TMB607 (unboosted with ritonavir) acts as a standalone drug, will be the promising candidate to inhibit the SARS-CoV-2 M^{pro}, it may be considered as a repurposed

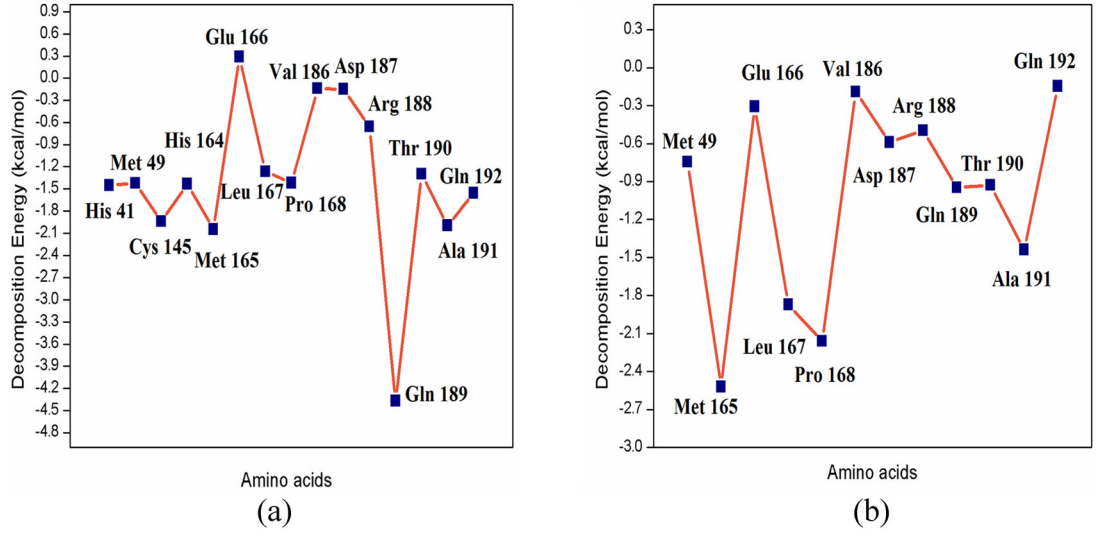

Figure 5. Decomposition free energy plots of (a) TMB607-SARS-CoV-2 M^{pro} and (b) TMC310911-SARS-CoV-2 M^{pro} complexes during the MD simulations.

drug to treat the devastating COVID-19 viral disease after in vitro and clinical studies. The results also provide key information useful for further drug designing.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

References

Aanouz, I., Belhassan, A., El-Khatabi, K., Lakhlifi, T., El-Ldrissi, M., & Bouachrine, M. (2020). Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations. *Journal of Biomolecular Structure and Dynamics, 6,* 1–9. https://doi.org/10.1080/07391102.2020.1758790

Adeoye, A. O., Oso, B. J., Olaoye, I. F., Tijjani, H., & Adebayo, A. I. (2020). Repurposing of chloroquine and some clinically approved antiviral drugs as effective therapeutics to prevent cellular entry and replication of coronavirus. *Journal of Biomolecular Structure and Dynamics,* 1–11. https://doi.org/10.1080/07391102.2020.1765876

Al-Khafaji, K., Al-Duhaidahawil, D., & Tok, T. T. (2020). Using integrated computational approaches to identify safe and rapid treatment for SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics,* 1–9. https://doi.org/10.1080/07391102.2020.1764392

Andrew, J., & Ben, L. (2011). Adaptive stochastic methods for sampling driven molecular systems. *Journal of Chemical Physics*, *135*, 084125. https://doi.org/10.1063/1.3626941

Bacha, U., Barrila, J., Velazquez-Campoy, A., Leavitt, S. A., & Freire, E. (2004). Identification of novel inhibitors of the SARS coronavirus main protease 3CL^{pro}. *Biochemistry*, *43*(17), 4906–4912. https://doi.org/10.1021/bi0361766

Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., Di Nola, A., & Haak, J. R. (1984). Molecular dynamics with coupling to an external bath. *The* Journal of Chemical Physics, *81*(8), 3684–3690. https://doi.org/10.1063/1.448118

Bolcato, G., Bissaro, M., Pavan, M., Sturlese, M., & Moro, S. (2020). Targeting the Coronavirus SARS-CoV-2: Computational insights into the mechanism of action of the protease inhibitors Lopinavir, Ritonavir and Nelfinavir. *Researchsquare*. https://doi.org/10.21203/rs.3.rs-20948/v1

Boopathi, S., Poma, A. B., & Kolandaivel, P. (2020). Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. *Journal of Biomolecular Structure and Dynamics,* 1–10. https://doi.org/10.1080/07391102.2020.1758788

Case, D. A., Babin, V., Berryman, J. T., Betz, R. M., Cai, Q., Cerutti, D. S., Cheatham, T. E., Darden, T. A., Duke, R. E., Gohlke, H., Goetz, A. W., Gusarov, S., Homeyer, N., Janowski, P., Kaus, J., Kolossváry, I., Kovalenko, A., Lee, T. S., Le Grand, S., … Kollman, P. A. (2014). *AMBER 14*. University of California.

Chen, L. R., Wang, Y. C., Lin, Y. W., Chou, S. Y., Chen, S. F., Liu, L. T., Wu, Y. T., Kuo, C. J., Chen, T. S. S., & Juang, S. H. (2005). Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *15*(12), 3058–3062. https://doi.org/10.1016/j.bmcl.2005.04.027

Daniel, R. R., & Cheatham, T. E. (2013). PTRAJ and CPPTRAJ: Software for processing and analysis of molecular dynamics trajectory data. *Journal of Chemical Theory and Computation*, *9*(7), 3084–3095. https://doi.org/10.1021/ct400341p

Das, S., Sarmah, S., Lyndem, S., & Roy, A. S. (2020). An investigation into the identification of potential inhibitors of SARS-CoV-2 main protease using molecular docking study. *Journal of Biomolecular Structure and Dynamics,* 1–11. https://doi.org/10.1080/07391102.2020.1763201

Dassault Systems BIOVIA. (2017). *Discovery studio modeling environment, release*. Dassault Systems BIOVIA.

Delano, W. L. (2002). *PyMol molecular graphics system*. Delano Scientific.

Elfiky, A. (2020). A SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: An *in silico* perspective. *Journal of Biomolecular Structure and Dynamics,* 1–9. https://doi.org/10.1080/07391102.2020.1761882

Elmezayen, A. D., Al-Obaidi, A., Şahin, A. T., & Yelekçi, K. (2020). Drug repurposing for coronavirus (COVID-19): Insilico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. *Journal of Biomolecular Structure and Dynamics,* 1–13. https://doi.org/10.1080/07391102.2020.1758791

Enmozhi, S. K., Raja, K., Sebastine, I., & Joseph, J. (2020). Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: An in silico approach. *Journal of Biomolecular Structure and Dynamics,* 1–7. https://doi.org/10.1080/07391102.2020.1760136

Fenghua, W., Cheng, C., Wenjie, T., Kailin, Y., & Haitao, Y. (2016). Structure of main protease from human coronavirus NL63: Insights for wide spectrum anti-coronavirus drug design. *Scientific Reports*, *6*, 22677. https://doi.org/10.1038/srep22677

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Montgomery, Jr., J. A., Vreven, T., Kudin, K. N., Burant, J. C., Millam, J. M., Iyengar, S. S., Tomasi, J., Barone, V., Mennucci, B., Cossi, M., Scalmani, G., Rega, N., Petersson, G. A., … Pople, J. A. (2004). Gaussian, Inc., Wallingford CT. *Gaussian 03, Revision C. 02*. Gaussian.

Glenn, J. M., Adam, H., & Tuckerman, M. E. (1999). Molecular dynamics algorithms for path integrals at constant pressure. *The* Journal of Chemical Physics, *110*(7), 3275–3290. https://doi.org/10.1063/1.478193

Gyebi, G. A., Ogunro, O. B., Adegunloye, A. P., Ogunyemi, O. M., & Afolabi, S. O. (2020). Potential Inhibitors of Coronavirus 3-Chymotrypsin-Like Protease (3CL^{pro}): An *in silico* screening of Alkaloids and Terpenoids from African medicinal plants. *Journal of Biomolecular Structure and Dynamics*, 1–13. https://doi.org/10.1080/07391102.2020.1764868

Hossam, M. A., Walid, F E M., Masudur, R., & Hatem, A E. (2020). Insights into the recent 2019 novel coronavirus (SARS-CoV-2) in light of past human coronavirus outbreaks. *Pathogens*, *9*(186), 1–15. https://doi.org/10.3390/pathogens9030186

https://clinicaltrials.gov/ct2/show/NCT00838162.

https://clinicaltrials.gov/ct2/show/NCT03110549.

https://www.the-scientist.com/news-opinion/flu-and-anti-hiv-drugs-show-efficacyagainstcoronavirus-67052.

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD-Visual Molecular Dynamics. *Journal of Molecular Graphics*, *14*(1), 33–38. https://doi.org/10.1016/0263-7855(96)00018-5

Islam, R., Parves, M. R., Paul, A. S., Uddin, N., Rahman, M. S., Mamun, A. A., Hossain, M. N., Ali, M. A., & Halim, M. A. (2020). A. molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics,* 1–12. https://doi.org/10.1080/07391102.2020.1761883

Jacobson, M. P., Pincus, D. L., Rapp, C. S., Day, T. J. F., Honig, B., Shaw, D. E., & Friesner, R. A. (2004). A hierarchical approach to all-atom protein loop prediction. *Proteins*, *55*(2), 351–367. https://doi.org/10.1002/prot.10613

Jinzi, J., Wu, B. R. S., Milot, G., Ge, M., Dandache, S., Forte, A., Pelletier, I., Dubois, A., Belanger, O., & Panchal, C. (2006, September 27–30). Pl-100, a next generation protease inhibitor against drug-resistant HIV: In vitro & in vivo metabolism. In *46th Annual ICAAC Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francosco, CA.

Joshi, R. S., Jagdale, S. S., Bansode, S. B., Shankar, S. S., Tellis, M. B., Pandya, V. K., Chugh, A., Giri, A. P., & Kulkarni, M. J. (2020). Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. *Journal of Biomolecular Structure and Dynamics,* 1–16. https://doi.org/10.1080/07391102.2020.1760137

Khan, R. J., Jha, R. K., Amera, G. M., Jain, M., Singh, E., Pathak, A., Singh, R. P., Muthukumaran, J., & Singh, A. K. (2020). Targeting SARS-CoV-2: A systematic drug repurposing approach to identify promising inhibitors against 3C-like proteinase and 2′-O-ribose methyltransferase.

Journal of Biomolecular Structure and Dynamics, 1–14. https://doi.org/10.1080/07391102.2020.1753577

Khan, S. A., Zia, K., Uddin, S. A. R., & Ul-Haq, Z. (2020). Identification of chymotrypsin-like protease inhibitors of SARS-CoV-2 *via* integrated computational approach. *Journal of Biomolecular Structure and Dynamics,* 1–10. https://doi.org/10.1080/07391102.2020.1751298

Kumar, D., Kumari, K., Jayaraj, A., Kumar, V., Kumar, R. V., Dass, S. K., Chandra, R., & Singh, P. (2020). Understanding the binding affinity of noscapines with protease of SARS-CoV-2 for COVID-19 using MD simulations at different temperatures. *Journal of Biomolecular Structure and Dynamics,* 1–14. https://doi.org/10.1080/07391102.2020.1752310

Linlin, Z., Daizong, L., Xinyuanyuan, S., Ute, C., Christian, D., Lucie, S., Stephan, B., Katharina, R., & Rolf, H. (2020). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science, 368* (6489), 409–412. https://doi.org/10.1126/science.abb3405.

Li-Sheng, W., Yi-Ru, W., Da-Wei, Y., & Qing-Quan, L. (2020). A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. *International Journal of Antimicrobial Agents, 55* (6), 105948. https://doi.org/10.1016/j.ijantimicag.2020

Lobo-Galo, N., Terrazas-López, M., Martínez-Martínez, A., & Díaz-Sánchez, A. G. (2020). FDA-approved thiol-reacting drugs that potentially bind into the SARS-CoV-2 main protease, essential for viral replication. *Journal of Biomolecular Structure and Dynamics,* 1–9. https://doi.org/10.1080/07391102.2020.1764393

Lv, Z., Chu, Y., & Wang, Y. (2015). HIV protease inhibitors: A review of molecularselectivity and toxicity. *HIV/AIDS - Research and Palliative Care*, *7*, 95–104.

Minah, P., Alex, R. C., Jue, T. L., Yinxiaohe, S., & Borame, L. D. (2020). A Systematic Review of COVID-19 Epidemiology Based on Current Evidence. *Journal of Clinical Medicine*, *9*, 967. https://doi.org/10.3390/jcm9040967

Muralidharan, N., Sakthivel, R., Velmurugan, D., & Gromiha, M. M. (2020). Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. *Journal of Biomolecular Structure and Dynamics,* 1–6. https://doi.org/10.1080/07391102.2020.1752802

Onufriev, A., Bashford, D., & Case, D. A. (2000). Modification of the generalized born model suitable for macromolecules. *The Journal of Physical Chemistry B*, *104*(15), 3712–3720. https://doi.org/10.1021/jp994072s

Pant, S., Singh, M., Ravichandiran, V., Murty, U. S. N., & Srivastava, H. K. (2020). Peptide-like and small-molecule inhibitors against Covid-19. *Journal of Biomolecular Structure and Dynamics,* 1–10. https://doi.org/10.1080/07391102.2020.1757510

Parr, R. G., & Yang, W. (1989). *Density functional theory of atoms and molecules*. Oxford.

Stellbrink, H. J., Arasteh, K., Schürmann, D., Stephan, C., Dierynck, I., Smyej, I., Hoetelmans, R. M. W., Truyers, C., Meyvisch, P., Jacquemyn, B., Mariën, K., Simmen, K., & Verloes, R. (2014). Antiviral activity, pharmacokinetics, and safety of the HIV-1 Protease Inhibitor TMC310911, coadministered with ritonavir, in treatment-naive HIV-1-infected patients). *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *65*(3), 283–289. https://doi.org/10.1097/QAI.0000000000000003

Su, S., Wong, G., Shi, W., Liu, J., Lai, A. C. K., Zhou, J., Liu, W., Bi, Y., & Gao, G., F. (2016). Epidemiology, genetic recombination, and pathogenesis of coronaviruses. *Trends in Microbiology*, *24*(6), 490–502. https://doi.org/10.1016/j.tim.2016.03.003

Tang, B., Bragazzi, N. L., Li, Q., Tang, S., Xiao, Y., & Wu, J. (2020). An updated estimation of the risk of transmission of the novel coronavirus (2019-nCov). *Infectious Disease Modelling*, *5*, 248–255. https://doi.org/10.1016/j.idm.2020.02.001

Umesh, K., D., Selvaraj, C., Singh, S. K., & Dubey, V. K. (2020). Identification of new anti-nCoV drug chemical compounds from Indian spices exploiting SARS-CoV-2 main protease as target. *Journal of Biomolecular Structure and Dynamics,* 1–9. https://doi.org/10.1080/07391102.2020.1763202

Wahedi, H. M., Ahmad, S., & Abbasi, S. W. (2020). Stilbene-based natural compounds as promising drug candidates against COVID-19. *Journal of Biomolecular Structure and Dynamics,* 1–10. https://doi.org/10.1080/07391102.2020.1762743

Xiaoyu, X., Hongwei, Y., Haitao, Y., Fei, X., Zhixin, W., Wei, S., Jun, L., Zhe, Z., Yi, D., Qi, Z., Xuejun, C. Z., Ming, L., Mark, B., & Zihe, R. (2008). Structures of two coronavirus main proteases: Implications for substrate binding and antiviral drug design. *Journal of Virology*, *82*(5), 2515–2527. https://doi.org/10.1128/JVI.02114-07

Yamamoto, N., Matsuyama, S., & Hoshino, T. (2020). Nelfinavir inhibits replication of severe acute respiratory syndrome coronavirus 2 in vitro. *bioRxiv*. https://doi.org/10.1101/2020.04.06.026476

Yamamoto, N., Yang, R., Yoshinaka, Y., Amari, S., Nakano, T., Cinatl, J., Rabenau, H., Doerr, H. W., Hunsmann, G., Otaka, A., Tamamura, H., Fujii, N., & Yamamoto, N. (2004). HIV protease inhibitor nelfinavir inhibits replication of SARS-associated coronavirus. *Biochemical and Biophysical Research Communications*, *318*(3), 719–725.

Zhou, L., Liu, Y., Zhang, W., Wei, P., Huang, C., Pei, J., Yuan, Y., & Lai, L. (2006). Isatin compounds as noncovalent SARS coronavirus 3C-like protease inhibitors. *Journal of Medicinal Chemistry*, *49*(12), 3440–3443. https://doi.org/10.1021/jm0602357

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G. F., & Tan, W. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *The New England Journal of Medicine*, *382*(8), 727–733. https://doi.org/10.1056/NEJMoa2001017