Exploring the effect of ritonavir and TMC-310911 on SARS-CoV-2 and SARS-CoV main proteases: potential from a molecular perspective

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Materials & methods: We used an integrated computational algorithm to explore the binding mechanism of TMC-310911/ritonavir (RVT) with SARS-CoV-2 and SARS-CoV main proteases. Results: RVT and TMC-310911 had favorable interactions with the proteases, and these high interactions are facilitated by some significant residues such as Asn133, Gly195 and Gln192. Our study further implicated two important rings in the structure of RVT as a possible chemical culprit in its therapeutic activity. Conclusion: Although there are conflicting clinical results on the therapeutic potency of RVT in the treatment of coronavirus disease 2019, our findings provided molecular insight into the binding mechanism of TMC-310911 and RVT with SARS-CoV-2 and SARS-CoV main proteases.

Lay abstract: As coronavirus (CoV) disease 2019 spreads, there is an urgent need to develop drugs that can halt the spread, manage and perhaps cure this disease. We repurposed two antiretroviral drugs that target the same protein as SARS-CoV-2 (the virus that causes coronavirus disease 2019). Our findings revealed that these two drugs demonstrated favorable activity on SARS-CoV-2 protein.
been reported to have helped patients overcome the infection through boosting their immune system [8]; however, currently, no vaccines or drugs are available for the treatment of this disease [11]. The global spread of COVID-19 and the absence of vaccines or drugs have necessitated the demand of drug repurposing. Crucial proteins that have gained attention in the development of potential COVID-19 drugs include SARS-CoV-2 main proteases (COV), RNA-dependent RNA polymerase, RNA binding N terminal domain of nucleocapsid protein, viral ion channel, 2′O-ribosemethyltransferase and human angiotensin-converting enzyme 2 receptor [12–14]. Several existing drugs such as remdesivir, chloroquine, hydroxychloroquine, camostatmesylate, lopinavir, etc., have been considered for targeting different phases of the virus life cycle.

Ritonavir (RVT) is an antiretroviral drug used in the treatment of advanced HIV, it is used as a protease inhibitor and also used to boost other protease inhibitors [15]. It is sometimes used in combination with antiretroviral drugs such as nucleotide reverse transcriptase inhibitor or nucleoside [15,16]. An in vivo study found a reduced risk of mortality and hypoxia in 41 SARS-CoV patients who were administered a combination of lopinavir/RVT and ribavirin, when compared with controls administered ribavirin alone [17,18]. A different study from Korea revealed that there was a significant reduction in CoV titers upon lopinavir-RVT administration [19]. Contradictory, a combination of lopinavir and RVT was investigated in a controlled trial study, patients with COVID-19 were administered either lopinavir-RVT 400/100 mg orally twice daily plus standard of care, or standard of care alone, there was no therapeutic benefit observed upon administration [20]. There are at least three randomized clinical trials currently been carried out to determine the therapeutic efficacy of a combination of lopinavir and RVT [21]. Similarly, ASC-09 also referred to as TMC-310911 is a protease inhibitor that is still under clinical studies to treat HIV infection. It has shown in vitro activity against the HIV strains that have developed resistance to other protease inhibitors [22]. There is currently an ongoing clinical trial that seeks to evaluate the safety and efficiency of a combination of ASC09/RVT on CoV infection (clinicaltrials.gov/ct2/show/NCT04261907) [23].

In this study, we repurposed two existing HIV-protease inhibitors RVT and TMC-310911 (ASC09) to target SARS-CoV-2 main protease (COV) and SARS-CoV main protease (SARS) to explore their possible mode of inhibition.

**Structure preparation & dynamic studies**

The starting structures of COV and SARS were obtained from Protein Data Bank with PDB ID 6LU7 [24] and 5N19 [25], respectively. Cocrystallized molecules identified with the proteins were deleted and the addition of missing residues was performed using modeler [26]. RVT and TMC-310911 were retrieved from PubChem, the 2D structures were converted to 3D structures and optimized using B3LYP/6-311++G(d,p) [27] level of Gaussian16 [28]. The final structures were saved as Mol2 files. Molecular docking of the proteases with the inhibitors was carried out using AutoDock Vina [29] inbuilt in UCSF chimera [30]. AutoDock Vina is software developed by O’Trott of Molecular Graphics Laboratory at the Scripps Research Institute [29]. Validation of the docking results was carried out by redocking multiple times. COV and SARS were prepared for docking by the removal of water and cocrystallized ligands. Hydrogens were added and optimization of the hydrogen-bonding network was carried out with the aid of Avogadro software [31]. The clean COV and SARS structures were saved for docking. During the docking process, we allotted partial chargers called Gasteiger to the ligands. The AutoDock [32] GUI provided by the Molecular Graphics Laboratory tool was utilized to define the AutoDock atomic types [33]. The defined parameter grid dimensions used in docking TMC-310911 with COV and SARS were center (x = -17.56, Y = 0.29 and Z = -21.46) and size (X = 14.21, Y = 16.06 and Z = 13.19), while center (x = -17.19, Y = 0.44 and Z = -21.95) and size (X = 14.21, Y = 16.58 and Z = 11.86) were used for docking RVT with COV and SARS. The remaining values were set to default. Molecular dynamic simulation run was carried out with the aid of Amber 19 [34] software using the FF14SB force field [35]. The general Amber force field and restrained electrostatic potential were used in describing the atomic charges of RVT and TMC-310911. Leap variant present in Amber 19 was used for system neutralization and hydrogen atoms addition [36]. The system was solvated with an orthorhombic box of TIP3P water molecules surrounding all protein atoms at a distance of 9 Å [27]. System minimization was carried out first with a 2000 step minimization utilizing a restraint potential of 500 kcal/mol. Second, we used a 10,000-step full minimization process without restraint. Afterwards, the system was gradually heated at a temperature of 0–300 k at 50 ps. The system solutes are kept at a potential harmonic restraint of 10 kcal mol⁻¹ Å⁻² and collision frequency of 1.0 ps⁻¹. Next, the equilibration of 500 ps was carried out. The temperature and pressure were kept constant at 300 k and 1 bar (isobaric-isothermal ensemble, constant temperature and pressure using Berendsen barostat).
Each step of the simulation was run for 2 fs and an single-precision floating-point precision model was adopted. The simulations were kept at constant temperature and pressure, and Langevin thermostat at collision frequency of 1.0 ps\(^{-2}\). Six systems were set up for molecular dynamics (MD) simulations. These systems are unbound COV, COV bound to TMC-310911 (COV\_TMC), COV bound to RVT (COV\_RVT), unbound SARS, SARS bound to TMC-310911 (SARS\_TMC) and SARS bound to RVT (SARS\_RVT). PTRAJ variant of Amber 19 was adopted for further analysis which included root mean square deviation (RMSD) and radius of gyration (RoG) [37]. The data plots were then made with ORIGIN analytical tool and visualization was done using UCSF Chimera [38].

### Results

**Mechanism of inhibition of ritonavir & TMC-310911 on SARS-CoV-2 & SARS CoV main proteases**

RVT and TMC-310911 (TMC) like any other HIV protease inhibitors work by inhibiting proteases found in the liver, intestine and other places [40,41]. The binding of RVT to the active site of HIV proteases prevents cleavage of the viral polyproteins subsequently leading to inactive and noninfectious viral components [15]. Similarly, TMC-310911 works following this pharmacokinetic and pharmacodynamic route [22]. Therefore, we explored the mechanism of inhibition and structural perturbative effect of RVT and TMC-310911 binding to COV and SARS. MM/PBSA has been widely employed in the drug development and computer-aided drug designs space, it estimates the binding strength between an inhibitor and a protein [42,43]. COV\_RVT, SARS\_RVT, COV\_TMC and SARS\_TMC had binding free energy of -29.46, -32.34, -32.29 and -47.19 kcal/mol, respectively. Findings from this analysis revealed that the RVT and TMC-310911 had a favorable binding interaction with the COV and SARS (Table 1).

### Table 1. Calculated binding free energy (kcal/mol) of the studied complexes.

| Complexes       | Δ\( G_{\text{bind}} \) (±SEM) | ΔG\text{ele,sol} (±SEM) | ΔG\text{complex, sol} (±SEM) | ΔG\text{gas} (±SEM) | ΔE\text{int} (±SEM) |
|-----------------|-------------------------------|------------------------|-----------------------------|-------------------|---------------------|
| COV\_RVT       | -40.90 (±0.18)                | -35.64 (±0.58)         | -5.26 (±0.81)               | -5.39 (±0.05)     | -18.82 (±0.55)      |
| SARS\_RVT      | -49.71 (±0.33)                | -40.35 (±0.65)         | -9.35 (±0.84)               | -6.16 (±0.04)     | -16.83 (±0.61)      |
| COV\_TMC       | -42.41 (±0.39)                | -41.21 (±0.16)         | -18.5 (±1.44)               | -5.25 (±0.04)     | -15.80 (±0.01)      |
| SARS\_TMC      | -55.38 (±0.52)                | -14.95 (±1.04)         | -20.49 (±1.38)              | -6.51 (±0.06)     | 16.43 (±1.07)       |

\( \Delta E_{\text{ele}} \): Electrostatic energy; \( \Delta E_{\text{vdW}} \): Van der Waals energy; \( \Delta G_{\text{ele,sol}} \): Desolvation energy; \( \Delta G_{\text{gas}} \): Total gas-phase energy; \( \Delta G_{\text{complex, sol}} \): Nonpolar desolvation energy; \( \Delta G_{\text{gas}} \): Solvation energy; COV: Coronavirus; COV\_RVT: SARS-CoV-2 main protease bound to RVT; COV\_TMC: SARS-CoV-2 main protease bound to TMC-310911; RVT: Ritonavir; SARS\_RVT: SARS-CoV main protease bound to RVT; SARS\_TMC: SARS-CoV main protease bound to TMC-310911; SEM: Standard error of the mean.

## Future Directions

Further studies are needed to validate these findings in vitro and in vivo models. Additionally, exploring the potential for combination therapy involving ritonavir and TMC-310911 with other antiviral drugs could provide insights into efficacy and pharmacokinetic interactions. Understanding the specific binding modes and structural changes induced by these inhibitors may also guide the development of next-generation antiviral drugs.
Furthermore, from Table 1, it is observed that the various free energy components facilitated this strong interaction. Most especially the electrostatic and van der Waals interactions observed in the gas phase of COV-TMC and SARS-TMC.

We used a representative snapshot and active site residue decomposition to further explore the time-wise bond interaction occurring between major residues in the active site of COV/SARS and TMC-310911/RVT. In the COV_RVT system, Asn133, Gly195 and Gln192 elicited hydrogen bond interactions, Phe185 formed π-sulphur interaction with the S atom present in RVT ring (Figure 1A). The decomposition of this interaction also showed that favorable energy was contributed by residues possessing energy contributions greater than -0.5 kcal/mol. Ala194, which formed a π-alkyl bond with RVT, contributed the highest total, van der Waal, and electrostatic energies to the overall binding (Figure 1B). Decomposing the active site residues of the SARS_RVT system revealed that Met49 and Gln189 contributed the most to the overall binding of RVT to SARS. In comparison to the COV_RVT system, SARS_RVT had higher ΔG_{bind}, this could be as a result of the high sigma bond formed by
Exploring the effect of ritonavir & TMC-310911 on SARS-CoV-2 & SARS-CoV main proteases

Pro168, Met49 and Cys145 (Figure 1C). Binding of TMC-310911 to COV and SARS demonstrated an overall favorable binding when compared with RVT binding. This is evident by the high energy decomposition of the individual active site residues. Asn142 and Asp187 elicited a high hydrogen bond, while Ser46, Thr45 and Met 49 formed a pi-alkyl bond with TMC-310911 (Figure 2).

To further explore the possible mechanism of the action of RVT on SARS-CoV-2 main protease, we examined the interaction trend of two important rings in RVT in the course of the simulation run. Interestingly, these two rings formed strong interaction with residues embedded inside the active site. These interactions sequestered RVT deeper into the active site (Figure 3).
COV & SARS perturbative effect upon ritonavir & TMC-310911 binding

To understand the structural perturbation of COV and SARS upon RVT and TMC-310911 binding, we used RMSD and RoG to characterize the structural events in the proteins in the course of the simulation. In the course of the 200 ns simulation run, the six systems attained structural stability early in the simulation run with COV, COV\_TMC, COV\_RVT, SARS, SARS\_TMC and SARS\_RVT having an average RMSD value of 3.43, 3.34, 2.77, 1.85, 2.56 and 2.00\(\text{Å}\), respectively (Figure 4A). The average RMSF value of COV, COV\_TMC, COV\_RVT, SARS, SARS\_TMC and SARS\_RVT are 1.19, 1.19, 1.06, 1.50, 1.57 and 1.77\(\text{Å}\), respectively. Likewise, COV, COV\_TMC, COV\_RVT, SARS, SARS\_TMC and SARS\_RVT had average RoG values of 22.53, 21.89, 21.92, 22.41, 21.85 and 22.42\(\text{Å}\), respectively (Figure 4B).

Discussion

Sequence similarity is described as a measure of the empirical relationship that exists between sequences, it establishes the propensity of sequences evolving from a common ancestor. Joshi et al. have demonstrated that COV has higher sequence similarity with SARS when compared with MERS-CoV main protease [44]. In this study, RVT and TMC-310911 were repurposed to bind with COV and SARS. Although there are conflicting results on the therapeutic effectiveness of RVT and TMC-310911 in the treatment of COVID-19. For instance, in a clinical trial study, the combination of lopinavir and RVT was investigated in a controlled trial study, patients with COVID-19 were administered either lopinavir-RVT 400/100 mg orally twice daily plus standard of care or standard of care alone, there was no therapeutic benefit observed upon administration [20]. RMSD is a commonly used quantitative parameter employed to estimate the similarity between two superimposed structures. RMSD can be computed for different components of a biomolecule. In MD simulation, the RMSD is often calculated for the \(\text{Ca}\) of the entire protein structure, for example, those found in the loop, active site and perhaps transmembrane helices. Many types of researches have used RMSD as a measure of protein stability and equilibration. RMSF is defined as the measure of the atomic displacement of a single or a group of atoms relative to the starting or reference structures, averaged over the number of atoms [45]. RoG is a function used to define the distribution of atoms of a protein around its axis. The most significant parameter used in the prediction of protein compactness is RoG [46]. Insight from RMSD, RMSF
and RoG revealed the dynamical events occurring upon TMC-310911 and RVT binding to COV and SARS. The binding score derived from docking of RVT and TMC-310911 to COV and SARS revealed that the COV-RVT had a binding score of -7.09 kcal/mol, SARS_RVT, COV_TMC and SARS_TMC had -8.12, -8.00 and -9.09 kcal/mol, respectively. Similarly, an increasing trend was observed in the binding free energy of the systems as computed by MM/PBSA. COV_RVT, SARS_RVT, COV_TMC and SARS_TMC had binding free energy of -29.46, -32.34, -32.29 and -47.19 kcal/mol, respectively. Nukoolkarn et al. used MD simulation to explore the binding of RVT to SARS-CoV protease, from their study, the total binding energy of -45.3 kcal/mol was observed [47]. Upon binding of RVT to SARS-CoV-2 main protease, the structural compactness of the COV_TMC, COV_RVT system were lesser than that of the COV system, similar trend was observed in the SARS_TMC and SARS_RVT systems.
Conclusion
Presently, there is no available clinical antiviral compound (or drug) with therapeutic evidence that cures COVID-19 and several strategies are being considered to treat this disease, including repurposing drugs that are active against SARS-CoV and MERS-CoV. Other strategies such as inhibitors of viral and host protease, host-directed therapies and the use of antibodies are being currently explored. In this study, we explored the inhibitory effect of RVT and TMC-310911 on COV and SARS. Results from this study revealed that RVT and TMC-310911 had strong binding interaction with COV and SARS. Two rings were found to be crucial to the binding of RVT to COV and SARS. These two rings could be further explored in the development of inhibitors specifically tailored to target SARS-CoV-2 proteases.

Future perspective
We employed integrated computational algorithms and force field protein–ligand dynamics calculations to repurpose and explore the possible mechanism of inhibition of RVT and TMC-310911 upon binding to COV and SARS. Findings from this study can facilitate a new frontier in structure-based design of highly effective and tailored inhibitors of SARS-CoV-2 main protease in the treatment of CoV infections.

Summary points
- Upon ritonavir (RVT) binding to SARS coronavirus 2 (SARS-CoV-2) main protease (COV), Asn133, Gly195 and Gln192 elicited hydrogen bond interactions, while Phe185 formed π-sulphur interaction with the S atom present in the RVT ring.
- RVT and TMC-310911 had strong interactions with the proteases, and these high interactions are facilitated by some significant residues found in their active site.
- Despite having similar residues in their active sites, COV elicited ligand interactions with dissimilar residues as SARS-CoV protease.
- Two rings were found to be crucial to the binding of RVT to COV and SARS-CoV main proteases.

Author contributions
OS Soremekun conceived the idea. OS Soremekun, KF Omolabi and AT Adewumi carried out the experiment. OS Soremekun and KF Omolabi contributed to writing the manuscript. MES Soliman proofread the manuscript and approved the research.

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Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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