Abiraterone Acetate Attenuates SARS-CoV-2 Replication by Interfering with the Structural Nucleocapsid Protein

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Abstract
The drug repurposing strategy has been applied to the development of emergency COVID-19 therapeutic medicines. Current drug repurposing approaches have been directed against RNA polymerases and viral proteases. Recently, we found that the inhibition of the interaction between the SARS-CoV-2 structural nucleocapsid (N) and spike (S) proteins decreased viral replication. In this study, drug repurposing candidates were screened by in silico molecular docking simulation with the SARS-CoV-2 structural N protein. In the ChEMBL database, 1994 FDA-approved drugs were selected for the in silico virtual screening against the N terminal domain (NTD) of the SARS-CoV-2 N protein. The tyrosine 109 residue in the NTD of the N protein was used as the center of the ligand binding grid for the docking simulation. In plaque forming assays performed with SARS-CoV-2 infected Vero E6 cells, atovaquone, abiraterone acetate, and digoxin exhibited a tendency to reduce the size of the viral plaques without affecting the plaque numbers. Abiraterone acetate significantly decreased the accumulation of viral particles in the cell culture supernatants in a concentration-dependent manner. In addition, abiraterone acetate significantly decreased the production of N protein and S protein in the SARS-CoV-2-infected Vero E6 cells. In conclusion, abiraterone acetate has therapeutic potential to inhibit the viral replication of SARS-CoV-2.

Key Words: Abiraterone acetate, Docking simulation, Drug repurposing, Nucleocapsid protein, SARS-CoV-2, Virtual screening

INTRODUCTION
Since infection with severe acute respiratory syndrome CoV-2 (SARS-CoV-2) was first reported in Wuhan City, China in December 2019 (Wu et al., 2020; Zhou et al., 2020a), the pandemic coronavirus disease 2019 (COVID-19) has caused serious challenges for the public health and medical systems worldwide (https://covid19.who.int/). SARS-CoV-2 is an enveloped virus with a plus-stranded RNA genome (varies from 29.8 kb to 29.9 kb) and belongs to lineage B beta-coronaviruses (Khailany et al., 2020). SARS-CoV-2 maintains its structure with four major structural protein complexes: spike (S) glycoprotein, nucleocapsid (N) phosphoprotein, membrane (M) glycoprotein and small envelope (E) glycoprotein (Satarker and Nampoothiri, 2020). It is known that angiotensin-converting enzyme 2 (ACE2) is a receptor for SARS-CoV-2 S protein for mediating viral attachment/membrane fusion and that transmembrane protease serine 2 (TMPRSS2) contributes to viral entry into host cells (Datta et al., 2020; Matsuyama et al., 2020; Zeidler and Karpinski 2020). In this regard, the therapeutic strategy against SARS-CoV-2 infection targeting the entry process is directed towards the development of neu-
tralizing antibodies, fusion inhibitors, and protease inhibitors (Hoffmann et al., 2020; Jiang et al., 2020; Kandeel et al., 2021).

The N protein is essential to the viral RNA packaging and modulation of cellular responses as a multifunctional protein (Perdikari et al., 2020). The large RNA genome of SARS-CoV-2 is associated with the N protein to form a helical nucleocapsid (Perdikari et al., 2020). The N protein consists of three segments containing two structural domains: the N-terminal domain (NTD), C-terminal domain (CTD), and an intrinsically disordered central Ser/Arg (SR)-rich linker (LKR) (Fig. 1). The NTD of the SARS-CoV-2 N protein interacts with the viral RNA by a hydrogen-bond-forming moiety at the base recognition site. Unlike the NTD of the human coronavirus OC43 (HCoV-OC43) N protein, the NTD of the SARS-CoV-2 N protein has a unique structure that evades steric hindrance at the binding site for a branching phosphate group to maintain a high affinity with the RNA genome (Kang et al., 2020). The CTD of the SARS-CoV-2 N protein forms a dimer and has a higher affinity for the first seven ribonucleotides of the SARS-CoV-2 genome compared to previously known affinities of N protein CTDs of other coronaviruses to longer ssRNA or ssDNA (Zhou et al., 2020b; Zinzula et al., 2020). Recently, we reported a direct interaction between the S and N proteins of MERS-CoV and SARS-CoV-2. The CTD of the S protein interacts with the N protein in MERS-CoV-infected, SARS-CoV-2-infected cells (Kim et al., 2021b; Park et al., 2021b) and HCoV-OC43-infected cells (Kim et al., 2022). Taken together, the N protein of SARS-CoV-2 has gained attention as a target to inhibit the replication of SARS-CoV-2.

Drug repurposing is one of the effective strategies when the development of novel therapeutics is emergent, especially in a pandemic situation. The SARS-CoV-2 N protein is an RNA-binding protein which is essential for both viral RNA replication and viral RNA genome packaging by forming helical ribonucleoproteins (Wu et al., 2020). In this study, the drug repurposing strategy was applied to screen therapeutic drugs to inhibit the interaction between the NTD of the SARS-CoV2 N protein and the SARS-CoV-2 genomic RNA by computational docking simulation. The anti-viral activity of the drug repurposing candidates was evaluated with plaque reduction assays using SARS-CoV-2 infected cells.

**MATERIALS AND METHODS**

**Computational docking simulation of the SARS-CoV-2 N protein NTD**

The protein structures for the molecular docking simulation were obtained from the Research Collaboratory for Structural Bioinformatics Protein Database (RCSB PDB). PDB entry codes for the NTD of the SARS-CoV2 N protein were 6M3M, 6VYO, 6WKP, and 7CDZ. The assembly modeling for the protein structure prediction with AlphaFold was performed by the web-based server exploiting ColabFold (Jumper et al., 2021). For the docking simulation, monomeric forms were isolated from multimeric NTD structures. The monomeric NTD structure was constructed based on the public cryo-electron-tomography model of SARS-CoV-2 ribonucleoproteins (EMD-30429) composed of five N protein dimers, each fitted into head-to-tail reverse L-shaped densities (Yao et al., 2020). All monomers were further processed for the docking simulation using the PyMol software (Schrödinger, LLC, New York, NY, USA) and MGLTools (The Scripps Research Institute, La Jolla, CA, USA). The chemical library for the US FDA-approved drugs was constructed as SMILES strings from the ChEMBL Database (https://www.ebi.ac.uk/chembl/) using selection filters to search for phase 3 approved and phase 4 drugs. Constructed SMILES strings were further processed to remove metals and inorganic components in the drugs. After excluding duplicated compounds, 1994 SMILES strings were obtained for the dock-

![Fig. 1. The ligand binding site of SARS-CoV-2 N protein for the computational docking simulation.](https://doi.org/10.4062/biomolther.2022.037)
ing simulations with the N protein NTD monomeric structures. SMILES strings were converted into the three-dimensional (3D) PDB file format for the docking simulations using the OpenBabel software and MGLTools. The molecular modeling simulation was performed using Autodock Vina version 1.1.2 (The Scripps Research Institute). Free energy-minimized docking simulations were performed, and ligand-binding modes were visualized using PyMOL and BIOVIA Discovery Studio (Dassault Systèmes, Velizy-Villacoublay, France).

Cell culture and virus amplification
African green monkey kidney Vero E6 cells and human airway epithelial Calu-3 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Vero E6 cells and Calu-3 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific), 25 mM HEPES, 100 U/mL penicillin, and 100 µg/mL streptomycin in a 5% CO₂ incubator at 37°C. SARS-CoV-2 (hCoV-19/South Korea/KCDC03/2020, NCCP43326) was provided by the National Culture Collection for Pathogens (Osong, Korea). The virus amplification was performed as described elsewhere (Park et al., 2019; Kandeel et al., 2020). To amplify the virus, 2×10⁶ Vero E6 cells (6-well plates, Corning, NY, USA) were cultured in a CO₂ incubator overnight. The 6-well plates were washed with phosphate-buffered saline (PBS), and SARS-CoV-2 in PBS at a MOI of 0.01 was inoculated into each well. Then, the cells were incubated for 1 h with shaking at 15-20 min intervals in a CO₂ incubator. After infection, the suspensions were removed, and DMEM containing 2% FBS (2 mL) was added to each well, and the plates were incubated at 37°C in a CO₂ incubator. After 3 days of cultivation, the cell culture supernatants were harvested, and cell debris was removed by centrifugation at 2,000 rpm for 10 min at 4°C. The virus titer was quantified by plaque assay, and aliquots (5×10⁶ plaque forming unit (pfu)/mL) of the virus were stored at −70°C. SARS-CoV-2 amplification and cell culture procedures were performed according to biosafety level 3 (BSL-3) conditions in the Hallym Clinical and Translational Science Institute in accordance with the recommendation of the Institutional Biosafety Committee of Hallym University (Chunchon, Korea).

Virus quantification
SARS-CoV-2 quantification was performed as described elsewhere (Kim et al., 2021a; Park et al., 2021a). First, 7×10⁶ cells/well of Vero E6 cells were cultured in 6-well plates overnight in 5% CO₂ incubator. Then, the cells were washed with PBS and inoculated with 10-fold serial dilutions of SARS-CoV-2. After a 1 h incubation with shaking at 20 min intervals in a CO₂ incubator, suspensions were removed, and the wells were overlaid with 3 mL DMEM/F12 medium (Thermo Fisher Scientific) containing 2% Oxoid agar and N-p-Tosyl-L-phenylalanine chloromethyl ketone (TPCK, 1 µg/mL)-treated trypsin (Sigma-Aldrich, Saint Louis, MO, USA). The plates were incubated in a CO₂ incubator for 72 h at 37°C and then stained with 0.1% crystal violet for 1 h to evaluate plaque formation.

Plaque reduction assay
First, 7×10⁶ Vero E6 cells/well were plated on six-well plates for 12 h. Then, SARS-CoV-2 (200 pfu/well) was mixed with each chemical at a final concentration of 10 µM or 1 µM, and Vero E6 cells in each well were treated with the mixtures. After a 1 h incubation with shaking at 20 min intervals in a CO₂ incubator, the supernatant was removed, and the wells were overlaid with 3 mL DMEM/F12 medium (Thermo Fisher Scientific) containing 2% Oxoid agar and TPCK (1 µg/mL)-treated trypsin (Sigma-Aldrich). Finally, 72 h after infection, the plaques formed in each well were observed with crystal violet staining.

Investigation of SARS-CoV-2 replication in the presence of the chemicals
Vero E6 cells (5×10⁴ cells/well 6-well plates) were cultured overnight. The cells were infected with SARS-CoV-2 in PBS (0.1 MOI) for 1 h with shaking at 20 min intervals in a CO₂ incubator at 37°C, and then, 2 mL of DMEM containing 2% FBS were added to each well. After a 3 h incubation in a CO₂ incubator at 37°C, the cells were treated with 0.1% DMSO, atovaquone (Sigma-Aldrich), abiraterone acetate (Sigma-Aldrich), digoxin (Sigma-Aldrich), or β-Estradiol 3-benzoate (Sigma-Aldrich) in a dose-dependent manner, and the plates were incubated for an additional 48 h. Supernatants of the virus-infected cells were collected, and virus replication was quantified using the plaque formation assay.

Cell viability assay
Vero E6 cells (1×10⁵ cells/well) were plated onto 96-well plates in DMEM containing 10% FBS for 12 h. Then, the cells were incubated with atovaquone, abiraterone acetate or digoxin at the indicated concentrations for 48 h in DMEM containing 2% FBS. The cells were then treated with 10 µL Cell Counting Kit-8 (CCK-8) solution (Dojindo Molecular Technology, Kumamoto, Japan) and incubated at 37°C, the cell culture supernatants were harvested, and cell debris was removed by centrifugation at 2,000 rpm for 10 min at 4°C. The virus titer was quantified by plaque assay, and aliquots (5×10⁶ plaque forming unit (pfu)/mL) of the virus were stored at −70°C. SARS-CoV-2 amplification and cell culture procedures were performed according to biosafety level 3 (BSL-3) conditions in the Hallym Clinical and Translational Science Institute in accordance with the recommendation of the Institutional Biosafety Committee of Hallym University (Chunchon, Korea).

Confocal images
The effects of the chemicals on the expression of the N protein and S protein in SARS-CoV-2-infected cells were investigated by indirect immunofluorescence and confocal microscopy. Vero E6 cells were plated on coverslips in 12-well plates overnight and then infected with SARS-CoV-2 (0.1 MOI) in PBS with shaking at 15-20 min intervals in a CO₂ incubator for 1 h at 37°C. After washing with PBS, the cells were cultured in 2 mL of DMEM containing 2% FBS for a 3 h incubation at 37°C, and then, the cells were treated with 0.1% DMSO, abiraterone acetate (10 µM) or digoxin (10 µM). After a 48 h incubation in a CO₂ incubator, the cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100, and then blocked with 3% BSA. The permeabilized cells were incubated with rabbit anti-SARS-CoV-2 Spike protein polyclonal antibody (anti-SARS-CoV-2 S, Ab, Cat. No. 40592-T62, Sino Biological, Vienna, Austria) or mouse anti-SARS-CoV-2 S protein monoclonal antibody (anti-SARS-CoV-2 S, Ab, Cat. No. 40413-MM05, Sino Biological) for 2 h. The cells were then washed with PBS and incubated with Alexa Fluor 488-conjugated secondary antibody (Thermo Fisher Scientific) for 1 h. The nuclei were stained with Hoechst 33258. The slides were examined using a Carl Zeiss LSM710 microscope (Carl Zeiss, Oberkochen, Germany).

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Statistical analysis
Results are shown as the mean ± standard deviation. Differences between the samples were analyzed using an unpaired, 2-tailed nonparametric t-test of significance (Instat; GraphPad Inc., San Diego, CA, USA). p-values<0.05 were considered statistically significant.

RESULTS
Docking simulation for targeting the NTD of the SARS-CoV-2 N protein
The NTD of the SARS-CoV-2 N protein directly binds to RNA molecules by stacking interactions between aromatic amino acids and nucleobases (Kang et al., 2020). For the molecular docking simulation, the putative ligand binding sites of four PDB N protein NTD structures, 6M3M, 6VYO, 6WKP, and 7CDZ, were defined as a 30 Å³ size grid box centered on tyrosine (TYR) residue 109 which is the important residue for the RNA binding affinity of SARS-CoV-2 N proteins (Kang et al., 2020) (Fig. 1B). In this study, the drug repurposing library was constructed as SMILES strings from the US FDA-approved drugs in the ChEMBL Database. The average binding free energies of the repurposed drug candidates to the energy-minimized docking models for the four PDB NTD structures were calculated and used to prioritize the compounds for biological analysis. In addition, the qualitative selection criteria were used for filtering false positive compounds out in the experimental validation analysis by considering the interaction with the TYR109 residue of the SARS-CoV-2 N protein. After removing the compounds lacking an interaction with TYR109, the US FDA-approved drugs in the repurposing library were listed by rank based on the averaged free energy value (Table 1, Supplementary Table 1). Next, extensive literature studies for the top 100 ranked compounds were performed to set the priorities for evaluating their anti-viral activity against SARS-CoV-2. Through this process, several compounds were excluded due to the well-established antiviral activities found in prior reports or the therapeutic unsuitability of the compounds. For instance, proscillaridin was excluded because of the prior reports for its antiviral activity (Jeon et al., 2020). In addition, venetoclax was excluded because it had been reported that it could worsen the symptoms of COVID-19 in chronic lymphocytic leukemia (CLL) patients (Fürstenau et al., 2020).

Screening by the viral plaque reduction assay
The anti-viral activities of twelve FDA-approved drug candidates were evaluated by the plaque reduction assay with SARS-CoV-2. Each FDA-approved drug was mixed with SARS-CoV-2 (200 pfu) at a final concentration of 1 or 10 µM, and then, Vero E6 cells were treated with the mixture. In the plaque reduction assay results, no chemicals significantly reduced the number of plaques. However, three drugs, atovaquone, abiraterone acetate, and digoxin, tended to reduce the size of the SARS-CoV-2 viral plaques in the preliminary screening assays (Fig. 2A-2C). In the best optimized docking models, atovaquone, abiraterone acetate, and digoxin interacted with the TYR109 residue of the N protein NTD (Fig. 3). In the energy-optimized model, atovaquone interacted with the TYR109 residue via π-π stacking interactions whereas abiraterone acetate and digoxin interacted with the TYR109 residue via hydrophobic interactions.

Table 1. Analysis of N-protein docking results and prioritized compound sets

| Compounds          | 6M3M  | 6VYO  | 6WKP  | 7CDZ  | average  |
|--------------------|-------|-------|-------|-------|----------|
| Ergotamine         | –8.1  | –7.1  | –7.4  | –7.8  | –7.6 ± 0.4 |
| Abiraterone        | –7.1  | –7.4  | –6.9  | –7.3  | –7.2 ± 0.2  |
| Digoxin            | –7.1  | –7.2  | –7.1  | –7.2  | –7.2 ± 0.1  |
| Lumacaftor         | –7    | –7.2  | –7.1  | –7.1  | –7.1 ± 0.1  |
| Eltrombopag        | –7.5  | –7.4  | –6.6  | –7    | –7.1 ± 0.4  |
| Abiraterone acetate| –7    | –7.4  | –6.8  | –7.3  | –7.1 ± 0.3  |
| Proscillaridin     | –7.4  | –7    | –6.8  | –7.2  | –7.1 ± 0.3  |
| Atovaquone         | –6.9  | –7.4  | –6.8  | –6.9  | –7.0 ± 0.3  |
| Olaparib           | –7.2  | –6.5  | –6.9  | –7    | –6.9 ± 0.3  |
| Fluspirilene       | –7.1  | –7    | –6.7  | –6.4  | –6.8 ± 0.3  |
| Difenoxin          | –6.8  | –6.6  | –6.6  | –6.9  | –6.7 ± 0.2  |
| Quinestrol         | –6.9  | –7.3  | –6.2  | –6.5  | –6.7 ± 0.5  |
| Norethynodrel      | –6.8  | –7    | –6.2  | –6.8  | –6.7 ± 0.3  |
| Venetoclax         | –7.3  | –6.3  | –6.5  | –6.5  | –6.7 ± 0.4  |

Fig. 2. Screening of the FDA-approved drugs against SARS-CoV-2 infection in Vero E6 cells. The plaque reduction assay was performed with 12 FDA-approved drugs. SARS-CoV-2 was mixed with 10 µM or the indicated concentrations of each compound, and then, the mixture was used to infect Vero E6 cells for 1h. The supernatant was removed and overlaid with 3 ml DMEM/F12 medium containing 2% Oxoid agar and TPCK (1 µg/mL)-treated trypsin. After a 72 h infection, the plaques were observed by staining with crystal violet. These data show only 5 representatives of the 18 FDA-approved drugs. (A) Effect of atovaquone (10 µM) and β-estradiol 3-benzoate (10 µM) on SARS-CoV-2 infection. (B) Effect of abiraterone acetate (10 µM) on SARS-CoV-2 infection. (C) Effect of digoxin on SARS-CoV-2 infection at each concentration.
raterone acetate and digoxin bound to the TYR109 residue with π-alkyl interactions. Notably, abiraterone acetate formed an additional hydrogen bond with the THR91 residue of the NTD and was surrounded by the binding pocket residues including TYR109 (Fig. 3E).

After the COVID-19 pandemic, many drug reposition studies have been reported for FDA-approved drugs. Although abiraterone acetate (Yuan et al., 2020) and digoxin (Cho et al., 2020) along with atovaquone (Farag et al., 2020) were evaluated as SARS-CoV-2 antiviral drugs in vitro, the effects of abiraterone acetate and digoxin were controversial, especially in reducing the number of plaques. The plaque reduction assay is cost-effective for screening purposes, but it has a limitation in determining the anti-viral activity of chemicals targeting viral structural proteins like SARS-CoV-2 N proteins because anti-replicative anti-viral drugs may not affect the entry of the virus into cells.

Investigation of the antiviral activity by counting virus particle numbers in the cell culture supernatant

To overcome the limitation of screening for SARS-CoV-2 replication inhibitors by the plaque reduction assay, quantitative analysis was performed by measuring the number of virus particles produced in culture supernatants using the plaque assay. When Vero E6 cells were infected with a 0.1 MOI of SARS-CoV-2 and then treated with the drug candidates for 48 h, atovaquone had no effect on the viral secretion into the supernatants (Fig. 4A). Notably, abiraterone acetate reduced the virus particles in the culture supernatants in a concentration-dependent manner (Fig. 4B). Specifically, the plaque formation was significantly reduced by above two logarithmic scales in the presence of 10 μM of abiraterone acetate. Digoxin also significantly reduced the accumulation of virus particles in the culture supernatants (Fig. 4C). To exclude a non-specific effect of the drugs in cell-based assays, the cell viability was evaluated with CCK-8-based cytotoxicity assays. The cell viability of the Vero E6 cells was unaffected when exposed to up to 50 μM of atovaquone and abiraterone acetate (Fig. 4D). However, digoxin exhibited a significant cytotoxicity against Vero E6 cells even at much lower concentrations. Therefore, the significant inhibitory effect of abiraterone acetate on virus particle accumulation in the cell culture supernatants was not associated with non-specific cytotoxicity.

Effects of the drugs on SARS-CoV-2 N protein and S protein production

In the measurement of the live virus in the culture supernatant, abiraterone acetate exhibited a concentration-dependent inhibitory activity (Fig. 4). To investigate the inhibitory mechanism in detail, we examined the effects of abiraterone acetate on SARS-CoV-2 N protein and S protein expression in Vero E6 cells. Vero E6 cells were infected with SARS-CoV-2 (0.1
Fig. 4. Effect of the FDA-approved drugs on the replication of SARS-CoV-2 and cell viability. (A-C) Vero E6 cells were infected with a 0.1 MOI of SARS-CoV-2 in 6-well plates and then treated with 0.1% DMSO, atovaquone (A), abiraterone acetate (B) or digoxin (C) in a dose-dependent manner at 3 h after virus infection. Supernatants of the virus-infected cell cultures were collected at 48 h after virus infection. Viral replication was quantified by plaque formation assay. *p<0.05, **p<0.01. (D) Effect of the FDA-approved drugs on the cell viability of Vero E6 cells. Vero E6 cells were cultured in DMEM medium containing 2% FBS with the indicated concentrations of each FDA-approved drug for 48 h. The cells were incubated with CCK-8 solution, and then, soluble formazan was measured using a microplate reader.

MOI) and then treated with atovaquone and abiraterone acetate. The immunofluorescence data of the confocal images showed that abiraterone acetate significantly reduced the expression of the S and N proteins in the SARS-CoV-2-infected Vero E6 cells (Fig. 5). However, atovaquone did not affect the expression of the S and N proteins in the infected cells (Fig. 5). Conclusively, abiraterone acetate has a therapeutic potential to inhibit the viral particle formation of SARS-CoV-2 by interfering with the interaction between viral RNAs and SARS-CoV-2 structural N proteins.

DISCUSSION

Although people are being currently vaccinated against SARS-CoV-2 in many countries, there is an unmet medical need to find potential therapeutics using drug-repurposing strategies. Large scale screening has been performed to find FDA-approved drugs that inhibit SARS-CoV-2 replication (Jeon et al., 2020; Liu et al., 2020; Riva et al., 2020; Weston et al., 2020; Yuan et al., 2020). Many studies have investigated proteases such as TMPRSS2 (Hoffmann et al., 2020), cathepsin B/L (Padmanabhan et al., 2020), main protease (Mpro) (Sternberg et al., 2020) and papain-like protease (PLpro) (Klemm et al., 2020) as potential therapeutic targets for the treatment of SARS-CoV-2 infection. Therefore, there have been many published reports concerning protease inhibitors inhibiting SARS-CoV-2 replication screened from FDA-approved drugs. Currently, drug-repurposing studies targeting viral structural proteins are less available in the literature.

The N protein of coronavirus is essential to the virus assembly through direct interaction with the viral RNA. Therefore, chemicals interfering with the interaction between the viral RNA and the N protein have therapeutic potential against SARS-CoV-2 infection by directly affecting viral replication. In this study, we screened candidate chemicals capable of inter-
acting with N proteins from FDA-approved drugs through in silico docking analyses with four PDB N protein NTD structures, 6M3M, 6VYO, 6WKp, and 7CDZ. The conventional in silico screening is based on the energy-minimized docking models of test chemicals (Forli et al., 2016). To select candidate drugs, the interaction between the TYR109 residue of the N protein and a test chemical was introduced as an additional selection filter. We tested 12 FDA-approved drugs in the SARS-CoV-2 plaque reduction assay to validate the in silico screening result. However, none of the 12 candidate drugs reduced the number of the SARS-CoV-2 viral plaques. Atovaquone, abiraterone acetate, and digoxin were likely to reduce the size of the viral plaques. This result can be explained in that the drug targeting the SARS-CoV-2 structural N protein cannot inhibit the cellular entry of SARS-CoV-2 but can inhibit viral replication. For the development of an optimal method to evaluate drug candidates against structural proteins essential in viral replication, we treated the cells with the candidate drugs for 3 h after SARS-CoV-2 infection and measured the production of viral progeny particles. As a result, we confirmed that abiraterone acetate significantly inhibited the amount of virus progeny in cell culture supernatants. However, atovaquone and digoxin had no effect on the viral particle accumulation in the supernatants. Notably, it was reported that digoxin significantly inhibited the replication of SARS-CoV-2 (Cho et al., 2020). Digoxin is currently being prescribed to manage atrial fibrillation, atrial flutter, and heart failure as an inhibitor of sodium potassium adenosine triphosphatase (Na+/K+ ATPase) (Kjeldsen et al., 2002). In our study, digoxin exhibited a severe cytotoxicity in SARS-CoV-2-infected Vero E6 cells. Further studies should be directed to resolve the conflicting results to use digoxin as a therapeutic drug against SARS-CoV-2 infection.

The medical use of abiraterone acetate is to treat prostate cancer (Yin and Hu, 2014). Abiraterone acetate is an active metabolite of abiraterone and inhibits cytochrome P450 17A1, also called 17α-hydroxylase, and functions as an antagonist of the androgen receptor (Yin and Hu, 2014). In this regard, it is necessary to check whether the inhibition of CYP17A1 or androgen function contributes to the inhibitory effect of abiraterone acetate on SARS-CoV-2 replication through the inhibition of N protein functions. In conclusion, abiraterone acetate has therapeutic potential by inhibiting viral replication in SARS-CoV-2.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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