Early combination treatment with existing HIV antivirals: an effective treatment for COVID-19?

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Research Article

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Abstract

Since no effective therapy exists, we aimed to test existing HIV antivirals for combination treatment of Coronavirus disease 19 (COVID-19). Our molecular docking findings suggest that lopinavir, ritonavir, darunavir, and atazanavir activated interactions with the key binding sites of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) protease with a better Ki for lopinavir, ritonavir, and darunavir. Furthermore, we evidenced the ability of remdesivir, tenofovir, emtricitabine, and lamivudine to be incorporated in SARS-CoV-2 RNA-dependent RNA polymerase in the same protein pocket where poses the corresponding natural nucleoside substrates with comparable Ki and activating similar interactions. In principle, the four antiviral nucleotides might be used effectively against SARS-CoV-2. The combination of a protease inhibitor and two nucleoside analogues should be evaluated in clinical trials for the treatment of COVID-19.

1. Introduction

Several cases of an unknown severe acute respiratory syndrome (SARS) have been reported in Wuhan (Hubei Province, China) at the end of December 2019 [1]. On the 7th of January, a new Coronavirus, possibly responsible for the SARS cases in Wuhan, was detected [2]. Peng Zhou *et al.* were the first to obtain the full-length genome sequences of the virus that shares 79.5% sequence with the SARS-CoV and shows a 96% homology to a bat Coronavirus. For this reason, they hypothesized a bat origin of SARS-CoV-2 [3].

The replicase complex is believed to be comprised of up to 16 viral subunits and a number of cellular proteins. Besides RNA-dependent RNA polymerase (RdRp), RNA helicase, and protease activities based on their physiological role, coronavirus proteases can be classified into accessory and main proteases. The firsts are responsible for cleaving the more divergent N-proximal pp1a/pp1ab regions at two or three sites. The main proteases (M^{pro}), instead, cleave the major part of the polyproteins at 11 conserved sites and also release the conserved key replicative functions, such as RdRp, helicase, and three of the RNA processing domains [4,5]. The structural conformation of SARS-CoV-2 M^{pro} obtained by resolution of the crystal structure has been recently described by Zhang *et al.* [6]. The SARS-CoV-2 M^{pro} is a homodimer whose each monomer is tightly joined with each other and comprising Glu166 and Thr285 residues. Each monomer possesses three domains that are highly similar to those represented in the crystal structure of SARS-CoV. The substrate-binding sites of SARS-CoV-2 M^{pro} were identified after lead optimization and according to the favored interactions of an *in vitro* (mice and human) effective peptidomimetic inhibitor co-crystallized which SARS- CoV-2 M^{pro}. Information on active sites had identified Cys145 and His41 as amino acids likely involved in the catalytic site whereas Thr190 and Gln189 as amino acids characterizing a bulky and small hydrophilic pocket, respectively. Glu166 and Thr285 residues represent the interaction site of the dimerization of the enzyme that is necessary for catalytic activity.

The RdRp, also known as non-structural protein 12 (nsp12), catalyzes the synthesis of viral RNA and thus plays a central role in the replication and transcription cycle of the COVID-19 virus, possibly with the

assistance of nsp7 and nsp8 as co-factors. Two structures of SARS-CoV-2 RdRp has recently appeared in the literature. Gao Y *et al.* describes interactions with conventional antiviral nucleos(t)ides co-crystallized with RdRp (resolution 2.9 Å) where, unfortunately, metal atoms and RNA template, both responsible for virus replication, are missing in the protein [7]. Immediately after, Yin *et al.* published the crystal structure of RdRp protein bounded to the template-primer RNA with the active form of remdesivir, a known coronavirus inhibitor[8]. In virtue of the presence of viral RNA primer, the latter crystal structure (resolution 2.5 Å) provides more reliable information at the atomic level on drug design and repositioning of conventional drugs targeting viral RdRp.

There is high sequence conservation in the RdRp across CoVs. For example, the RdRp of SARS-CoV-2 has 99.1% similarity and 96% amino acid identity to that of SARS-CoV[9]. The SARS-CoV-2 domain adopts the conserved architecture of the viral polymerase family and is featured by three subdomains containing catalytic cations. In the central cavity of a polymerase, three elements take place in viral replication reaction; the nucleos(t)ide triphosphate interacts with the template-primer RNA providing to the final nascent strand.

Challenges to CoV antivirals development are multiple. The replication of positive-sense RNA virus genomes is generally characterized by high error rates, high viral yields, short replication times, and abundant homologous and non-homologous recombination[10]. As a result, genome mutants with varying degrees of fitness are generated.

Since different approaches have been tried so far, but no definitive treatment or effective vaccination exists. Thanks to the common features shared between different coronavirus, the repositioning of conventional antivirals effective on SARS-CoV and MERS-CoV could be a valuable pharmacological approach in finding antiviral agents selective for SARS-CoV-2 infection. Very recently, some articles on this topic using *in silico* studies have been published; nevertheless, the resolution of the crystal protein and the computational approach play a crucial role in finding reliable molecular docking and thus the best ligand[11–13].

Our research aimed to perform molecular docking studies on high-resolved crystal structure of SARS-CoV-2 M^{pro} and SARS-CoV-2 RdRp in order to investigate potential combination antiviral treatment for COVID-19 using widely available drugs used to treat HIV infection. For this purpose, we decided to test four HIV protease inhibitors (lopinavir, ritonavir, darunavir, and atazanavir) and to compare remdesivir, a promising drug for COVID-19 treatment available only for intravenous use, with other orally administered nucleos(t)ide analogues (tenofovir, emtricitabine, and lamivudine).

2. Materials And Methods

2.1 In silico docking study, computational equipment, and software

The crystal structures of SARS-CoV-2 M^{pro} (PDB ID: 6Y2F, in the monoclinic crystal form co-crystallized with peptidomimetic inhibitor O6K, resolution: 1.95 Å) [6] and SARS-CoV-2 RdRp (PDB ID: 7BV2, co-

crystalized with remdesivir monophosphate, resolution: 2.50 Å) [14] were released from the RCSB protein databank (www.rcsb.org). SARS-CoV-2 RdRp (PDB ID: 7BV2) was crystallized with remdesivir trisphosphate, experimentally recognized as the active form of remdesivir. In the crystal structure, remdesivir trisphosphate appears co-crystallized as monophosphate with the diphosphate residue, bonded to magnesium of the protein Mg1004, is being positioned close to remdesivir monophosphate with which activate interaction POP1003.

The starting proteins were prepared, stripping the crystallographic water molecules and ligands.

Hydrogen atoms were added to the structures using ADT of the MGLTools 1.5.7rc1 module[15].

All the ligands were constructed by using the standard molecule deposited in PubChem. Gasteiger-Marsili charges method was applied to assign all ligands and proteins [16].

Computational modeling experiments were carried out on an HP8100 Workstation and EXXACT Tensor Workstation TWS-1686525-AMB with the Cuda platform.

Binding of the compounds was performed using AutoDock 4.2.6 docking programs [17,18] Lamarckian genetic algorithm (LGA) was used for structures docking. In the case of the blind docking procedure of protease, the LGA was defined through a centered grid, with coordinates: x = -4.876, y = -0.822, z = 11.992, of 126 × 126 × 126 grid points in x, y, z dimensions, respectively, spacing 0.555 Å. The protocol of LGA was set to 100 runs, with a population size of 150 individuals and the maximum number of generations and energy evaluations of 27,000 and 25,000,000, respectively.

For the standard docking focused on the binding site of the protein, a grid with 60 × 60 × 60 points in x, y, z dimensions were selected for SARS-CoV-2 M^{pro}, and a grid size of 50 x 50 x 50 points in x,y,z dimension was selected for SARS-CoV-2 RdRp, respectively. For both proteins, the grid spacing was set to 0.375 Å.

All inhibitors were docked with all bonds completely free to rotate.

Free energy of ligand binding (EFEB, ΔG), the estimated inhibition constant (E.I.C., K_i) for each ligand was calculated according to equation (1)

$$K_i = \exp[(\Delta G \times 1000)/(R \times T)] \quad (1)$$

Where ΔG is the docking estimated free energy, R (gas constant) = 1.98719 cal/(K × mol), and T = 298.15 K.

Graphical representation of the poses derived from the docking calculation and hydrophobic interactions were obtained using MGLTools 1.5.7rc1, Chimera 1.13.1, and LigPlot+ software [19,20].

3. Results

3.1 Computational studies

The reliability of the docking approach was verified on SARS-CoV-2 M^{pro} by stripping the peptidomimetic inhibitor O6K from 6Y2F.pdb crystal structure and by considering O6K as a normal ligand. Two experiments were carried out for the validation of the protocol, O6K was forced into the conformation of the crystal by blocking rotatable bonds for two different dockings, the first on the site with the standard grid used for this series of experiments (60 x 60 x 60, spacing 0.375 Å) the second with the maximum grid and spacing such as to cover the entire macromolecule (126 x 126 x 126, spacing 0.555 Å). After repositioning of O6K into the protein, the new ligand location was in accord with the original X-ray structure, with only minimal conformational changes, therefore confirming the reliability of the system (100% overlaid on the site, 50% in the Blind Docking) as depicted in Figure S1.

From docking studies on O6K ligand and SARS-CoV-2 M^{pro}, a set of amino acid residues able to activate the best interactions with the pharmacophore lead was identified (Table 1). Cys145, Ser144, Gly143 and His41 point to the catalytic site, the latter amino acid residue is being characterized by a restricted lipophilic pocket. Both Thr190 and Gln189 occupy a large hydrophobic site whereas Glu166 and Thr285 residues are located on the interaction site of enzyme dimerization that is necessary for catalytic activity. Four conventional antiviral drugs, namely lopinavir, ritonavir, darunavir, and atazanavir, were selected for docking intro 6Y2F crystal structure. Structurally, all of them are stereocontrolled peptidomimetics because they mimic a natural peptide. They are characterized by substituents with different lipophilicity and structural flexibility that influence the interactions protein-ligand and the conformational state of the molecule. To a different extent, all conventional antiviral drugs activated interactions with the protease active sites that, theoretically, would interfere with the function of SARS-CoV-2 M^{pro}. Ritonavir, lopinavir, and darunavir had the best Ki estimated 15.13, 37.44, and 56.76 nM for each of them, whereas atazanavir ki was 1.10 μM, identifying a minor virtual interaction with SARS-CoV-2 M^{pro}.

Figure S2 and Table S1 represent hydrophobic and H-bond interactions, respectively, predicted for the four antiviral drugs activated with SARS-CoV-2 M^{pro}. At least five H-bonds were estimated for each drug, evidencing the high affinity with the protein. Lopinavir interacted with more key amino acids with both hydrophobic and H-bond interactions such as Cys145, Ser144, and Gly143, involved in the catalytic site, with Thr190 and Gln192, located on the bulky hydrophilic site and with Glu166, representative of the interaction site of protein dimerization. Differently, from the other drugs, atazanavir activates H-bond with oxygen and nitrogen of Thr26 and hydrophobic interaction with Thr25, two amino acids far from the theoretical active pocket site. In Figure 1, an image of the best poses of each drug in the active sites of SARS- CoV-2 M^{pro} is being represented.

In SARS-CoV-2 RdRp (PDB ID: 7BV2) crystal structure, remdesivir trisphosphate (remdesivir-3P) appears co-crystallized as monophosphate partially bonded to U20 and U10 of uracil base and A11 of adenine base[8]. The crystal sets the situation when diphosphate residue has been just released; it is being bonded to magnesium atom M1004 of the protein and activates interaction POP1003 with remdesivir monophosphate. The ligand engages interactions with Thr688 and Asp761 of the protein and with A11, U10, and U20. In our docking approach, remdesivir monophosphate and the diphosphate residues were

extracted from the crystal structure. After, remdesivir, tenofovir, lamivudine, and emtricitabine, all of them as triphosphate (3P), were launched for molecular docking in order to find the right pose among the generated conformations. Nearly complete superimposed poses were reached with co-crystallized remdesivir monophosphate and remdesivir-3P with the contemporary alignment of the nucleos(t)ide bases (Figure 2). Adenosine triphosphate (adenosine-3P) and cytosine triphosphate (cytosine-3P) were also added to the simulation study because they represent the natural substrate used by RdRp to replicate the viral RNA-based genome. 7BV2 RNA crystal possesses uracil base alignment suitable for the complementary adenosine nucleos(t)ide base; thus, when cytosine-3P and cytosine nucleos(t)ide antivirals were docked, uracil was replaced with guanine base, the complementary base of cytosine.

The best interactions of adenosine-3P, tenofovir-3P, and remdesivir-3P with the amino acids residue of SARS-CoV-2 RdRp have been represented in Table 2, where Ki reached a magnitude of 10^{-18}M. Remdesivir-3P and tenofovir-3P activate similar interactions with the polymerase and comparable to those of remdesivir monophosphate co-crystallized with SARS-CoV-2 RdRp (7BV2 crystal). Conversely to the crystal where remdesivir monophosphate is covalently bonded to the primer strand of RNA, in our study remdesivir-3P appeared perfectly aligned with the nucleos(t)ide base with which attivated strong interactions. Remdesivir monophosphate Tenofovir-3P and remdesivir-3P interact with the same viral RNA bases even though remdesivir-3P provides additional interactions with Asp623, Ser682, Thr687, and Asn691 of the protein and more lipophilic and H-bond interactions (Figure S3 and Table S2). Figure 3 represents the best poses of adenosine-3P, tenofovir-3P, and remdesivir-3P in the active pocket of the viral polymerase.

As estimated for adenosine-analogues antivirals, also lamivudine-3P and emtricitabine-3P, two cytosine-analogues antivirals, target the active pocket of the polymerase and with the same pose as cytosine-3P does (Figure 4). Except for Asp760, lamivudine-3P interacts with the same set of amino acids, RNA bases, and magnesium cations of emtricitabine-3P and cytosine-3P, all of them with significantly low Ki (Table 2). Conversely, lamivudine-3P activates more H-bond with the polymerase in comparison with emtricitabine-3P, whereas both activate analogues lipophilic interactions with the protein (Table S2 and Figure S4).

4. Discussion

The World Health Organization (WHO) declared Coronavirus disease 19 (COVID-19) a public health emergency of international concern and declared the pandemic on the 12th of March, 2020. Since the start of the pandemic, the number of reported cases in the world is 6,152,160 with 371,700 deaths as of the 1st of June 2020 [21]. The pandemic has been described as the worst health and economic threat after world war two.

The clinical picture of COVID-19 is characterized by a broad spectrum of presentations ranging from asymptomatic to life-threatening disease[22,23]. The clinical course has been divided into three stages: the first is the early (mild) infection with minor symptoms, second (moderate) stage characterized by

pulmonary involvement with or without hypoxia and a third stage, the most severe, that is characterized by extra-pulmonary systemic hyper-inflammatory syndrome [24]. The first stage is characterized by the replication of SARS-CoV-2, mainly in the upper respiratory tract, and represents the best moment to start an antiviral treatment in order to avoid the progression of COVID-19.

Our *in silico* results suggest that lopinavir, ritonavir, darunavir, and atazanavir activated interactions with the key binding sites of SARS-CoV-2 protease with a better Ki for lopinavir, ritonavir, and darunavir. Although atazanavir appears the less effective *in silico*, it would not rule out pharmacokinetics and pharmacodynamics of the molecule under human trials. These four drugs have been extensively used as part of combination antiretroviral treatment of HIV infection for more than a decade [25–27]. In the clinical use in HIV-infected patients, ritonavir is currently used only at a low dose as a pharmacokinetic booster in combination with lopinavir, darunavir, and atazanavir.

A randomized, controlled, open-label study reported on the efficacy and safety of lopinavir/ritonavir for treating hospitalized adults with severe COVID-19 [28]. In this trial, 99 patients received lopinavir/ritonavir and 100 standard care for 14 days. Of note, the median interval time between symptoms onset and randomization was 13 days. No statistically significant difference in time to clinical improvement was observed between arms. However, 28 days mortality was lower in the lopinavir/ritonavir group (19.2%) compared to the control group (25%).

Furthermore, patients on lopinavir/ritonavir had significantly shorter stay (6 vs. 11 days) in the intensive care unit (ICU) in respect of the control group. Overall, faster clinical recovery and reduced mortality were observed in those treated within 12 days of symptom onset. Gastrointestinal complaints were more common in the lopinavir/ritonavir group, whereas serious adverse events did not differ between the two arms. These results suggest that lopinavir/ritonavir was associated with a faster clinical recovery in those who started early and with a shorter duration of ICU stay.

The efficacy of lopinavir/ritonavir is currently also tested in the “Recovery” trial against other three different single drug approaches (dexamethasone, hydroxychloroquine, or azithromycin) [29].

The combination of darunavir/cobicistat is currently under evaluation as monotherapy compared to conventional treatment for COVID-19 in a small open-label randomized clinical trial (30 participants in the two arms, NCT04252274). Unfortunately, no real-life data are available for darunavir and atazanavir in the treatment of COVID-19.

Remdesivir is reported to act via chain termination of nascent viral RNA in Ebola virus infection [30] and is also effective against a broad spectrum of human and pre-epidemic zoonotic CoVs and potently inhibits replication of SARS-CoV and MERS- CoV in primary human airway epithelial cultures [31,32].

One potential drawback of remdesivir monotherapy is represented by the possible selection of resistance, which is mediated by RdRp residues F480L and V557L in SARS-CoV, resulting in a 5-fold shift in half-maximal inhibitory concentration (IC_{50}) [32]. Importantly, the two remdesivir resistance mutations, alone

or together, conferred increased sensitivity to inhibition by β-D-N4-hydroxycytidine (NHC), a novel potential broad-spectrum antiviral, suggesting unique patterns of resistance for single drugs [9].

More recently, remdesivir has been shown to effectively inhibit SARS-CoV-2 replication in Vero E6 cells [33] and to interact with RdRp by forming two H-bonds with Trp509 and His381, π-cation contacts with Phe504, and hydrophobic interactions with Lys508, Leu401, Asn386, and Ser384[11]. It is currently under evaluation as a potential monotherapy treatment for COVID-19 in at least nine randomized clinical trials. Wiliamson B *et al.* showed in the animal model that very early administration of remdesivir to rhesus macaques was able to reduce virus titers in bronco-alveolar lavage significantly and that the viral load was significantly lower in lungs of remdesivir-treated animals than in controls [34]. However, the authors admit that the results were difficult to translate in real life of patients' management because remdesivir was administered 12 hours after virus inoculation that in human disease is coincident with an asymptomatic incubation period. Furthermore, remdesivir is available only for intravenous administration that is logistically impossible to implement for home treatment, especially on a large scale.

Fifty-three inpatients with severe COVID-19 were enrolled in compassionate use of remdesivir study. After a median follow-up of 18 days, 36 patients (68%) showed an improvement in respiratory parameters; of these, 17 (57%) who were receiving mechanical ventilation have been extubated. Overall, 25 patients (47%) were discharged, whereas seven (13%) died. Among patients receiving invasive ventilation, the mortality was 18% and 5% among those not [35]. A recent double-blind, randomized, placebo-controlled trial enrolled adults hospitalized with Covid-19 pneumonia. Patients were randomized to receive either remdesivir or placebo for up to 10 days. Preliminary results from 1059 patients indicated that those treated with remdesivir had a median recovery time of 11 days, in respect of 15 days in those who received a placebo (rate ratio for recovery, 1.32; 95% CI, 1.12 to 1.55; P<0.001). Mortality by 14 days was 7.1% with remdesivir and 11.9% with placebo (hazard ratio for death, 0.70; 95% CI, 0.47 to 1.04) using Kaplan Meier estimates. Serious adverse events were less frequently reported in the remdesivir group compared to placebo [36].

The combination of tenofovir/emtricitabine *vs.* hydroxychloroquine is currently under evaluation in a randomized clinical trial conducted in Spain as a preventive regimen in healthcare professionals exposed to COVID-19 patients. Therefore, no nucleos(t)ide analogues combinations are under evaluation in clinical trials for the treatment of COVID-19.

Our in silico studies evidenced the ability of remdesivir, tenofovir, lamivudine, and emtricitabine to be incorporated, as active forms, in SARS-CoV-2 RdRp in the same protein pocket where poses the corresponding natural nucleos(t)ide substrates with comparable Ki value and activating similar interactions. In principle, the four antiviral nucleos(t)ides might be used effectively against SARS-CoV-2. To maximize the antiviral effect on SARS-CoV-2 RdRp, a cytosine-based antiviral nucleos(t)ide could be associated with an adenosine-based one.

Tenofovir, emtricitabine, and lamivudine are nucleos(t)ide analogues already widely available in the market as antiretroviral drugs. These medications have been extensively used in HIV-infected patients

showing a favorable safety profile, especially when considering the limited duration of COVID-19 treatment [37–39]. Furthermore, these drugs are available for oral administration and show minimal drug-drug interactions, thus representing ideal potential candidates for both out and inpatients treatment in the early stage COVID-19. The possibility to treat patients with effective combinations at home is of crucial importance given the possible further waves of the pandemic in the upcoming months while waiting for a protective vaccine.

From lessons learned from other viral infections, including HIV and HCV, the combination of drugs with different targets may reduce more rapidly SARS-CoV-2 viral load, the probability of interhuman transmission and selection of resistance to single medications. Furthermore, early antiviral treatment has been associated with better clinical outcomes in COVID-19 patients in a large observational study conducted in China[40].

5. Conclusion

Based on the available literature and our experimental results, we propose that a combination of a boosted protease inhibitor (lopinavir/r, darunavir/r or atazanavir/r) and two nucleos(t)ide analogues (tenofovir disoproxil or tenofovir alafenamide + emtricitabine or lamivudine) started early in the course of the disease (ideally within the first seven days) and administered for a short time (5-20 days depending on the severity of the disease) should be evaluated as soon as possible in clinical trials for the treatment of COVID-19.

Declarations

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Author contributions: GM, PAS, ADV, GD, AD, RND participated in the formulation or evolution of research goals and aims of the study; GD, AD, RND were responsible for data curation; GM, PAS, ADV, RND, AD, GD analyzed the data; RND, AD performed the experiments and created the models; GM, PAS and GD were responsible for the research activity and execution; GD provided the resourches for the conduction of the experiments including the computational study and molecular modelling; GM, PAS, GD were responsible for the overall supervision of the research; RND, AD, GD were responsible for verification of the overall replication/reproducibility of experiments; GM and GD were responsible for visualization and data presentation; GM and GD drafted the initial manuscript; GM, PAS, ADV, GD, AD, RND critical reviewed, revised and approved the final manuscript.

Competing interests: GM has been advisor for Gilead Sciences, Janssen and Merck Sharp and Dohme and ViiV Healthcare and has received speakers' honoraria from Gilead Sciences, Merck Sharp and Dohme, Janssen and ViiV Healthcare; all other authors declare they have no potential conflict of interest to disclose.

Data and materials availability: All data is available in the main text or in the supplementary materials. All data used in the analysis are available to any researcher for purposes of reproducing or extending the analysis upon request.

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Tables

Table 1. Estimated interactions of antiviral drugs with the amino acids residues of SARS-CoV-2 M^{pro}.

Active Metabolite	n° run	Active Torsion	M.B.E.[a]	E.F.E.B.[b]	E.I.C., Ki[c]	Interactions
Atazanavir	100	19	-8.13	-8.13	1.10,µM	Thr24,Thr25,Thr26,Leu27,His41, Thr45,Ser46, Met49, Leu141,Gly143, Cys145,His163,His164,Met165, Glu166, Leu167,Pro168,Gln189, Thr190,Gln192
Darunavir	100	14	-9.89	-9.89	56.76, nM	His41,Met49,Phe140,Leu141,Asn142, Gly143,Ser144, Cys145,His163,His164 Met165,Glu166,His172,Asp187, Arg188,Gln189,Thr190,Gln192
Ritonavir	100	19	-10.13	-10.13	37.44, nM	His41,Met49,Phe140,Leu141,Asn142, Gly143,Ser144,Cys145,His163,His164, Met165,Glu166,Leu167,Pro168, His172, Asp187,Arg188,Gln189, Thr190,Gln192
Lopinavir	100	16	-9.34	-10.67	15.13, nM	His41,Met49,Leu141,Asn142,Gly143, Ser144,Cys145,His163,His164,Met165, Glu166,Leu167,Pro168,Asp187, Arg188,Gln189,Thr190,Ala191,Gln192

[a]M.B.E.: Mean Binding Energy, [b]E.F.E.B.: Estimated Free Energy of Binding, [c]E.I.C., Ki: Estimated Inhibition Constant, Ki.

Table 2. Estimated interactions of adenosine-3P (ATP), tenofovir-3P and remdesivir-3P, cytosine-3P (CTP), emtricitabine-3P and lamivudine-3P with the amino acids residues of SARS-CoV-2 RdRp[a]

Active Metabolite	% cluster	M.B.E.[b]	E.F.E.B.[c]	E.I.C., Ki[d]	Interactions
ATP	19	-19.42	-20.89	483.59, aM	MG1,MG2,U10,A11,U20,Lys545,Arg553, Asp618,Tyr619
Tenofovir-3P	11	-20.04	-21.86	95.15, aM	MG1,MG2,U10,A11,U20,Asp760,Asp761
Remdesivir-3P	8	-21.91	-24.01	2.51, aM	MG1,MG2,U10,A11,U20,Asp623,Ser682, Thr687,Asn691,Asp760,Asp761
CTP	14	-20.16	-21.44	192.32, aM	MG1,MG2,G10,U20,Ser682,Thr687, Asp760,Asp761
Emtricitabine-3P	15	-21.33	-22.86	17.51, aM	MG1,MG2,G10,U20,Arg553,Ser682, Thr687,Asn691
Lamivudine-3P	9	-21.34	-23.00	13.83, aM	MG1,MG2,G10,U20,Ser682,Thr687, Asp760,Asp761

[a]in 7BV2 crystal of SARS-CoV-2 RdRp, uracil was replaced with guanine base for interactions with CTP, emtricitabine-3P, and lamivudine-3P.
[b]M.B.E.: Mean Binding Energy,
[c]E.F.E.B.: Estimated Free Energy of Binding,

Supplementary Materials

Figures S1-S4

Tables S1-S2

Figures

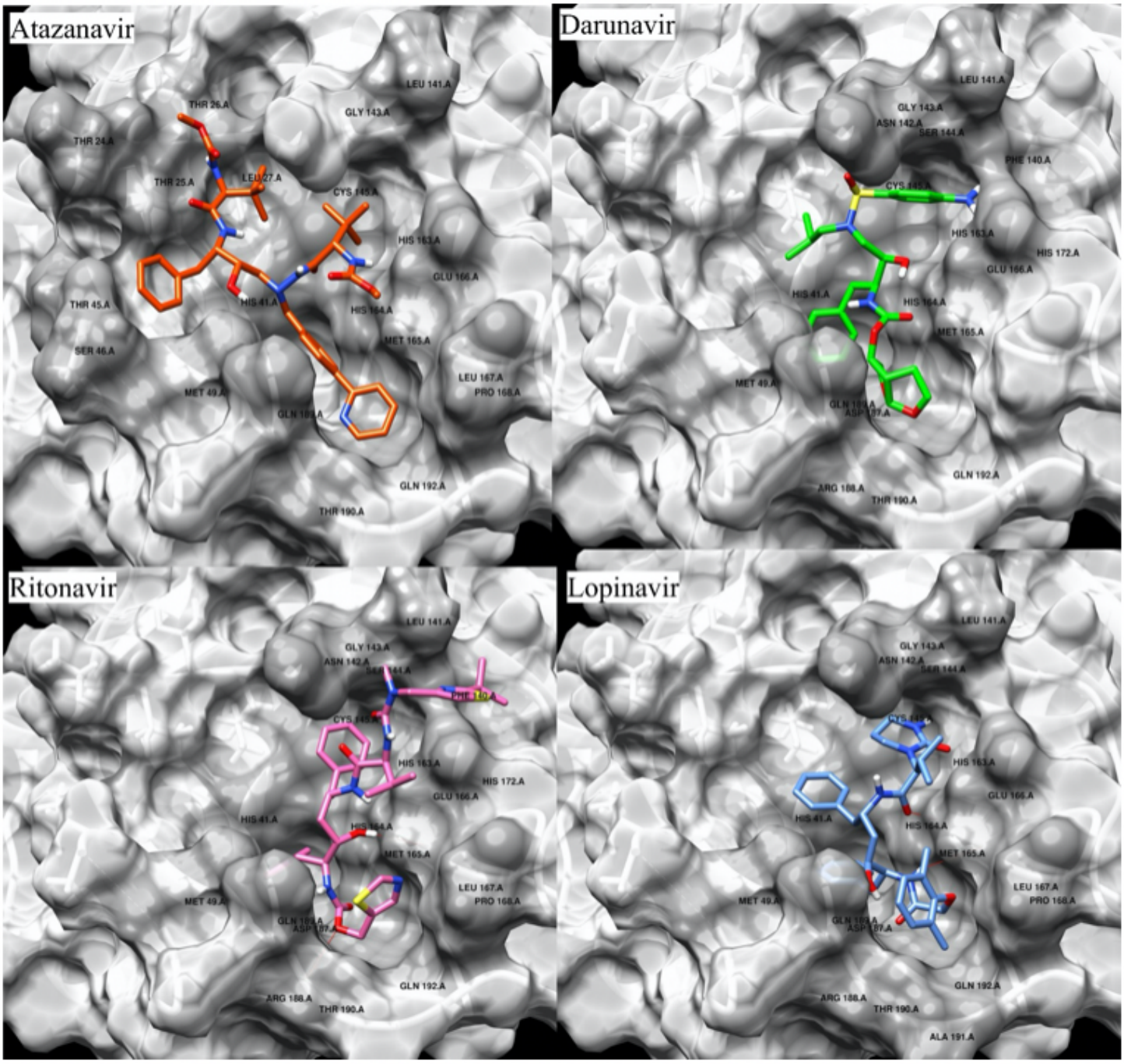

Figure 1

The best fit of atazanavir, darunavir, lopinavir, and ritonavir to the active sites of SARS CoV-2 Mpro after molecular docking. The dark grey spots represent lipophilic pockets.

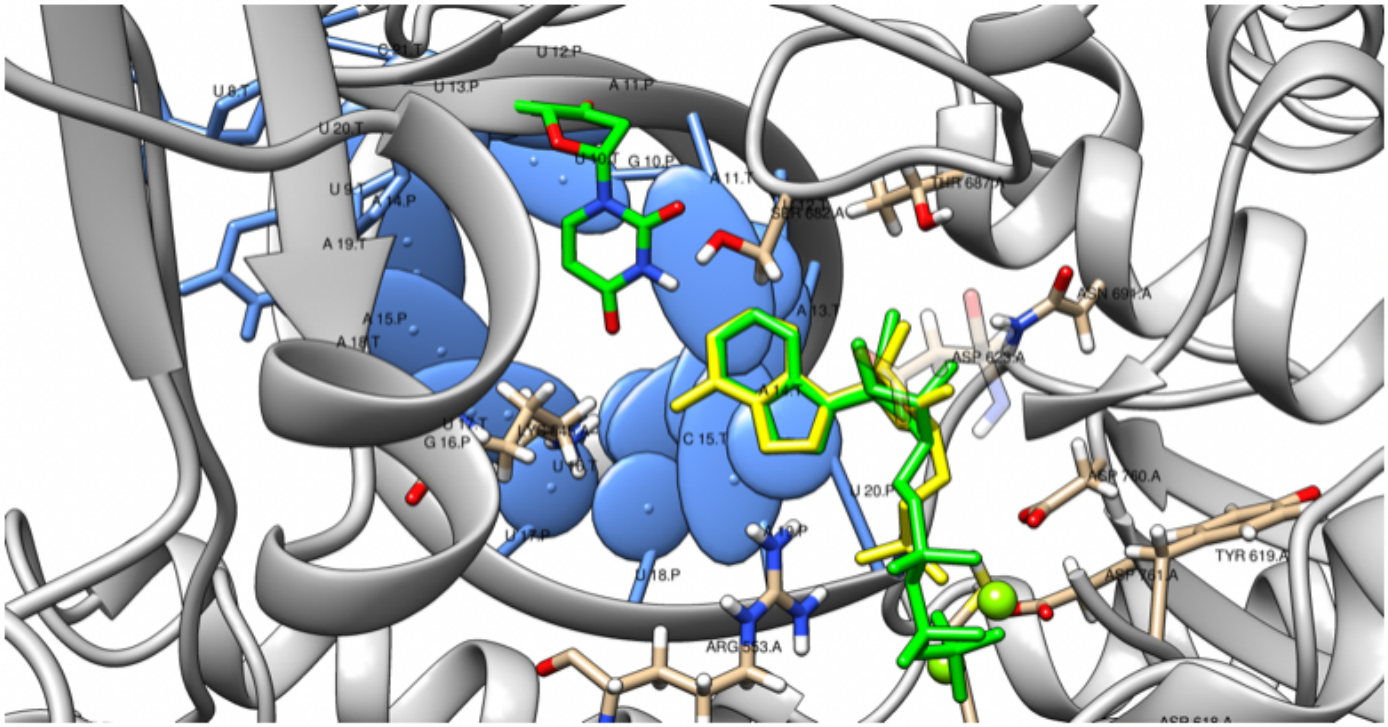

Figure 2

Remdesivir monophosphate from 7BV2 (yellow) and molecular docking of remdesivir-3P (light green).

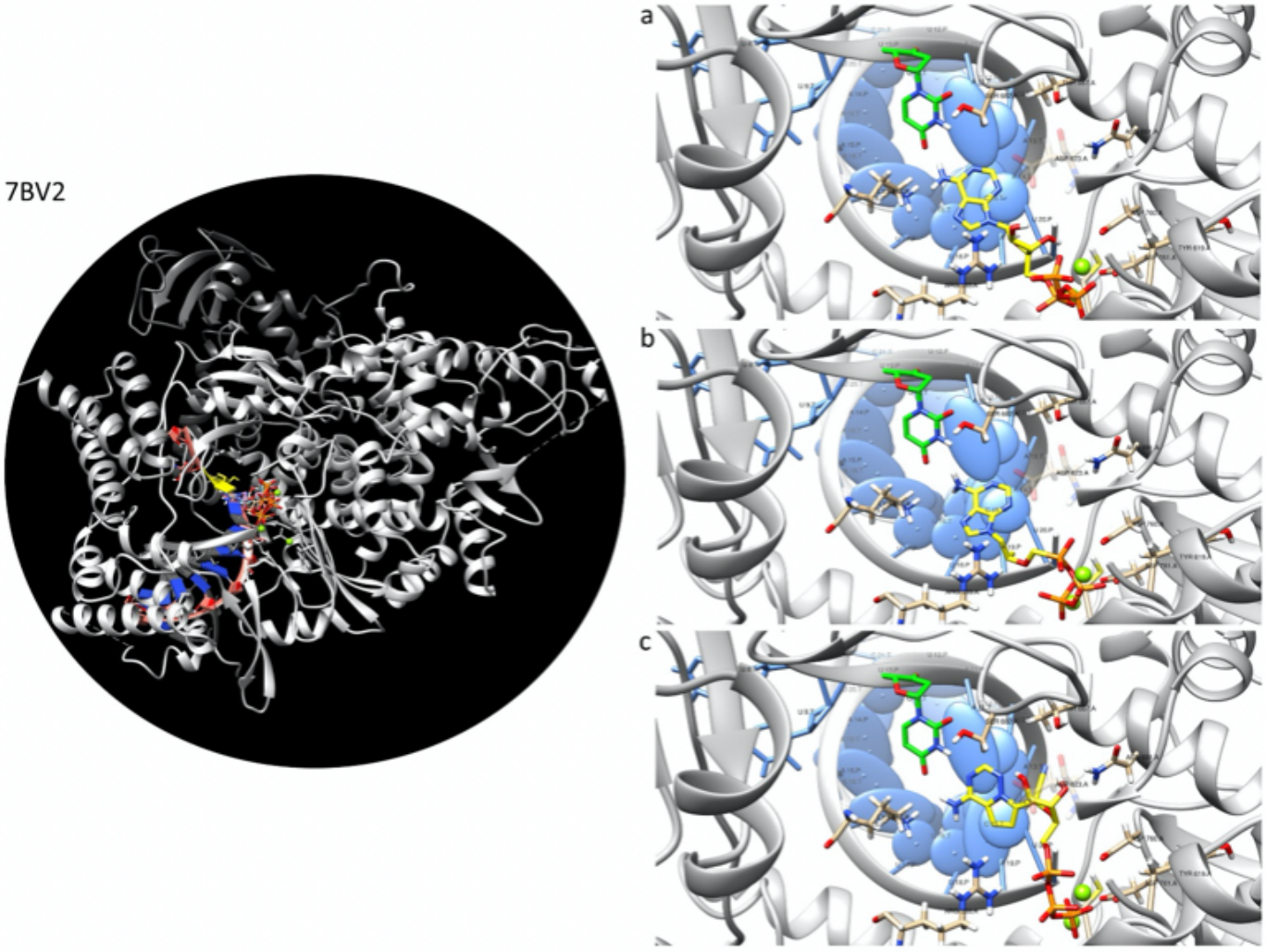

Figure 3

The best fit for the active sites of SARS CoV-2 RdRb after molecular docking with adenosine-3P and nucleoside antivirals (yellow). Left: 7BV2 crystal where uracil (green) points to the active sites for interactions with adenosine-3P and antiviral adenosine analogues, right: adenosine-3P (a), tenofovir-3P (b), remdesivir-3P poses in the active sites of the polymerase (c).

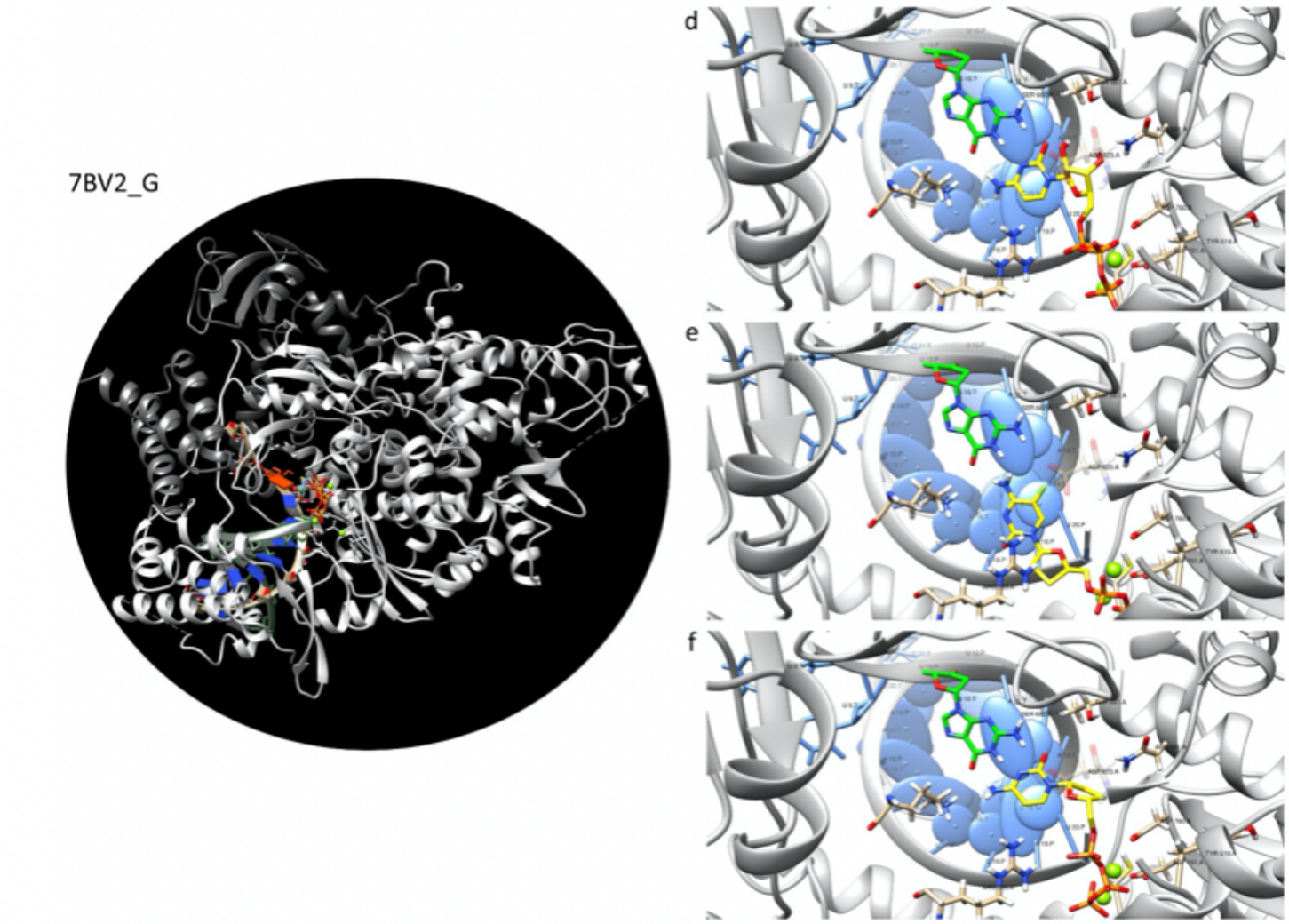

Figure 4

The best fit for the active sites of SARS CoV-2 RdRb after molecular docking with cytosine-3P and nucleoside antivirals (yellow). Left: 7BV2_G, from crystal 7BV2 where uracil was replaced with guanine (green) (G) for interactions with cytosine-3P and antiviral cytosine analogues, right: cytosine-3P (d), lamivudine-3P (e), emtricitabine-3P (f) poses in the active sites of the polymerase.

Supplementary Files

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- MadedduetalSupplementaryMaterialsSRs.docx