

The screening and evaluation of potential clinically significant HIV drug combinations against the SARS-CoV-2 virus

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ABSTRACT

Spike glycoprotein is essential for the reproduction of the SARS-CoV-2 virus, and its inhibition using already approved antiviral drugs may open new avenues for treatment of patients with the COVID-19 disease. Because of that we analyzed the inhibition of SARS-CoV-2 spike glycoprotein with FDA-approved antiviral drugs and their double and triple combinations. We used the VINI in silico model of cancer to perform this virtual drug screening, showing HIV drugs to be the most effective. Besides, the combination of cobicistat-abacavir-rilpivirine HIV drugs demonstrated the highest in silico efficacy of inhibiting SARS-CoV-2 spike glycoprotein. Therefore, a clinical trial of cobicistat-abacavir-rilpivirine on a limited number of COVID-19 patients in moderately severe and severe condition is warranted.

1. Introduction

It is still unclear where the SARS-CoV-2 virus originated: we also have no scientific confirmation that this virus has jumped from an animal species to human. However, we do have confirmation of the transmission of SARS-CoV-2 virus by humans to both dogs and cats. Specifically, the World Health Organization (WHO) data describes two dogs in Hong Kong and one cat in Belgium, which were virus-contaminated from humans, but this was synonymous with COVID-19 infection in these animals. Shortly after the first confirmed cases of infection with the SARS-CoV-2 coronavirus [1], COVID-19 spread rapidly to almost all continents of the world, becoming a pandemic in a very short time [2]. The number of patients confirmed to be infected

with SARS-CoV-2 is increasing rapidly, and in the mid of January 2021 there were more than 93 million confirmed cases according to WHO [3]. The end of this pandemic cannot yet be foreseen, and to make matters worse, the estimated mortality caused by this virus is from about 1% up to as high as 12% in the epidemic epicenters [4].

Vaccination is recognized as the most important goal [5], but even that will not completely solve the problem of this pandemic. This is due to the fact that there are strains of SARS-CoV-2 virus constantly emerging, and immunity acquired to one strain does not guarantee immunity to another strain [6].

Consequently, in order to reduce the number of deaths and long term health consequences, efforts by the world scientific community and the pharmaceutical industry need to be focused on finding effective drugs

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and therapies against COVID-19 as quickly as possible. Finding new antiviral drugs is generally a costly and time-consuming process, and often limited by our understanding of biology [7]. Therefore, it is justified to consider the repurposing of existing drugs to cure new diseases [8], as these drugs already have well-established doses and regimens, known side effects, and methods of preventing or mitigating such effects. Equally important, the optimal approach to synthesizing existing drugs is known, so in the case of increased demand for a certain drug, it is easier, faster, and less expensive to expand existing production capacities than to design and build new ones [9]. Repurposing existing drugs to treat diseases can provide important benefits, enabling the administration of multiple drugs at the same time. For example, by using combinations of drugs, with which the efficacy against a specific mechanism of the pathogen is greater than the efficacy of any individual drug, it would be possible to achieve better therapeutic effects. Another approach is to combine several drugs, with each drug acting on a different mechanism of the pathogen [10]. Their combined use may reduce the potential for the pathogen to develop resistance [11]. This is now a standard in the treatment of many serious diseases, including cancer [12], bacterial infections [13], and human immunodeficiency virus (HIV) infections [14].

Up until now, various approaches have been suggested. One is to use available angiotensin receptor 1 (ATR1) blockers, such as losartan, thereby preventing SARS-CoV-2 from attaching to angiotensin-converting enzyme 2 (ACE2) on the human cell with its spike glycoprotein [15]. The other approach is to directly block the SARS-CoV-2 spike glycoprotein with a combination of existing drugs, preventing the virus from attaching to the human cell. Kaletra (lopinavir-ritonavir combination) alone or in combination with α -interferon, reverse transcriptase inhibitor DESCOPY (emtricitabine with tenofovir alafenamide fumarate), oseltamivir, and guanosine analog and reverse transcriptase inhibitor ribavirin, are being tested on SARS-CoV-2 patients. Trials with ritonavir plus ASC09, umifenovir, and remdesivir are either planned [16] or have already been performed. Antiviral drugs such as favipiravir, chloroquine, and nucleotide analog remdesivir are also under investigation [17]. In our research, we use the second approach, by identifying combinations of available antiviral drugs that could efficiently inhibit the spike glycoprotein of the virus.

Given the research and clinical findings to date, we decided to systematically investigate the possibility of administering pre-existing antiviral drugs and their combinations to treat COVID-19. As a tool, we used the VINI in silico model of cancer [18]. This model currently runs on the supercomputer "Bura" at Rijeka University and performs virtual drug screening [19] on KEGG diseases' metabolic pathways [20]. The high accuracy of this model in predicting the efficacy of cancer drugs and their combinations against the various types of cancer has been confirmed by comparison of the computed results with in vitro NCI-60 data and clinical trials [18,21]. Moreover, the VINI model is versatile, and can be used either for virtual drug screening on nearly all diseases described by KEGG metabolic pathways, or for only one specific protein. In our work, we decided to use the VINI model for a virtual drug screening on a specific structural SARS-CoV-2 protein, called spike glycoprotein.

Like other coronaviruses, SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins; the N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope [22] (see Fig. 1).

Although all four of these structural proteins are potential drug targets, we restricted our investigation to the spike protein, a glycoprotein that allows for the binding of SARS-CoV-2 to ACE2 (an angiotensin converting enzyme 2) and the transfer of viral RNA material to the host cell [23]. The first three-dimensional structures of spike glycoproteins were deposited by the same author's team at www.rcsb.org [24] on March 06, 2020. In our study, we used one of these structures, designated 6VXX [25], as a target for the virtual drug screening.

In this research we have achieved the following results:

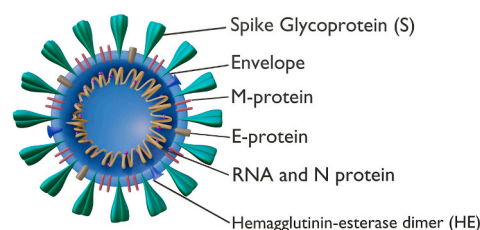


Fig. 1. Schematic presentation of SARS-CoV-2 virus, depicting its four structural proteins.

1. We computed binding free energies of 44 FDA-approved small-molecule antiviral drugs and 5 interferon antiviral drugs with SARS-CoV-2 spike proteins.
2. Based on the computed results, we concluded that HIV antiviral drugs and their combinations could be a good drug candidates for COVID-19.
3. To the best of our knowledge, we are the first to perform in silico modelling of inhibition of spike glycoprotein using drug cocktails (up to 3 drugs),
4. Our analysis has shown that the combination of cobicistat-abacavir- rilpivirine HIV drugs could be one of the best inhibitors of the SARS spike protein and a potent candidate for further clinical trials.

The rest of the paper is structured as follows: Section 2 describes the used methodology, software packages, and workflows used to perform computational tasks and analyze the results. The computed free binding energies and inhibition efficiencies of single, double, and triple drug combinations are presented in Section 3. Finally, the obtained results and predicted efficacy of drug combinations are discussed, with some final conclusions given in Section 4.

2. Methodology

In our research, we perform virtual screening of the efficacy of existing and approved antiviral drugs and their combinations on the SARS-CoV-2 spike glycoprotein. The task of virtual drug screening is to find drugs or chemical compounds (ligands) with high free binding energy (in further text abbreviated ΔG) to target molecules (receptors). Some chemical compound or approved drug with higher ΔG to the target may have a chance to be a potent therapeutic because it better inhibits the target. The VINI model uses the AutoDock Vina [26] to calculate ΔG [27] between proteins and small molecules. For calculating ΔG of the protein-protein complexes, the VINI model uses the Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approach [28]. In the VINI model the MMPBSA approach is implemented via the Hex docking tool [29], Gromacs molecular dynamics package [30], APBS (Adaptive Poisson-Boltzmann Solver) [31], and the g_mmpbsa [32] tool. Unlike calculating ΔG between protein and small molecule, calculating ΔG between two proteins in the VINI model is a computationally very demanding task. In the first step, Hex builds a complex composed of these two proteins, after which Gromacs performs the molecular dynamics simulation of that complex. The trajectories of atoms generated by Gromacs simulation are inputs for g_mmpbsa. Finally, g_mmpbsa calls APBS to compute ΔG . According to the chemical convention, ΔG values are negative, expressed in kcal/mol units. Thus, when it comes to free binding energy, the higher the free binding energy corresponds to the lower ΔG value. Consequently, a lower ΔG value means better receptor inhibition. Receptors can be any kind of chemical compounds, but the most common receptors in biological processes are proteins. Unlike receptors, drug candidates are small molecules of several to several dozen atoms, but are also proteins.

The research is divided into 4 stages:

2.1. Data gathering

We identified approved generic antiviral drugs from the U.S. Food and Drug Administration (FDA) portal. At the time of writing, there were 50 such drugs. Of these, 44 are small molecule drugs, 5 are interferon drugs, and one drug is sinecatechin, a specific aqueous extract of green tea leaves from *Camellia sinensis*.

From the these 44 small molecule drugs, we identified 22 drugs indicated for HIV, 6 for influenza virus (IFV), 6 for herpes simplex virus (HSV), 4 for hepatitis B virus (HBV), 4 for cytomegalovirus (CMV), and 2 for respiratory syncytial virus (RSV). The 3D structures of these molecules and interferon were fetched from the DrugBank [39]. For the SARS-CoV-2 spike glycoprotein we used the 6VXX structure file from the RCSB portal.

2.2. Computing the efficacy of single drugs

We computed the binding energies and estimated the inhibition of the SARS-CoV-2 spike glycoprotein with 49 FDA-approved antiviral drugs with the VINI model. For 44 small molecule drugs, the VINI model used the AutoDock Vina software, which performs docking and binding free energy calculation between the SARS-CoV-2 spike glycoprotein and each small-molecule. Finally, the VINI model used the MMPBSA procedure for five interferon drugs. Of all these 49 drugs, most HIV drugs have shown to express high inhibition of SARS-CoV-2 spike glycoprotein (see Results section), while other antiviral drugs mainly show weak or no efficacy.

2.3. Computing the efficacy of drug combinations

In order to reduce the very high time and resources demands for computing double and triple combinations for all drugs, we decreased the number of drugs to be used in this stage. We proceeded with HIV drugs only, as ΔG s of almost all other antiviral drugs to the SARS-CoV-2 spike glycoprotein were larger than -7.0 kcal/mol, thus considered as not significant.

Due to possible adverse drug interactions, we limited our search for cocktail therapies with up to three individual drugs. When computing ΔG of double and triple drug combinations, the result depends on the order the drugs have been applied to the spike glycoprotein. For example, the ΔG of protein P first binding with drug A and then with drug B is different from the ΔG of the same protein binding first with drug B and then with drug A. Symbolically, this can be written as:

$$\Delta G(P, A, B) \neq \Delta G(P, B, A) \quad (1)$$

The total number of single, double, and triple combinations with N drugs can be computed as follows:

$$M = N + N^2 + N^3 \quad (2)$$

In order to compare the drugs as well as the combinations of drugs, we defined the efficiency (E) of a particular combination of drugs or a single drug as the absolute sum of the lowest (min) and highest (max) total binding energy:

$$E = |\min(\Delta G) + \max(\Delta G)| \quad (3)$$

In the case of single drugs, the min and max ΔG s are equal; therefore, efficiency is defined as the absolute value of $2\Delta G$.

2.4. Examining the toxicity of drug combinations

Drug-to-drug interaction (D2D) can cause serious adverse health effects. Therefore, in this stage, we performed D2D analysis of the 10 HIV drug combinations showing the highest inhibition of spike glycoprotein (see Fig. 3 in the Results section). Analysis was performed using Medscape drug-drug interaction software and LexiComp Drug

Interactions database [33]. Drug combinations that are not recommended without reducing the dosage of the individual drugs are discarded, and given no further consideration as a potential remedy for the COVID-19 disease.

3. Results

In the first step, we computed the ΔG s of the 44 FDA approved antiviral drugs (excluding 5 interferon drugs and sinecatechin) using the VINI model. The results of these calculations are presented in Fig. 2.

Sinecatechin is a topical ointment prepared from green tea leaves and not a single chemical compound and the VINI model is unable to process it. Therefore, we omitted sinecatechin from further analysis.

In order to study the efficacy of interferon drugs, we provided to the VINI model the 1ITF structure from RCSB [34]. We have chosen this structure because it describes human interferon alpha, which is the active ingredient in each of the 5 FDA-approved antiviral interferon drugs with the following generic names: Peginterferon alfa-2a, Peginterferon alfa-2b, Interferon alfa-2a, Recombinant, Interferon alfacon-1, and Interferon alfa-2b. However, Gromacs failed to process the complex of spike glycoprotein and human interferon alpha. We tried another structure of the spike glycoprotein from RCSB, 7CN9 [35], but with the same result. The SARS-CoV-2 spike glycoprotein is a heavily glycosylated, large and complex protein, with three protomers, each of them composed of 1260 amino acids. These protomers twist around each other, forming a triple helical structure. In our opinion, that creates this complexity, which is further increased by the complexity of human interferon; this stopped Gromacs from carrying out MD simulations. Because these protomers have very similar structures, we used only one of these protomers in further computations. This time, Gromacs succeeded in performing MD simulation of a single protomer in a complex with interferon, but warned that this complex may be unstable.

Because of this instability, g_mmpbsa failed to perform MMPBSA calculation of spike glycoprotein and interferon complex. We tried to carry out this calculation with another tool, the MMPBSA software from the AmberTools software suite [36]. MMPBSA from AmberTools uses both GB (Generalized-Born) and PB (Poisson-Boltzmann) method for ΔG calculation. The PB method provides slightly more accurate results than GB [37]. On the other side, the computational time required for the PB method is significantly longer than for the GB method. In our case, the GB calculation lasted 0.62 h and the PB calculation 9.72 h. This was an important finding in this study, that the GB method, when performing on a HPC (High Performance Cluster), can be very useful in a coarse virtual drug screening over a very large chemical spaces. The mean value of ΔG for interferon-spike glycoprotein complex calculated with GB method was -26.8 kcal/mol, while that calculated with PB method was -39.57 kcal/mol.

The ΔG of interferon-spike glycoprotein complex is about 3.9 times higher than the ΔG of saquinavir-spike glycoprotein complex, HIV drug with a highest ΔG to the spike glycoprotein. However, interferon, unlike saquinavir and other small molecule antiviral drugs, is a large molecule with an approximate weight of 19260 g/mol. Therefore, ΔG of -39.57 kcal/mol is not high enough to ensure its stable binding to a protomer in this complex. That is a reason why Gromacs warned that this complex may be unstable.

The size and weight of a molecule are also important for an overall inhibitory potential of a certain antiviral drug in which that molecule is an active ingredient. To show this, let us define TIP (total inhibitory potential) of a certain compound per unit volume V as the product of the number N of its molecules in that volume with its ΔG to the virus:

$$TIP = (N * \Delta G) / V \quad (4)$$

Furthermore, the number of molecules N in a unit volume is inversely proportional to its molecular weight M , where C is the coefficient of proportionality:

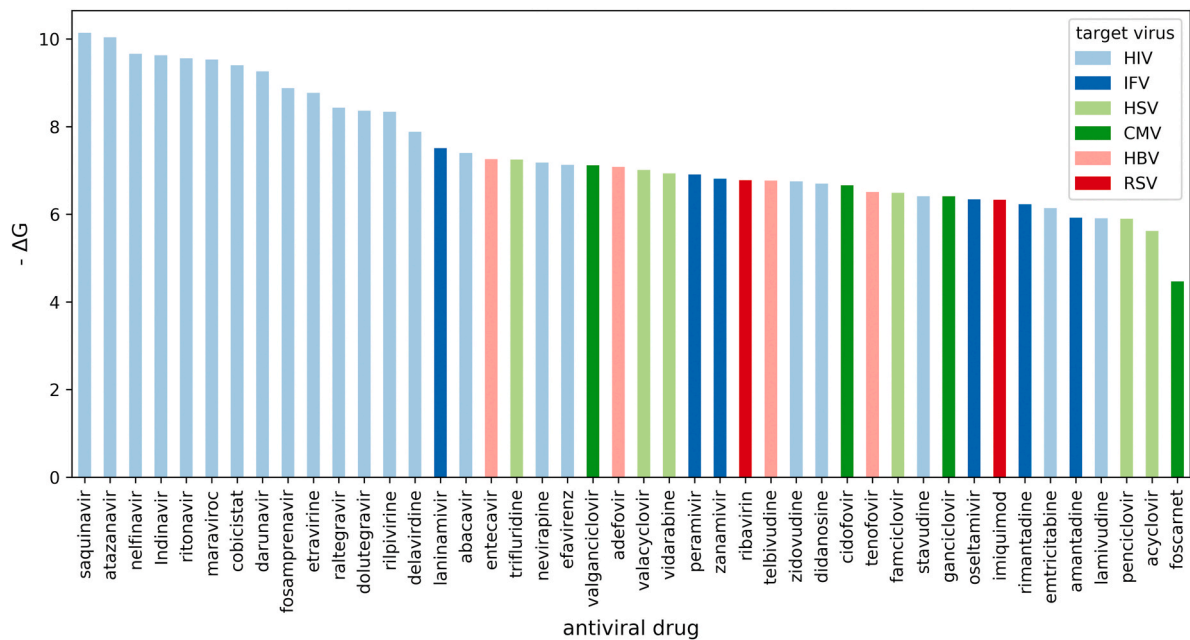


Fig. 2. Calculated ΔG s of 44 small-molecule antiviral drugs with SARS-CoV-2 spike glycoprotein. A highest ΔG to the spike glycoprotein is expressed by saquinavir, and a lowest by foscarnet, a cytomegalovirus drug. The drugs for which ΔG is higher than -7.0 kcal/mol are considered to have minor or no inhibition of the SARS-CoV-2 spike glycoprotein. Exact values of ΔG s for these drugs are given in Table 1 in supplementary files.

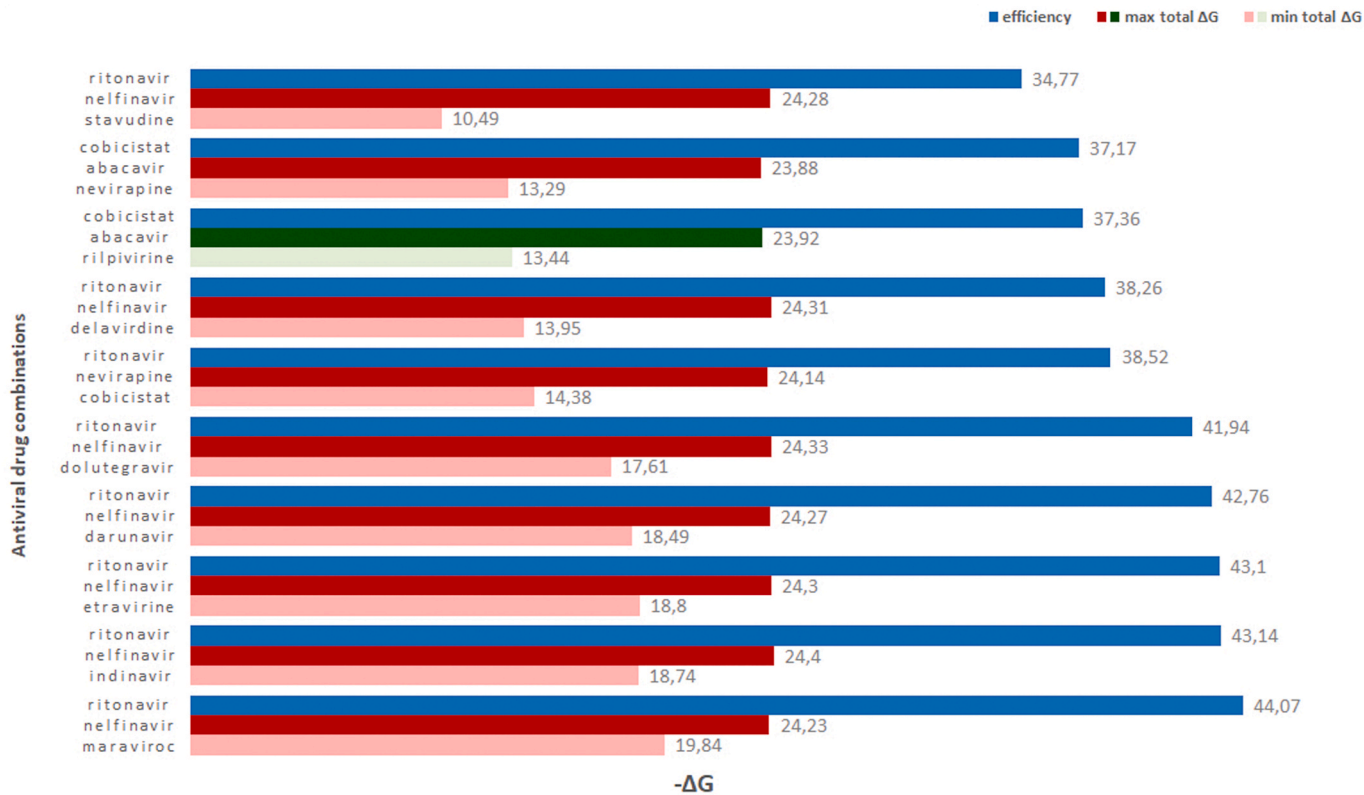


Fig. 3. Ten combinations with the highest inhibition of SARS-CoV-2 spike glycoprotein. All details of experimental results for these 10 combinations are given in Table 2, in supplementary materials.

$$N = (C \cdot V) / M \quad (5)$$

By substituting N from (5) in (4) one obtains:

$$TIP = C \cdot (\Delta G / M) \quad (6)$$

For interferon with $M = 19260$ g/mol and $\Delta G = -39.57$ kcal/mol, (6) will reduce to:

$$TIP_i = C_i \cdot 0.002 \quad (7)$$

and for the small molecule drug saquinavir with $M = 670.84$ g/mol and $\Delta G = -10.14$ kcal/mol, (6) will reduce to:

$$TIP_s = C_s \cdot 0.015 \quad (8)$$

Finally, from (7) and (8):

$$TIP_i = TIP_s \cdot (C_i / C_s) \cdot 0.13 \quad (9)$$

If C_i and C_s are equal, then the total inhibitory potential of interferon is about 7.69 times smaller than the total inhibitory factor of saquinavir. However, for different molecules, C_i and C_s will have different values, and these values can be determined experimentally. Regardless of that fact, equation (9) indicates that interferon, despite its much higher ΔG , has significantly lower total inhibitory potential than saquinavir, and consequently, other small molecule antiviral drugs.

Besides, clinical trials to date have not confirmed any efficacy of interferon in the treatment of COVID-19 patients, with its use further increasing their mortality rate [38]. Whether ineffectiveness and increased mortality are due to the possible adverse effects of interferon on the already overreacted immune system in COVID-19 patients, or some other processes are responsible for this, is difficult to assess at this time. Based on the results from Gromacs, previous consideration, and the results from clinical trials, we decided to drop interferon from further analysis.

From equation (2) and because AutoDock Vina simulation between SARS-CoV-2 spike glycoprotein and each drug was performed 10 times, it follows that for all single, double, and triple combinations of $N = 44$ drugs, the total number of required simulations would be $10 \cdot M$, which is a total of 871.640 different drug-protein combinations. We estimated that computing binding energies for all these combinations, on the computing infrastructure available for this study, would take more than two weeks. Such a long calculation is unacceptable given the situation caused by the SARS-CoV-2 virus, which requires rapid development of new drugs. To decrease computational time, we limited the scope of virtual drug screenings to HIV drugs only. The main basis for such a decision is that most HIV drugs have a binding energy to the SARS-CoV-2 glycoprotein higher than other antiviral drugs (Fig. 2) thus making them better candidates for investigating the impact of their double and triple

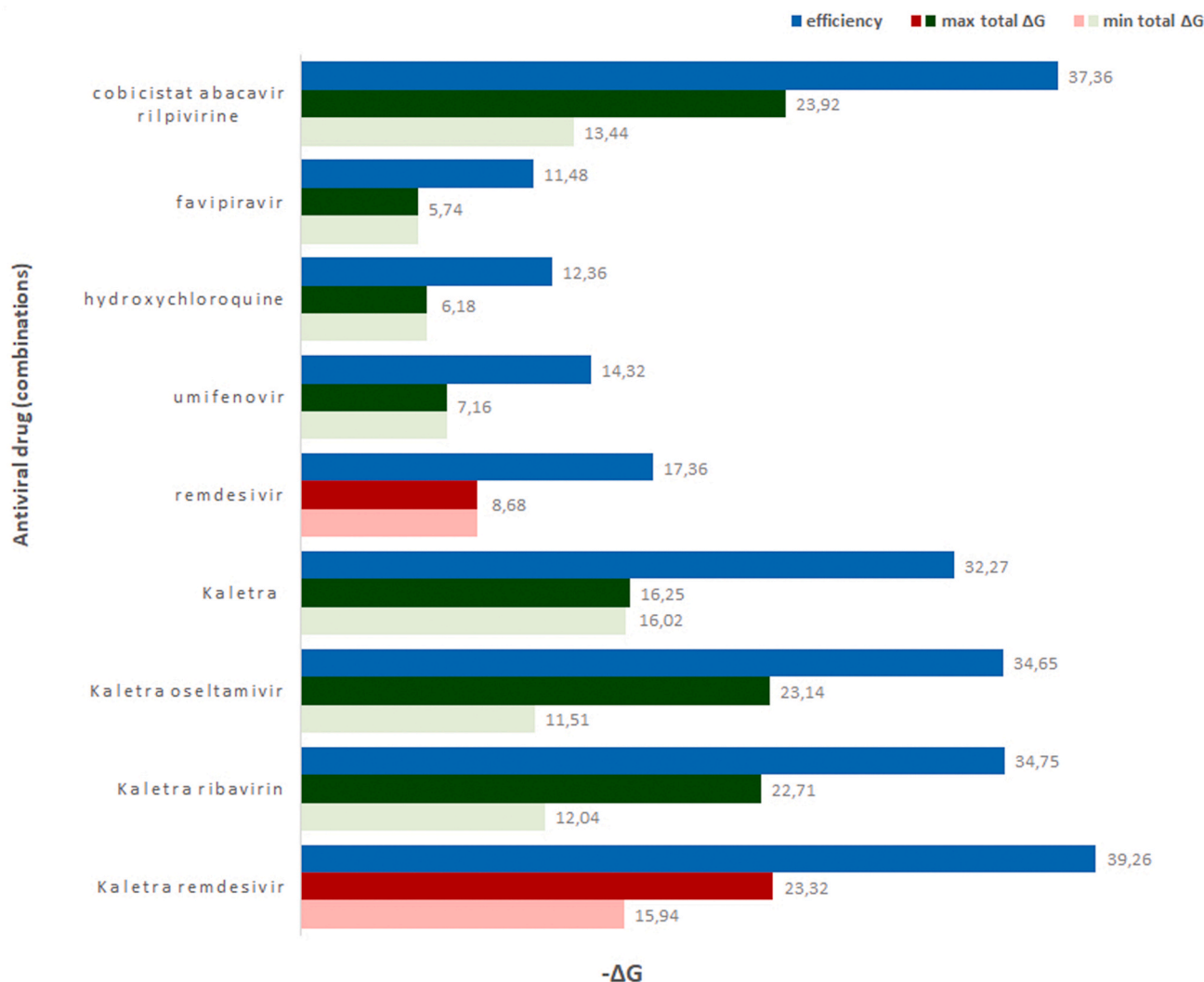


Fig. 4. Results of the relative efficacy of cobicistat-abacavir-rilpivirine cocktail against cocktails and single drugs already used to treat COVID-19, or are under investigation. Details of experimental results are given in Table 3, in supplementary materials.

combination on that virus.

Consequently, the ΔG values for all double and triple combinations of HIV drugs were computed, and 10 combinations with the highest inhibition of SARS-CoV-2 spike glycoprotein were observed. The results of these calculations are shown in Fig. 3.

All calculated values refer to the ΔG between SARS-CoV-2 glycoprotein and the drugs, which are given in kcal/mol units. Drug-drug interactions and possible side effects of single drugs were examined using Medscape [40] and Lexicomp [33] drug interaction checkers. Combinations falling into category D (it is not recommended to administer these combinations without reducing the dosage of the individual drugs) are indicated in red. The combination cobicistat-abacavir-rilpivirine is highlighted in green, as both Medscape and Lexicomp found no serious interaction effects. This means that no dosage reduction of the individual drugs is required (category C). In order to compare the relative efficacy of the cobicistat-abacavir-rilpivirine cocktail in inhibiting the SARS-CoV-2 spike glycoprotein against cocktails and single drugs already used to treat COVID-19 (or under investigation), we computed ΔG for the following combinations and compounds: Kaletra alone, Kaletra in combination with oseltamivir, remdesivir and ribavirin, and hydroxychloroquine, remdesivir, favipiravir and umifenovir alone. Results of this comparison are presented in Fig. 4.

The cocktails and single drugs that fall into category C are indicated in green. This category does not require a reduction in doses of the individual drugs in combination. The combination of Kaletra with remdesivir and remdesivir alone are highlighted in red: although remdesivir has recently been approved by the FDA, phase 3 clinical trials are still ongoing.

The predicted efficacy of the cobicistat-abacavir-rilpivirine combination, computed as in (3), is higher than the predicted efficacy of other cocktail therapies currently used or investigated, to our knowledge, against SARS-CoV-2. The computed gain of the cobicistat-abacavir-rilpivirine cocktail in inhibiting the SARS-CoV-2 spike glycoprotein over Kaletra is 13.62%, over Kaletra with oseltamivir 7.25%, and over Kaletra with ribavirin 6.98%. The gain over single drugs either used or planned to be used is much higher, is 69.27% over favipiravir, and 61.67% over umifenovir. The gain over hydroxychloroquine is not relevant, as this drug works on a completely different basis, i.e., by suppressing the immune system's overreaction. Only the predicted efficacy of Kaletra with a remdesivir is higher than the cobicistat-abacavir-rilpivirine combination, equaling 4.83%. However, remdesivir is not generally approved drug, and is still under investigation in ongoing clinical trials.

From the results, one can discern a slight fluctuation of ΔG results for the same receptor-ligand pair in different experiments. Thus, in the first experiment ΔG for indinavir was -9.63 kcal/mol (Table 1), and in the second experiment -9.50 kcal/mol (Table 2). Such fluctuations are expected and related to the stochastic nature of AutoDock Vina, which initiate simulations of the same receptor-ligand pair from different, randomly selected initial coordinates.

4. Discussion and conclusions

The SARS-CoV-2 spike glycoprotein is a key factor in binding of the virus to angiotensin-converting enzyme (ACE2), allowing the virus to transmit its RNA material into the host cell, and hence its further reproduction. Effective inhibition of the SARS-CoV-2 spike glycoprotein can slow or even completely inhibit the reproduction of the SARS-CoV-2 virus, increasing chances of the host immune system to fight it. The results of our study provide scientific confirmation of the high efficacy of some combinations of approved HIV drugs, including compounds still under investigation, for the inhibition of SARS-CoV-2 spike glycoprotein. Application may pave the way for new, more effective therapies than those currently used in the treatment of COVID-19.

The ten most effective HIV drug combinations, predicted by the VINI

model as the most effective in inhibiting SARS-CoV-2 spike glycoprotein, highlights the cobicistat-abacavir-rilpivirine combination as standings out. Ranked eighth regarding the efficiency, for its relatively low toxicity and acceptable drug-drug interactions, it is suitable at standard doses of individual drugs without the need for adjustment.

The predicted efficacy of this combination in the inhibition of SARS-CoV-2 spike glycoprotein is greater than the efficacy of the drug combinations or individual drugs, which, to the best of our knowledge, have been tried, or are planned to be tried in the treatment of COVID-19 (Kaletra (lopinavir-ritonavir combination), Kaletra in combination with oseltamivir, Kaletra in combination with ribavirin, as well as hydroxychloroquine, favipiravir and umifenovir as single drugs).

The predicted efficacy of the cobicistat-abacavir-rilpivirine combination is slightly lower than the efficacy of the Kaletra-remdesivir combination. However, the efficacy of remdesivir is still under investigation and its' possible side effects and interactions with lopinavir and ritonavir are not known well.

From the obtained results, it can be concluded that the order of action of individual drugs (in some combination) on the SARS-CoV-2 spike glycoprotein does matter. As such, the highest ΔG of the cobicistat-abacavir-rilpivirine combination on the glycoprotein is achieved when it first binds to cobicistat, then to abacavir, and at the end to rilpivirine. This indicates that a 3-time daily regimen in which cobicistat is used first, then abacavir, and last rilpivirine, could increase the effectiveness of this combination therapy. However, a detailed pharmacokinetic analysis of the effects of individual drugs will be required to evaluate the benefits of such a regimen, which goes beyond the scope of this study.

Therefore, a clinical trial of cobicistat-abacavir-rilpivirine with a limited number of COVID-19 patients, who are in moderately severe and severe condition, is warranted.

Author contributions

Tomić D: Development of the VINI in silico model of cancer and its extension towards COVID-19 disease. Screening antiviral drugs and their combinations for their inhibition of the SARS-CoV-2 spike glycoprotein. Prepared a first draft version of the manuscript. Contributed to the preparation of revised paper.

Davidović D: Discussed the results and contributed to the writing. Contributed to the preparation of revised paper.

Szasz A.M: Discussed the results and contributed to the writing.

Rezeli M: Discussed the results and contributed to the writing.

Pirkić B: Analysis of possible routes of SARS-CoV-2 virus transmission from humans to animals and vice versa. Discussed the results and contributed to the writing.

Petrik J: Produced the original picture of SARS-CoV-2. Screening and analysis of potential clinically significant drug - drug interactions.

Baćić Vrca V: Screening and analysis of potential clinically significant drug - drug interactions.

Jandel V: Discussed the results and contributed to the writing.

Lipić T: Preparation of graphics.

Karolj S: Discussed the results and contributed to the writing.

Josip M: Preparation of graphics.

Milković Periša M: Discussed the results and contributed to the writing.

Šojat Z: Discussed the results and contributed to the writing.

Medved Rogina B: Discussed the results and contributed to the writing.

Data availability statement

The datasets generated during this study (compressed archive 1 GB in size) can be found in the full-text institutional repository of the Ruđer Bošković Institute, <http://fulir.irb.hr/6161/>. The latest release of the VINI in silico model of cancer is available at <https://github.com/draskot/Vini>.

Declaration of competing interest

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

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Appendix A. Supplementary data

Three tables (Table 1, Table 2, and Table 3) were uploaded separately in the submission. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.imu.2021.100529>.

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