Rationale Based Selection and Prioritization of Antiviral Drugs for COVID-19 Management

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Abstract

Infection with SARS-CoV-2 has resulted in COVID-19 pandemic and infected more than 5 million individuals with around 0.35 million deaths worldwide till May 2020 end. Several efforts are on in search of therapeutic interventions, but the preferred way is drug repurposing due to the feasibility and urgency of the situation. To select and prioritize approved antiviral drugs and drug combinations for COVID-19, 61 antiviral drugs having proven safety profile in humans were subjected to virtual screening for binding to three select targets namely human angiotensin-converting enzyme receptor-2 receptor-binding domain (hACE-2) involved in virus entry, SARS-CoV-2 RNA dependent RNA polymerase (RdRp) responsible for viral RNA replication and SARS-CoV-2 main protease (M^{Pro}) causing proteolytic processing of viral polyprotein slab. Targeting multiple 'disease pathogenesis specific proteins' within a close network of interaction or having dependent functionality can provide effective intervention. Ledipasvir, Daclatasvir, Elbasvir, Paritaprevir, Rilpivirine and Indinavir were identified as candidate drugs of interest for COVID-19 based on a derived combined activity score, pharmacokinetic and pharmacodynamic parameters. Ledipasvir and Daclatasvir and their approved marketed combination with Sofosbuvir emerged as leading candidate drugs/drug combinations for SARS-CoV-2. These candidates have the potential for the antiviral activity for SARS-CoV-2 infection better than the investigational drug Remdesivir and other antiviral drugs/drug combinations being evaluated. These drugs/combinations merit systematic fast track preclinical and clinical evaluation for COVID-19 management. The present work brings back attention to the potential usefulness of approved antiviral drugs/drug combinations, commonly available with established safety profile, currently not in focus for COVID-19. It provides a rationale based approach for the selection of drugs with potential antiviral activity against SARS-CoV-2 highlighting the desired properties.

Keywords: Antiviral, COVID-19, hACE-2, main protease, RNA dependent RNA polymerase, SARS-CoV-2, Drug Repurposing

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection has resulted in the current COVID-19 pandemic. Worldwide this disease has infected more than 5 million individuals till end of May 2020 with a mortality rate ranging from 5 to 10%[1]. Currently, there are no specific antiviral drugs or vaccines available for the treatment and management of COVID-19. Thus, drug repurposing offers a potential and rapid solution for the mitigation of COVID-19. There are several efforts ongoing to reposition approved and marketed drugs. These repurposed drugs either aim to attain antiviral effect (viral replication cycle) or control symptoms of the disease. Amongst these two strategies, we focused on rationale based identification and prioritization of effective antiviral drugs for COVID-19. Clinical trials of antiviral drugs Lopinavir/Ritonavir, Remedesivir, Favipiravir, Umifenovir, Oseltamavir, Danoprevir and Darunavir are ongoing[2]. Several other non-antiviral drugs are also being evaluated for antiviral effects against SARS-CoV-2. Examples include Chloroquine, Hydroxychloroquine, Nafamostat, Camostat and Ivermectin[3]. Combinations of antiviral drugs are often used in the management of viral infections. In our quest for identifying an effective intervention for SARS-CoV-2, we have explored a rationale based approach for selection and prioritization of approved anti-viral drugs or drug combinations for COVID-19. Targeting multiple virus pathogenesis specific proteins within a close network of interaction or having dependent functionality can provide effective intervention. Based on the literature evidence we selected three key targets within a close network of interaction for the evaluation. Human angiotensin-converting enzyme receptor-2 receptor-binding domain (hACE-2) involved in virus entry, SARS-CoV-2 RNA dependent RNA polymerase (RdRp) for the viral RNA replication and SARS-CoV-2 Main Protease (M^{Pro}) for virus maturation[4].

SARS-CoV-2 requires expression of the cellular receptor hACE-2 to infect cells[5,6]. The full-length hACE-2 contains a structural transmembrane domain, which anchors its extracellular domain to the plasma membrane. The extracellular domain has been demonstrated as a receptor for the spike (S) protein of SARS-CoV and recently, for the SARS-CoV-2. During infection, the S protein is cleaved into subunits, S1 and S2[6]. S1 binds ACE-2, whereas S2 anchors the S protein to the viral membrane[7,8]. SARS-CoV-2 binds to hACE-2 with higher affinity (~10 fold) than other coronaviruses and is an important target for intervention[5].

Furthermore, the viral genome replication process is an important intervention step for anti-viral activity including SARS-CoV and SARS-CoV-2. Coronaviruses use a multiprotein complex to replicate their RNA based genomes. The cleavage of viral polyproteins (ORF1a and ORF1b) produces a set of non-structural proteins (NSPs). Of these, RNA-dependent RNA polymerase (RdRp or NSP12) catalyzes the synthesis of

structural proteins that are known to play an important role in the replication and transcription cycle of the virus. RdRp is the primary target for nucleoside analogues antiviral inhibitors such as Remdesivir, an investigational drug under evaluation for SARS-CoV-2 infection in clinics[9,10].

Main Protease (M^{Pro}) of coronaviruses have been studied extensively for drug discovery. They are papain-like proteases involved in the self-maturation and processing of viral replicase enzymes[11,12]. Due to their key role in virus replication, inhibition of these proteases emerges as an important drug target. Furthermore, with their very low similarity with human proteases, inhibitors of M^{Pro} are found to be very less cytotoxic[11,13].

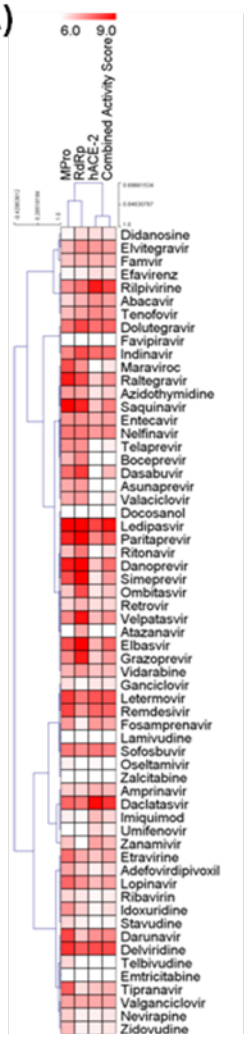
Drugs or drug combinations impacting the upstream step of viral entry as well as viral replication and maturation intracellularly have the potential for effective antiviral activity against SARS-CoV-2. Sixty-one marketed small-molecule antiviral drugs were evaluated *in-silico* for their potential to bind to these targets of interest. Reported crystal structures of these targets served as templates for virtual screening. Binding energy to the respective target was expressed as Kcal/mole. Lower binding energy is indicative of high-affinity binding. A combined activity score was assigned based on binding energy to targets of interest and our perspective of their relevance. The high combined activity score is indicative of the potential for high-affinity binding at all three targets of interest. Combined activity scores of shortlisted candidates along with their reported pharmacokinetic and pharmacodynamic activity was evaluated to prioritize and select lead drug candidates for effective prophylactic or therapeutic application for COVID-19 management.

2. Results and Discussion

Virtual screening of 61 approved antiviral drugs demonstrates several approved antivirals have the potential to intervene SARS-CoV-2 cycle in humans at multiple steps and can potentially offer avenues to manage COVID-19. In particular, amongst antivirals the focus for COVID-19 has been on Lopinavir/Ritonavir (Kaletra)[14,15], Favipiravir[16], Umifenovir *and* Remdesivir (Investigational drug)[9,17,18]. Remdesivir, a small molecule nucleotide analogue, has shown broad-spectrum antiviral activity against RNA viruses in several families including C Coronaviridae (such as SARS-CoV, MERS-CoV and strains of bat coronaviruses capable of infecting human respiratory epithelial cells). Remdesivir is a drug with evidence for a decrease in viral load in preclinical and clinical studies for which human safety has been demonstrated. The US Food and Drug Administration (FDA) reviewed the investigational new drug (IND) application filed by the company for Remdesivir to treat COVID-19 and granted investigational new drug authorization to study the drug in February 2020. Interest in Remdesivir, Gilead's investigational drug amid the ongoing coronavirus pandemic has been high. The New England Journal of Medicine in mid-April published an analysis indicating that

over two-thirds of a small group of severely ill COVID-19 patients saw their condition improve after treatment with Remdesivir[18]. The impact of viral load reduction on clinically relevant outcomes like reducing hospital stay or decreasing mortality is yet to be demonstrated in clinics conclusively. It has been suggested that Remdesivir has activity beyond RdRp inhibition[10]. This promising investigational drug was used as a reference for comparison in our study. Remdesivir exhibited good binding to all three targets of interest. The binding energy was -8.2, -7.2 and -7.8 Kcal/mole for M^{Pro}, RdRp and hACE-2, respectively **(Figure 1)**. The combined activity score for Remdesivir was better than all other above-mentioned drugs. Rank order for combined activity score for these drugs in clinics is Remdesivir (7.8) > Darunavir (7.4) ≥ Lopinavir/Lopinavir metabolites (7.1/7.4) ≥ Favipravir/Favipiravir metabolite (6/7.2) > Oseltamvir (6). Remdesivir also exhibited a combined activity score better than other non-antiviral drugs being repurposed for antiviral effect against SARS-CoV-2. Binding energy, combined activity scores for these drugs and their relevant metabolites along with a brief overview and comments are captured in **Supplementary Data S1.**

Figure 1 (A) (B)



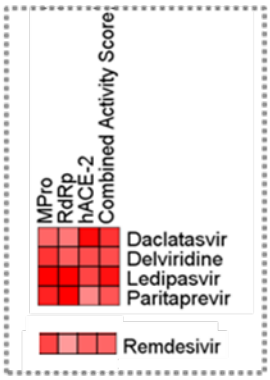


Figure 1. (A) Hierarchical clustered Heatmap illustration for the comparative analysis of binding score and the combined score of 61 approved antivirals (B) Rank product analysis of binding score indicating top molecules from screen and Remdesivir as a positive control.

Supplementary Data S2 provides binding energy for M^{Pro}, RdRp and hACE-2 and combined activity score for 61approved antiviral drugs. The range of binding energy of approved antiviral drugs exhibiting the best binding energy for the respective target is outlined in **Table 1.**

Drugs exhibiting very low binding energy (< -9 Kcal/mole) with the targets are predicted to have high affinity. Thus, such a drug scan potentially provides effective intervention at the proposed target at low concentrations. Drugs exhibiting this property are Ledispavir (RdRp and M^{Pro}), Elbasvir (RdRp), Danoprevir (RdRp), Saquinavir (M^{Pro}) and Paritaprevir (RdRp). These drugs are therefore shortlisted for detailed scrutiny for repurposing. To identify the promising drug candidates impacting upstream step of viral entry as well as viral replication and maturation, drugs with binding energy to hACE-2 comparable or lower (-7.8 ± 0.5 Kcal/mole) than for Remdesivir and the combined activity score equivalent (7.8 ± 0.5) or greater than Remdesivir was shortlisted for detailed evaluation. The shortlisted drugs based on these properties are listed in **Table 2.** Ledipasvir and Daclatasvir exhibited strong ACE-2 binding (≤ -8.2 Kcal/mole) and combined activity score better than Remdesivir (> 8.2).

Ledipasvir and Daclatasvir are direct-acting antivirals used as part of combination therapy to treat chronic Hepatitis C, an infectious liver disease caused by infection with the Hepatitis C Virus (HCV)[19–21]. It inhibits Non-structural Protein 5A (NS5A) involved in viral RNA replication and assembly of HCV virions. Ledipasvir is available as a fixed-dose combination with Sofosbuvir (Harvoni), and this combination is approved by the FDA in October 2014 with or without Ribavirin for HCV genotype 1, 4, 5 and 6 infections[21,22]. Its use has also been proven successful in HCV patients infected with HIV. Daclatasvir was FDA approved in July 2015 for use with Sofosbuvir (Sovaldi) with or without Ribavirin to treat HCV genotype 1 and 3 infections[21,23]. Sofosbuvir is a nucleotide analogue inhibitor of the hepatitis C virus NS5B polymerase, a key enzyme mediating HCV RNA replication.

Ledipasvir emerges as a lead candidate for drug repurposing. It has significantly better combined activity against all three targets as compared to Remdesivir. Its combined activity score and binding with RdRp and M^{Pro} is high. Its binding energy is < -9 Kcal/mole for RdRp and M^{Pro} with good binding for hACE-2 (-8.2 Kcal/mole). **Daclatasvir** exhibits the strongest binding to hACE-2 amongst 61 antiviral drugs evaluated. Its combined activity score is next to Ledipasvir. The tight binding of the spike protein of SARS-CoV-2 to hACE-2 has been reported and intervention of this tight binding could be a key determinant for

antiviral activity against SARS-CoV-2. This target was, therefore, given higher weightage in combined activity score determination over M^{Pro} and RdRp. Daclatasvir is, therefore also shortlisted as a lead candidate. Their interaction with the binding pocket of targets has been depicted in **Figure 2**. The pharmacokinetic properties of these drugs are well worked out and an overview of the drugs is summarized in **Table 3**.

Figure 2

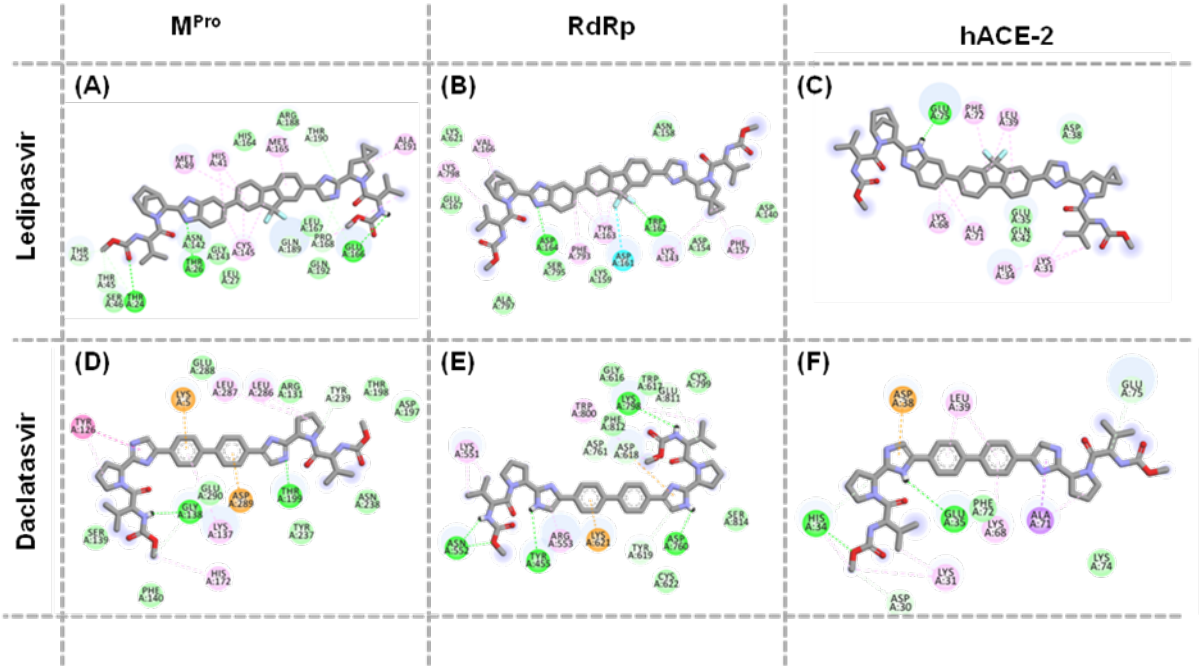


Figure 2. Ledipasvir interaction with active site residues of (A) M^{Pro} (B) RdRp and (C) hACE-2. It is followed by an interaction map of Daclatasvir with (D) MPro (E) RdRp and (F) hACE-2. It has been observed that both lead molecules make several strong contacts with binding site residues of targets.

Remdesivir (GS-5734) is a prodrug of the parent adenosine analogue GS-441524, which is efficiently delivered intracellularly. GS-441524 is metabolized to an active nucleoside triphosphate (NTP) intracellularly by the host cell[10]. Triphosphate metabolites of Remedesivir and GS-441524 exhibits good activity against intracellular targets, RdRp and M^{Pro}. The same is captured in **Supplementary Data S1**. GS-441524, which has access to extracellular target hACE-2 has significantly less affinity for this target. GS-441524 has a long half-life (half-life of 14h in Non-human primate and 20h in humans) suggestive of prolonged engagement of intracellular targets, whereas Remdesivir as parent molecule has a short half-life (0.4 h in non-human primate-NHP) and thus effective hACE-2 inhibition for a shorter duration **(Table 3)**. Protein binding data for Remdesivir is yet not available in the public domain.

Both Ledipasvir and Daclatasvir are not extensively metabolized. The median terminal half-life is 47 h for Ledipasvir. Daclatasvir has a half-life of 12 to 15 h. These drugs, therefore, have the potential for a prolonged effect on extracellular target hACE-2 as compared to Remdesivir. Effective intracellular concentrations in host cells for engagement of targets RdRp and M^{Pro} is evident from a mechanistic understanding of known antiviral activity against HCV. Therefore, Ledipasvir and Daclatasvir are selected as lead candidate drugs for repurposing for COVID-19.

Remdesivir has to be administered intravenously, whereas Ledipasvir, Daclatasvir are administered orally. Clinically, Ledipasvir and Daclatasvir are given in combination with Sofosbuvir. Sofosbuvir also exhibits a high combined activity score of 7.5. Approved antiviral drug combinations also merit evaluation for COVID-19 management. While Ledipasvir and Daclatasvir are highly protein-bound drugs (> 99%), Sofosbuvir exhibits moderate protein binding (65%) suggesting better free drug concentration availability at extracellular hACE-2 target. Apart from potency at hACE-2, free drug concentration availability could be a critical component for effective intervention.

Regarding this combination, however, it is important to point out that sofosbuvir has a short circulating half-life of 0.4 h as a parent molecule (like Remdesivir). Sofosbuvir is metabolized intracellularly into its active triphosphate form GS-46103 (2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate). This is then converted to dephosphorylated metabolite GS331007 subsequently. GS-331007 has an elimination half-life of 27h. Sofosbuvir, therefore, provides prolonged effect at intracellular targets such as RdRp and M^{Pro}. GS - 331007 which is accessible for extracellular target hACE-2, does not exhibit good binding to hACE-2 **(Supplementary Data S3)**.

While Daclatasvir and Sofosbuvir combination with Ribavarin is in use for HCV, the addition of Ribavirin to the regimen is not recommended for SARS-CoV-2 as Ribavarin does not exhibit good combined activity score (6.2) (**Supplementary Data S2)**[22]. Daclatasvir and Asunaprevir combination is approved in Japan for HCV. The combined activity score for Asunaprevir is low (5.7) and is not the preferred combination for SARS-CoV-2 as per our evaluation.

Other drug candidates that also merit attention are drugs with combined activity scores and hACE-2 binding comparable to/or better than Remdesivir. Rilpivirine, Delviridine, Paritaprevir, Letermovir and Dolutegravir have a combined activity score ≥ Remdesivir (7.8 to 8.3)[24]. All these drugs act directly and thus have the ability to bind to extracellular target hACE-2. These drugs also have high protein binding and therefore offer no distinct advantage over lead candidate drugs Ledipasvir and Daclatasvir **(Supplementary Data S4)**. These drugs, however, have the potential to offer efficacy comparable to or better than Remdesivir. Rilpivirine exhibits significantly greater binding to hACE-2 as compared to

Remdesivir. Furthermore, Paritaprevir also shows significantly greater binding to RdRp and M^{Pro} as compared to Remdesivir.

Rilpivirine and Delviridine are non-nucleoside reverse transcriptase inhibitors (NNRTI) which are approved for HIV-1 infections[25]. Rilpivirine was FDA approved in 2011. Dolutegravir is an HIV-1 integrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell in HIV. Dolutegravir was FDA approved in 2013 and has an excellent safety profile[26]. In the year, 2017, Dolutegravir in combination with Rilpivirine, was approved as part of the first complete treatment regimen with only two drugs HIV-1 (Juluca)[27]. This combination is recommended for the antiviral effect against SARS-CoV-2. Delavirdine was FDA approved in 1997 and is recommended only as second-line therapy and has inconvenient dosing schedule. Therefore, Delviridine is not selected for further evaluation.

Paritaprevir is a new generation direct-acting antiviral medication used as part of combination therapy to treat chronic HCV. It is well tolerated and in combination prescribed as first-line therapy for HCV genotypes 1a, 1b and 4. It inhibits NS3/4A serine protease of HCV involved in the maturation of viral particles. For HCV, Paritaprevir is often used in combination with other antivirals such as Ombitasvir, Dasabuvir, Ritonavir, and Ribavirin[28]. A low combined activity score was observed for these drugs- Ombitasvir (6.7), Dasabuvir (6.8), Ritonavir (6.4), and Ribavirin (6.2). These drugs are unlikely to contribute to antiviral activity against SARS-CoV-2 and combination with these drugs is not recommended. Combination with low dose Ritonavir should, however, is considered to boost levels of Paritaprevir for SARS-CoV-2.

Drugs exhibiting combined activity scores comparable to Remdesivir are Indinavir, Sofosbuvir, Darunavir, Abacavir and Tenofovir. Some critical features for potential antiviral effect against SARS-CoV-2 are highlighted. Indinavir is a directly acting drug with a half-life of 1.8 ± 0.4h, moderate protein binding (60%) and hACE-2 binding energy comparable to Remdesivir (-7.7 Kcal/mol). Indinavir has the potential to inhibit hACE-2 more effectively due to the availability of higher free drug concentration at the key target hACE-2. This is prescribed in combination with other HIV drugs. Based on its safety profile, Indinavir has been shortlisted as a candidate drug of interest. Fixed-dose combination of Indinavir with low dose Ritonavir is available for HIV. Ritonavir, with a poor combined activity score, is unlikely to contribute as an active antiviral moiety against SARS-CoV-2[29]. Ritonavir inhibits the hepatic metabolism of Indinavir and thus prolongs its half-life and this combination should be considered for SARS-CoV-2. The development of novel combinations and formulations of this drug for intravenous use in critically ill patients merits attention.

Darunavir, though a directly acting drug, has high protein binding and therefore offers no distinct mechanistic advantage over lead candidate drugs Ledipasvir and Daclatasvir or candidate drugs such as Rilpivirine or Paritaprevir (**Supplementary Data S1**).

Abacavir is another molecule with moderate protein binding (50%) and a short half-life of 1.54 ± 0.63 h. Parent molecule exhibits binding energy of -7.3 Kcal/mol with the hACE-2 suggests the potential for effective intervention. Intracellularly, Abacavir is converted by cellular enzymes to the active metabolite Carbovir triphosphate. The combined activity score for Abacavir is 7.4. Carbovir triphosphate has good binding energy for intracellular viral targets M^{Pro} and RdRp **(Supplementary Data S3)**[30]. Abacavir (ABC) is a powerful nucleoside analogue reverse transcriptase inhibitor (NRTI) with activity against HIV-1. Abacavir is often prescribed in combination with drugs such as Lamivudine, Zidovudine and Dolutegravir for HIV-1. The combined activity scores are 5.3 and 6.3 for Lamivudine and Zidovudine, respectively. Dolutegravir exhibits a good combined activity score (7.8) which is comparable to Remdesivir (7.8). Dolutegravir exhibits high protein binding and half-life of 14 h. Combination of Abacavir with Dolutegravir thus is preferred over its combination with Lamivudine or Zidovudine for COVID-19.

Tenofovir exhibits low protein binding (< 0.7% plasma) and has a terminal half-life of 17 h. Tenofovir disoproxil fumarate is the fumarate salt of the prodrug tenofovir disoproxil[31]. Tenofovir disoproxil is absorbed and converted to its active form, tenofovir, a nucleoside monophosphate analogue. Tenofovir is then converted, tenofovir diphosphate, by constitutively expressed enzymes in the cell[32]. Tenofovir diphosphate does not exhibit good binding potential for RdRp and M^{Pro}. This drug, in our view, does not merit prioritization over other highlighted candidate drugs for COVID-19. This drug is a component of multiple drug combinations approved for HIV-1, which include Emtricitabine, Efavirenz, Lamivudine and Cobicitat which exhibit low combined activity scores and thus are unlikely to contribute to antiviral activity against SARS-CoV-2[33]. Its reported combination with Rilpivirine is preferred but does not offer an advantage over other shortlisted candidate drug/drug combinations.

Elbasvir with distinctly high binding to RdRp (-10 Kcal/mole) and combined activity score of 7.8 which is comparable to Remdesivir, has the potential for effective intervention for SARS-CoV-2 replication and maturation. Its binding energy for M^{Pro} is -8.1 Kcal/mole. This drug has a half-life (geometric mean) of 24 h and is shortlisted as a candidate drug for COVID-19.

Elbasvir, is a direct-acting antiviral approved as part of combination therapy to treat HCV. It inhibits HCV NS5A protein[34]. It is available as a fixed-dose combination with Grazoprevir and is used with or without Ribavarin[21]. Ribavarin exhibits a low combined activity score (6.2). Grazoprevir exhibits a combined activity score of 7.2 and exhibits low

binding energy for RdRp (-8.7 Kcal/mole). The fixed-dose combination of Elbasvir with Grazoprevir without Ribavarin is suggested for COVID-19.

Danoprevir, an investigational drug for COVID-19 has a combined activity score of 7.7 which is comparable to Remdesivir. It exhibits distinctly better binding for M^{Pro} (-8.7 Kcal/mole) and RdRp (-9.8 Kcal/mole) as compared to Remdesivir. This drug, however, exhibits poor binding for hACE-2. Danoprevir, thus offers no distinct advantage over lead candidate drug Ledipasvir. Outcomes with drugs such as Ledipasivir from preclinical and clinical studies from efficacy perspective will help delineate the criticality of targeting hACE-2. Half-life and protein binding information is not available for Danoprevirin the public domain. Danoprevir is an NS3/4A protease inhibitor approved for HCV[21]. Clinical outcomes from ongoing clinical trials for COVID-19 will be of interest.

Saquinavir exhibits high binding to M^{Pro} (-9.0 Kcal/mole) but has a combined activity score (7.5) lower than Remdesivir. This drug has poor bioavailability and is administered intravenously like Remdesivir. Saquinavir is a highly protein-bound with low binding for hACE-2 and often given in combination with other drugs. This drug is not shortlisted for further perusal. Saquinavir is approved for the treatment of advanced HIV-1.

Raltegravir though has low binding energy for M^{Pro} (-8.7 Kcal/mole), it has a combined activity score (7.3) is lower than Remdesivir (7.8). It exhibits poor binding at hACE-2. Therefore, Raltegravir is not shortlisted as a candidate drug for COVID-19. This drug is the HIV integrase inhibitor approved by the FDA in 2007 for HIV in combination with other drugs.

In silico studies have identified two non-antiviral drugs Ergotamine[35] and Ubrogepant[36] with very low binding energy for hACE-2. Both these drugs are approved for their use in migraine. The binding energy for these non-antiviral drugs was also evaluated in our screen **(Supplementary Data S5).** These molecules exhibited the potential to bind to all three targets. Binding energy at key target of interest hACE-2 for Ubrogepant was low (-7.0 Kcal/mole). The binding energy of Ergotamine for all three targets was comparable to the identified lead candidate Ledipasvir **(Supplementary Data S5)**. Daclatasvir exhibited better binding at hACE-2 as compared to Ergotamine. It is important to note that Ubrogepant and Ergotamine are known to act on G-protein coupled receptors extracellularly. Thus, unlike anti-viral drugs, their ability to engage intracellular targets such as M^{Pro} and RdRp is not demonstrated. The reported *in silico* study had identified Ergotamine as a promising candidate amongst the study of 1785 drugs[35]. Commonly prescribed drugs for cardiovascular indications such as ACE inhibitors and angiotensin receptor antagonists do not exhibit binding to hACE-2[37]. This further strengthens the case for the prioritization of Daclatasvir and Ledipasvir as lead candidates for COVID-19. Therefore, Ubrogepant and

Ergotamine do not merit prioritization over the shortlisted anti-viral lead candidate or candidate drugs for COVID-19.

Recent studies have implicated that furin (human cell protease) is involved in the cleavage of the Spike (S) protein of SARS-CoV-2. S2 protein is cleaved by TMPRSS2, a human cell surface serine protease, resulting in membrane fusion[6,38]. These enzymes merit attention along with attention for hACE-2 as dependent functionality which can provide effective intervention upstream of viral replication and maturation. Camostat and Nafamostat are serine protease inhibitors in clinical practice for a long time in Japan for chronic pancreatitis. Camostat has been demonstrated to inhibit TMPRSS2. Nafamostat can prevent the fusion of the envelope of the virus with the host cell surface proteins, the first step in SARS-CoV-2 infection, at one-tenth of the concentration required by Camostat[5,39]. Camostat and Nafamostat are being considered for drug repurposing for COVID-19. Their combinations with anti-viral drugs with high binding for hACE-2 such as Daclatasvir, Rilpivirine has the potential to offer synergistic effects. The findings also highlight that search for drugs or druggable molecules with potential for high-affinity binding (binding energy ≤ -9.4 Kcal/mole) at hACE-2 along with RdRp and M^{Pro} binding should continue. Moderate protein binding with prolonged half-life will be desirable for this potential therapeutics. We have recently reported multi-targeted ligands with a high affinity for all three select targets binding energy < -9 Kcal/mole suggesting identification of such molecules is a realistic goal[4].

Our findings provide a scientific rationale for the need to focus on shortlisted approved antiviral and their select combinations for systematic preclinical evaluation for COVID-19 on priority. These drug/drug combinations have the potential to exhibit activity better than Remdesivir and several other anti-viral drugs in clinics. Further, these drugs also offer the advantage of the ease of oral administration. Evaluation of these drugs at the proposed targets M^{Pro} and RdRp activity and ability to intervene interaction of SARS-CoV-2 spike protein with hACE-2 will be an important step towards the evaluation of the proposed concept.

Given the current scenario of COVID-19, these findings provide the rationale for prioritization of compassionate use and clinical evaluation of shortlisted lead candidates Ledipasvir and Daclatasvir and their combination with Sofosbuvir in critically ill COVID-19 patients or select high-risk group until the data from the systematic preclinical evaluation for these drugs are available. These drugs have the potential for better antiviral effect against SARS-CoV-2 than several other antiviral drugs/drugs being repurposed for COVID-19 management and investigational drug Remdesivir.

3. Conclusion

Based on the virtual screening of antiviral drugs, we have identified drugs with the potential to bind to multiple targets-SARS-CoV-2 main proteases (M^{Pro}) and RNA dependent RNA polymerase (RdRp) and human angiotensin-converting enzyme receptor-2 receptor-binding domain(hACE-2) **(Figure 3)**. These drugs have the potential for effective antiviral activity against SARS-Co-2 by impacting virus entry, replication and maturation and can be repurposed for COVID-19. Amongst several drugs under clinical evaluation for COVID-19, with focus on antiviral activity, Remdesivir, an investigational drug exhibited best-combined activity score indicating its potential to bind to the targets of interest.

Figure 3

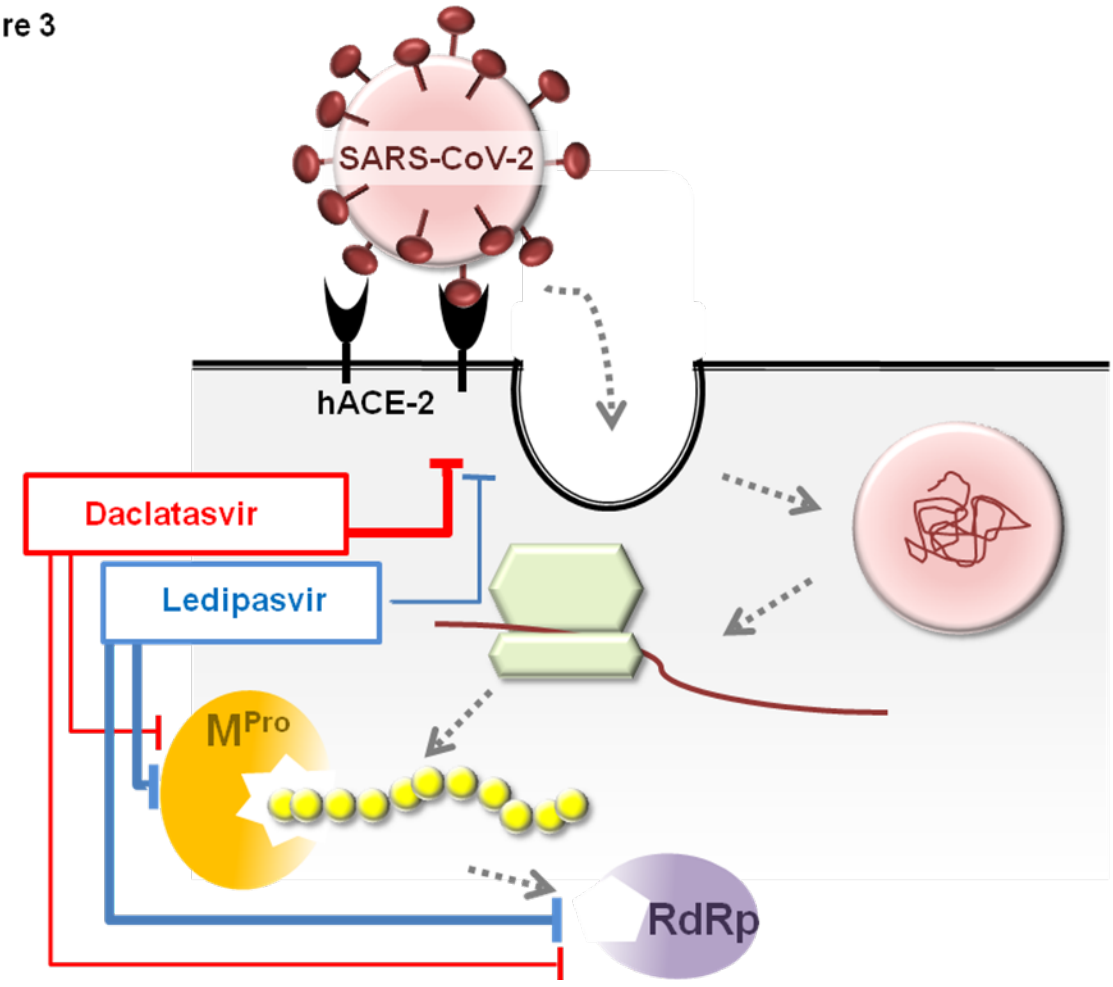


Figure 3. Schematic representation of predicted actions of Ledipasvir and Daclatasvir against multiple targets in SARS-CoV-2. Ledipasvir targeting prominently M^{Pro} and RdRp indicated by the thick blue lines and binding moderately to hACE-2 illustrated by the relatively thin blue line. In the case of Daclatasvir, binding is strong with hACE-2 and is indicated by a thick red line. Daclatasvir binds moderately to M^{Pro} and RdRp which is indicated by thin red lines

Ledipasvir, Daclatasvir, Elbasvir, Paritaprevir, Rilpivirine and Indinavir were identified as candidate drugs of interest-based on combined activity score, pharmacokinetic and

pharmacodynamic parameters. Ledipasvir and Daclatasvir the directly acting antiviral drugs emerged as lead candidate drugs with combined activity scores better than Remdesivir and prolonged half-life ensuring prolonged extracellular hACE-2 engagement along with RdRp and M^{Pro}. Remdesivir has to be administered intravenously, whereas Ledipasvir and Daclatasvir and other drugs selected through this screening can be administered orally. These antiviral molecules and their preferred approved combination(s) with established safety profile have the potential for efficacy better than Remdesivir and other drugs/drug combinations in use or being evaluated for COVID-19. These should thus be considered for systematic fast track preclinical and clinical evaluation for COVID-19 management.

Given the current scenario of COVID-19, our findings provide a scientific rationale for **Ledipasvir's and Daclatasvir's combination with Sofosbuvir** (Harvoni) currently approved for clinical use in HCV genotype-1 in COVID-19 patients for compassionate use and clinical evaluation until the systematic evaluation of these drugs is undertaken. Recently, initiation of clinical trials with Ledipasvir and Daclatasvir in combination with Sofosbuvir for COVID-19 has been reported. Based on our study we recommend prioritization and aggressive perusal of clinical evaluation of these drug combinations. Outcomes from these trials will be awaited with interest.

4. Methods

4.1 **Library preparation**

The library of approved antivirals was prepared using available structures from PubChem[40]. All the molecules were checked for stereochemical properties and then converted to *.pdbqt format using Autodock Tools [41]. This library was used for further docking studies. A detailed methodology is as described earlier[4].

4.2 **Preparation of the target molecules**

Crystal structure of liganded M^{Pro} (PDB ID: 6Y2F) and hACE-2 complexed with viral spike protein (PDB ID: 6VW1) was downloaded from the RCSB Protein DataBank[7,11]. Water and other heteroatoms were deleted from these structures. Grid for M^{Pro} was set around active site residues H41 and C145 with dimension on 36 x 56 x 40Å using the AutoGrid program of AutoDock Tools [41]. The protein is converted to *.pdbqt for further docking studies. A similar process was performed on the SARS-CoV2 RdRp crystal structure (PDB: 7BTF) with a grid around RNA binding pocket with a dimension of 34 x 34 x 36Å [9]. Furthermore, hACE2 was prepared for docking, with a grid dimension of 20 x 38 x 24Å around the viral spike protein recognition residues (K31, E35, D38, M82, K353)[7]. These target molecules were then further used for virtual screening.

4.3 **Virtual screening using Autodock vina**

Prepared receptor molecules from the custom-made libraries were set for the virtual screening by AutoDock Vina based Lamarckian Genetic Algorithm (LGA) parameter for ligand tethering of the proteins using 10 runs criteria [42]. Top hits of ligands were selected based on their docking score against all targets namely, M^{Pro}, RdRp and hACE2. A comparison of the docking score for these targets is represented as a heatmap of the binding score using MeV software (http://www.tm4.org/). Ligands with high solubility and bioavailability were further taken for the interaction analysis. Ligand binding position and interaction analysis were performed using PyMOL visualization software (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC) and two-dimensional ligand interaction images are made using Biovia Discovery Studio 4.5 (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016).

4.4 Combined Activity Score

Based on our perspective of literature evidence relative weightage was assigned to the three targets as follows M^{Pro} = 20%; RdRp = 20% and hACE-2 = 60%. The combined activity score is calculated as [(Binding energy M^{Pro} *0.2)+(Binding energy RdRp *0.2)+(Binding Energy hACE-2*0.6)]. Combined activity score of the drugs which are converted to active di or triphosphate metabolites intracellularly is calculated taking hACE-2 of the parent molecule and binding energy for metabolites for RdRp and M^{Pro}. A combined activity score for triphosphate metabolites that do not have access to extracellular target hACE-2 is not calculated. A combined activity score of ± 0.4 will not be considered significantly different.

REFERENCES

1 WHO. Coronavirus disease. *World Heal Organ* 2020; **2019**: 2633.

2 Thorlund K, Dron L, Park J, Hsu G, Forrest JI, Mills EJ. A real-time dashboard of clinical trials for COVID-19. *Lancet Digit Heal* 2020; **7500**: 2019–20.

3 Shaffer L. 15 drugs being tested to treat COVID-19 and how they would work. *Nat Med* 2020. DOI:10.1038/d41591-020-00019-9.

4 Joshi RS, Jagdale SS, Bansode SB, *et al.* Discovery of Potential Multi-Target-Directed Ligands by Targeting Host-specific SARS-CoV-2 Structurally Conserved Main Protease$. *J Biomol Struct Dyn* 2020; : 1–16.

5 Hoffmann M, Kleine-Weber H, Schroeder S, *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; : 1–10.

6 Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 2020; **181**: 281-292.e6.

7 Shang J, Ye G, Shi K, *et al.* Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020; : 1–8.

8 Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol* 2016; **3**: 237–61.

9 Gao Y, Gao Y, Yan L, *et al.* Structure of the RNA-dependent RNA polymerase from COVID-19 virus. 2020; **7498**: 1–9.

10 Amirian ES, Levy JK. Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. *One Heal* 2020; **9**: 100128.

11 Zhang L, Lin D, Sun X, *et al.* Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science* 2020; **3405**: 1–9.

12 Krichel B, Falke S, Hilgenfeld R, Redecke L. Processing of the SARS-CoV pp1a / ab nsp7 – 10 region. 2020; **0**: 1009–19.

13 Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CLpro) Structure: Basis for design of anti-SARS drugs. *Science (80-)* 2003; **300**: 1763–7.

14 Cao B, Wang Y, Wen D, *et al.* A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med* 2020; : 1–13.

15 Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 Protease against COVID-19. *J Biomol Struct Dyn* 2020; : 1–7.

16 Chen C, Huang J, Cheng Z, *et al.* Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. *medRxiv* 2020; : 2020.03.17.20037432.

17 Imbert I, Guillemot J-C, Bourhis J-M, *et al.* A second, non-canonical RNA-dependent RNA polymerase in SARS Coronavirus. *EMBO J* 2006; **25**: 4933–42.

18 Beigel JH, Tomashek KM, Dodd LE, *et al.* Remdesivir for the Treatment of Covid-19 - Preliminary Report. *N Engl J Med* 2020; : 1–12.

19 Kumari R, Nguyen MH. Fixed-dose combination of sofosbuvir and ledipasvir for the treatment of chronic hepatitis C genotype 1. *Expert Opin Pharmacother* 2015; **16**: 739–48.

20 Belema M, Nguyen VN, Bachand C, *et al.* Hepatitis C Virus NS5A Replication Complex Inhibitors: The Discovery of Daclatasvir. *J Med Chem* 2014; **57**: 2013–32.

21 Diseases L, Society ID, Present A, Updated L. AASLD/IDSA HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. *Clin Liver Dis* 2018; **12**: 117.

22 Younossi ZM, Stepanova M, Marcellin P, *et al.* Treatment with ledipasvir and sofosbuvir improves patient-reported outcomes: Results from the ION-1, -2, and -3 clinical trials. *Hepatology* 2015; **61**: 1798–808.

23 INDICATIONS AND USAGE DAKLINZA is indicated for use with sofosbuvir for the treatment of patients with chronic hepatitis C virus (HCV) genotype 3 infection [see Dosage and Administration (2) and Clinical Limitations of Use : Sustained virologic respo. 2015; : 1–24.

24 Xia Q, Radzio J, Anderson KS, Sluis-Cremer N. Probing nonnucleoside inhibitor-induced active-site distortion in HIV-1 reverse transcriptase by transient kinetic analyses. *Protein Sci* 2007; **16**: 1728–37.

25 Fernández-Montero JV, Vispo E, Anta L, de Mendoza C, Soriano V. Rilpivirine: a next-generation non-nucleoside analogue for the treatment of HIV infection. *Expert*

Opin Pharmacother 2012; **13**: 1007–14.

26 Fantauzzi A, Mezzaroma I. Dolutegravir: Clinical efficacy and role in HIV therapy. *Ther Adv Chronic Dis* 2014; **5**: 164–77.

27 Zamora F, Ogbuagu O. Dolutegravir / rilpivirine for the treatment of HIV-1 infection. 2018; : 215–24.

28 Klibanov OM, Gale SE, Santevecchi B. Ombitasvir/Paritaprevir/Ritonavir and Dasabuvir Tablets for Hepatitis C Virus Genotype 1 Infection. *Ann Pharmacother* 2015; **49**: 566–81.

29 Hull MW, Montaner JSG. Ritonavir-boosted protease inhibitors in HIV therapy. *Ann Med* 2011; **43**: 375–88.

30 Faletto MB, Miller WH, Garvey EP, St. Clair MH, Daluge SM, Good SS. Unique intracellular activation of the potent anti-human immunodeficiency virus agent 1592U89. *Antimicrob Agents Chemother* 1997; **41**: 1099–107.

31 Fung HB, Stone EA, Piacenti FJ. Tenofovir disoproxil fumarate: A nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. *Clin Ther* 2002; **24**: 1515–48.

32 Delaney IV WE, Ray AS, Yang H, *et al.* Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. *Antimicrob Agents Chemother* 2006; **50**: 2471–7.

33 Gilead Sciences. Gilead Sciences Reference ID : 4218165 Gilead Sciences Reference ID : 4218165. 2018; : 1–34.

34 Bell AM, Wagner JL, Barber KE, Stover KR. Elbasvir/grazoprevir: A review of the latest agent in the fight against hepatitis C. *Int J Hepatol* 2016; **2016**. DOI:10.1155/2016/3852126.

35 Vishal M, Pravin D, Himani G, Nilam V, Urvisha B, Rajesh P. Drug Repurposing of Approved Drugs Elbasvir, Ledipasvir, Paritaprevir, Velpatasvir, Antrafenine and Ergotamine for Combating COVID19. *chemRxiv* 2020. DOI:10.26434/chemrxiv.12115251.v1.

36 Omotuyi O, Nash O, Ajiboye B, *et al.* The Disruption of SARS-CoV-2 RBD/ACE-2 Complex by Ubrogepant Is Mediated by Interface Hydration. *Preprints* 2020. DOI:10.20944/PREPRINTS202003.0466.V1.

37 Cure E, Cumhur Cure M. Angiotensin-converting enzyme inhibitors and angiotensin

receptor blockers may be harmful in patients with diabetes during COVID-19 pandemic. *Diabetes Metab Syndr Clin Res Rev* 2020; **14**: 349–50.

38 Hasan A, Paray BA, Hussain A, *et al.* A review on the cleavage priming of the spike protein on coronavirus by angiotensin-converting enzyme-2 and furin. *J Biomol Struct Dyn* 2020; : 1–13.

39 Yamamoto M, Kiso M, Sakai-Tagawa Y, *et al.* The anticoagulant nafamostat potently inhibits SARS-CoV-2 infection. 2020; : 1–19.

40 Kim S, Chen J, Cheng T, *et al.* PubChem 2019 update: improved access to chemical data. *Nucleic Acids Res* 2018; **47**: D1102–9.

41 Morris G, Huey R. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J …* 2009; **30**: 2785–91.

42 Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010; **31**: 455–61.

Table 1: Drug Candidates Exhibiting Strong Binding to M^{Pro}/RdRp/hACE-2.

Target	Binding Energy Range (Kcal/mole)	Drug	Binding Energy (Kcal/mole)	Combined Activity Score
M^{Pro}	-3.2 to 10.2	**Remdesivir**	-8.2	7.8
		Ledipasvir	**-9.4**	8.8
		Saquinavir	**-9.0**	7.5
		Danoprevir	-8.7	7.7
		Raltegravir	-8.7	7.3
RdRp	-3.2 to -9.4	**Remdesivir**	-7.2	7.8
		Ledipasvir	**-10.2**	8.8
		Elbasvir	**-10**	7.8
		Danoprevir	**-9.8**	7.7
		Paritaprevir	**-9.4**	8.0
hACE-2	-2.8 to -8.9	**Remdesivir**	-7.8	7.8
		Daclatasvir	**-8.9**	8.4
		Rilpivirine	-8.7	8.2
		Ledipasvir	-8.2	8.8

Drugs exhibiting low binding energy to either M^{Pro}/RdRp/hACE-2 are shortlisted.

Binding energy ≤-8.9 Kcal/mole are highlighted.

Remdesivir- the reference drug is highlighted.

Table 2: Binding energy and combined activity scores for approved anti-viral drugs exhibiting hACE-2 binding better than or comparable to Remdesivir

	Drug	M^{Pro}	RdRp	hACE-2	Combined
		Binding Energy (Kcal/mole)			Activity Score
	Combined Activity Score Significantly Better Than Remdesivir				
1	Ledipasvir	-9.4	-10.2	-8.2	8.8
2	Daclatasvir	-7.8	-7.6	-8.9	8.4
	Combined Activity Score Comparable to Remdesivir				
1	Rilpivirine	-7.3	-7.7	-8.7	8.2
2	Delviridine	-8.4	-7.9	-8.1	8.1
3	Paritaprevir	-8.6	-9.4	-7.4	8.0
4	Letermovir	-8.3	-7.6	-8.0	8.0
5	Dolutegravir	-7.6	-8.2	-7.7	7.8
6	**Remdesivir***	**-8.2**	**-7.2**	**-7.8**	**7.8***
7	Indinavir	-7.4	-8.1	-7.7	7.7
8	Sofosbuvir*	-7.6	-7.3	-7.6	7.5
9	Darunavir	-8.2	-6.7	-7.3	7.4
10	Abacavir*	-6.5	-6.9	-7.3	7.4*
11	Tenofovir*	-6.8	-7.1	-7.4	7.1*

(*) These drugs are converted intracellularly to active metabolites. The binding energy of known key active metabolite present intracellularly was considered for M^{Pro} and RdRp for the combined activity score determination.

Reference drug Remdesivir has been highlighted.

Table 3: Pharmacokinetic Properties of Lead candidates and Reference Drug

	Remdesivir*	Ledipasvir	Daclatasvir
Status **Indication** **(*In clinics)**	Investigational SARS-COV-2	Approved Chronic HCV genotype 1a,1b,4,5 &6 infection in combination with Sofosbuvir (Harvoni)	Approved Chronic HCV genotype 1,3 and 4infection in combination with Sofosbuvir, ribavirin or interferon
The key known target for the approved indication	RdRp inhibition by triphosphate metabolite (NTP)	Prevent hyper-phosphorylation of NS5A	Prevent hyper-phosphorylation of NS5A
Bio-availability	Not Available	76%	67%
Protein Binding	Not Available	>99.8 %	99%
Elimination Half-life	0.4h Parent (Non-human primate (NHP) 20h for NTP metabolite in humans,14 h in NHP	47 h (Median terminal)	12–15 h
Metabolism	Not Available	No detectable metabolism Excretion – unchanged in faeces	Faecal (53% as unchanged drug), kidney

Remdesivir* - Reference Drug is highlighted.

Supplementary Data S1: Binding Energy and Combined Activity Scores of Drugs Being Repurposed For COVID-19 (Comparison with Remdesivir)

	Drug/Metabolite	Binding Energy (Kcal/mole)			Combined Activity Score
		M^{Pro}	RdRp	hACE-2	
	A. Antiviral Drugs&their Metabolites For COVID-19				
1	Lopinavir	-7.8	-7.3	-6.8	7.1
	Lopinavir M1 Metabolite	-7.5	-7.3	-7.4	7.4
	Lopinavir M2 Metabolite	-7.1	-7.1	-7.6	7.4
	Lopinavir M3/M4 metabolite(s)	-6.8	-7.5	-5.2	6.0
2	Ritonavir	-6.8	-7.6	-5.8	6.4
3	Favipiravir*	-4.8	-5.4	-5.0	6.0*
	Favipiravir-ribofuranosyl-5'triphosphate	-7.7	-7.3	-7.0	7.2
4	Umifenovir	-5.8	-5.8	-6.5	6.2
5	Oseltamivir	-6.2	-5.6	-6.1	6.0
6	Darunavir	**-8.2**	-6.7	-7.3	7.4
7	Cobicistat	-7.2	-7.0	-6.6	6.8
	Other Drugs Being Repurposed For COVID-19				
1.	Chloroquine	-5.6	-5.6	-5.6	6.1
2	Hydroxycholoroquine	-5.3	-5.6	-6.0	5.8
3	Ivermectin	**-8**	**-9.3**	-5.9	7.0
4	Doxycycline	-7.2	**-8.2**	-6.6	7.0
5	Azithromycin	-7.0	**-8.2**	-5.7	6.5
6	Camostat	-6.1	-6.3	-7.3	6.9
7	Nafamostat	-7	-6.9	**-8.1**	7.6
8	Famotidine	-4.6	-5.1	-5.2	5.1
9.	Nitazoxanide	-5.9	-6.4	-6	6.1
	Remdesivir (GS-5734)*	-8.2	-7.2	**-7.8**	**7.8***

NTP of GS-5734 & GS-441524	**-7.2**	**-7.6**	-6.5
GS-441524 (Metabolite of Remdesivir)	-6.9	-6.3	-6.5

(*) These drugs are converted intracellularly to active metabolites. The binding energy of known key active metabolite present intracellularly was considered for the combined activity score.

NTP – Active NeucleosideTriphosphate. The binding energy lower thanRemdesivir (hACE-2)/its active metabolite (M^{Pro} and RdRp) are highlighted.

Remdesivir exhibits combined activity score better than other antiviral drugs reported in clinics.Rank order for combined activity score for drugs in clinics is Remdesivir (7.8*)>Darunavir (7.4) ≥ Lopanavir/Lopinavir metabolites M1&M2 (7.1/7.4) ≥Ritonavir (6.4)≥Favipiravir/Favipiravir metabolite (6*/7.2)>Umifenovir (6.2) ≥ Oseltamvir (6.0)

Use of Lopinavir /Ritonavir combination for COVID-19 has been extensively reported. Lopinavir exhibits binding to M^{Pro} and RdRp comparable to Remdesivir but binding to hACE-2 was comparatively weak. Ritonavir is a potent CYP3A inhibitor which that increases the plasma concentration of Lopinavir, a drug extensively metabolized by CYP3A4. Our findings suggest the minimal contribution of Ritonavir as active moiety against SARS-CoV-2. It is of interest to note that circulating metabolites of Lopinavir M1 and M2 exhibits good combined activity score and may contribute to efficacy against SARS-CoV-2. A randomized open-label trial in China of some 200 hospitalized patients did not find the drug to be more effective than standard of care(Ref) but further clinical trials are pending. Umifenofir, is used in Russia and China as prophylaxis for influenza virus A and B. In our screen it exhibited very low combined activity score of 6.2. A study comparing Lopinavir /Ritonavir and Uminofir reported Uminofirtobe more effective at reducing viral loads in patients whereas, trial in another preprint found neither treatment to be effective at improving outcomes for patients with mild to moderate COVID-19.

Darunavir is a second-generation protease inhibitor used in combination with other protease inhibitor drugs as well as Ritonavir for the effective management of HIV-1 infection. It was initially approved by the FDA in 2006. Darunavir is being studied as a possible treatment for SARS-CoV-2, due to *in vitro* evidence supporting its ability to combat this infection. Clinical trials are underway and are expected to conclude in August 2020[2]. In our screen, Darunavir exhibited combined activity score comparable to Remdesivir.

Cobicistat is a CYP3A inhibitor indicated to increase the systemic exposure of drugs with extensive CYP3A4 metabolism such as Darunavirand is used in combination with other

antiretroviral agents in the treatment of HIV-1 infection. Like Ritonavir as active moiety is unlikely to contribute to activity against SARS-CoV-2.

Chloroquine and Hydroxychloroquine are polymerase inhibitors classically used as antimalarial drugs. In malaria, they inhibit heme polymerase, causing accumulation of toxic heme in the parasite. In COVID-19, its proposed mechanism is inhibition of glycosylation of host receptors and breaking down of production of viral proteins by inhibiting endosomal acidification. In our screen, these drugs exhibited low binding to all the targets of interest. These drugs are subject of more than 30 different clinical trials, evidence for beneficial effects above standard treatment is lacking.

Ivermectin, a drug used as a broad-spectrum anti-parasitic drug known to act by binding to parasite glutamate-gated chloride ion channels. Interestingly, in our screen, Ivermectin exhibited strong binding to SARS-CoV-2 RdRp with binding energy < -9.0 Kcal/mole and SARS-CoV-2 M^{Pro} with the binding energy of -8 Kcal/mole which is lower than the binding energy of observed intracellular active metabolite of Remdesivir for the respective target. In an observational multicenter study more than 1000 patients (still under review), administration of Ivermectin was associated with lower death rate and shorter hospital stays. Binding of Ivermectin to these SARS-COV-2 targets can explain observed beneficial effects in COVID-19 patients. These findings support the focus on RdRp and selected for screening. With very weak binding to hACE-2 receptor binding domain, its combined activity score is, however, lower than that of Remdesivir.

Camostat and Nafamostat are serine protease inhibitors in clinics with demonstrated treatments for pancreatitis and other diseases. Camostat is a narrow spectrum serine protease inhibitor in clinical practice for a long time in Japan for the treatment of the remission of acute symptoms of chronic pancreatitis. Nafamostat is a wide spectrum protease inhibitor used in cardiology like Aprotinin. Camostat has been demonstrated to inhibit TMPRSS2. Nafamostat can prevent the fusion of the envelope of the virus with the host cell surface proteins, the first step in SARS-CoV-2 infection, at one-tenth of the concentration required by Camostat mesylate (Foypan)[5,39]. Camostat and Nafamostat are being considered for drug repurposing for COVID-(ref). Our screen highlights its potential binding to hACE-2 suggesting access to the critical site for the interdependent step of membrane fusion. The difference in activity between Camostat and Nafamostat reflected in the difference in their binding energy to hACE-2. Combined activity score for Nafamstat was comparable to Remdesivir.

Use of Tetracyclines and Azitromycin has been suggested in COVID-19 in combination with other drugs. Doxycycline exhibited good binding to SARS-CoV-2 RdRp and M^{Pro} with binding

better/comparable at these targets providing the potential rationale for their inclusion in combination antiviral therapy for SARS-CoV-2.

Drugs in clinics with a focus on antiviral effect but known to have known to bind to other target proteins are not expected to exhibit binding to these targets. Nitazoxanide, an anti-infective with efficacy in parasitic and bacterial and viral infections, is known to act on nucleocapsid N protein is therefore not expected to exhibit binding to these targets of interest. Famotidine, a drug with unknown mechanism though was evaluated is not expected to bind to the targets of interest. Combined activity score for these drugs at targets of interest was low.

Intravenous Remdesivir was studied for the treatment of Ebola virus disease, in which it was adequately tolerated but was less effective than several monoclonal antibody therapeutics. Remdesivir has been in focus for COVID-19. Amongst the several drugs being considered for COVID-19 which aim for antiviral effect against SARS-CoV-2, Remdesivirexhibts the **best combined activity** score in our screen indicating the potential for high-affinity binding to all targets of interest. It also exhibited the lowest binding energy to hACE-2 amongst several drugs being considered for repurposing for antiviral activity (**Supplementary Data S1**) indicating the **highest affinity for hACE-2.**

This drug has demonstrated potent inhibition of SARS-CoV-2 replication in human nasal and bronchial airway epithelial cells. In a non-lethal rhesus macaque model of SARS-CoV-2 infection, early Remdesivir administration is reported to have significant antiviral and clinical effects (reduced pulmonary infiltrates and virus titres in bronchoalveolar lavages vs vehicle only). Remdesivir was, therefore, used as a reference for selection and prioritization of antiviral drugs for SARS-CoV-2 infection.

In a randomised, double-blind, placebo-controlled clinical trial assessing the effect of intravenous Remdesivir in adults admitted to hospital with severe COVID-19 with intention-to-treat population, the primary endpoint of time to clinical improvement was not significantly different between groups but was numerically shorter in the Remdesivir group than the control group.

Supplementary Data S2: Binding, Energy and Combined Activity Score Of Approved Anti-viral Drugs

	Drug	Binding Energy (Kcal/mol)			Combined Activity Score
		M^{Pro}	RdRp	hACE-2	
1	Daclatasvir	-7.8	-7.6	**-8.9**	8.4
2	Rilpivirine	-7.3	-7.7	-8.7	8.2
3	Ledipasvir	**-9.4**	**-10.2**	-8.2	8.8
4	Delviridine	-8.4	-7.9	-8.1	8.1
5	Letermovir	-8.3	-7.6	-8.0	8.0
6	Remdesivir	-8.2	-7.2	-7.8	7.8
7	Dolutegravir	-7.6	-8.2	-7.7	7.8
8	Indinavir	-7.4	-8.1	-7.7	7.7
9	Sofosbuvir	-7.6	-7.3	-7.6	7.5
10	Paritaprevir	-8.6	**-9.4**	-7.4	8.0
11	Tenofovir	-6.8	-7.1	-7.4	7.2
12	Darunavir	-8.2	-6.7	-7.3	7.4
13	Abacavir	-6.5	-6.9	-7.3	7.1
14	Nelfinavir	-7.6	-7.6	-7.2	7.4
15	Elvitegravir	-6.8	-7.3	-7.2	7.1
16	Fosamprenavir	-7.2	-6.2	-7.2	7.0
17	Zanamivir	-6.7	-6.0	-7.1	6.8
18	Valganciclovir	-7.1	-7.0	-7.0	7.0
19	Elbasvir	-8.1	**-10.0**	-6.9	7.8
20	Entecavir	-7.3	-7.3	-6.9	7.1
21	Famvir	-6.7	-6.9	-6.9	6.9
22	Amprinavir	-6.6	-6.5	-6.9	6.8
23	Velpatasvir	-7.4	-8.8	-6.8	7.3
24	Lopinavir/ritonavir	-7.8	-7.3	-6.8	7.1
25	Imiquimod	-6.2	-6.1	-6.8	6.5
26	Danoprevir	-8.7	**-9.8**	-6.7	7.7
27	Saquinavir	**-9.0**	-8.6	-6.7	7.5
28	Etravirine	-7.7	-7.0	-6.7	7.0
29	Tipranavir	-7.9	-6.4	-6.7	6.9
30	Vidarabine	-6.9	-7.2	-6.7	6.8
31	Raltegravir	-8.7	-8.1	-6.6	7.3
32	Grazoprevir	-7.4	-8.7	-6.6	7.2
33	Umifenovir	-5.8	-5.8	-6.5	6.2

34	Retrovir	-6.5	-6.9	-6.4	6.5
35	Didanosine	-6.1	-6.4	-6.4	6.3
36	Efavirenz	-6.1	-6.2	-6.4	6.3
37	Simeprevir	-8.1	**-9.0**	-6.3	7.2
38	Ombitasvir	-6.7	-8.1	-6.3	6.7
39	Azidothymidine	-7.2	-7.1	-6.3	6.6
40	Ganciclovir	-6.4	-6.1	-6.3	6.3
41	Adefovirdipivoxil	-7.1	-6.6	-6.2	6.5
42	Nevirapine	-6.6	-6.2	-6.2	6.3
43	Zidovudine	-6.8	-6.2	-6.2	6.3
44	Idoxuridine	-7.3	-6.6	-6.1	6.4
45	Ribavirin	-6.5	-6.3	-6.1	6.2
46	Oseltamivir	-6.2	-5.6	-6.1	6.0
47	Dasabuvir	-7.7	-8.3	-6.0	6.8
48	Stavudine	-6.5	-6.2	-6.0	6.1
49	Telbivudine	-6.5	-5.7	-6.0	6.0
50	Valaciclovir	-7.0	-7.2	-5.9	6.4
51	Ritonavir	-6.8	-7.6	-5.8	6.4
52	Zalcitabine	-5.8	-5.3	-5.7	5.6
53	Emtricitabine	-6.0	-5.3	-5.5	5.6
54	Telaprevir	-7.3	-7.3	-5.4	6.2
55	Lamivudine	-5.4	-5.1	-5.4	5.3
56	Maraviroc	-8.1	-7.3	-5.3	6.3
57	Atazanavir	-6.0	-7.0	-5.3	5.8
58	Boceprevir	-6.9	-7.4	-5.1	5.9
59	Asunaprevir	-7.0	-7.2	-5.1	5.9
60	Favipiravir	-4.8	-5.4	-5.0	5.0
61	Docosanol	-3.2	-3.5	-2.8	3.0

Binding energy ≤-8.9 Kcal/mole is highlighted.

Rank order of drugs is based on their binding energy for hACE-2

Supplementary Data S3: Binding Energy and Combined Activity Scores of Remdesivir, Sofosbuvir, Abacavir, Tenofovir and Their Metabolites

Drug/Metabolite	Binding Energy (Kcal/mole)			Combined Activity Score
	M^{Pro}	RdRp	hACE-2	
Remdesivir (GS-5734)	-8.2	-7.2	**-7.8**	7.8*
Active Triphosphate of GS-5734 & GS-441524	**-7.2**	**-7.6**	-6.5	
GS-441524	-6.9	-6.3	-6.5	6.5*
Sofosbuvir	-7.6	-7.3	**-7.6**	7.5*
GS-461203 (Active triphosphate of Sofosbuvir)	**-7.3**	**-7.4**	-7.4	
GS-331007	-6.0	-5.9	-5.8	5.9
Abacavir	-6.5	-6.9	-7.3	7.4*
Carbovir triphosphate (Activetriphosphateof Abacavir)	**-7.7**	**-7.4**	-7.4	
Tenofovir	-6.8	-7.1	**-7.4**	7.1*
Tenofovir Monophosphate	-6.5	-6.4	-6.0	
TenofovirDiphosphate	**-6.3**	**-6.8**	-6.6	

(*) These drugs are converted intracellularly to active metabolites. The binding energy of known key active metabolite present intracellularly was considered for the combined activity score.

Values considered for combined activity score for such drugs are highlighted

Supplementary Data S4: Pharmacokinetic Properties of Select Drugs of Interest

(A)

	Rilpivirine	**Delviridine**	**Paritaprevir**	**Letermovir**	**Dolutegravir**
Status	Approved	Approved		Approved	
Indication (*In clinics	HIV- type 1	HIV- type 1	HCV-1a &1b in Combination	CMV Prophylaxis	HIV post exposure prophylaxis
The key known target for the approved indication	Non-nucleoside reverse transcriptase inhibitor (nNRTI)	Non-nucleoside reverse transcriptase inhibitor (nNRTI)	NS3/4A serine protease inhibition	DNA terminase complex inhibitors	Integrase Inhibitor
Bio-availability	Orally bioavailable	85%	Orally bioavailable	94% (healthy subjects)	Orally bioavailable
Protein Binding	>99%	98%	97–98.6%	99%	>98.9%
Elimination Half-life	38h	5.8h	5.5h	12h	~14h
Metabolism	Hepatic (CYP3A4)	Hepatic (CYP3A4- and CYP2D6-mediated)	Hepatic,CYP3A4 (major) & CYP3A5(minor)	A minor degree of metabolism through UGT1A1/1A3	UGT1A1 and CYP3A4
Excretion	Excreted fecally (85%, 25% as unchanged drug)	Renal (51%) andfecal (44%)	Feces (88%), Urine (8,8%)	In feces (70% Unchanged)	Feces (53%) and urine (18.9%)

(B)

	Indinavir	**Sofosbuvir***	**Darunavir**	**Elbasvir**	**Abacavir***
Status	**Approved**	**Approved**	**Approved**	**Approved**	**Approved**
Indication (*In clinics)	HIV-1 in combination with other drugs	Hepatitis C	HIV-1 in combination with ritonavir	HCV1a, 1b, and 4 in combination with other drugs	HIV-1 infection, in combination with other antiretroviral agents
Key known target for the approved indication	Protease Inhibitor	Triphosphate metabolite (NTP) is a defective substrate for RdRp(NS5B)	Protease Inhibitor	Inhibitor of NS5B Structural Protein	Nucleoside analogue reverse transcriptase inhibitor by diphosphate metabolite(NRTI)
Bio-availability	~65%	Orally Bioavailable	37% (without ritonavir), 82% (with ritonavir)	32% Absolute Bioavailability	83% (Tablet)
Protein Binding	60%	61-65%	95%	>99%	50%
Elimination Half-life	1.8 ± 0.4 h	0.4h (Parent) Metabolite 20h	15h	24h	1.54±0.63 h
Metabolism	Hepatic via CYP3A4	Hepatic Cathepsin and Esterase	Hepatic (CYP3A4)	(CYP3A4)	
Excretion	<20% Unchanged in urine	Urine (80%), feces (14%), and respiration (2.5%);	Faeces (80%), urine (14%)	Faeces (90%), urine (<1%)	Urine [carboxylic acid (30%) glucronide metabolite (36%)] Feces (16%)

Supplementary Data S5: Binding Energy of Select Non-Antiviral Drugs: Comparison with Lead Antiviral Candidates.

Drug	Binding Energy (Kcal/mole)			Combined Activity Score
	M^{Pro}	RdRp	hACE-2	
Ubrogepant	-8.4?	-8.6?	-7.0	
Erogotamine	-10.3?	-10.0?	-8.3	
Ledipasvir	-9.4	-10.2	-8.2	8.8
Daclatasvir	-7.8	-7.6	-8.9	8.4
Remdesivir	-8.2	-7.2	-7.8	7.8*

? Target engagement not evident. Combined activity score for these drugs is not calculated. Antiviral drugs are highlighted.

(*) This drug is converted intracellularly to active metabolites. The binding energy of known key active metabolite present intracellularly was considered for the combined activity score.