Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Drug repurposing against SARS-CoV-2 using computational approaches

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has generated a critical need for treatments to reduce morbidity and mortality associated with this disease. However, traditional drug development takes many years, which is not practical solution given the current pandemic. Therefore, a viable option is to repurpose existing drugs. The structural data of several proteins vital for the virus became available shortly after the start of the pandemic. In this review, we discuss the importance of these targets and their available potential inhibitors predicted by the computational approaches. Among the hits identified by computational approaches, 35 candidates were suggested for further evaluation, among which ten drugs are in clinical trials (Phase III and IV) for treating Coronavirus 2019 (COVID-19).

Keywords: COVID-19; SARS-CoV-2; Drug repurposing; Computational approaches; Molecular docking

Introduction

From the first report of COVID-19 in December 2019, the pandemic has spread out around the world; intensive research is underway to find suitable and effective therapies for the treatment and prevention of the coronavirus infection and the complications caused by the disease. As reported by the COVID-19 Vaccine Tracker, to date, 22 vaccines developed around the world to prevent COVID-19 have received regulatory approval. There are another 91 vaccine candidates in various stages of clinical trials (as of September 30, 2021).[1] Although vaccination is an effective measure, the continuous mutation of the virus means that such an approach does not provide a 100% guarantee of protection against infection.[2] At the time of writing (September 2021), 12 variants of SARS-CoV-2 had been reported, with the latest being omicron.[3] In addition, vaccine rollouts have been hampered by those deciding against vaccination. Additionally, there is also a preference for orally administered therapeutics rather than for intravenous therapy. Small molecules are more likely to be administered orally than vaccines. Given that the traditional development of drugs is a time-consuming and expensive procedure, repurposing of known drugs is a viable and promising alternative.[4]

In recent years, molecular docking has been widely used in drug design[5,6] because it improves the efficiency of the drug design process.[7] In recent reports,[8,9] authors highlighted the importance of computational approaches for faster drug discovery, especially during a pandemic. Computational approaches allow virtual screening of larger libraries in the time available, reduce the cost of identifying hits, and increase the chances of finding the desired drugs. Molecular docking makes it possible

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https://doi.org/10.1016/j.drudis.2022.02.004

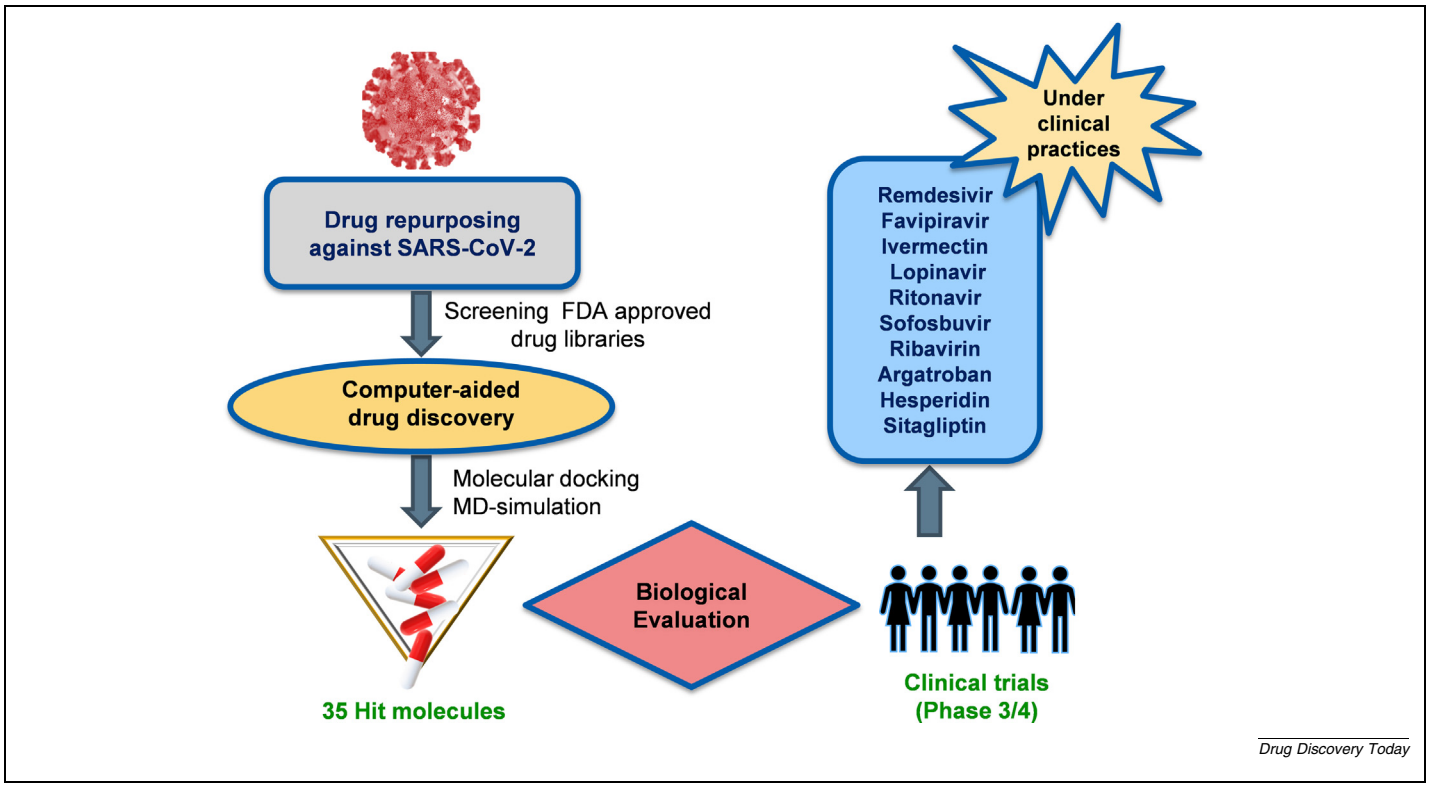


FIGURE 1
Flowchart representing drug repurposing through computational approaches delivering a few clinically important leads as therapeutics against SARS-CoV-2 infection.

to recognize a new set of compounds that could have high therapeutic value, to predict ligand-based interactions, and to determine structure–activity relationships.[10] The mechanisms of SARS-CoV-2 invasion, infection, and replication are now well documented; moreover, many bimolecular targets involved in this mechanism have been identified as promising for the creation of antiviral agents. Thus, all the necessary data are available to begin computational inquiries into the possibility of repurposing existing drugs to tackle COVID-19.[11]

Zaslavsky *et al.* recently reported developments in computational approaches for known drugs against SARS-CoV-2, discussing drugs showing high virtual potency.[12] In this review, we discuss the function and potential of key proteins and their targets, which could be effective against SARS-CoV-2. Additionally, we highlight computational studies of approved, clinical, and preclinical candidates being screened against each viral target. We also report candidates predicted by computational approaches that are now approved therapeutics against COVID-19 or are being currently evaluated in clinical trials (Fig. 1).

Drug targets against SARS-CoV-2

Crucial processes involved during a viral infection (i.e., virus entry and replication) are identified as key intervention events for antiviral agents. Entry into the host cells includes viral adsorption to the cell surface, penetration into endosomes, and fusion of viral and lysosomal membranes. Thus, compounds disrupting the proteins involved in these stages have potential as antivirals. Here, we discuss the potential viral targets being probed as efficient intervention points against SARS-CoV-2.

Spike glycoprotein

The Spike (S) protein is responsible for SARS-CoV-2 entry through binding to human angiotensin-converting enzyme 2 (hACE2; the functional receptor for SARS-CoV-2).[13] S protein mediates membrane fusion with the help of its two subunits (S1 and S2).[14] Qian *et al.* revealed that most S protein entered 293/hACE2 cells via endocytosis. Their results also suggested that the S protein enhances the spread of the virus by triggering protease-independent and receptor-dependent syncytium formation.[15] There is a furin site in S protein but its role in viral propagation remains to be determined. Thus, targeting the S protein could be helpful in minimizing the virus spread, which would ultimately hinder the rapid spread of COVID-19.

Nonstructural proteins

After viral entry, the virus releases its genomic RNA into the host cell cytoplasm, which is further converted into polyproteins (pp1a and 1ab). These polyproteins are then cleaved by viral proteases into a total of 16 nonstructural proteins (nsps; proteolytic processing), which could serve as essential targets against SARS-CoV-2.[16] Out of these 16 nsp, some have crucial roles in virus propagation and replication, as discussed below.

3CLpro (Mpro; Main protease; nsp5) is responsible for cleaving the polyprotein at 11 alternate sites to generate numerous nsps, which are essential for viral replication. The amino acid

residues His41 and Cys145 form catalytic dyads for 3CLpro; it also has a substrate-binding site positioned between domains I and II.[17] Chen *et al.* reported that the polypeptide chain of novel coronavirus is 306 amino acids long.[18] Based on recent reports, Mpro is essential for viral assembly and maturation and, thus, is a vital drug target against SARS-CoV-2.

RNA-dependent RNA polymerase (RdRp; nsp12) is a core component of the virus because it is used for genome replication and viral gene transcription.[19] Although nsp12 exhibits minimal catalytic activity, it requires support from other cofactors (nsp7 and nsp8) to display significant polymerase activities.[20] Carmer *et al.* revealed that the active site of RdRp, comprising cofactors, nsp12, and the RNA template, mimics the replicating enzyme.[21] The authors reported binding between the first turn of RNA and the active-site cleft of nsp12, whereas nsp8 (two copies) was positioned near the second turn of RNA by binding to opposite sides to that of the cleft. This forms positively charged 'sliding poles' because of helical extensions in nsp8, which are involved in replicating the long genome of coronaviruses. Thus, nsp12-nsp7-nsp8 is known as the core component for virus RNA replication and RdRp is a vital drug target against COVID-19.

The papain-like protease (PLpro; nsp3) of novel coronavirus shares 83% sequence similarity with that of earlier reported SARS-CoV. Recently, Dikic *et al.* reported that these two proteases display distinct preferences for the host substrate; PLpro of the novel coronavirus is responsible for cleaving the ubiquitin-like interferon-stimulated gene 15 protein (ISG15), whereas ubiquitin chains are preferentially targeted by PLpro of SARS-CoV.[22] Furthermore, the authors analyzed the crystal structure of the protein in complex with ISG15 and revealed that PLpro forms specific interactions with the latter. Thus, PLpro is an important drug target to inhibit SARS-CoV-2 because it is responsible for the generation of the replicase complex and supports the spread of the virus.

Apart from nsp3 (PLpro), nsp5 (Mpro), and nsp12 (RdRp), various other nsps are also known to have important roles in replication or host immune system regulation of novel coronavirus. Nsp1 activates NFAT signaling and immune activation in SARS-CoV and a similar function is expected, but not yet explored, for nsp1 of this novel coronavirus because both are closely related.[23] Nsp9 is a dimeric protein that is responsible for the reproduction of RNA; thus, any disruption of its dimerization could be a vital strategy to tackle COVID-19.[24] Recently, it was suggested that nsp15 could be a target for overcoming the burden of SARS-CoV-2 because it has the ability to evade detection by the host immune system.[24]

Reprofiling of existing drugs against SARS-CoV-2

Repurposing drugs targeting the S protein

Egieyeh *et al.* computationally studied several peptides that can act as potential inhibitors of the S protein.[25] The authors investigated 1070 drugs and found two to have excellent predicted binding affinity. Sar9 Met (O2)11-Substance P showed a binding free energy of −10.63 kcal/mol and BV2 of −17.32 kcal/mol (Table 1; **1**, **2**). The binding free energy value of −35.05 kcal/mol in absence of BV2 peptide drug suggests that BV2 has a crucial role in limiting the interaction between the S protein and the hACE2 receptor. They also performed the molecular dynamics (MD) simulation (100 ns) for both complexes, which indicated the stability of the Substance P–hACE2 complex with a root mean square deviation (RMSD) of 3.49 Å. Likewise, through virtual screening methods, Tian-zi *et al.*[26] explored the S protein as a target to minimize the spread of SARS-CoV-2. The authors considered ~15 000 molecular candidates to carry into docking studies. Among US Food and Drug Administration (FDA)-approved drugs, digitoxin (used to treat heart failure) showed excellent binding energy, −8.7 kcal/mol (Table 1; **3**). Bisindigotin, from Traditional Chinese Medicine System Pharmacology (TCMSP), displayed excellent results with a binding free energy of −8.3 kcal/mol (Table 1, 4).

Qiao *et al.*[27] also performed computational studies to find potential inhibitors of the SARS-CoV-2 S protein. They selected numerous FDA-approved drugs from the Binding Database. Among the selected drugs, ergotamine and amphotericin b demonstrated promising results with a docking score of −8.8 and −8.3 kcal/mol, respectively (Table 1; **5**, **6**). However, the validation of docking studies through MD simulation was not explored.

Oliveira *et al.*[28] virtually screened a library of 9091 drugs against the S protein. Among them, 24 drug candidates (binding affinity <−8.1 kcal/mol) were selected as top-ranked hits from the docking results. The authors then performed MD simulation for the three hit ligands complexed with the protein. They found that R403, R405, Y449, L455, G496 and Y505 were the residues interacting for longest period, with Lig8970 (Table 1; **7**) displaying the best results, with a binding score of −40.43 kcal/mol.

Maffucci *et al.*[29] virtually screened a library of 3000 drugs against the S protein through computational approaches. They initially carried out molecular docking studies, followed by short MD simulations to shortlist top-ranked candidates. Polymyxin B demonstrated significant affinity towards the S protein RBD-binding site (Table 1; **8**).

Repurposing drugs targeting 3CLpro

Arun *et al.*[30] used 4600 drugs (ligands) from the superDRUG2 databank to predict candidates for drug repurposing. They generated an e-pharmacophore model with the help of the available crystal structure of 3CLpro and shortlisted 1000 ligands for further screening. Docking was performed using the GLIDE module from the Schrodinger software. Hits were identified following high-throughput virtual screening and binding free energy. The results indicated a strong binding affinity for binifibrate and bamifylline, with a binding free energy of −69.04 and −63.19 Kcal/mol, respectively (Table 2; **1**, **2**). The stability of interactions between the protein–ligand complexes was assessed by MD simulation at 100 ns. However, the FDA recently withdrew binifibrate from the market because of adverse effects.[30]

Deshpande *et al.*[31] screened various antiviral drugs against multiple proteins through docking and reported lopinavir as lead against 3CLpro with a binding score of −9.9 kcal/mol (Table 2; **3**). Remdesivir showed the second highest binding efficiency with 3CLpro (−9.7 kcal/mol) and interacted with similar residues to lopinavir within the active site (Table 2; **4**). However, validation studies are required to determine the impact of this binding on protein function.

TABLE 1

Chemical structure, name, docking or binding score, binding free energy, platform, and Protein Data Bank ID for best-reported drug candidates against the S protein of SARS-CoV-2.

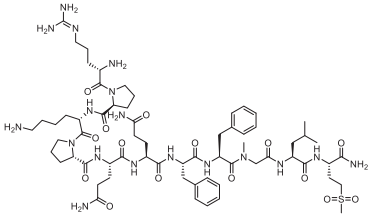
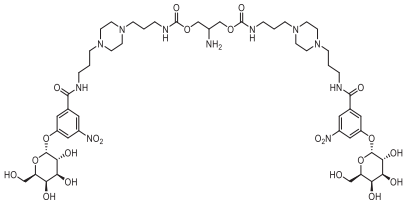
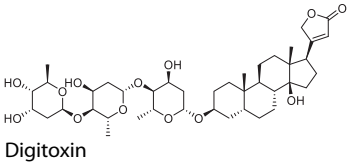
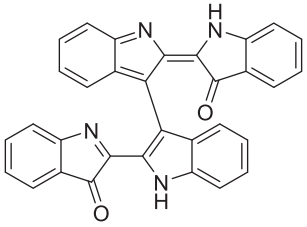
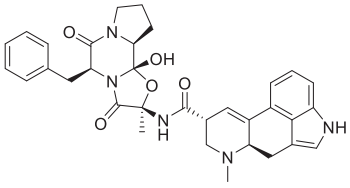
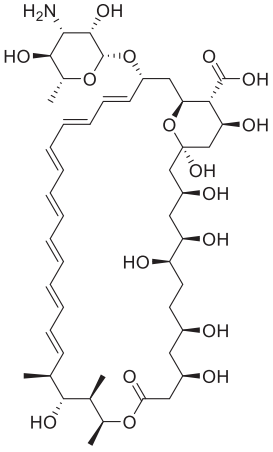
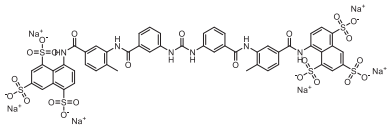
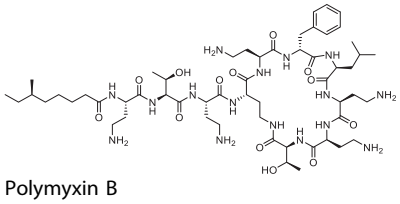
Entry no.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA; kcal/mol)	Platform (Protein Data Bank ID)	Refs
1	 Sar9 Met (O2)11-Substance P	–	−10.63	Molecular operating environment (MOE) (6LZG)	25
2	 BV2	–	−17.32	MOE (6LZG)	25
3	 Digitoxin	−8.7	–	Autodock; AMBER16 (6LZG)	26
4	 Bisindigotin	−8.3	–	Autodock; AMBER16 (6LZG)	26
5	 Ergotamine	−8.8	–	Autodock; (6ACD)	27
6		−8.3	–	Autodock; (6ACD)	27

TABLE 1 (*CONTINUED*)

Entry no.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA; kcal/mol)	Platform (Protein Data Bank ID)	Refs
7	Amphotericin b  Lig8970	−8.7	−40.43	Autodock; GROMACS (5X5B, 6ACG, 5I08)	28
8	 Polymyxin B	−99.4	−164.3	PLANTS; AMBER (6M0J)	29

A comparison study of the 3D structure of 3CLpro was carried out with seven other proteases (central enzymes that act as drug targets for antiviral therapy) by Eleftheriou and co-authors.[32] Docking analysis of 34 protease inhibitors with 3CLpro revealed that the best results were obtained for telaprevir and boceprevir (HCV protease inhibitors) with a free energy of binding of −10.05 and −9.15 Kcal/mol, respectively (Table 2; **5**, **6**). A thrombin inhibitor, argatroban (−9.03 kcal/mol; Table 2; **7**) and a DPP-4 inhibitor, sitagliptin (−8.80 kcal/mol; Table 2; **8**) were found to be the best in their respective enzyme category. Recently, Ma *et al.*[33] reported that boceprevir inhibits the enzymatic activity of 3CLpro with an IC_{50} of 4.13 mM, and inhibited viral replication in cell culture with an EC_{50} of 1.90 mM. These results highlight the therapeutic potential of boceprevir, which provides a promising starting point for *in vivo* studies.

Kumar *et al.*[34] virtually screened 77 approved drugs against 3CLpro of SARS-CoV-2. From molecular docking results, ten drugs were selected that displayed a binding score <−8.0 kcal/mol. The authors then carried out MD simulations for the top three drugs (Table 2; **9**–**11**). All three drugs exhibited good molecular interactions with the targeted protein. Lastly, the authors evaluated the *in silico* inhibition constant (K_i) for each candidate, finding that a lopinavir–ritonavir combination displayed the best K_i score of 16 nM. However, these computational results need to be validated by biological assays and detailed structural investigations.

Tsuji[35] performed virtual screening of bioactive scaffolds (approved, clinical, and preclinical drugs) obtained from the ChEMBL data base to evaluate their potential against 3CLpro. The author used RDOCK and Autodock Vina as platforms to perform the study. To filter the library, thresholds were set on both the platforms: a RDOCK score threshold of ≤−50 kcal/mol and a Autodock Vina score threshold of ≤−10 kcal/mol. The procedure helped to reduce the drug library of more than 1 million compounds to 64 potential inhibitors. Docking simulations for the top-ranked compounds with 3CLpro indicated that 28 drug candidates displayed good docking interactions. However, eszopiclone was the only approved drug (for neuropsychiatric disease) among the shortlisted bioactive scaffolds with a docking score of <−10 kcal/mol (Table 2; **12**); docking simulations revealed that the carbonyl moiety of the drug was close to the catalytic dyad. Overall, the strategy followed by Tsuji could be beneficial for shortlisting effective drugs from a large library.

Keretsu *et al.*[36] performed modeling studies to recognize potential inhibitors of 3CLpro from the MEROPS database and reported that 15 compounds displayed higher binding affinity compared with an α-ketoamide inhibitor. Among them, saquinavir (Table 2; **13**) exhibited important H-bond interactions with residues at the S1 subsite, whereas other investigational drugs (aclarubicin, TMC-310911, and faldaprevir) formed a smaller number of H-bond interactions. Therefore, saquinavir should be evaluated further in biological studies.

Several other reports have been published using an approved drug library to identify potent inhibitors of 3CLpro. Saquinavir (Table 2; **14**),[27] hesperidin (Table 2; **15**),[37] mitoxantrone (Table 2; **16**),[38] raltegravir (Table 2; **17**),[39] and paritaprevir (Table 2; **18**)[40] are some of the inhibitors of 3CLpro identified virtually from the screened libraries.

Apart from individual docking, the combination of scaffolds was also proposed to tackle COVID-19. Muralidharan *et al.*[41] studied the synergistic effect of lopinavir, oseltamivir, and ritonavir against 3CLpro with the help of sequential docking. Interestingly, the combination results showed much better binding affinity compared with individual drugs. Furthermore, MD simulations indicated that the complex of the main protease and three drugs remained stable during a simulation period of 100 ns. Hence, this strategy could be applied to other potential inhibitors to determine successful therapeutic strategies to combat COVID-19. The synergistic effect of different drug combinations against SARS-CoV-2 was also studied in detail by Bobrowski and co-authors.[42] The authors used computational approaches to select 73 combinations among 23 approved drugs that could have a potent activity profile against SARS-CoV-2. All combinations were tested in culture for their efficacy against SARS-CoV-2. The results indicated 16 synergistic and eight antagonistic combinations. In a similar report, Jin *et al.*[43] developed a model using machine learning to explore the effect of combination therapies on SARS-CoV-2. The model emphasized two types of interaction: drug–target and target–disease. The designed model was validated and showed remdesivir–reserpine and

TABLE 2

Chemical structure, name, docking or binding score, binding free energy, platform, and Protein Data Bank ID for best-reported drug candidates against the main proteases (Mpro or 3CLpro) of SARS-CoV-2.

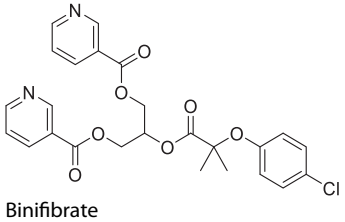
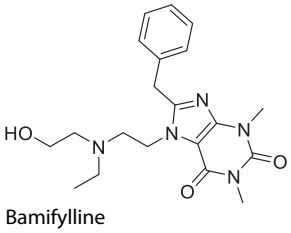
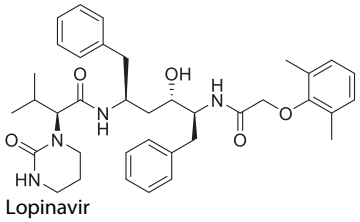
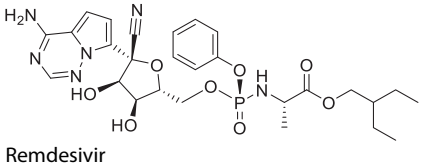
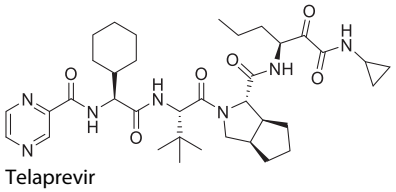
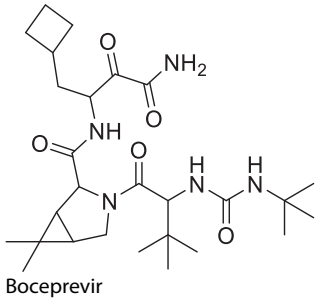
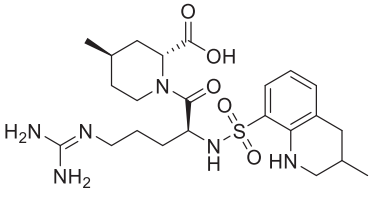
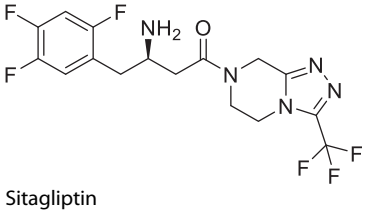
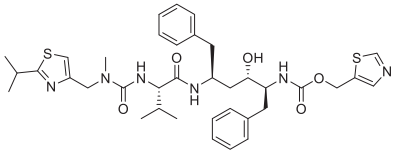
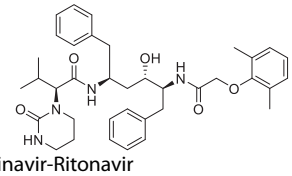
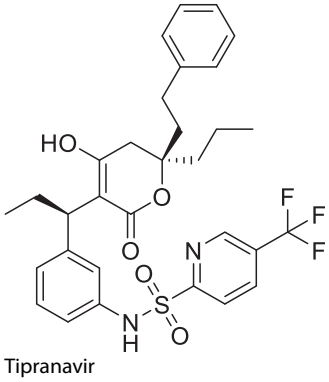
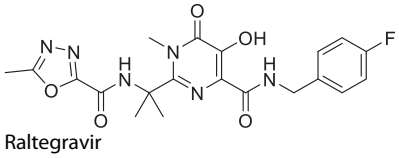
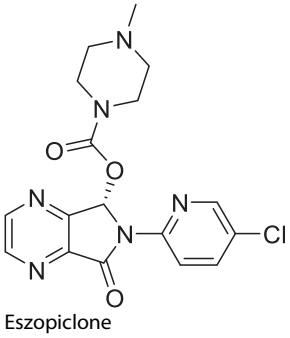
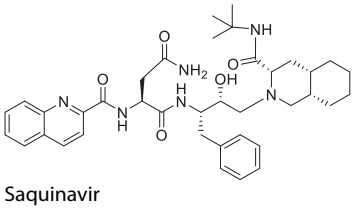
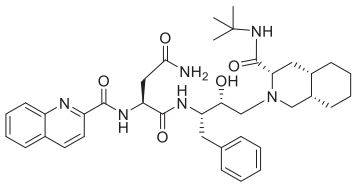
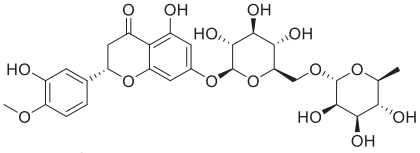
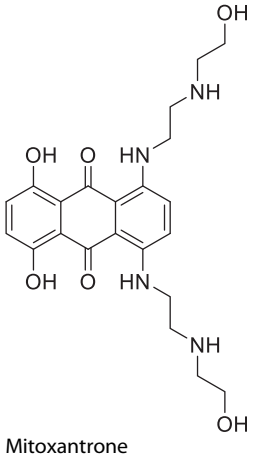
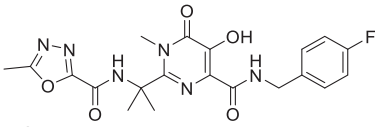
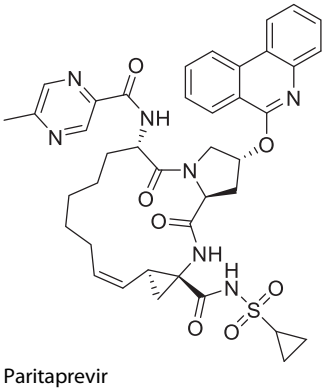
Entry No.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (Protein Data Bank ID)	Refs
1	 Binifibrate	–	–69.04	Schrodinger (6 W63)	30
2	 Bamifylline	–	–63.19	Schrodinger (6 W63)	30
3	 Lopinavir	−9.9	–	PyRx 0.8 (6Y84)	31
4	 Remdesivir	−9.7	–	PyRx 0.8 (6Y84)	31
5	 Telaprevir	−10.05	–	Autodock (6LU7)	32
6	 Boceprevir	−9.15	–	Autodock (6LU7)	32
7		−9.03	–	Autodock (6LU7)	32

TABLE 2 (*CONTINUED*)

Entry No.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (Protein Data Bank ID)	Refs
8	Argatroban 	−8.80	–	Autodock (6LU7)	32
9	Sitagliptin 	−10.6	–	Autodock (6Y2F)	34
	Lopinavir-Ritonavir 				
10	 Tipranavir	−8.7	–	Autodock (6Y2F)	34
11[a]	 Raltegravir	−8.3	–	Autodock (6Y2F)	34
12	 Eszopiclone	−10.0	–54.504	Autodock (6Y2G)	35
13[b]	 Saquinavir	−9.1	–125	Autodock; GROMACS (6LU7)	36

(continued on next page)

TABLE 2 (*CONTINUED*)

Entry No.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (Protein Data Bank ID)	Refs
14[b]	 Saquinavir	−9.5	–	Autodock (6LU7)	27
15	 Hesperidin	−8.3	–	Autodock (6LU7)	37
16	 Mitoxantrone	–	–43.5854	Schrodinger (6LU7)	38
17[a]	 Raltegravir	−9.0	–	Autodock (6LU7)	39
18	 Paritaprevir	−10.9	–	Autodock (6Y2E)	40

[a] Chosen as top candidate in two studies.
[b] Chosen as top candidate in two studies.

remdesivir–IQ-1S as drug combinations with potent synergistic effects against SARS-CoV-2 in culture. Thus, the synergistic effect of approved drugs could be beneficial in finding a potent chemical–chemical combination to fight COVID-19.

Repurposing drugs targeting RdRp

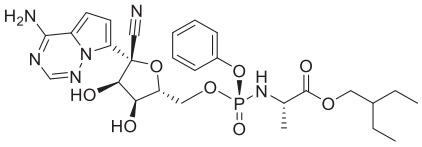
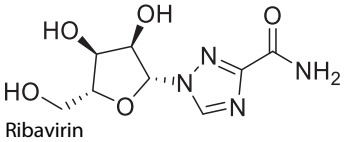
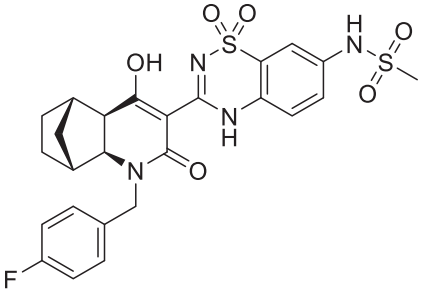
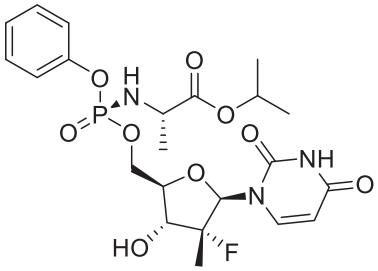
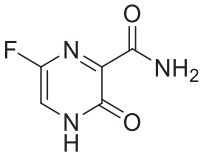
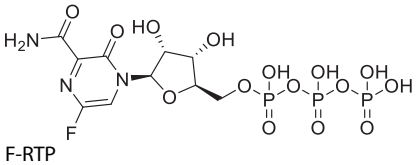
Elfiky *et al.*[44] carried out molecular docking to evaluate various direct-acting antiviral (DAA) drugs against the RdRp enzyme. In total, 24 compounds were taken into consideration for the study, of which five were FDA approved and 13 were clinical trial drug candidates against HCV. The authors also selected cinnamaldehyde and thymoquinone as negative controls, which do not have an affinity for RdRp. Among the FDA-approved drugs, remdesivir and ribavirin displayed a higher binding affinity with RdRp, with a binding score of −7.6 and −7.8 kcal/mol, respectively (Table 3; **1**, **2**). Setrobuvir was the top-ranked candidate

among 13 clinical trial compounds with a binding score of −9.3 kcal/mol (Table 3; **3**). Sofosbuvir displayed a binding score of −7.5 kcal/mol (Table 3; **4**) and is on the WHO's List of essential medicines. Therefore, apart from FDA-approved drugs, clinical trial candidates could also be explored as potent drug inhibitors of the RdRp enzyme.

Sada *et al.*[45] used computational approaches to evaluate the interactions between favipiravir (an antiviral agent for the treatment of influenza) and the RdRp enzyme of SARS-CoV-2. The authors studied molecular interactions of favipiravir and its active form [i.e., favipiravir ribofuranosyl-5′-tripho sphate (F-RTP)]. The binding score between F-RTP and RdRp was −6.6 kcal/mol (Table 3; **6**), whereas favipiravir displayed a score of −4.0 kcal/mol (Table 3; **5**), indicating the higher affinity of the active form. The experimental EC_{50} of favipiravir against SARS-CoV-2 in cell culture was 61.88 μM, which was higher compared to its EC_{50} against influenza virus (0.030–0.46 μg/ml). Therefore, a higher concentration of favipiravir is required to inhibit SARS-CoV-2 compared with that required for influenza.

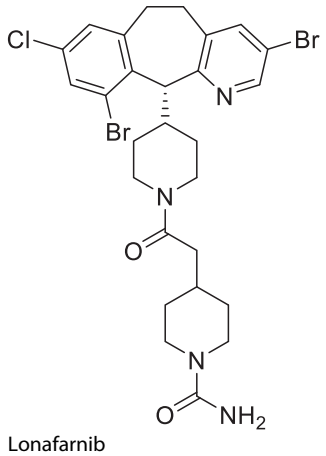
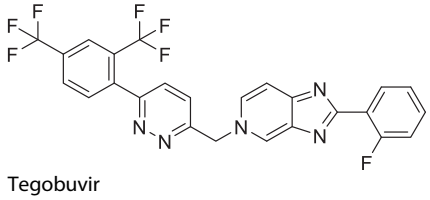
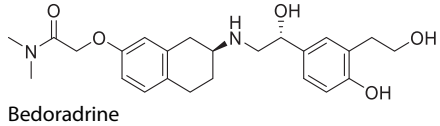
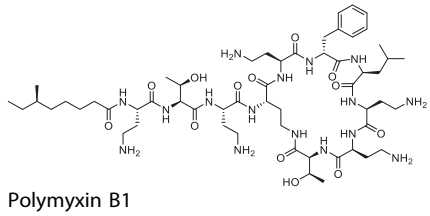
TABLE 3

Chemical structure, name, docking or binding score, binding free energy, platform, and Protein Data Bank ID for best-reported drug candidates against the RdRp protein of SARS-CoV-2.

Entry no.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (Protein Data Bank ID)	Refs
1	 Remdesivir	−7.6	–	Autodock (6NUR)	44
2	 Ribavirin	−7.8	–	Autodock (6NUR)	44
3	 Setrobuvir	−9.3	–	Autodock (6NUR)	44
4	 Sofosbuvir	−7.5	–	Autodock (6NUR)	44
5	 Favipiravir	−4.0	–	Autodock (6 M71, 7BV2)	45
6	 F-RTP	−6.6	–	Autodock (6 M71, 7BV2)	45

(continued on next page)

TABLE 3 (*CONTINUED*)

Entry no.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (Protein Data Bank ID)	Refs
7	 Lonafarnib	−7.6	−25.53	Vina 1.1.2 (7BW4)	46
8	 Tegobuvir	−7.8	−24.44	Vina 1.1.2 (7BW4)	46
9	 Bedoradrine	−8.961	−84.58	Schrodinger (6 M71)	47
10	 Polymyxin B1	−12.646	–	Schrodinger (6 M71)	48

Ruan *et al.*[46] studied the nsp12–nsp7–nsp8 complex because nsp7 and nsp8 are essential viral cofactors for blocking nsp12. The authors downloaded ~7964 drugs from the ZINC database, and screened them against nsp12–nsp8 and nsp12–nsp7. Among all the drugs, the top 20 and top 10 from each nsp12–nsp7 and nsp12–nsp8 interface were selected, showing a docking score < −7.5 kcal/mol. Subsequently, the authors performed a simulation trajectory of 5 ns and calculated the binding free energy for hits, shortlisting a total of eight candidates from both interfaces. Lonafarnib (Table 3; **7**), with a binding free energy of −25.53 kcal/mol, was most active drug candidate for the nsp12–nsp7 complex. Calculated binding free energies for the nsp12–nsp8 complex system suggested tegobuvir to have the highest binding free energy (Table 3; **8**; −24.44 kcal/mol).

Ribaudo *et al.*[47] performed virtual screening of 8815 molecules. Based on molecular docking and the binding energy results, the top-10 compounds were shortlisted and bedoradrine was identified as hit with a binding free energy score of −84.58 kcal/mol (Table 3; **9**). The results suggested kinase inhibitor drug candidates as potential RdRp inhibitors subject to *in vitro* and *in vivo* validation.

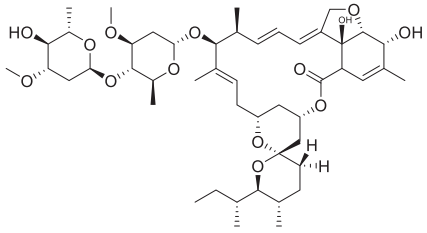
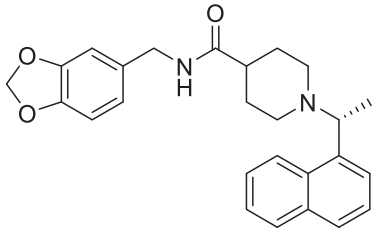
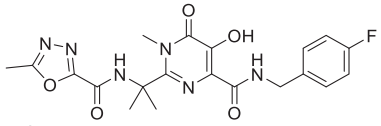
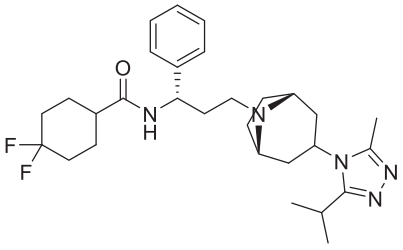
Ahmad *et al.*[48] screened 7922 FDA-approved drugs and, based on the docking score, the top-ranked molecules were shortlisted and analyzed for further studies. Some of the drugs showed significant results by binding with both forms (holoenzyme and core) of RdRp. Among the drugs that showed a binding affinity with both the forms of RdRp, Polymyxin B1 (Table 3; **10**) exhibited the strongest binding affinity with the RdRp complex with two salt bridges with Asp760 and Asp761, and two H-bonds with Arg555 amino acid residues. Targeting both the forms of RdRp could be a crucial strategy for finding potent inhibitors of SARS-CoV-2.

Repurposing drugs targeting PLpro and other nsps of SARS-CoV-2

A library of compounds was explored computationally against multiple proteins of SARS-CoV-2, including PLpro, by Marak and co-authors.[49] The results indicated that the antiparasitic drug ivermectin B1a showed the highest affinity toward the targeted enzyme among all the explored antiparasitic drugs (docking score: −8.3 kcal/mol; Table 4; **1**). Ivermectin B1a was analyzed for its interactions with PLpro and showed H-bonds

TABLE 4

Chemical structure, name, docking or binding score, binding free energy, platform, and Protein Data Bank ID for best-reported drug candidates against PLpro and other nsps of SARS-CoV-2.

Entry no.	Compound	Docking/binding score (kcal/mol)	Binding free energy (MM-GBSA) (kcal/mol)	Platform (protein; Protein Data Bank ID)	Refs
1	 Ivermectin B1a	−8.3	–	Autodock (PLpro; 6W9C)	49
2	 GRL-0667	−7.0	–	Autodock (PLpro; self-developed model)	50
3	 Raltegravir	−8.3	–	Autodock; SwissDock; GROMACS (nsp16; 6W4H)	51
4	 Maraviroc	−9.73	–	Autodock; SwissDock; GROMACS (nsp16; 6W4H)	51

with residues (Asp164, Gly_266, and Tyr268), and pi-alkyl interactions with residues (Pro247 and Tyr268).

Elfiky *et al.*[50] constructed a model for PLpro of SARS-CoV-2, in an attempt to virtually screen drug molecules. The authors reported GRL-0667 as the best candidate based on its binding score (Table 4; **2**; −7 kcal/mol). The binding score for GRL-0667 against PLpro of SARS-CoV-2 was found to be in a similar range to that of Middle East respiratory syndrome (MERS)-CoV (−7.62 ± 0.65 kcal/mol) but was slightly less when compared with SARS-CoV (−9.3 kcal/mol).

In a study related to the discovery of potential therapeutics against nsp16, a virtual drug repurposing approach was adopted by Tazikeh-Lemeski and co-authors.[51] The authors initially screened approved drugs using as a positive control (Sinefungin) and drugs exhibiting similar scores to Sinefungin were selected for further studies. They also selected four nucleosides (raltgravir, maraviroc, favipiravir, and prednisolone) and analysed their binding energies and molecular docking interactions. The drug molecules displaying lower binding energy (or higher binding affinity) were considered for MD simulation. The results indicated that Raltegravir and Maraviroc (Table 4; **3**,**4**) are more potent molecules than sinefungin, as evidenced by strong binding affinity toward the active site of the enzyme.

An unique approach was adopted by Kumar *et al.*[24] while targeting a bunch of key proteins (nsp3, nsp9, nsp12, and nsp15) of novel coronavirus through a library of approved drugs. A multi-targeting approach demonstrated that diosmin was the one among all the screened compounds, which showed potential binding affinity to all the targeted proteins.

Another distinctive approach was adopted by Zhou *et al.*,[52] which was a network-based virtual screening of about 2000 FDA approved downloaded from several databases. The authors have calculated Z-score and permutation test (P), and computationally identified 135 drugs with a minimum parameter ($Z < -1.5$ and $P < 0.05$), showing interactions with human coronavirus. The gene set enrichment analysis (GSEA) score for each drug was calculated and used as an indication of the bioinformatics validation of the selected drugs. Drug candidates with GSEA scores ranging from 0 to 3 met the criteria for a specific drug. After a detailed evaluation of all the parameters, three potent drugs were prioritized for SARS-CoV-2 (mesalazine, sirolimus, and equilin). Among 135 drugs identified, mesalazine was

demonstrated to be the top network-predicted repurposable drug, with a Z-score of −2.44. Overall, the study indicated the importance of network-based pharmacology methodologies for the fast discovery of drug candidates, which could provide promising leads for tackling COVID-19.

Discussion

This overview of using drug repurposing to find efficient therapeutics against SARS-CoV-2 has shown that this approach is an effective way of highlighting lead compounds for further investigation. We found 35 drugs with the potential to become such a therapy based on the results of molecular docking and MD approaches. All 35 drugs were predicted to bind to one of the four viral targets: S protein, Mpro, RdRp, and PLpro. To assess the predictive power of molecular docking for drug repurposing, we collected data (see Table S1 in the supplemental information online) on clinical trials in Phases III and IV for the found docking hits (Tables 1–4) using the NIH US National Library of Medicine database Clinical Trials (https://clinicaltrials.gov/) and the WHO International Clinical Trials Registry Platform (WHO ICTRP, https://trialsearch.who.int).

From Table S1 in the supplemental information online, it is evident that ten of the hits found through molecular docking and MD are being, or have been, evaluated in Phase III trials. Remdesivir (36 studies) and ivermectin (40 studies) have the highest number of registered trials and are currently being evaluated or have cleared Phase IV (four trials for remdesivir and nine trials for ivermectin) as monotherapy or in various combinations. Thus, we can conclude that computational approaches used for drug repurposing are a vital tool in events such as a pandemic to highlight quickly, directions for research when available data is limited.

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.

Acknowledgments

This work was supported by the Defence Research & Development Organization (ERIP/202110007/M/01/1789), Government of India. Financial support from National Academy of Science of Ukraine within the project “Directed synthesis, chemical transformations and properties of new nitrogen-containing heterocyclic compounds, is also acknowledged. S.K. is thankful to CSIR, New Delhi, India for providing a senior research fellowship (08/700(0003)/2018-EMR-I).

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