Déjà vu: Stimulating open drug discovery for SARS-CoV-2

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In the past decade we have seen two major Ebola virus outbreaks in Africa, the Zika virus in Brazil and the Americas and the current pandemic of coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). There is a strong sense of déjà vu because there are still no effective treatments. In the COVID-19 pandemic, despite being a new virus, there are already drugs suggested as active in *in vitro* assays that are being repurposed in clinical trials. Promising SARS-CoV-2 viral targets and computational approaches are described and discussed. Here, we propose, based on open antiviral drug discovery approaches for previous outbreaks, that there could still be gaps in our approach to drug discovery.

Introduction

The two major Ebola virus outbreaks in Africa [1], the Zika virus in Brazil [2] and the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3], which was declared by the World Health Organization (WHO) as a pandemic [4,5], have all occurred in the past decade. In each case there is a strong sense of déjà vu for those scientists involved in drug discovery. A lack of available antivirals to treat the infected patients leads to a clamoring to test anything available, and some pharmaceutical companies charge in to offer their drugs. We also seem to see similar patterns in response across outbreaks. There is a rush to be 'first' and this sense of priority might not lead to the best or even any outcome for patients. Always, there is the immediate proposal to create a

vaccine and pronouncements that this will be available in a short time or by the end of the year of the actual outbreak in question; and it never happens within these optimistic artificial deadlines. Again, we have experienced this with the current outbreak. For Ebola the vaccine was ready for the second outbreak and has now been approved [6] .
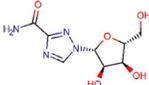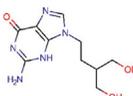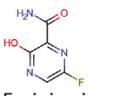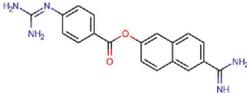
Governments are out to calm their populations while, at the same time, needing to be seen to do something that will vanquish the virus. In the case of SARS-CoV-2 it results in pneumonia [7] and shares aspects of pathology and pathogenesis with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [8]. SARS-CoV-2, SARS-CoV and MERS-CoV belong to the same family *Coronaviridae* and genus *Betacoronavirus*. Several drug companies immediately made their antivirals available. For example, Abbvie made their HIV approved drug lopinavir/ritonavir combination available to the Chinese government [9]. This

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TABLE 1

Compounds screened *versus* SARS-CoV-2: Vero E6 cells were infected with nCoV- 2019BetaCoV/Wuhan/WIV04/2019 at a multiplicity of infection (MOI) of 0.05 [14]

Molecule	Class	EC$_{50}$ (µM)	CC$_{50}$ (µM)	SI	FDA status
Ribavirin	Antiviral for hepatitis C	109.5	>400	3.65	Approved
Penciclovir	Antiviral for herpesvirus	95.96	>400	4.17	Approved
Favipiravir	Broad-spectrum antiviral	61.88	>400	6.46	Experimental
Nafamostat	Anticoagulant agent	22.50	>100	4.44	Approved
Nitazoxanide	Anthelmintic agent	2.21	35.53	16.76	Approved
Remdesivir	Antiviral agent	0.77	>100	129.87	Phase II/III
Chloroquine	Antimalarial agent	1.13	>100	88.50	Approved
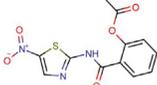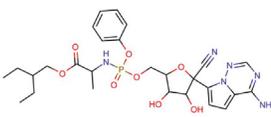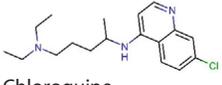
drug had previously been suggested as promising based on combination study results from a marmoset study [10]. Publications have already appeared describing the use of this drug in a patient in Korea, significantly decreasing the viral load [11]. Simultaneously, Gilead made available remdesivir, which had previously failed in clinical trials for Ebola [12] but had recently shown activity against MERS in rhesus macaques [13]. Remdesivir, chloroquine and several other drugs were tested *in vitro* against SARS-CoV-2 (Table 1) and shown to be active [14]. Some researchers had also suggested as early as January 2020 what treatment options might be most likely and these included lopinavir/ritonavir, remdesivir, favilavir, arbidol, as well as a broad array of nucleoside analogs, neuraminidase inhibitors, peptides, RNA synthesis inhibitors, anti-inflammatory drugs and traditional Chinese medicines [15–17].

The need for other treatment options will be important as we learn more about its transmission [18] and as we see increases in the number of travel-related infections [19]. Already in the past weeks we have seen the rapid spread to Italy and from there to many other countries including Brazil and beyond. Such virus outbreaks are greatly straining the healthcare systems of affected countries and will test every aspect of our infrastructure, supply chains and preparedness [20]. This raises the question of what we can learn from our past drug discovery efforts in an attempt to develop additional drugs for SARS-CoV-2 more efficiently. The acceleration of global SARS-Cov-2 data generation, effectively just

in a couple of months, has been remarkable. However, scientific information gaps that impede progress toward small-molecule treatments can still be perceived. Many of these persist from when they were first outlined for Ebola back in 2015 [21]. We can thus make some 'then-and-now' comparisons (see Supplementary material Figs. S1–S4 and Table S1 online) as well as share our own experiences of research on these virus outbreaks.

Ebola drug discovery

Our efforts in Ebola drug discovery initially started in 2014 with a computational pharmacophore analysis of a small number of *in vitro* Ebola active compounds [22]. This was followed by identifying and summarizing all the FDA-approved drugs that could be used against the virus [23,24] and highlighting various strategies for the next virus outbreak [21]. These steps preceded a pivotal point for us in developing machine learning models for the Ebola virus derived from *in vitro* data [25,26].

An early drug [22] identified by these screens was the antimalarial amodiaquine, which was subsequently shown to be associated with decreased mortality [27], as the drug (artesunate/amodiaquine) was used for malaria treatment in some Ebola patients, whereas others took a different malaria medicine (artemether/lumefantrine) [27]. Our Ebola machine learning models were used to select three molecules for *in vitro* testing [28]. We identified pyronaridine, tilorone and quinacrine as having good *in*
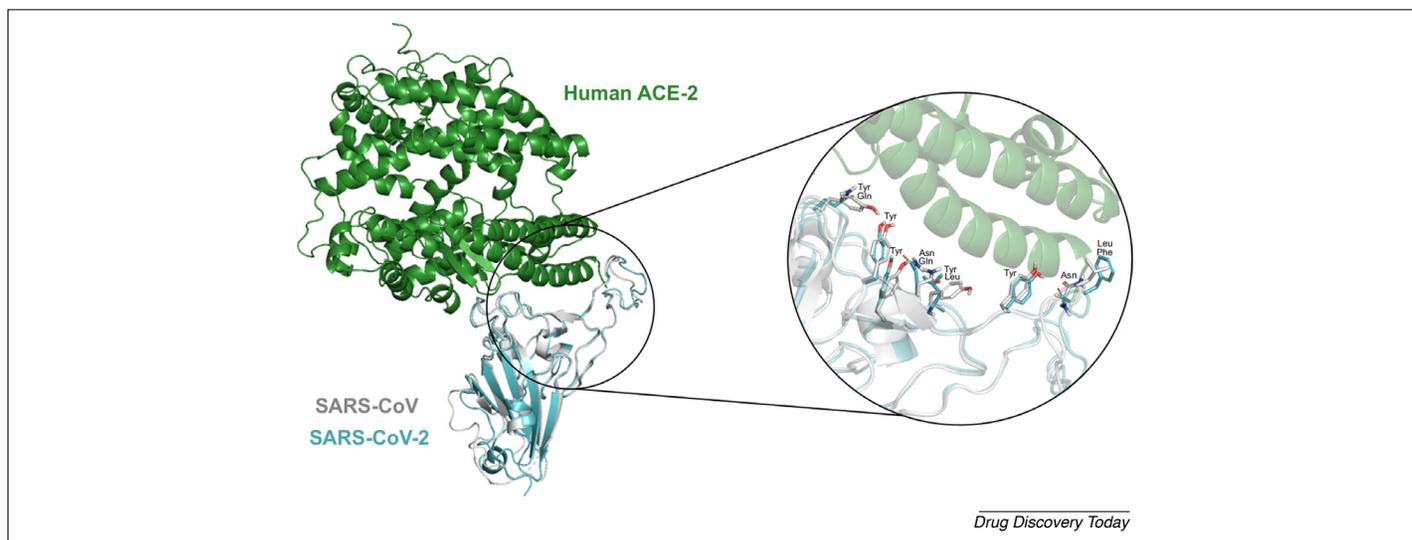
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FIGURE 1

Superposition of SARS-CoV and SARS-CoV-2 spike proteins highlighting the spike–ACE2 interface binding pocket. Zoom in at the residues of SARS-CoV-2 spike S1 Asn167, Tyr169, Tyr133, Tyr129, Gln178, Phe166, Leu135 and Gln173 and the correspondent SARS-CoV spike S1 Asn473, Tyr475, Tyr440, Tyr436, Tyr484, Leu472, Tyr442 and Asn479.
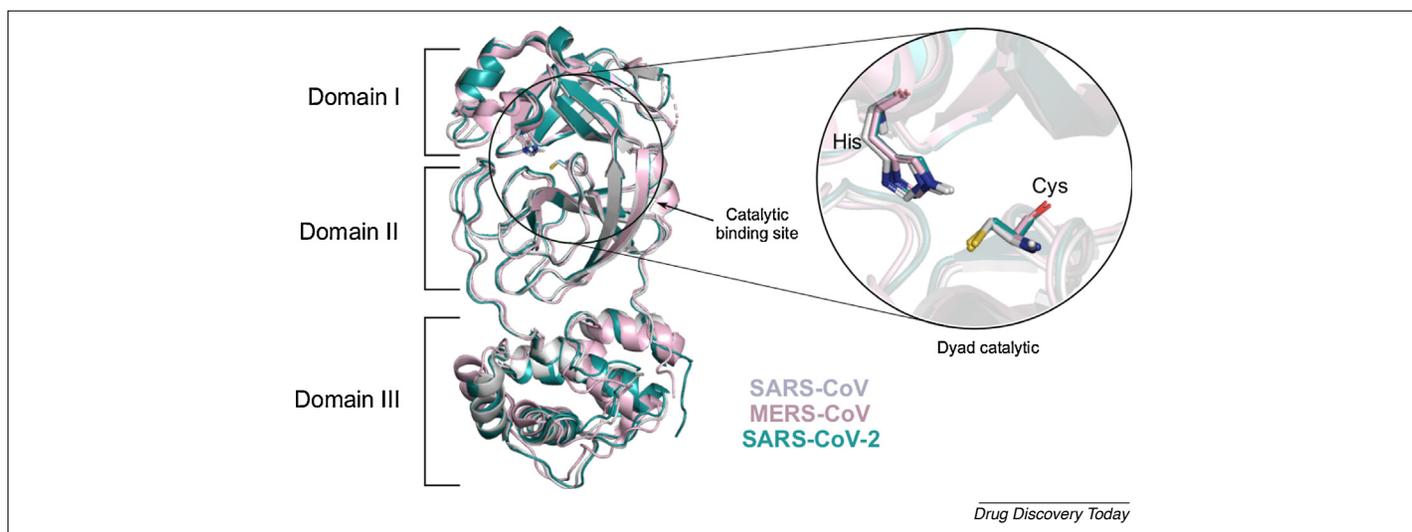

FIGURE 2

Superposition of SARS-CoV, MERS-CoV and SARS-CoV-2 main proteases, and respective catalytic binding site. SARS-CoV (PDB ID 1Q2W) and SARS-CoV-2 (PDB ID 6LU7) share the same residues at the catalytic site: His41–Cys145, and MERS-CoV (PDB ID 5WKL) His41 and Cys148, situated between domains I and II.

vitro activity (nM) against the Ebola virus [28]. These preliminary data enabled us to obtain funding from the NIH to take one of the compounds through *in vivo* testing. We also leveraged NIH support to a collaborator to test the other two compounds as well. These molecules were then each tested in the mouse model for Ebola infection and demonstrated significant efficacy [29–31]. Pyronaridine is currently being pursued in larger animal models of Ebola virus infection. Working on computational models through to *in vitro* testing happened in the space of a few months, whereas it took several years to obtain funding for our first mouse studies. This is by no means a streamlined approach to drug discovery but it was cost effective for the amount of data ultimately generated and led to revitalized interest in these molecules.

The OpenZika drug discovery experience

In 2016 we pulled together a team of researchers in Brazil and the USA to provide some suggestions for an open drug discovery effort for the Zika virus. These included various computational strategies to repurpose molecules and docking into the Zika proteins [32]. We also described resources and molecules that could be prioritized for testing. This was followed by our homology modeling of every Zika virus protein [33] weeks before the first crystal structures for Zika proteins were released. All of this work was performed in the open and it attracted the attention of the IBM World Community Grid team who requested we submit a proposal to them for a Zika project using their distributed computing facilities. This

TABLE 2

Compounds identified with *in vitro* activity against MERS or SARS [63,64]; additional molecules can be found in [61]
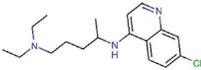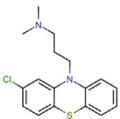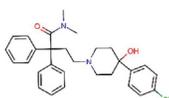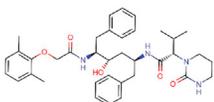
Molecule	Class	MERS EC$_{50}$ (µM)	MERS CC$_{50}$ (µM)	MERS SI	SARS EC$_{50}$ (µM)	SARS CC$_{50}$ (µM)	SARS SI	Ref.	FDA status
Chloroquine	Antimalarial	3.0	58.1	19.4	4.1	>128	31	[66]	Approved
Chlorpromazine	Antipsychotic	4.9	21.3	4.3	8.8	24.3	2.8	[66]	Approved
Loperamide	Antidiarrheal	4.8	15.5	3.2	5.9	53.8	9.1	[66]	Approved
Lopinavir	Antiviral for HIV	8.0	24.4	3.1	17.1	>32	>2	[66]	Approved
Emetine	Anti-protozoal and vomiting inducer	0.014			0.051			[67]	Approved
Chloroquine diphosphate	Antimalarial	6.275			6.538			[67]	Yes
Hydroxychloroquine sulfate	Antimalarial	8.279			7.966			[67]	Yes
Mefloquine	Antimalarial	7.416			15.553			[67]	Yes
Amodiaquine	Antimalarial	6.212			1.274			[67]	Approved
Aloxistatin	Cysteine protease inhibitor	1.275			0.761			[67]	Experimental
Gemcitabine	Antineoplastic agent	1.216			4.957			[67]	Approved
Tamoxifen	Antineoplastic agent	10.117			92.886			[67]	Approved
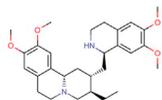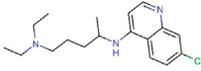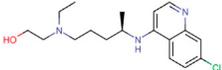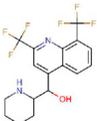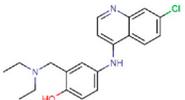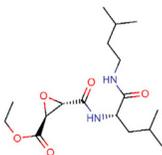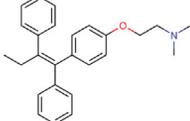
TABLE 2 (*Continued*)

Molecule	Class	MERS EC$_{50}$ (μM)	MERS CC$_{50}$ (μM)	MERS SI	SARS EC$_{50}$ (μM)	SARS CC$_{50}$ (μM)	SARS SI	Ref.	FDA status
Toremifene	Antineoplastic agent	12.915			11.969			[67]	Approved
Terconazole	Antifungal agent	12.203			15.327			[67]	Approved
Triparanol	Cholesterol-lowering agent	5.283						[67]	Withdrawn
Anisomycin	Antibacterial agent	0.003			0.191			[67]	Experimental
Cycloheximide	Antifungal agent	0.189			0.043			[67]	Experimental
Homoharringtonine	Antineoplastic agent	0.0718						[67]	Approved
Benztropine	Anticholinergic agent	16.627			21.611			[67]	Approved
Fluspirilene	Antipsychotic agent	7.477			5.963			[67]	Approved
Thiothixene	Antipsychotic agent	9.297			5.316			[67]	Approved
Fluphenazine	Antipsychotic agent	5.868			21.431			[67]	Approved
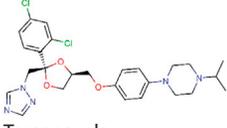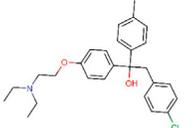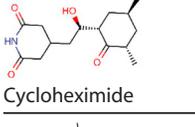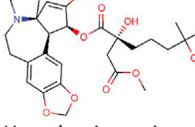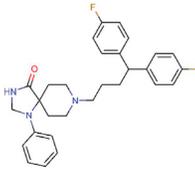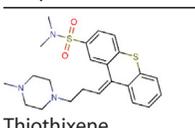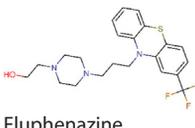
TABLE 2 (*Continued*)

Molecule	Class	MERS EC$_{50}$ (μM)	MERS CC$_{50}$ (μM)	MERS SI	SARS EC$_{50}$ (μM)	SARS CC$_{50}$ (μM)	SARS SI	Ref.	FDA status
Astemizole	Antihistamine agent	4.884			5.591			[67]	Withdrawn
Chlorphenoxamine	Anticholinergic and antihistamine agent	12.646			20.031			[67]	Approved
Thiethylperazine	Antipsychotic agent	7.865						[67]	Approved
Triflupromazine	Antipsychotic agent	5.758			6.398			[67]	Approved
Clomipramine	Antidepressant agent	9.332			13.238			[67]	Approved
Imatinib	Antineoplastic agent	17.689			9.823			[67]	Approved
Dasatinib	Antineoplastic agent	5.468			2.100			[67]	Approved
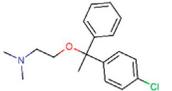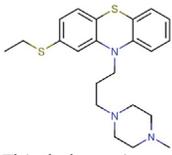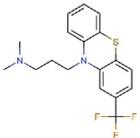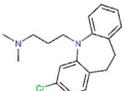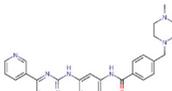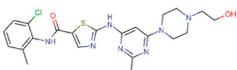
became the OpenZika project led by our team in Brazil and the USA and it involved the docking of millions of molecules from the ZINC database in all Zika virus and related flavivirus protein crystal structures [34] . We have since also performed further modeling of Zika proteins [35] and an analysis of the first 2 years following the outbreak [36]. Our most recent efforts have led to the discovery of an inhibitor for NS3 helicase [37].

What we can learn from the OpenZika project to streamline future open science projects would be as follows. We would pick fewer targets to dock molecules into as the OpenZika project created billions of docking results for tens of targets that will take many years to process. We would also suggest that it is best to work with high-resolution crystal structures *versus* homology models as soon as possible. We would also start with a library of commercially available drugs and antivirals, this would massively limit the number of molecules docked to a few thousand because we found that docking of the ZINC library available at that time led to compounds that were not readily available. Focused libraries are interesting options to perform target-directed virtual screening. Examples are ChemDiv [38] and Asinex [39] databases, which have specific subsets for protease inhibitors, for example facilitating and accelerating the experimental screening. During OpenZika we learnt that having multiple biology labs committed to doing testing *in vitro* is also very important to have access to experimental results more quickly to validate the computational approach. Working with the IBM World Community Grid did not include any funding. We found it very difficult to obtain NIH funding to support this project from the very beginning (because it was computational) and also attempted small business grants with academic collaborators without success, even though we were leveraging massive computational resources through the World Community Grid. We would recommend that open efforts like this need some funding for *in vitro* validation. Millions of dollars have been invested by the NIH to fund other Zika research projects since the 2016 outbreak and there is as yet no small molecule of note in the drug discovery pipeline [36]. There is still therefore an unmet medical need for treatments for Zika virus and computational efforts could aid this and be cost-effective.

SARS-CoV-2 drug discovery efforts
The whole genome sequences for SARS-CoV-2 were isolated from patients from several countries, such as Brazil, Canada, China, Germany, Korea and USA, and were made rapidly available at the Global Initiative on Sharing All Influenza Data (GSAID) platform
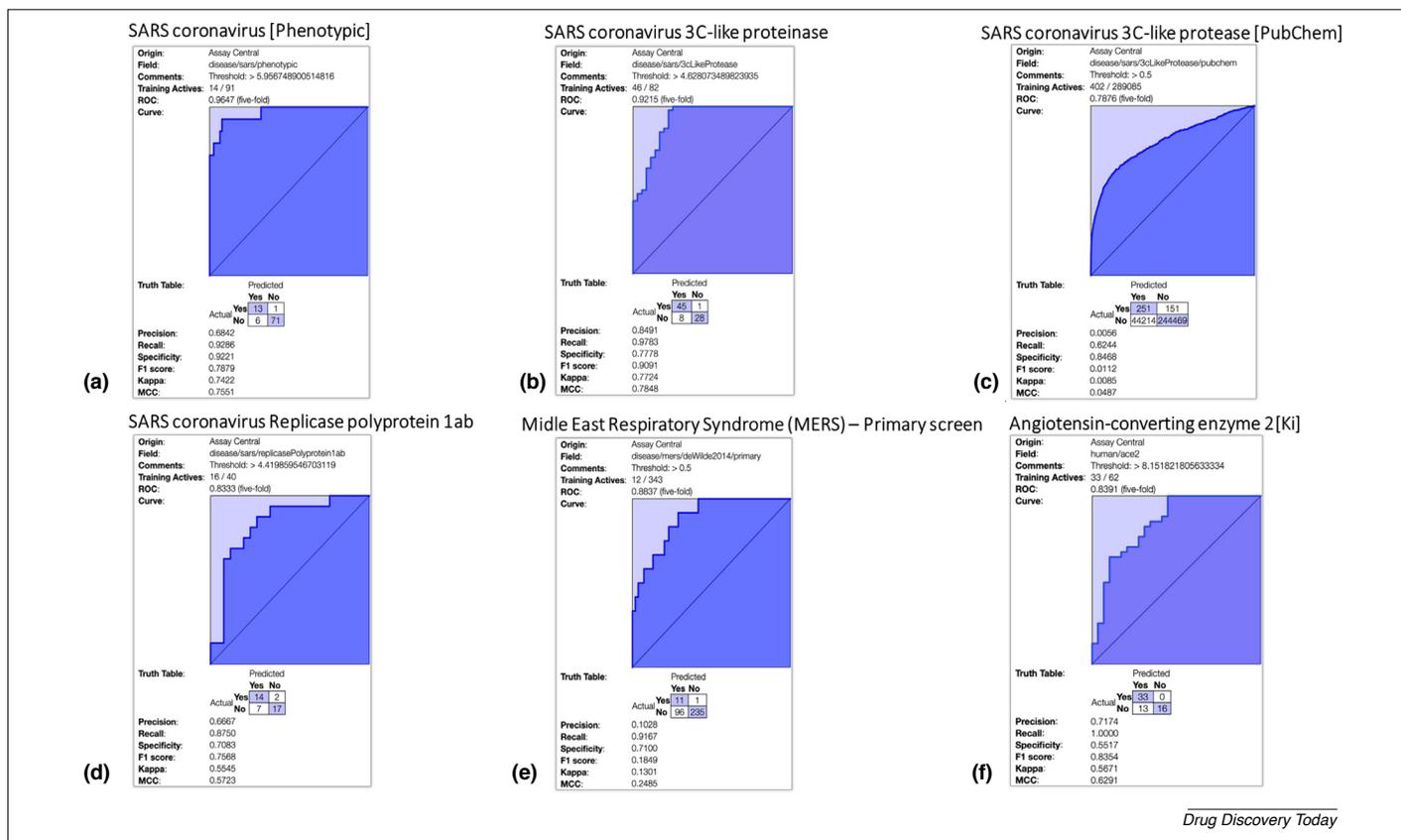
FIGURE 3

Fivefold cross validation ROC plots for Bayesian machine learning models using ECFP6 fingerprints for SARS and MERS datasets which can be used for scoring and selecting compounds to test: **(a)** SARS phenotypic screen; **(b)** SARS 3C-like proteinase; **(c)** SARS 3C-like proteinase primary screen from PubChem; **(d)** SARS replicase polyprotein 1ab; **(e)** MERS phenotypic screen; **(f)** angiotensin-converting enzyme 2

[40]. There were 135 genomes sampled between December 2019 and February 2020. The samples were found to be closely related with few mutations relative to a common ancestor. Preliminary genetic analysis indicates that the Brazilian genome differs by three mutations to the Wuhan reference strain [41]. Two of these mutations are shared with its closest sequence–the Germany strain [41].

In the space of a few weeks after the acknowledgement of the latest coronavirus outbreak, several laboratories started to deposit homology models for the main viral protease [42,43], and then for all the SARS-CoV-2 proteins using the server I- TASSER [44]. Others performed docking in these models [45] or developed their own models to understand how the virus enters cells by modeling the Spike (S) protein and human angiotensin-converting enzyme 2 (ACE2) protein interaction [46]. At the time of writing there were structures available for the SARS-CoV-2 main protease in complex with an inhibitor N3 (PDB ID 6LU7) [47] and S1 and S2 subunits of Spike glycoprotein receptor-binding domain up (6VSB), the post fusion core subunit (6LXT) and the HR2 domain (6LVN) [48,49]. A useful overview is provided by searching the entire virus sequence against the Protein Data Bank (PDB) (see Supplementary material Fig. S2 online). In comparison, for Zika it took several months from the beginning of the outbreak until there was a single protein available in the PDB [50].

The Spike glycoprotein is responsible for the fusion of the viral membrane with the host-cell membrane. During virus maturation, it is cleaved to its subunits: S1, which attaches the virion to the cell membrane by interacting with the host receptor; and S2, which mediates the fusion of the virion and cellular membranes. A recent study reports that SARS-CoV-2 Spike protein binds ACE2 with higher affinity than SARS-CoV [48].

Because there was only one Cryo-EM structure of SARS-CoV-2 Spike protein with low resolution (3.46 Å) without the human ACE2 protein [48] at the time of writing this paper, we preferred to build a model by homology modeling (see Supplementary material methods online). Using our SARS-CoV-2 Spike protein model and the available SARS-CoV Spike protein crystal structure (PDB: 2AJF), we aligned both structures to compare the residues in the Spike–ACE2 interface residues for both viruses. As can be seen in Fig. 1, the residues for SARS-CoV are Asn473, Tyr475, Tyr440, Tyr436, Tyr484, Leu472, Tyr442 and Asn479 and for SARS-CoV-2 are Asn167, Tyr169, Tyr133, Tyr129, Gln178, Phe166, Leu135 and Gln173. Moreover, some SARS-CoV-2 residues, such as Gln178, Phe166, Leu135 and Gln173, are not conserved but have the same physical–chemical features, except the residues SARS-CoV-2 Gln178 (polar) and SARS Tyr484 (hydrophobic) (Fig. 1).

The main protease (M^{pro}) of coronaviruses is a cysteine protease that participates in the viral replication, cleaving the polyprotein.
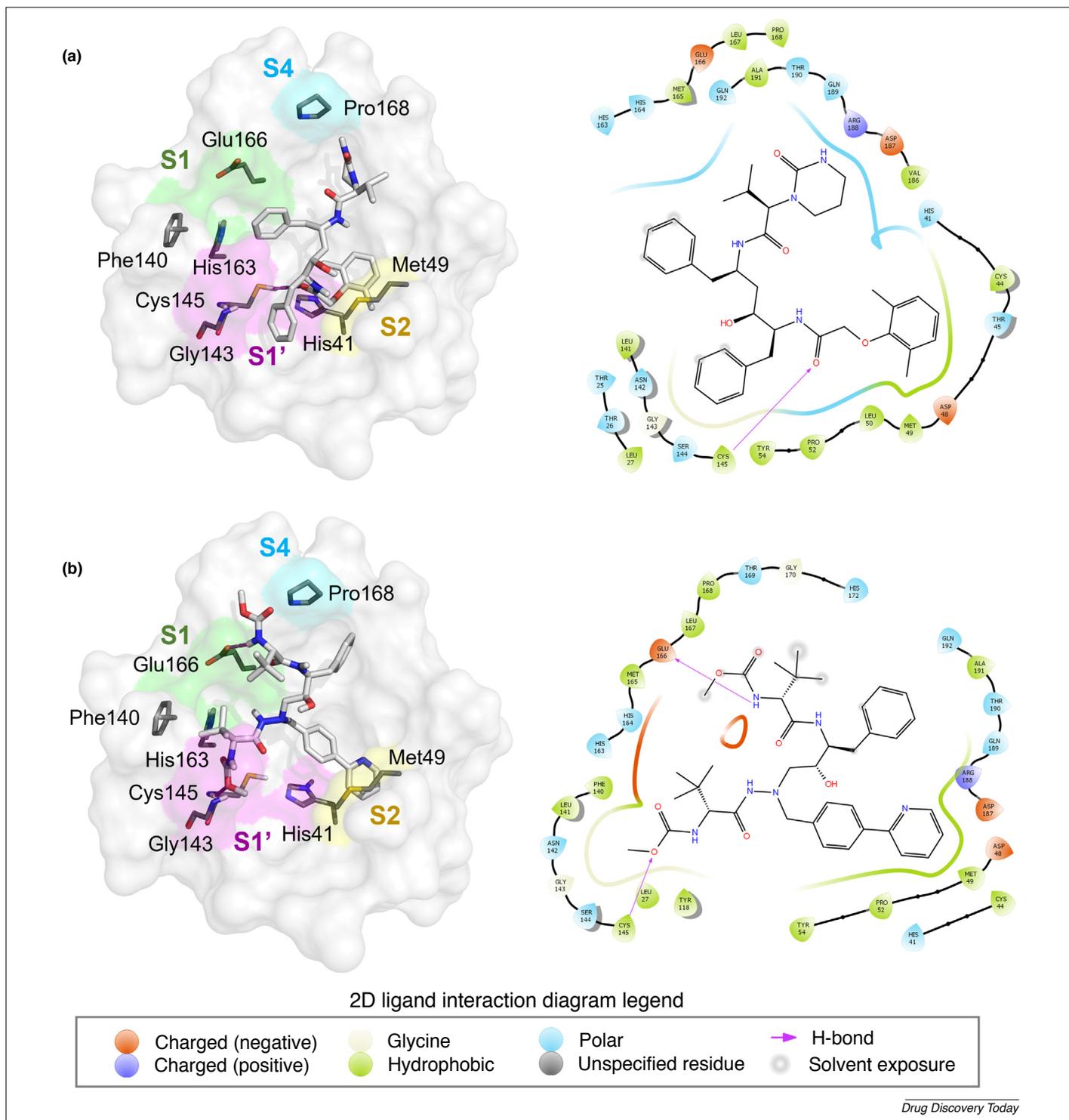
FIGURE 4

Intermolecular 3D and 2D interactions of (a) lopinavir and (b) atazanavir with the main protease structure of SARS-CoV-2 (PDB ID 6LU7) obtained by docking. In the 3D representation (left), H-bonds are presented as magenta dashed lines and the color code of carbon, oxygen, nitrogen and hydrogen atoms are grey, red, blue and white, respectively. The S1, S1', S2, and S4 pockets are presented as green, magenta, yellow, and blue surfaces, respectively. The PyMol software [79] was used for the visual inspection of 3D docking poses and to render the 3D molecular images.

M^{pro} is organized into three domains and is active in a homodimer form [51]. This protease recognizes peptide substrate P4, P2, P1 and P1′ positions through the S4, S2, S1 and S1′ protein subsites, which are critical for substrate binding [53]. The SARS-CoV-2 M^{pro} S4 pocket is formed by amino acid residue Pro168, the S2 pocket by Met49, the S1 pocket by Ser1, Phe140, His163 and Glu166 and S1′ pocket by His41, Cys145 and Gly143 residues [80]. We aligned the crystal structures of M^{pro} of SARS-CoV, MERS-CoV and SARS-CoV-2 to

TABLE 3

Docking results for SARS-CoV-2 Spike–ACE2 model against an FDA-approved drug database

Drug name	Drug original class	Docking score (kcal mol^{-1})
Rizatriptan	Antimigraine agent	−7.49
Dasabuvir	Antiviral for hepatitis C	−7.09
Pravastatin	Lipid-lowering agent	−6.17
Empagliflozin	Hypoglycemic agent	−6.37

compare the structures and the residues of the catalytic site. The catalytic site for SARS-CoV and SARS-CoV-2 are the same: His41–Cys145 [52], whereas for MERS-Cov it is His41–Cys148 [52] (Fig. 2). This catalytic site is localized between the interface of domain I (residues 8–101) and II (102–184). The third domain (residues 201–303) is C-terminal, and consists of a loop region, required for the dimerization [53–56]. Recently, it has been reported that the structures of M^{pro} of SARS-CoV and SARS-CoV-2 have high identity of 96% [43]. The function and the structural equivalence of M^{pro} of SARS-CoV, MERS-CoV and SARS-CoV-2 demonstrate that the coronavirus proteases are promising targets for development of broad-spectrum antivirals in these viruses.

MERS and SARS repurposing

Drug repurposing offers a potentially faster approach to identify drugs that are already approved for other uses and might work for the disease of interest [57–59]. This type of strategy was attempted for MERS-CoV and SARS-CoV [60,61] and the repurposing of host-based therapies has also been proposed for coronaviruses [62]. Several studies have screened a few hundred molecules and tested the activity against MERS-CoV and SARS-CoV (Table 2) [63,64]. The first screen of 348 FDA-approved drugs identified four FDA-approved drugs including chloroquine, chlorpromazine, lopera-mide and lopinavir [63]. At the same time, a second study screened 290 compounds and identified 27 FDA- and non-FDA-approved drugs including chloroquine among the many hits [64], and the Abl2 kinase inhibitors such as imatinib which impacts the early stages of infection after internalization and endosomal trafficking. This latter molecule appears to work by inhibiting fusion at the viral membrane [65]. Chloroquine is widely used for malaria and loperamide is used for diarrhea. These represent drugs that are readily available in many locations around the world and for which physicians are also familiar with the side-effect profile. The ligand datasets from these and other screens can be used to apply machine learning methods to produce predictive models that can then be used to screen compound libraries and suggest new compounds to test. As an example, we have developed several models for ACE2, SARS-CoV and MERS-CoV using our Assay Central software [66–69] which uses ECFP6 descriptors and the Bayesian algorithm (Fig. 3, the experimental details are described in the Supplementary material online; the Bayesian models are also available at www.assaycentral.org). The advantage of this approach is that the method is fast, does not require crystal structures and it enables small-molecule structures to be scored against many models simultaneously.

Repurposing collections of compounds

As a well-established principle any SARS-CoV-2 relevant enzyme assay, protein–protein interaction antagonism or viral infectivity determination can be run against a collection of approved drugs. However, it is neither trivial to get a definitive list of these structures *in silico* prepared for docking or to acquire these as a physical collection ready for screening (even though there are various repurposing subsets available from vendors). Notwith-standing, an approximation to a definitive approved set can be selected in PubChem as the intersect between curated drug resources such as DrugBank, DrugCentral and the IUPHAR/BPS Guide to PHARMACOLOGY, with totals of 9135, 4012 and 7674 molecules, respectively. The three-way compounds-in-common total is 1119 chemical identifiers (CID). PubChem indicates that 1197 molecules are available from chemical vendors. Relaxing the stringency of the repurposing provides triage steps that can certainly be useful for docking and/or machine learning or other modeling efforts. In this case the union of all three drug curation sources is 16,468. Other drug-centric solutions include the selection of 9527 international non-proprietary names (INN) CID. The majority of these will have either been progressed to Phase I testing or are close to it.

Computational approaches to accelerate SARS-CoV-2 drug discovery

An alternative to screening every approved drug is to take one class of compounds. For example we have now taken the opportunity to dock FDA-approved HIV-1 protease inhibitors (amprenavir, ataza-navir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir and tipranavir) and hepatitis C NS3/4A pro-tease inhibitors (asunaprevir, boceprevir, grazoprevir, paritaprevir, simeprevir and telaprevir) into the M^{pro} structure of SARS-CoV-2. The docking was performed with two different commercial dock-ing software products: Biovia Discovery Studio and Schrödinger Glide [70–73], and the best docking results were compared (see Supplementary material Table S2 online). After medicinal chem-istry visual inspection, the results of both docking approaches indicated that azatanavir and lopinavir may be of most interest for *in vitro*/and/or *in vivo* testing (Fig. 4). Lopinavir (Fig. 4a) (LigandFit score of 191.196) may interact with M^{pro} by hydrogen bonding with Cys145 residue of the S1' pocket. The aromatic ring of lopinavir also formed hydrophobic interactions with residues Cys44, Met49 (S2 pocket), Leu50, Pro52, Tyr54, and Cys145 (S1' pocket). In relation to atazanavir (Fig. 4b) (LigandFit score of 175.497), hydrogen bonds were established between residues Cys145 and Glu166 both from S1' pocket and nitrogen atoms of carbamate groups. Similar hydrophobic interactions observed with lopinavir occurred also with atazanavir, with residues Met49 (S2 pocket), Pro52 and Tyr54 (Fig. 4). In China and South Korea lopinavir 200 mg/ritonavir 50 mg has already been used for the treatment of COVID-19. From the day after lopinavir/ritonavir administration, the viral load started to decrease and no detectable or little coronavirus titers have been observed since then [11]. Lopinavir, co-formulated with ritonavir is commercially available and known as Kaletra® around the world–it is on formularies for HIV. By contrast, atazanavir is less commonly used. Kaletra® can be widely produced and distributed quickly through existing channels but it will be important to ensure HIV patients do not
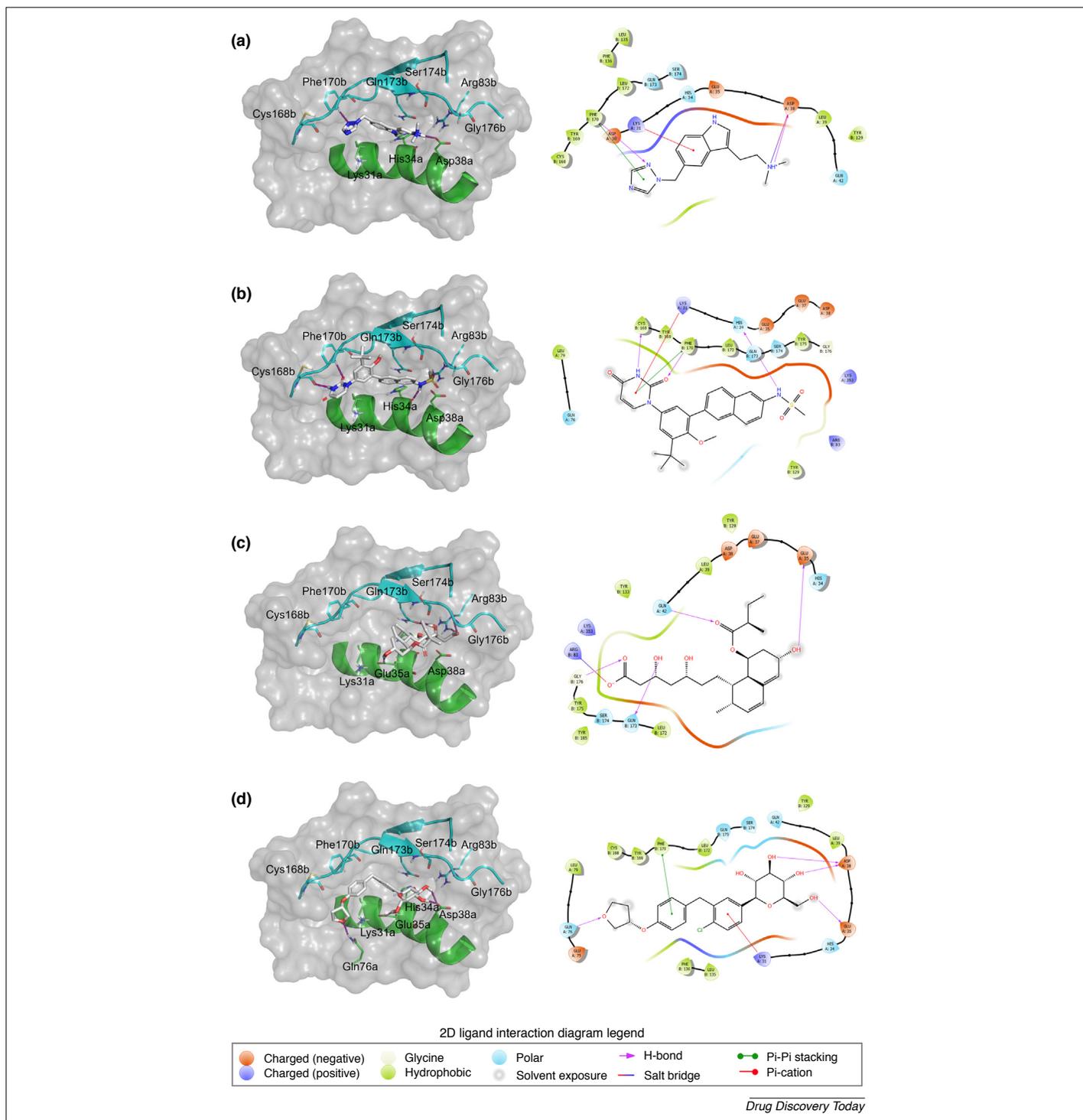
FIGURE 5

3D and 2D intermolecular interactions of rizatriptan **(a)**, dasabuvir **(b)** , pravastatin **(c)** and empagliflozin **(d)** with the spike–ACE2 interface. In 3D representation (left), the SARS-CoV-2 Spike (S1 subunit) is colored in light blue and human ACE2, in green. Hydrogen bonds are presented as magenta dashed lines and the color code of carbon, oxygen, nitrogen and hydrogen atoms is grey, red, blue and white, respectively. The PyMol software [71,73,79] was used for the visual inspection of 3D docking poses and to render the 3D molecular images.

lose access to the medications they need. It is also a medication that clinicians are familiar with in terms of side effects, unlike other less commonly used medications. A broader analysis of all FDA-approved drugs docked in this protease structure could point to further drugs worth testing.

We have also performed docking calculations of SARS-CoV-2 spike glycoprotein complexed with the host ACE2 protein against all FDA- approved drugs (~2400 molecules) using Schrödinger Glide software [70–73] . Because there was only one Cryo-EM structure of SARS-CoV-2 Spike protein with low resolution
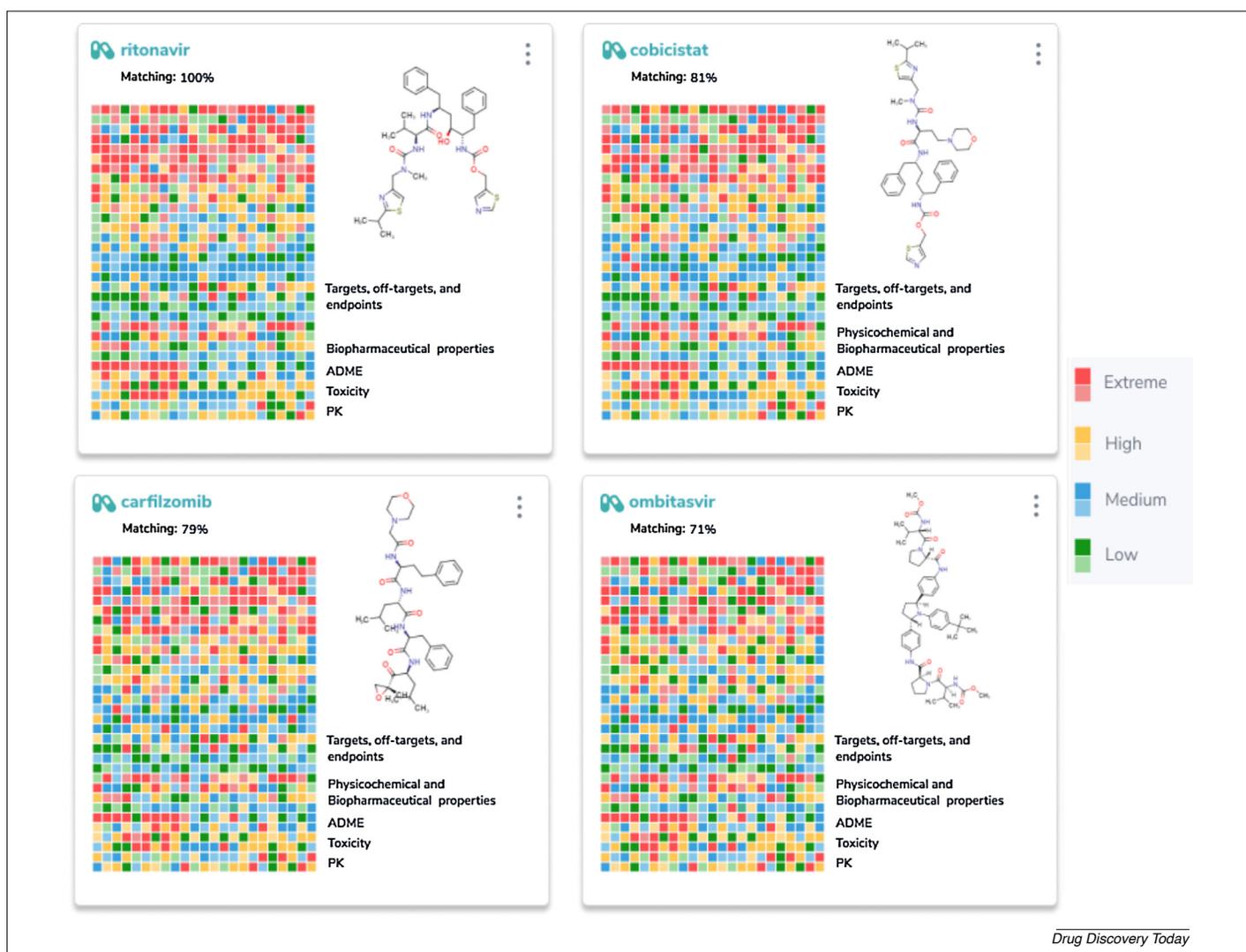
FIGURE 6

Molecular and biological signature profiles generated for ritonavir compared with other drugs, using artificial intelligence to create thousands of decision-makers that mimic a biological response through the interaction of a small molecule with multiple enzyme targets, off-targets and *in vitro* and *in vivo* endpoints. The drugs cobicistat, carfilzomib and ombitasvir presented the higher matching scores (81–71%) with ritonavir.

(3.46 Å) without the human ACE2 protein [48] at the time of doing this analysis, we preferred to build a model by homology modeling, because lower-resolution structures (3 Å or higher) show the basic contours of the protein chain and the atomic structure must be inferred. We built the SARS-CoV-2 Spike subunit S1 model using the SwissModel server [74]. The template used was the SARS-CoV Spike S1 protein (PDB ID 2GHV) [75], which has resolution of 2.2 Å and 73.22% of sequence identity with SARS-CoV-2 Spike protein. After the protein refinement, we added the human ACE2 protein to the model, based on the SARS-CoV Spike–ACE2 structure (PDB ID 2AJF) [76], and built the full SARS-CoV-2 Spike S1–ACE2 model (see Fig. S5 in Supplementary material online). The quality statistics of the modeled SARS-CoV-2 Spike–ACE2 were good: 96.53% of the residues lie in the most favorable regions of the Ramachandran plot and the Molprobity score was 1.66. Our SARS-CoV-2 Spike–ACE2 model is available (see Supplemental material PDB file online). Subsequently, we performed docking-based virtual

screening with a grid centered at the Spike–ACE2 interface. As can be seen in Table 3, docking scores indicated that rizatriptan (an antimigraine agent, Glide score = −7.49 kcal mol^{-1}), dasabuvir (an antiviral for hepatitis C, Glide score = −7.09 kcal mol^{-1}), pravastatin (a lipid-lowering agent, Glide score = −6.17 kcal mol^{-1}) and empagliflozin (a hypoglycemic agent, Glide score = −6.37 kcal mol^{-1}) may be of interest for *in vitro* testing. Details of intermolecular interactions of these drugs with the interface of Spike–ACE2 are shown in Fig. 5. In summary, these drugs form π-cation interactions with Lys31a (ACE2's residue), π–π interactions with Phe170b (Spike's residue), and H-bonds with Glu35a and Asp38a residues of ACE2, Gly176b and Ser174b (residues of Spike). In good agreement with our results, a previous study suggests that an early and high dose of a statin (such as pravastatin) could be crucial to surviving MERS-CoV infection [77]. These suggested drugs could be validated in future *in vitro* experiments with SARS-CoV-2 cell-based assays and with Spike-ACE2 enzymatic assays.

Modern drug discovery efforts try to eliminate inefficiency by increasing knowledge, through extensive utilization of generated data to guide new experiments. Therefore, we have also taken a drug repurposing approach based on polypharmacology using a method called the 'molecular and biological signature' [78]. This is a profiling approach, guided by artificial intelligence, capable of presenting the complex relationships between the chemical substance with molecular targets, cells and/or organs. This could create a more complete understanding of the 'multi-biological profile' caused by the drug analyzed in response to a disease. As an example, we have applied the drugs ritonavir [9], favipiravir, ribavirin and chloroquine as scaffolds to find new potential molecular and biological signature profiles, to search for non-obvious, mechanism-of-action-based associations between drugs, molecular targets and diseases (Fig. 6; and Supplementary material Fig. S6 and Table S3 online). Comparing the molecular and biological signature of ritonavir with other drugs, we found that the drugs cobicistat, carfilzomib and ombitasvir presented the highest matching scores (81–71%), suggesting that those drugs could be of further interest for SARS-CoV-2.

Concluding remarks

Although there are already several drugs being assessed clinically for SARS-CoV-2, there is still a need to identify additional treatments as alternatives (to offset drug shortages) and possibly discover molecules with increased efficacy, safety and cost-effectiveness. We have previously proposed and applied computational drug discovery approaches such as machine learning and docking to aid in the discovery of new candidate molecules for Ebola and Zika, respectively. What we have learnt in these efforts can be applied to SARS-CoV-2 as we have demonstrated here. However, the research in this case has moved at a much faster pace initially, delivering a first crystal structure in just a few weeks after first public notification of the virus. Companies have mobilized to bring their drugs to patients and clinicians have used prior *in vitro* research that proposed compounds active against MERS-CoV and SARS-CoV to suggest treatments for SARS-CoV-2. We currently do not clinically use any broad-spectrum antivirals in the USA but they would be invaluable in situations like this for SARS-CoV-2 and other viruses. Identifying medications with side-effect profiles known by clinicians that are already produced at scale and available at local pharmacies around the world will allow more patients to be treated, especially as travel restrictions and quarantines could reduce production and distribution of certain drugs. It will remain important that those who need these medications at baseline, such as HIV patients who take ritonavir/lopinavir, are able to continue to access the medications they need or else there is a risk of poor clinical outcomes and antiviral resistance in these

patients. Although other viruses such as the flu might have killed more patients in the USA this season so far, the crippling financial impact of SARS-CoV-2 remains to be seen globally. If past Ebola, ZIKV, MERS-CoV and SARS-CoV outbreaks are anything to go by this SARS-CoV-2 pandemic will be far more significant. Having multiple effective treatments available for SARS-CoV-2 that can be rapidly delivered to patients could save lives. We can also supplement these treatments by a combination of computational screening with machine learning and docking to select additional drugs to test as proposed herein and this can be repeated for future virus outbreaks. The sense of déjà vu will probably be repeated again and again with the next virus outbreaks until we develop broader-spectrum antivirals. Each time we hope to be better prepared. Hope however does not save lives. Effective antiviral treatments do, and we need to have them ready in the future.

Conflict of interest

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2020. 03.019.

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