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Full length article

In silico identification of strong binders of the SARS-CoV-2 receptor-binding domain

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ABSTRACT

The world is currently witnessing the spread of the deadly severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes the coronavirus disease 2019 (COVID-19). In less than three months since the first cases were reported, the World Health Organization declared it a pandemic disease. Although several treatment and prevention strategies are currently under investigation, a continuous effort to investigate and develop effective cures is urgently needed. Thus, we performed molecular docking and structure-based virtual screening of libraries of approved drugs, antivirals, inhibitors of protein-protein interactions, and one million other small molecules to identify strong binders of the SARS-CoV-2 receptor-binding domain (RBD) that might interfere with the receptor recognition process, so as to inhibit the viral cellular entry. According to our screening and selection criteria, three approved antivirals (elbasvir, grazoprevir, and sovaprevir) and 4 other drugs (hesperidin, pamaqueside, diosmin, and sitogluside) were identified as potent binders of the RBD. The binding of these molecules involved several RBD residues required for the interaction of the virus with its cellular receptor. Furthermore, this study also discussed the pharmacological action of the 4 non-antiviral drugs on hematological and neurological disorders that, in addition to inhibiting the viral entry, could be beneficial against the neurological disorders identified in COVID-19 patients. Besides, six other small-molecules were identified, with no pharmacological description so far, exhibiting strong binding affinities to the RBD that we believe worth being investigated as inhibitors of the SARS-CoV-2-receptor interaction.

1. Introduction

The coronavirus disease 2019 (COVID-19) is a severe viral respiratory infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Huang et al., 2020). The infection has rapidly spread across the globe; in less than three months following the detection of the first cases, the World Health Organization (WHO) has declared it a pandemic disease (on March 11th, 2020). By July 10th, 2020, the WHO situation report-172 indicated that more than 12 million confirmed cases had been reported globally with more than 25% of the cases in the US alone; the total deaths caused by the disease reached 551 046 cases, mainly in the Americas and Europe (50% and 37%, respectively) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/).

SARS-CoV-2 is an enveloped single-stranded RNA virus belonging to the genus *beta-coronavirus* of the large family of coronaviruses. The coronaviruses are the largest known RNA viruses, widely distributed in the animal kingdom, and when infecting humans they cause a wide range of disorders such as respiratory, neurologic, and gastrointestinal diseases (Weiss and Leibowitz, 2011; Xu et al., 2020). The life cycle of coronaviruses (all viruses in general) starts with the recognition of a specific cellular receptor that allows entry into the host cells, and the identification of such receptor and the moiety of the virus that interacts with them is a crucial step towards the understanding of the virus

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pathogenesis and towards the development of effective means to stop the infection at its early stages. SARS-CoV-2 initiates its cellular entry through the recognition and binding to the human angiotensin-converting enzyme 2 (ACE2) protein present on the surface of the host cells (Hoffmann et al., 2020; Lan et al., 2020; Walls et al., 2020). This binding is mediated by the viral spike protein, which forms clove-shaped trimers on the viral surface and contains a large ectodomain, a single-pass transmembrane anchor, and a short intracellular tail (Lan et al., 2020; Walls et al., 2020). The spike protein ectodomain consists of a receptor-binding subunit S1 and a membrane-fusion subunit S2; the S1 subunit contains distinct N-terminal and C-terminal domains, and the latter forms the receptor-binding domain (RBD) that binds to the ACE2 receptor (Hulswit et al., 2016; Walls et al., 2020; Yan et al., 2020).

Given the severity of the COVID-19 pandemic, it is extremely urgent to develop effective treatment and prevention strategies. To develop such cures (whether it is drug repurposing or development of new drug-leads), it is important to target the key steps in the virus life cycle such as entry into the host cells, RNA replication and transcription, proteolytic processing of viral proteins, virion assembly and release of new viruses, and/or host processes that are essential for disease onset and progression (Guy et al., 2020). Thus, the present work aimed to identify strong binders of the spike protein RBD that potentially could interfere with the binding of this latter to the ACE2 receptor, and therefore, inhibiting the SARS-CoV-2 cellular entry. To this end, the structure-based virtual screening approach (Batool et al., 2019) was adopted using the recently-determined crystal structure SARS-CoV-2 RBD and different libraries of already known drugs, antiviral molecules, inhibitors of protein-protein interactions, and one million other compounds.

2. Materials and methods

2.1. Structural model

The 3D structure of the RBD of the spike protein of SARS-CoV-2 was prepared from the crystal structure of the SARS-CoV-2 receptor-binding domain (PDB ID: 6W41) retrieved from the Protein Data Bank.

2.2. Prediction of the binding pockets

The AADS server (http://www.scfbio-iitd.res.in/dock/ActiveSite_new.jsp) was used to predict the active sites on the SARS-CoV-2 RBD. The AADS predicts all the possible binding pockets within a protein, based on its tertiary structure with a 100% accuracy (Singh et al., 2011). SARS-CoV-2 RBD was submitted to the AADS server, and the results were visualized with PyMol to select the binding pockets near the receptor-binding motifs, so as to narrow the screening to this region.

2.3. Virtual screening

a) MTiOpenScreen webserver was used for screening against two of its integrated libraries: Drugs-lib containing 7173 purchasable drugs and iPPI-Lib containing 51 232 drug-like molecules with properties likely to be efficient to inhibit protein-protein interactions (Labbe et al., 2015). The binding grid was defined according to the residues previously reported as critical for the interaction with the ACE2 receptors (Wang et al., 2020; Yan et al., 2020). From the 4500 docking combinations produced, the best 100 molecules (according to the binding energies) were selected and visually examined using the PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC.
b) After the prediction of the binding pockets using the AADS server, those located near the receptor-binding motifs were selected as binding sites, and another screen was performed using the RASPD software (Soni et al., 2013). This second screening was conducted against a library of a million small molecules obtained from the ZINC database and implemented in the RASPD software.

2.4. Molecular docking

A set of 21 antiviral drugs that could bind the SARS-CoV-2 RBD has been previously identified using a predicted 3D model (before the availability of the high-resolution structure of the RBD in the Protein Data Bank). Besides, another molecular docking was performed using the crystal structure of SARS-CoV-2 RBD against these antiviral molecules using PyRx software (Dallakyan and Olson, 2015).

2.5. Calculation of drug-likeness parameters

Swiss-ADME (http://www.swissadme.ch) (Daina et al., 2017) was used to compute physicochemical descriptors and to predict ADME parameters, pharmacokinetic properties, drug-likeness and medicinal chemistry friendliness of the small molecules predicted as the best ligands from other than Drug-Lib.

3. Results

3.1. Selection of the structural model of the SARS-CoV-2 RBD

Several high-resolution structures of the SARS-CoV-2 RBD are currently available at the Protein Data Bank. In the present work, the SARS-CoV-2 RBD in complex with human antibody CR3022 (PDB ID: 6W41) was selected to perform the structure-based virtual screening and molecular docking. The selection of this particular model is based on the following reasons:

1) It is very likely that the binding of the ACE2 receptor to the RBD could affect, to some extent, the flexibility and the exposition of the RBD residues (especially interface residues). Thus, using an RBD structural model obtained from the RBD-ACE2 complex to identify ligands that bind to the RBD at the ACE2-binding interface could affect the reliability of the ligand-binding predictions. Consequently, we opted for an RBD structure obtained from other than RBD-ACE2 complexes.
2) The epitope of the CR3022 antibody does not overlap with the ACE2-binding site (Yuan et al., 2020). Although it is not a neutralization epitope, it is very likely to be targeted by the host humoral response, and the binding of such antibodies could also trigger conformational changes on the overall flexibility of the RBD residues (especially interface residues). Therefore, the RBD structure in the 6W41 model presents an RBD unbound to the ACE2 receptor but with the changes that might occur after interacting with the host immune system.
3) The binding of the CR3022 antibody occurs only when the RBD is in the “up” conformation (Yuan et al., 2020), the same conformation allowing the interaction with the ACE2 receptor (Walls et al., 2020; Yan et al., 2020). Therefore, using this RBD model allows a more reliable prediction of potential inhibitors of the RBD-ACE2 interaction.
4) Finally, the CR3022 antibody cannot neutralize SARS-CoV-2 in in-vitro assay while synergetic effects on neutralization of SARS-CoV have been reported for CR3022 and other RBD-targeted antibodies (Ter Meulen et al., 2006). Therefore, similar synergy to neutralize the SARS-CoV-2 could be achieved by the humoral response against CR3022 epitope and strong binders of the SARS-CoV-2 RBD (binding to the receptor-binding motifs of the RBD).

3.2. Screening against the inhibitors of protein-protein interaction library (iPPI-Lib)

In the MTiOpenScreen server output, the hits are annotated ‘Accepted’ or ‘Intermediate’; the first annotation indicates that the compound does not contain toxic or pan-assay interference compounds (PAINS) groups while the second annotation suggests that the compound does not contain PAINS groups but toxicophores belonging to the

low-risk toxicity category. Therefore, the best 100 hits were retrieved for further analysis, from which, the compounds with the 'Intermediate' annotation were discarded leaving 29 accepted small molecules as potential safe binders (Table S1). Then, the selected molecules were visualized in PyMol for the analysis of the binding position. All of the compounds were bound to one of the three binding pockets illustrated in Fig. 1B and C, namely sites 1, 2, and 3. Since site 3 was far from the receptor-binding motif, the molecules binding to this site were eliminated (four molecules).

Recently, Yan and coworkers (Yan et al., 2020) reported that the two

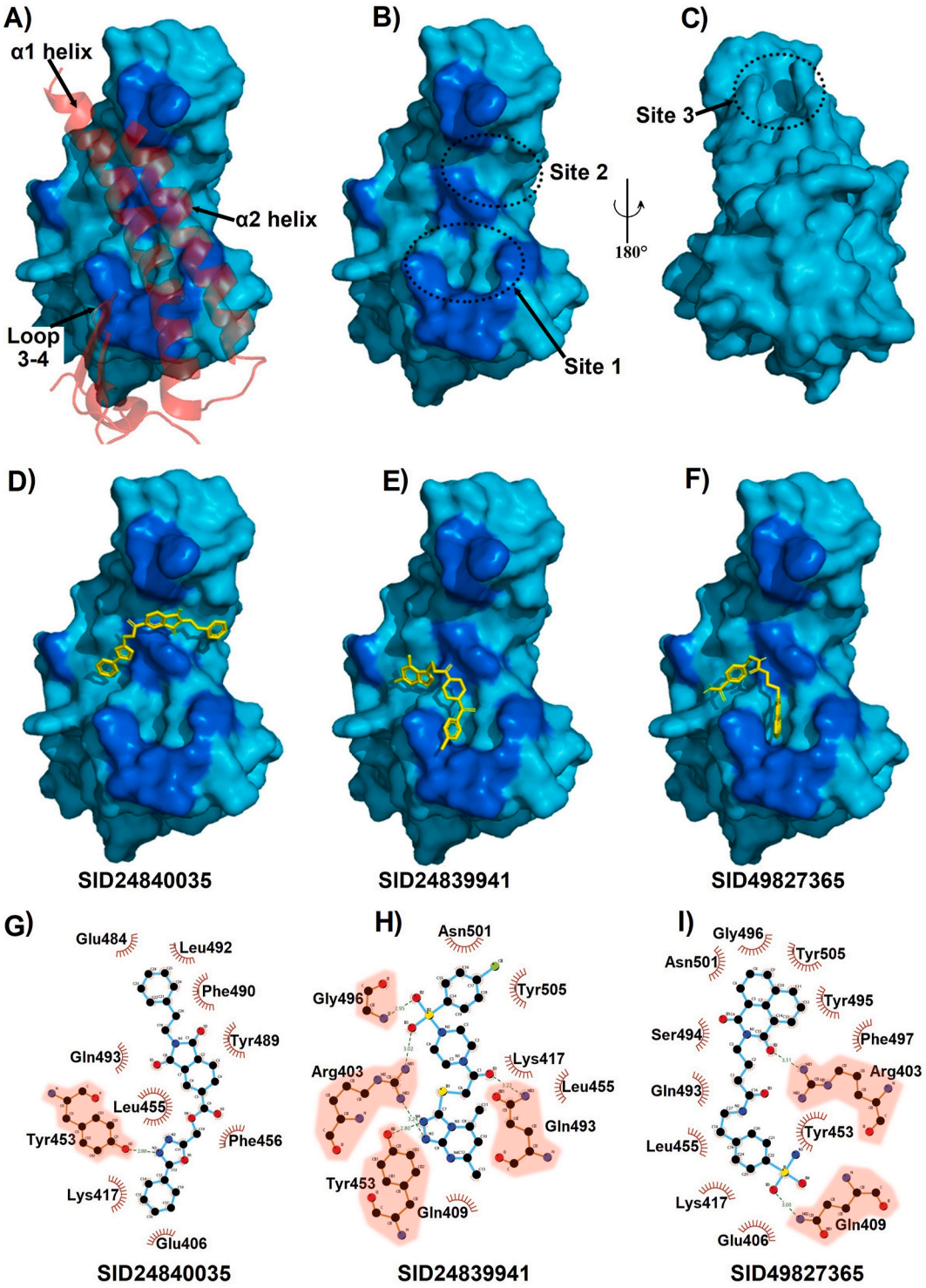

Fig. 1. Binding positions of the small molecules identified from the iPPI-Lib: A) Surface representation of the SARS-CoV-2 RBD with the bound ACE2 receptor (only the α1 helix, the α2 helix and the loop 3–4 of ACE2 are illustrated in transparent red cartoon), and the key binding residues of the RBD are exhibited in blue; B and C) Surface representation of the SARS-CoV-2 RBD showing the three ligand-binding sites; D, E and F) Binding positions of the selected ligands (yellow) on the SARS-CoV-2 RBD and their PubChem SID identification numbers are indicated under the structure representation; G, H, and I) 2D representation of the molecular interaction between the selected ligands and the residues of the SARS-CoV-2 RBD, the residues forming hydrogen bonds with the ligands are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ends of the RBD bridge interact with the N and C termini of the α1 helix, small areas on the α2 helix, and with the loop 3–4 of the ACE2 receptor, while the middle segment of α1 reinforces the interaction by engaging polar contacts (Fig. 1A). The engaged residues in these interactions were Lys417, Tyr449, Tyr453, Leu455, Lys474, Phe486, Asn487, Tyr489, Gln493, Gln498, Thr500, Asn501, Gly502, and Tyr505 (Wang et al., 2020; Yan et al., 2020), hereafter referred to as key-binding residues. Accordingly, a final refinement of the compound selection was performed based on the binding position of the molecules as visualized in PyMol, and the best candidates were selected if they could bind in a way interfering with the binding of the α1 helix of the ACE2 receptor.

The best-selected candidates were the compounds with PubChem database Substance identifiers (SID): 24840035, 24839941, and 49827365 (Fig. 1D, E, and F). The SID24840035 compound binds to Site 2 and interact with 10 residues of the RBD, mostly with hydrophobic interactions, and only one hydrogen bond with Tyr453 residue (Fig. 1D and G). The SID24839941 and SID49827365 compounds both bind to Site 1 and interact with 9 and 13 RBD residues, respectively (Fig. 1E, F, H, and I). The SID24839941 molecule forms 5 hydrogen bonds with four different residues while SID49827365 forms only two with two different residues. All three molecules interact with two of the key binding residues: Lys417 and Tyr453. Besides, SID24840035 and SID24839941 both form hydrogen bonds with Tyr453, and the binding of SID24839941 and SID49827365 engages another key-binding residue: Asn501.

3.3. Screening against the one million molecules library

Using the Automated Version of Active Site Prediction (AADS) server (Singh et al., 2011), nine binding pockets were identified in the SARS-CoV-2 RBD. To focus on the identification of only potential binding molecules that might disrupt RBD-ACE2 inrectation, the two pockets located near the receptor-binding motif were selected, as shown in Fig. 2A; then, the screening against the One Million Molecules library integrated into the RASPD software was performed (Soni et al., 2013). With a binding energy cut-off of −10 kcal/Mol, a total of 6086 hits were identified, from which the first 200 molecules with the least binding energy (from −12.1 kcal/Mol to −13.7 kcal/Mol) were selected for molecular docking. Furthermore, the docking results were processed as follows: a total of 1782 poses were generated and then classified according to their binding energies; poses involving the minimized-energy structures of the ligands (root-mean-square deviation = 0) with a binding energy of −7 kcal/Mol or less were visualized in PyMol; next, the molecules binding to the receptor-binding motif of SARS-CoV-2 RBD, in way interfering with the binding of the α1 helix of ACE2, were selected; finally, the 60 molecules selected so far (Table S2) were submitted to Swiss-ADME server (Daina et al., 2017), and the list of the best candidates was refined according to the drug-likeness scores using the Lipinski rule of five and the PAINS (Baell and Holloway, 2010) and Brenk filters (Brenk et al., 2008) for the identification of potentially problematic fragments.

Following the above procedure, 10 lead molecules were selected, as listed in Table 1. The analysis of the docked complexes indicated that the binding of these ligands engaged between 8 and 11 residues of the RBD. The binding of three of these ligands (ZINC14998051, ZINC1299985, and ZINC14996176) was only through hydrophobic interactions while the other molecules formed 1 to 4 hydrogen bonds. Three compounds ZINC20993095, ZINC22917729, and ZINC9191993 seem to be more promising than the other molecules. They form 4, 3, and 2 hydrogen bonds with the RBD residues, respectively, and their interaction involves several key-binding residues (Fig. 2 and Table 1).

3.4. Screening against the Drug-Lib

The screening against the Drug-Lib molecules has also been performed using the MTiOpenScreen server. After the visualization of the first 300 docking poses, 40 different compounds were selected as potential inhibitors of the RBD-ACE2 interaction (Table 2). As described above, the selection of these drugs was based on their binding position to the RBD (in a way interfering with the binding of α1 helix of the ACE2 receptor, as illustrated in Fig. 1). Among the selected drugs, 11 are anticancer and antitumor drugs and 6 were Ergot derivatives with indications for the treatment of migraine disorders and some idiopathic neurological diseases. Importantly, five of the molecules were antiviral drugs used for the treatment of hepatitis C (HCV): Elbasvir, Ledipasvir, Grazoprevir, Ravidasvir, and Velpatasvir. According to the binding energies, the HCV drug Elbasvir was the highest-ranked binder (−9 kcal/Mol) interacting with 16 residues of the RBD but with only 3 hydrogen bonds. However, according to the number of hydrogen bonds formed during the binding, the bioflavonoid Hesperidin comes in the first place with 13 hydrogen bonds and interacting with 13 residues of the SARS-

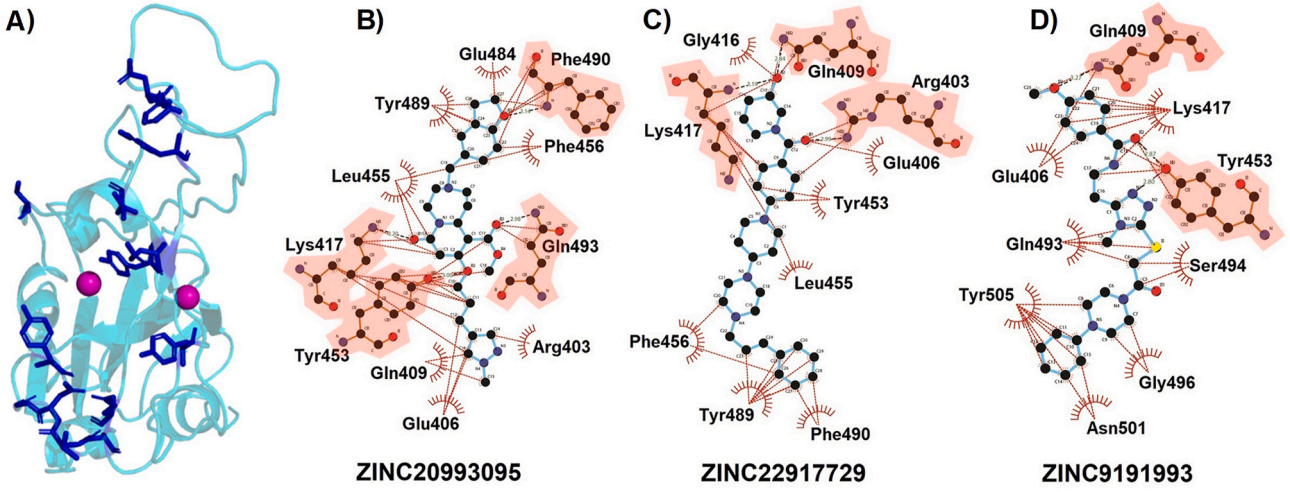

Fig. 2. Representation of the molecular interaction between the SARS-CoV-2 RBD and ligands selected from the One Million Molecules library. A) Top view of the SARS-CoV-2 RBD presented as a cartoon representation with the key receptor-binding residues exhibited as blue sticks; the two spheres represent the two pockets' centers identified by the AADS server and used to perform the screening against the One Million Molecules library integrated into the RASPD software. B, C, and D) 2D representation of the molecular interaction between the selected ligands (ZINC20993095, ZINC22917729, and ZINC9191993) and the residues of the SARS-CoV-2 RBD, the residues forming hydrogen bonds with the ligands are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
List of the 10 ligands selected from the One Million Molecules library.

Rank in the docking results	Ligands	Binding energy (kcal/Mol)	Total engaged residues	Key receptor-binding residues	Hydrogen bonds
14	ZINC14998051	−8.3	10	Lys417, Tyr453, Leu455, Tyr489, Gln493	0
70	ZINC14884913	−7.4	11	Tyr453, Leu455, Tyr489, Tyr505	1
71	ZINC14952536	−7.4	8	Leu455, Phe486, Tyr489	1
77	ZINC1299985	−7.3	9	Tyr449, Leu455, Gln493	0
93	ZINC19921140	−7.2	10	Tyr449, Tyr453, Gln493, Asn501, Tyr505	1
94	ZINC20993095	−7.2	11	Lys417, Tyr453, Leu455, Tyr489, Gln493	4
95	ZINC22917729	−7.2	10	Lys417, Tyr453, Leu455, Tyr489	3
107	ZINC9191993	−7	9	Lys417, Tyr453, Gln493, Asn501, Tyr505	2
111	ZINC14744864	−7	11	Lys417, Tyr453, Leu455, Gln493, Tyr505	1
112	ZINC14996176	−7	8	Lys417, Tyr453, Leu455, Gln493, Asn501, Tyr505	0

CoV-2 RBD, followed by Pamaqueside, Diosmin, Sitogluside, and Etoposide that form 9, 8, 8, and 7 hydrogen bonds, respectively (with binding energies of −7.9, −8.1, −8, −8, and −7.9 kcal/Mol, respectively) (Table 2).

3.5. Molecular docking using antiviral drugs

A list of 21 antiviral drugs was generated earlier when we used a predicted structural model of the SARS-CoV-2 for the virtual screening. After the determination of high-resolution structures of the RBD, these drugs were used to re-perform the molecular docking. The list comprises 9 drugs against Human Immunodeficiency Virus (4 approved and 5 under investigation), 10 drugs against HCV (6 approved and 4 under investigation), one approved drug against Smallpox, and one drug against Cytomegalovirus still under investigation. The molecular docking results indicated that the binding energies varied from −8.4 to −6.1 kcal/Mol (Table S3). Next, the 14 drugs with binding energies less than −7 kcal/Mol were visualized in PyMol to analyze the binding positions, the engaged residues, and the hydrogen and hydrophobic bonds. Finally, 8 molecules were selected as potential binders, as presented in Fig. 3 and Fig. S1. Besides, two of these antivirals were also identified in the Drug-Lib screening (Elbasvir and Grazoprevir). Interestingly, Sovaprevir was identified as the strongest binder with a binding energy of −7.7 kcal/Mol, interacting with 11 RBD residues and forming 7 hydrogen bonds. Two of these hydrogen bonds were formed with the key binding residues Lys417 and Tyr453; among the other engaged residues, five have been also reported important for the interaction of the SARS-CoV-2 RBD with the ACE2 receptor (Leu455, Tyr489, Gln493, Asn501, and Tyr505).

4. Discussion

The life cycle of a virus mainly starts with its entry to the target cells, which is mediated through the recognition of specific receptor(s). In the case of the SARS-CoV-2 infection, considerable efforts have been made to identify its receptor and the related recognition mechanism. Such efforts determined that the SARS-CoV-2 recognizes and binds to the ACE2 receptor with high affinity through its spike protein RBD (Lan et al., 2020; Shang et al., 2020; Walls et al., 2020; Wang et al., 2020; Yan et al., 2020). The determination of the high-resolution structure of the RBD-ACE2 complex also revealed that the RBD interacts mainly with the arch-shaped α1 helix of the ACE2 and to a minor extent with the α2 helix and the loop connecting the β3 and β4 antiparallel strands of ACE2 (Yan et al., 2020); the residues of the RBD playing a pivotal role in the interaction have been identified (Wang et al., 2020; Yan et al., 2020). Accordingly, these key residues were used in the present study to set the docking grid on the RBD to emphasize the screening for molecules that bind the interface recognized by the ACE2 receptor. Besides, in the analysis for potential drug-leads in the results, the selection of compounds that bind to the RBD in a way interfering with the binding of the ACE2 α1 helix was further stressed, even though the binding to other pockets in the RBD could affect the binding to the ACE2 receptor (through structural modification upon ligand binding).

Considering the severity of the COVID-19 pandemic, there is an unprecedented global effort (at least in this century) for the development of effective therapeutic and preventive approaches. The fastest adopted approach was to repurpose approved drugs that have been developed for other indications by exploiting their pharmacological and toxicological data to speed up clinical trials. Among these drugs, three has been considered on the basis that they can block the viral entry into the target cells, suggesting that the recombinant human ACE2 was regarded as a potential drug against COVID-19 by acting as a decoy to disrupt SARS-CoV-2 binding to its ACE2 receptor (Monteil et al., 2020). Moreover, both camostat and nafamostat are also investigated as drugs blocking the viral entry through the inhibition of the transmembrane protease serine-type 2 (TMPRSS2) required for the proteolytic processing of the SARS-CoV-2 spike glycoprotein (Hoffmann et al., 2020; Shaffer, 2020).

Several antiviral drugs were or are currently considered for the treatment of COVID-19: human immunodeficiency virus protease inhibitors lopinavir and ritonavir that a trial on using them in severe COVID-19 patients revealed that no benefit was observed with the treatment beyond standard care (Cao et al., 2020), remdesivir and favipiravir that target the viral RNA-dependent RNA polymerase (Hoffmann et al., 2020), and helicase inhibitors amenamevir or pretelivir (Guy et al., 2020). It should be noted that these antivirals were selected because they can affect the SARS-CoV-2 life cycle by acting on different steps of the cycle other than receptor recognition. Our results suggested that other antivirals could effectively bind to the SARS-CoV-2 RBD, which might in turn inhibit the ACE2 binding, especially elbasvir and grazoprevir used as a combined therapy for the treatment of hepatitis C (Asante-Appiah et al., 2017; Asselah et al., 2020). Their binding to the SARS-CoV-2 RBD involves several key residues required for the RBD-ACE2 complex formation. Besides, another computational target-based drug repurposing study also reported that elbasvir could target SARS-CoV-2 main protease (Wang, 2020). Adding this to our results suggests that the elbasvir-grazoprevir combination could act on two different steps (if not more) of the SARS-CoV-2 lifecycle, making it

Table 2
List of the best binders to the SARS-CoV-2 RBD as predicted by the MTiOpenScreen server from Drug-Lib.

Rank[1]	Drug	ZINC ID	Binding energy	Engaged[2] residues	H bonds[3]	Indication[4]
84	Hesperidin	ZINC248139800	−7.9	13	13	Blood vessel conditions
39	Pamaqueside	ZINC257972190	−8.1	11	9	Not available
51	Sitogluside	ZINC118922613	−8	14	8	Not available
58	Diosmin	ZINC4098512	−8	11	8	Venous disease
100	Etoposide	ZINC100023538	−7.9	10	7	Anticancer
34	Uk432097	ZINC150664074	−8.1	16	6	Not available
30	Venetoclax	ZINC150338755	−8.2	13	5	Anticancer
72	Teniposide	ZINC77313309	−7.9	14	5	Anticancer
10	Metergotamine	ZINC72266819	−8.5	10	4	Migraine Disorders
15	Mk3207	ZINC43203371	−8.5	10	4	Migraine Disorders
35	Cadazolid	ZINC43195938	−8.1	11	4	Antibacterial
36	Bencianol	ZINC4214953	−8.1	9	4	Not available
76	Fluazuron	ZINC2570819	−7.9	9	4	Not available
1	Elbasvir	ZINC150588351	−9	16	3	Anti-viral (HCV)
13	Ledipasvir	ZINC150338819	−8.5	10	3	Antiviral (HCV)
18	Ergotamine	ZINC52955754	−8.4	8	3	Migraine Disorders
28	Alpha-Ergocryptine	ZINC59796556	−8.2	11	3	Neurological diseases
29	Golvatinib	ZINC43195317	−8.2	11	3	Anticancer
46	Gedatolisib	ZINC49757175	−8.1	11	3	Anticancer
47	Abamectin-component-b1a	ZINC245224132	−8.1	14	3	Anthelmintic
61	Mk-0893	ZINC95574316	−8	14	3	Type 2 Diabetes
90	Lumacaftor	ZINC64033452	−7.9	10	3	Cystic fibrosis
95	Proscillaridin	ZINC118915484	−7.9	9	3	Anticancer
2	R428	ZINC51951669	−9	12	2	Anticancer
21	Dihydroergocristine	ZINC3947495	−8.3	9	2	Neurological diseases
31	Losulazine	ZINC4216779	−8.2	9	2	Not available
52	Chir-265	ZINC18710085	−8	11	2	Anticancer
56	Grazoprevir	ZINC95551509	−8	12	2	Antiviral (HCV)
68	Irinotecan	ZINC1612996	−8	13	2	Anticancer
77	Velpatasvir	ZINC220902773	−7.9	14	2	Antiviral (HCV)
78	Posaconazole	ZINC3938482	−7.9	12	2	Antifungal
85	Beta-Ergocryptine	ZINC100071818	−7.9	11	2	Neurological diseases
89	Omipalisib	ZINC43208634	−7.9	11	2	Anticancer
6	Bolazine	ZINC8214506	−8.6	9	1	Androgen/anabolic steroid
16	Entrectinib	ZINC43204146	−8.5	9	1	Anticancer
33	Ditercalinium	ZINC4215707	−8.2	10	1	Not available
55	Farglitazar	ZINC49639808	−8	13	1	Not available
57	Ravidasvir	ZINC150607150	−8	13	1	Antiviral (HCV)
80	Amg-900	ZINC43208325	−7.9	12	0	Not available
99	Zoliflodacin	ZINC145806066	−7.9	8	0	Gonorrhoea

1: Rank of the ligand-RBD complex in the MTiOpenScreen output; 2: Number of the RBD residues engaged in the interaction with the ligand; 3: Number of hydrogen bonds formed in ligand-RBD complex; 4: indication of the drug as indicated in DrugBank database (https://www.drugbank.ca/).

interesting for repurposing to treat COVID-19. Furthermore, our results also indicated the HCV protease inhibitor sovaprevir is quite interesting for repurposing since it binds the RBD by forming 7 hydrogen bonds and engaging 11 residues of the RBD, among which 7 were previously reported crucial for RBD-ACE2 binding.

We also identified from the drug library hesperidin, pamaqueside, diosmin, sitogluside as strong binders of the RBD with binding energies below −7.9 kcal/Mol and by forming several hydrogen and hydrophobic bonds with the RBD key residues. Recent findings indicated that neurological complications, especially coagulopathy-related stokes, are common in COVID-19 patients (Hess et al., 2020; Terpos et al., 2020; Zhou et al., 2020). The identified RBD-binders hesperidin and diosmin, alone or in combination, are indicated mainly for the treatment of venous disease symptoms (Steinbruch et al., 2020). However, other interesting effects on stroke-related disorders in preclinical studies have been reported for both hesperidin (Gaur et al., 2011; Ikemura et al., 2012; Qin et al., 2020) and diosmin (Delbarre et al., 1995; Liu et al., 2014). Likewise, sitogluside also exhibited some effects on neurological disorders (Chung et al., 2016; Ji et al., 2017; Zhang et al., 2020). Adding to this the ability of these molecules to bind the RBD (possible inhibition of SARS-CoV-2-ACE2 interaction), makes these molecules very interesting for exploration for the possibility of repurposing against SARS-CoV-2 infection.

It is worth noting that our results also revealed that several ergot derivatives presented an appreciable binding affinity to the SARS-CoV-2 RBD (Table 2). Such drugs have a long history of pharmacological uses for the treatment of a wide range of disorders, especially neurological ones such as migraines (Schardl et al., 2006). Therefore, they are also worth investigating for possible dual action on COVID-19 due to possible inhibition of receptor recognition through binding to the RBD and participation in reducing the neurological complications observed in COVID-19 patients (Di Gennaro et al., 2020).

Apart from the drugs discussed above, 6 other small molecules, with no pharmacological description so far, were also identified in this study. They exhibited promising results as RBD binders, standing, therefore, as potential inhibitors of SARS-CoV-2-ACE2 interaction.

In conclusion, Guy and coworkers (Guy et al., 2020) pointed out that before herd immunity against COVID-19 is established, it is likely that several seasons of this disease could occur in the future. Therefore, research of treatment and prevention strategies are urgently needed to manage the ongoing pandemic and to counteract future occurrences. In the present work, the virus entry was regarded as a key step for combating the SARS-CoV-2 infection, and several potential binders of the virus RBD that might inhibit its binding to its receptor ACE2 were identified. This would hopefully merge into the rapidly growing body of literature on COVID-19 to help find the best treatment of symptomatic cases while waiting for the development of an effective vaccine.

CRediT authorship contribution statement

Nouredine Behloul: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review &

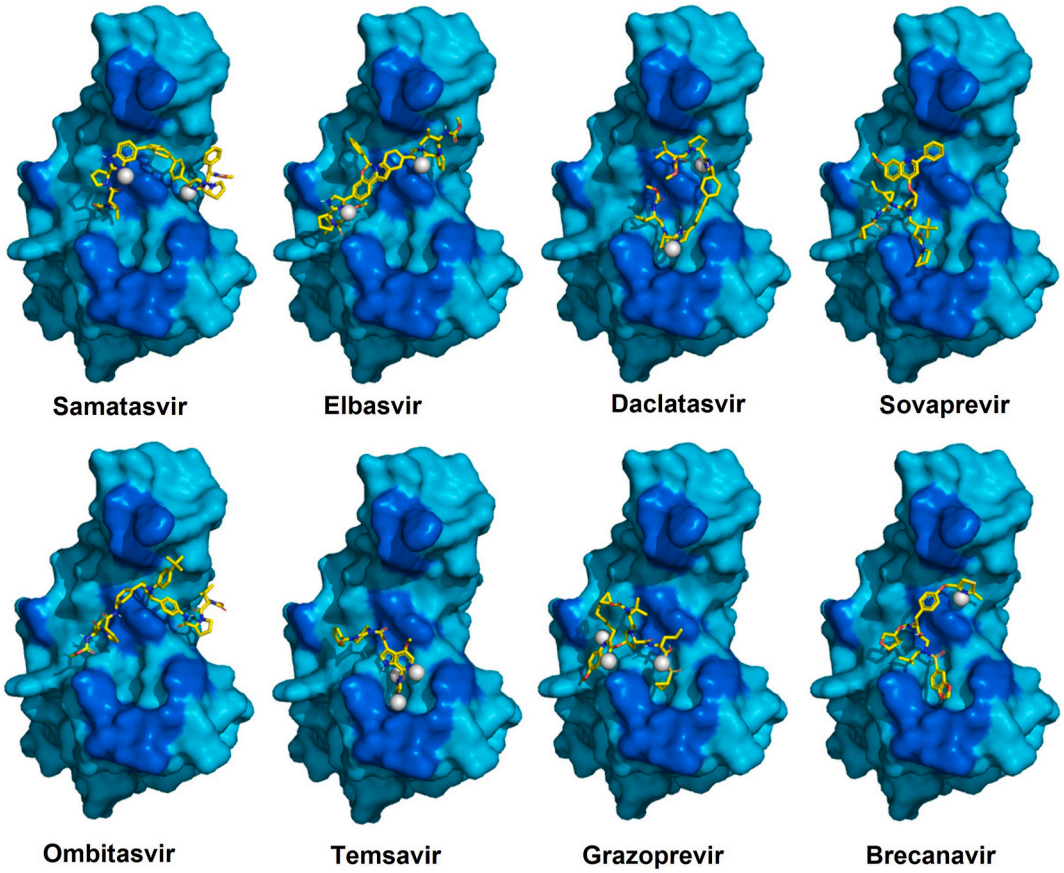

Fig. 3. Binding positions of the selected antiviral drugs on the SARS-CoV-2 RBD. SARS-CoV-2 RBD is represented as a surface with the key receptor-binding residues depicted in blue; the antiviral molecules are shown in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

editing. **Sarra Baha:** Methodology, Data curation, Formal analysis, Writing - review & editing. **Yuqian Guo:** Methodology, Formal analysis. **Zhifang Yang:** Conceptualization, Supervision, Resources. **Ruihua Shi:** Conceptualization, Supervision, Writing - review & editing. **Jihong Meng:** Conceptualization, Methodology, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors have no competing interests to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejphar.2020.173701.

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