Drug Repurposing for Covid-19: Discovery of Potential Small-Molecule Inhibitors of Spike Protein-ACE2 Receptor Interaction Through Virtual Screening and Consensus Scoring

Sachin P. Patil^{1,§}, Jeremy Hofer^{1,2}, Pedro J. Ballester³, Elena Fattakhova¹, Juliette DiFlumeri¹, Autumn Campbell^{1,4}, Michael Oravic^{1,4}

1. NanoBio Laboratory, Department of Chemical Engineering, Widener University, Chester, PA, USA.

2. Department of Computer Science, Widener University, Chester, PA, USA.

3. Cancer Research Center of Marseille (CRCM), INSERM U1068, Marseille, France.

4. Department of Biomedical Engineering, Widener University, Chester, PA, USA.

§ Corresponding address: One University Place, Kirkbride Hall, Chester, Pennsylvania, USA:
Tel.: 1-610-4994492, Fax: 1-610-4994059, Email: spatil[at]widener.edu

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Abstract

Objective

There is an increased interest in drug repurposing against Covid-19 (SARS-CoV-2) as its spread has significantly outpaced development of effective therapeutics. Our aim is to identify approved drugs that can inhibit the interaction of SARS-CoV-2 spike protein with human angiotensin-converting enzyme 2 (ACE2) that is critical for coronavirus infection.

Methods

The published crystal structure of SARS-CoV-2 spike protein-ACE2 receptor interaction was first analyzed for druggable binding pockets. The binding interface was then probed by an integrated virtual screening protocol executed by a high-performance computer cluster, involving docking and consensus scoring using various machine-learning, empirical and knowledge-based scoring functions. The consensus-ranked lists of screened drugs were generated via 'rank-by-rank' and 'rank-by-number' schemes.

Findings

Although spike protein and ACE2 lacked druggable pockets in their unbound forms, they presented a well-defined pocket when bound together. Accordingly, we identified many drugs with high binding potential against this protein-protein interaction pocket. Importantly, several antivirals against two major (+)ssRNA viruses (HCV and HIV) constituted major group of our top hits, of which Atazanavir, Grazoprevir, Saquinavir, Simeprevir, Telaprevir and Tipranavir could be of most importance for immediate experimental/clinical investigations. Additional notable hits included many anti-inflammatory/antioxidant, antibiotic/antifungal, and other relevant compounds with proven activity against respiratory diseases, further emphasizing robustness of our current study. Notably, we also discovered Maraviroc, the only FDA-approved drug capable of targeting virus-host interaction and blocking HIV entry.

Conclusion

Our newly identified compounds warrant further experimental investigation against SARS-CoV-2 spike-ACE2 interaction, which if proven effective may present much-needed immediate clinical potential against Covid-19.

Introduction

Since detection of the first case of an infection on December 8, 2019, the novel coronavirus 2019 (Covid-19 or SARS-CoV-2) has spread to 213 countries and territories, infecting more than 7 million people worldwide and claiming a staggering 404,396 lives as of June 9, 2020 [1]. This new SARS-CoV-2 coronavirus belongs to the broader coronavirus family comprising total seven known human coronaviruses such as common 229E, HKU1, NL63, OC43 coronaviruses and notorious MERS-CoV (Middle East Respiratory Syndrome) and SARS-CoV (Severe Acute Respiratory Syndrome) coronaviruses [2]. These are enveloped viruses that contain a single positive-stranded RNA and cause a wide variety of respiratory, gastrointestinal and neurological diseases in humans [3]. In comparison to other human coronaviruses, the fatality rate of SARS-CoV-2 is relatively low [3]. The transmission rate of SARS-CoV-2, however, is estimated to be significantly higher, infecting first 1,000 patients in merely two days, leading to its declaration as a pandemic by the World Health Organization (WHO) within less than two months of its onset.

Currently there are no FDA-approved drugs available against SARS-CoV-2 as well as SARS-CoV and MERS-CoV, besides the ‘exceptional approval’ and the ‘emergency use authorization’ of Remdesivir against SARS-CoV-2 in Japan and the United States, respectively. Thus, there is an unprecedented need to develop effective therapies against these epidemic coronaviruses in general. In this context, several major targets are proposed for SARS-CoV-2 drug discovery such as nonstructural protein 14 and 20 complex (nsp14-nsp20) [4], main protease (M_{Pro}), transmembrane protease serine 2 (TMPRSS2) [5] and RNA-dependent RNA polymerase (RdRp) [6], involved in coronavirus RNA replication and transcription process. Another target of therapeutic importance involves protein-protein interaction between coronavirus spike protein receptor-binding domain (RBD) and angiotensin-converting enzyme 2 (ACE2) entry receptor that is central to coronavirus-host interactions. Considering the urgent medical need, the specific drug discovery emphasis is on repurposing already approved drugs against these targets, which may present immediate clinical potential against this global health emergency. In this regard, several approved drugs including Remdesivir (RdRp inhibitor), Lopinavir/Ritonavir (antiviral drugs), Camostat (TMPRSS2 inhibitor), as well as Chloroquine/Hydroxychloroquine (antimalarials) and Azithromycin (antibiotic) are currently undergoing clinical trials to determine whether they offer sufficient benefits as anti-Covid-19 agents. In addition, numerous studies have been published so far reporting other promising compounds against Covid-19 identified by different computational and high throughput approaches [7-10].

In this study, we present computational investigation of drugs approved in at least one jurisdiction worldwide for their potential utility against the SARS-CoV-2 viral uptake through ACE2 interaction. Specifically, total 91 approved drugs are identified as putative agents against SARS-CoV-2 through their potential role in inhibiting the SARS-CoV-2 spike protein-ACE2 receptor interaction. These drugs, if experimentally validated to inhibit this pathological protein-protein interaction, may present immediate repurposing opportunity for Covid-19 treatment and thus warrant further characterization and development by broader scientific community.

Materials and Methods

Virtual screening with AutoDock Vina

The crystal structure of the SARS-CoV-2 spike protein receptor-binding domain bound with the human ACE2 entry receptor (PDB ID: 6M0J, resolution 2.45 Å) was downloaded from the protein data bank (PDB) and processed using the AutoDock Tools utility [11]. The two chains were then investigated both individually and in their bound form for potential druggable pockets and sub-pockets using DoGSiteScorer web server [12]. The approved drug subset downloaded from ZINC15 database [13] was used for the virtual screening against these appropriate protein binding pockets (3,278 drugs approved in major jurisdictions worldwide). Owing to the multiple potential structural entries associated with each ligand in ZINC databases, there were total 5,587 ZINC ligand entries in this database. The ligand and protein atoms were converted into PDBQT format. The AutoDock Vina docking algorithm was then used to carry out structure-based docking of these drug molecules to the spike-ACE2 binding interface. Default docking parameters were used and the docked ligands were ranked according to their best docking score values.

Consensus scoring

To decrease the potential false positives and thus increase the hit rate, the best docked conformer of each compound was further reevaluated with multiple scoring functions, viz. DSX (knowledge-based), X-Score (empirical), and NNScore and RF-Score-VS (machine-learning) [14-18]. To create a consensus-ranked list of the screened drug molecules across five scoring functions, the best score associated with a given scoring function was used for each ligand via 'rank-by-rank' and also 'rank-by-number' schemes [19]. The rank-by-rank method is straightforward, whereby the consensus rank of each ligand is an average of the rank values from the individual scoring functions. In contrast, in the rank-by-number scheme the binding scores of each scoring function were first scaled to unit variance and centered, as described previously [20]. The consensus score for each ligand is then determined by taking average of scaled scores across the 5 individual scoring functions mentioned above. We implemented automated scripts on a high-performance parallel computing cluster to carry out all these virtual screening and scoring analyses.

Molecular dynamics

To investigate potential molecular interactions of the top hits within the target pocket, their top-ranked vina docking complexes were subjected to the post-docking structural optimization using the default relaxation protocol in the Desmond Molecular Dynamics package. The relaxation protocol included two stages of minimization (restrained and unrestrained) followed by four stages of short molecular dynamics runs with gradually diminishing restraints and increasing temperature. The optimized ligand-protein complex structures were visually inspected using Schrodinger's Maestro GUI, for complementarity to the binding site and interactions with surrounding amino acid residues.

Results and Discussion

The human infections by SARS coronaviruses including CoV-2 are caused by the interactions of the viral spike proteins with the receptors expressed on human cells viz. the Angiotensin Converting Enzyme 2 (ACE2) [21]. ACE2 is expressed on lung, heart, kidney, testis and gut cell membranes [22]. ACE2 being the critical gatekeeper of viral entry into different human cells, this spike-ACE2 interaction represents one of the most important therapeutic targets for the development of drugs and vaccines against coronaviruses [23]. It should be noted that ACE2 is critical not only for viral entry into the cell and replication, but also important for defense against heart and lung injury [24]. Thus, inhibitors of ACE2 enzymatic activity could potentially worsen the ability of the lung to defend itself from damage caused by the virus. In contrast, we wish to report here the discovery of approved drugs as putative small-molecule inhibitors of spike-ACE2 protein-protein interaction, through structure-based virtual screening followed by consensus scoring. Such inhibitors of this pathological protein-protein interaction may prove highly effective against Covid-19 through limiting the coronavirus entry and infection of the host cells.

Although some computational drug repurposing studies against this challenging target have been published so far, most of them utilized homology models of these proteins owing to the lack of experimental structure at that time [25-29]. In contrast, our virtual screening approach utilized the most recent X-ray crystal structure of SARS-CoV-2 spike receptor-binding domain bound with the human ACE2 entry receptor [30]. This high-resolution X-ray structure offers the closest representation of the atomic level understanding towards the initial step of infection by SARS-CoV-2 coronavirus. As the spike protein is considered to be central to promoting host attachment and protein-mediated membrane fusion of the virus, blocking its receptor-binding domain by a ligand may provide an effective way to inhibit its entry into human cells.

In this context, we first explored the human ACE2 interaction domain on the isolated SARS-CoV-2 spike protein receptor-binding domain to identify any potential druggable pockets suitable to bind such small-molecule ligands, using DoGSiteScorer server [12]. Unfortunately, the ACE2 interaction domain on SARS-CoV-2 spike protein completely lacked such druggable binding pockets, thus rendering it to be unsuitable for virtual screening efforts. This was also the case with the isolated ACE2 protein interface where spike protein receptor-binding domain binds. The observed lack of any druggable binding pockets on these two proteins at their individual binding surfaces should be taken into account when analyzing the data from previous studies that carried out docking against them separately [26-29, 31-33]. In contrast, these binding partners in their bound form presented a well-defined binding pocket with the DoGSiteScorer druggability score of 0.71, thus offering an induced binding cleft that is optimum for binding putative small-molecule modulators of this important protein-protein interaction (**Figure 1**). We hypothesize that such interface-binding molecules, especially those with potential to exert disproportionate interaction affinity towards one protein partner over another, may prove effective in destabilizing the host-virus interactions.

Therefore, with the aim of discovering such small-molecule inhibitors targeting this spike-ACE2 interface pocket, we docked ~5,587 approved drugs into this pocket using the Autodock Vina algorithm. The top docking hits showed good binding scores to the spike-ACE2 interface pocket, with the best score being -11.1 Kcal/mol for ZINC72206342. Although these promising

AutoDock Vina scores further support druggability of this targeted pocket as assessed by the DoGSiteScorer algorithm, it should be noted that Vina scoring function has ~2.85 Kcal/mol standard error [34]. This is a general limitation associated with all available scoring functions to a varying degree, making use of any one of them individually prone to enriching the false positives in a given virtual screening campaign.

Therefore, we utilized consensus scoring approaches, whereby multiple scoring functions are applied to re-score and re-rank the best docked conformer of each of the docked ligands. Such consensus scoring approach has been shown to be more robust and accurate than any single scoring procedure, thus leading to significant improvement of the hit rates in virtual library screening [19]. Importantly, consensus scoring has been shown to be independent of the target receptor under consideration, the docking method used, and also the scoring functions applied. Also, use of even a moderately small number of scoring functions (three or four) is shown to be sufficient to improve the screening results significantly. Thus, in addition to default AutoDock Vina, we used four additional scoring functions spanning knowledge-based (DSX), empirical (X-Score) and machine-learning scoring functions (NNScore and RF-Score-VS) [14-18]. We then combined these data from multiple scoring functions using the rank-by-rank and rank-by-number strategies, in an effort to enrich the virtual screening hit list [20]. The consensus hit lists containing top 100 each of the approved drugs thus obtained by these two ranking schemes are presented in **Tables 1** and **2**. As can be seen, many of the drugs are common to both lists.

We then combined these two sets of data, leading to a final consensus hit list that contained total 141 ZINC ligand entries representing 91 unique approved drug molecules (**Table 3**). When grouped according to their action mechanism or target, the known antivirals were identified to be the most prominent group of our hit list (total 14 antiviral drugs). This is very promising and in line with a recently published comprehensive analysis that showed antivirals together with antibiotics and corticosteroids were the most frequently administered drug classes to the first 9,152 reported cases of Covid-19 [35]. The antivirals discovered here included approved drugs against two other major enveloped (+) ssRNA viruses, viz. HIV (Atazanavir, Cobicistat, Indinavir, Saquinavir and Tipranavir) and HCV (Asunaprevir, Daclatasvir, Elbasvir, Glecaprevir, Grazoprevir, Ombitasvir, Simeprevir, Telaprevir, Voxilaprevir), further supporting their potential use to treat Covid-19. Notably, all four ZINC database structure entries for Saquinavir were short-listed as the top hits against this protein-protein interaction target. It is noteworthy that majority of our identified drugs are novel suggestions against Covid-19. In comparison, other antiviral drugs studied against Covid-19 previously included Acyclovir, Darunavir, Favipiravir, Ganciclovir, Lopinavir-Ritonavir combination, Oseltamivir, Remdesivir, Ribavirin and Umifenovir [36-38]. Thus, our newly identified antiviral drugs warrant urgent addition to the arsenal for *in vitro* testing against Covid-19.

In addition to these antiviral drugs, other major groups of compounds identified here as potential inhibitors of spike protein-ACE2 receptor interaction involved several anti-inflammatory/antioxidant (~14%), antibiotic/antifungal (~13%), anticancer (~15%), anticonvulsant/antipsychotic (~5%), and antihypertensive (~4%) drugs. Although our innate immune system is vital in controlling the replication and infection of coronavirus [26], overactivated immune system leads to inflammatory cytokine storm in many patients, resulting in fatal collateral tissue damage [39]. Thus, anti-inflammatory and antioxidant drugs identified in

the present study may prove helpful against overactivated immune response observed in Covid-19 patients. In this context, clinical trials are ongoing to test efficacy of various immunosuppressant drugs against Covid-19, including two specific IL-6 inhibitors, viz. Tocilizumab by Roche and Sarilumab by Regeneron. Notably, one of our top hits is Everolimus, which is an effective macrolide immunosuppressant drug.

Furthermore, several antibiotic and antifungal drugs we discovered here may prove effective against Covid-19. A macrolide antibiotic Azithromycin is already being investigated against Covid-19 owing to its excellent activity in inhibiting viral replication *in vitro* [40]. Interestingly, one of our antibiotic hits Telithromycin has been proven to be effective against community-acquired pneumonia caused by streptococcus pneumoniae [41], indicating potential application to treat coronavirus pneumonia. Also, two of our top antifungal hits Itraconazole and Posaconazole, are known to be effective in treating lung infections [42]. Moreover, three anticonvulsant drugs we discovered are metabolites of Valproate, which is currently being investigated against Covid-19 owing to its anti-inflammatory and intrinsic antiviral properties [43].

Three antiparasitic drugs also appeared in our hit list, viz. Ivermectin, Spinosad and Voacamine. Spinosad is an effective, natural product-based insecticide used to treat head lice and has low mammalian toxicity. Voacamine is approved for use as an antimalarial drug in many countries worldwide. Furthermore, Ivermectin is an approved drug with nuclear transport inhibitory activity that has been shown to be effective in significantly inhibiting Covid-19 virus replication *in vitro* [44].

Antitumor drugs also formed a major group of our hit list, including several chemotherapeutics, a proteasome inhibitor Carfilzomib and three selective tyrosine kinase inhibitors, viz. AFN911 (a metabolite of Imatinib), Brigatinib and Ponatinib. It is noteworthy that Imatinib has previously been demonstrated to inhibit both SARS-CoV and MERS-CoV replication *in vitro* [45]. Interestingly, Imatinib was shown to specifically interfere with SARS-CoV spike protein-induced membrane fusion of virus with host cells [46]. Taken together, our current data may suggest similar activity against SARS-CoV-2 also, through potential inhibition of spike protein-ACE2 receptor interaction, as shown in the present study.

Antihypertensives are another group of drugs we identified as top hits that are of direct relevance to Covid-19. These include angiotensin-converting enzyme/ACE inhibitors (Fosinopril and Reserpinin) and angiotensin receptor blockers/ARBs (Candesartan and Saralasin). Such inhibitors of renin-angiotensin-aldosterone system (RAAS) were initially thought to be contraindicated in Covid-19, owing to their potential role in possibly increasing the ACE2 levels that may aid in SARS-CoV-2 viral entry into host cells [47]. This is, however, largely based on preclinical studies, and the human clinical data in support of this is clearly lacking. In contrast, ACE2 is known to offer protection against cardio and lung injury, and SARS-CoV-2 is actually found to downregulate ACE2 expression after exploiting it for cell entry [47]. In this context, clinical trials are ongoing to test the efficacy of ACE2 restoration against Covid-19 through administration of RAAS modulators, including recombinant ACE2 and the ARB Losartan (ClinicalTrials.gov IDs: NCT04287686, NCT04312009, NCT04311177). Here, it is noteworthy that the RAAS inhibitors identified in our present study may prove to be dually beneficial against Covid-19 by not only increasing the expression of lung- and cardio-protective ACE2, but also

simultaneously blocking its interaction with SARS-CoV-2 spike protein as indicated by our *in silico* data.

Other promising drugs identified here included antihistamines (Astemizole, Ternadin), antipsychotics (Aripiprazole, Pipotiazine), leukotriene receptor antagonists (Montelukast, Zafirlukast), pulmonary surfactants (Palmitoyloleoyl-phosphatidylglycerol, Colfosceril palmitate), a chemokine receptor antagonist (Maraviroc), an antithrombotic (Vorapaxar), and a cardiac glycoside (Digoxigenin bisdigitoxoside). Out of these, Astemizole has been shown to significantly inhibit replication of SARS-CoV-2 as well as MERS-CoV and SARS-CoV [45, 48], while Pipothiazine is structurally similar to another phenothiazine antipsychotic drug Chlorpromazine that is active against MERS-CoV and SARS-CoV [48]. Also, Montelukast and Zafirlukast are approved for treating chronic obstructive pulmonary disease. Furthermore, pulmonary surfactants Colfosceril palmitate and Palmitoyloleoyl-phosphatidylglycerol are beneficial in patients with respiratory distress syndrome [49]. Importantly, another of our top compound Maraviroc is the only FDA-approved drug of its kind that is known to target virus-host interaction and human cell entry, through blocking interaction of HIV-1 gp120 protein with CCR5 expressed on T-cells [36]. To the best of our knowledge, our study is the first to indicate possible use of Maraviroc against SARS-CoV-2 cell entry through potential inhibition of spike protein-ACE2 receptor interaction.

To investigate potential molecular interactions of our top hits at the spike-ACE2 binding interface, their top-ranked docking complexes were subjected to the post-docking optimization. The optimized ligand-protein complex structures thus obtained for 20 most promising drugs are shown in **Figure 2**. As expected, reasonable binding poses were found in all cases, demonstrating that these drugs fit inside the binding pocket well. As can be seen, these compounds made multiple interactions with several amino acid residues near the binding pocket on both binding proteins. By making these key hydrogen bonding and hydrophobic interactions at the binding interface, these molecules may efficiently inhibit the spike protein-mediated fusion of the virus with the host cell membrane, leading in turn to blocking the first step of viral infection in terms of viral cell entry.

Conclusions

In summary, we have described a successful structure-based discovery of several approved drugs as potential inhibitors of spike protein-ACE2 receptor interaction. In addition to inhibition of viral host entry through blocking this critical protein-protein interaction, the primary inherent pharmacological actions of our newly discovered drugs may provide additional therapeutic avenues for treating viral infection-associated pneumonia, inflammation and oxidative damage. Some of these drugs are also suggested to bind to other important Covid-19 targets. For example, Atazanavir and Saquinavir are predicted to bind to coronavirus main protease M_{Pro} [9, 28, 50], while Itraconazole is predicted to bind to RdRp, another important target involved in viral RNA synthesis and replication [26]. Such multi-targeting drugs may not only increase the therapeutic efficacy, but also significantly decrease the possibility of drug resistance development by this highly infectious disease. Therefore, we wish to make these readily available compounds public here, with the aim of facilitating their urgent *in vitro* evaluations leading to rapid clinical development of effective drugs against Covid-19.

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Conflicts of interest

The authors declare no conflict of interest.

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Table 1. Top 100 hits from a database of approved drugs, as identified by *rank-by-rank* consensus scoring scheme utilizing five different scoring functions.

Rank	ZINC ID	Name	Rank	ZINC ID	Name	Rank	ZINC ID	Name
1	11677911	Bisectrizole	35	896717	Zafirlukast	69	150338703	Carbetocin
2	3914596	Saquinavir	36	3941496	Atazanavir	70	14879961	Leutein
3	150588351	Elbasvir	37	150339325	Hydroxyitraconazole	71	85537142	Aclarubicin
4	72190231	Posaconazole	38	49783788	Valrubicin	72	11677890	Bemotrizinol
5	26664090	Saquinavir	39	4074875	Candesartan	73	685933138	Rifapentine
6	29416466	Saquinavir	40	150339328	Hydroxyitraconazole	74	3831511	Ternadin
7	27990463	Lomitapide	41	68204830	Daclatasvir	75	4097309	Fosinopril
8	169621210	Voacamine	42	3934128	Temoporfin	76	261527149	Ivermectin
9	11677894	Bemotrizinol	43	85536932	Cabazitaxel	77	3995811	Faslodex
10	26985532	Saquinavir	44	95551509	Grazoprevir	78	601274	Astemizole
11	150601177	Ombitasvir	45	95617640	Imatinib metabolite	79	95617639	AFN911
12	100022637	Tipranavir	46	936069565	Glecaprevir	80	85540202	Asunaprevir
13	100016058	Tipranavir	47	72131401	Hydroxyitraconazole	81	96006024	Spinosad
14	253387884	Hydroxyitraconazole	48	4097344	Itraconazole	82	52955754	Ergotamine
15	60392779	Posaconazole	49	118915215	Digoxigenin bisdigitoxoside	83	43131420	Fostamatinib
16	8214644	Pentagastrin	50	118915214	Digoxigenin bisdigitoxoside	84	3992480	Telaprevir
17	22448696	Indinavir	51	118915216	Digoxigenin bisdigitoxoside	85	85555528	Vinblastine
18	49841054	Carfilzomib	52	4214612	Valrubicin	86	4097185	Reserpinin
19	218037687	Voxilaprevir	53	95617679	Telithromycin	87	21981272	Hydroxynefazodone
20	96006020	Paclitaxel	54	95618786	Zafirlukast	88	94303244	Desacetylvinblastine
21	17653971	Canthaxanthin	55	95617638	Imatinib metabolite	89	85537014	Cobicistat
22	28639340	Posaconazole	56	11677901	Bemotrizinol	90	85536958	Vinorelbine
23	164528615	Glecaprevir	57	49918330	Valrubicin	91	205224698	Fosnetupitant
24	238809663	Atracurium besylate	58	95564895	Aripiprazole Lauroxil	92	204073689	Vorapaxar
25	6845076	B-Carotene	59	85537078	Vinflunine	93	3817234	Maraviroc
26	4097427	Candesartan	60	40164432	Lutein	94	100003902	Maraviroc
27	3938482	Posaconazole	61	101160855	Maraviroc	95	148723177	Brigatinib
28	49918329	Valrubicin	62	95618827	Hydroxyitraconazole	96	53683271	Dalfopristin
29	253632968	Simeprevir	63	169369935	3'-p-Hydroxypaclitaxel	97	3995616	Ergoloid
30	164760756	Simeprevir	64	11616852	Valrubicin	98	28232746	Valrubicin
31	95618745	RPR123142	65	85537091	Proglumetacin	99	28232755	Valrubicin
32	95618746	RPR123142	66	3831151	Montelukast	100	261139420	Alcurnium
33	150339320	Hydroxyitraconazole	67	68202099	Sonidegib			
34	169621209	Voacamine	68	36701290	Ponatinib			

Table 2. Top 100 hits from a database of approved drugs, as identified by *rank-by-number* consensus scoring scheme utilizing five different scoring functions.

Rank	ZINC ID	Name	Rank	ZINC ID	Name	Rank	ZINC ID	Name
1	26664090	Saquinavir	35	230123065	Valproic Acid CoA	69	261527149	Ivermectin
2	11677911	Bisotrizole	36	27990463	Lomitapide	70	95617679	Telithromycin
3	3914596	Saquinavir	37	8215434	Flavin adenine dinucleotide	71	85537068	Pralmorelin
4	85537091	Proglumetacin	38	238809665	Atracurium besylate	72	164528615	Glecaprevir
5	150588351	Elbasvir	39	72190231	Posaconazole	73	4097427	Candesartan
6	150601177	Ombitasvir	40	95617677	Pentagastrin	74	3995811	Faslodex
7	26985532	Saquinavir	41	238809664	Atracurium besylate	75	85555528	Vinblastine
8	169368439	Saralasin	42	261494704	Tricrocin	76	22448696	Indinavir
9	29416466	Saquinavir	43	96006013	Cisatracurium	77	95615286	Senktide
10	261527202	Rifapentine	44	118915215	Digoxigenin bisdigitoxoside	78	253630390	Ivermectin
11	11677894	Bemotrizinol	45	118915214	Digoxigenin bisdigitoxoside	79	261494615	Fidaxomicin
12	238809663	Atracurium besylate	46	118915216	Digoxigenin bisdigitoxoside	80	85574641	Ubiquinol
13	685933138	Rifapentine	47	8214658	Pipotiazine	81	3992480	Telaprevir
14	3941496	Atazanavir	48	169621228	Rifapentine	82	28639340	Posaconazole
15	85537089	Proglumetacin	49	169621210	Voacamine	83	164760756	Simeprevir
16	261106255	Senktide	50	96006015	Atracurium besylate	84	72131401	Hydroxyitraconazole
17	49841054	Carfilzomib	51	96006020	Paclitaxel	85	96006024	Spinosad
18	150339320	Hydroxyitraconazole	52	8552309	Palmitoyl-oleoyl-phosphatidylglycerol	86	3938482	Posaconazole
19	8214644	Pentagastrin	53	95564895	Aripiprazole	87	936069565	Glecaprevir
20	245224178	Crocine	54	3831151	Montelukast	88	85427689	Ubidecarenone
21	11677890	Bemotrizinol	55	100022637	Tipranavir	89	252286706	Ivermectin
22	169289386	Saralasin	56	100016058	Tipranavir	90	4097309	Fosinopril
23	150339052	Nabumetone	57	95618745	RPR123142	91	253632968	Simeprevir
24	85537142	Aclarubicin	58	95618746	RPR123142	92	85536932	Cabazitaxel
25	238809662	Atracurium besylate	59	1530755	Probucol	93	95618786	Zafirlukast
26	261494702	Tricrocin	60	261106252	Senktide	94	96061888	Roxithromycin
27	96006014	Atracurium besylate	61	6845076	B-Carotene	95	17653971	Canthaxanthin
28	252441679	Tylosin tartrate	62	261494703	Tricrocin	96	40164432	Lutein
29	150340074	Sorbitan trioleate	63	8214373	Colfosceril palmitate	97	253975480	Rifaximin
30	261494569	3-ene-Valproic acid CoA	64	169677008	Everolimus	98	169369935	3'-p-Hydroxypaclitaxel
31	229984054	4-ene-Valproic acid CoA	65	8220175	Zeaxanthin	99	95551509	Grazoprevir
32	150338703	Carbetocin	66	85537014	Cobicistat	100	253387884	Hydroxyitraconazole
33	11677901	Bemotrizinol	67	3938642	Thymopentin			
34	261106254	Senktide	68	49918329	Valrubicin			

Table 3. The combined consensus hit list of total 91 unique approved drugs, predicted to target SARS-CoV-2 spike protein-ACE2 receptor interaction.

No.	Drug Name	ZINC IDs	Drug Class/Target
1	Asunaprevir	85540202	Antivirals
2	Atazanavir	3941496	
3	Cobicistat	85537014	
4	Daclatasvir	68204830	
5	Elbasvir	150588351	
6	Glecaprevir	164528615, 936069565	
7	Grazoprevir	95551509	
8	Indinavir	22448696	
9	Ombitasvir	150601177	
10	Saquinavir	3914596, 26664090, 29416466, 26985532	
11	Simeprevir	253632968, 164760756	
12	Telaprevir	3992480	
13	Tipranavir	100022637, 100016058	
14	Voxilaprevir	218037687	
15	B-Carotene	6845076	Antioxidants
16	Canthaxanthin	17653971	
17	Crocin	245224178	
18	Flavin adenine dinucleotide	8215434	
19	Lutein	14879961, 40164432	
20	Tricrocin	261494702, 261494704, 261494703	
21	Ubidecarenone	85427689	
22	Ubiquinol	85574641	
23	Zeaxanthin	8220175	
24	Nabumetone	150339052	Anti-inflammatory
25	Proglumetacin	85537091, 85537089	
26	Fostamatinib	43131420	
27	Aclarubicin	85537142	Antibiotics
28	Dalfopristin	53683271	
29	Fidaxomicin	261494615	
30	Rifapentine	685933138, 261527202, 169621228	
31	Rifaximin	253975480	
32	Roxithromycin	96061888	

33	Telithromycin	95617679	Antibiotics
34	Tylosin tartrate	252441679	
35	Valrubicin	49918329, 49783788, 4214612, 49918330, 11616852, 28232746, 28232755	
36	Hydroxyitraconazole	253387884, 150339320, 150339325, 150339328, 72131401, 95618827	Antifungals
37	Itraconazole	4097344	
38	Posaconazole	72190231, 60392779, 28639340, 3938482	
39	3'-p-Hydroxypaclitaxel	169369935	Anticancer drugs
40	AFN911	95617640, 95617638, 95617639	
41	Brigatinib	148723177	
42	Cabazitaxel	85536932	
43	Carfilzomib	49841054	
44	Desacetylvinblastine	94303244	
45	Faslodex	3995811	
46	Paclitaxel	96006020	
47	Ponatinib	36701290	
48	RPR123142	95618745, 95618746	
49	Sonidegib	68202099	
50	Vinblastine	85555528	
51	Vinflunine	85537078	
52	Vinorelbine	85536958	
53	Fosinopril	4097309	Antihypertensives (ACE Inhibitors and angiotensin-receptor blockers)
54	Reserpinin	4097185	
55	Candesartan	4097427, 4074875	
56	Saralasin	169368439, 169289386	
57	Ivermectin	261527149, 253630390, 252286706	Antiparasitic drugs
58	Spinosad	96006024	
59	Voacamine	169621210, 169621209	
60	Alcuronium	261139420	Neuromuscular blocking agents
61	Atracurium besylate	238809663, 238809662, 96006014, 238809665, 238809664, 96006015	
62	Cisatracurium	96006013	

63	3-ene-Valproic acid CoA	261494569	Anticonvulsants
64	4-ene-Valproic acid CoA	229984054	
65	Valproic Acid CoA	230123065	
66	Montelukast	3831151	Leukotriene receptor antagonists
67	Zafirlukast	896717, 95618786	
68	Palmitoyl-oleoyl-phosphatidylglycerol	8552309	Pulmonary surfactants
69	Colfosceril palmitate	8214373	
70	Astemizole	601274	Antihistamines
71	Ternadin	3831511	
72	Aripiprazole Lauroxil	95564895	Antipsychotics
73	Pipotiazine	8214658	
74	Lomitapide	27990463	Hypolipidemic agents
75	Probucol	1530755	
76	Bemotrizinol	11677894, 11677901, 11677890	UV absorbers
77	Bisotrizole	11677911	
78	Carbetocin	150338703	Oxytocic
79	Digoxigenin bisdigitoxoside	118915215, 118915214, 118915216	Cardiac glycoside
80	Ergoloid	3995616	Vasodilator
81	Ergotamine	52955754	Alpha-1 selective adrenergic agonist
82	Everolimus	169677008	Immunosuppressant
83	Fosnetupitant	205224698	Antiemetic
84	Hydroxynefazodone	21981272	Antidepressant
85	Maraviroc	101160855, 3817234, 100003902	Chemokine receptor antagonist
86	Pentagastrin	8214644, 95617677	Gastrin analogue
87	Pralmorelin	85537068	Growth hormone-releasing peptide 2
88	Senktide	261106255, 261106254, 261106252, 95615286	Neuromedin K3 (NK3) receptor agonist
89	Temoporfin	3934128	Photosensitizer
90	Thymopentin	3938642	Immunostimulant (against HIV)
91	Vorapaxar	204073689	Inhibitor of protease-activated receptor

Figure 1. The SARS-CoV-2 spike protein-ACE2 receptor interface (PDB ID: 6M0J). The binding interfaces (shown in white rectangles) of isolated (A) ACE2 receptor and (B) spike protein receptor-binding domain lack any druggable pockets. (C) Together in their bound form, however, they present a well-defined, tunnel-like binding pocket (shown in white circle) with druggability score of 0.71, making it suitable for binding a small-molecule modulator of therapeutic importance against Covid-19.

Figure 2. The predicted binding modes of top 10 representative hits at the SARS-CoV-2 spike protein-ACE2 receptor binding interface, and their associated 2D ligand–protein interaction diagrams: (1) Atazanavir, (2) Grazoprevir, (3) Saquinavir, (4) Simeprevir, (5) Telaprevir, (6) Tipranavir, (7) Aclarubicin, (8) Hydroxyitraconazole, (9) AFN911 (Imatinib metabolite), (10) Sonidegib, (11) Vinorelbine, (12) Fosinopril, (13) Candesartan, (14) Saralasin, (15) Voacamine, (16) Atracurium besylate, (17) Zafirlukast, (18) Astemizole, (19) Maraviroc, (20) Senktide.

(1) Atazanavir:

(2) Grazoprevir:

Figure 2 (continued)

(3) *Saquinavir*:

(4) *Simeprevir*:

Figure 2 (continued)

(5) *Telaprevir*:

(6) *Tipranavir*:

Figure 2 (continued)

(7) *Aclarubicin*:

(8) *Hydroxyitraconazole*:

Figure 2 (continued)

(9) AFN911 (Imatinib metabolite):

(10) Sonidegib:

Figure 2 (continued)

(11) Vinorelbine:

(12) Fosinopril:

Figure 2 (continued)

(13) *Candesartan*:

(14) *Saralasin*:

Figure 2 (continued)

(15) *Voacamine*:

(16) *Atracurium besylate*:

Figure 2 (continued)

(17) *Zafirlukast*:

(18) *Astemizole*:

Figure 2 (continued)

(19) *Maraviroc*:

(20) *Senktide*: