Drug repurposing and polypharmacology to fight SARS-CoV-2 through the inhibition of the main protease

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Abstract

There is an urgent need to develop therapeutic options to fight the outbreak of a novel Coronavirus (SARS-CoV-2), which causes a disease named COVID-19 and is spreading rapidly around the world. Drug repurposing can significantly accelerate the identification of drug candidates suitable for clinical evaluation. Moreover, drugs with polypharmacological effects may increase antiviral activity and/or counteract severe disease complications concurrently affecting COVID-19 patients. Herein, we present the results of a computational drug repurposing campaign in search for potential inhibitors of the main protease of SARS-CoV-2. To this aim, the complete DrugBank database, including drug metabolites, was docked to the recently solved crystal structure of the SARS-CoV-2 M^{pro} and the results were post-processed by using our *in-house* tool BEAR. Here we report 32 promising drugs that could be repositioned to fight SARS-CoV-2. Some of them have already entered clinical trials against COVID-19, thus supporting our results, but the vast majority of the selected compounds is new and has never been considered before. For each repurposed compound its therapeutic relevance and the potential beneficial polypharmacological effects that may arise thanks to its original therapeutic indication are thoroughly discussed.

Introduction

At present, we are facing with one among the most devastating pandemic crisis in human history: the coronavirus disease 2019 (COVID–19), which has to date affected more than 2 million people and is responsible for almost 170000 deaths[1]. Unfortunately, neither a vaccine nor therapeutic options are currently available to prevent or cure such infection, which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV–2)[1,2].

SARS-CoV–2 is a positive-sense single-stranded RNA virus belonging to the β-genus of the *Coronaviridae* family[3], and is closely related to coronaviruses responsible for the SARS and MERS outbreaks that spread in 2003 and 2012, respectively[4]. Although outbreaks deriving from coronavirus infections appear to be recurrent[5], no specific antiviral drugs are currently available for treating these diseases. Therefore, major efforts are now being focused on developing a vaccine as well as effective drugs to treat infected patients[6].

At least three biological targets are now under investigation to develop SARS-CoV–2 specific antiviral compounds, *i.e.* the RNA-dependent RNA polymerase, the spike protein, and the main protease, for which crystal structures have been very recently solved[7]. In the meanwhile, many clinical trials based on antivirals developed for other infections are now ongoing, together with experimentation of drugs meant to reduce inflammation and severe respiratory complications[6]. Clinical investigations include the antiviral drugs favipiravir, originally developed in Japan to fight *influenza* and found to be effective also against Ebola virus (ClinicalTrials Identifiers: NCT04349241, NCT04303299, NCT04310228), and remdesivir, an Ebola virus RNA-dependent RNA polymerase prodrug inhibitor found to be effective also against MERS and SARS (currently under evaluation for COVID–19 in more than 10 clinical trials)[8–10]. Chloroquine and hydroxychloroquine are under investigation, the latter being approved under Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA) for the treatment of COVID–19[11]. The monoclonal antibodies tocilizumab and sarilumab directed against the interleukin–6 receptor (IL–6R) have entered clinical trials (ClinicalTrials Identifiers: NCT04322773, NCT04332913) for the same purpose.

The rapid spread of the SARS-CoV–2 pandemics and the lack of specific antiviral drugs suggest that drug repurposing should be the preferred way for rapidly selecting suitable candidates for clinical testing. In fact, these compounds are well characterized and already possess optimized pharmacokinetics and safety profiles[12]. However, drugs under investigation are currently limited in number and often redundant, and none of them originate from trials on a specific SARS-CoV–2 target. Therefore, a rational target-based repositioning campaign is expected to disclose additional and valuable drug candidates to be used either alone or in combination. Moreover, considering the severe life-threatening disease complications, polypharmacological drugs, *i.e.,* single drug molecules that combine antiviral activity with *e.g.* anti-inflammatory or antithrombotic activity may be particularly useful[13]. To this end, in this study we performed an extensive structure-based virtual screening campaign to identify 32 top-candidate approved or experimental drugs able to potentially inhibit the SARS-CoV–2 Main Protease (M^{pro}). The DrugBank database[14], including drug metabolites, was docked in the M^{pro} enzyme and the results were post-processed with BEAR[15], an *in-house* developed screening tool with a well documented ability to refine virtual screening results[16]. The candidate selection process took into special

consideration the analysis of drug annotations and reported biological activity information to grasp for possible favorable polypharmacological effects arising from the original therapeutic indication. Because of their fitting to the SARS-CoV–2 main protease active site, the reported drugs should be readily repurposed to elicit an antiviral response.

Results

The SARS-CoV-2 Main Protease (M^{pro}), also known as 3C-like protease, is a relevant target for drug repurposing because it plays a critical role in the maturation of the viral particle[17]. A computational screening workflow (Figure 1) was devised to identify drug candidates able to bind and inhibit the SARS-CoV-2 Main Protease.

To this aim, the complete DrugBank database, including drug metabolites, was docked into the crystal structure of the SARS-CoV-2 Main Protease (PDB ID: 6LU7), using Glide (Schrodinger Suite), and the results were ranked according to the Glide Score scoring function. The 2000 top ranking compounds were post-processed with BEAR[15], an *in-house* developed tool that performs the molecular mechanics and molecular dynamics refinement of the docking pose and rescores ligands according to more accurate MM-PBSA and MM-GBSA binding free energy predictions. Then, the selection of the best candidates was made according to i) docking and post-docking scores, ii) visual inspection of the protein-ligand complexes, and iii) analysis of drug annotations and literature information to repurpose known drugs or clinical candidates or experimental compounds as M^{pro} inhibitors that may benefit of their original therapeutic indications to reduce severe COVID-19 complications, while inhibiting viral particle maturation (beneficial polypharmacology[13]).

The most promising compounds resulting from the screening are reported in Table 1, along with their DrugBank IDs, the predicted docking and binding free energy scores, the rank position in the screening outcome, the therapeutic targets and original indications, and the PDB ID of the crystal structure of each drug in complex with its original target, when available. The 2D chemical structures of the candidates are reported in Figure S1 (see Supplementary information). A detailed discussion of the selected drug candidates, divided into candidates to drug repurposing, candidates to drug repurposing with beneficial polypharmacology, and candidates to drug repurposing based on active drug metabolites, is reported in the following paragraphs.

Candidates to drug repurposing

The drugs herein reported (either approved or investigational) are excellent examples of possible candidates for repositioning as SARS-CoV-2 M^{pro} inhibitors. As an evidence of the reliability of our results, several of the proposed candidates are viral protease inhibitors, which in some cases are already under study as COVID-19 therapeutic agents.

Saquinavir (DB01232) is an effective anti-retroviral drug used for AIDS treatment, targeting HIV-1 protease[18]. As discussed below, two major saquinavir metabolites (M2 and M10) were predicted to bind to the SARS-CoV-2 M^{pro} with a higher affinity with respect to the parent compound.

The hydroxymethyl ketones **EXPT02467** (cruz-2, DB02128) and **EXPT02989** (cruz-1, DB01871) are two reversible inhibitors of Cruzipain, a cysteine-type endopeptidase of *Tripanosoma cruzi*[19]. The structures of their complexes with the Cruzipain target are available with PDB IDs: 1ME3 and 1ME4, respectively. Another interesting Cruzipain inhibitor emerging from our screening is **WRR-204** (EXPT03235, DB04502), which is an irreversible inhibitor (PDB ID: 1EWO).

MMI-175 (DB02378) is an experimental drug that inhibits β-secretase (BACE-1)[20], one of the two aspartic proteases responsible for the generation of amyloid-β peptides in the neurons. As such, drugs blocking this enzyme may help slowing down Alzheimer's disease progression[21]. According to the predicted pose (Figure 2, panel *a*) and the binding affinities, this compound is expected to efficiently bind to the SARS-CoV-2 enzyme. The ability of this compound to cross the blood brain barrier would be of high interest for COVID-19 treatment[22]. In fact, previous studies have reported the presence of coronavirus particles in the CNS and their potential association with neurologic manifestations in patients[22,23].

JE-2147 (EXPT01956, DB02668) is an HIV-1 protease inhibitor, whose X-ray crystal structure was deposited (PDB ID: 1KZK). It is remarkably potent against several common resistant strands[24].

EH58 (EXPT01332**,** DB03063**)** is a potent inhibitor of Plasmepsin 2, an aspartic protease in the food vacuole of *Plasmodium falciparum* (PDB ID: 1LF3), provided with antimalarial activity (K_i=100 nM)[25]. Another Plasmepsin 2 inhibitor worth of consideration, even if predicted scores are lower than those of EH58, is **RS370** (DrugBank ID: DB04378, PDB ID: 1LF2), with a reported K_i of 30 nM[26].

EXPT00713 (DB03648) is a *P. falciparum* formylmethionine deformylase inhibitor with a reported activity of 130 nM[27]. As already mentioned, other antimalarial drugs, such as chloroquine and hydroxychloroquine, are under the spotlight of COVID-19 researches[11].

QF34 (EXPT02729, DB04353) is a pseudopeptide inhibitor of several variants of HIV-1 and HIV-2 proteases[28], and even of some highly resistant mutants. The compound was also crystallized with the HIV-1 protease (PDB IDs: 1IZH, 1IZI).

Compounds **I2** (DB04692), **N1** (DB04710) and **N3 (**PRD_002214, DB04595), three experimental inhibitors of the SARS-CoV M^{pro} reported in 2005, could also be valuable candidate inhibitors of the SARS-CoV-2 M^{pro}. Compounds I2, N1, and N3 were co-crystallized in complex with SARS-CoV M^{pro} (PDB IDs: 2D2D, 1WOF, and 2AMQ, respectively). Notably, compound N3 has been very recently confirmed to bind and inhibit the SARS-CoV-2 M^{pro}, the crystal structure of its complex being the starting point of our virtual screening (PDB ID: 6LU7)[29].

Rupintrivir (AG-7088, DB05102) is a potent irreversible rhinovirus 3C and 3C-Like protease inhibitor in development for use against human rhinoviral (HRV) infections that has been recently co-crystallized with its target (PDB ID: 6KU8)[30]. Rupintrivir has subsequently shown to be a broad-spectrum antiviral agent, acting against picornavirus, norovirus and coronavirus proteases[31]. Although the drug failed to meet the requirements of the Phase II clinical trials for the treatment of common cold, it was reconsidered in 2003 as a good candidate for the inhibition of SARS-CoV M^{pro}, as it shares homology with the HRV-C 3C protease[32].Therefore, rupintrivir is a very interesting candidate for testing against COVID-19, as recently suggested also by Liu *et al*[33].

Larazotide (DB05645) is a peptidic inhibitor of paracellular permeability, investigated for the treatment of autoimmune diseases, such as diabetes mellitus type I, and gastrointestinal diseases and disorders[34]. Larazotide acetate is known to prevent immunologic changes induced by gluten consumption in patients with celiac disease. We predict that larazotide might bind to the SARS-CoV-2 M^{pro} protease very efficiently.

Compound **WRR-183** (DB08732) is a α,β-epoxyketone that irreversibly inhibits the SARS-CoV M^{pro} (PDB code 2OP9)[35]. This compound is predicted to bind the SARS-CoV-2 M^{pro} tightly and with a similar pose, the epoxide being in close proximity to the Cys residue. Moreover, WRR-183 and especially its C-2 (R) epoxide isomer **WRR-182** are highly active against SARS-CoV spike-mediated entry[36]. Therefore, they show the potential to block SARS-CoV at two different steps of the replication cycle, *i.e.* viral entry and particle assembly[36].

Birinapant (DB11782) is an inhibitor of apoptosis proteins as XIAP, currently under investigation against solid tumors[37]. Birinapant was crystallized in complex with XIAP (PDB ID: 4KMP). XIAP stops apoptotic cell death induced either by viral infection or by overproduction of caspases[38]. In a recent study, birinapant treatment resulted in a rapid clearance of detectable *Hepatitis B Virus* (HBV) genetic material from serum[39]. Combination treatment with birinapant and entecavir (a reverse transcriptase inhibitor) is non-toxic and leads to a quicker clearance with respect to treatment with either drugs alone[39]. Therefore, birinapant modulates a host cell protein involved in viral persistence and may be useful to eliminate rather than merely control the SARS-CoV-2 infection.

Difelikefalin (formerly known as CR-845, DB11938) is a highly selective agonist of the κ-opioid receptor[40]. It is an analgesic opioid peptide that acts peripherally, under investigation for the treatment of acute and post-operative pain and, more recently, chronic pruritus[40]. Difelikefalin is currently in two Phase II clinical trials for the treatment of pruritus in atopic dermatitis and biliary cholangitis (Clinical Trials Identifiers: NCT04018027 and NCT03995212). The peripheral analgesic activity of the compound, together with the potential SARS-CoV-2 M^{pro} activity, may prove beneficial to COVID-19 patients experiencing peripheral neurologic symptoms and pain.

Ipamorelin (DB12370) is a selective agonist of the growth hormone (GH) secretagogue receptor increasing GH levels in plasma[41]. It was investigated for the treatment of gastrointestinal hypomotility disorders (Clinical Trials Identifiers: NCT01280344, NCT00672074).

Candidates to drug repurposing with beneficial polypharmacology

Polypharmacological ligands are extremely interesting in drug repurposing, because they offer the potential for higher efficacy and a combination of synergistic effects[13]. Therefore, for each top-ranking compound we investigated whether a possible beneficial polypharmacological effect may arise thanks to the reported biological activities and original therapeutic indications.

Caspofungin (DB00520) is an antifungal echinocandine possessing a cyclic peptide core. The reported mechanism of action involves inhibition of β-glucane synthase, which affects the fungal cell wall[42]. The *in silico* results returned a promising pose in complex with the SARS-CoV-2 protease, suggesting a possible antiviral activity. It is worth to note that caspofungin is the first-choice treatment for *Pneumocystis* pneumonia, one of the most serious secondary opportunistic mycotic infections that many severely ill COVID-19 patients tend to develop[43].

Enalkiren (DB03395) belongs to the class of direct renin inhibitors. By mimicking the transition state of angiotensin, enalkiren is able to block the first step of the renin-angiotensin system[44]. It has been recently reported that SARS-CoV-2 binds to the widespread angiotensin-converting enzyme 2 (ACE2) to enter target cells[45], and that levels of serum angiotensin II are considerably increased in COVID-19 patients[46]. Therefore, the modulation of the renin-angiotensin system by enalkiren, coupled with inhibition of the SARS-CoV-2 M^{pro}, might exhibit beneficial effects to treat COVID-19. According to our analyses, enalkiren is well accommodated within the SARS-CoV-2 M^{pro} binding site (Figure 2, panel *b*). Interestingly, another very recent computational study based on a different workflow also identified enalkiren as a potential candidate for the SARS-CoV-2 M^{pro}[33], further supporting its selection as a promising candidate for COVID-19 treatment.

The **Calpain inhibitor IV** (ZLLYCH$_2$F, DB04653), a covalent inhibitor of the Calpain-1 cysteine protease (PDB ID: 1ZCM)[47], is a top ranking candidate with peculiar characteristics. In our docked structure, the reactive methylene group of the compound is in close proximity to the Cys residue of the SARS-CoV-2 M^{pro} active site. This means that a covalent bond providing specificity and higher affinity over other proteases can potentially be formed. Interestingly, in 2004 calpain inhibitor IV resulted as an active agent against SARS-CoV[48]. Calpain regulates the activity of proteins that are part of processes influencing neuronal plasticity, cognition and neurodegeneration[49]. In particular, Calpain-1 is a calcium-activated cysteine protease that plays an important role in neutrophil motility and is a potential target for intervention in inflammatory diseases[50]. Interestingly, sarilumab and tocilizumab IL-6 antagonists and anakinra (IL-1 antagonist), which were approved for inflammatory diseases, are now under evaluation for their effectiveness against COVID-19 (Clinical Trials Identifiers: NCT04315298, NCT04317092, NCT04306705, NCT04315480, NCT04339712).

Ethylsulfonamide-D-Trp-Gln-p-aminobenzamidine (DB04758) was designed to potently inhibit factor VIIa (FVIIa), whose complex with tissue factor (TF) starts the extrinsic coagulation cascade[51]. Compared with other anti-thrombotic agents, the specific targeting of the extrinsic coagulation provides lesser risks of bleedings. SARS-CoV-2 infection often causes dramatic consequences to the circulatory system[52,53]. Preliminary reports include thrombocytopenia, elevated d-dimer levels, prolonged prothrombin time, and disseminated intravascular coagulation[54]. Our *in silico* findings suggested that DB04758 could also bind with high affinity to the SARS-CoV-2 M^{pro}. Therefore, this molecule might exhibit a dual activity against two crucial aspects of the virus infection.

Z-LY-CMK (DB07571) is a covalent inhibitor of the ATP-dependent Clp protease proteolytic subunit (ClpP)[55]. Interestingly, the ClpP enzyme has recently gained attention as a promising drug target for antibiotics development[56]. If confirmed, the possibility of this compound to act both as an antimicrobial agent and SARS-CoV-2 inhibitor would be particularly useful to treat secondary bacterial infections, potentially affecting COVID-19 patients.

The thrombin inhibitor **BM51.1011** (DB07934) is particularly interesting, as thrombotic complications appear to be an important issue in patients affected by COVID-19[54]. As the pandemic is spreading, the reported coagulation disorders in COVID-19 patients

and in previous SARS and MERS patients should be carefully addressed. The molecule is reported to bind to the original target (thrombin, PDB ID: 1UVS).

Cobicistat (DB09065) is a CYP3A blocker, co-administered with HIV-1 protease inhibitors (*e.g.* elvitegravir, darunavir, and atazanavir), which has no activity on the HIV-1 enzyme, but protects protease modulators against liver degradation[57]. However, we predicted a high binding affinity to the SARS-CoV-2 Main Protease (Figure 2, panel *c*). Such dual CYP3A/SARS-CoV-2 M^{pro} inhibition, if demonstrated, would provide a particularly effective antiviral compound against SARS-CoV-2.

Delparantag (formerly known as PMX-60056, DB12955**)** is a top scoring candidate for all the scoring functions. This molecule reverses the anticoagulation effects of heparin by binding to the pentasaccharide group of unfractionated heparin (UFH) and low-molecular-weight heparins (LMWH)[58]. Like protamine sulphate, which shares the same mechanism, delparantag should be administered in the minimal quantity required to antagonize heparin-associated bleeding. Heparin has raised increasing attention for its ability to prevent blood coagulation in COVID-19 patients affected by severe pneumonia and concomitant anti-inflammatory effects due to reduction of IL-6[52]. However, a recent study showed that among a cohort of COVID-19 patients at high risk of venous thromboembolism, 11% of them also had a high risk of bleeding[59]. Patients with COVID-19 can rapidly develop severe or critical vascular diseases, which can affect both venous thromboembolism and bleeding status[60]. Therefore, controlling venous thromboembolism and bleeding risks regularly is essential. Deparantag may be an effective tool to mitigate bleeding risks while eliciting antiviral activity due to inhibition of the SARS-CoV-2 M^{pro}.

Remdesivir (DB14761) is a nucleotide prodrug acting on the Ebola virus RNA dependent RNA polymerase and Replicase polyprotein 1ab[61]. The drug was subsequently found to be able to inhibit MERS and SARS viruses[10]. It is currently under clinical trials to treat COVID-19. The prodrug itself is well suited to dock and bind to the protease (Figure 2, panel *d*). Therefore, it may block viral maturation outside of the nucleus. Another nucleotide prodrug of this kind is **tenofovir alafenamide** (DB09299), a reverse transcriptase inhibitor (HIV-1) and DNA polymerase inhibitor (HSV2)[62].

Candidates to drug repurposing based on active drug metabolites

A unique feature of our repurposing strategy regards the inclusion of metabolites among screened compounds. Although they are usually discarded in drug repurposing studies, major metabolites can provide extremely interesting results. For this reason, here we describe some of the top scoring metabolites in our screening. Remarkably, many of these metabolites were shown to bind to the active site of the SARS-Cov-2 M^{pro} with higher affinity with respect to their parent drug.

Ritonavir and **lopinavir**, two HIV-1 protease inhibitors that are administered in combination[63], are now clinically investigated against COVID-19[64]. Based on our *in silico* analyses, neither of them seems to interact favourably with the SARS-CoV-2 M^{pro}, although the **N-desmethyl metabolite M7** of **ritonavir** (DBMET00084) was demonstrated to better interact with the protein thanks to its free urea group that establishes favourable hydrogen bonds with the SARS-CoV-2 M^{pro}.

Saquinavir decahydroisoquinoline metabolites M2 (DBMET01550 and DBMET01549), and **t-butyl hydroxyl M10** (DBMET01548) were predicted to strongly bind to the SARS-CoV-2 M^{pro} active site according to the docking scores. Interestingly, while saquinavir metabolites are inactive against HIV-1 protease[18], they seem to be more potent than saquinavir itself against the SARS-CoV-2 M^{pro}. This finding may be relevant for the pharmacokinetics and dosing of this antiviral for the treatment of COVID-19.

The glucuronide metabolite DBMET02115 (major active metabolite) of **ezetimibe** (DB00973) was predicted to establish favourable interactions with the protease active site. Interestingly, the metabolite is predicted to bind the protease with an affinity higher than that of the drug itself. Considering that this cholesterol-lowering drug is rapidly and extensively metabolized to its active glucuronate form in the liver and intestine[65], the metabolite would be readily available to inhibit the viral target.

Discussion

The goal of the present study was to perform a systematic drug repurposing screening of compounds of the DrugBank database for their ability to bind the SARS-CoV-2 main protease. The performed analyses allowed the identification of 32 drugs or

experimental compounds, and among them 24 can be considered as “novel”, as they have not been included in current clinical trials yet. Interestingly, many of them could be able to combine a potential antiviral activity with, *e.g.*, antithrombotic or anti-inflammatory activity, due to their activity on the primary target and therapeutic indication. Such “polypharmacological” behaviour, if confirmed, would make the identified candidate drugs extremely attractive to be further evaluated for COVID-19 treatment. In fact, a single molecule would be able to concurrently exert an antiviral activity and mitigate or abolish COVID-19 comorbidities, whose severity often leads to patient’s death. Therefore, in this study the importance of these repurposed molecules is also discussed from a polypharmacologic perspective. Finally, we found a number of drug metabolites that appeared to bind tighter to the SARS-CoV-2 M^{pro} than the parent drugs (*e.g.*, saquinavir and ezetimibe metabolites). To the best of our knowledge, drug repurposing based on drug (major) metabolites represents a novel approach, which may offer additional and valuable opportunities to repurpose candidate drugs through modulation of *in vivo* pharmacokinetics. Given the dramatic need for therapeutic options for COVID-19, our work can suggest some key repositioning, while at the same time proposing effective candidates.

Methods

Structure-based calculations

The recently reported 6LU7 crystal structure of the SARS-CoV-2 Main Protease[29] was first collected from Protein Data Bank (accessed on March 17th, 2020), and then prepared for the *in silico* screenings by using the *Protein Preparation Wizard* (Schrödinger release 2020-1)[66]. Defaults parameters were used during the protein preparation. Atom types and connectivity issues were fixed, hydrogen atoms were added, and interaction geometries were optimized. The co-crystallized water molecules and the peptide-like PRD_002214 inhibitor were retained during the protein preparation process, while removed in the following docking and post-processing phases.

Docking calculations were performed in the active site of the prepared 6LU7 crystal structures by using Glide (Schrödinger release 2020-1) with the Standard Precision (SP) mode[67]. In particular, the receptor grid was first generated on the coordinates of the co-crystallized PRD_002214 ligand, with a box of (10 Å × 10 Å × 10 Å) dimensions (default settings). Then, the docking protocol was validated by redocking the co-crystallized ligand into its parent crystal structure, with satisfactory results.

Approved drugs, clinical and preclinical candidates, and metabolites were first downloaded from the DrugBank database (www.drugbank.ca, accessed on March 17th, 2020), and then prepared for the structure-based calculations by using the *LigPrep* utility (Schrödinger release 2020-1)[68]. In particular, ionization states and tautomers at pH equal to 7±2 were first generated for each ligand in the screening database, and then minimized according to the OPLS3e force field. Stereoisomers were also generated for the DrugBank ligands with undefined chiralities. Afterwards, the pre-treated compounds were screened with the validated docking protocol. Finally, the predicted poses were visually inspected and the first 2000 top-scoring ligand-protein complexes were further processed with the BEAR post-docking tool[15].

The BEAR protocol consists of three steps based on molecular mechanics (MM) minimization and molecular dynamics cycles, followed by more accurate binding free energy estimation of the refined complex with the MM-PBSA and MM-GBSA methods[15]. Further details on the BEAR post-processing procedure, which was demonstrated to considerably increase the prediction performances in several virtual screening campaigns, are reported in reference 16.

A final step of visual inspection of the refined complexes and their comparison with the corresponding poses predicted by Glide, aided to a final selection of potential candidates for SARS-CoV-2 Main Protease inhibition. Analysis of data annotation and literature search returned top candidates with a potentially beneficial polypharmacology profile.

Declarations

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Contributions

LP performed all calculations. All authors participated to data analysis, compound selection, and manuscript editing. GR conceived and coordinated the study, and wrote the initial draft.

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Ethics declarations

Competing interests

The author declares no competing interests.

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Table

Table 1: Drug candidates selected from the computational repurposing campaign.

DrugBank ID	Compound Name	Scores			RANK #			PDB Codes	Primary Targets (UNIPROT ID)	Therapeutic Indications
		Glide	BEAR GB	BEAR PB	Glide	BEAR GB	BEAR PB			
Candidates to drug repurposing										
DB01232	Saquinavir	-9.8	-48.2	-20.1	47	768	1101	1HXB	HIV-1 protease (O90777)	HIV-1 Infection
DB01871	Cruz-1; EXPT02989	-9.3	-62.2	-39.7	90	206	76	1ME4	Cathepsin F (Q9UBX1); Cruzipain (P25779)	Chagas disease (Experimental)
DB02128	Cruz-2; EXPT02467	-9.4	-62.7	-40.4	82	187	65	1ME3	Cruzipain (P25779)	Chagas disease (Experimental)
DB02378	MMI-175; EXPT02196	-12.0	-82.7	-62.5	3	20	1	1XS7	β-secretase 1 (P56817)	Alzheimer's disease (Experimental)
DB02668	JE-2147; EXPT01956	-8.7	-64.3	-45.7	199	153	25	1KZK; 1MSM; 1MSN	HIV-1 protease (O90777)	HIV-1 infection (Experimental)
DB03063	EH58; EXPT01332	-12.2	-65.4	-46.4	2	129	21	1LF3	Plasmepsin-2 (P46925)	Malaria (Experimental)
DB03648	EXPT00713	-11.2	-73.2	-40.0	8	48	72	1RL4	Formylmethionine deformylase (Q8I372)	Malaria (Experimental)
DB04353	QF34; EXPT02729	-10.0	-60.9	-39.7	35	229	77	1IZH; 1IZI	HIV-1 protease (O90777)	HIV infection (Experimental)
DB04378	RS370; EXPT02746	-9.0	-50.6	-28.9	121	612	511	1LF2	Plasmepsin-2 (P46925)	Malaria (Experimental)
DB04502	WRR-204; EXPT03235	-9.0	-63.8	-33.1	118	165	257	1EWO	Cruzipain (P25779)	Chagas disease (Experimental)
DB04595	PRD_002214; N3	-9.9	-58.4	-35.8	42	292	151	6LU7	SARS-CoV-2 main protease	SARS-CoV-2 infection (experimental)
DB04692	I2	-10.2	-69.8	-49.0	25	74	11	2D2D	3C-like proteinase (P0C6X7)	SARS-CoV infection (experimental)
DB04710	N1	-9.1	-64.0	-34.3	117	159	195	1WOF; 2AMP	3C-like proteinase (P0C6X7)	SARS-CoV infection (Experimental)
DB05102	AG7088; Rupintrivir	-9.4	-64.1	-34.4	78	155	192	6KU8	Rhinovirus 3C protease (E5D8F2)	Human rhinoviral HRV infections (Experimental)
DB05645	Larazotide	-8.7	-67.1	-52.1	183	99	6		Zonulin receptor (P00738)	Coeliac disease (Experimental)
DB08732	WRR-183	-9.5	-52.1	-29.9	65	526	441	2OP9	SARS-CoV replicase polyprotein 1a (P0C6U8); SARS-CoV replicase polyprotein 1ab (P0C6X7)	SARS-CoV infection (Experimental)
DB11782	Birinapant	-9.6	-69.2	-19.9	60	82	1111	4KMP	Baculoviral IAP repeat-containing protein 3 (Q13489)[a]; Baculoviral IAP repeat-containing protein 2 (Q13490)[a]	Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukemia (CMML) treatment
DB11938	Difelikefalin	-11.1	-77.9	-34.0	9	32	217		κ-opioid receptor (P41145)	Acute Pain and Postoperative Pain treatment (Investigational)
DB12370	Ipamorelin	-10.9	-78.7	-42.4	12	30	45		Ghrelin/growth hormone secretagogue receptor (Q92847)	Gastrointestinal Dysmotility and Ileus treatment (Investigational)
Candidates to drug repurposing with beneficial polypharmacology										
DB00520	Caspofungin	-8.8	-60.8	-10.1	164	233	1581		1,3-b-glucan synthase	Antifungine

									component FKS1 (A2QLK4)	
DB03395	Enalkiren	-9.7	-67.9	-45.3	56	89	27		Renin (P00797)	Agent Acting on the Renin-Angiotensin System [b]
DB04653	Calpain inhibitor IV; $ZLLYCH_2F$	-9.7	-66.9	-42.5	52	100	44	1ZCM	Calpain-1 catalytic subunit (P07384)	Aging and aging-related diseases (Experimental)
DB04758	Ethylsulfonamide-D-Trp-Gln-p-aminobenzamidine	-10.6	-66.6	-44.1	17	106	1581	1WUN	Coagulation factor VII (P08709)	Anticoagulant (Experimental)
DB07571	Z-LY-CMK	-9.2	-53.7	-29.6	99	444	464	2FZS	ATP-dependent Clp protease proteolytic subunit (P0A6G7)	Potential antimicrobial agent (Experimental)
DB07934	BM51.1011	-8.8	-66.4	-49.3	155	111	10	1UVS	Prothrombin (P00734)	Anticoagulant (Experimental)
DB09065	Cobicistat	-11.9	-87.2	-60.5	4	12	3		Cytochromes: P450 3A4 (P08684)[a]; P450 3A5 (P20815)[a]; P450 3A43 (Q9HB55)[a]; P450 3A7 (P24462)[a]	(HIV) Infections given in combination
DB12955	Delparantag; PMX-60056	-13.1	-108.7	-48.1	1	1	14		Heparin antagonist[c]	Angioplasty, coronary artery disease, percutaneous coronary intervention (Experimental)
DB14761	Remdesivir	-8.9	-62.6	-35.8	132	189	152		Replicase Polyprotein 1ab (P0C6X7); RNA-directed RNA polymerase L (Q05318)	Ebola virus, SARS-CoV, MERS-CoV and SARS-CoV2 infections (Investigational)
Candidates to drug repurposing based on active drug metabolites										
DBMET00084	Ritonavir M7[a]	-7.6	-62.5	-49.7	935	194	9		HIV-1 protease (O90777)	HIV-1 infection[a]
DBMET01548	Saquinavir M10[a]	-10.5	-66.0	-38.9	20	119	90		HIV-1 protease (O90777)	HIV-1 infection[a]
DBMET01549	Saquinavir M2	-10.5	-67.7	-40.2	19	92	68		HIV-1 protease (O90777)	HIV-1 infection[a]
DBMET01550	Saquinavir M2	-10.5	-62.6	-31.8	18	192	320		HIV-1 protease (O90777)	HIV-1 infection[a]
DBMET02115	Ezetimibe glucuronide	-8.7	-39.6	-13.5	184	1336	1458		Niemann-Pick C1-like protein 1 (Q9UHC9)	Cholesterol lowering compound

Notes:

Compound DB04595, which is a SARS-CoV-2 M^{pro} inhibitor emerged in a previous computational screening, is shown in red.

For metabolites, therapeutic targets and indications of their parent drugs are shown.

Glide, BEAR GB and BEAR PB scores are reported in kcal/mol.

Bibliographic references of the crystal structures are reported in Table S1 of the Supplementary information.

[a] Data retrieved from: ChEMBL, accessed on April 20th, 2020.

[b] Data retrieved from: https://www.drugs.com, accessed on April 20th, 2020.

[c] Data retrieved from: KEGG, accessed on April 23rd, 2020

Figures

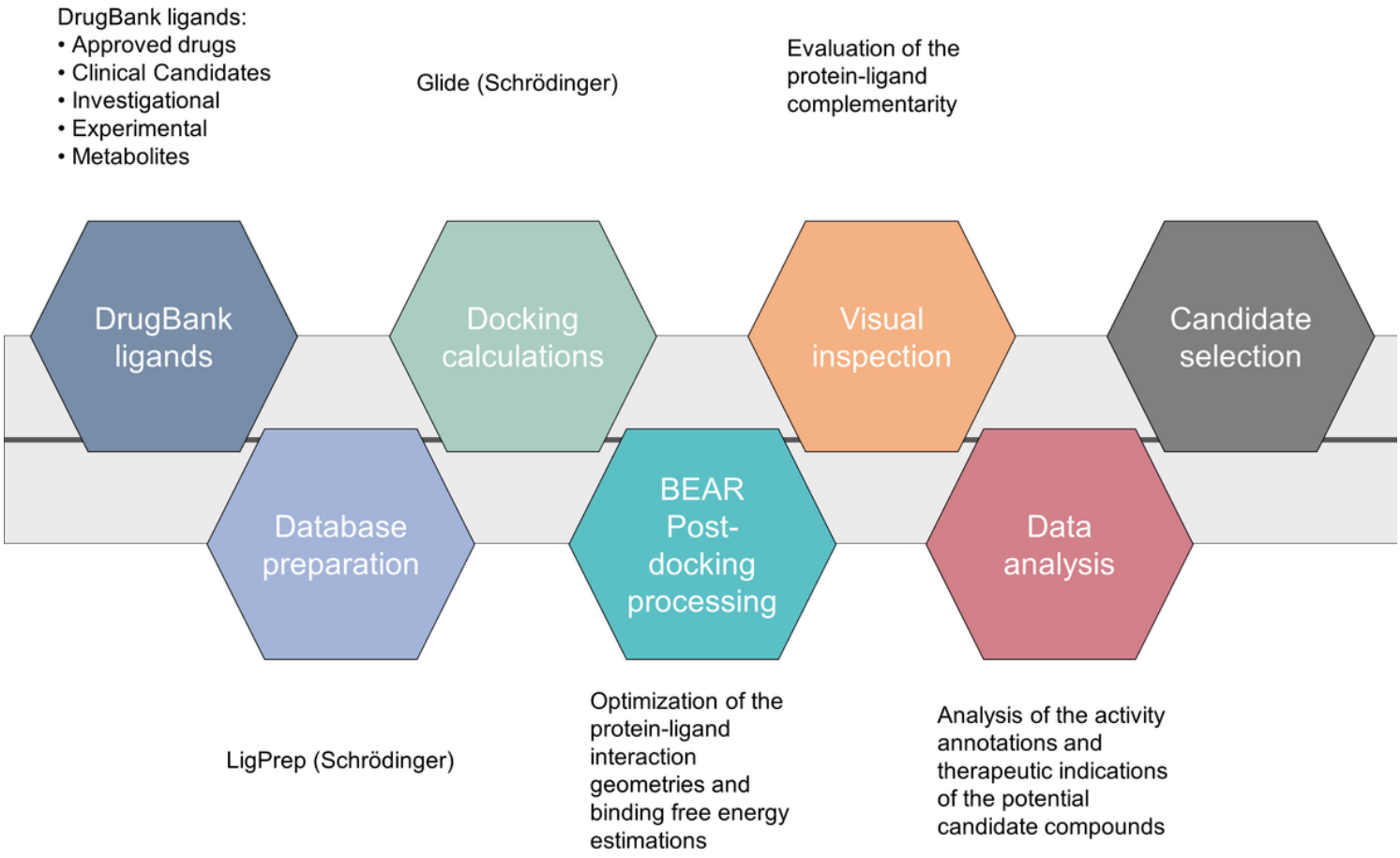

Figure 1

Computational workflow for the repurposing screening of SARS-CoV-2 3C-like protease inhibitors.

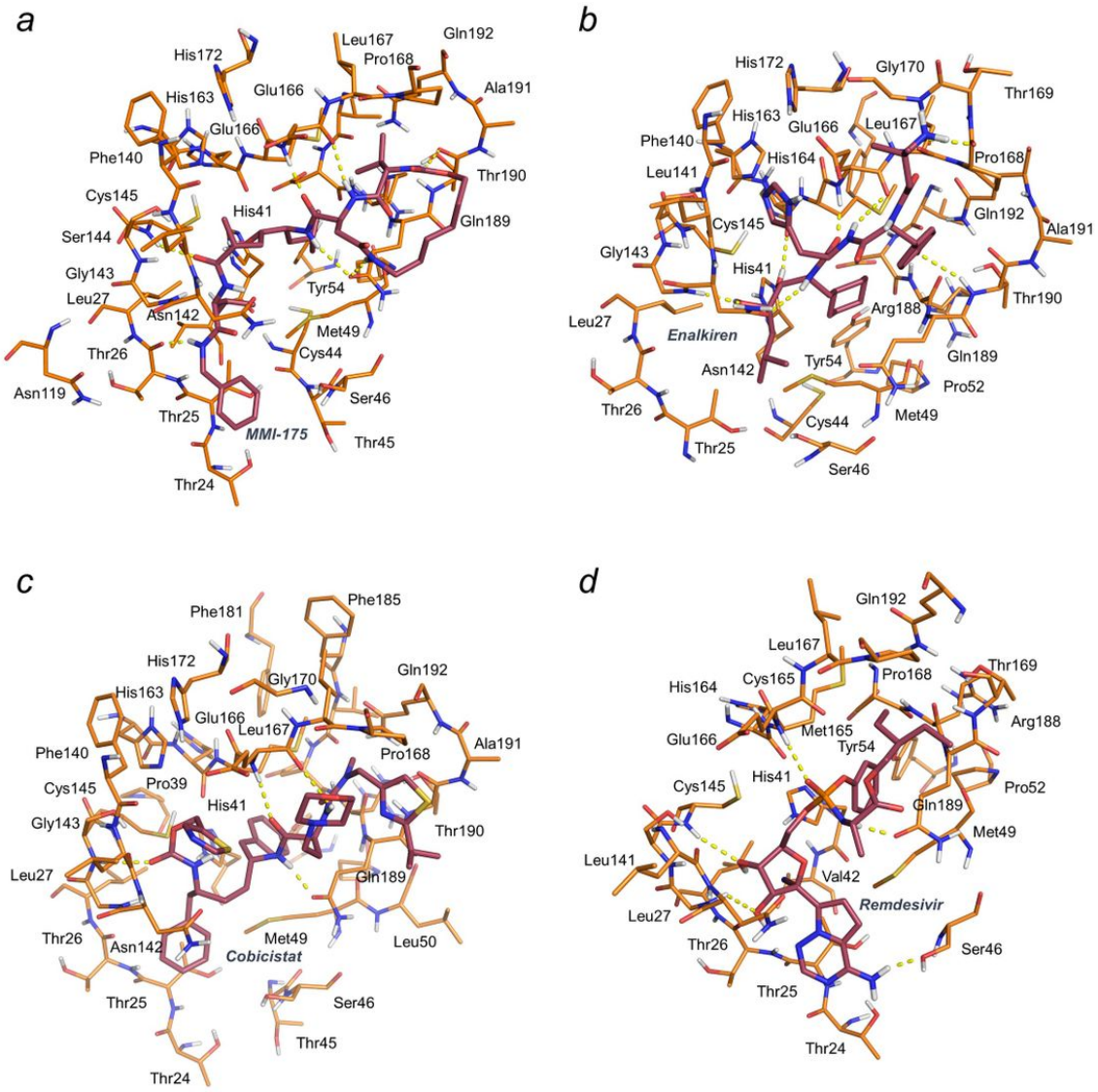

Figure 2

Binding mode of MMI-175 (panel a), Enalkiren (panel b), Cobicistat (panel c) and Remdesivir (panel d) into the 6LU7 crystal structure. The SARS-CoV-2 Mpro binding site residues and the potential repurposed compounds are represented in orange and raspberry sticks, respectively. The image was created with PyMol (The PyMOL Molecular Graphics System, Version 2.1.1, Schrödinger, LLC).

Supplementary Files

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