Subject Section

Potential covalent drugs targeting the main protease of the SARS-CoV-2 coronavirus

Sen Liu[1,2,*], Qiang Zheng[1,2] and Zhiying Wang[1,2]

[1]National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Key Laboratory of Industrial Fermentation (Ministry of Education), Hubei University of Technology, Wuhan 430068, China, [2]Institute of Biomedical and Pharmaceutical Sciences, Hubei Key Laboratory of Industrial Microbiology, Hubei University of Technology, Wuhan, 430068, China.

*To whom correspondence should be addressed.

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Abstract

Motivation: Since December 2019, the newly identified coronavirus SARS-CoV-2 has caused a massive health crisis worldwide and resulted in over 70,000 COVID-19 infections so far. Clinical drugs targeting SARS-CoV-2 are urgently needed to decrease the high fatality rate of confirmed COVID-19 patients. Traditional de novo drug discovery needs more than 10 years, so drug repurposing seems the best option currently to find potential drugs for treating COVID-19.

Results: Compared with traditional non-covalent drugs, covalent drugs have attracted escalating attention recent years due to their advantages in potential specificity upon careful design, efficiency, and patient burden. We recently developed a computational protocol named as SCAR for discovering covalent drugs. In this work, we used the SCAR protocol to identify possible covalent drugs (approved or clinically tested) targeting the main protease (3CLpro) of SARS-CoV-2. We identified 11 potential hits, among which at least 6 hits were exclusively enriched by the SCAR protocol. Since the preclinical or clinical information of these identified drugs is already available, they might be ready for being clinically tested in the treatment of COVID-19.

Contact: senliu.ctgu@gmail.com

1 Introduction

Starting December 2019, an outbreak of pneumonia of unknown cause took place in Wuhan, Hubei province of China (Chaolin Huang *et al.*, 2020). In a short time, Chinese authorities rapidly isolated and characterized a novel coronavirus closely related to the SARS-CoV that caused the outbreak of a severe acute respiratory syndrome 18 years ago in China (Zhou *et al.*, 2020). The newly identified coronavirus was initially represented by 2019-nCoV, but formally named as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) by the International Committee on Taxonomy of Viruses (ICTV) on February 12th, 2020. Meanwhile, the disease caused by this virus was named as COVID-19 by the World Health Organization (WHO). Although China has adopted unprecedented policies to control the spread of the virus including temporarily "shutting down" the Wuhan City on January 23rd, 2020, SARS-CoV-2 has resulted in over 70,000 COVID-19 patients and more than 2,000 fatalities worldwide by February 20th, 2020. With an estimated case fatality rate of 2%-3% and the growing patient numbers, SARS-CoV-2 poses a serious health threat to the whole world (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/).

Unfortunately, specific clinical treatments for the rapidly escalating international crisis caused by SARS-CoV-2 are very limited, which is one of the reasons of the high mortality rate. Due to the deficiency of targeted drugs, the current clinical treatment of COVID-19 focuses on supportive care and symptom relief. Remdesivir, an experimental drug developed to treat the Middle East respiratory syndrome coronavirus (MERS-CoV), has been reported to be effective in treating several COVID-19 cases, but a systematic clinical trial of this drug is still ongoing in Wuhan, China (ClinicalTrials.gov Identifier: NCT04257656). Similarly, chloroquine, lopinavir/ritonavir, and many other drugs have also been reported to be potentially effective, but supportive clinical data are not available for all (Maxmen, 2020). Therefore, it is of great value to identify more potential drugs targeting SARS-CoV-2 to save COVID-19 patients.

In the traditional process, the development of a drug will need over 15 years from target identification, target validation, hit discovery, lead optimization, and preclinical and clinical trials (Kaitin, 2010). Therefore, it is virtually not possible to develop de novo drugs in the time frame needed to impact the current SARS-CoV-2 crisis. Hence, the most feasible approach is to find potential cures from clinical drugs by drug repurposing (also known as drug repositioning, reprofiling, redirecting, or rediscovering). Drug repurposing can rapidly expand target disease indications of an existing drug while saving time and money, since the data for human pharmacokinetics, safety and the preclinical results are already available (Cha *et al.*, 2018). Successful examples of drug repurposing include the use of sildenafil in treating erectile dysfunction and the anti-cancer uses of thalidomide (Pushpakom *et al.*, 2019).

Previously, we described a computer-aided drug discovery protocol named as SCAR (steric-clashes alleviating receptors) (Ai *et al.*, 2016) for the screening of covalent inhibitors of target proteins enlightened by the in silico protein design strategy (Liao *et al.*, 2015). Realizing the potential use of SCAR in discovering both covalent and non-covalent inhibitors, we recently demonstrated that SCAR is also quite efficient in drug repurposing (Y. Zhang *et al.*, 2020). Compared with non-covalent drugs, covalent drugs have the following advantages (Mah *et al.*, 2014): (i) covalent drugs have better biochemical efficiency since they are more competitive than many non-covalent endogenous substrates and co-factors; (ii) covalent drugs cause lower patient burden and delay the emergence of drug resistance due to lower and less frequent dosing; (iii) covalent drugs might have better target specificity by reacting with a non-conserved nucleophilic amino acid with careful designs (Cuesta *et al.*, 2020). Therefore, recent years have witnessed the resurgence of the discovery of covalent drugs. As a result, we set out to use SCAR to identify possible covalent drugs targeting SARS-CoV-2 by drug repurposing in this work.

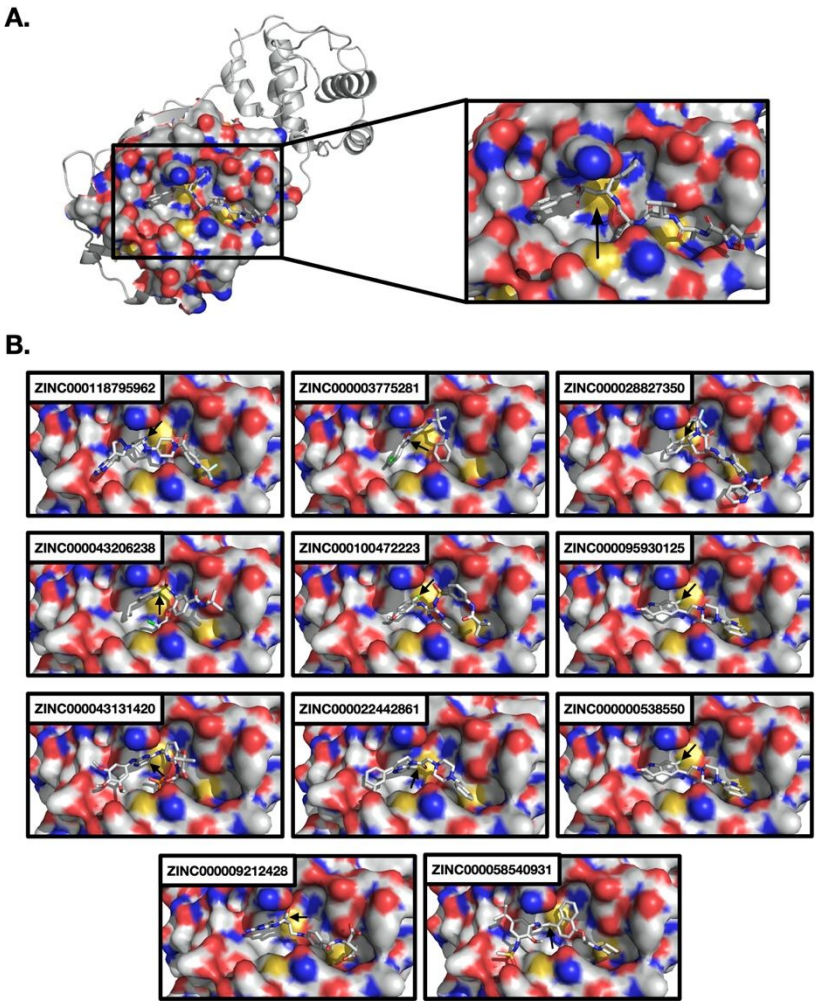

Fig. 1. The putative binding poses of the candidate hits from the SCAR protocol. (A) The structure of the substrate binding pocket of the SARS-CoV-2 3CLpro (PDB ID: 6LU7). The protein pocket is shown in surface and the inhibitor in sticks. The covalent bond is indicated by the arrow. (B) The binding poses of the identified hits. The putative reactive atoms in the drugs are indicated by black arrows. The protein without the SCAR mutation (C145G) was used for representation, and the yellow surface close to the arrow represents Cys145. The steric conflicts between the reactive atoms and Cys145 are expected, which can be eliminated by the covalent bonding between the drug and Cys145.

Table 1. The potential drugs that might be repurposed as covalent inhibitors of the SARS-CoV-2 3CLpro from the SCAR strategy. Docking score and conformation rank are listed for the identified pose. Atom distance is the distance between the putative reactive atom of the drug and Cys145-SG. SCAR enriching score is calculated as the top docking score of the drug docked to the wild-type 3CLpro minus the listed docking score.

ZINC ID	Atom distance (Å)	Docking Score	Pose rank	Warhead	Drug name	CAS Number	Drugbank ID	Approved or Investigational treatment	SCAR enriching score
ZINC000118795962	2.0	-9.0	7	-CN (Furber *et al.*, 2014)	Itacitinib	1334298-90-6	DB12154	Melanoma, endometrial cancer, B-cell malignancies, etc.	-0.6
ZINC000003775281	2.5	-8.9	1	-CN	Oberadilol	114856-44-9	Not available	Heart failure; hypertension	0.5
ZINC000028827350	1.2	-8.8	2	-Ph-F (Shannon *et al.*, 2014)	Telcagepant	781649-09-0	DB12228	Migraine	0.1
ZINC000043206238	1.6	-8.7	1	-Ph-Cl (Shannon *et al.*, 2014)	Vidupiprant	1169483-24-2	DB12272	Asthma	0.2
ZINC000000472223	1.3	-8.5	6	-Ph-Cl	Pilaralisib	934526-89-3	DB11772	Cancer	-0.4
ZINC000095930125	1.3	-8.4	1	-Ph-F	Poziotinib	1092364-38-9	DB12114	Breast cancer; adenocarcinoma of lung	0.8
ZINC000043131420	1.2	-8.2	4	-Ph-F	Fostamatinib	901119-35-5	DB12010	Rheumatoid arthritis; Immune Thrombocytopenic Purpura (ITP)	0.1
ZINC000022442861	0.6	-8.1	9	-CN	CL-275838	115931-65-2	Not available	Cognition enhancer	-0.9
ZINC000000538550	1.1	-8.0	1	-Ph-Cl	Ziprasidone	146939-27-7	DB00246	Schizophrenia; bipolar disorder	0.0
ZINC000009212428	2.2	-8.0	1	-C=O (L. Zhang *et al.*, 2016)	Leucal/Folinic acid	58-05-9	DB00650	Toxic effects of methotrexate and pyrimethamine	0.6
ZINC000058540931	2.7	-8.0	1	-N-CO-CO- (Barrett *et al.*, 2004)	ITX5061	1252679-52-9	Not available	Rheumatoid arthritis; hepatitis C Infection	-0.1

2 Methods

2.1 Preparation of the in silico compound library

The structure files of the compounds were downloaded as mol2 files from the ZINC15 database (http://zinc15.docking.org). As described by ZINC15, the 3D conformations were protonated at physiological pH and biologically relevant tautomers were generated for each molecule (Sterling and Irwin, 2015). The "in-trials" catalog (2019-04-22 version) was downloaded, which contains 5811 approved or investigational (clinically tested but not approved) drugs worldwide. MGLTools (version 1.5.6) was used to generate the PDBQT files from the mol2 files for docking.

2.2 Structure optimization of the protein

The SARS-CoV-2 3CLpro structure was downloaded from PDB (PDB ID: 6LU7). This is a complex structure of the SARS-CoV-2 3CLpro and an inhibitor covalently bonding to Cys145. The structure was energetically minimized in Rosetta (Leaver-Fay *et al.*, 2011) by applying harmonic distance and angle constraints on the bonding atoms in the inhibitor and Cys145. The backbone of the protein was fixed during the minimization. The lowest score model was chosen from 1000 models. For SCAR docking, the Cys145 was mutated to Gly. MGLTools (version 1.5.6) was used to generate the PDBQT file for docking.

2.3 In silico docking and analysis

The computational docking process was similar as previously described (Ai *et al.*, 2016; Liao *et al.*, 2015). Briefly, AutoDock Vina (Trott and Olson, 2010) (version 1.1.2) was used to dock the small molecules to the substrate binding pocket of the SARS-CoV-2 3CLpro. The docked poses were then manually evaluated by docking score, ranking, and the distance between the reactive atom and the sulfur atom of Cys145 in the original structure as previously described (Ai *et al.*, 2016).

3 Results

In SARS-CoV, the 3C-like proteinase (3CLpro) is the main protease, which cleaves the large replicase polyprotein 1a (pp1a) and pp1ab to produce non-structural proteins (NSPs) for the transcription and replication of the virus (Zumla *et al.*, 2016). Therefore, 3CLpro is a key drug target in inhibiting SARS-CoV. The recent data showed that the

SARS-CoV-2 3CLpro is highly similar with the SARS-CoV 3CLpro both in sequence and structure (PDB ID: 6LU7). Previous studies demonstrated that Cys145 is a key residue in the active site of 3CLpro (Changkang Huang *et al.*, 2004), which makes this residue be an attractive target for covalent bonding of covalent 3CLpro inhibitors. Cysteine is also a most popular target of covalent inhibitors because its high intrinsic reactivity at physiological pH permits the use of relatively unreactive electrophiles (Cuesta *et al.*, 2020). Meanwhile, targeting cysteine renders the high selectivity of covalent inhibitors due to the low prevalence of cysteine in the proteome (Cuesta *et al.*, 2020).

To repurpose potential covalent drugs targeting the SARS-CoV-2 3CLpro, we firstly optimized the X-ray complex structure of this protein with its inhibitor using the macromolecule modeling suite Rosetta (Leaver-Fay *et al.*, 2011) (Figure 1A). As demonstrated in our previous work, this structural optimization could benefit the docking result (Ai *et al.*, 2016). Next, we mutated Cys145 to Gly as required by the SCAR protocol to prepare the docking target (Ai *et al.*, 2016). The "in-trials" dataset (10083 compounds/conformations) obtained from ZINC15 was filtered by the generally used warhead groups targeting cysteine (5010 compounds/conformations) before it was used in the docking process (represented as "SCAR-dock"), among which 1253 were left for manual checking after score filtering (-8.0).

As listed in Table 1, we identified 11 potential covalent inhibitors of the 3CLpro of SARS-CoV-2 following the SCAR protocol described previously (Ai *et al.*, 2016). These hits contain five different covalent warhead groups suitable for targeting cysteine, which represents diverse structural options. As shown in Figure 1B, the putative covalent poses of the identified hits fit in the binding pocket reasonably. In each drug, the distance between the putative nucleophilic atom and the SG atom of Cys145 is shown in Table 1. In 6LU7 (PDB ID), the distance between the covalent carbon atom of the inhibitor and the SG atom of Cys145 is 1.8 Å. Therefore, the proximity between these two atoms indicates high potential for covalent bonding between the drug and Cys145.

To investigate how the SCAR strategy helped enriching these hits, we re-docked these compounds to the SARS-CoV-2 3CLpro without mutating Cys145 (represented as "regular-dock"). For quantitative comparison, we took the lowest score of each compound in the regular-dock results. Then we compared the difference between this lowest score with the score of the same compound in the SCAR-dock results (represented as "SCAR enriching score"). A note is that we used the score of the pose oriented for covalent bonding in SCAR-dock, which is the lowest score only if the rank of the conformation is 1 (Table 1). As shown in Table 1, for 6 out of these 11 compounds (54.5%), the SCAR-dock protocol generated lower scores (higher affinities, corresponding to positive SCAR enriching scores). This result might indicate that the SCAR protocol can generate better binding poses than the regular docking protocol if the compounds can covalently bind to Cys145, although experimental validations will be necessary. Base on the parameters in Table 1, we suggest the following ones might have higher priorities: Telcagepant, Vidupiprant, Poziotinib, and Fostamatinib.

In summary, we have identified eleven approved or investigational drugs that might be repurposed to covalently inhibit the 3CLpro of SARS-CoV-2. According to our previous studies, the hits from the SCAR protocol could have different reactive (bonding) activities. One reason is that the SCAR protocol does not consider the reactive activity of the warhead in a compound. The other reason is that the reactive activity of a warhead is affected by the attached scaffold, and the prediction of a warhead's reactive activity is still a profoundly challenging issue. Considering the worldwide health risk posed by SARS-CoV-2, although wet experiments are needed to validate our computational hits, we hope our work will provide more options for the rapid identification of targeted drugs to inhibit SARS-CoV-2 and save COVID-19 patients in time.

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Conflict of Interest: none declared.

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