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Discovery of anti-SARS-CoV-2 agents from 38 Chinese patent drugs toward respiratory diseases *via* docking screening

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Abstract

The 2019 novel coronavirus (2019-nCoV) causes novel coronavirus pneumonia (NCP). Given that approved drug repurposing becomes a common strategy to quickly find antiviral treatments, a collection of FDA-approved drugs can be powerful resources for new anti-NCP indication discoveries. In addition to synthetic compounds, Chinese Patent Drugs (CPD), also play a key role in the treatment of virus related infections diseases in China. Here we compiled major components from 38 CPDs that are commonly used in respiratory diseases and docked them against two drug targets, ACE2 receptor and viral main protease (M^{pro}). According to our docking screening, 10 antiviral components, including hesperidin, saikosaponin A, rutin, corosolic acid, verbascoside, baicalin, glycyrrhizin, mulberroside A, cynaroside, and bilirubin, can directly bind to both host cell target ACE2 receptor and viral target M^{pro}. From a combination of the docking results, the natural abundance of the substances, and botanical knowledge, we proposed that artemisinin, rutin, glycyrrhizin, cholic acid, hyodeoxycholic acid, puerarin, oleanic acid, andrographolide, matrine, codeine, morphine, chlorogenic acid, and baicalin (or Yinhuang Injection containing chlorogenic acid and baicalin) might be of value for clinical trials during a 2019-nCov outbreak. In addition, the result found that most of the top 10 compounds show inhibited M^{pro}/$3CL^{pro}$ activity.

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Introduction

The 2019 novel coronavirus (2019-nCov), named as the Wuhan coronavirus [the pneumonia caused by it is now named as novel coronavirus pneumonia (NCP)], is a positive-sense, single-strand RNA coronavirus[1]. To date, global infections of 2019-nCov surge past 40,000 (WHO website). Given that drug repurposing is the common strategy to search antiviral treatments, several approved drugs were reported to benefit patients[2]. Besides synthetic compounds, natural products, especially Chinese Patent Drug (CPD), also play a key role in the treatment of virus related infectious diseases in China. We emphasize the antiviral qualities of CPDs despite the possibility that their processes are linked to immune control. In this study, we assembled major components from 38 CPDs that are frequently used in respiratory diseases and docked them against two drug targets, ACE2 receptor and viral M^{pro}.

Like severe acute respiratory syndrome-related coronavirus (SARS-CoV), the 2019-nCoV attaches to host cells through S protein and angiotensin converting enzyme 2 (ACE2) receptor interaction[3]. The catalytic inhibitor of ACE2 receptor is likely to induce a conformational change of ACE2, therefore blocking the interaction between S protein and ACE2 receptor[4]. S protein of 2019-nCoV is not currently available but the structure of ACE2 receptor is well-known[5]. Thus ACE2 receptor was selected to quickly identify entry inhibitors of 2019-nCoV using marketed CPDs-derived natural products.

In addition to entry inhibitors, the replication inhibitors are also good strategies for antiviral drug discovery and development[6]. Given that 2019-nCoV is a (+)SS RNA virus, its M^{pro} is likely to be required to mediate viral replication and transcription through extensive cleavage of two replicase polyproteins. Therefore inhibition of viral M^{pro} might block virus replication[7]. The researchers reported the crystal structure of M^{pro} of 2019-nCoV (PDB: 6LU7) and several drug repurposing docking screening studies were reported[5,8]. To date, one of the best-characterized drug targets among coronaviruses is the M^{pro} and many M^{pro} inhibitors were discovered[9–20]. Here, in order to search for antiviral replication agents, we docked a natural product database to the M^{pro}.

Due to the limited time and lack of the available 2019-nCoV in hand, it is impossible to develop novel compounds against 2019-nCoV by biological screening. We here used docking screening to identify natural products from marketed CPDs that inhibit both virus entry and replication, therefore providing a potential prevention/treatment alternative against 2019-nCoV.

Materials and methods

Molecular docking

The major components of each herb in the selected 38 CPDs were collected as the ligands, and all the ligands were in PDBQT format. The protein model 1R4L was selected as ACE2 receptor

docking model while 6LU7 was selected as M^{pro} docking model. Both PDB files of protein models were fetched from Protein Data Bank. The docking screenings were conducted by using AutoDock Vina v.1.0.2. The docking parameters for AutoDock Vina were kept at their default values. The grid box was 25 Å × 25 Å × 25Å, encompassing the catalytic pocket. The binding modes were clustered through the root mean square deviation (RMSD) among the Cartesian coordinates of the ligand atoms.

Ligands preparation for docking screening

For ligand library establishment, the SMILE format of phytochemicals was compiled from Pubchem. The SMILES format of compounds was converted to PDB format by CORINA online service (www.molecular-networks.com/online_demos/corina_demo). The PDB format of compounds was then converted to PDBQT format by AutoDock Tools 1.5.6 (The Scripps Research Institute, CA, USA).

Target proteins preparation for docking screening

For receptor preparation, crystal structures were obtained from the Protein Data Bank. Both ligands and water molecules in target proteins were removed by Chimera 1.7mac (UCSF Resource for Biocomputing, Visualization, and Informatics, CA, USA). The hydrogen and Kollman Charges were then added to the target protein by AutoDock Tools 1.5.6 (The Scripps Research Institute, CA, USA). The atoms of target protein were assigned as AD4 type, and the modified protein was converted to PDBQT format for docking screening.

Docking parameters validation

The docking parameters for AutoDock Vina were kept to their default values. The grid box was 25 Å × 25 Å × 25Å, encompassing the inhibitor binding pocket. The docking results were ranked by the binding free energy. We extracted the inhibitors from original protein models for parameter validation. Our docking simulation showed that the predicted conformations of inhibitors are close to the experimental conformations of inhibitors. Furthermore, the inhibitors exhibited high binding scores.

Chemicals

Hesperidin, saikosaponin A, rutin, corosolic acid, verbascoside, baicalin, glycyrrhizin, mulberroside A, cynaroside, and bilirubin were purchased by Shanghai Bidepharmatech Co.,Ltd (Shanghai, China).

M^{pro}/$3CL^{pro}$ inhibitory assay

In vitro M^{pro}/$3CL^{pro}$ activity assay were performed by using M^{pro}/$3CL^{pro}$ Inhibitor Screening Kit (Beyotime, Cat No. P0312S, China). Briefly, 2019-nCoV M^{pro}/$3CL^{pro}$ was diluted by Assay Buffer, then pre-incubated with compounds for 10 min at 37 °C, then substrate was added for another 5 min incubation at 37 °C. The optical density (OD) values were thereafter measured with the excitation wavelength at 360 nm and the emission wavelength at 460 nm respectively by Microplate Reader (BioTek, Synergy 2). The data were analyzed using GraphPad Prism5 (GraphPad Software Inc.). Ebselen was positive control. All the tests were performed in triplicate.

Statistical analysis

fThe statistical data were obtained from biological triplicates. Statistical analysis was performed by t by ANOVA for multiple groups. * $p < 0.05$ was considered significant difference; ** $p < 0.01$ was considered very significant difference.

Results and discussion

A total of 38 marketed CPDs (Table 1) containing 93 herbs used for the treatment of respiratory diseases were selected. Totally we docked 95 components (Supplemental Table S1 & S2) and the top 10 hits are summarized in Table 2. All of them provide good binding affinities against both two targets. The key residues for each ligand binding were also summarized in Table 3, Fig. 1 and Supplemental Fig. S1[9].

Analysis of the predicted binding energy results from Table 2, it was found that the top 10 antiviral components are hesperidin, saikosaponin A, rutin, corosolic acid, verbascoside, baicalin, glycyrrhizin, mulberroside A, cynaroside, and bilirubin, and their binding sites toward 6LU7 and 1R4L are listed in Table 3 & Supplemental Table S1. A close analysis found that 19 compounds directly bind to ACE2 receptor with high affinities (docking score < −10 kcal/mol), these compounds are hesperidin, saikosaponin A, mulberroside A, rutin, bilirubin, verbascoside, vincetoxicoside B, baicalin, prim-*O*-glucosylcimifugin, corosolic acid, cynaroside, orientin, corynoline, astragaloside A, protostemonine, ilexgenin A, amygdalin, paeoniflorin, and ursolic acid (Supplemental Table S1). Whereas, in M^{pro}

Table 1. Commercial names of 38 Chinese patent drugs (CPDs).

No.	CPDs
1	Fengre Ganmao Granules
2	Xiaochaihu Granules
3	Qingkailing Capsules
4	Jinlianhua Capsules
5	Zhongganling Capsules
6	Lianhua Qingwen Capsules/Granules
7	Lanqin Oral Solution
8	Qingwen Jiedu Tablets
9	Fangfeng Tongsheng Pills
10	Shuanghuanglian Oral Solution
11	Huoxiang Zhengqi Oral Solution
12	Huoxiang Zhengqi Capsules
13	Maxing Zhike Syrup
14	Choulingdan Oral Solution
15	Erding Capsules
16	Zhiganjia Granules
17	Kanggan Granules
18	Kangbingdu Granules
19	Kangbingdu Oral Emulsion
20	Kangbingdu Capsules
21	Fufang Banlangen Granules
22	Ganmao Shufeng Capsules/Granules
23	Ganmao Qingre Granules
24	Fufang Jinyinhua Granules
25	Yinqiao Jiedu Pills/Granules
26	Vitamin C Yinqiao Tablets
27	Fufang Yinqiao Anfen Capsules
28	Xiasangju Granules
29	Vitamin C Effervescent Tablets
30	Xiaoer Ganmao Granules
31	Banlangen Granules
32	Qingkailing Oral Solution
33	Yinqiao Jiedu Granules
34	Fufang Yinqiao Anfen Vitamin C Tablets
35	Ganmao Soft Capsules
36	Fenghan Ganmao Granules
37	Qiangli Pipa Syrup
38	Fufang Anwanan Tablets

Table 2. Natural products from CPDs docking results.

Ligand	Docking score (kcal/mol)		
	6LU7	1R4L	SUM
Hesperidin	−8.5	−11.4	−19.9
Saikosaponin A	−8.8	−11	−19.8
Rutin	−8.9	−10.7	−19.6
Corosolic acid	−8.8	−10.2	−19
Verbascoside	−8.4	−10.6	−19
Baicalin	−8.4	−10.5	−18.9
Glycyrrhizin	−8.9	−9.9	−18.8
Mulberroside A	−7.7	−11	−18.7
Cynaroside	−8.4	−10.2	−18.6
Bilirubin	−7.8	−10.7	−18.5

docking screening, 12 phytochemicals, rutin, glycyrrhizin, dipsacoside B, saikosaponin A, corosolic acid, puerarin, morusin, hesperidin, polyphyllin I, verbascoside, baicalin, and cynaroside have been identified as potential M^{pro} inhibitors (docking score ≤ –8.4 kcal/mol), indicating their potential for 2019-nCoV. Notably, artemisinin, berberine, rutin, glycyrrhizin, chlorogenic acid, baicalin, cholic acid, hyodeoxycholic acid, puerarin, oleanic acid, andrographolide, catalpol, matrine, codeine, morphine, caffeic acid, α-asarone, α-pinene, and taurine are commercially available with good supply (already marketed drugs). In addition, a combination of their docking results, natural abundance, and traditional knowledge from their source herbs allows us to recommend artemisinin, rutin,

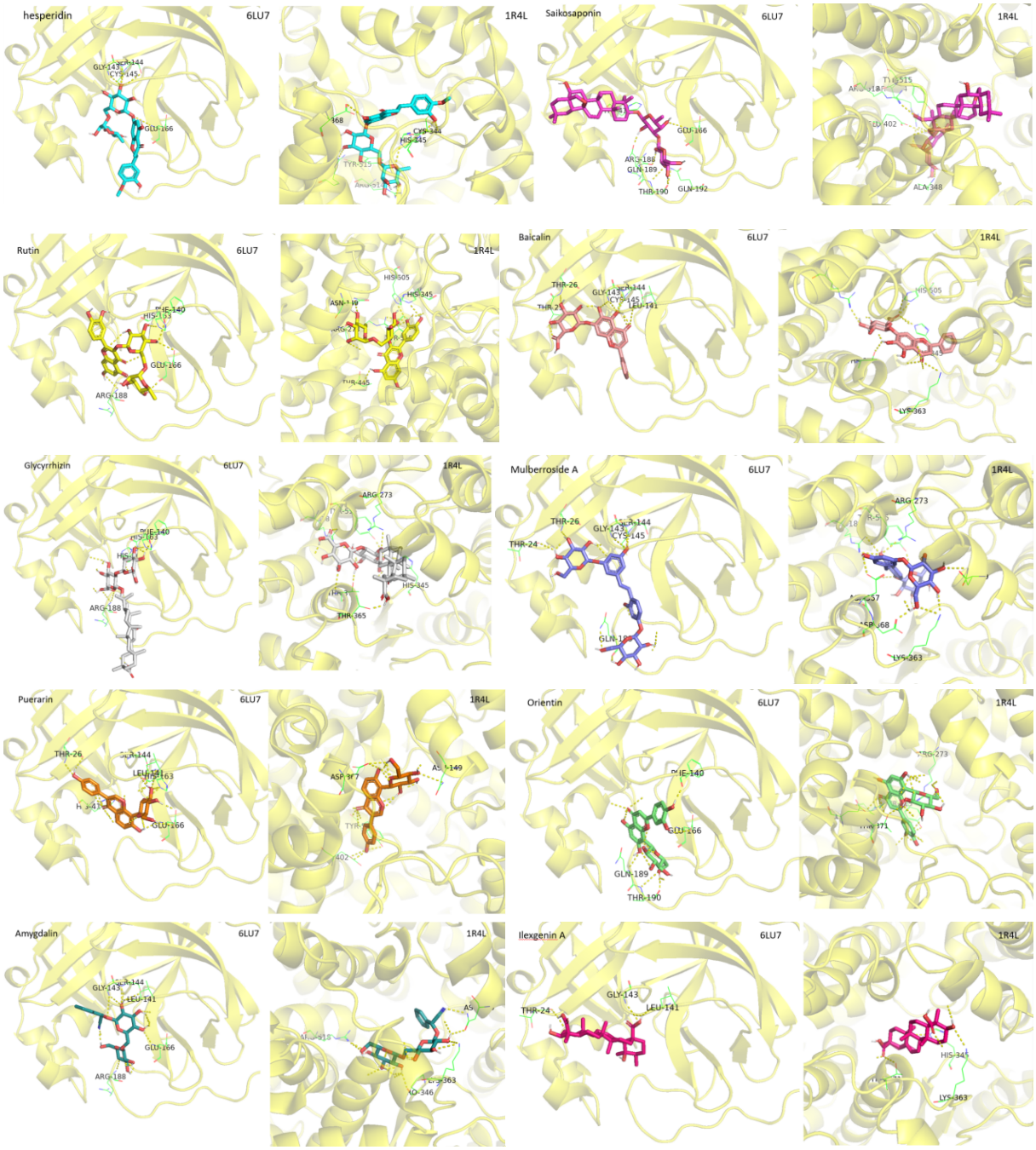

Fig. 1 The docking diagrams for potential inhibitor binding.

Table 3. Key residues for potential inhibitor binding.

Ligand	Key residues	
	6LU7	1R4L
Hesperidin	Gly143, Ser144, Cys145, Glu166	Cys344, His345, Asp368, Arg514, Tyr515, Arg518
Saikosaponin A	His41, Glu166, Arg188, Gln189, Thr190, Gln192	Ala348, Glu402, Arg514, Tyr515, Arg518
Rutin	His163, Phe140, Glu166, Arg188	Asn149, Arg273, His345, Thr445, His505, Tyr515
Corosolic acid	Gly143, Ser144, Cys145	Lys363, Thr371
Verbascoside	Phe140, Gly143, Glu166, Thr190, Gln192	Ser128, Glu145, Asn277, Cys344, His345, Arg518
Baicalin	Thr25, Thr26, Leu141, Gly143, Ser144, Cys145	His345, Lys363, Thr371, His505, Arg518
Glycyrrhizin	Phe140, His163, His164, Arg188	Arg273, His345, Thr365, Thr371, Tyr515, Arg518
Mulberroside A	Thr24, Thr26, Gly143, Ser144, Cys145, Gln189	Asn149, Arg273, Lys363, Asp367, Asp368, Tyr515, Arg518
Cynaroside	Thr24, Thr25, Thr26, Gly143	Asn149, Pro346, Lys363, Asp368
Bilirubin	Leu141, Ser144, His163, Gln189	Thr371, Glu406, Tyr515

glycyrrhizin, chlorogenic acid, baicalin, cholic acid, hyodeoxycholic acid, puerarin, oleanic acid, andrographolide, matrine, codeine, and morphine for clinical trials during a 2019-nCoV outbreak. Yinhuang Injection, a marketed drug in China, might be also worth recommendation because it is mainly composed of chlorogenic acid and baicalin. In addition, the results of Supplemental Table S2, in combination with the literature data, indicate the natural sources of these active compounds with relatively high content. Basically, around 34 compounds are present in natural sources at more than 1% (g/g), which are, respectively, hesperidin, baicalin, glycyrrhizin, puerarin, amygdalin, paeoniflorin, berberine, arctiin, forsythiaside A, chlorogenic acid, geniposide, tectoridin, timosaponin BII, dryocrassin, oleanic acid, genistein, trisalbaspidin ABA, daidzein, andrographolide, rosmarinic acid, quercetin (source plant: *Sophorae Flos*), curcumin (source plant: *Curcumae Longae Rhizoma*), dipsacoside B (source plant: *Lonicerae Dasystylae Flos*), rutin (source plant: *Potentilla chinensis*), and harpagide (source plant: *Ajuga pantantha*). This natural abundance information in combination with the docking results and the medicinal values of the source herbs suggests that the plants or herbs or their extracts with the above enriched active compounds might be valuable for fighting against 2019-nCoV. Although the content of magnolol, lobetyolin, pulegone, citrulline, L-menthol, 6-gingerol, catalpol, caffeic acid, and *trans*-cinnamaldehyde is also more than 1%, it might be not from either their docking resluts or botanical knowledge (Supplemental Table S2). Despite the fact that the other herbs or CPDs are not found to be active toward 2019-nCoV, this doesn't mean that they are not useful for NCP because only limited compounds in herbs were selected which doesn't exclude the fact that more compounds or their analogues in herbs of CPDs are active. In addition, the principles of formulating Chinese herbal prescriptions, include eliminating evil and strengthening the body resistance, therefore, we couldn't exclude that these CPDs do work against NCP *via* regulating the immune system.

The top 10 compounds of molecular docking were tested *in vitro* M^{pro}/$3CL^{pro}$ activity by using M^{pro}/$3CL^{pro}$ Inhibitor Screening Kit (Fig. 2)[21]. The results showed that except for mulberroside A, the remaining nine compounds had potential activity at 40 μM concentration. To some extent, the accuracy of molecular docking is verified, but these studies are superficial, and more in-depth studies are needed to prove the therapeutic potential of these compounds.

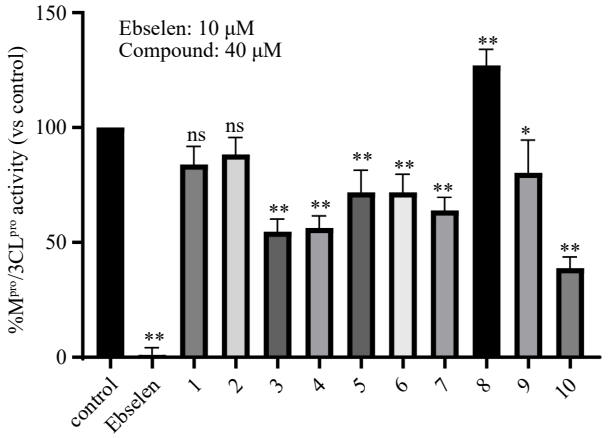

Fig. 2 Inhibitory effects of the top 10 antiviral components from AGE against SARS-CoV-2 M^{pro}. (1 = Hesperidin, 2 = saikosaponin A, 3 = rutin, 4 = corosolic acid, 5 = verbascoside, 6 = baicalin, 7 = glycyrrhizin, 8 = mulberroside A, 9 = cynaroside, and 10 = bilirubin).

Conclusions

We analyzed 38 CPDs and selected representative pharmacodynamic substances in each CPD as the target natural compounds. The 95 natural compounds by docking screening showed that some of the structures had good binding ability for protein model 1R4L and 6LU7, which partly explains the effectiveness of these substances against SARS-CoV-2. In addition, experimental verification found that the most of the top 10 compounds are shown to inhibit M^{pro}/$3CL^{pro}$ activity. This findings provide a basis and guidance for traditional Chinese medicine to fight against the SARS-CoV-2 and find effective natural compounds from them.

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Conflict of interest

The authors declare that they have no conflict of interest. Yong-Xian Cheng is the Editorial Board members of *Medicinal*

Plant Biology. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and his research groups.

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