Repurposing the natural compound for antiviral during an epidemic

-a case study on the drug repurpose of natural compounds to treat COVID-19

Zhihao Wang[1,2*], Chi Xu[3*], Bing Liu[1,2#], Nan Qiao[3#]

[1]BioBank, The First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi, 710061, China.
[2]Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, SW7 2AZ London, United Kingdom.
[3]Laboratory of Health Intelligence, Huawei Technologies Co., Ltd

*These authors contributed equally

#Correspondence : qiaonan3@huawei.com; bliu2018@xjtu.edu.cn

Key words: COVID-19, *in silico* docking, natural compounds, bicuculline, lobeline

Abstract

The pandemic caused by the novel coronavirus SARS-CoV-2 is rapidly spreading and infecting the population on the global scale, it is a global health threat due to its high infection rate, high mortality and the lack of clinically approved drugs and vaccines for treating the disease (COVID-19). Utilising the published structures and homologue remodelling for proteins from SARS-CoV-2, an *in silico* molecular docking based screening was conducted and deposited in the Shennong project database. The results from the screening could be used to explain the clinical observation of repurposing the Ritonavir and Lopinavir to treat patients in the early stage of COVID-19 infection, and the prescription of Remdisivir in the United States as the therapy. Additionally, this molecular docking identified natural compound candidates for drug repurposing. This *in silico* molecular docking screen may be used for the initatial evaluation and rationalisation for drug repurposing of other potential candidates, especially other natural compounds from traditional Chinese medicines.

Introduction

Since the emergence of a novel coronavirus (SARS-CoV-2) from Wuhan, China, in December 2019, it has caused over 130,000 cases of human infections and with over 4,000 deaths globally and was declared as a pandemic by World Health Organisation[1]. The virus spreads rapidly from the transmission among human, and the symptoms caused by the virus (abbreviated "COVID-19") ranges from mild coughs and fevers to severe pneumonia and potentially life-threatening symptoms[2].

The novel SARS-CoV-2 virus belongs to the lineage B of the *betacoronavirus* genus and shares high genomic similarity to other two coronaviruses from the same lineage: the Severe acute respiratory syndrome coronavirus (SARS-CoV; about 79% sequence identity) and the Middle East respiratory syndrome coronavirus (MERS-CoV; about 50%)[3]. These viruses are enveloped, positive-sense, single-stranded RNA viruses with a genome of 29891 nucleotides that comprises 12 putative open reading frames (orf) translating to the synthesis of viral structural and non-structural proteins[4,5]. Four structural proteins which include Spike (S), membrane (M), envelop (E), and the nucleocapsid (N) proteins are crucial for the maturation of SARS-CoV-2[5,6]. The E and M proteins are essential for the viral assembly, while the N protein is responsible for viral RNA synthesis[7].

Based on the decade-long studies on the SARS-CoV and other coronaviruses, the envelop-attached trimetric glycoprotein Spike protein is recognised to meditate the infection of human cells by first binding to the host receptor angiotensin-converting enzyme 2 (hACE2) and the subsequent virus-cell membrane fusion[8]. The S protein can be divided into two functional domains. The first domain S1 facilitates the attachment to the host cell using its receptor-binding domain (RBD), which is located on the tip of the spike trimer[9], whereas the second subunit S2 is responsible for the fusion of viral and cellular membranes. The S1 is cleaved sequentially from the second domain at two sites. The first is at the S1/S2 site, and the S1 subunit remains non-covalently bound to stabilise the second subunit. The other cleave site lies within S2 domain[10], and the cleavage is facilitated by host proteases such as cathepsin L and serine protease TMPRSS2 during cell entry[11–13]. The membrane fusion between the virus and the ACE2 receptor is proposed to be activated by the cleavage that induced extensive and irreversible conformational changes[13,14].

The Spike protein for SARS-CoV and SARS-CoV-2 share 76.5% identity in their primary sequences, and they also share a high degree of structural homology[15,16]. The S1 subdomain in S of SARS-CoV-2 also contains the RBD (residue 319 to 591), and within it, the core structure and the receptor-binding motif (RBM) that is proposed to interact with hACE2 receptor via the same mechanism as that of SARS-CoV. Residue Q493 of the SARS-CoV-2 (N479 in SARS-CoV) in its receptor-binding-domain (RDB) was reported to interact with K31 on human ACE2 receptor, and residue N501 (T487 in SARS-CoV) formed interaction with K353 on hACE2[17,18], suggesting S protein of SARS-CoV-2 is capable of recognising and binding with hACE2 and infecting human cells. Analysis of the kinetics of this interaction revealed that SARS-CoV-2 S protein had a binding affinity that is about 10 to 20-fold higher than that of SARS-CoV S protein[19], which may contribute to the high transmission rate observed for the COVID-19.

As an essential step in viral entry and replication, S protein and the human ACE2 receptor have been seen as targets for potential therapeutic approaches[20], since blocking the initial entry of the virus is proposed to be a successful strategy in viral containment. As a result, laboratories around the world have been focusing on developing spike-protein based vaccines. However,

the lengthy duration of clinical trials for vaccines would only provide long term solution, and the repurposing of existing and approved drugs would be the necessary short-term solution for this race against time to combat the global pandemic[21]. Among the lists of existing drugs and naturally-occurring small molecules, the active ingredients from traditional Chinese medicines (TCM) such as "Shuang Huang Lian" and "Lian Hua Qing Wen" have been reported to suppress viral infection *in vitro*, and drinking tea has shown to inhibit viral replication by researchers from Zhejiang disease control unit, China. Additionally, the usages of TCM are also recommended by the State Administration of Traditional Chinese Medicine to treat the disease[22]. Although the underlying biochemical mechanisms remain unclear, these reports may provide clues in searching for remedies from natural compounds found in many traditional Chinese medicines. Active ingredients from TCM and other natural compound are good candidates for clinical repurposing for COVID-19, as many of the naturally-occurring active ingredients have undergone thorough toxicity and dosage trials (presented in *Chinese Pharmacopoeia,* 10th Edition).

Using the published complex structures of S protein of SARS-CoV-2 and hACE2, solved by several groups around the world[19,23], and the structure homologues of other viral proteins based on the proteins from SARS-CoV, researchers have created a database (Shennong Project[24]) to *in silico* simulate the binding of existing drugs from Drugbank[25] to all the proteins from SARS-CoV-2. The Shennong project examines the *in silico* interactions using the existing drugs and small molecules in the Drugbank to provide clues and potential targets for the repurposing of the existing drug to combat the current COVID-19 global pandemic.

We conducted a preliminary search using the "Shennong" database to investigate the potential candidates to bind with S1 of SARS-CoV-2, with the focus on naturally-occurring compounds from TCM and other plants, to inhibit its interaction with hACE2. We also examined and compared the effectiveness of several active small molecules from TCM and tea in terms of binding to the S protein to clinically prescribed drugs used to treat COVID-19. It is essential to highlight that the work reported is based on and limited to the *in silico* simulations from "Shennong" database, so the conclusion presented in this report shall be scrutinised by *in vitro* and *in vivo* experiments.

Results

Sequence alignment of S protein of SARS-CoV-2 and SARS-CoV

By aligning the sequence of the S protein of SARS-CoV and that of SARS-CoV-2, it was seen that most of the differences occurred in the S1 subdomain (the N-terminal domain). Most of the residues in the S2 subdomain were conserved, except for the insertion of four residues in between S1/S2 junction (residue 679 to 682) that introduced an "RRAR" furin cleavage site(reference) which was not observed in SARS-CoV S protein (Figure 1). The S1/S2 junction is disordered in the reported structures, in aligning with its proposed function as a cleave site.

Within the S1 subdomain, the RBD (residue 319 to 591) and the RBM (residue 437 to 508 on S of SARS-CoV-2) had only one insertion (G483, located at the loop facing away from hACE2 in EM structure), and the majority of the residues were conserved. Thus both viral proteins were able to interact with hACE2. The substitutions of the residues in the RBM presumably increased the binding affinity toward hACE2.

Figure 1: The sequence alignment between the Spike protein of SARS-CoV (S_SARS_COV) and that of SARS-CoV-2 (S_SARS-COV-2). A: The alignment for the entire S protein and the majority of the differences occurred in the N-terminal S1 domain. B: The alignment in the RBD and the RBM of the S1 subdomain and the S1/S2 cleave site. S protein of SARS-CoV-2 contains an insertion to include the furin cleave site.

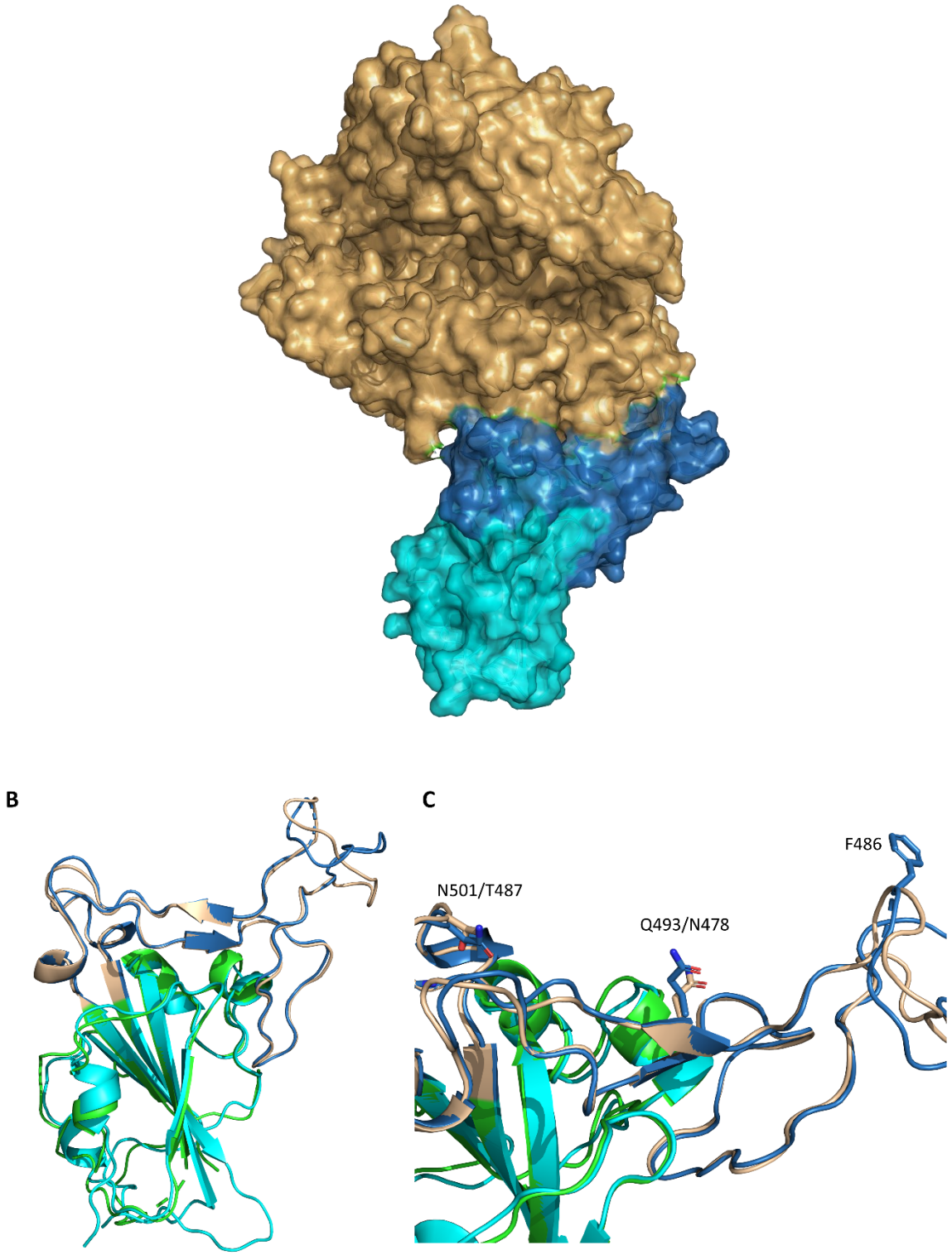

Figure 2: cryo-EM structure of hACE2 in complex with S of SARS-CoV-2. A: The cryo-EM structure of hACE2 (shown in wheat surface) in complex with SARS-CoV-2 S protein (shown in cyan surface), with the RBM highlighted in blue. B: the RBD of S SARS-CoV-2 S protein (cyan) superimposed with the RBD of SARS-CoV S protein (green), while the RBM (blue in SARS-CoV-2 S and wheat in SARS-CoV S) are highlighted. The structures are almost identical. C: The detailed view of the binding interface on the RBM. The residues (the residues in SARS-CoV-2 S/residues in SARS-CoV S) involved in the interactions are labelled.

Potential naturally-occurring candidate for binding with S protein

In the "Shennong" database that used either the published viral protein structures or homologue models built from existing structures from SARS-CoV as the templates, 8506 existing drugs from the Drugbank were docked into the protein using autodock Vina[26]. The respective binding energies were then calculated and ranked so that the drug with the most negative binding energy was ranked the highest and appeared as the top hit. ITI-214, a potential phosphodiesterase I inhibitor for treating Parkinson's Disease, having a binding energy of -8.1 kcal/mol, was the top in the list of drugs (Table 1).

Amongst the 8506 drugs in the system, most of the small molecules were synthetic drugs intended for the treatment of various diseases, and are currently under clinical trials. In terms of naturally occurring small molecules, lobeline, extracted from *Lobelia chinensis* that has been used as traditional Chinese medicine, was the highest-ranked drug out of natural products (-7.1 kcal/mol, 94th, Table 1). In addition, natural products from other plants such as bicuculline (the active ingredient in *Corydalis ambigua*, -5.2 kcal/mol), Epigallocatechin gallate (EGCG, from black and green tea, -5.1 kcal/mol) ranked in the middle of the list, whereas theophylline and caffeine (from coffee and tea, -3.4 kcal/mol and -3.2 kcal/mol respectively) ranked near the bottom (Table 1).

In comparison, ritonavir and lopinavir, included in the anti-HIV drug Kaletra clinically prescribed to treat COVID-19 from the guideline from China[27], were also searched in the Shennong database to examine the binding energy. Both drugs showed high binding affinity towards Nsp15 protein, a non-structural, viral endoribonuclease[28,29]. Ritonavir had binding energy of -8.4 and ranked 26th among the drugs (-5.4 kcal/mol, ranked 2956 for S protein), whereas lopinavir had binding energy of -9.3 kcal/mol and ranked 3rd for Nsp15 (-5.9 kcal/mol, ranked 1461 for S protein). Additionally, the novel nucleotide prodrug under development Remdesivir prescribed as the treatment for the first patient in the USA[30,31] was also searched in the database. Its binding energies to S was found to be -5.9 kcal/mol ranked 1687 for S, Table 1, and that to Nsp15 was -6.5 kcal/mol, ranked 2680 (Table 2).

Drug Name	Binding Energy (kcal/mol)	Rank
ITI-214	-8.1	1
Metergoline	-8.0	3
Lobeline	-7.1	94
Lopinavir	-5.9	1461
Remdesivir	-5.9	1687
Ritonavir	-5.4	2956
Bicuculline	-5.2	3932
Epigallocatechin gallate (EGCG)	-5.1	4331
theophylline	-3.4	8075
caffeine	-3.2	8212

Table 1: Table of selected drugs docked with S protein of SARS-CoV-2 and their respective binding energy and ranking.

Drug Name	Binding Energy (kcal/mol)	Rank
Saquinavir	-9.9	1
Phthalocyanine	-9.7	2
Lopinavir	-9.3	3
Ritonavir	-8.4	26
Epigallocatechin gallate (EGCG)	-7.5	355
Bicuculline	-7.2	741
Lobeline	-7.0	1014
Remdesivir	-6.5	2680
Theophylline	-4.8	7159
Caffeine	-4.5	7610

Table 2: Table of selected drugs docked with Nsp15 protein of SARS-CoV-2 and their respective binding energy and ranking.

In silico model for binding of small molecules with S protein

In the existing X-ray crystal structures containing the small molecules, the interaction between the proteins and ligands predominantly involved pi-interaction between the heteroaromatic/aromatic component of the chemical molecules and the aromatic residues of π proteins, and on top of that, the hydrophobic interactions among the ligands and the non-polar residues of proteins helped to stabilise the complex. For instances, EGCG formed π-π interaction with nearby Y24 and H41 residues seen in the X-ray crystal structure of influenza strain pH1N1 2009 Polymerase subunit PA endonuclease in complex with EGCG[32] (PDB: 4AWM, Figure 3A), lobeline formed π-π interaction with W53 as seen from the X-ray crystal structure of α7-AChBP in complex with lobeline[33] (PDB: 5AFH, Figure 3B) and bicuculline established interaction with Y212 as seen from the X-ray crystal structure of Glycine binding protein in complex with bicuculline[34] (PDB 5OBH, Figure 3C).

In the docking models for the small molecules with S protein (Figure 4), the binding site for the small molecules was located in the RBM with the ACE2 receptor. The interaction was π-π stacking with the aromatic residue (F455) and a few hydrophobic interactions with the residues with hydrophobic side chains. Lobeline formed a stronger interaction (lower binding energy), due to the better position and the consequent strong π-π interactions with F421 and F455 of S protein (Figure 5C). EGCG and bicuculline could only form π-π interactions with F455 (Figure 5A and B, respectively) that resulted in weaker binding. Caffeine and theophylline have even weaker interaction, as there were no π-π-interaction at all (Figure 5D).

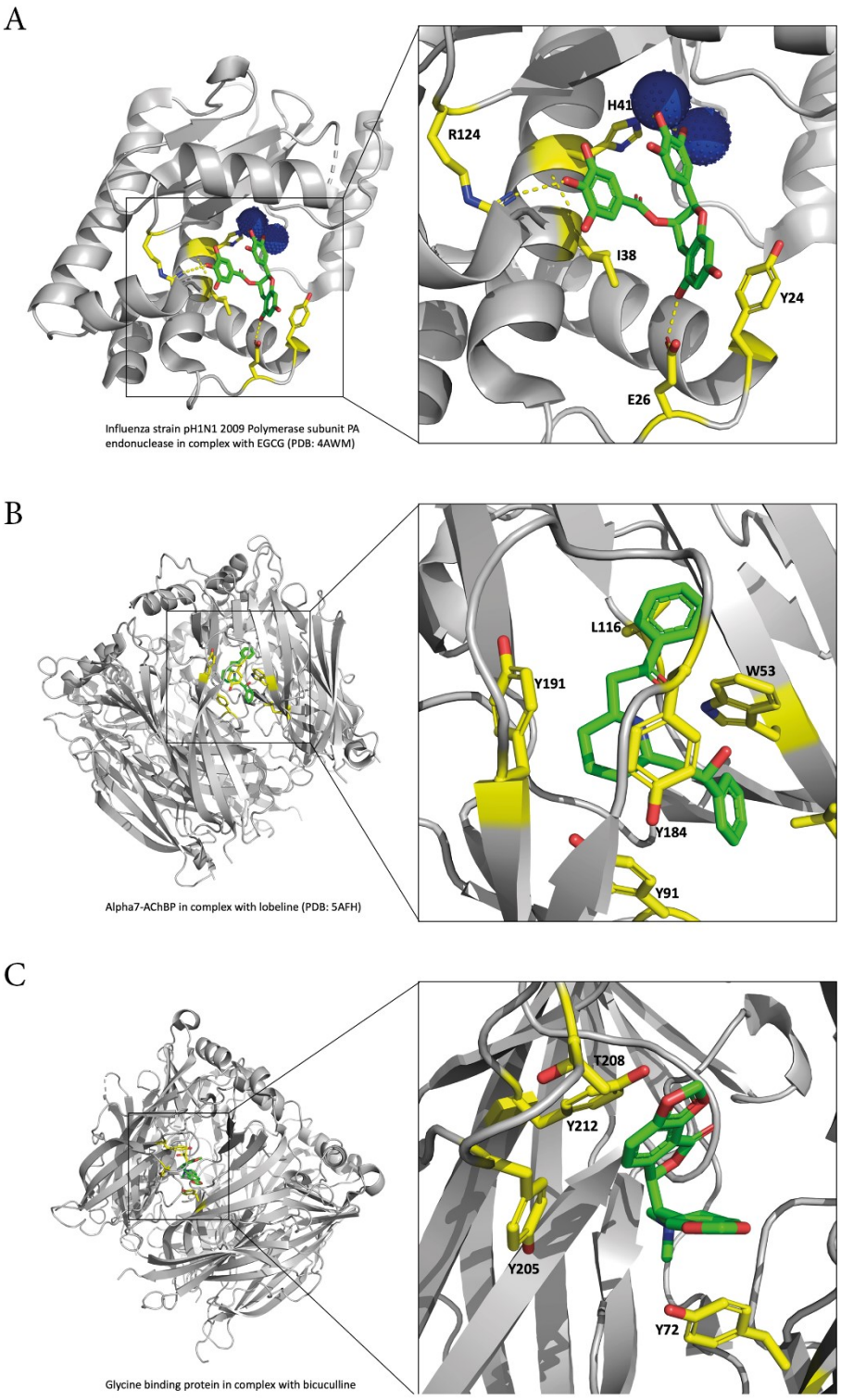

Figure 3: X-ray crystal structures of proteins (grey cartoon) in complex with EGCG, lobeline and bicuculline (green sticks), respectively. (A): X-ray crystal structure of Influenza strain pH1N1 2009 polymerase subunit PA endonuclease in complex with EGCG and the side chains involved in the binding are R124, I38, H41, E26 and Y24. (B): X-ray crystal structure of Alpha7-AChBP in complex with lobeline and the side chains involved in the binding are Y191, L116, W53, Y184 and Y91. (C): X-ray crystal structure of glycine binding protein in complex with bicuculline, and the side chains involved in binding are Y205, T208, Y212 and Y72.

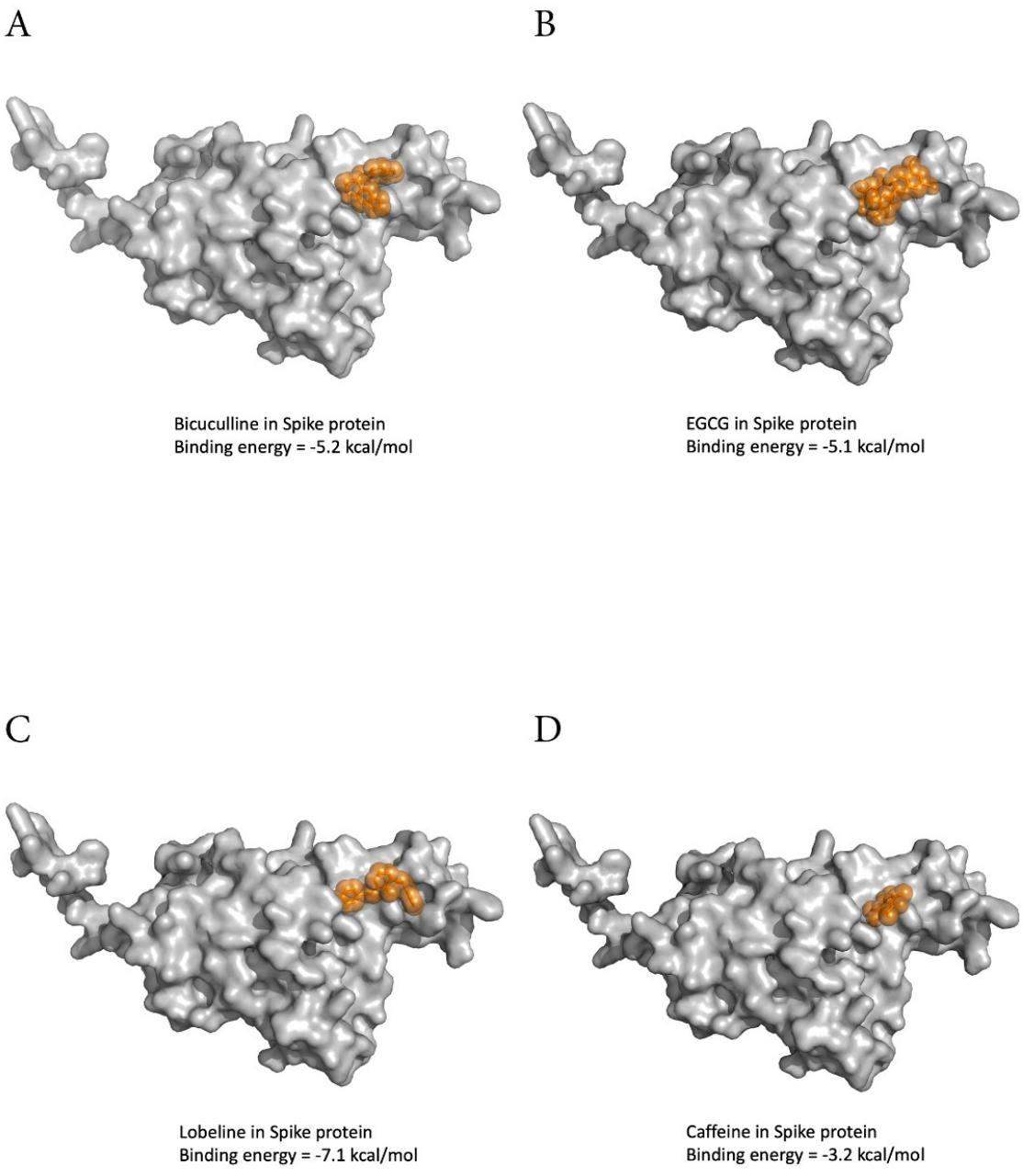

Figure 4: Docking models of small molecules(orange sphere) in the RDB of SARS-CoV-2 S protein (grey surface) with the respective binding energy. The binding sites are near the RBM so that they could inhibit the binding of Spike protein to human ACE2 receptor. A: Bicuculline docked in S protein with the binding energy of -5.2 kcal/mol. B: EGCG docked in S protein with the binding energy of -5.1 kcal/mol. C: lobeline docked in S protein with the binding energy of -7.1 kcal/mol. D: caffeine docked in S protein with the binding energy of -3.2 kcal/mol.

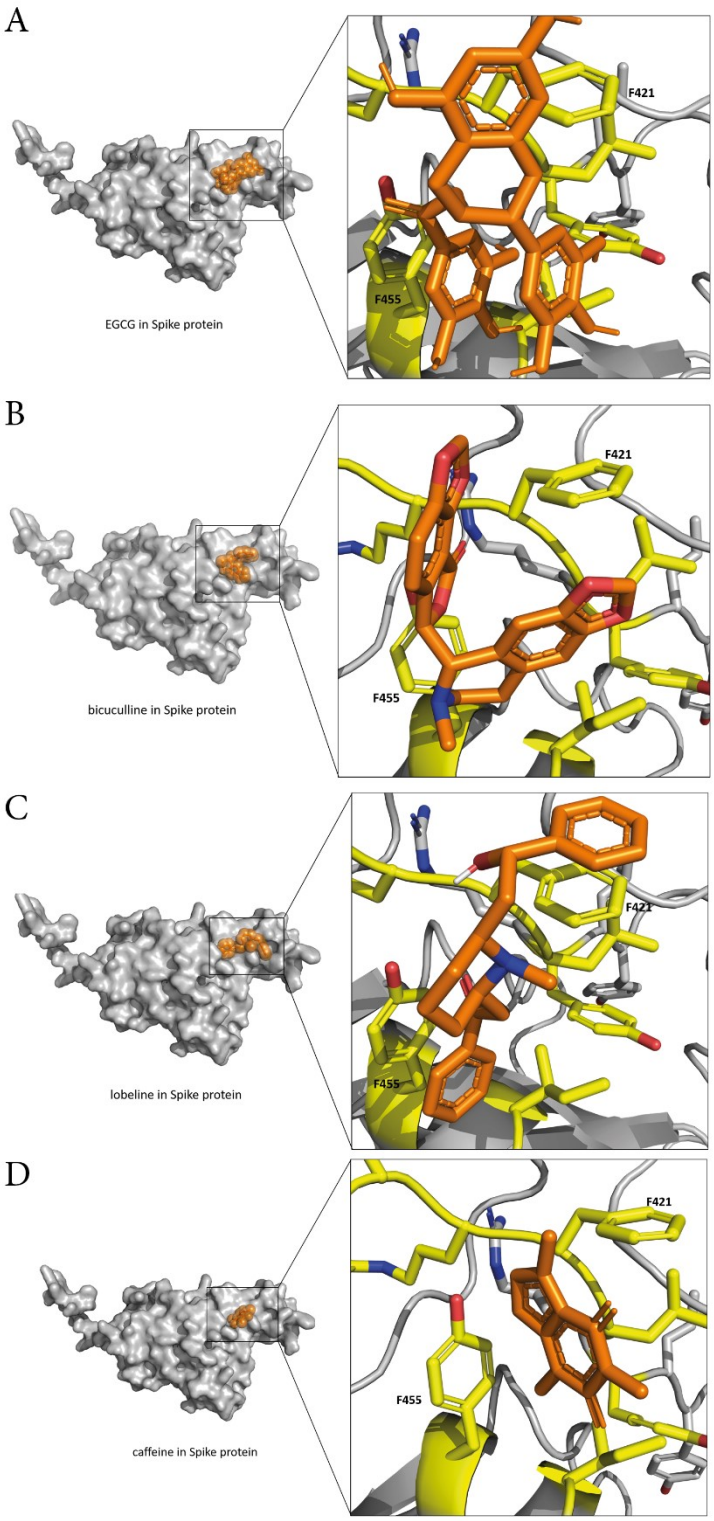

Figure 5: Docking models of small molecules(orange sphere) in SARS-CoV-2 Spike protein (grey surface) shown in detail. The residues near the binding pocket are shown in yellow. The key residue in all interactions is F455 that forms π-π interactions with the aromatic ring of the small molecules except caffeine. Lobeline has lower binding energy due to the additional π-π-interaction with F421 residue nearby.

In silico model for other viral proteins

To further investigate the effect of tea as the potential treatment for COVID-19 suggested in the report by researchers from Zhejiang disease control unit, China, the caffeine molecule was docked with all other viral proteins of SARS-CoV-2 as well as human ACE2 receptor. Among all the proteins, caffeine showed the highest binding to Nsp16 protein (-5.6 kcal/mol) but ranked 6323 among all the drugs for Nsp16, and the binding energies for other proteins were high and insignificant (Figure 6). Theophylline, being very similar in chemical structure, showed similar binding energies to the viral proteins. EGCG, on the other hand, showed stronger interaction with all but Nsp1, Nsp 7 and Nsp 3 proteins than that of caffeine and theophylline. The binding energies of EGCG for Nsp15, Nsp10, Nsp13 were ranked in the top 1000 among the list of drugs for respective viral proteins. For various viral proteins in complex with lobeline, the binding energies with S protein, hACE2 receptor, Nsp10, Nsp8, Nsp 12, Nsp14 and Nsp16 were ranked above 1000, while for bicuculline the binding energies with Nsp16, Nsp13, Nsp10, Nsp14, Mpro, Nsp8, Nsp15, hACE2 were ranked 1000 (Table 3).

Epigallocatechin gallate (EGCG)		
Target protein	Binding Energy (kcal/mol)	Rank
Nsp15	-7.5	355
Nsp10	-7.3	376
Nsp13	-8.3	453
Bicuculline		
Target protein	Binding Energy (kcal/mol)	Rank
Nsp16	-9.0	14
Nsp13	-9.4	39
Nsp10	-7.3	468
Nsp14	-9.7	562
Mpro	-8	675
Nsp8	-5.5	700
Nsp15	-7.2	741
hACE2	-5.5	947
Lobeline		
Target protein	Binding Energy (kcal/mol)	Rank
S	-7.1	94
hACE2	-5.7	487
Nsp10	-7.2	528
Nsp8	-5.5	609
Nsp12	-7.5	779
Nsp16	-7.6	844

Table 3: Table of drugs docked with viral proteins of SARS-CoV-2 and their binding energies of those ranked in the top 1000 among the list of drugs for the respective viral protein.

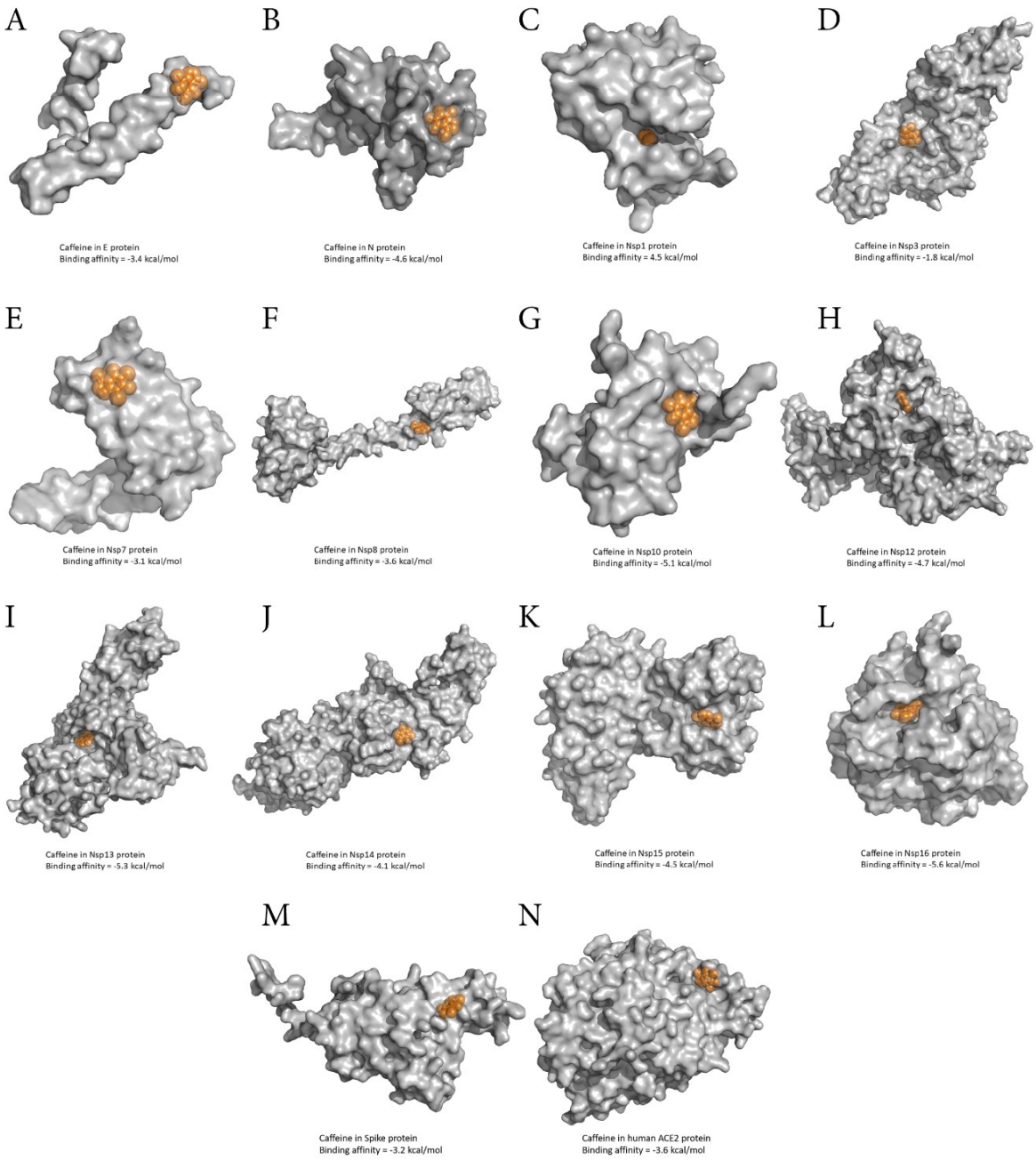

Figure 6: Docking models of caffeine (orange sphere) in several SARS-CoV-2 viral proteins (A to M) (grey surface) and human ACE2 receptor (N). caffeine showed the highest binding to Nsp16 protein (-5.6 kcal/mol) but ranked 6323 among all the drugs for Nsp16, whereas the binding to other viral proteins showed high binding energies.

Discussion

The *in silico* models for molecular docking can provide a rapid and inexpensive evaluation of binding mechanism and energies of the potential drug candidates, and the estimated binding energies can be ranked so the compounds can assist the rationalisation of drugs selection. However, the works based on the models are only indicative not conclusive, due to the limitations that arose from the nature of the *in silico* molecular dockings. For instance, the simulations are mostly conducted under vacuum condition to reduce the computational workload and time, which allowed a higher degree of freedom for proteins and ligands. These factors could lead to high false-positive rates and low correlation between the calculated and experimental binding energies. Nonetheless, the estimates of binding energies from the molecular docking can provide a comparative analysis of the potential drug candidates, thus assisting in the drug selection and repurposing as the treatment for COVID-19 pandemic.

Repurposing of existing drugs to treat COVID-19 is advantageous over the development of new drug discovery, due to the de-risk of the active compound that potentially decrease the development time and cost[35]. Drug repurposing has been serendipitous, and the systematic approach in this process has only been developed in recent years by the aid of increasing computing capability[36,37]. With the ongoing COVID-19 pandemic, the *in silico* Shennong database employed *in silico* docking models based on the published structural data of the viral proteins to predict and assist on the process of drug repurposing.

The cocktail of lopinavir and ritonavir have been prescribed as the treatment in the guidance from China with positive results for early treatment[27]. The results from of lopinavir and ritonavir docked in viral proteins (-9.3 and -8.4 kcal/mol and ranked 3rd and 26th respectively for Nsp15) are constant with the effectiveness of the drugs in the treatment of COVID-19 from the preliminary clinical data[27]. In the Drugbank database, there are potential drugs with stronger binding affinities toward the viral proteins. However, most of them are experimental or still under development, so their evaluations on the toxicity and dosage are still unclear. Therefore, repurposing of these drugs would require time and would not be plausible to be used as COVID-19 treatment in the short term.

Among the lists of potential candidates, natural compounds such as Lobeline, Bicuculline and EGCG appeared to have relative high binding affinities toward viral proteins Table 3). The effect of relative high binding affinity of EGCG with nsp15, nsp10 and nsp13 proteins (≤-7.3 kcal/mol) could be the reason for the observation of inhibition of viral replication in cells treated with tea, although additional *in vitro* and *in vivo* experiments using pure EGCG with varying concentration should be conducted to verify its effect. Conversely, the effect of caffeine/theophylline from the tea on the viral inhibition is likely to be negligible, due to the low binding affinities (Figure 6). The concentration of EGCG in tea, however, is present in small quantities in tea leaves that are unlikely to meet the minimum effective level. Hence drinking tea alone is unlikely to treat COVID-19. Bicuculline showed high binding affinity toward nsp16, nsp13, nsp10, nsp14, Mpro and nsp15 (≤-7.2 kcal/mol, Table 3), and also ranked in top 1000 in the drugs for docking with hACE2. Lobeline also showed high binding affinity to viral proteins, especially for S protein (-7.1 kcal/mol), which ranked 94th for all drugs in Drugbank (Table 3).

Taken these binding affinities to the viral protein, especially S protein, altogether, bicuculline and lobeline can be considered good potential candidates for the treatment of COVID-19, and

the further *in vitro* and *in vivo* experiments are required to investigate the molecular mechanism of binding and the consequent inhibition, as well as to verify the effectiveness as a treatment for COVID-19. Additionally, the *in silico* docking models can be used in conjunction with other natural product drug discovery computational tools[38,39] to examine the effect of other active ingredients from TCM that are under investigation to use as a remedy and provide clues for the systemic approach of the repurposing of the compounds from TCM as the alternative treatment for COVID-19.

Conclusion

With the COVID-19 pandemic deteriorates daily on the global scale, the search for treatment is a race against time to save lives and economic losses and the screening for an effective drug with low health risks is a matter of urgency. The computer-aided simulations on docking of small molecules into viral proteins have provided the rations for drug repurposing. From the Shennong database, Lobeline and Bicuculline could potentially interact with multiple viral proteins, and more specifically inhibit the viral entry facilitated by the binding of the S protein to human ACE2 receptor. The predicted binding and the resultant binding affinity provide clues for the search of treatment and lay the rations for *in vitro* and *in vivo* experiment to speed up the process of finding a cure to combat this global pandemic.

References

(1) World Health Organisation. (2020) Coronavirus disease 2019 (COVID-19) Situation Report -53.

(2) Centers for Diseases Control and Prevention. (2020) Symptoms for Coronavirus Disease 2019.

(3) Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., Chen, J., Meng, Y., Wang, J., Lin, Y., Yuan, J., Xie, Z., Ma, J., Liu, W. J., Wang, D., Xu, W., Holmes, E. C., Gao, G. F., Wu, G., Chen, W., Shi, W., and Tan, W. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet 395*, 565–574.

(4) Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., and Zhang, Y.-Z. (2020) A new coronavirus associated with human respiratory disease in China. *Nature 579*, 265–269.

(5) Chan, J. F.-W., Kok, K.-H., Zhu, Z., Chu, H., To, K. K.-W., Yuan, S., and Yuen, K.-Y. (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microbes Infect. 9*, 221–236.

(6) Shang, W., Yang, Y., Rao, Y., and Rao, X. (2020) The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. *npj Vaccines 5*, 18.

(7) Schoeman, D., and Fielding, B. C. (2019) Coronavirus envelope protein: current knowledge. *Virol. J. 16*, 69.

(8) Li, F. (2016) Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu. Rev. Virol. 3*, 237–261.

(9) Li, F. (2015) Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies. *J. Virol.* (Goff, S. P., Ed.) *89*, 1954 LP – 1964.

(10) Millet, J. K., and Whittaker, G. R. (2014) Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *Proc. Natl. Acad. Sci. 111*, 15214 LP – 15219.

(11) Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., Steffen, I., Tsegaye, T. S., He, Y., Gnirss, K., Niemeyer, D., Schneider, H., Drosten, C., and Pöhlmann, S. (2011) Evidence that TMPRSS2 Activates the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Membrane Fusion and Reduces Viral Control by the Humoral Immune Response. *J. Virol. 85*, 4122 LP – 4134.

(12) Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M., and Taguchi, F. (2010) Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *J. Virol. 84*, 12658–12664.

(13) Markus Hoffmann, 1, 13*Hannah Kleine-Weber, 1, 2, 13, Simon Schroeder, 3, 4Nadine Krüger, 5, 64Tanja Herrler, 7Sandra Erichsen, 8, 9Tobias S. Schiergens10, Georg Herrler, 5Nai-Huei Wu, 55Andreas Nitsche, 11Marcel A. Müller, 3, 4, 12Christian Drosten, 3, 4, 14*. (2020) SARS-CoV-2cell entry depends on ACE2 and TMPRSS2 and is blocked by a 1clinically-proven protease inhibitor. *Cell* 1–10.

(14) Millet, J. K., and Whittaker, G. R. (2015) Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus Res. 202*, 120–134.

(15) Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., Zhong, W., and Hao, P. (2020) Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci. 63*, 457–460.

(16) Li, F., Li, W., Farzan, M., and Harrison, S. C. (2005) Structure of SARS Coronavirus Spike Receptor-Binding Domain Complexed with Receptor. *Science (80-.). 309*, 1864 LP – 1868.

(17) Wu, K., Peng, G., Wilken, M., Geraghty, R. J., and Li, F. (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *J. Biol. Chem. 287*, 8904–8911.

(18) Wan, Y., Shang, J., Graham, R., Baric, R. S., and Li, F. (2020) Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *J. Virol.* JVI.00127-20.

(19) Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., Graham, B. S., and McLellan, J. S. (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (80-.). 367*, 1260 LP – 1263.

(20) Zhang, H., Penninger, J. M., Li, Y., Zhong, N., and Slutsky, A. S. (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.*

(21) Harrison, C. (2020) Coronavirus puts drug repurposing on the fast track. *Nature*.

(22) Cui, H.-T., Li, Y.-T., Guo, L.-Y., Liu, X.-G., Wang, L.-S., Jia, J.-W., Liao, J.-B., Miao, J., Zhang, Z.-Y., Wang, L., Wang, H.-W., and Wen, W.-B. (2020) Traditional Chinese medicine for treatment of coronavirus disease 2019: A review. *Tradit. Med. Res. 5*, 65–73.

(23) Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*.

(24) Xu, C., Ke, Z., Liu, C., Wang, Z., Liu, D., Zhang, L., Wang, J., He, W., Xu, Z., Li, Y., Yang, Y., Huang, Z., Lv, P., Wang, X., Han, D., Li, Y., Qiao, N., and Liu, B. (2020) Systemic in Silico Screening in Drug Discovery for Coronavirus Disease (COVID-19) with an Online Interactive Web Server.

(25) Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., Chang, Z., and Woolsey, J. (2006) DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res. 34*, D668–D672.

(26) Trott, O., and Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem. 31*, 455–461.

(27) Jin, Y.-H., Cai, L., Cheng, Z.-S., Cheng, H., Deng, T., Fan, Y.-P., Fang, C., Huang, D., Huang, L.-Q., Huang, Q., Han, Y., Hu, B., Hu, F., Li, B.-H., Li, Y.-R., Liang, K., Lin, L.-K., Luo, L.-S., Ma, J., Ma, L.-L., Peng, Z.-Y., Pan, Y.-B., Pan, Z.-Y., Ren, X.-Q., Sun, H.-M., Wang, Y., Wang, Y.-Y., Weng, H., Wei, C.-J., Wu, D.-F., Xia, J., Xiong, Y., Xu, H.-B., Yao, X.-M., Yuan, Y.-F., Ye, T.-S., Zhang, X.-C., Zhang, Y.-W., Zhang, Y.-G., Zhang, H.-M., Zhao, Y., Zhao, M.-J., Zi, H., Zeng, X.-T., Wang, Y.-Y., Wang, X.-H., and Evidence-Based Medicine Chapter of China International Exchange and Promotive Association for Medical

and Health Care (CPAM), for the Z. H. of W. U. N. C. M. and R. T. (2020) A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil. Med. Res. 7*, 4.

(28) Bhardwaj, K., Liu, P., Leibowitz, J. L., and Kao, C. C. (2012) The coronavirus endoribonuclease Nsp15 interacts with retinoblastoma tumor suppressor protein. *J. Virol. 86*, 4294–4304.

(29) Deng, X., Hackbart, M., Mettelman, R. C., O'Brien, A., Mielech, A. M., Yi, G., Kao, C. C., and Baker, S. C. (2017) Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages. *Proc. Natl. Acad. Sci. 114*, E4251 LP-E4260.

(30) Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., Spitters, C., Ericson, K., Wilkerson, S., Tural, A., Diaz, G., Cohn, A., Fox, L., Patel, A., Gerber, S. I., Kim, L., Tong, S., Lu, X., Lindstrom, S., Pallansch, M. A., Weldon, W. C., Biggs, H. M., Uyeki, T. M., and Pillai, S. K. (2020) First Case of 2019 Novel Coronavirus in the United States. *N. Engl. J. Med. 382*, 929–936.

(31) Routh, J. (2020) NIH clinical trial of remdesivir to treat COVID-19 begins. *Natl. Institutes Heal.*

(32) Kowalinski, E., Zubieta, C., Wolkerstorfer, A., Szolar, O. H. J., Ruigrok, R. W. H., and Cusack, S. (2012) Structural Analysis of Specific Metal Chelating Inhibitor Binding to the Endonuclease Domain of Influenza pH1N1 (2009) Polymerase. *PLOS Pathog. 8*, e1002831.

(33) Spurny, R., Debaveye, S., Farinha, A., Veys, K., Vos, A. M., Gossas, T., Atack, J., Bertrand, S., Bertrand, D., Danielson, U. H., Tresadern, G., and Ulens, C. (2015) Molecular blueprint of allosteric binding sites in a homologue of the agonist-binding domain of the α7 nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. 112*, E2543 LP-E2552.

(34) Dawson, A., Hunter, W. N., and Jones, M. Crystal structure of glycine binding protein in complex with bicuculline. *To be Publ.*

(35) Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., and Pirmohamed, M. (2019) Drug repurposing: progress, challenges and recommendations. *Nat. Rev. Drug Discov. 18*, 41–58.

(36) Govindaraj, R. G., Naderi, M., Singha, M., Lemoine, J., and Brylinski, M. (2018) Large-scale computational drug repositioning to find treatments for rare diseases. *npj Syst. Biol. Appl. 4*, 13.

(37) Oprea, T. I., Nielsen, S. K., Ursu, O., Yang, J. J., Taboureau, O., Mathias, S. L., Kouskoumvekaki, L., Sklar, L. A., and Bologa, C. G. (2011) Associating Drugs, Targets and Clinical Outcomes into an Integrated Network Affords a New Platform for Computer-Aided Drug Repurposing. *Mol. Inform. 30*, 100–111.

(38) Kim, E., Choi, A., and Nam, H. (2019) Drug repositioning of herbal compounds via a machine-learning approach. *BMC Bioinformatics 20*, 247.

(39) Romano, J. D., and Tatonetti, N. P. (2019) Informatics and Computational Methods in Natural Product Drug Discovery: A Review and Perspectives . *Front. Genet.* .