 doi:10.20944/preprints202001.0358.v3

Potential natural compounds for preventing SARS-CoV-2 (2019-nCoV) infection

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Abstract

SARS-CoV-2 (2019-nCoV), a novel coronavirus, caused the pneumonia outbreak in China and continue to expand. The host receptor for 2019-nCoV Angiotensin-converting enzyme 2 (ACE2), is the same as the host receptor for SARS-CoV. Targeting ACE2 holds the promise for preventing and inhibiting 2019-nCoV infection. Chinese Medicine herbs could be a valuable pool for identifying active compounds for treating infection of 2019-nCoV. In this study, we summarize several active compounds, including baicalin, Scutellarin, Hesperetin, Nicotianamine and glycyrrhizin that could have potential anti-2019-nCoV effects. We conduct molecular docking to predict their capacity for binding ACE2, which may prevent the 2019-nCoV infection. We propose that these selected compounds worth further investigation for preventing 2019-nCoV.

Keywords: SARS-CoV-2 (2019-nCoV), Baicalin, Scutellarin, Hesperetin, Nicotianamine, Glycyrrhizin

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Introduction

SARS-CoV-2 (2019-nCoV), a novel coronavirus, caused the pneumonia outbreak in Wuhan city, Hubei Province, China and subsequently expands. The original pneumonia cases were linked to a large seafood and animal market in Wuhan. This is an emerging, rapidly evolving situation. One genome sequence (WH-Human_1) of the 2019-nCoV was first released on Jan 10, 2020, and subsequently, five additional Wuhan CoV genome sequences were released (Zhang, 2020; Shu and McCauley, 2017). By comparing to the genomes of SARS-CoV and MERS-CoV, the WH-human_1 genome has a better sequence homology toward the genomes of SARS-CoV than that of MERS-CoV. By using structural modeling of its S-protein, scientists suggest a strong interaction of human ACE2 molecules with 2019-nCoV (Xu et al. 2020). ACE2 is a type I integral membrane protein, with its active site domain exposed to the extracellular surface of cells. ACE2 has been demonstrated to be a functional receptor for the SARS-coronavirus (CoV) (Kuhn et al., 2006). Michael Letko et al. showed that the 2019-nCoV receptor-binding domain (RBD) was capable of entering cells expressing human ACE2, but not any of the other receptors, further confirming that human ACE2 is the receptor for the recently emerging 2019-nCoV (Letko & Munster, 2020). As the host cell receptor is critical

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for the virus entry, targeting ACE2 holds the promise for preventing infection of 2019-nCoV infection.

The extracellular region of human ACE2 enzyme is composed of two domains, one is zinc metallopeptidase domain and the other is C terminus. Zinc metallopeptidase domain consists of ~42% residues (residues 19-611). While C terminus has ~48% to human collectrin (residues 612-740) (Zhang, 2001). The metallopeptidase domain divides into two subdomains. The residue from 19-102, 290-397, 417-430 are composed of first subdomain. The other is consists of residue from 103-289, 398-416, 431-615. One prominent α-helix connects the two subdomains and forms part of the floor of the canyon. The main secondary structure of ACE2 is α-helical segments, which is make up ~62% of metallopeptidase domain. Only six β-structure segments in the ACE2. The zinc-binding site is located near the bottom and on one side of the large active site cleft (subdomain I side), nearly midway along its length. The zinc is coordinated by His^{374}, His^{378}, Glu^{402}, and one water molecule. Proline and leucine are two residues that preferred the P_1 position with a partiality for hydrophobic residues in P_1' position. Regarding to the hydropathy of ACE2, the side chain of Phe^{274}, Pro^{346}, Thr^{371}, Met^{360} and disulfide linkage of Cys^{344} and Cys^{361} provided a hydrophobic environment. The bioactive compound and functional

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peptide might interfere with viral spike protein binding to ACE2 through binding to ACE2 active site and making large conformational change.

The development of drugs for targeting ACE2 and treating 2019-nCoV could be time-consuming, and the safety of newly-developed drugs could be a major concern, which needs time for testing. Therefore, it seems unrealistic to synthesize new drugs and tests for safety and toxicity within such a limited time when the infection is growing fast. Traditional Chinese Medicine has been practiced in China for thousands of years, and Chinese medicine licorice was suggested to be promising for treating SARS (Pilcher, 2003). Considering the low toxicity and availability, screening active compounds from Chinese herbal medicine for targeting the ACE2 receptor could be a potential strategy for treating 2019-nCoV. In this mini-review, we summarize the potential natural compounds that could target ACE2 for the potential treatment of 2019-nCoV. By using molecular docking, we proposed that the five natural compounds, including **baicalin, Scutellarin, Hesperetin, glycyrrhizin and Nicotianamine (Figure 1)** are potential compounds that target the ACE2 receptor and exert anti-virus effects for preventing 2019-nCoV infection.

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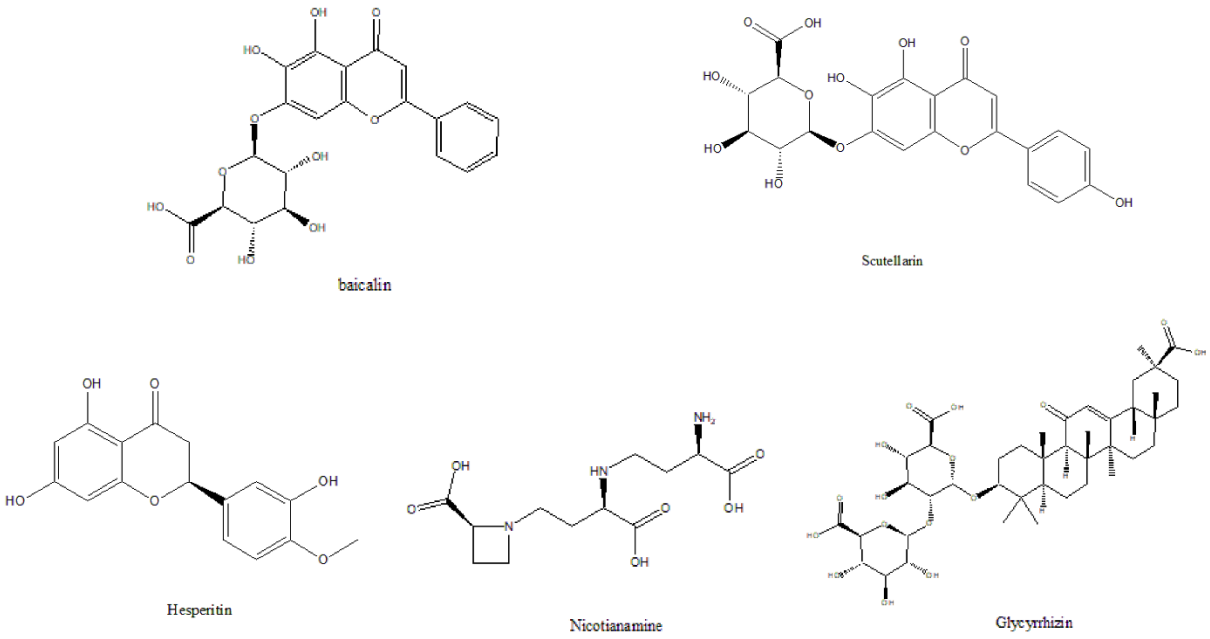

Figure 1, Chemical structure of baicalin, Scutellarin, Hesperetin, glycyrrhizin and Nicotianamine.

Natural compounds candidates for 2019-nCoV treatment

In the following session, we will summarize these five natural compounds that may have therapeutic effects against 2019-nCoV infection. To generate putative binding poses, we used the AutoDock Vina software package with the default scoring

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function (Trott O, Olson AJ, 2010). In the AutoDock Vina configuration files, the parameter num_modes was set to 1000 and exhaustiveness to 100. We identified the receptor-binding pocket based on the structures of ACE2 proteins. We chose all the rotatable bonds in ligands to be flexible during the docking procedure, and we kept all the protein residues inside the binding pockets rigid. We assigned the Gasteiger atomic partial charges and converted all receptors and ligands to the PDBQT format using the AutoDockTools package. We did not use explicit hydrogens either for the receptors or for the ligands.

Baicalin

Baicalin is extracted and purified from the Chinese medicinal plant Scutellaria baicalensis Georgi (Chinese name: Huang Qin). Baicalin has broad therapeutic effects, including anti-oxidative stress, anti-inflammation, anti-apoptosis (Chen et al., 2017; Ishfaq et al., 2019). Scientists have been shown baicalin's antiviral activities for SARS coronavirus using the foetal rhesus kidney-4 (fRhK-4) cell line, with an EC50 12.5 ug/ml at 48 hours, and selectivity index more 4 to 8 (Chen et al., 2004). The plaque reduction assay showed that baicalin has an EC 50 of 11ug/ml (Chen et al., 2004). Those results suggest that baicalin has anti-SARS effects. Since the 2019-nCoV shared similarity with the SARS virus, we suspect that baicalin may

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also show anti-virus effects on 2019-nCoV. In addition, a study showed that baicalin could inhibit ACE, with an IC50 value of 2.24 mM in vitro (Deng et al., 2012). However, whether baicalin can bind to ACE2 is not yet studied. Therefore, we use the molecular docking to test the possibility of baicalin binding to the ACE2 receptor, which may subsequently block the entry of 2019-nCoV. The docking result shows that baicalin may have strong binding to the ACE2 enzyme (Figure 2), with an estimated ΔG (kcal/mol) -8.46. , and the potential binding site at ASN-149, ARG-273, HIS-505. The binding site is located on the hydrophobic region of ACE2. Based on the anti-SARS activity and its potential binding to ACE2, we suggest that baicalin is one of the potential candidates for 2019-nCoV treatment. Given the low toxicity of baicalin, its efficacy on anti-2019nCoV worth further investigation.

A

B

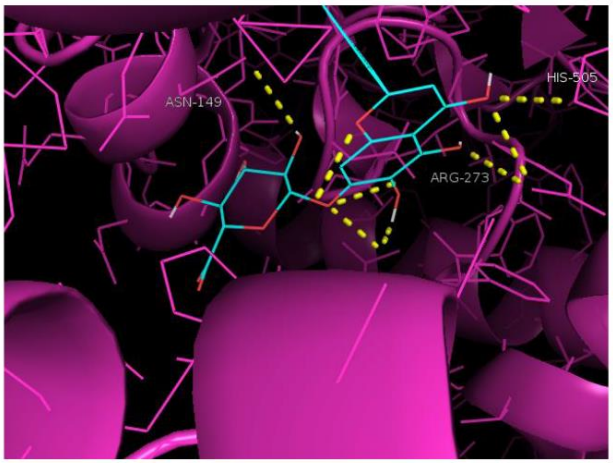

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Figure 2, molecular docking result of baicalin to ACE2 enzyme.

Scutellarin

Scutellarin is another active compound from Chinese Medicine Erigeron breviscapus (Vant.) Hand Mazz, which showed broad pharmacological effects, including anti-oxidant, anti-inflammation, vascular relaxation, anti-platelet, anti-coagulation (Wang and Ma, 2018). A study showed that scutellarin treatment could reduce the expression and activity of ACE in brain tissue *in vivo* (Wang et al., 2016). The IC50 value of scutellarin against ACE was 48.13 ± 4.98 μM (Wang et al., 2016). However, whether scutellarin could inhibit ACE2 is not yet reported. Here we conduct a molecular docking and find that scutellarin has the potential to bind to ACE2, with estimated ΔG (kcal/mol) -14.9, with binding site GLU-495, UNK-957, ARG-482 (Figure 3). Therefore, it's worthwhile to test whether scutellarin could inhibit ACE2 and block the infection of 2019-nCoV.

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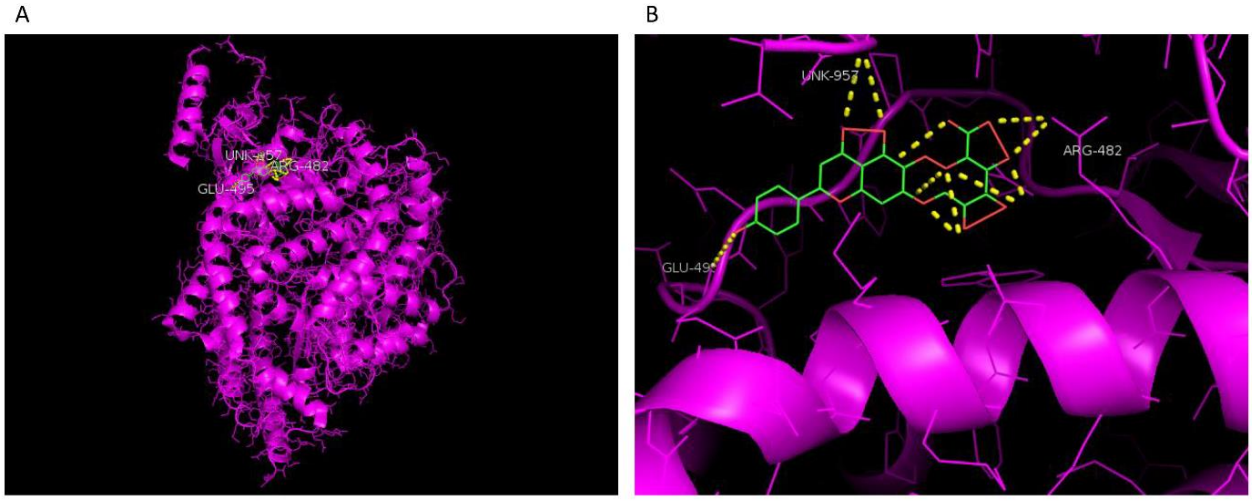

Figure 3, molecular docking result of Scutellarin to ACE2 enzyme.

Hesperetin

Hesperetin is a bioflavonoid compound abundant in Chinese Medicine citrus aurantium and Citri Reticulatae Pericarpium. Hesperetin dose-dependently inhibited cleavage activity of the 3C-like protease (3CLpro) of SARS-coronavirus in cell-free and cell-based assays, with an IC50 8.3 uM (Lin et al., 2005). Whether Hesperetin could inhibit 2019-nCoV replication is not yet investigated. To understand whether Hesperetin has the potential to inhibit ACE2, we conduct the molecular docking of Hesperetin to the ACE2 enzyme. The results showed that Hesperetin has the potential biding to ACE2 with an estimated ΔG (kcal/mol) -8.3, with binding cites TYR-613, SER-611, ARG-482, GLU-479 (Figure 3). This result

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suggests that Hesperetin may bind to ACE2, therefore, block the infection of 2019-nCoV.

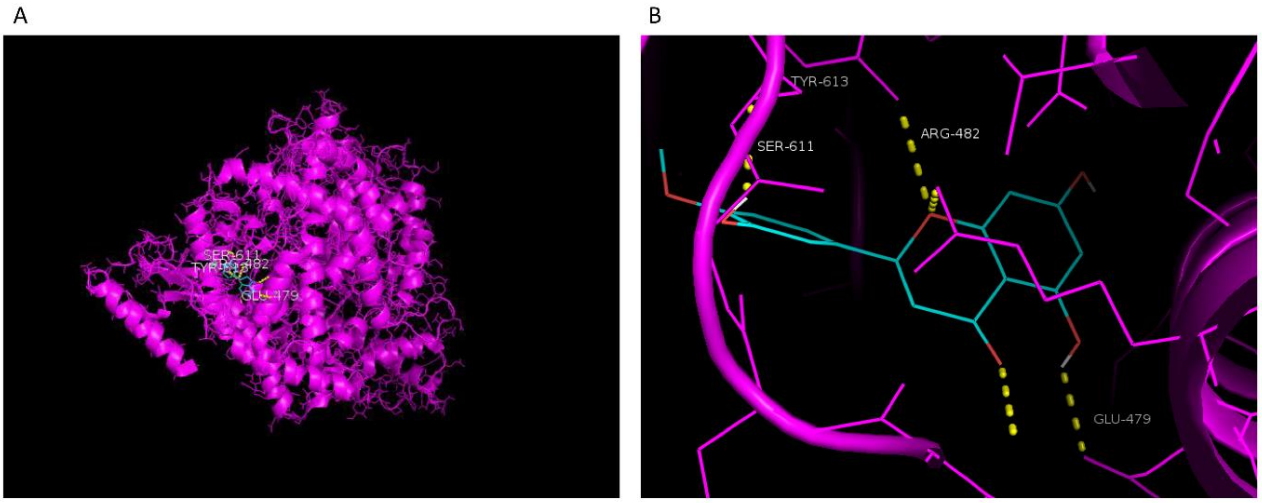

Figure 4, molecular docking result of Hesperetin to ACE2 enzyme.

Nicotianamine

Nicotianamine is rich in soybean (Takenaka, 2009). Toshihiro et al. reported that nicotianamine is a potent inhibitor of ACE2, with an IC_{50} value of 84 nM. The authors screened ACE2 inhibitors from various foodstuffs and found that soybean contained vigorous ACE2 inhibitory activity. They isolated the active compound "soybean ACE2 inhibitor" (ACE2iSB), which was identical to nicotianamine by direct comparison with a standard compound. We conducted the molecular docking of Nicotianamine to the ACE2 enzyme, and the results showed that Nicotianamine has

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the potential biding to ACE2 with an estimated ΔG (kcal/mol) -5.1, with binding cites ARG-518, GLU-406, SER-409, GLN-522, GLN-442 (Figure 4). Of which, ARG-518 is a residue located in the α-helix that might contribute to the conformational change of ACE2. Since ACE2 is critical for the 2019-nCoV infection, we hypothesize that nicotianamine may block the infection of 2019-nCoV through inhibiting ACE2, which needs further investigation.

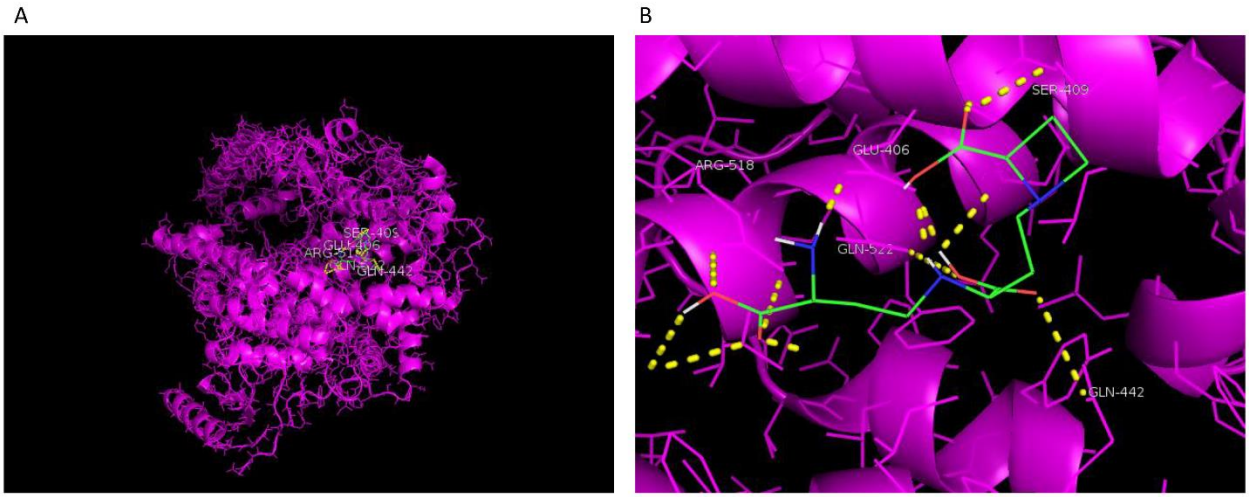

Figure 5, molecular docking result of Nicotianamine to ACE2 enzyme.

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Glycyrrhizin

Glycyrrhizin is another plant product isolated from Chinese Medicine herb licorice root (Glycyrrhiza radix), a herb that is promising for SARS treatment (Pilcher, 2003). Glycyrrhizin is used for treating chronic hepatitis and is relatively non-toxic. *In vitro* study showed that glycyrrhizin has anti-SARS-CoV effects. It inhibited viral adsorption and penetration and was most effective when administered both during and after the viral adsorption period (Cinatl et al., 2003). Chemical modifications increased the antiviral potency of glycyrrhizin, but also increased the cytotoxicity. Thus the selectivity index was reduced as compared with that of glycyrrhizin (selectivity index: $\geqslant$65) (Hoever et al., 2005). Whether glycyrrhizin has anti-2019-nCoV effects need further investigation. Our docking results showed that glycyrrhizin has the potential biding to ACE2 with an estimated ΔG (kcal/mol) - 9, with the binding cites ARG-559, GLN-388, ARG-393, ASP-30 (Figure 5). Based on the hydropathy of ACE2, the predict binding site of glycyrrhizin is located near the hydrophobic site. GLN-388 and ARG-393 are close to the zinc metallopeptidase that might regulate the activity of ACE2 in cells. Given the low toxicity of glycyrrhizin, its anti-virus effects on SARS and its potential interaction with ACE2, it's worthwhile to test its efficacy against 2019-nCoV infection.

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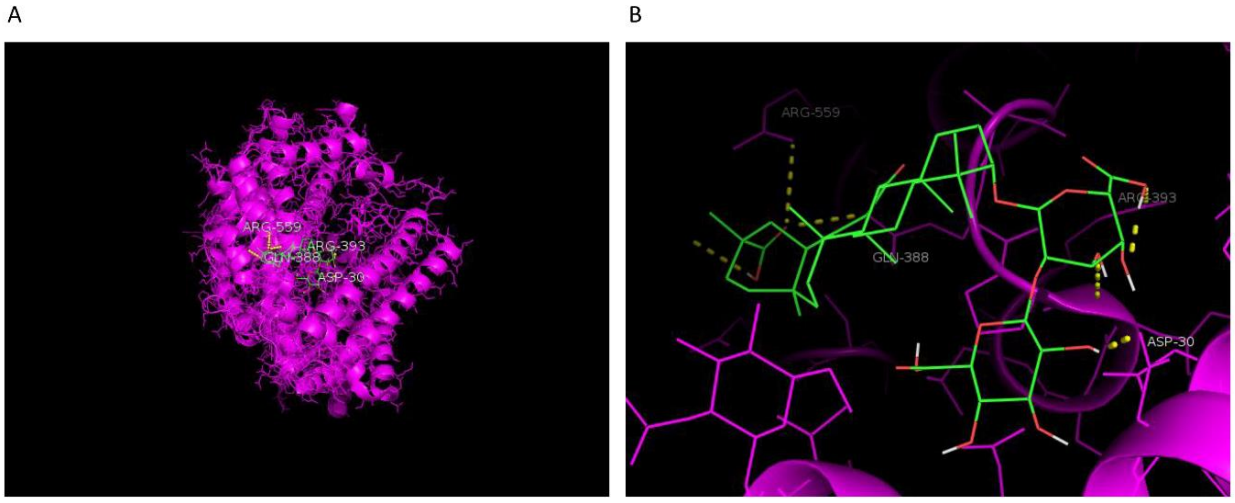

Figure 6, molecular docking result of glycyrrhizin to ACE2 enzyme.

Summary

Drug development for treating 2019-nCoV is timely important due to its rapid expansion. Vaccine development could take a long time to complete, and its safety needs to be verified. Synthesized agents for blocking ACE2 also needs to test its toxicity. Chinese Medicine is applied for anti-virus treatment for a long time, and active compounds from Chinese Medicine may be applied for the 2019-nCoV. Due to the low toxicity and availability of some active compounds from Chinese Medicine, it is worthwhile to select potential candidates for 2019-nCoV treatment.

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Since 2019-nCoV share some common sequence with SARS-CoV, and used the same host receptor ACE2, we review the potential active compounds for anti-SARS-CoV, and at the same time, predict the binding affinity of those compounds to bind ACE2. In this study, by using the molecular docking and reviewing the literature, we propose for the first time that baicalin, Scutellarin, Hesperetin, Nicotianamine, glycyrrhizin has the potential to bind to ACE2 and block the entry of 2019-nCoV. Further studies are needed to verify our results and test the anti-2019-CoV effects of these compounds.

Notes

Dr. Hansen Chen is a Postdoctoral Fellow in Department of Neurosurgery, School of Medicine, Stanford University.

Mr. Qiaohui Du is a Ph.D. student in School of Chinese Medicine, The University of Hong Kong

Funding

The authors did not receive grants for this study

Acknowledgment

We thank Dr. Xingmiao Chen (From Shanghai, China) for her support and suggestions

Conflict of interest

None

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