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In silico screening of chalcones and their derivatives as potential inhibitors of spike proteins and ACE2 enzymes for SARS-CoV-2 treatment

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Abstract:

The COVID-19 pandemic causing acute respiratory syndrome is a significant public health problem. Drugs that can treat this disease are currently a high priority. The SARS-CoV-2 spike protein and human ACE2 enzyme receptor, which both play important roles in virus entry into the host cell, are promising therapeutic targets for inhibiting viral infection. This research evaluates the potential of chalcone compounds to inhibit the spike proteins and ACE2 enzymes through molecular docking *in silico* approaches. Based on the ChemFaces database, we collected 92 chalcone compounds. These compounds were further docked to target the active sites of spike protein and human ACE2. After comparing the binding energies of the 92 compounds to artemisinin, ribavirin, and lopinavir, which have inhibitory activity to these protein targets of SARS-CoV-2, we chose 20 out of the 92 compounds that had a higher ability to inhibit the protein targets than the reference inhibitors. Next, five phytochemical compounds with the best binding energy were selected, which included flavanomarein, sarcandrone B, sarcandrone A, calyxin H, and sieboldin. Then, Lipinski's 5 rule was used to evaluate the drug-like properties of these compounds. Predictive ADME/tox filtering tests were also applied to the top docked compounds. The results suggest that sarcandrone B has good pharmacokinetic properties, which should be further explored as an anti-SARS-CoV-2. To confirm these findings, experimental studies are recommended.

Keywords: **chalcones, human ACE2, *in silico*, molecular docking, SARS-CoV-2, spike protein.**

Classification number: **3.3**

Introduction

Coronaviruses are a broad group of viruses that may cause moderate to severe respiratory illnesses in humans and also may infect many different species. Since the novel coronavirus designated as SARS-CoV-2 was first discovered in Wuhan city, Hubei province, China, the scientific community as well as the human race have had to confront the extraordinary task of finding a cure to this disease. This new coronavirus illness has spread rapidly around the world as it is highly transmissible. As of 20 August, 2021, there have been 209,558,900 reported cases and 4,398,234 deaths globally. In Vietnam, 312,611 cases and 7,150 deaths have been reported [1]. One of the biggest concerns is that the symptoms of the disease are often very diverse and can manifest differently in each patient. Clinical symptoms are usually noticed 5 to 6 days after infection, but the incubation period can be up to 14 days [2]. Those who are infected with SARS-CoV-2 have signs of viral pneumonia such as fever, cough, and chest pain as well as dyspnoea and bilateral infiltration in severe situations, which is similar to SARS and MERS patients.

SARS-CoV-2 has a 29.9 kb-size positive-sense RNA genome. It is composed of 14 open reading frames (ORFs), which encodes for a total of 27 proteins divided into structural and non-structural proteins (NSPs) [3]. Covering the surface of SARS-CoV-2, spike proteins are an important protein to viral entering because SARS-CoV-2 employs the receptor binding domain (RBD) of its glycosylated S protein to engage the cell-specific surface receptors and trigger membrane fusion [4]. This includes binding to the human Angiotensin Conversion enzyme

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(hACE2) followed by human protease proteolytic activation [5]. Owing to the roles of the spike protein and ACE2 enzyme in the mechanism of SARS-CoV-2 cell entry, considerable attention has been paid to these major targets for medications and vaccines treating COVID-19 [5].

Due to the few side effects and great health advantages of phytochemicals, anti-infection treatments based on plants have attracted health researchers throughout the world [6]. Indeed, the development of plant-based medicines for their antibacterial, antivirus, anticancer, and antioxidant properties are being continually explored [7, 8]. Chalcones are a group of polyphenolic compounds derived from plants that belongs to the flavonoid family. Many studies indicated that some chalcones have a variety of antioxidant, antimicrobial, antimycobacterial, and antileishmanial properties [9-11]. Chalcones also exhibit inhibitory activity against the tobacco mosaic virus, HIV, herpes simplex virus, and dengue virus [11-13]. In that context, there is some existing evidence about the anti-SARS-CoV-2 potential of chalcones and their derivatives [14, 15]. Our research focuses on the virtual screening of chalcones and their derivatives against SARS-CoV-2-related spike protein viruses and human ACE2 enzymes. The structures were all collected from ChemFaces database, which is a professional high purity natural products manufacturer.

Materials and methods

Ligands preparation

The ligand structures were collected from ChemFaces for the SARS-CoV-2 spike protein and the human ACE2 target involved 92 bioactive compounds. The structures were downloaded from PubChem in .sdf format and then converted into 3D structures in PDB format using MOE software. Structure Data Format (SDF) structures of the reference inhibitors (artemisinin, ribavirin, lopinavir) were retrieved from the PubChem database. After that, they were optimised by Avogadro software using Conjugate Gradients and converted to .pdbqt format using Autodock Tools software.

Retrieval and preparation of protein structure

Three-dimensional images of the SARS-CoV-2 spike protein (PDB ID: 6VSB) and human ACE2 (PDB ID: 1R4L) were retrieved from the Protein Data Bank RCSB [16, 17]. The co-crystal ligand XX5 in the structure of human ACE2 was used to evaluate and optimize the docking model. All water molecules and co-crystals were removed from the protein molecule using Discovery Studio Visualizer 4.0 software, while missing hydrogen atoms were added using Autodock Vina before regenerating the active site using MGL Autodock Tools 1.5.6 software. The active site of the SARS-CoV-2 spike protein and human ACE2 were identified as (x, y, z) = (40.003, 6.069, 28.538) (grid box size 12 Å×10 Å×13 Å); (x, y, z) = (204.457, 199.799, 246.898) (grid box size = 50 Å×50 Å×50 Å). The protein was saved in .pdbqt format to prepare for the docking program.

Lipinski's rule of five

Lipinski's rule of five helps to compare drug-like and non-drug-like molecules [18]. Lipinski's rule of five is popularly used to evaluate the potential molecular to become a therapeutic drug. This rule acts as a filter to screen promising compounds with particular pharmacological drugs.

We used the online tool to evaluate Lipinski's rule of five [19]. The chemical structures were downloaded from the PubChem database and set at pH 7.0.

Molecular docking study

AutoDock Vina performed an initial simulated screening of 92 bioactive compounds against SARS-CoV-2 spike protein and human ACE2. The molecular interactions between compounds that have good free binding energies and molecular targets were viewed using Discovery Studio Visualizer 2020.

Prediction of ADMET by computational analysis

The physicochemical efficiencies of the compounds were analysed by *in silico* ADMET profiling. An ADMET profile involves five parameters: absorption, distribution, metabolism, excretion, and toxicity, which all play a significant role to demonstrate the likelihood of success of turning a compound into a drug. Drug absorption depends on many factors including membrane permeability, intestinal absorption, levels of skin permeability, substrate, or inhibitor of P-glycoprotein. Drug distribution is influenced by variables such as CNS permeability, the blood-brain barrier (logBB), and the volume of distribution (VDss). Based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4), metabolism is expected. Based on the total clearance model and the renal OCT2 substrate, excretion is expected. Based on AMES toxicity, hepatotoxicity, and skin sensitization, the toxicity of drugs is expected. These criteria have been determined and their standard ranges have been tested for compliance. ADMET profiling was predicted using the pkCSM tool [20]. The canonical SMILES molecular structures of collected compounds were retrieved from PubChem [21].

Results

Evaluation of the docking model

Before screening compounds, the docking model needs to be evaluated for its accuracy. The results after docking the co-crystal ligand produced an RMSD value of 0.777 Å. This value satisfies the condition that RMSD is less than 1.5 Å, indicating that the results of molecular docking on the target are reliable. The results of re-docking and the interaction between the co-crystallised ligand with the human ACE2 are shown as shown in Figs. 1 and 2.

Molecular docking of compounds with the target protein

We docked 92 bioactive compounds retrieved with SARS-CoV-2 spike protein and human ACE2. To evaluate the compounds' abilities to inhibit target proteins, we compared the docking scores of the ligands with artemisinin, ribavirin, and lopinavir. Artemisinin has also been demonstrated by M. Sehailia, et al. (2020) [22] to act as a spike protein inhibitor. Recently, the effectiveness of lopinavir, an inhibitor of the proteases, and ribavirin, a nucleoside analogue to eliminate the removal of SARS-

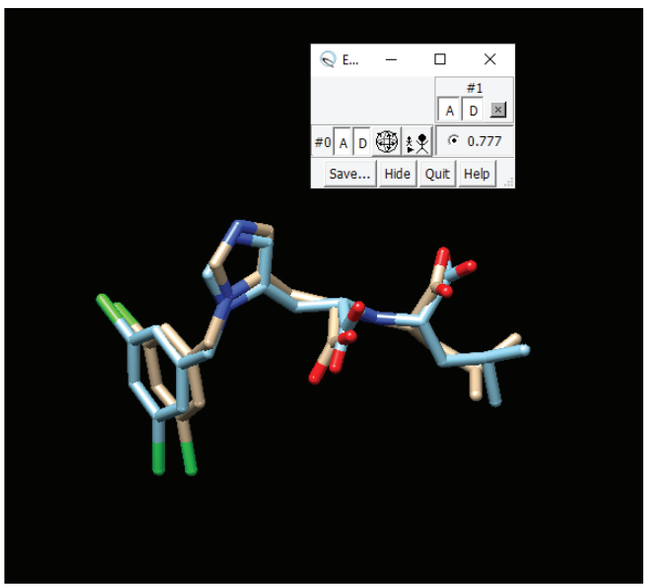


Fig. 1. Co-crystallised ligand re-dock results of 1R4L.

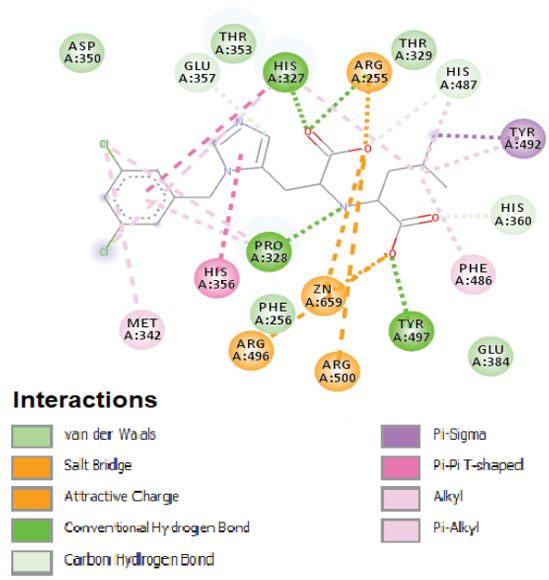
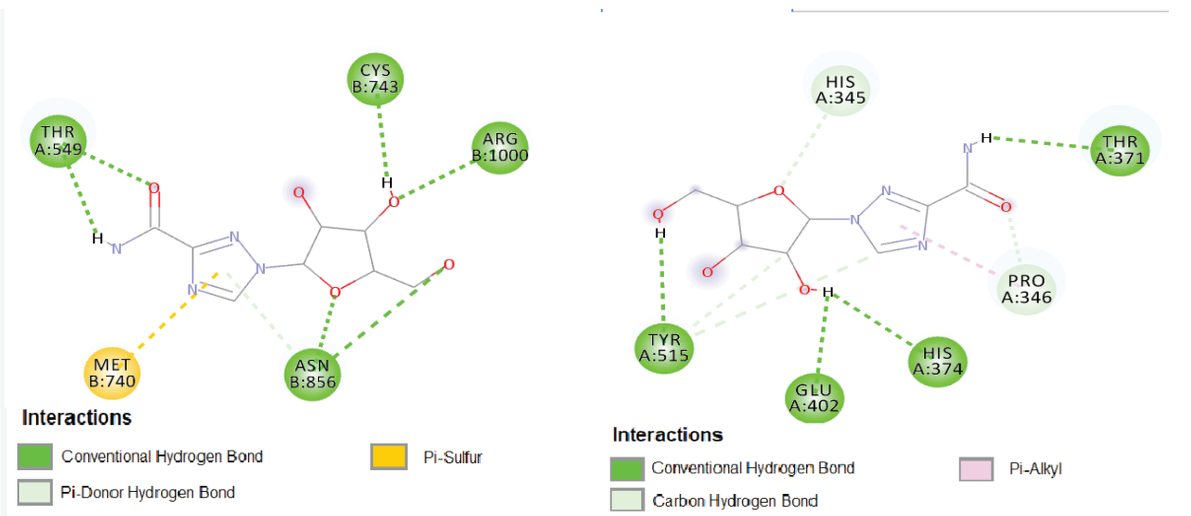


Fig. 2. 2D representation of the interaction of co-crystallised ligand XX5 with human ACE2.



(A) (B)

Fig. 3. Interactions between (A) ribavirin and spike protein and (B) ribavirin and human ACE2.

CoV-2, has been documented [23-26]. Artemisinin had binding affinities of -7.3 kcal/mol for the spike protein. The binding energies of lopinavir and ribavirin were found to be -6.3 and -5.7 kcal/mol, respectively, when docked in the spike protein and -6.7 and -7.6 kcal/mol when docked in the human ACE2. Figure 3 shows the interaction between the reference inhibitors and targets.

A hit list of 20 bioactive compounds was defined based on the negative and low value of ΔG and compared with the reference inhibitors (Table 1).

Table 1. The docking results of 20 hit phytochemicals and reference compounds with spike protein and human ACE2.

No.	Name	Binding energy with spike protein (kcal/mol)	Binding energy with human ACE2 (kcal/mol)	No.	Name	Binding energy with spike protein (kcal/mol)	Binding energy with human ACE2 (kcal/mol)
1	Isoliquiritin	-8.3	-8.4	13	Phlorizin dihydrate	-8.1	-7.8
2	Flavanomarein	-8.9	-9.4	14	Isodorsmanin A	-8	-8.5
3	Sarcandrone B	-9.4	-10.3	15	Calyxin H	-9	-6.2
4	Sarcandrone A	-9.8	-3.4	16	Isoliquiritoside	-8.1	-8.7
5	Trilobatin	-8	-8.4	17	Xanthohumol I	-8.2	-8.3
6	Morachalcone A	-7.9	-8.2	18	Xanthohumol L	-8.1	-8.3
7	Trilobatin 2"-acetate	-8.8	-8.3	19	Xanthohumol B	-7.9	-8.1
8	Nothofagin	-8.3	-8.7	20	Sieboldin	-8.4	-8.8
9	Bavachromene	-8.2	-8.1	**R1**	**XX5**		**-7.5**
10	Kuraridine	-8.2	-7.8	**R2**	**Artemisinin**	**-7.9**	
11	Marein	-8.5	-8.6	**R3**	**Ribavirin**	**-6.3**	**-6.7**
12	Neoisoliquiritin	-8.6	-8.5	**R4**	**Lopinavir**	**-5.7**	**-7.6**

From these, the top five bioactive compounds with the most negative binding energies were chosen, which were flavanomarein, sarcandrone B, sarcandrone A, calyxin H, and sieboldin. It was observed that all these compounds were the topmost docked compound to the spike protein as well as human ACE2. The results show that the ligands majorly interacted with the residues through hydrogen bonds and π-anion bonds.

Lipinski's rule of five

Lipinski's rule of five helps to distinguish between drug-like and non-drug-like molecules. It predicts with high probability the drug-like effectiveness or failure of molecules complying with 2 or more of the following rules: molecular mass (MW) below 500 Dalton; high lipophilicity (LogP does not exceed 5); no more than 5 donors of hydrogen bonds (HBD); no more than 10 acceptors of hydrogen bonds (HBA1); and molar refractivity (MR) should be between 40-130. The top five bioactive compounds are listed in Table 2. These compounds satisfy more than 2 criteria. Next, we focus on analysing the pharmacokinetic properties of these drugs including absorption, distribution, metabolism, excretion, and toxicity.

Table 2. The result of Lipinski's rule of five.

No.	Name	Molecular weight	HBD	HBA1	logP	MR	Drug-likeness
1	Flavanomarein	450	7	11	-0.3114	104.59	Yes
2	Sarcandrone B	554	3	8	6.381006	153.61203	Yes
3	Sarcandrone A	554	3	8	6.381006	153.61203	Yes
4	Calyxin H	566	5	7	6.485604	162.271667	Yes
5	Sieboldin	452	8	11	-0.4968	106.589844	Yes

Prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile.

The prediction of absorption, distribution, metabolism, excretion, and toxicity profile of the five selected drugs are shown in Table 3.

Table 3. The result of the ADMET profile.

Properties	Flavanomarein	Sarcandrone B	Sarcandrone A	Calyxin H	Sieboldin
Absorption					
Water solubility (log mol/l)	-3.082	-3.608	-3.447	-2.912	-2.777
Caco-2 permeability (log P_{app} in 10^{-6} cm/s)	0.487	-0.323	-0.343	-0.877	-1.175
Intestinal absorption (human) (%)	40.359	100	100	78.977	22.15
Distribution					
VDss (human) (log l/kg)	0.915	-2.186	-2.199	-1.682	1.154
BBB permeability (log BB)	-1.428	-1.235	-1.287	-1.235	-1.567
CNS permeability (log PS)	-4.267	-3.039	-2.969	-2.742	-4.178
Metabolism					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	No	Yes	Yes	Yes	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	Yes	Yes	Yes	No
CYP2C9 inhibitor	No	Yes	Yes	Yes	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	Yes	Yes	Yes	No
Excretion					
Total clearance (log ml/min/kg)	0.011	0.34	0.322	0.202	-0.158
Renal OCT2 substrate	No	No	No	No	No
Toxicity					
AMES toxicity	Yes	No	No	No	No
Hepatotoxicity	No	No	No	No	No
Skin sensitisation	No	No	No	No	No
Minnow toxicity (log mM)	5.937	-0.18	-2.32	-0.349	6.787

The first property is the absorption process in which human intestinal absorption (HIA) and human colon adenocarcinoma-2 cell line (Caco-2) are two important parameters that determine the absorption of the drug. A substance is considered poorly absorbed if the percentage absorbed in the human intestine is less than 30% [27]. The results show that sieboldin was poorly absorbed with an absorbance of 30%. Three compounds, sarcandrone B, sarcandrone A, and calyxin H, are well absorbed in the intestine especially sarcandrone A and B, which have a maximum absorption percentage of 100%. The Caco-2 cell line consists of human colon adenocarcinoma cells. A compound has high Caco-2 permeability if it has P_{app}>8×10^{-6} cm/s, i.e., logPapp>0.9 [27]. The results of all five compounds had a Caco-2 permeability less than 0.9.

Secondly, the distribution of a substance is expressed through several parameters including lipid-solubility, concentration, as well as the binding capability to plasma proteins, transfer proteins, and lipid-solubility. The steady-state volume of distribution (VDss) is the theoretical volume to which the total dose of a drug should be uniformly distributed to obtain the same plasma concentrations [27]. The higher the VDss, the more the drug is distributed in tissue rather than plasma. Compounds were said to be well distributed to tissues if logVDss>0.45 and poorly distributed if logVDss<-0.15 [27]. The two compounds flavanomarein and sieboldin distributed well to tissues with logVDss values of 0.915 and 1.154, respectively. The remaining three compounds were poorly distributed. The capability of a drug to cross into the brain is a factor to consider to help reduce toxicities and side effects or to improve the effectiveness of drugs whose pharmacological activity is within the brain [27]. The blood-brain barrier ability of all compounds was poor because their logBB values were all less than -1. Compounds are considered to enter the central nervous system (CNS) in the presence of logPS>-2 [27]. The results showed that all five compounds failed to enter the CNS because their logPS values were less than -2.

Cytochrome P450 plays an important role in the metabolism of many drugs. P450 inhibitors can significantly alter the pharmacokinetics of these drugs. The results showed that the two flavanomarein compounds and sieboldin, are not substrates of CYP2D6, CYP3A4 and are not inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The compounds sarcandrone A, B, and calyxin H are substrates of CYP2D6, CYP3A4 and are inhibitors of CYP2C19, CYP2C9, and CYP3A4.

Regarding elimination, we predicted total clearance and likelihood as a renal OCT2 substrate. The resulting total clearance is shown in Table 3. Organic Cation Transporter 2 (OCT2) is a renal absorption transporter that plays an important role in renal processing and clearance of drugs and endogenous compounds [27]. All substances are not OCT2 substrates.

The toxicity prediction results for the five compounds showed that only flavanomarein caused AMES toxicity (mutagenicity). The remaining compounds are not hepatotoxic, mutagenic, and have no skin toxicity.

Discussion

In this study, we screened 92 chalcones compounds on the spike protein and ACE2 targets of the SARS-CoV-2 virus. The results obtained 5 compounds with the most negative free binding energy including sarcandrone A and B, flavanomarein, calyxin H, and sieboldin.

Sarcandrone A and sarcandrone B are flavan-chalcone dimers isolated from *Sarcandra hainanensis*. From the results of evaluating the HIV-1 inhibitory ability of C.M. Cao, et al. (2009) [28] obtained IC_{50} values of sarcandrone A and B at 18.05 and 25.27 mM, respectively. In our study, the docking results showed that sarcandrone A and sarcandrone B are the two compounds with the most negative free binding energy on the SARS-CoV-2 spike target. Sarcandrone A has a free binding energy of -9.8 kcal/mol and -3.4 kcal/mol corresponding to the two targets of SARS-CoV-2 spike protein and human ACE2 enzyme. Meanwhile, sarcandrone B inhibits these two targets with good free binding energies of -9.4 and -10.3 kcal/mol, respectively, to the spike protein and ACE2 enzyme. These two compounds have similar structures that differ mainly in the position of the -OH and -OCH_3 groups on the (D) ring. This may be the reason for sarcandrone A's poor ability to inhibit the ACE2 target with a free binding energy of -3.4 kcal/mol. In addition, ADMET results for both compounds were quite positive with 100% intestinal absorption without hepatotoxicity, skin toxicity, or AMES toxicity. Therefore, these are two potential natural compounds for the treatment of COVID-19.

Calyxin H is isolated from *Alpinia katsumadai* and *Alpinia blepharocalyx* [29, 30]. Our research results show that calyxin H strongly inhibits SARS-CoV-2 spike proteins with a free binding energy of -9.0 kcal/mol, which is much more negative than the positive controls artemisinin, ribavirin, and lopinavir. The pharmacokinetic prediction also shows that the compound is well soluble in water, does not cross the blood-brain barrier, does not penetrate the central nervous system, and has little toxicity. Therefore, we can see the great potential of

calyxin H in inhibiting the spike protein of SARS-CoV-2.

Flavanomarein is the main compound in *Coreopsis tinctoria*, which has been shown to have good antioxidant, antidiabetic, antihyperlipidemic, and antihypertensive effects [31-34]. However, the antiviral effects of flavanomarein have not been studied. The docking results of flavanomarein on the spike protein of SARS-CoV-2 and the human ACE2 enzyme gave good results. Specifically, flavanomarein inhibited the spike protein and ACE2 receptor with binding energies of -8.9 and -9.4 kcal/mol, respectively. However, the intestinal absorption of this compound is not high at a little more than 40%. Flavanomarein has no skin toxicity and no hepatotoxicity but causes AMES toxicity.

Sieboldin is a dihydrochalcone compound present in several species of *Malus*. Sieboldin exhibits cytotoxicity to cancer cell lines and an antioxidant capacity [35]. This compound also showed inhibitory potential on both important targets of SARS-CoV-2 with binding energies of -8.4 and -8.8 kcal/mol for the spike protein and ACE2 enzyme, respectively. In addition, sieboldin is well distributed to tissues, is not metabolised in the liver, and has little toxicity. However, the absorption of sieboldin in the human intestine is poor at only 22.15%.

As soon as SARS-CoV-2 appeared, scientists began aggressively searching for drugs to treat COVID-19. The *in silico* approach was chosen to screen a large number of substances for potential compounds in a short time. As a result, many compounds with the ability to inhibit SARS-CoV-2 have been found such as amentoflavone, epigallocatechin gallate, and kaempferol [36-38]. However, the five potential compounds that we screened are relatively new and unpublished compounds with the ability to inhibit two targets: ACE2 and spike protein of the SARS-CoV-2 virus. The *in silico* method has high reliability, but it also inevitably leads to the discovery of the failure of non-potential compounds. Therefore, *in vivo* and *in vitro* studies should be conducted.

Conclusions

In this study, we found five natural chalcone compounds, flavanomarein, sarcandrone B, sarcandrone A, calyxin H, and sieboldin, that strongly inhibited the spike protein and ACE2 receptor of the SARS-CoV-2 virus. Among them, sarcandrone B has the most potential due to its strong inhibition of these 2 targets, its drug-like properties, good pharmacokinetic parameters, and low toxicity. Therefore, further tests on the ability of sarcandrone B to inhibit SARS-CoV-2 need to be conducted.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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