

Discovery of alliin as a putative inhibitor of the main protease of SARS-CoV-2 by molecular docking

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ABSTRACT

The outbreak of viral pneumonia caused by the novel coronavirus SARS-CoV-2 that began in December 2019 caused high mortality. It has been suggested that the main protease (Mpro) of SARS-CoV-2 may be an important target to discover pharmaceutical compounds for the therapy of this life-threatening disease. Remdesivir, ritonavir and chloroquine have all been reported to play a role in suppressing SARS-CoV-2. Here, we applied a molecular docking method to study the binding stability of these drugs with SARS-CoV-2 Mpro. It appeared that the ligand–protein binding stability of the alliin and SARS-CoV-2 Mpro complex was better than others. The results suggested that alliin may serve as a good candidate as an inhibitor of SARS-CoV-2 Mpro. Therefore, the present research may provide some meaningful guidance for the prevention and treatment of SARS-CoV-2.

METHOD SUMMARY

Molecular docking was used to assess the binding stability of various drugs with SARS-CoV-2 main protease (Mpro). PyMOL software was used to align the protein structures of SARS-CoV Mpro (ID: 5C5N) and SARS-CoV-2 Mpro (ID: 6LU7).

KEYWORDS:

alliin • molecular docking • remdesivir • ritonavir • SARS-CoV-2 Mpro

Since December 2019, many cases of viral pneumonia have been found in Wuhan City (Hubei Province, China), all of which were diagnosed with viral pneumonia and pulmonary infection. On 11 February 2020, the new coronavirus causing the pneumonia epidemic in Wuhan was officially named SARS-CoV-2 by the International Committee on Taxonomy of Viruses.

Viral pneumonia is inflammation of the lungs caused by a viral infection of the upper respiratory tract that spreads downward; it is an inhalation infection, transmitted via person-to-person droplets and contact. Viruses that cause viral pneumonia are common and include influenza viruses, parainfluenza virus, cytomegalovirus, adenovirus, rhinovirus and coronavirus. An outbreak of infectious atypical pneumonia in 2002–2003 was caused by the SARS virus SARS-CoV, a coronavirus [1,2].

It has been reported in the literature that the source of SARS-CoV-2 may be derived from bats. Moreover, homology analysis has shown that the similarity of the entire genome sequence of SARS-CoV-2 to SARS-CoV and the Middle East Respiratory Syndrome virus was 79.0 and 51.8%, respectively. The highest similarity was with the SARS-like coronavirus (SL-CoV ZC45 and ZXC21) carried by two chrysanthemum bats found in Zhoushan in 2005, ranging from 87.6 to 87.7%. The number of novel coronavirus infections is on the rise, which is a matter of great concern. Scientific research institutions and pharmaceutical companies around the world are committed to developing new vaccines and therapeutic drugs for SARS-CoV-2. There are two types of known drugs which are often discussed and may offer therapeutic benefit at present. One is ritonavir, an inhibitor of HIV protease; according to research, SARS and SARS-CoV-2 have a protease target similar to that of HIV. The other is remdesivir, a new nucleoside analog antiviral drug against Ebola. Chloroquine, a drug used for the treatment of malaria and rheumatic diseases, has also been proved to inhibit virus replication and has been shown to play a role in suppressing SARS-CoV-2 in cellular experiments [3].

The complex phytochemistry of garlic (*Allium sativum*) has often been studied. A large number of recent studies have identified the functional actions of garlic on cardiovascular disease [4] and cancer [5,6]. Furthermore, garlic has been demonstrated to possess immunomodulation, antiinflammatory [7], antimicrobial [8], antioxidant [9] and antiviral properties [10]. Alliin is the main active component of garlic and a wide range of biological activities of garlic are shown to originate from this compound.

SARS-CoV-2 main protease (Mpro), also known as chymotrypsin-like protease, is primarily responsible for cleaving polyproteins, while a papain-like protease also aids in the process. In an effort to better investigate inhibitors of SARS-CoV-2 Mpro, a molecular docking

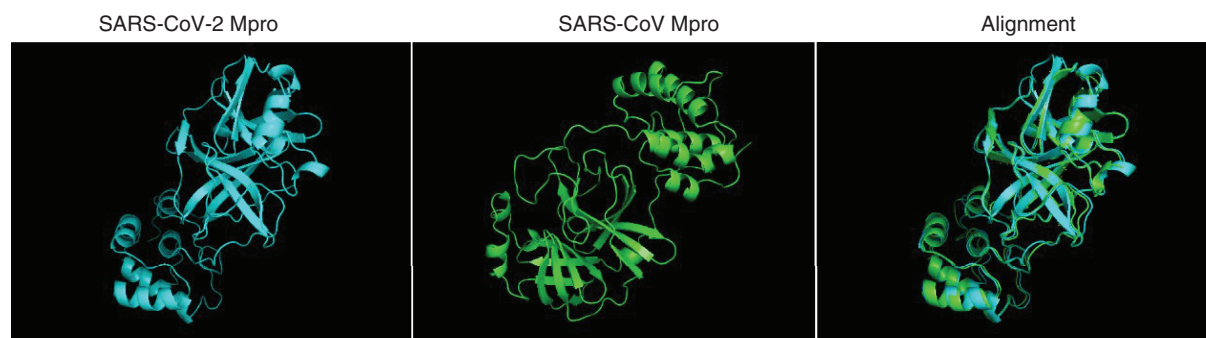


Figure 1. Protein alignment by PyMOL.

Table 1. Molecular docking results of SARS-CoV-2 main protease and severe acute respiratory syndrome coronavirus main protease.

Receptor	Ligand	Score	RMSD
SARS-CoV-2 Mpro	remdesivir	-9.4339	3.1145
SARS-CoV-2 Mpro	ritonavir	-8.9094	1.8379
SARS-CoV-2 Mpro	chloroquine	-7.0456	1.3582
SARS-CoV-2 Mpro	alliin	-12.6107	1.5180
SARS-CoV Mpro	alliin	-4.9641	1.6326

Mpro: Main protease; RMSD: Root-mean-square deviation; SARS-CoV: Severe acute respiratory syndrome coronavirus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

approach was used to examine the docking interaction between the three inhibitors (remdesivir, ritonavir and chloroquine) and SARS-CoV-2 Mpro. In addition, alliin was screened out to dock with SARS-CoV-2 Mpro, which may provide clues to the prevention and treatment of SARS-CoV-2.

Materials & methods

Computational methods

Structures of SARS-CoV Mpro (ID: 5C5N) and SARS-CoV-2 Mpro (ID: 6LU7) were obtained from the Protein Data Bank. The protein structures were aligned using the PyMOL software (PyMOL Molecular graphics System, Version 1.4).

Molecular docking

Molecular docking is an efficient way to investigate the noncovalent binding of macromolecules or a macromolecule (receptor) and a small molecule (ligand) [11,12]. Molecular docking was performed using the molecular operating environment docking software (MOE 2015). We obtained the sdf structure formats of ligands from the PubChem database: remdesivir (CID: 121304016), ritonavir (CID: 392622), chloroquine (CID: 2719) and alliin (CID: 87310). Then the proteins were quick prepared. At the end of docking, the binding modes of four ligands to SARS-CoV-2 Mpro and SARS-CoV Mpro were observed to identify the critical residues.

Results & discussion

To understand the relationship between SARS-CoV-2 and SARS-CoV, we aligned the protein sequences of the two viruses. Figure 1 shows the coincidence of their 3D proteins; the root-mean-square deviation value between them is 0.585 Å, which shows that the two viruses' Mpros are similar.

According to reports of the Wuhan Virus Research of the Chinese Academy of Sciences and the Institute of Toxicology and Pharmaceuticals of the Academy of Military Medical Sciences, remdesivir, ritonavir and chloroquine might play a role in suppressing SARS-CoV-2 at the cellular level. In this study, we used a molecular docking method to study the binding of these drugs with SARS-CoV-2 Mpro and compared the potential inhibitory effects of alliin on SARS-CoV-2 Mpro and SARS-CoV Mpro.

The molecular docking results revealed a high affinity between SARS-CoV-2 Mpro and the four ligands, as shown in Table 1; the molecular docking scores of SARS-CoV Mpro and alliin are also listed. As shown in Figure 2A, remdesivir was found to interact with SARS-CoV-2 Mpro at Asn-142, Ser-144 and His-41 with two H-bonds and one arene H-bond. Figure 2B shows that ritonavir was observed to interact with SARS-CoV-2 Mpro at Met-49, Glu-166 and Thr-26 with two H-bonds and one arene H-bond. As shown in Figure 2C, chloroquine was found to be interacting with SARS-CoV-2 Mpro at His-41 and Met-49 with two arene H-bonds. Finally, as shown in

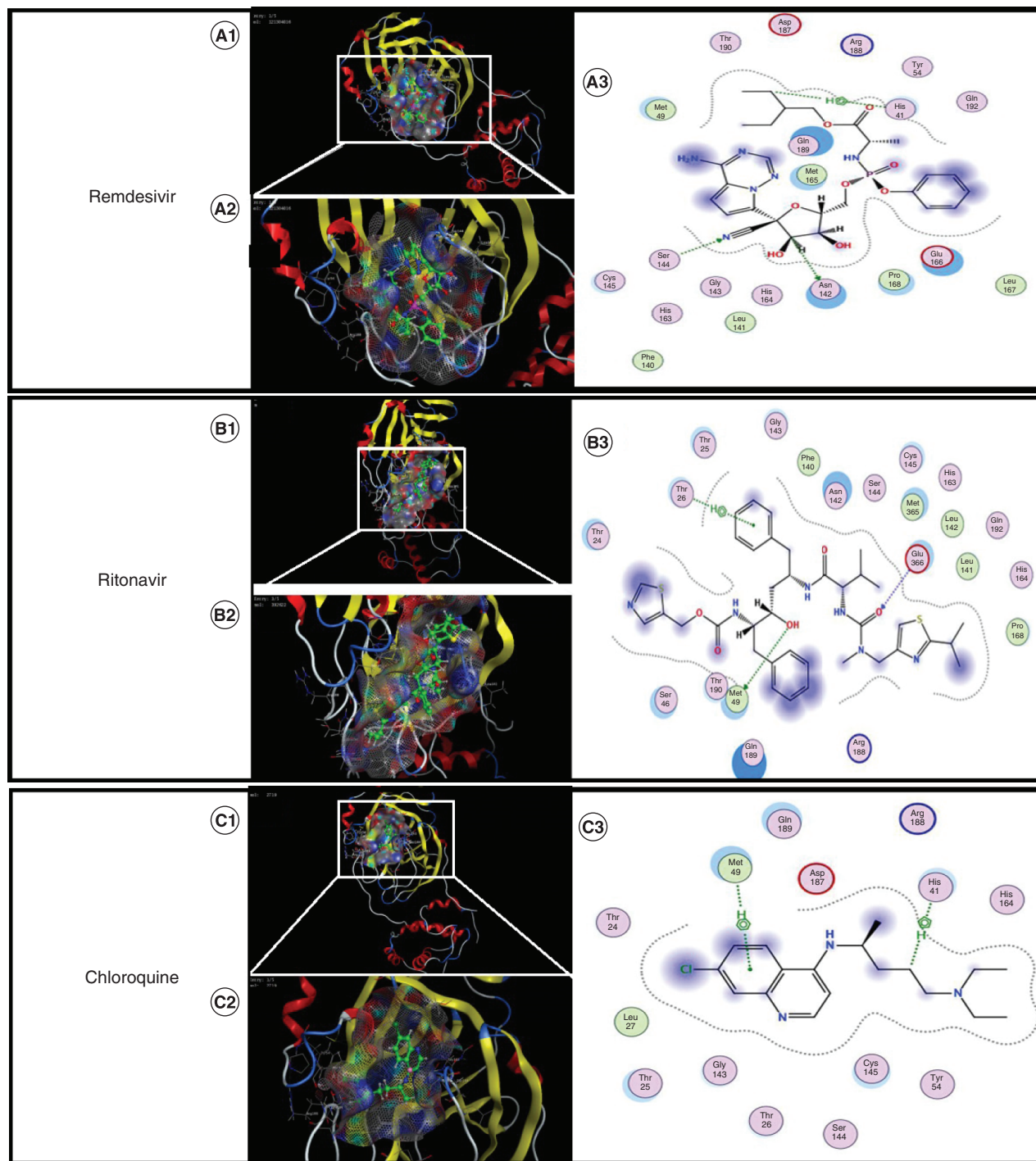


Figure 2. Interactions of known drugs with hydrophobic pockets of SARS-CoV-2 main protease. (A) 1 and 2 show interactions of remdesivir with hydrophobic pockets of SARS-CoV-2 Mpro; 3 shows molecular interactions between remdesivir and SARS-CoV-2 Mpro. (B) 1 and 2 show interactions of ritonavir with hydrophobic pockets of SARS-CoV-2 Mpro; 3 shows molecular interactions between ritonavir and SARS-CoV-2 Mpro. (C) 1 and 2 show interactions of chloroquine with hydrophobic pockets of SARS-CoV-2 Mpro; 3 shows molecular interactions between chloroquine and SARS-CoV-2 Mpro.

Mpro: Main protease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

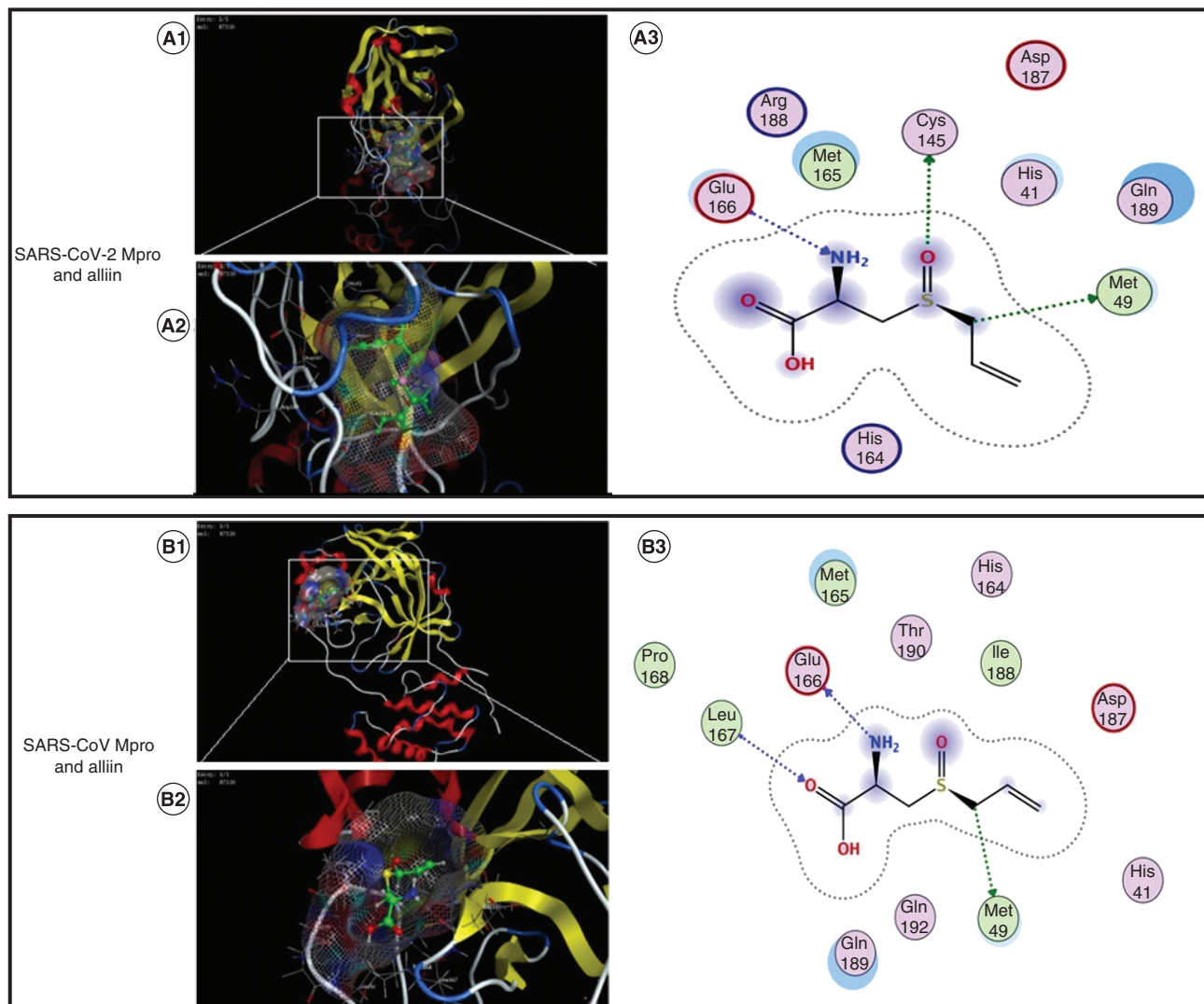


Figure 3. Interactions of alliin with hydrophobic pockets of SARS-CoV-2 main protease and severe acute respiratory syndrome coronavirus main protease. (A) 1 and 2 show interactions of alliin with hydrophobic pockets of SARS-CoV-2 Mpro; 3 shows molecular interactions between alliin and SARS-CoV-2 Mpro. (B) 1 and 2 show interactions of alliin with hydrophobic pockets of SARS-CoV Mpro; 3 shows molecular interactions between alliin and SARS-CoV Mpro.

Mpro: Main protease; SARS-CoV: Severe acute respiratory syndrome coronavirus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Figure 3, alliin was found to interact with SARS-CoV Mpro at Leu-167, Met-49 and Glu-166 with three H-bonds; for SARS-CoV-2 Mpro, the observed docking sites of alliin were Cys-145, Met-49 and Glu-166 with three H-bonds.

It has been reported that inhibiting the main protease would help to restrict viral maturation, thereby reducing the SARS-CoV-2 infection in humans [13]. The active site of SARS-CoV-2 Mpro is located in the gap between domains I (residues 8–101) and II (residues 102–184) and has a Cys–His catalytic dyad (Cys145 and His41) [14,15]. A recent article indicated that montelukast, a potential drug acting on SARS-CoV-2 Mpro, was well fitted into the active pocket of Mpro, in which hydrophobic amino acids (Thr24, Leu27, His41, Phe140, Cys145, His163, Met165, Pro168 and His172) compose a relatively hydrophobic environment to contain the compound and stabilize its conformation [16]. In another study, Qamar *et al.* revealed that SARS-CoV-2 has a Cys–His catalytic dyad (Cys-145 and His-41) consistent with the SARS chymotrypsin-like protease (Cys-145 and His-41) [17]. The authors indicated that 5,7,3',4'-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone, a potential candidate for treatment of SARS-CoV-2, formed strong hydrogen bonds with the catalytic dyad residues (Cys-145 and His-41) and had significant interactions with the receptor-binding residues Thr24, Thr25, Thr26, Cys44, Thr45, Ser46, Met49, Asn142, Gly143, His164, Glu166 and Gln189 [18]. Consistent with this, in our study alliin was well fitted into the active pocket of SARS-CoV-2 Mpro, containing many amino acids, such as His41, His164, Met165, Asp187, Arg188 and Gln189, which formed significant interactions with

the compound and stabilized its conformation. In addition, the docking scores indicated the strength of the ligand–protein complexes, with lower values indicating greater stability. It can therefore be inferred that the binding stability of the alliin and SARS-CoV-2 Mpro ligand–protein complex was better than that of the others tested.

Conclusion

The lack of effective anti-SARS-CoV-2 agents is a current problem and there is urgent demand to discover anti-SARS-CoV-2 inhibitors to combat this deadly disease. SARS-CoV-2 Mpro is an important target for the design of therapeutically useful drugs. In the present study, alliin was screened out to dock with SARS-CoV-2 Mpro and the mechanisms of alliin and known inhibitors were investigated. The results suggested that alliin may be a good candidate for the prevention and treatment of SARS-CoV-2.

Future perspective

Our results are based on *in silico* analysis. We have not conducted further *in vivo* and *in vitro* antiviral experiments yet, because we want to share the results with other researchers as quickly as possible. In the next study, we will be close to *in vivo* and *in vitro* evaluations and will prepare for clinical trial applications.

Author contributions

T Li designed and revised the work. B Cheng analyzed the data and wrote the manuscript. Both authors approved the final version of the manuscript.

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Financial & competing interests disclosure

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