Virol Curr Res
Volume 5:2, 2021

Virology: Current Research

Research Article

Open Access

# Investigation of Cepharanthine Binding with Viral Proteins Reveal its Potential Targets against Coronavirus

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## Abstract

The outbreak of Corona Virus Disease 2019 (COVID-19) caused by SARS-CoV-2 is becoming a worldwide problem. We previously reported that cepharanthine (CEP) demonstrated strong anti-coronavirus effects, however, the mechanism underlying CEP's anti-coronavirus effects remains unknown. We herein performed Surface Plasmon Resonance (SPR) to investigate the biological influence of CEP on different proteins of SARS-CoV-2. Meanwhile, molecular docking study was used to screen the potential binding sites of CEP on the virus. The binding of CEP to the nsp13 helicase with a $K_d$ of $3.806*10^{-6}$ M shows that helicase is a relatively strong possible target of CEP. Besides, CEP could bind to the viral main proteinase (3CLpro) that contributes to the intervention of polypeptide cleavage. We also compared the potential binding pockets and binding affinity on viral spike proteins (S1 and S2 subunits) at both open and closed states. Our study revealed that CEP exerts its anti-coronavirus effects at viral genomic RNA replication, transcription, translation and viral invasion levels, providing a theoretical basis for the development of CEP as a promising anti-coronavirus drug.

**Keywords:** SARS-CoV-2 • Coronavirus • Spike protein • 3CLpro • Helicase • Cepharanthine • Molecular docking

## Abbreviations

COVID-19: Corona Virus Disease 2019; CEP: Cepharanthine; SPR: Surface Plasmon Resonance; ORF: Open Reading Frame; $3CL^{pro}$: Main proteinase; S protein: Spike protein; WHO: World Health Organization; Nsp: Nonstructural Protein; ACE2: Angiotensin Converting Enzyme 2; SBDD: Structure-Based Drug Design; BIAcore: Molecules Biomolecular Interaction Analysis core; EDC: 1-Ethyl-3-(3-dimethylaminopropyl) Carbodiimide Hydrochloride; NHS: N-Hydroxysuccinimide; BP: Binding Pocket.

## Introduction

The outbreak of Corona Virus Disease 2019 (COVID-19) caused by SARS-CoV-2, which was identified as a novel coronavirus [1,2] has spread around the world and has serious impact on all aspects of people's lives. The World Health Organization (WHO) reported over 79,000,000 confirmed infected patients, and 1,761,381 SARS-CoV-2 related deaths globally (as of December 29th, 2020) [3]. It is urgent to study and develop new drugs against SARS-CoV-2 [4-6].

Nsp5 protease (also known as 3C-like protease, $3CL^{pro}$), is a key enzyme for 2019-nCoV replication. It is also encoded by the polypeptide and responsible for processing the polypeptide into functional proteins [7,8]. Nsp13 helicase is thought to play crucial roles in many aspects of the viral life cycle which is essential for viral replication [9-12]. The transmembrane viral spike (S) protein locates on the surface of SARS-CoV-2 virus, forming a homotrimer that protrudes out from the surface of the virus body. The transfer of genetic materials of viruses into host cells occurs upon the binding of S protein with its receptor Angiotensin-Converting Enzyme-2 (ACE2) expressing cells [13,14].

Cepharanthine (CEP) is a natural alkaloi (Figure 1), which has been widely used to treat many of the acute and chronic diseases. Recent evidences showed that CEP could be used as a potential antiviral agent for the prevention and treatment of infective diseases including its re-purposing use for coronavirus related diseases [6,15-18]. Therefore, it is of great interest to investigate the anti-coronavirus mechanism of CEP.

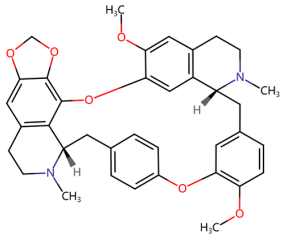

**Figure 1.** Molecular structure of Cepharanthine (CEP).

To investigate the mechanisms underlying the anti-coronavirus effects of CEP, we carried out the molecules Biomolecular Interaction Analysis core (BIAcore) [19-22] of three kinds of proteins (spike, $3CL^{pro}$ and helicase). Besides, we conducted interaction simulation between CEP and the viral S proteins (S1 and S2 subunits), $3CL^{pro}$ and helicase using molecular docking approach. Molecular docking as a Structure-Based Drug Design (SBDD) method makes the best use of the receptor structure information, providing the potential interactions between a ligand and a particular binding site [23-25]. The results showed that CEP exerted its anti-coronavirus effects on different viral proteins.

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**Received:** January 05, 2021; **Accepted:** January 19, 2021 ; **Published:** January 26, 2021

# Materials and Methods

## Binding kinetics of CEP with SARS-CoV-2 spike, 3CL$^{pro}$, and helicase using BIAcore binding assay

Cepharanthine was purchased from MedChemExpress company (Monmouth Junction, NJ, USA), SARS-CoV-2 helicase and 3CL protein were purchased from novoprotein (Shanghai, China), SARS-CoV-2 S1 and S2 protein were purchased from sino biological company (Peking, China). To measure the binding affinities of CEP to SARS-CoV-2 S1, S2, 3CL$^{pro}$ and helicase, SPR technology based BIAcore T200 biosensor was used (BIAcore AB, Uppsala, Sweden) in our study. The S1, S2, 3CL and helicase proteins from SARS-CoV-2 were separately diluted to a final concentration of 20 μg/mL in 10 mM sodium acetate buffer (pH 4.5) and immobilized to the CM5 sensor-chip by using the standard primary amine coupling method to about 6809, 8888, 10125.4 and 18547.7 Response Units (RU) respectively. Running buffer containing 1XPBS-P and 5%DMSO was used during immobilization procedure. The surface was activated by injecting a 1:1 mixture containing 0.2 M EDC and 50 mM NHS for target level of 10000 RU at a flow rate of 20 μL/min. Proteins were injected separately and the surface was then blocked by injecting 1 M ethanolamine at pH 8.5 for seven minutes. All the screening assays were performed over the unmodified dextran surface and the proteins surface. SPR assay was performed at 25°C.

To test the dose-dependent effects of CEP binding to the proteins, a series of dilution of CEP from 0 μM to 100 μM were prepared for the binding assay. A proper control for each dilution regarding pH value, the concentrations of DMSO and substances in buffers were carefully considered. Each sample assay consisted of a 180-s buffer injection and a 300-s dissociation phase, and the blank injection was used to check the carryover effects. The signal was adjusted for non-specific binding of the samples to dextran matrix by subtracting the signal in the reference channel from the signal in the active channel.

## Molecular docking of CEP to SARS-CoV-2 spike, 3CL$^{pro}$, nsp13 helicase

**Preparation of molecular structure of CEP for docking analysis:** We downloaded the SMILES of CEP (CHEMBL code CHEMBL449782) on CHEMBL website [26], and generated its 3D conformations with parameters set by default using OpenEye software [27-29], and then we generated energy-optimized 3D structures of CEP molecule (ligand) by adding Gasteiger charges and merged non-polar hydrogens using AutoDockTools software [30]. As shown in Figure 2, there were six different types of 3D molecular structures of CEP conformations obtained with slight differences in side chains.

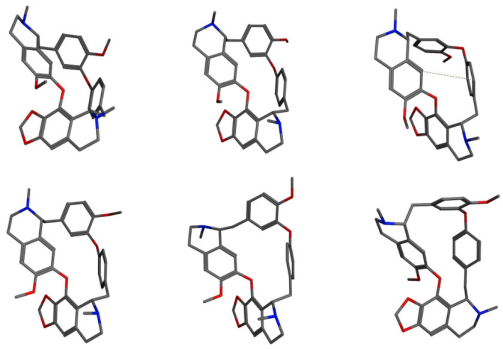

**Figure 2.** The structure of CEP (CHEMBL code: CHEMBL449782) and its six different conformations. Atoms in red represents O atom, and blue represents N atom.

**Structure of SARS-CoV-2 spike, 3CL$^{pro}$ and nsp13 helicase:** For S protein, considering there are two known states of viral S proteins, which might be different from each other in CEP binding, we therefore performed molecular docking using the original separate chains of structures at open (PDB code: 6VYB) and closed state ( PDB code: 6VXX) of SARS-CoV-2.14 S protein contains two subunits (S1 and S2), as shown in Figures 3 and 4, it was reported that the coronavirus infected host cell through the binding of RBD to ACE2 receptor on host cell [31]. Recently published structures 3CLpro (PDB code: 6XMK) [32], nsp13 helicase (PDB code: 6XEZ) [33] with high resolution were used for docking studies, both structures contained the ligand and the protein.

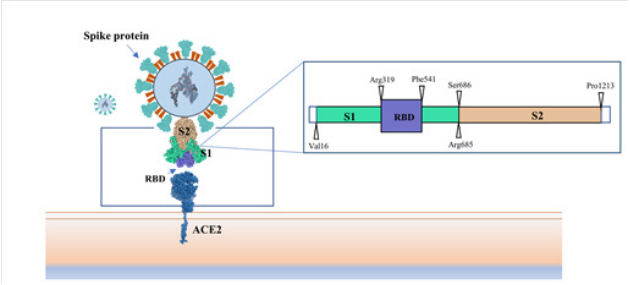


**Figure 3.** Schematic diagram of coronavirus using spike protein to enter host cells by binding to ACE2. S1 indicates V16-R685 with RBD located at; S2 indicates S686-P1213.

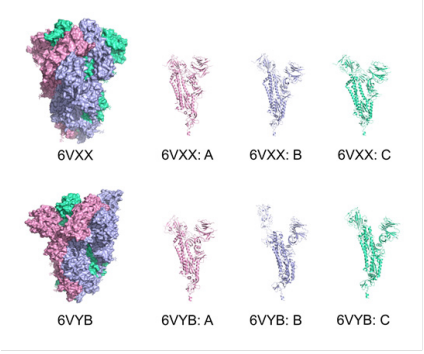


**Figure 4.** Structures of S protein of SARS-CoV-2. The structures of three chains of S proteins in closed state (PDB code: 6VXX ) and the structure of three chains of S proteins in open state (PDB code: 6VYB). Molecular docking analyses were conducted with CEP and separated chains.

**Preparation of protein structures for docking analysis:** To prepare the protein structures for docking, we first examined the chains of protein structure, and then cleared all water molecules and other ligands. For 3CL$^{pro}$ and nsp13 helicase structures, we retained the ligand in the possible binding sites for the following binding pockets detection. For all protein structures, we treated termini as neutral, added H-atoms to the unoccupied valence of heavy atoms and repaired backbone and side chains, finally carried out pre-docking minimization.

**Docking mode and parameters:** The detection of the binding pocket is a key process in protein-ligand docking analysis, The SYBYL-X 2.0 software package has its advantage from this point [34-38]. We used multi-channel functionality to detect potential active site cavities in S protein with protomol bloat and protomol_threshold specified at default values. Since there was a ligand in the active site in the structure of 3CL$^{pro}$, nsp13 helicase and RdRp, the ligand mode was used to find the binding pocket. We then selected the Surflex-Dock GeomX docking mode, which can perform flexible processing on ring molecules, optimized energy before and after docking, and used soft grid. For small molecules, we generated top 200 energy-optimized 3D structures, and kept maximum number of 20 poses per ligand. Other parameters were adopted using default settings.

The Surflex scoring function [39,40], which is based on the binding affinities of protein-ligand complexes, takes into account several terms, including hydrophobic, polar, repulsive, entropic solvation and crush. During docking process, the putative poses of molecules are scored. We can easily get the predicted $K_d$ according to the meaning represented by

the total score. If the score is above 0, it means there is a possibility the ligand will bind to the receptor. When the scores is below 0, we take it as no binding at all [41].

$$\text{Total score} = -\log_{10}(K_d)$$

$$K_d = 10^{(-\text{Total score})}$$

We kept the top 20 docking poses. All three chains of S protein from each structure were used to perform docking simulation with CEP. We intended to find some common binding pockets (or positions) that had a higher possibility to be the actual binding sites and potential binding pockets even if they were not detected in all the three chains. For other proteins, we intended to observe the binding affinity of CEP and the binding pockets in these proteins.

## Results and Discussion

To investigate whether CEP could inhibit coronavirus entry, we performed the binding assay of CEP with the recombinant proteins S1 and S2 using the Surface Plasmon Resonance (SPR) assay. The results demonstrated that CEP could bind to SARS-CoV-2 subunits S1 and S2 in a dose-dependent manner (Figures 5A and 5B). According to the experimental data, we found that the experimental $K_d$ value of CEP with S1 was 3.766 × $10^{-4}$ M and that of with S2 was 3.863 × $10^{-5}$ M, the Rmax were 354.3 RU and 9.865 RU, $Chi^2$ were 0.0286 $RU^2$ and 0.0338 $RU^2$ of S1 and S2, respectively.

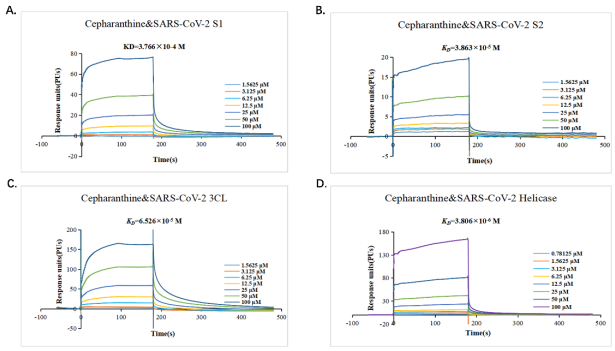


**Figure 5.** The binding kinetics of CEP with different viral proteins. Sensorgrams of (A) CEP&SARS-CoV-2 S1 subunit. (B) CEP&SARS-CoV-2 S2 subunit. (C) CEP&$3CL^{pro}$. (D) CEP&nsp13 helicase. Experiments were performed with CEP at different concentrations.

In addition, we need further research to explore the exact binding sites. We simulated the binding affinity of CEP with the chain A of S protein using computational tools to predict its binding pockets on S protein. In the closed state of S protein (6VXX: A), we found that there were 11 possible Binding Pockets (BPs), among which there were 4 BPs with their total binding affinity scores over 5, and 8 BPs forming outstanding hydrogen bond with CEP. Further analysis revealed that the strongest interaction between CEP with chain A of S protein (6VXX: A) were at BPa3 with highest total binding affinity score 5.92 and hydrogen bond (H-bond) score 2.10 (Binding pocket C in Figure 6A). We then analyzed the binding affinity of CEP with chain B and chain C of S protein (6VXX: A and 6VXX: C), respectively. We found that there were 7 BPs in Chain B and 14 BPs in Chain C which could bind with CEP. The Binding Pocket 3 on chain B (BPb3) and binding pocket 11 on chain C (BPc11) showed the highest binding affinity with CEP in the corresponding chains, respectively (Table 1). The H-bond residues formed between CEP with three chains of closed S protein were also listed in Table 1.

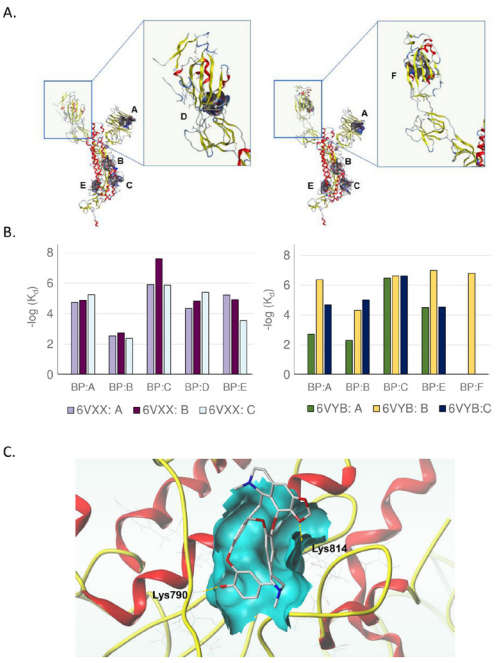


**Figure 6.** The binding pockets and binding affinity of CEP with coronavirus S protein. (A) There are five common binding pockets (BPs) in each chain of 6VXX, among which BP: B, BP: C and BP: E locate on S2 subunit, BP: A and BP: D locate on S1 subunit. When the S protein is in the closed state, CEP can bind to the S1 subunit in the BP: D binding pocket, whereas when the S protein is in the open state, CEP no longer binds to BP: D and instead will bind to the BP: F binding site. (B) Comparison of the predicted binding affinity of CEP with S proteins at different binding pockets. (C) A representative interaction between CEP and BP: C with chain C of S protein at closed state (6VXX). Dashed yellow line represents the H-bonds formed between CEP with residues of Lys790, and with Lys814 of S protein.

Among those binding pockets of CEP with S proteins, we noticed that there were five common binding pockets of CEP on these chains and we marked them as BP: A, BP: B, BP: C, BP: D and BP: E (Figure 6A and Table 1). We compared these five common binding pockets and their binding parameters with CEP. As was shown in Figure 6B, the Binding Pockets C (BP:C) of CEP on the chains of S protein exhibited the highest binding score, indicating that the predicted $K_d$ was lower than $10^{-5}$ M, whereas all the predicted $K_d$ values in BP: A and BP: D were between $10^{-4}$ M and $10^{-5}$ M. The binding $K_d$ of chain C (6VXX: C) on position BP: E with CEP was less than $10^{-4}$ M, suggesting that CEP could weakly binds to that position. Our results also showed that the binding affinity of CEP to S protein was the weakest at the binding pocket B (BP: B) when S protein was in the closed state (6VXX).

In the open state of S protein (6VYB), we discovered that there were 9 possible BPs of CEP with chain A (6VYB: A), 10 BPs with chain B (6VYB: B), 12 BPs with chain C (6VYB: C) (Supplementary Table S1 and Figure S1). Compared to its closed state, no obvious conformational changes could be seen in chain A and chain C of S proteins. Interestingly, chain B exhibits a very significant conformational is also significantly different in the two states. Molecular docking data demonstrated that a binding pocket change when switching from a closed to an open state, and the corresponding combination of CEP and chain B F (BP: F) was found in the open state of S protein, with the disappearance of BP: D, a strong binding pocket of CEP, named as BP: F appears on Chain B (Figures 6A and 6B), which locates at Receptor Binding Domain (RBD) of S protein. As we have known, RBD on S1 subunit is necessary for SARS-CoV-2 to interact with its receptor [42], we therefore reasoned that CEP may exert its antiviral effects by interfering

**Table 1.** Simulated molecular binding affinity of CEP with S protein closed state (6VXX) and the key residues involved H-bond formation.

| Chain name | Binding pocket (BP) | Total score (-log $K_d$) | H-bond | H-bond residues |
|---|---|---|---|---|
| 6VXX: A | a1 (A)* | 4.76 | 1.0 | Asn121, Ser172 |
| | a2 (B)* | 2.56 | 0.02 | ** |
| | a3 (C)* | 5.92 | 2.1 | Lys814, Lys790, Thr791 |
| | a4 (D)* | 4.35 | 0.00 | ** |
| | a5 (E)* | 5.24 | 0.98 | Asp80 |
| | a6 | 4.42 | 1.12 | Thr732 |
| | a7 | 4.66 | 0.04 | ** |
| | a8 | 4.8 | 1.05 | Thr572 |
| | a9 | 5.68 | 1.14 | His1048 |
| | a10 | 4.67 | 1.27 | Lys964 |
| | a11 | 5.59 | 1.77 | Arg1000 |
| 6VXX: B | b1 (A)* | 4.88 | 0.89 | Ser172, Asn121, Arg190 |
| | b2 (B)* | 2.76 | 0.00 | ** |
| | b3 (C)* | 7.62 | 2.97 | Lys790Ser875 |
| | b4 (D)* | 4.84 | 0.00 | ** |
| | b5 (E)* | 4.94 | 1.43 | Arg1107 |
| | b6 | 4.08 | 0.17 | Ser305 |
| | b7 | 3.49 | 1.08 | Gly142 |
| 6VXX: C | c1 (A)* | 5.26 | 0.34 | Asn121 |
| | c2 (B)* | 2.39 | 0.09 | ** |
| | c3 (C)* | 5.88 | 2.23 | Lys814, Lys790 |
| | c4 (D)* | 5.42 | 0.01 | ** |
| | c5 (E)* | 3.55 | 0.00 | ** |
| | c6 | 5.89 | 1.06 | Thr1006 |
| | c7 | 3.39 | 0.00 | ** |
| | c8 | 4.06 | 0.00 | ** |
| | c9 | 5.50 | 0.46 | Arg1000 |
| | c10 | 4.58 | 0.00 | ** |
| | c11 | 7.34 | 0.22 | Gln1106 |
| | c12 | 3.89 | 0.00 | ** |
| | c13 | 4.13 | 1.01 | Arg577 |
| | c14 | 5.75 | 0.00 | ** |

*indicates the common BPs in three chains, there are five common BPs, namely A, B, C, D, and E. **no strong hydrogen bonding interaction.

with the entry of viruses into host cells through the formation of binding pockets with the viral S protein.

As described above, the viral S protein contains two subunits, S1 and S2. Based on the results of simulation, the binding pocket of CEP to the S protein, BP: A and BP: D (or the binding pocket of BP: D shifted to BP: F at the open state), is located on the S1 subunit, and BP: B, BP: C and BP: E are located on the S2 subunit. Our molecular docking simulation data suggested that the predicated $K_d$ values of CEP binding to S1 were at $10^{-4}$-$10^{-5}$ M on an averaged level, whereas the $K_d$ values of CEP with S2 were less than $10^{-5}$ M on an averaged level, all of which were consistent with the experimental $K_d$ values. Although the protein binding assay using recombinant S protein subunits to test the binding affinity of CEP with S protein could not be used to compare the differences in binding to CEP in the open or closed state of the S protein, the experiments results still provided strong evidence to confirm that CEP does bind to S protein. Meanwhile, it demonstrated the reliability of our molecular docking method for predicting affinity between CEP and S proteins.

Previous study showed that CEP could exert its anti-coronavirus effects by acting on the viral S protein to inhibit virus attachment and entry into the host cell. [6,13]. Our current results provided favourable supporting evidence for previous work suggesting that CEP interferes with the entry of the virus into the cell by interacting with S protein although whether the binding of CEP with S protein could affect binding of viral S protein to ACE2 receptor of host cells need further study.

Protein $3CL^{pro}$ is thought to be the protease to cleave the polypeptide ORF1ab, allowing the ORF1ab protein to produce 16 non-structural proteins (nsp1-16) that promote viral replication and hijack the normal function of host cells. Therefore, the protein $3CL^{pro}$ has been taken as a target for anti-coronavirus drug development. SPR assay was used to test the binding affinity of CEP and $3CL^{pro}$, showing that the $K_d$ value was $6.526*10^{-5}$ M, the Rmax was 203.9 RU and the $Chi^2$ was 0.952 $RU^2$. Through molecular docking analysis, we found a binding pocket on protein $3CL^{pro}$ where CEP formed two strong hydrogen bonds with residues Glu166 and Gln189, respectively (Figure 7A). The predicted binding affinity of CEP with $3CL^{pro}$ (Total score=5.0872, predicted $K_d$=$8.181*10^{-6}$ M) is consistent with the data obtained from experimental protein binding analysis by BIAcore (Figure 5C). We speculated that the binding of CEP to protein $3CL^{pro}$ might interfere the cleavage of polypeptide ORF1ab, resulting in indirect down-regulation of virus replication and transcription.

Nsp13 helicase is considered a critical component for coronavirus replication and shares the highest sequence conservation, highlighting their importance for viral viability. In our study, BIAcore test showed the $K_d$ was $3.806*10^{-6}$ M (Figure 5D), the Rmax was 21.03 RU and the Chi2 was 0.133 RU2, which meant CEP had high binding affinity of the protein. After that, we used docking study on CEP and SARS-CoV-2 helicase, found the predicted $K_d$ was $3.160*10^{-6}$ M (Total score=5.50). As we observed the interactions between CEP and the binding pocket (Figure 7B), CEP formed hydrogen bonds with His290, Arg442 and Arg443, these hydrogen bonds made great contributions to the strong interaction between the two. With our computational and biological study on CEP and helicase active site, we think that CEP can bind at this pocket and interfere with the protein activity, which influence viral replication and some other aspects of coronavirus life cycle.

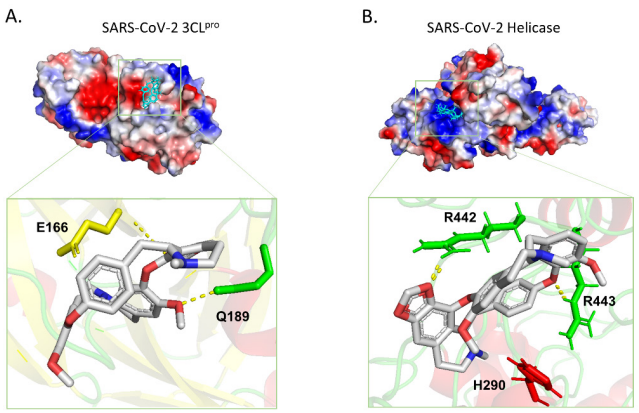


**Figure 7.** Visualization of representative interactions of CEP with $3CL^{pro}$ and helicase). (A) Docking analysis visualization of CEP and $3CL^{pro}$ binding. CEP formed two evident hydrogen bonds with residues Glu166 and Gln189 when CEP was predicted at this pose. (B) CEP binds to nsp13 helicase at its active site, three residues (His290, Arg442, Arg443) can form evident hydrogen bonds with CEP. Dashed yellow line represents the H-bonds.

## Conclusion

The global epidemic of COVID-19 caused by the novel coronavirus SARS-CoV-2 has seriously affected every aspect of people's lives, and with the continuous mutation of the virus, the epidemic is likely to recur every year. In view of this serious situation, it is urgent to develop effective anti-coronavirus drugs. Our previous drug screening data by using over 2400 clinical approved drugs suggested that CEP exerts excellent anti-coronaviral effects both on GX_P2V (the most homologous coronavirus to SARS-CoV-2) and SARS-CoV-2 (unpublished result).

Protein binding assay using BIAcore revealed that CEP could bind to viral S protein at S1 and S2 subunits, $3CL^{pro}$, and helicase. Among these proteins, CEP showed a relatively strong binding affinity to helicase. Docking study also confirmed the binding of CEP to the S protein at different sites with a slight difference when S protein is at open and closed states, and we studied the interactions of CEP to $3CL^{pro}$ and helicase at the active binding pockets. Our computational results were consistent with SPR results which indicated CEP might exert its anti-coronavirus effects not only at viral entry level, but also genome replication and polypeptide processing levels. Although further study needs to be done to decipher the detailed anti-coronavirus mechanism of CEP, our data provided a theoretical basis for the development of CEP as a promising anti-coronavirus drug.

## Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Authors' Contributions

W An. conceived, designed and supervised this study. D. Huo did molecular binding simulation and wrote the manuscript. H. Xu performed the CEP binding kinetics using BIAcore binding assay. W. An, A. Yan and Y. Tong supervised, edited, corrected and proofread the full contents of the paper. All authors read and approved the final manuscript.

## Acknowledgements

This research was supported by a grant from The National Key Research and Development Program of China (2018YFA0903000), the National Natural Science Foundation of China (81672001 and 21675010), Fundamental Research Funds for Central Universities (No. BUCTRC201917, No. BUCTZY2022) and China MoST Emergency Project on COVID-19 (2020YFC0840800).

## References

1. Alexander, Gorbalenya, Baker Susan, Baric Ralph, and de Groot RJ, et al. "The Species Severe Acute Respiratory Syndrome Related Coronavirus: Classifying 2019-nCoV and Naming it SARS-CoV-2." *Nat Microbiol* 5(2020): 536-544.
2. Fan, Wu, Zhao Su, Yu Bin, and Chen YM, et al. "Author Correction: A new Coronavirus associated with Human Respiratory Disease in China." *Nat* 580(2020):E7.
3. World Health Organization. Coronavirus-2019 (Accessed on Dec 29, 2020).
4. Amin, Abdul, and Jha Tarun. "Fight Against Novel Coronavirus: A Perspective of Medicinal Chemists." *Eur J Med Chem* 12(2020):112559.
5. Chengyuan, Liang, Tian Lei, Liu Yuzhi, and Hui Nan, et al. "A Promising Antiviral Candidate Drug for the COVID-19 Pandemic: A Mini-Review of Remdesivir." *Eur J Med Chem* 6(2020):112527.
6. Fan, Hua-Hao, Li-Qin W, Wen-Li L, and Xiao-Ping A, et al. "Repurposing of Clinically Approved Drugs for Treatment of Coronavirus Disease 2019 in a 2019-Novel Coronavirus-Related Coronavirus Model." *Chin Med J* 133(2020):1051-1056.
7. Denison, MR, Zoltick PW, Leibowitz JL, and Pachuk CJ, et al. "Identification of Polypeptides Encoded in Open Reading Frame 1b of the Putative Polymerase Gene of the Murine Coronavirus Mouse Hepatitis Virus A59." *J Virol* 65(1991):3076-3082.
8. Ziebuhr J, Herold J, and Siddell SG. "Characterization of a Human Coronavirus (Strain 229E) 3C-like Proteinase Activity." *J Virol* 69(1995):4331-4338.
9. Rong, Zhang, Li Y, Cowley TJ, and Steinbrenner AD, et al. "The nsp1, nsp13, and M Proteins Contribute to the Hepatotropism of Murine Coronavirus JHM. WU." *J Virol* 89(2015):3598-3609.
10. Leonie C, Van Dinten, van Tol H, Gorbalenya AE, and Snijder EJ. "The Predicted Metal-Binding Region of the Arterivirus Helicase Protein is involved in Subgenomic mRNA Synthesis, Genome Replication, and Virion Biogenesis." *J Virol* 74(2000):5213-5223.
11. Konstantin A, Ivanov, and Ziebuhr J. "Human Coronavirus 229E Nonstructural Protein 13: Characterization of Duplex-Unwinding, Nucleoside Triphosphatase, and RNA 5′-Triphosphatase Activities." *J Virol* 78(2004):7833-7838.
12. G van, Marle, van Dinten LC, Spaan WJ, and Luytjes W, et al. "Characterization of an Equine Arteritis Virus Replicase Mutant Defective in Subgenomic mRNA Synthesis." *J Virol* 73(1999):5274-5281.
13. Shunxin, Wang, Guo F, Liu K, and Wang H, et al. "Endocytosis of the Receptor-Binding Domain of SARS-CoV Spike Protein together with Virus Receptor ACE2." *Virus Res* 136(2008):8-15.
14. Alexandra C, Walls, Park YJ, Tortorici MA, and Wall A, et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." *Cell* 181(2020):281-292.
15. Dong Eon, Kim, Min JS, Jang MS, and Lee JY, et al. "Natural Bis-Benzylisoquinoline Alkaloids-Tetrandrine, Fangchinoline, and Cepharanthine, Inhibit Human Coronavirus OC43 Infection of MRC-5 Human Lung Cells." *Biomolecules* 9(2019):696.
16. Kouki, Matsuda, Hattori S, Komizu Y, and Kariya R, et al. "Cepharanthine Inhibited HIV-1 Cell–Cell Transmission and Cell-Free Infection Via Modification of Cell Membrane Fluidity." *Bioorg Med Chem Lett* 24(2004):2115-2117.
17. Moshe, Rogosnitzky, and Danks R. "Therapeutic Potential of the Biscoclaurine Alkaloid, Cepharanthine, for a Range of Clinical Conditions." *Pharmacol Rep* 63(2011):337-347.
18. Dwight L, McKee, Sternberg A, Stange U, and Laufer S, et al. "Candidate Drugs Against SARS-CoV-2 and COVID-19." *Pharmacol Res* 29(2020):104859.

19. Esa, Stenberg E, Persson B, Roos H, and Urbaniczky C. "Quantitative Determination of Surface Concentration of Protein with Surface Plasmon Resonance Using Radiolabeled Proteins." *J Colloid Interface Sci* 143(1991):513-526.

20. Robert, Karlsson. "SPR for Molecular Interaction Analysis: A Review of Emerging Application Areas." *J Mol Recognit* 17(2004):151-161.

21. Rukang, Zhang, Wang J, Zhao L, and Liu S, et al. "Identification of Novel Inhibitors of Histone Acetyltransferase hMOF through High throughput Screening." *Eur J Med Chem* 157(2018):867-876.

22. Ilaria, Massarelli, Bianucci AM, Chiellini F, and Eidelman C, et al. "Computational Modeling and Surface Plasmon Resonance analyses in the Assessment of Peptide Ligands Interacting with Fibrin $\gamma$ (312–324) Epitope." *Eur J Med Chem* 44(2009):2128-2134.

23. Natasja, Brooijmans, and Kuntz ID. "Molecular Recognition and Docking Algorithms." *Ann Rev Biophys Biomol Struct* 32(2003):335-373.

24. Evanthia, Lionta, Spyrou G, K Vassilatis D, and Cournia Z. "Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances." *Curr Top Med Chem* 14(2014):1923-1938.

25. Leonardo G, Ferreira, Ricardo N. Dos Santos, Glaucius Oliva, and Adriano D Andricopulo. "Molecular Docking and Structure-based Drug Design Strategies." *Molecules* 20(2015):13384-13421.

26. Anna, Gaulton, Hersey A, Nowotka M, and Bento AP, et al. "The ChEMBL Database in 2017." *Nucleic Acids Res* 45(2017):D945-D954.

27. OMEGA 3.1.0.3: OpenEye Scientific Software. (Accessed on October 14, 2020).

28. Benjamin A, Ellingson, Geballe MT, Wlodek S, and Bayly CI, et al. "Efficient Calculation of SAMPL4 Hydration Free Energies using OMEGA, SZYBKI, QUACPAC, and Zap TK." *J Comput Aided Mol Des* 28(2014):289-298.

29. Paul CD, Hawkins, Skillman AG, Warren GL, and Ellingson BA, et al. "Conformer Generation with OMEGA: Algorithm and Validation Using High Quality Structures from the Protein Databank and Cambridge Structural Database." *J Chem Inf Model* 50(2010):572-584.

30. Garrett M, Morris, Huey R, Lindstrom W, and Sanner MF, et al. "AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility." *J Comput Chem* 30(2009):2785-2791.

31. Donald J, Benton, Wrobel AG, Xu P, and Roustan C, et al. "Receptor Binding and Priming of The Spike Protein of SARS-CoV-2 for Membrane Fusion." *Nat* 588(2020):327-330.

32. Athri D, Rathnayake, Zheng J, Kim Y, and Perera KD. "3C-like Protease Inhibitors Block Coronavirus Replication in vitro and Improve Survival in MERS-CoV–Infected Mice." *Sci Transl Med* 12(2020): eabc5332.

33. Chen J, Malone B, Llewellyn E, and Grasso M. "Structural Basis for Helicase-Polymerase Coupling in the SARS-CoV-2 Replication-Transcription Complex." *Cell* 182(2020):1560-1573.

34. Fulong, Wu, Lv Q, Wang Z, and Li D, et al. "3D-QSAR, Molecular Docking and Molecular Dynamics Studies of 2, 4-Diarylaminopyrimidine Analogues (DAAP Analogues) as Potent ALK Inhibitors." *Lett Drug Des Discov* 14(2017):270-286.

35. SYBYL-X 2.0, TRIPOS, Inc. (A Certara Company), 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.

36. Shrinivas D, Joshi, Kumar D, More UA, and Aminabhavi TM. "Docking, CoMFA, and CoMSIA Analyses of Phenoxy Triazole Derivatives as Enoyl-ACP Reductase Inhibitors for Escherichia Coli." *Med Chem Res* 23(2014):4932-4955.

37. Shrinivas D, Joshi SD, Dixit SR, and Kirankumar MN, et al. "Synthesis, Antimycobacterial Screening and Ligand-Based Molecular Docking Studies on Novel Pyrrole Derivatives Bearing Pyrazoline, Isoxazole and Phenyl Thiourea Moieties." *Eur J Med Chem* 107(2016):133-152.

38. Saba Kauser, Shaik, Kamble RR, Somagond SM, and Devarajegowda HC, et al. "Tetrazolylmethyl Quinolines: Design, Docking Studies, Synthesis, Anticancer and Antifungal Analyses." *Eur J Med Chem* 128(2017):258-273.

39. Gregory L, Warren, Andrews CW, Capelli AM, and Clarke B, et al. "A Critical Assessment of Docking Programs and Scoring Functions." *J Med Chem* 49(2006):5912-5931.

40. Ajay N, Jain. "Scoring Functions for Protein-Ligand Docking." *Curr Protein Pept Sci* 7(2006):407-420.

41. Ajay N, Jain. "Scoring Noncovalent Protein-Ligand Interactions: A Continuous Differentiable Function Tuned to Compute Binding Affinities." *J Comp Aided Mol Des* 10(1996):427-440.

42. Gregory J, Babcock, Esshaki DJ, Thomas WD and Ambrosino DM. "Amino Acids 270 to 510 of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein are Required for Interaction With Receptor." *J Virol* 78(2004):4552-4560.

**How to cite this article:** Huo, Donghui, Wenlin An, Huan Xu, and Aixia Yan, et al. "Investigation of Cepharanthine Binding with Viral Proteins Reveal its Potential Targets against Coronavirus." *Virol Curr Res* 5 (2021): 124