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Article

Discovery of Natural Products to SARS-CoV-2 with Comparative Molecular Docking and Moderate Cell Proliferation Inhibition In Vitro

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Abstract: Based on screening in computational biology and biological in vitro assays, five natural products isolated from extracts of the herbal medicine toad skin, such as cinobufagin (CBFi), bufalin (BFi), arenobufagin (ABFi), telocinobufagin (TBFi), bufotalin (BFTi), were subjected to molecular docking calculations with the use of SARS-CoV-2 main protease (PDB 6LU7 and 7BTF) and top-scoring ligand-receptor complexes were obtained. The results showed that the binding energy of ABFi to the 3CL protein was -17.044kcal/mol, which was higher than CBFi and TBFi. However, the binding energy of ABFi to the RdRp protease was -23.250 kcal/mol, which was much lower than that of CBFi and TBFi, EVEN lower than that of ABFi to the 3CL protein. ABFi also has polar interactions with amino acids such as Glu811, Ser814, Ser681 and Thr680 of RdRp enzyme. The results revealed that ABFi had a moderate inhibitory effect on the cell proliferation of SARS-CoV-2 in vitro, with an inhibition rate of 61.12%, even weaker than Remdesivir. This new discovery provides us with new ideas for in-depth studies on the development of natural products with this class of structural generalizations as inhibitors of SARS-CoV-2, and provides an experimental basis for the next step of mechanistic studies.

Keywords: Natural products; molecular docking, antiviral activity; SARS-CoV-2; cell proliferation inhibition

1. Introduction

Traditional Chinese medicines (TCMs) is widely involved in the treatment of COVID-19, traditional medicines were found to help 90% of the 214 individuals who were administered recover1. In addition, certain traditional herbal treatments protected healthy people from SARS-CoV-2 infections[1,2] and enhanced the care of individuals with moderate or severe signs. Several plant-based therapeutic regimens have been suggested to alleviate the COVID-19 symptoms[3–5]. Different forms of terpenoids appear to have potential effects in inhibiting viral replication[6,7] and might be used in future investigations[8–13]. Natural products are physiologically active and as such a source of new molecular entities drugs (NMEs)[14–16] can be very instructive in accelerating the development of new drugs[17]. Natural products can be used as preventative[18] and therapeutic agents[19] in the battle against coronavirus20, according to prior research[21–23]. The integrative TCMs[24] and Western medicine treatment in relieving symptoms, improving hypoxemia, or accelerating the absorption of lung lesions[25], might provide a positive references for patients with COVID-19. References[26–29] on the mechanism in the treatment of COVID-19 by TCMs based on

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network pharmacology had greatly promoted the its reliability and safety[30,31]. The development of drugs with specific anti-SARS-CoV-2 viruses from thousands of natural active products remains a tedious but significant endeavour that deserves continuous research and exploration by drug scientists.

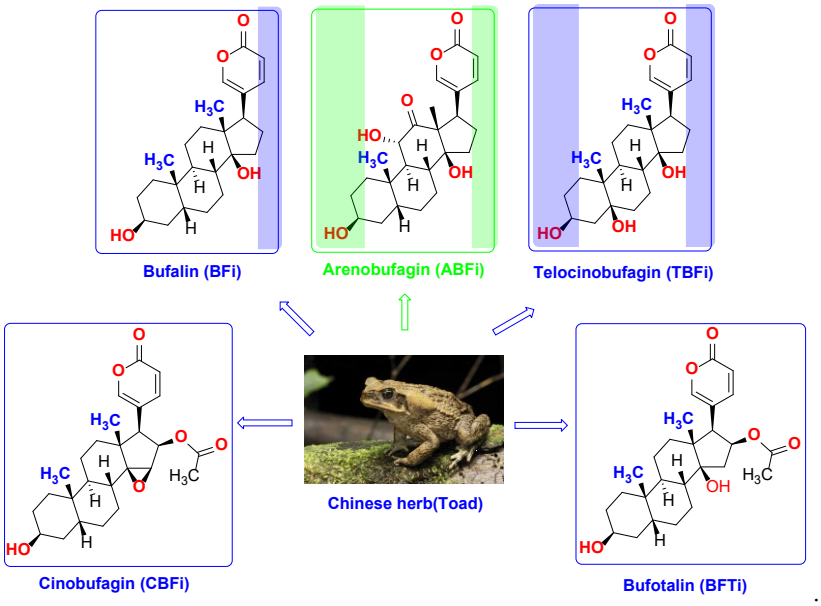

Figure 1. Structure of the Natural Products from Chinese Herbs(Toad).

The antiviral effect of Huachansu injection (Chinese name: Chansu)[32] is the mechanism of nucleoside antiviral blocking viral RNA or DNA replication, especially against hepatitis B virus[33,34]. In addition, Huachansu injection is widely used in the treatment of human respiratory tract infections or viral pneumonia[35,36]. A clinical study of Huachansu injection combined with penicillin in the treatment of acute bronchitis in children was reported[37–40]. Chansu were reported[41,42] as the main antiviral drug used for poultry respiratory infections caused by coronaviruses[43], such as pigs, ducks and chickens[44–47].

In recent years, the screening of natural product molecules for pharmacological activity by molecular docking tests has been increasingly reported, which is especially valuable to be utilised in natural product drug discovery, and can greatly shorten the drug development time[48–51]. The exploration of natural compounds with anti-SARS-CoV-2 activity via inhibition of SARS-CoV-2 Mpro was reported by Bharadwaj[52]. Herein, several natural products (CBFi, BFi, ABFi, TBFi, BFTi) (Figure 1) were subjected to molecular docking calculations with the use of SARS-CoV-2 main protease (PDB 6LU7 and 7BTF). According to the computational results, the cell proliferation activity test of the selected natural products (CBFi, ABFi, TBFi) to SARS-CoV-2 was carried out through the use of the BetaCoVIJS02/Human/2019 strain of SARS-CoV-2 and Vero-E6 cells. The docking results showed that the binding energy of ABFi to the RdRp protease was -23.250 kcal/mol, which was much lower than that of CBFi and TBFi, EVEN lower than that of ABFi to the 3CL protein. The molecular docking results of ABFi to the RdRp protease of SARS-CoV-2 was helpful to analyze the cell proliferation results of ABFi to SARS-CoV-2 with a strong inhibitory effect. The proliferation results revealed that ABFi had a stronger inhibitory effect on the cell proliferation of SARS-CoV-2 than others in vitro, with an inhibition rate of 61.12%. The structural optimization of the molecule to improve the inhibition rate of cell proliferation and the search for a suitable animal model to obtain experimental data on the cell proliferation of ABFi on the RdRp protease of SARS-CoV-2 are in the next plan.

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2. Results and Discussion

2.1 Molecular Docking Results of Compounds with 3CL

The binding energy of CBFi to 3CL was -21.460 kcal/mol, which was lowest than the binding energy of TBFi (-18.939 kcal/mol), BFTi (-17.787 kcal/mol), ABFi (-17.044 kcal/mol), BFi (-11.912 kcal/mol) (Table 1). Due to binding energy is one of the main bases for reflecting the ability of a compound molecule to bind to a protein, the binding ability with 3CL is revealed as follow: CBFi > TBFi > BFTi > ABFi > BFi. CBFi was binding to the substrate binding pocket of 3CL hydrolase, which was mainly composed of P1, P2 and P3 pocket regions. The structure of CBFi occupied the P1 and P3 pockets, however, the P2 pocket is not occupied by the inhibitory group which might be one of the reasons why the binding energy of CBFi to 3CL is higher (Figure 2). The carbonyl group on the a ring(words was labelled in the red by Figure 3c) and the hydroxyl group on the e ring (words was labelled in the red by Figure 3c) of CBFi form hydrogen bonds with Cys145 and Thr190 of 3CL, respectively. CBFi also forms hydrophobic interactions with Phe140, Leu141, Pro168, Asn142, Glu166, Gln189, His163 and Ser144 or other amino acids have polar roles (Figure 3).

Table 1. Docking binding energy of each compound and 3CL

Cmp	**CBFi**	BFi	**ABFi**	**TBFi**	BFTi
Binding Energy (kcal/mol)	-21.460	-11.912	-17.044	-18.939	-17.787

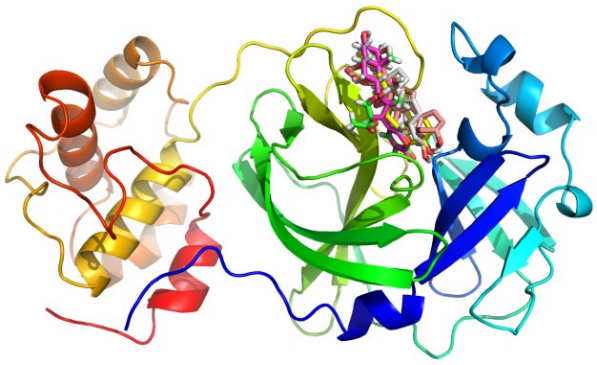

Figure 2. The compound binds to the substrate binding pocket of 3CL hydrolase

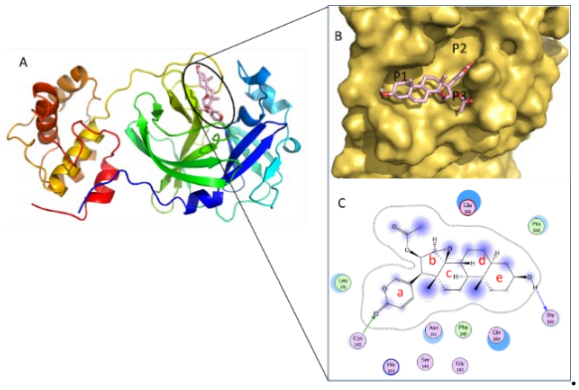

Figure 3. The compound binds to the substrate binding pocket of 3CL hydrolase. (A). The binding site of CBFi and 3CL; (B). The binding surface of CBFi and 3CL; (C). The specific binding mode of CBFi and 3CL

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2.2 Molecular Docking Results of Compounds with RdRp

The binding energy of ABFi to RdRp protein was lowest, which was -23.250 kcal/mol, the binding energy of BFi to RdRp protein was the highest, which was -8.949 kcal/mol (Table 2). ABFi was binding to the substrate NTP binding pocket of RNA (Figure 4). The carbonyl group on the c-ring of ABFi has metal chelation with the hydroxyl group and the two Mg2+ of the RdRp enzyme (Figure 5). The hydroxyl group adjacent to the b and c-rings (words was labelled in the red by Figure 5c) forms a hydrogen bond with Arg553 and Arg555. ABFi also has a polar effect with amino acids such as Glu811, Ser814, Ser681 and Thr680.

Table 2. Docking binding energy of compounds and RdRp of SARS-CoV-2.

Cmp	**CBFi**	BFi	**ABFi**	**TBFi**	BFTi
Binding Energy (kcal/mol)	-19.450	-8.949	-23.250	-23.019	-14.378

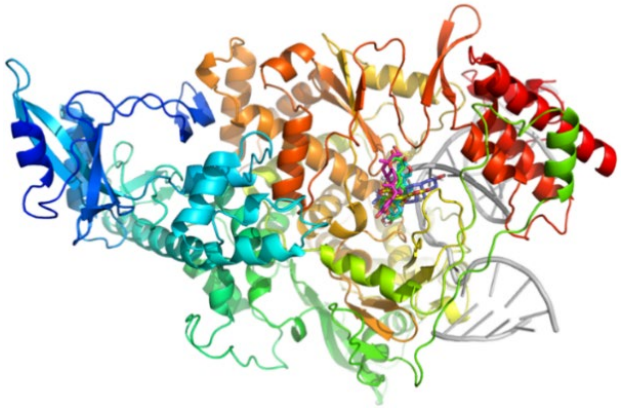

Figure 4. The compound binds to the NTP binding site of RdRp

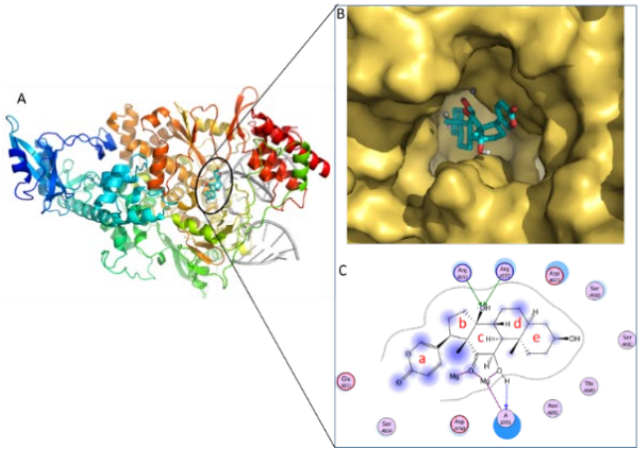

Figure 5. The compound binds to the NTP binding site of RdRp. (A). The binding site of ABFi and RdRp;(B). The binding surface of ABFi and RdRp;(C). The specific binding mode of ABFi and RdRp

2.3 In Vitro Proliferation Inhibition of SARS-CoV-2

We found that the ethylene oxide structure formed by O on the carbon atoms of the b,c ring bridge of CFBi (Figure 6) increases the spatial effect, so that the carbonyl O atoms on the a ring and the OH atoms of the e ring maximally form hydrogen bonds with the amino acids in the pocket, which has a certain functional effect and contributes to the improvement of the inhibition of cell proliferation activity.

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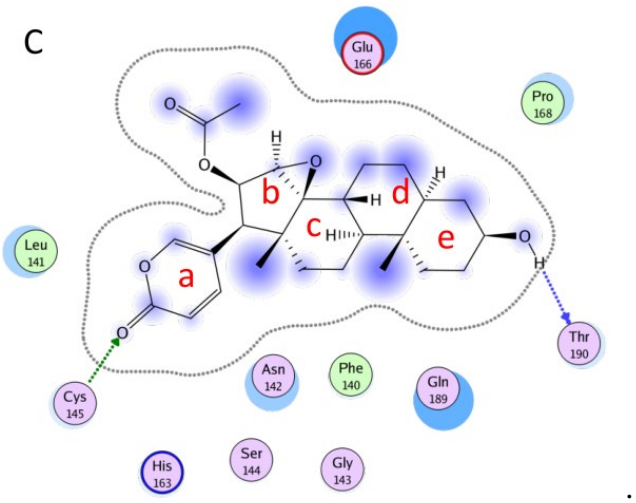


Figure 6. The specific binding mode of CBFi and RdRp

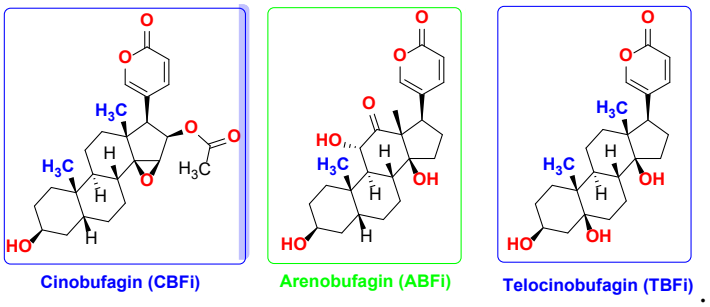
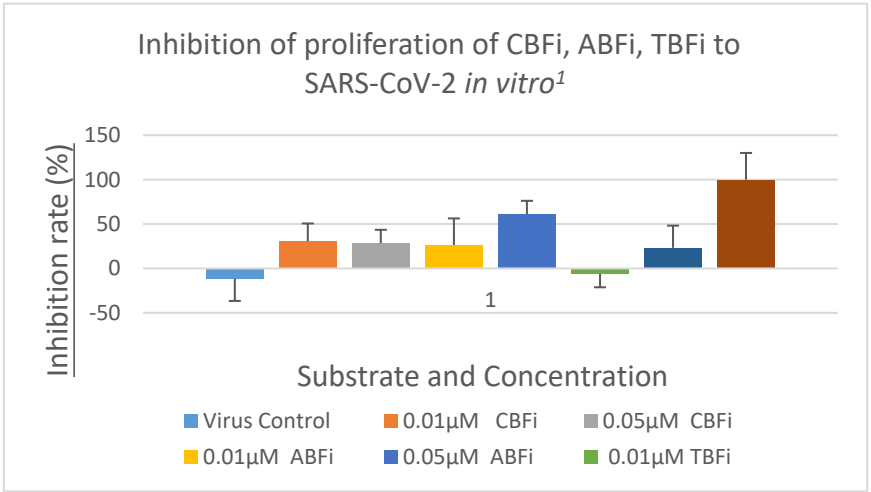


Figure 7. The structure of selected Natural Products used to Proliferation inhibition of SARS-CoV-2 in vitro

This inference, which we can get verification in the docking test of ABFi,TBFi, is one of the reasons why we chose these three natural products for cell proliferation assay (Figure 7); another reason is that the special requirements of Bio-Safety Level 3 Lab (BSL-3) are needed to carry out the cell proliferation inhibition assay of SARS-CoV-2 viruses, which leads to the cost of the expensive inability to perform cell proliferation inhibition assays for five natural products.



[1] 5 μM Remdesivir

Figure 8. Inhibition of proliferation of CBFi, ABFi, TBFi to SARS-CoV-2 in vitro.

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The MTT (5 mg/mL) 20 μl was added into the 96-well culture plate and continued to culture at 37 ℃ for 4 h. Culture medium was replaced by 100 μL DMSO to each well to dissolve crystallization for 20 min. The absorbance values were measured at 490 nm and the proliferation inhibition rate of each group were calculated respectively. The results showed that CBFi, ABFi and TBFi had no obvious toxic effects on the growth of Vero-E6 cells at the concentrations of 0.01 μM and 0.05 μM, . The inhibitory effect of ABFi on the proliferation of new coronavirus in vitro was about 61.12 % (Figure 8).

2.4 Results of Cytotoxicity assay of Compounds on MDCK Cells

The original solution was used as the mother liquor of the test drug, frozen and stored in -20℃ refrigerator for spare, when used, the MEM cell maintenance solution (1% fetal bovine serum, 2μg/ml TPCK-treated trypsin, 100U/ml penicillin and streptomycin) was diluted into 6-8 concentrations according to a 4-fold gradient to carry out the evaluation of the efficacy and toxicity of the test.

Compounds showed significant cytotoxicity against MDCK cells at higher dose conditions, with TC50 in the range of 0.02-24.1 μM; the positive control drug oseltamivir showed TC50 > 40 μg/mL against MDCK cells; the positive control drug ribavirin showed TC50 of 50 μg/mL against MDCK cells; and the experimental results of the respective samples are detailed in Table 3.

Table 3. Docking binding energy of compounds and RdRp of SARS-CoV-2.

Sample name	TC50	IC50	SI
CBFi	0.07	0.01	7.10
BFi	0.71	0.04	17.75
ABFi	0.09	0.01	11.25
TBFi	**0.29**	**0.01**	**48.00**
BFTi	0.02	0.04	0.48

[1] TC50: half toxic concentration of the drug; IC50: half inhibitory concentration of the drug on the virus; SI: selection index, SI = TC50/IC50.

3. Materials and Methods

The structural formulae of the five natural products (CBFi, BFi, ABFi, TBFi, BFTi) were modelled using Schrodinger 2015-3 software, calculated using Meastro, then subjected to ligand preparation, and finally proton hydrogenation and conformational optimisation under pH 7.0 ± 2.0, OPLS3 force field conditions. The chiral characteristics of the original compound are maintained under the conditions, and each molecule can produce up to 32 conformations. The crystal structures of 3CL (PDB ID: 6LU7) and RdRp (PDB ID: 7BTF) of SARS-CoV-2 were obtained from the PDB database, and receptor preparation was also performed in Schrodinger 2015-3 (calculated using Meastro, then ligand preparation), including removal of water molecules, hydrogenation. Hydrogen bonds were optimized by the PROPKA method at pH 7.0, and OPLS3 force field was used to optimize the protein with restricted energy so that the RMSD of heavy atoms converged to 0.3 Å. The 3CL protein of SARS-CoV-2 was used to the amino acids within 15 Å around the original ligand in 6LU7 as the active site to generate a grid file; the RdRp enzyme of SARS-CoV-2 was used to the NTP binding pocket as the docking site Generate a grid file. The Schrodinger 2015-3 (calculated using Meastro, then Glide Docking module) was used for molecular docking, and SP (Standard Precision) was used as the scoring function. After docking, energy optimization was performed to calculate the binding energy of compounds and receptors, and to analyze compounds and receptors.

The culture of SARS-CoV-2 and the infection experiment of Vero cells were completed in Bio-Safety Level 3 Lab (BSL-3) of the fifth Institute of The Academy of Military Medical Sciences. Vero E6 cells was plated into a 96-well dishes and in an atmosphere of 5% CO2 at 37°C. Discarding the

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medium when Vero E6 cells grow into a monolayer. After rinsing by fresh DMED, the cells were co-incubated with the virus and compound to be measured. Briefly, a total of nine groups were setting, normal Vero E6 cells control, virus control (negative control), CBFi (0.01 μM, 0.05 μM) groups, ABFi (0.01 μM, 0.05 μM) groups, TBFi (0.01 μM, 0.05 μM) groups and remdesivir (5 μM, positive control) group.

4. Conclusions

The carbonyl group on the c-ring of ABFi in the substrate NTP binding pocket of RNA has metal chelation with the hydroxyl group and the two Mg2+ of the RdRp enzyme. It formed hydrogen bonds with Arg553 and Arg555 by the hydroxyl group adjacent to the b and c rings, so its binding energy barrier with the receptor is lower, its binding energy is -23.250 kcal/mol. The binding energy of TBFi is -23.019 kcal/mol due to the substrate binding P1, P2 and P3 pocket of 3CL hydrolase of SARS-CoV-2, and the structure of CBFi occupied the P1 and P3 pockets. The carbonyl group on the a ring and the hydroxyl group on the e ring of CBFi form hydrogen bonds with Cys145 and Thr190 of 3CL. Respectively, the hydrophobic interactions with Phe140, Leu141 and Pro168 was formed and a polar effect with Asn142, Glu166, Gln189, His163 and Ser144 and other amino acids might be one of the reasons for the higher binding energy of CBFi than that of ABFi. CBFi, ABFi, TBFi were used to the cell inhibition test of SARS-CoV-2 with the BetacoVIJS02/Human/2019 under the condition of 0.05μM concentration. The highest cell proliferation inhibition rate of ABFi was up to 61.12%, better than the CBFi, but which was slightly worse than the positive control drug.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: title; Table S1: title; Video S1: title.

Author Contributions: Conceptualization, Y.S.W.; methodology, Y.S.W. and Z.B.; software, X.P.L.; validation, Y.S.W. and Y.L.; data curation,W.Z.X., G.S., C.Z.Y., L.S.N.; writing—original draft preparation, Y.S.W., D.H.Z., Z.J.M. and H.X.M.; supervision, Y.S.W., Z.J.F., and T.H.; project administration,Y.S.W., W.X.H. and B.W.J.; funding acquisition, Y.S.W. and Z.B. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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