Medicinal Plant Biology

ARTICLE

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https://doi.org/10.48130/MPB-2023-0010

Medicinal Plant Biology **2023**, 2:10

Discovery of anti-SARS-CoV-2 agents from commercially available flavor *via* docking screening

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Abstract

Flavor and spice are largely consumed in food, cosmetics, and pharmaceutical industries. A novel coronavirus, recently named the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), was first identified in humans in Wuhan, China in 2019. This study is to examine whether flavor components can prevent humans from SARS-CoV-2 infection. Given that the drugable antiviral target ACE2 receptor and viral main protease (M^{pro}) were reported, 169 compounds were screened against these two targets by using autodock vina. According to our docking screening, 10 antiviral components, including glycyrrhizic acid, theaflavin 3,3'-digallate, agnuside, fenflumizole, angelicide, sageone, oleanic acid, benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate, glycerol ester of rosin, and endere S can directly bind to both host cell target ACE2 receptor and viral target M^{pro}, indicating their potential for SARS-CoV-2 treatment. In addition, experimental verification found that theaflavin 3,3'-digallate show significant inhibit M^{pro}/$3CL^{pro}$ activity.

Citation: Zhang J, Shen X, Li Y, Yan Y, Wang Y, et al. 2023. Discovery of anti-SARS-CoV-2 agents from commercially available flavor *via* docking screening. *Medicinal Plant Biology* 2:10 https://doi.org/10.48130/MPB-2023-0010

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), previously named as 2019 novel coronavirus (2019-nCoV), is a positive-sense, single-strand RNA coronavirus. SARS-CoV-2 caused an ongoing outbreak of coronavirus disease 2019 (COVID-19) occured in December 2019[1]. Given that the emergence of SARS-CoV-2, drug repurposing study was immediately conducted by both virtual screening and cell-based screening, which provided several promising antiviral agents from approved drugs[2]. Flavor and spice have been widely used in China and India since ancient times, some spice such as ginger and pepper were proposed to be beneficial in countering against dampness evil in the human body according to the philosophy of traditional Chinese medicine, however, whether these components do prevent virus infection is still questionable. Herein, contributions were made to identify potential anti-SARS-CoV-2 agents from flavor ingredients by employing molecular docking screening.

As expected, angiotensin converting enzyme 2 (ACE2) receptor of host cells could be directly bound to spike protein of SARS-CoV-2[3]. Inhibition of ACE2 catalytic pocket by small molecules could change the conformation of ACE2, indicating that ACE2 inhibitor could block SARS-CoV-2 entry[4,5]. Therefore, in this effort, the ACE2 receptor was used as a protein model in accomplishing a quick-identification of the entry inhibitors of SARS-CoV-2.

Suppression of viral replication is also an appropriate aspect for exploring antiviral drugs besides the blockade of viral entry[6]. Considering that SARS-CoV-2 M^{pro} is closely related to the processing of two replicase polyproteins which are required in mediating viral replication and transcription, the M^{pro} inhibitor could be seen as a sally port to discover antiviral drugs[7–15]. We consequently docked flavor components to SARS-CoV-2 M^{pro} (PDB: 6LU7) whose structure was clarified by crystal data[16–18], to explore more antiviral replication agents.

Materials and methods

Molecular docking

The three-dimensional structure of ligands (aroma oil components) were generated by CORINA online service (https://demos.mn-am.com/corina_interactive.html). The experiment process is the same as we previously described[19]. The docking results were ranked by the binding free energy. The binding results were graphically presented by using PyMOL1.3 (Schrödinger, LLC).

Ligands preparation for docking screening

For ligands library establishment, the SMILE format of phytochemicals was compiled from Pubchem. The SMILES format of compounds was converted to PDB format by CORINA online service (https://demos.mn-am.com/corina_interactive.html). The PDB format of compounds was then converted to PDBQT format by AutoDock Tools 1.5.6 (The Scripps Research Institute, CA, USA).

Target proteins preparation for docking screening

The crystal structure was obtained from the Protein Data Bank for receptor preparation. Chimera 1.7mac (UCSF Resource for Biocomputing, Visualization, and Informatics, CA, USA)

eliminated both ligands and water molecules from target proteins. AutoDock Tools 1.5.6 (The Scripps Research Institute, CA, USA) was then used to add hydrogen and Kollman Charges to the target protein. The target protein's atoms were assigned the AD4 type, and the changed protein was transferred to PDBQT format for docking screening.

Docking parameters validation

The docking parameters for AutoDock Vina were left at their defaults. The grid box was 25 Å × 25 Å × 25Å, encompassing the inhibitor binding pocket. The docking results were ordered according to the binding free energy. For parameter validation, we retrieved the inhibitors from the original protein models (Supplemental Fig. S1). Our docking simulation revealed that the predicted conformations of inhibitors are close to the experimental conformations of inhibitors. Furthermore, the inhibitors had a high binding score.

Chemicals

The following substances were purchased by Shanghai Bidepharmatech Co., Ltd (Shanghai, China): Glycyrrhizic acid, theaflavin 3,3'-digallate, oleanic acid, and benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate.

M^{pro}/$3CL^{pro}$ inhibitory assay

M^{pro}/$3CL^{pro}$ activity *in vitro* was measured by using the M^{pro}/$3CL^{pro}$ Inhibitor Screening Kit (Beyotime, Cat No. P0312S, China). In brief, 2019-nCoV M^{pro}/$3CL^{pro}$ was diluted by Assay Buffer, then pre-incubated with compounds for 10 min at 37 °C, then the substrate was added for another 5 min incubation at 37 °C. The optical density (OD) values were then measured using Microplate Reader (BioTek, Synergy 2) with the excitation wavelength at 360 nm and the emission wavelength at 460 nm, respectively. GraphPad Prism5 (GraphPad Software Inc.) was used to analyze the data. Ebselen was positive control. All experiments were carried out in triplicates.

Statistical analysis

The statistical data were obtained from biological triplicates. Statistical analysis was performed by t-Test and ANOVA for multiple groups. $p < 0.05$ was considered significant difference '*'; $p < 0.01$ was considered very significant difference '**'.

Results

One hundred and sixty nine flavor components in total were docked to two drug targets. The top 10 hits including glycyrrhizic acid, theaflavin 3,3'-digallate, agnuside, fenflumizole, angelicide, sageone, oleanic acid, benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate, glycerol ester of rosin, and endere S, are summarized in Tables 1 & 2. Interestingly, these components were also characterized in traditional Chinese medicine with the exception of fenflumizole and benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate. As for the ACE2 receptor, 11 out of 169 compounds exhibited good binding affinities (<−9 kcal/mol) are glycyrrhizic acid, theaflavin 3,3'-digallate, agnuside, fenflumizole, angelicide, sageone, oleanic acid, benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate, glycerol ester of rosin, endere S, and testosterone. Of which, glycyrrhizic acid and oleanic acid are triterpenoidal acids. Moreover, three out of 169 compounds, glycyrrhizic acid, theaflavin 3,3'-digallate, and agnuside, inhibit M^{pro} with strong binding affinities (<−9 kcal/mol). Of note, the properties of glycyrrhizic acid against SARS-CoV-2 have been pointed out in our previous investigation[19]. It is also interesting that theaflavin 3,3'-digallate which is a phenolic compound generated from ginger, was found to be interactive with both ACE2 receptor and M^{pro} with considerable biding affinities. It is worth mentioning that the current mainstream view about SARS-CoV-2 is the observation of inflammation storms leading to death. However, we believe that there should be typical 'free radical storm' or severe oxidative stress during SARS-CoV-2 in view of biomedical or chemical defense. Normally, inflammation and free radical including reactive oxygen species are powerful weapons for human body against evils. Our present finding of theaflavin 3,3'-digallate and previous results regarding to

Table 1. Top 10 flavor agents docking results.

Ligand	Binding energy		
	1R4L	6LU7	Sum
Glycyrrhizic acid	−9.6	−9.3	−18.9
Theaflavin 3,3'-digallate	−8.3	−10	−18.3
Agnuside	−9.6	−8	−17.6
Fenflumizole	−9.5	−7.7	−17.2
Angelicide	−9.7	−7.1	−16.8
Sageone	−9	−7.8	−16.8
Oleanic acid	−9.4	−7.3	−16.7
Benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate	−9.4	−7.1	−16.5
Glycerol ester of rosin	−9.4	−6.9	−16.3
Endere S	−9.4	−6.8	−16.2

Table 2. Key residues for the inhibitor binding.

Ligand	Key residues	
	1R4L	6LU7
Glycyrrhizic acid	Arg273, His345, Ala348, Thr365, Arg518	Phe140, Gly143, His163, Gln189
Theaflavin 3,3'-digallate	Asn149, Asn154, Arg273, Asn277, His345, Lys363, Thr365, Arg518	Ser46, Tyr54, Phe140, Ser144, Cys145, Gly143, His163, Glu166, Gln189, Thr190
Agnuside	His345, Thr371, Glu406, Arg518	Thr24, Thr45, Leu141, Gly143, Ser144, Cys145, Glu166
Fenflumizole	His345	None
Angelicide	None	Gly143, Ser144, Cys145
Sageone	Arg273, His345	Gly143, Ser144, Cys145
Oleanic acid	Glu406	none
Benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate	Arg273, His345, Thr445, Tyr515	Gly143, Ser144, Cys145, His163
Glycerol ester of rosin	Arg273, His374, Arg518	Gly143, His163
Endere S	Arg273, Arg518	Gly143, His163

phenolics as hits[19] prompted us to consider their effects against SARS-CoV-2 might be also associated with their antioxidant potency. With this rationale, we tentatively suggest that marketed antioxidants such as edaravone and intake of ginger with high content of phenolics might be beneficial for SARS-CoV-2 patients. Unfortunately, this hypothesis and therapeutic approach has been largely ignored during the SARS-CoV-2 outbreak. Last but not the least, it is not surprising that the other flavor agents are not hits with super good binding energy (<−10 kcal/mol) (Supplemental Table S1) due to the difficulty of their relatively simple chemical structures in occupying the whole catalytic pocket and provide high binding affinities.

In addition, glycyrrhizic acid, theaflavin 3,3'-digallate, oleanic acid, and benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate were tested *in vitro* M^{pro}/$3CL^{pro}$ activity by using M^{pro}/$3CL^{pro}$ Inhibitor Screening Kit (Fig. 1)[20]. The results showed that all four compounds exhibited inhibitory activity, with theaflavin 3,3'-digallate being the strongest, and further, we tested theaflavin 3,3'-digallate and ebselen with IC_{50} values of 16.56, 0.33 μM (Fig. 2), respectively. The current study verifies the consistency of molecular docking and experimental results, but further studies need to be performed.

Conclusions

Drug repurposing is a common strategy to fight novel coronavirus. However, most of drug repurposing studies are about FDA approved drugs. Flavor ingredients were widely used to prevent plague in ancient China and India, and are commercially available in abundance. We were curious whether flavor ingredients can also prevent SARS-CoV-2 at this time. According to docking screening, we found that flavor ingredients including glycyrrhizic acid, theaflavin 3,3'-digallate, and agnuside are most likely to directly bind to both viral M^{pro} and ACE2 receptor, lending a hand for countering against SARS-CoV-2. In addition, experimental verification found that glycyrrhizic acid, theaflavin 3,3'-digallate, oleanic acid, and benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate show inhibit M^{pro}/$3CL^{pro}$ activity and are worth further study.

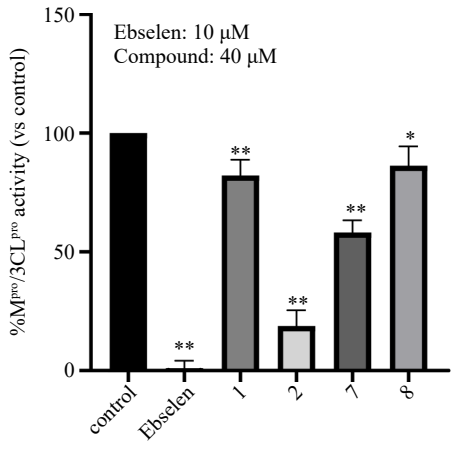

Fig. 1 Inhibitory effects of compounds from AGE against SARS-CoV-2 M^{pro}. (1 = glycyrrhizic acid, 2 = theaflavin 3,3'-digallate, 7 = oleanic acid, and 8 = benzyl (3-fluoro-4-morpholine-4-yl phenyl) carbamate).

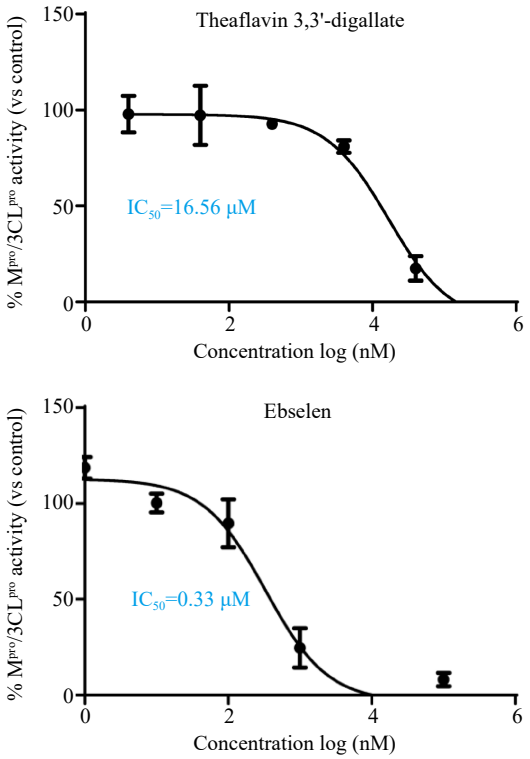

Fig. 2 IC_{50} of theaflavin 3,3'-digallate and ebselen for AGE against SARS-CoV-2 M^{pro}.

Acknowledgments

This study was supported by the National Science Fund for Distinguished Young Scholars (81525026) and National Natural Science Foundation of China (81903875).

Conflict of interest

The authors declare that they have no conflict of interest. Yong-Xian Cheng is the Editorial Board member of *Medicinal Plant Biology*. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and his research groups.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/MPB-2023-0010)

Dates

Received 7 January 2023; Accepted 25 June 2023; Published online 5 September 2023

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395:497−506
2. Wang M, Cao R, Zhang L, Yang X, Liu J, et al. 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Research* 30:269−71

3. Wan Y, Shang J, Graham R, Baric RS, Li F. 2020. Receptor recognition by the novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. *Journal of Virology* 94:e00127−20
4. Du L, He Y, Zhou Y, Liu S, Zheng B, et al. 2009. The spike protein of SARS-CoV-a target for vaccine and therapeutic development. *Nature Reviews Microbiology* 7:226−236
5. Towler P, Staker B, Prasad SG, Menon S, Tang J, et al. 2004. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. *The Journal of Biological Chemistry* 279:17996−8007
6. de Clercq E. 2002. Strategies in the design of antiviral drugs. *Nature Reviews Drug discovery* 1:13−25
7. Agost-Beltrán L, de la Hoz-Rodríguez S, Bou-Iserte L, Rodríguez S, Fernández-de-la-Pradilla A, et al. 2022. Advances in the development of SARS-CoV-2 M^{pro} inhibitors. *Molecules* 27(8):2523
8. Chen R, Gao Y, Liu H, Li H, Chen W, et al. 2022. Advances in research on 3C-like protease ($3CL^{pro}$) inhibitors against SARS-CoV-2 since 2020. *RSC Medicinal Chemistry* 14(1):9−21
9. Huff S, Kummetha IR, Tiwari SK, Huante MB, Clark AE, et al. 2022. Discovery and mechanism of SARS-CoV-2 main protease inhibitors. *Journal of Medicinal Chemistry* 65(4):2866−79
10. Amporndanai K, Meng X, Shang W, Jin Z, Rogers M, et al. 2021. Inhibition mechanism of SARS-CoV-2 main protease by ebselen and its derivatives. *Nature Communications* 12(1):3061
11. Sabbah DA, Hajjo R, Bardaweel SK, Zhong HA. 2021. An updated review on SARS-CoV-2 main proteinase (M^{Pro}): Protein structure and small-molecule inhibitors. *Current Topics in Medicinal Chemistry* 21(6):442−60
12. Narayanan A, Narwal M, Majowicz SA, Varricchio C, Toner SA, et al. 2022. Identification of SARS-CoV-2 inhibitors targeting M^{pro} and PL^{pro} using in-cell-protease assay. *Communications Biology* 5:169
13. Zhang L, Zhao H, Liu J, He L, Yu R, et al. 2022. Design of SARS-CoV-2 M^{pro}, PL^{pro} dual-target inhibitors based on deep reinforcement learning and virtual screening. *Future Medicinal Chemistry* 14(6):393−405
14. Silva RC, Freitas HF, Campos JM, Kimani NM, Silva CHTP, et al. 2021. Natural products-based drug design against SARS-CoV-2 M^{pro} $3CL^{pro}$. *International Journal of Molecular Sciences* 22(21):11739
15. Dou X, Sun Q, Xu G, Liu Y, Zhang C, et al. 2022. Discovery of 2-(furan-2-ylmethylene)hydrazine-1-carbothioamide derivatives as novel inhibitors of SARS-CoV-2 main protease. *European Journal of Medicinal Chemistry* 238:114508
16. Wit ED, van Doremalen NV, Falzarano D, Munster VJ. 2016. SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology* 14:523−34
17. Jin Z, Du X, Xu Y, Deng Y, Liu M, et al. 2020. Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature* 582:289−93
18. Hu Q, Xiong Y, Zhu G, Zhang Y, Zhang YW, et al. 2022. The SARS-CoV-2 main protease (M^{pro}): Structure, function, and emerging therapies for COVID-19. *MedComm* 3:e151
19. Yan Y, Shen X, Cao Y, Zhang J, Wang Y, et al. 2020. Discovery of anti-2019-nCoV agents from 38 Chinese patent drugs toward respiratory diseases via docking screening. *Preprints* 0:2020020254
20. Xiong Y, Zhu G, Zhang Y, Hu Q, Wang H, et al. 2021. Flavonoids in *Ampelopsis grossedentata* as covalent inhibitors of SARS-CoV-2 $3CL^{pro}$: Inhibition potentials, covalent binding sites and inhibitory mechanisms. *International Journal of Biological Macromolecules* 187:976−87