Ching-Yuan Wu   ORCID iD: 0000-0001-6172-6647

The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase

Jrhau Lung[1], Yu-Shih Lin[2], Yao-Hsu Yang[3,4,5], Yu-Lun Chou[6], Li-Hsin Shu [3], Yu-Ching Cheng [3],

Hung Te Liu [3], Ching-Yuan Wu [3, 5*]

[1] Department of Research and Development, Chiayi Chang Gung Memorial Hospital, Chiayi

Branch, Putzu, Taiwan

[2] Department of Pharmacy, Chiayi Chang Gung Memorial Hospital, Chiayi Branch, Putzu, Taiwan.

[3] Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chiayi Branch,

Putzu, Taiwan.

[4] Health Information and Epidemiology Laboratory of Chang Gung Memorial Hospital, Chiayi

Branch, Putzu, Taiwan.

[5] School of Chinese medicine, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan.

[6] Department of surgery, Kaohsiung Chang Gung Memorial Hospital

*Correspondence to: Ching-Yuan Wu, M.D., Ph. D.

Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital, Chiayi Branch,

Putzu, Taiwan.

School of Chinese medicine, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan.

No.6, W. Sec., Jiapu Rd., Puzi City, Chiayi County 613, Taiwan (R.O.C.).

Phone: +886 5 362-1000 Fax: +886 5 362-3002;

E-mail: smbepigwu77@gmail.com, smbepig@adm.cgmh.org.tw

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ABSTRACT

An outbreak of corona virus disease 2019 (COVID-19) occurred in Wuhan and it has rapidly spread to almost all parts of the world. For coronaviruses, RNA-dependent RNA polymerase (RdRp) is an important protease that catalyze the replication of RNA from RNA template and is an attractive therapeutic target. In this study, we

screened these chemical structures from traditional Chinese medicinal compounds proven to show anti-viral activity in SARS-CoV and the similar chemical structures through a molecular docking study to target RdRp of SARS-CoV-2, SARS-CoV and MERS-CoV. We found that theaflavin has a lower idock score in the catalytic pocket of RdRp in SARS-CoV-2 (−9.11 kcal/mol), SARS-CoV (−8.03 kcal/mol) and MERS-CoV (−8.26 kcal/mol) from idock. To confirm the result, we discovered that theaflavin has a lower binding energy of −8.8 kcal/mol when it docks in the catalytic pocket of SARS-CoV-2 RdRp by using the Blind Docking server. Regarding contact modes, hydrophobic interactions contribute significantly in binding and additional hydrogen bonds were found between theaflavin and RdRp. Moreover, one π -cation interaction was formed between theaflavin and Arg553 from the Blind Docking server. Our results suggest that theaflavin could be potential SARS-CoV-2 RdRp inhibitor for further study.

Keywords ： SARS-CoV-2, RNA-dependent RNA polymerase, theaflavin, traditional Chinese medicinal compounds

Introduction

An unprecedented outbreak of coronavirus disease 2019 (COVID-19) occurred in Wuhan, Hubei Province, China, in December 2019. It has since spread rapidly to almost all parts of China and many other countries. As at 3 March 2019, the incidence of COVID-19 continues to rise[1].

For coronaviruses, RNA-dependent RNA polymerase (RdRp) is an important enzyme that catalyzes the replication of RNA from RNA templates. Compared the sequence of RdRp in severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV and SARS-CoV-2 have remarkably similar sequences, and encode structurally similar structures of RdRp (Figure 1A, B) [2]. Moreover, RdRp of the three viruses has a huge and deep groove as an active site for the polymerization of RNA and the variations of residues in SARS-CoV-2 and SARS-CoV are distal to the active site[2] (Figure 1B). Remdesivir (GS-5734) is a 1 ´ -cyano-substituted adenosine nucleotide analogue inhibitor of RdRp and shows broad-spectrum

antiviral activity against several RNA viruses, including Ebola virus, SARS-CoV and MERS-CoV[3-5]. More importantly, one report indicated that remdesivir improved the critical condition of one COVID-19 patient[6]. Therefore, RdRp could be an attractive therapeutic target for SARS-CoV-2.

In China, many traditional Chinese medicinal compounds have been used to treat SARS-CoV and SARS-CoV-2[3]. However, the mechanisms of activity and the efficiency of these compounds remain unclear. Therefore, we screened the chemical structures of clinically used traditional Chinese medicinal compounds with proven antiviral activity against SARS-CoV and their similar chemical structures, using a molecular docking method to target RdRp of SARS-CoV-2.

Methods and Materials

Structure preparation

The 3D structure of RdRp of SARS-CoV-2 (NCBI Reference Sequence: YP_009725307.1), SARS-CoV (NCBI Reference Sequence: NP_828869.1) and MERS-CoV (NCBI Reference Sequence:

YP_009047223.1) were generated based on homologous modeling using Modeller[7] incorporated within the UCSF Chimera[8] and SWISS-MODEL[9].

Compound dataset collection

Eighty-three chemical structures from traditional Chinese medicinal compounds and their similar structures were retrieved from ZINC15 database.

Molecular docking and virtual screening

We used two molecular docking methods for analysis. First, molecular docking and virtual screening was performed using idock download from Github (https://github.com/HongjianLi/idock) in a local linux machine. Second, Blind Docking server was used for in silico prediction of the lowest free binding energy. Calculations were carried out with "Achilles" Blind Docking Server, available at: http://bio-hpc.eu/software/blind-docking-server/. The grid box encompassed by the active site (Val557) and surrounding amino acids around the catalytic pocket of SARS-CoV-2 RdRp was using for ligand

docking and virtual screening (Figure 1B). For each structure, nine docking posed were generated and the scores for the best docking poses of each structure were used for ranking.

Result

Screening of these chemical structures revealed that theaflavin (ZINC3978446, Figure 2A) has a lower idock score in the catalytic pocket of RdRp in SARS-CoV-2 (−9.11 kcal/mol), SARS-CoV (−8.03 kcal/mol) and MERS-CoV (−8.26 kcal/mol) (Figure 2B). The contact modes between theaflavin and RdRp of these viruses with the lowest idocking scores are illustrated in Figure 2C, 2D and 2E, respectively. Regarding contact modes by idock, hydrophobic interactions contribute significantly in binding and additional hydrogen bonds were found between theaflavin and Asp452, Arg553 and Arg624 of SARS-CoV-2 RdRp (Figure 2C), and between thealflavin with Thr440, Ser566, Ala569 and Asp644 of SARS-CoV RdRp (Figure 2D), and between thealflavin with Arg294, Thr292, Gln291, Leu427, Asn 390, Leu427 and Asp728 of MERS-CoV RdRp (Figure 2E).

Because theaflavin has the lowest idock score in the catalytic pocket of SARS-CoV-2 RdRp (−9.11 kcal/mol), we used the Blind Docking server to confirm the result. We found that theaflavin has a lower binding energy of −8.8 kcal/mol when it docks in the catalytic pocket of SARS-CoV-2 RdRp. The 2D and 3D contact modes between RdRp and theaflavin with the lowest binding energy are illustrated in Figure 2F and G. Regarding the contact modes by the Blind Docking server, hydrophobic interactions contribute significantly for binding. We observed additional hydrogen bonds and π-cation interaction between theaflavin and SARS-CoV-2 RdRp (Figure 2F and 2G). The contact mode showed hydrogen bonds were formed between theaflavin and Asp452, Lys545, Arg555, Thr556, Tyr619, Lys621, Cys622, Asp623, Arg624, and Asp760 of SARS-CoV-2 RdRp, near the active site of RdRp. In addition, one π-cation interaction was formed between theaflavin and Arg553 (Figure 2F and G).

Discussion

Many herbals and compounds used in traditional Chinese medicine have been screened as anti-SARS-CoV therapy [10-14]. Theaflavin, a

polyphenolic compound in black tea, is thought to be responsible for the medicinal value of black tea. Theaflavin and theaflavin gallate derivatives have shown broad-spectrum antiviral activity against several viruses, including influenza A and B viruses and hepatitis C virus [15,16].

Further, it has been shown that extracts from Pu'er tea and black tea, and theaflavin-3,3'-digallate and 3-isotheaflavin-3-gallate, in the theaflavins family, have potent inhibitory activity against SARS, by inhibiting SARS-CoV 3CL[pro] activity [17]. In this study, we found that theaflavin was able to dock in the catalytic pocket near the active site of RdRp in SARS-CoV-2, SARS-CoV and MERS-CoV. Further, theaflavin has a lowest idock score and lower binding energy in the catalytic pocket of SARS-CoV-2 RdRp in the two different molecular docking methods. We also found that theaflavin formed additional hydrogen bonds and π-cation interaction with the catalytic pocket of SARS-CoV-2 RdRp. This may explain why thealflavin had lower idock score than the other chemical structures that we screened. It is

possible that theaflavin could inhibit RdRp activity through blocking the active site in the groove.

Conclusion

Our results suggest that thealfavin could be used as a lead compound for developing a SARS-CoV-2 inhibitor that targets RdRp. However, the exact *in vivo* effect is still unclear, and further research is needed to confirm the mechanism whereby theaflavin target SARS-CoV-2.

Declarations

Acknowledgement

Availability of data and materials

All data and materials are contained and described within the manuscript.

Authors' contributions

J. L. performed the experiments; C.Y.W. conceived the idea and designed experiments and wrote manuscript. Y.H.Y., L.H.S., Y.C.C., H.T.L., Y.S.L. and J.L. analyzed the data. Y.L.C. revised English writing of the manuscript. All authors reviewed and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate
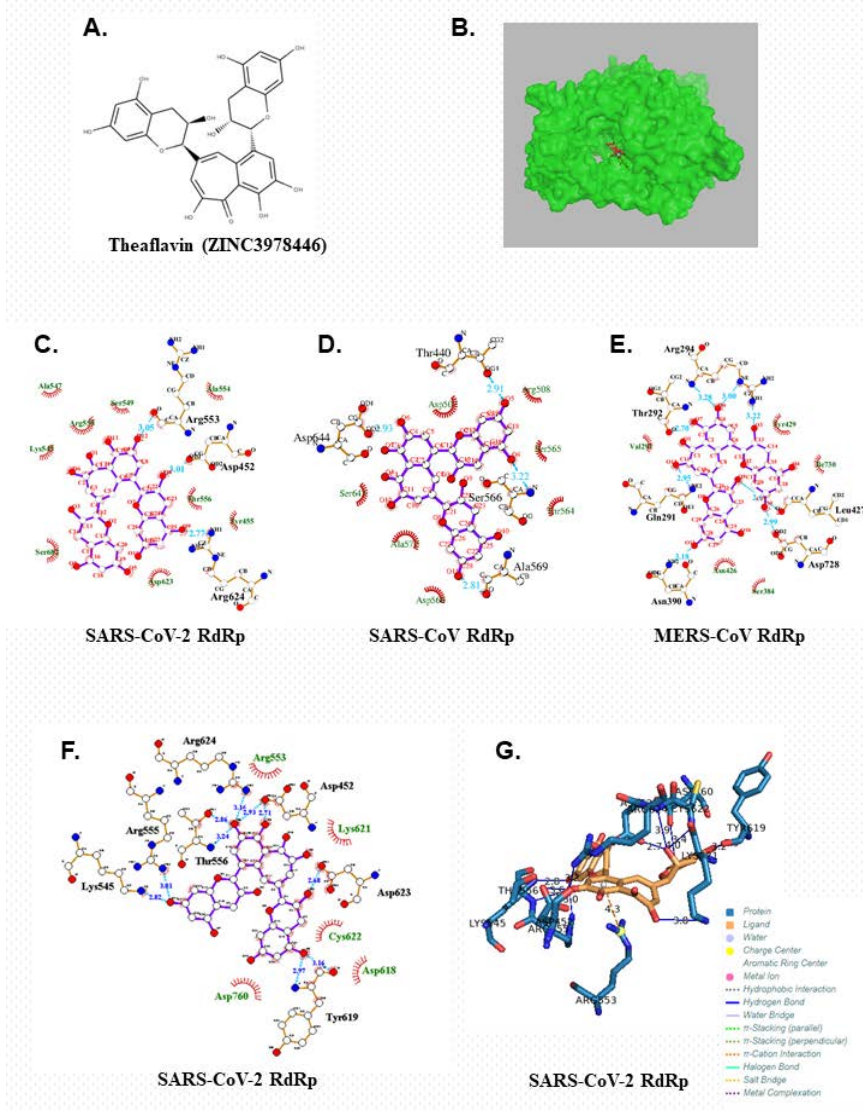
Not applicable.

Figures

Figure 1. (**A**) Sequence alignment for the amino acids of RdRp between SARS-CoV-2, SARS-CoV and MERS-CoV. (**B**) A modeled structure of SARS-CoV-2 RdRp, SARS-CoV RdRp and MERS-CoV RdRp and Grid box size for binding site using Modeller. The active site (Val557) and grid box size (light yellow) was showed for binding site.
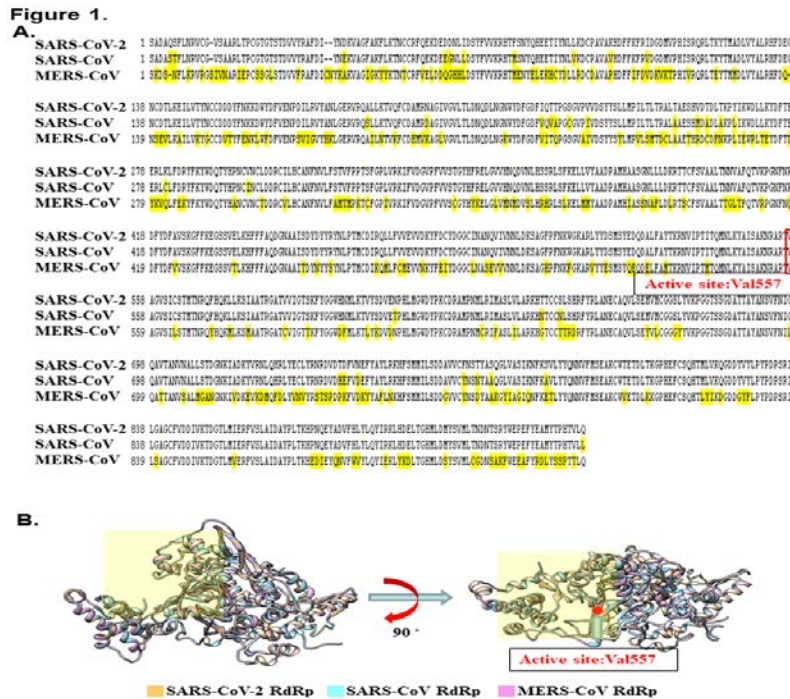
Figure 2. (**A**) The structure of theaflavin (ZINC3978446). (**B**) Red and green molecules represent crystallographic and predicted pose for theaflavin. (**C-E**) The contact model between theaflavin and SARS-CoV-2 RdRp (**C**), SARS-CoV RdRp (**D**) and MERS-CoV RdRp (**E**) are showed in 2D interaction diagram by idock. (**F**) The contact model between theaflavin and SARS-CoV-2 RdRp are showed in 2D interaction diagram by the Blind Dock server. Their relative distances between amino acid residues and theaflavin are analyzed and illustrated by LigPolt+. Carbon, oxygen, nitrogen, and fluoride molecules are marked as white, red, blue, and green circles, respectively. Covalent bonds in theaflavin and amino acid residues of RdRp are labeled in purple and orange solid lines, respectively. The light blue dot lines label the distance (in Å) of hydrogen bonds formed between the functional moieties of theaflavin and amino acid residues. Hydrophobic interactions between theaflavin and RdRp are depicted by the name of involving amino acid residues, which are labeled with dark green with dark red eyelashes pointing to the involved functional moiety of theaflavin. (**G**) The hydrogen bonds and π -cation interaction

established by theaflavin with the closest residues are showed through

Protein-Ligand Interaction Profiler (PLIP).

Figure 2.

A. Theaflavin (ZINC3978446)

B.

C. SARS-CoV-2 RdRp

D. SARS-CoV RdRp

E. MERS-CoV RdRp

F. SARS-CoV-2 RdRp

G. SARS-CoV-2 RdRp

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