



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Research paper

Discovery of chebulagic acid and punicalagin as novel allosteric inhibitors of SARS-CoV-2 3CL^{PRO}Ruikun Du^{a,b,c,1}, Laura Cooper^{d,1}, Zinuo Chen^a, Hyun Lee^e, Lijun Rong^{d,**}, Qinghua Cui^{a,b,c,*}^a College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, 250355, China^b Experimental Center, Shandong University of Traditional Chinese Medicine, Jinan, 250355, China^c Qingdao Academy of Chinese Medicinal Sciences, Shandong University of Traditional Chinese Medicine, Qingdao, 266122, China^d Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL, 60612, USA^e Department of Pharmaceutical Sciences, Center for Biomolecular Sciences, College of Pharmacy, Biophysics Core at Research Resources Center, University of Illinois at Chicago, Chicago, IL, 60607, USA

ARTICLE INFO

Keywords:

SARS-CoV-2

Chebulagic acid

Punicalagin

3CL^{PRO}

Allosteric inhibitor

ABSTRACT

The emerging SARS-CoV-2 infection is the cause of the global COVID-19 pandemic. To date, there are limited therapeutic options available to fight this disease. Here we examined the inhibitory abilities of two broad-spectrum antiviral natural products chebulagic acid (CHLA) and punicalagin (PUG) against SARS-CoV-2 viral replication. Both CHLA and PUG reduced virus-induced plaque formation in Vero-E6 monolayer at noncytotoxic concentrations, by targeting the enzymatic activity of viral 3-chymotrypsin-like cysteine protease (3CL^{PRO}) as allosteric regulators. Our study demonstrates the potential use of CHLA and PUG as novel COVID-19 therapies.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the virus that causes a pneumonia like illness pandemic called coronavirus disease 2019 (COVID-19). The disease has reached to almost every country in the world, posing a serious threat to global public health and the economy (Hamid et al., 2020). As of March 4, 2021, more than 114.43 million cases have been confirmed with 2.54 million deaths globally (<https://www.who.int/emergencies/diseases/novel-coronavirus-2019>). Encouragingly, 12 COVID-19 vaccines have been approved and globally available, and 22 vaccine candidates are in phase III clinical trials (<https://covid19.trackvaccines.org/vaccines/>). However, evidence is increasing that SARS-CoV-2 variants could evade immune responses triggered by either vaccines or previous infections, which may compromise the vaccine effectiveness (Callaway, 2021; Tegally et al., 2020). On the other hand, there is no specific antiviral therapy available for clinical usage, except remdesivir, of which the therapeutic effect is still under debate (Beigel et al., 2020). There is a pressing need to discover additional effective antivirals for the treatment of SARS-CoV-2 infection for use alone or in combination with already approved antiviral therapies.

Broad spectrum antiviral agents are valuable candidates of inhibitors against emerging viruses (Zhang et al., 2020b). To our knowledge, two hydrolyzable polyphenolics, chebulagic acid (CHLA, Fig. 1A) and punicalagin (PUG, Fig. 1B), have been well acknowledged as cost-effective and broad-spectrum antivirals. Previous studies have demonstrated CHLA and PUG can block interactions between cell surface glycosaminoglycan (GAG) and viral glycoproteins. Through this mechanism, they inhibit infections by diverse viruses that employ GAGs for host cell entry, such as herpes simplex virus type 1, human cytomegalovirus, and hepatitis C virus (Lin et al., 2011, 2013). Moreover, we recently identified that CHLA and PUG possess inhibitory effects against influenza viruses by targeting neuraminidase mediated virus release (Li et al., 2020b, 2020c).

In this study, we investigated the potent antiviral activity of CHLA and PUG against SARS-CoV-2.

2. Materials and methods

2.1. Virus and reagents

The clinical isolate of SARS-CoV-2 (SARS-CoV-2, Isolate USA-WA1/

* Corresponding author. College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, 250355, China.

** Corresponding author. Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL, 60612, USA.

E-mail addresses: Lijun@uic.edu (L. Rong), cuiqinghua1122@163.com (Q. Cui).

¹ Ruikun Du and Laura Cooper contributed equally to this work.

2020) was obtained from BEI Resources and manipulated in BSL3 containment at University of Illinois at Chicago (Chicago, IL). Compounds CHLA and PUG were purchased from MedChemExpress (MCE; Monmouth Junction, NJ, USA). The fluorescence resonance energy transfer (FRET)-based peptidic substrate (Dabcyl-KTSAVLQ/SGFRKME-Edans) was purchased from NJpeptide (Nanjing, China).

2.2. Antiviral assay and cytotoxicity assay

To examine the anti-SARS-CoV-2 activity of CHLA or PUG, plaque reduction assay was conducted. Briefly, Vero-E6 monolayers grown in 12 well plates were pre-treated with increasing concentrations of test compound for 1 h, followed by infection with SARS-CoV-2 (MOI of 0.0001) in the presence of test compounds. DMSO and remdesivir (3 μM) were used as negative and positive controls respectively. After 1-h incubation, the medium was replaced with fresh MEM containing 1.25% Avicel and test compound, and the plates were incubated for another 48 h at 37 °C and 5% CO₂. Then cells were fixed with 10% formalin and stained with 1% crystal violet to visualize plaques. The concentration required for the tested compound to reduce the plaque formation of the virus by 50% (the 50% effective concentration [EC₅₀]) was determined. To estimate the cytotoxicity of CHLA or PUG on VERO-E6 cells, Cell-Titer Glo® luminescent cell viability assay (Promega) was performed according to the manufacturer's instruction. The half of the cytotoxic concentration (CC₅₀) values were calculated from the percentages of cells whose viability was inhibited by CHLA or PUG at various concentrations.

2.3. Pseudotyped SARS-CoV-2 based entry inhibition assay

A lentiviral-based pseudovirus carrying the SARS-CoV-2 S protein (SARS-CoV-2pp) was prepared as previously described (Xia et al., 2020), while 293T cells transiently expressing human ACE2 and TMPRSS2 were adopted as target cells (Fig. S1A). The pseudovirus entry assay was conducted by inoculation of SARS-CoV-2pp to target cells in presence of increasing concentrations of test compound, with final concentrations ranged from 100 μM to 1.56 μM . After incubation for 48 h, luciferase activity was analyzed to monitor viral entry efficacy.

2.4. Enzymatic inhibition assay of 3-chymotrypsin-like cysteine protease (3CL^{pro})

The SARS-CoV-2 3CL^{pro} was prokaryotic expressed and purified as previously described with slight modification (Ma et al., 2020b). For enzymatic inhibition assay, the recombinant 3CL^{pro} (250 nM at a final concentration) was incubated with increasing concentrations of each

compound in 90 μL reaction buffer (50 mM Tris-HCl, pH 7.3, 1 mM EDTA) and incubated for 30 min (Dai et al., 2020). The reaction was initiated by adding 10 μL FRET-based peptidic substrate (Dabcyl-KTSAVLQ/SGFRKME-Edans) with a final concentration of 50 μM . The fluorescence signal was immediately measured every 20 s for 30 min with a Bio-Tek Synergy4 plate reader with filters for excitation at 336/20 nm and emission at 490/20 nm. The initial reaction velocities (V_0) of reactions were calculated to indicate the enzymatic activities. Three independent experiments were performed and IC₅₀ curves were analyzed using GraphPad Prism software.

2.5. In silico docking

For the molecular docking simulations, the 3CL^{pro} structure from SARS-CoV-2 (PDB code: 6m2n) was used. The three-dimensional (3D) structure of CHLA and PUG was obtained from PubChem Compound database (NCBI). Before docking, polar hydrogen atoms were added to the 3CL^{pro} protein using autodock tools. Docking was performed using a grid box covering the entire structure by AutoDock Vina software (Trott and Olson, 2010). After docking, the conformation of the compound was analyzed, and the 3D models were viewed with PyMOL.

3. Results

3.1. CHLA and PUG exhibit antiviral activity in vitro

In order to determine whether CHLA and PUG can inhibit replication of SARS-CoV-2, a plaque reduction assay was performed with authentic SARS-CoV-2 using remdesivir as positive control. As a result, both CHLA and PUG inhibited the plaque formation of SARS-CoV-2 in a dose-dependent manner (Fig. 2A), with EC₅₀ values of $9.76 \pm 0.42 \mu\text{M}$ and $7.20 \pm 1.08 \mu\text{M}$, respectively (Fig. 2B). To exclude the possibility that the inhibition of viral replication was due to compound-mediated cytotoxicity, a cell proliferation-based cytotoxicity assay was performed. As shown in Fig. 1B, the CC₅₀ values of CHLA and PUG were both around 100 μM , conferring selectivity index (SI[CC₅₀/EC₅₀]) of above 10 and 13, respectively. These results suggest CHLA and PUG exhibit *in-vitro* anti-SARS-CoV-2 activity.

3.2. CHLA and PUG do not block SARS-CoV-2 entry

Recently, two independent studies have demonstrated that cell surface GAGs such as heparan sulfate are involved in SARS-CoV-2 entry, by interacting with motifs within viral spike (S) glycoprotein (Clausen et al., 2020; Kim et al., 2020). Considering the aforementioned fact that CHLA and PUG can prevent interactions between GAGs and viral

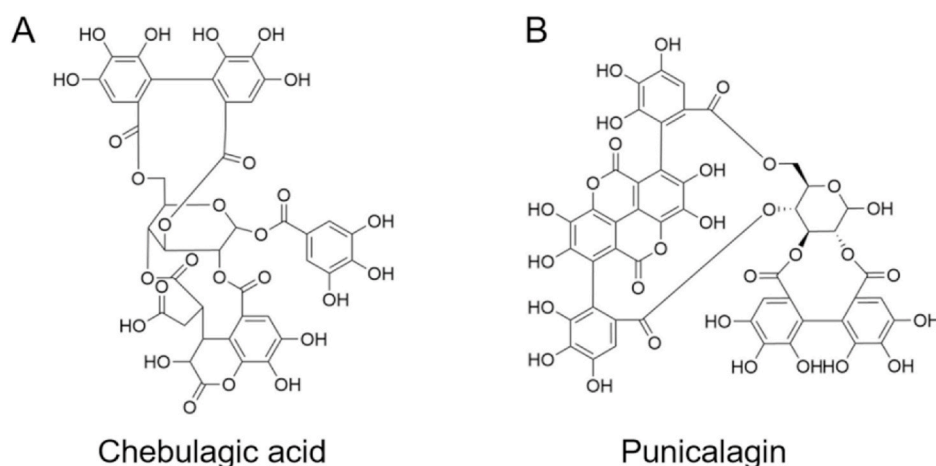


Fig. 1. The chemical structures of chebulagic acid (CHLA) and punicalagin (PUG).

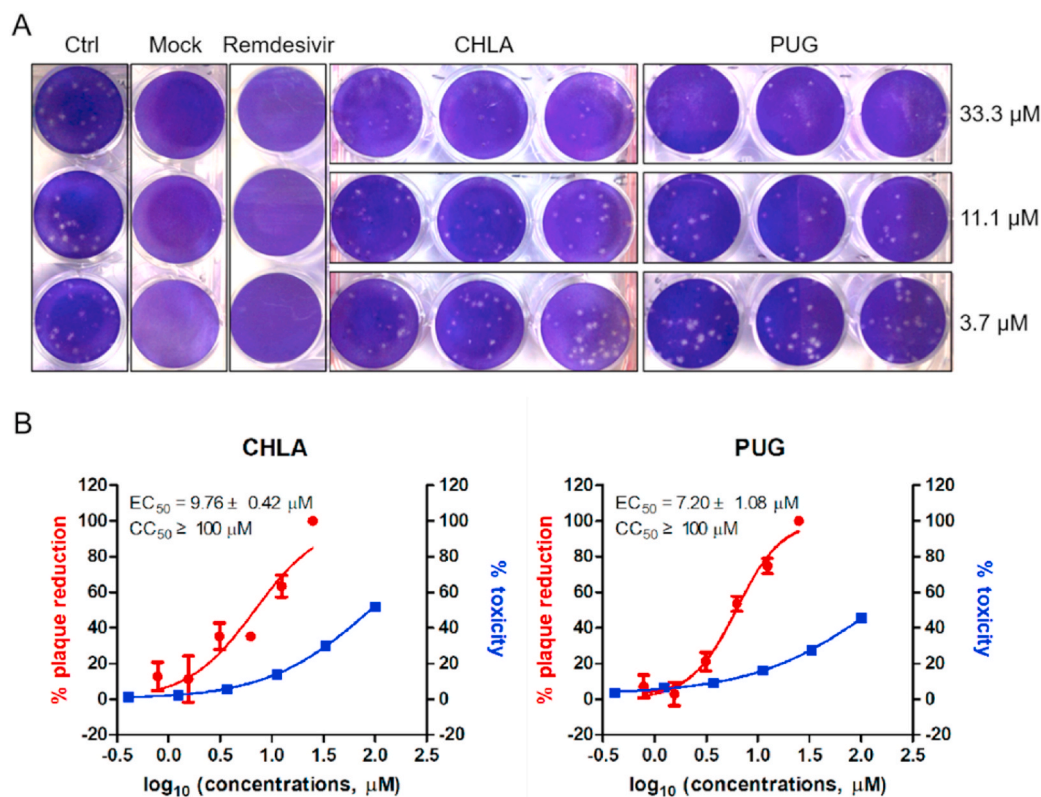


Fig. 2. CHLA and PUG inhibit SARS-CoV-2 replication. A. Plaque reduction assay of CHLA and PUG as well as remdesivir (3 μM) against authentic SARS-CoV-2 at indicated concentrations. B. Dose-dependent inhibition of CHLA and PUG on SARS-CoV-2 replication. Antiviral activity and cytotoxicity are shown in red and blue, respectively. The EC₅₀ and CC₅₀ are displayed in the upper left corner for each compound. The data represent mean ± standard deviation (SD) of the triplicate measurements. IC₅₀ values were determined by fitting the dose-response curves with four-parameter logistic regression in Prism GraphPad (version 8.1.2). All data was normalized to virus alone.

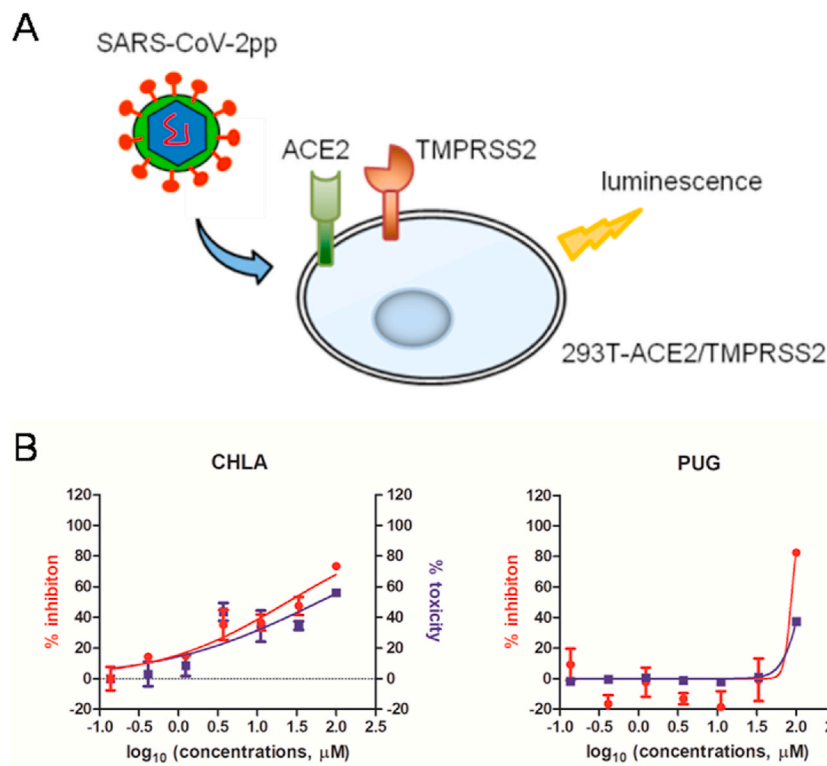


Fig. 3. Pseudoviral infection assay. A. representative diagram of pseudo-SARS-CoV-2 assay, where 293T cells transiently expressing ACE2 and TMPRSS2 were used as target cells. B. anti-pseudoviral activity and cytotoxicity are shown in red and blue, respectively. The data represent mean ± standard deviation (SD) of the triplicate measurements.

glycoproteins, we therefore first tested whether CHLA and PUG act by blocking SARS-CoV-2 entry using a pseudotyped SARS-CoV-2 (SARS-CoV-2pp). However, upon adding CHLA or PUG during infection of SARS-CoV-2pp into target 293T cells transiently expressing ACE2 and TMPRSS2 (Fig. 3A), neither CHLA nor PUG inhibited pseudovirus entry without interfering with target cell viability, indicating that CHLA and PUG inhibit SARS-CoV-2 replication by a mechanism other than preventing S-mediated viral entry (Fig. 3B).

3.3. CHLA and PUG exhibited inhibitory effects against 3CL^{pro} activity

The 3-chymotrypsin-like cysteine protease (3CL^{pro}) enzyme is one of the best characterized drug targets among coronaviruses (Zhang et al., 2020a). Along with the papain-like protease (PL^{pro}), 3CL^{pro} is an essential viral protease that processes the viral polyproteins, therefore, inhibiting the activity of this enzyme would block viral replication (Dai et al., 2020). Interestingly, an *in silico* study revealed that several hydrolyzable polyphenolics including CHLA are potential inhibitors of the SARS-CoV-2 3CL^{pro} (Khalifa et al., 2020). To address this, CHLA and PUG were subjected to a SARS-CoV-2 3CL^{pro} enzymatic inhibition assay. Upon mixture of 3CL^{pro} with a FRET-based peptidic substrate in reaction buffer, the substrate gets hydrolyzed. After hydrolysis, the fluorophore Edans is no longer affected by the quencher molecule Dabcyl, resulting in an increase of fluorescence signal with proper filters (Fig. S1A). Both CHLA and PUG dose-dependently reduced fluorescence production (Fig. S1B), with IC₅₀ values of 9.09 ± 0.87 μM and 4.62 ± 0.27 μM, respectively (Fig. 4). A fluorescent interference assay further illustrated that CHLA and PUG affected fluorescence only at higher concentrations (>50 μM, data not shown). Taken together, our study revealed that CHLA and PUG can block the enzymatic activity of 3CL^{pro}. Of note, CHLA showed little effect on the enzymatic activity of SARS-CoV-2 PL^{pro}, while PUG slightly inhibits the PL^{pro} activity with IC₅₀ of over 50 μM (Fig. S2). These data suggest that PUG is a potential dual-target SARS-CoV-2 inhibitor, although its inhibitory effect against the PL^{pro} is weak.

3.4. CHLA and PUG exhibited reversible, noncompetitive inhibition against 3CL^{pro}

To better understand the kinetic modes involved in the interaction of CHLA and PUG with SARS-CoV-2 3CL^{pro}, the proteolytic activity of the enzyme was measured using a series of enzyme concentrations and at various inhibitor concentrations. The substrate concentrations were held constant at sub-saturation levels. At each concentration of test inhibitor, the initial reaction velocities were plotted with the concentrations of 3CL^{pro}, and fitted to a line using linear regression analysis. As

indicated by Fig. 5A, as the concentration of either CHLA or PUG increases, the slope of the line decreases, suggesting that both CHLA and PUG are reversible inhibitors. In order to deduce the mode of binding, Lineweaver-Burk plots were carried out at a constant concentration of 3CL^{pro} (250 nM), increasing concentrations of substrate, and in absence or presence of various concentrations of CHLA or PUG. As a result, both CHLA and PUG exhibited noncompetitive modes of inhibition since all lines intercepting the x-axis (Fig. 5B).

3.5. Molecular docking

According to above enzymatic assays, both CHLA and PUG are noncompetitive allosteric inhibitors. In order to search potential allosteric binding sites, *in silico* docking of CHLA and PUG to 3CL^{pro} was thereafter performed using AutoDock Vina software. As depicted in Fig. 6, both CHLA and PUG may interact with the cleft between domain II and domain III within 3CL^{pro} with stable binding free energy. This binding site is located a little away from the substrate binding pocket, where His41 and Cys145 form the catalytic dyad (Dai et al., 2020).

4. Discussion

In the urgent campaign to develop SARS-CoV-2 therapeutics, natural products from medicinal plants have been an important source of new lead compounds exhibiting different mechanisms of action, such as shikonin (Li et al., 2020a), salvianolic acid C (Yang et al., 2020), cepharanthine (Rogosnitzky et al., 2020), lycorine (Zhang et al., 2020b), ginkgolic acid (Chen et al., 2021) and so on (Zhang et al., 2020c). We herein report two additional natural products, CHLA and PUG as novel SARS-CoV-2 inhibitors. By binding to SARS-CoV-2 3CL^{pro} at a pocket other than substrate binding site, CHLA and PUG act as allosteric inhibitors in reversible, noncompetitive manner.

There are two classes of inhibitors against a cysteine protease, covalent inhibitors and non-covalent ones. Development of covalent inhibitors is usually challenging due to their toxicity and lack of specificity resulting from covalent modification of untargeted cysteine residues, while encouraging strategies have been explored, for example, by rational design, to inhibit 3CL^{pro} via its covalent inhibition (Dai et al., 2020; Jin et al., 2020; Qiao et al., 2021).

On the other hand, a non-covalent inhibitor may specifically bind to the target proteins reversibly. Compared to covalent inhibitors, non-covalent inhibitors can often be optimized to diffuse through membranes and bind to target proteins with high affinity (Zhang et al., 2015). Moreover, non-covalent inhibitors are often more chemically stable than their covalent counterparts since they don't have reactive warheads, and undesirable toxic effects can be reduced for their binding to host

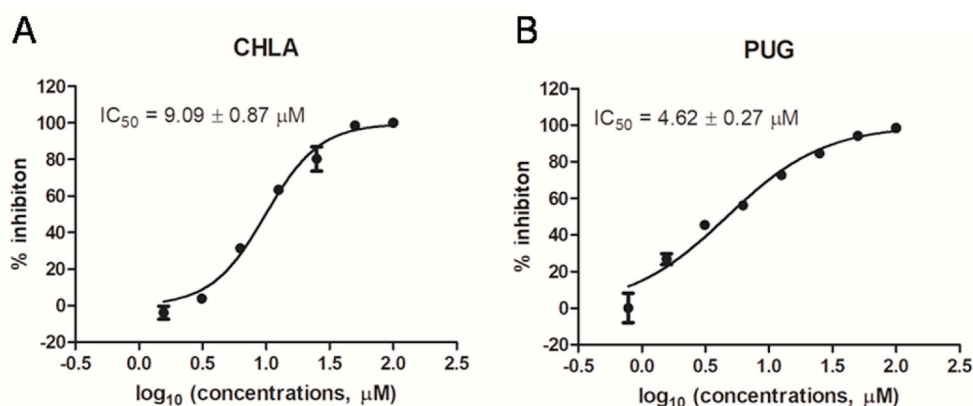


Fig. 4. Fluorescence resonance energy transfer (FRET)-based 3CL^{pro} enzymatic inhibition assay. The inhibitions of CHLA (A) and PUG (B) against 3CL^{pro} were determined using a FRET-based cleavage assay. For each compound, the IC₅₀ value is displayed in the upper left corner. The data represent mean ± standard deviation (SD) of the triplicate measurements.

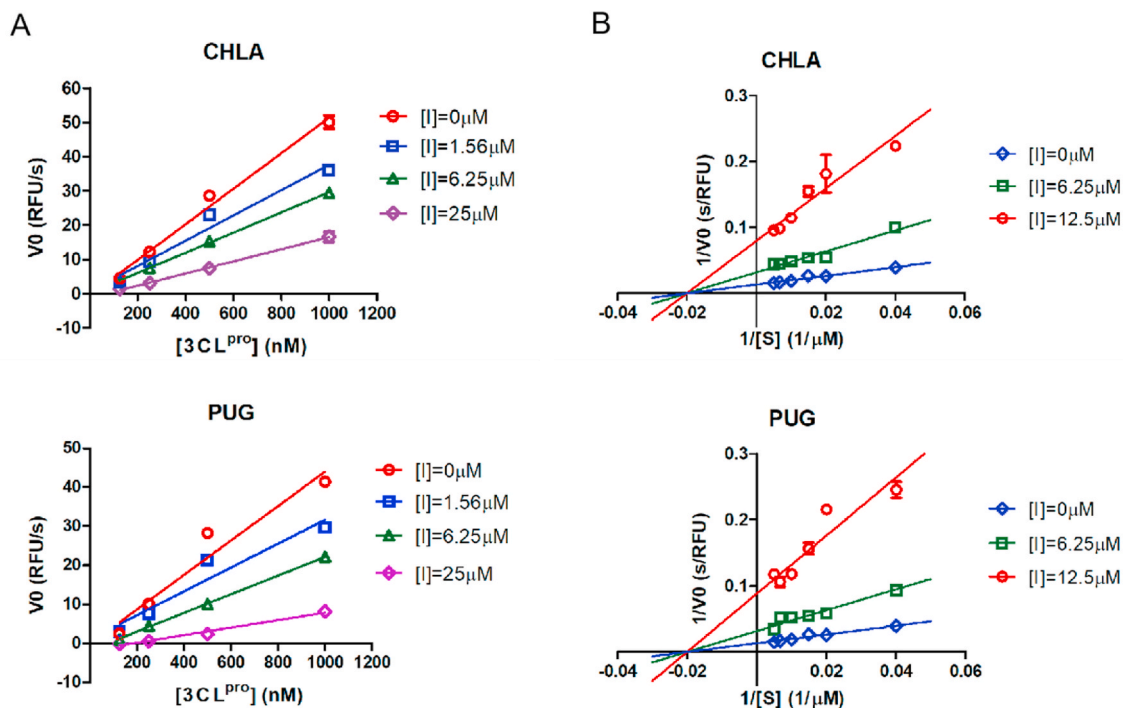


Fig. 5. Graphical determination of the type of enzymatic inhibition. A. Relationship of the enzymatic activity of SARS-CoV-2 3CL^{Pro} as function of enzyme concentrations at different concentration of CHLA or PUG. B. Lineweaver-Burk plot for inhibition of CHLA or PUG on SARS-CoV-2 3CL^{Pro}.

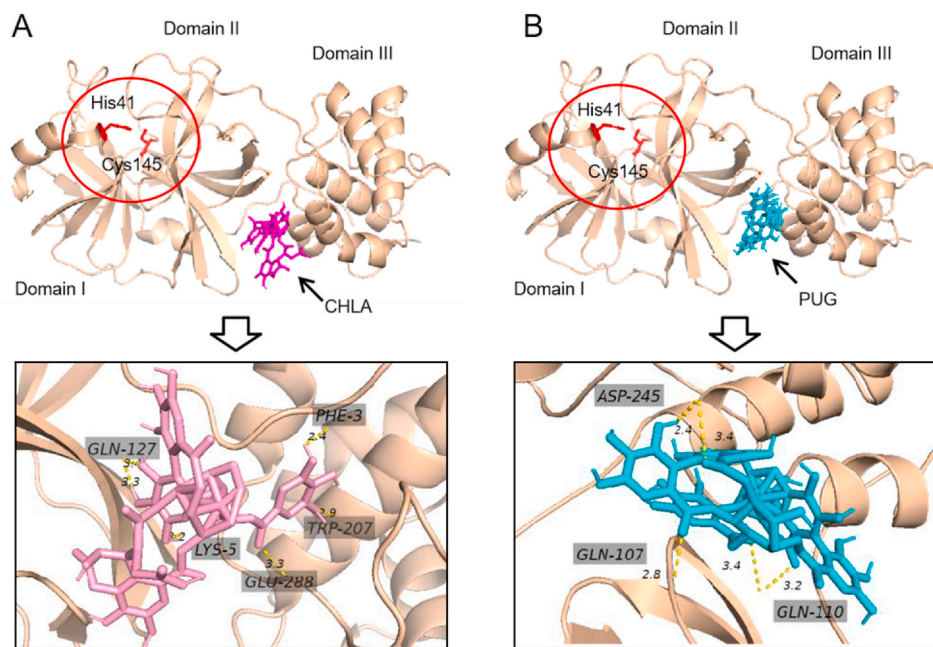


Fig. 6. Predicted binding modes of CHLA (A) and PUG (B) to 3CL^{Pro} (PDB ID: 6m2n). The red circle indicates the catalytic site. The SARS-CoV-2 3CL^{Pro} is present in wheat cartoon, while CHLA and PUG are shown as sticks. The bonds are represented as the dashed lines, with the bond length measured between heavy atoms.

proteins and nucleic acids are reversible (El-Baba et al., 2020).

Virtual screens have placed hundreds chemically diverse molecules in the active site of 3CL^{Pro}, which is the main target for a non-covalent inhibitor (Douangamath et al., 2020; Gyebi et al., 2020; Khalifa et al., 2020). Although most of the predicted inhibitors either were not subjected to antiviral assays or failed to inhibit SARS-CoV-2 replication, compound ML188 has been validated as SARS-CoV-2 inhibitor by competitively binding to 3CL^{Pro} with natural substrate (Lockbaum et al., 2021). Besides, a non-covalent inhibitor may also act by binding to

allosteric sites, which are away from the active site (El-Baba et al., 2020). Recently, a highly reactive pocket in the dimerization region at the domain III apex of SARS-CoV-2 3CL^{Pro} has been recognized as a possible allosteric site. Interestingly, our *in silico* docking data clearly reveal that both CHLA and PUG accommodates this pocket (Fig. 6).

Historically, in order to discover non-covalent cysteine protease inhibitors, reducing reagents were usually added in the enzymatic assay of cysteine protease to prevent nonspecific covalent modification of the catalytic cysteine (Ma et al., 2020a; Ratia et al., 2008). However, in the

case of CHLA and PUG, addition of 4 mM DTT in the enzymatic assay of SARS-CoV-2 3CL^{pro} shifted the IC₅₀ curves to right by 7–10 folds, suggesting significant loss of inhibitory effects (Fig. S3). This is inconsistent with our finding that CHLA and PUG are reversible, noncompetitive inhibitors of 3CL^{pro}. To address this discrepancy, we suspect that reducing reagents might induce slight conformational changes of 3CL^{pro}, which would not affect the detectable enzymatic activity, while the cavities where an allosteric inhibitor binds might be impaired. Although more evidence is required to support this hypothesis, the scientific community should be cautious to add reducing reagents in the enzymatic assay of cysteine protease if allosteric inhibitors were anticipated.

In summary, our study demonstrates CHLA and PUG as a novel class of SARS-CoV-2 inhibitors, by allosterically regulating the 3CL^{pro} activity.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr. Shibo Jiang and Dr. Lu Lu from Fudan University for kindly providing the plasmid expressing SARS-CoV-2 Spike glycoprotein. We thank Ms. Yanyan Wang for the docking analysis. This work was supported by (1) the Key R & D Project in Shandong Province, China (Grant No. 2020CXGC010505); (2) Shandong Provincial Natural Science Foundation, China (Grant No. ZR2020MH383); (3) The Social Benefiting Technology Program of Qingdao (Grant No. 21-1-4-rkjk-15-nsh).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.antiviral.2021.105075>.

References

- Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., Zingman, B.S., Kalil, A.C., Hohmann, E., Chu, H.Y., Luetkemeyer, A., Kline, S., Lopez de Castilla, D., Finberg, R. W., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T.F., Paredes, R., Sweeney, D.A., Short, W.R., Touloumi, G., Ly, D., Ohmagari, N., Oh, M.D., Ruiz-Palacios, G.M., Benfield, T., Fätkenheuer, G., Kortepeter, M.G., Atmar, R.L., Creech, C.B., Lundgren, J., Babiker, A.G., Pett, S., Neaton, J.D., Burgess, T.H., Bonnett, T., Green, M., Makowski, M., Osinusi, A., Nayak, S., Lane, H.C., 2020. Remdesivir for the treatment of covid-19 - final report. *N. Engl. J. Med.* 383, 1813–1826.
- Callaway, E., 2021. Fast-spreading COVID variant can elude immune responses. *Nature*.
- Chen, Z., Cui, Q., Cooper, L., Zhang, P., Lee, H., Chen, Z., Wang, Y., Liu, X., Rong, L., Du, R., 2021. Ginkgolic acid and anacardic acid are specific covalent inhibitors of SARS-CoV-2 cysteine proteases. *Cell Biosci.* 11, 45.
- Clausen, T.M., Sandoval, D.R., Spliid, C.B., Pihl, J., Perrett, H.R., Painter, C.D., Narayanan, A., Majowicz, S.A., Kwong, E.M., McVicar, R.N., Thacker, B.E., Glass, C. A., Yang, Z., Torres, J.L., Golden, G.J., Bartels, P.L., Porell, R.N., Garretton, A.F., Laubach, L., Feldman, J., Yin, X., Pu, Y., Hauser, B.M., Caradonna, T.M., Kellman, B. P., Martino, C., Gordts, P., Chanda, S.K., Schmidt, A.G., Godula, K., Leibel, S.L., Jose, J., Corbett, K.D., Ward, A.B., Carlin, A.F., Esko, J.D., 2020. SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell* 183, 1043–1057 e1015.
- Dai, W., Zhang, B., Jiang, X.M., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Peng, J., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., Liu, X., Xiao, G., Jiang, H., Rao, Z., Zhang, L.K., Xu, Y., Yang, H., Liu, H., 2020. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science* 368, 1331–1335.
- Douangamath, A., Fearon, D., Gehrtz, P., Krojer, T., Lukacik, P., Owen, C.D., Resnick, E., Strain-Damerell, C., Aimon, A., Abrányi-Balogh, P., Brandão-Neto, J., Carbery, A., Davison, G., Dias, A., Downes, T.D., Dunnett, L., Fairhead, M., Firth, J.D., Jones, S.P., Keeley, A., Keserü, G.M., Klein, H.F., Martin, M.P., Noble, M.E.M., O'Brien, P., Powell, A., Reddi, R.N., Skyner, R., Snee, M., Waring, M.J., Wild, C., London, N., von Delft, F., Walsh, M.A., 2020. Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease. *Nat. Commun.* 11, 5047.
- El-Baba, T.J., Lutowski, C.A., Kantsadi, A.L., Malla, T.R., John, T., Mikhailov, V., Bolla, J. R., Schofield, C.J., Zitzmann, N., Vakonakis, I., Robinson, C.V., 2020. Allosteric inhibition of the SARS-CoV-2 main protease: insights from mass spectrometry based assays*. *Angew Chem. Int. Ed. Engl.* 59, 23544–23548.
- Gyebi, G.A., Ogunro, O.B., Adegunloye, A.P., Ogunyemi, O.M., Afolabi, S.O., 2020. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CL^{pro}): an in silico screening of alkaloids and terpenoids from African medicinal plants. *J. Biomol. Struct. Dyn.* 1–13.
- Hamid, S., Mir, M.Y., Rohela, G.K., 2020. Novel coronavirus disease (COVID-19): a pandemic (epidemiology, pathogenesis and potential therapeutics). *New microbes and new infections* 35, 100679.
- Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., Yang, X., Bai, F., Liu, H., Liu, X., Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z., Yang, H., 2020. Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. *Nature* 582, 289–293.
- Khalifa, I., Zhu, W., Mohammed, H.H.H., Dutta, K., Li, C., 2020. Tannins inhibit SARS-CoV-2 through binding with catalytic dyad residues of 3CL^{pro}: an in silico approach with 19 structural different hydrolysable tannins. *J. Food Biochem.* e13432.
- Kim, S.Y., Jin, W., Sood, A., Montgomery, D.W., Grant, O.C., Fuster, M.M., Fu, L., Dordick, J.S., Woods, R.J., Zhang, F., Linhardt, R.J., 2020. Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions. *Antivir. Res.* 181, 104873.
- Li, J., Zhou, X., Zhang, Y., Zhong, F., Lin, C., McCormick, P.J., Jiang, F., Luo, J., Zhou, H., Wang, Q., Fu, Y., Duan, J., Zhang, J., 2020a. Crystal structure of SARS-CoV-2 main protease in complex with the natural product inhibitor shikonin illuminates a unique binding mode. *Sci. Bull.*
- Li, P., Du, R., Chen, Z., Wang, Y., Zhan, P., Liu, X., Kang, D., Zhao, X., Wang, L., Rong, L., Cui, Q., 2020b. Punicalagin is a neuraminidase inhibitor of influenza viruses. *J. Med. Virol.*
- Li, P., Du, R., Wang, Y., Hou, X., Wang, L., Zhao, X., Zhan, P., Liu, X., Rong, L., Cui, Q., 2020c. Identification of chebulinic acid and chebulagic acid as novel influenza viral neuraminidase inhibitors. *Front. Microbiol.* 11, 182.
- Lin, L.T., Chen, T.Y., Chung, C.Y., Noyce, R.S., Grindley, T.B., McCormick, C., Lin, T.C., Wang, G.H., Lin, C.C., Richardson, C.D., 2011. Hydrolyzable tannins (chebulagic acid and punicalagin) target viral glycoprotein-glycosaminoglycan interactions to inhibit herpes simplex virus 1 entry and cell-to-cell spread. *J. Virol.* 85, 4386–4398.
- Lin, L.T., Chen, T.Y., Lin, S.C., Chung, C.Y., Lin, T.C., Wang, G.H., Anderson, R., Lin, C.C., Richardson, C.D., 2013. Broad-spectrum antiviral activity of chebulagic acid and punicalagin against viruses that use glycosaminoglycans for entry. *BMC Microbiol.* 13, 187.
- Lockbaum, G.J., Reyes, A.C., Lee, J.M., Tilvawala, R., Nalivaika, E.A., Ali, A., Kurt Yilmaz, N., Thompson, P.R., Schiffer, C.A., 2021. Crystal structure of SARS-CoV-2 main protease in complex with the non-covalent inhibitor ML188. *Viruses* 13.
- Ma, C., Hu, Y., Townsend, J.A., Lagarias, P.I., Marty, M.T., Kolocouris, A., Wang, J., 2020a. Ebselen, disulfiram, carmofur, PX-12, tideglusib, and shikonin are nonspecific promiscuous SARS-CoV-2 main protease inhibitors. *ACS Pharmacol Transl Sci* 3, 1265–1277.
- Ma, C., Sacco, M.D., Hurst, B., Townsend, J.A., Hu, Y., Szeto, T., Zhang, X., Tarbet, B., Marty, M.T., Chen, Y., Wang, J., 2020b. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. *Cell Res.* 30, 678–692.
- Qiao, J., Li, Y.S., Zeng, R., Liu, F.L., Luo, R.H., Huang, C., Wang, Y.F., Zhang, J., Quan, B., Shen, C., Mao, X., Liu, X., Sun, W., Yang, W., Ni, X., Wang, K., Xu, L., Duan, Z.L., Zou, Q.C., Zhang, H.L., Qu, W., Long, Y.H., Li, M.H., Yang, R.C., Liu, X., You, J., Zhou, Y., Yao, R., Li, W.P., Liu, J.M., Chen, P., Liu, Y., Lin, G.F., Yang, X., Zou, J., Li, L., Hu, Y., Lu, G.W., Li, W.M., Wei, Y.Q., Zheng, Y.T., Lei, J., Yang, S., 2021. SARS-CoV-2 M(pro) inhibitors with antiviral activity in a transgenic mouse model. *Science*.
- Ratia, K., Pegan, S., Takayama, J., Sleeman, K., Coughlin, M., Baliji, S., Chaudhuri, R., Fu, W., Prabhakar, B.S., Johnson, M.E., Baker, S.C., Ghosh, A.K., Mesecar, A.D., 2008. A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16119–16124.
- Rogosnitzky, M., Okediji, P., Koman, I., 2020. Cepharanthine: a Review of the Antiviral Potential of a Japanese-approved Alopecia Drug in COVID-19. *Pharmacological Reports* : PR, pp. 1–8.
- Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D., Pillay, S., San, E.J., Msomi, N., Mlisana, K., von Gottberg, A., Walaza, S., Allam, M., Ismail, A., Mohale, T., Glass, A.J., Engelbrecht, S., Van Zyl, G., Preiser, W., Petruccione, F., Sigal, A., Hardie, D., Marais, G., Hsiao, M., Korsman, S., Davies, M.-A., Tyers, L., Mudau, I., York, D., Maslo, C., Goedhals, D., Abrahams, S., Laguda-Akingba, O., Alisoltani-Dehkordi, A., Godzik, A., Wibmer, C.K., Sewell, B.T., Lourenço, J., Alcantara, L.C.J., Pond, S.L.K., Weaver, S., Martin, D., Lessells, R.J., Bhiman, J.N., Williamson, C., de Oliveira, T., 2020. Emergence and Rapid Spread of a New Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) Lineage with Multiple Spike Mutations in South Africa. *medRxiv*.
- Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455–461.
- Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., Qin, C., Sun, F., Shi, Z., Zhu, Y., Jiang, S., Lu, L., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res.* 30, 343–355.
- Yang, C., Pan, X., Xu, X., Cheng, C., Huang, Y., Li, L., Jiang, S., Xu, W., Xiao, G., Liu, S., 2020. Salvianolic acid C potentially inhibits SARS-CoV-2 infection by blocking the formation of six-helix bundle core of spike protein. *Signal transduction and targeted therapy* 5, 220.
- Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K., Hilgenfeld, R., 2020a. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors. *Science* 368, 409–412.

- Zhang, S., Xue, X., Zhang, L., Zhang, L., Liu, Z., 2015. Comparative analysis of pharmacophore features and quantitative structure-activity relationships for CD38 covalent and non-covalent inhibitors. *Chem. Biol. Drug Des.* 86, 1411–1424.
- Zhang, Y.N., Zhang, Q.Y., Li, X.D., Xiong, J., Xiao, S.Q., Wang, Z., Zhang, Z.R., Deng, C. L., Yang, X.L., Wei, H.P., Yuan, Z.M., Ye, H.Q., Zhang, B., 2020b. Gemcitabine, lycorine and oxysophoridine inhibit novel coronavirus (SARS-CoV-2) in cell culture. *Emerg. Microb. Infect.* 9, 1170–1173.
- Zhang, Z.R., Zhang, Y.N., Li, X.D., Zhang, H.Q., Xiao, S.Q., Deng, F., Yuan, Z.M., Ye, H.Q., Zhang, B., 2020c. A cell-based large-scale screening of natural compounds for inhibitors of SARS-CoV-2. *Signal transduction and targeted therapy* 5, 218.