Molecules inhibit the enzyme activity of 3-chymotrypsin-like cysteine protease of SARS-CoV-2 virus

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Summary

SARS-CoV-2 has emerged as a world public health threat. Herein, we report that the clinical approved auranofin could significantly inhibit the activity of 3-chymotrypsin-like cysteine protease (Mpro or 3CLpro) of SARS-CoV-2. Phenyl isothiocyanate and gold cluster could perfectly inhibit 3CLpro of SARS-COV-2. Vitamin K3 could well suppress the activity of 3CLpro. For Mpro inhibition, IC50 of auranofin, Vitamin K3, phenyl isothiocyanate, gold cluster are about 0.51μM, 7.96μM, 3.68μM, 1.61μM, respectively. These compounds may be with potentials for treatment SARS-CoV-2 virus replication. Especially for FDA approved auranofin, it is an anti-inflammation drug in clinic, thus it may with strong potential to inhibit virus replication and suppress the inflammation damage in COVID-19 patients. Gold cluster is with better safety index and well anti-inflammation in vitro/vivo, therefore it is with potential to inhibit virus replication and suppress the inflammation damage caused by COVID-19 virus.

Introduction

A new coronavirus named as COVID-19 virus (also called SARS-CoV-2 virus) is responsible for the 2020 pandemic outbreak in the world. The viral 3-chymotrypsin-like cysteine protease (3CLpro, also called Mpro) enzyme controls this COVID-19 virus replication

and is essential for its life cycle. Therefore 3CLpro is drug target in the case of SARS-CoV-2. Our biochemistry studied revealed that the gold compounds such as auranofin, Isothiocyanate compounds such as phenyl isothiocyanate, vitamin K such as vitamin K3, and gold cluster such as glutathione coated gold cluster could well inhibit the activity of 3CLpro. These compounds could serve as potential anti-SARS-CoV-2 lead molecules for further drug studies to combat COVID-19.

Expression and purification of 3CLpro

Recombinant COVID-19 virus 3CLpro with native N and C termini was expressed in Escherichia coli and subsequently purified following the recently reported work (1). The full-length gene encoding COVID-19 virus Mpro was synthesized for Escherichia coli expression. Briefly, the expression plasmid was transformed into Escherichia coli cells and cultured in Luria Broth medium containing 100 μg/ml ampicillin at 37 °C. When the cells were grown to OD600 of 0.6-0.8, 0.5 mM IPTG was added to the cell culture to induce the expression at 16 °C. After 10 h, the cells were harvested by centrifugation at 3,000g. The cell pellets were resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 300 mM NaCl), lysed by high-pressure homogenization, and then centrifuged at 25,000g for 40 min. The supernatant was loaded onto Ni-NTA affinity column,

and washed by the resuspension buffer containing 20 mM imidazole. The His tagged Mpro was eluted by cleavage buffer (50 mM Tris-HCl pH 7.0, 150 mM NaCl) including 300 mM imidazole. Human rhinovirus 3C protease was added to remove the C-terminal His tag. The Mpro was further purified by ion exchange chromatography and size exclusion chromatography. The purified Mpro was stored in 50 mM Tris-HCl pH 7.3, 1 mM EDTA.

Inhibit Enzyme activity of Mpro in vitro

In order to characterize 3CLpro enzymatic activity, we used a fluorescence resonance energy transfer (FRET) assay (1, 2). To do this, a fluorescently labeled substrate, (EDNAS-Glu)-Ser-Ala-Thr-Leu-Gln-Ser-Gly-Leu-Ala-(Lys-DABCYL)-Ser, derived from the auto-cleavage sequence of the viral protease was chemically modified for enzyme activity assay (2). Potential inhibitors suppress COVID-19 virus Mpro were screened by the this enzymatic inhibition assay. Molecules picked from gold compounds library, gold cluster library (gold atoms stack together to form cluster and the cluster is stabled by organic molecule with thiol group, where thiol groups interact with gold cluster via Au-S bond), Isothiocyanate library, and vitamin K library were used. When the different compounds were added into the enzymatic reaction solution, the change of initial rates was calculated to evaluate their

inhibitory effect. The molecule inhibit Mpro activity reaction mixture included 0.5 μM protein, 20 μM substrate (EDNAS-Glu)-Ser-Ala-Thr-Leu-Gln-Ser-Gly-Leu-Ala-(Lys-DABCYL)-Ser) and molecules from afore mentioned compound library. The compounds of interest were defined as those with a percentage of inhibition over 50% compared with the reaction in the absence of inhibitor. IC50 values of four compounds were measured using 0.5 μM protein, 20 μM substrate and 9 different inhibitor concentrations of compounds. All experiments were performed in triplicate.

Results and discussion

For Mpro inhibition, IC50 of auranofin is about 0.51μM, data showed as following Figure 1. This is a very strong suppressor for Mpro. This result is a strong proof to well understand why auranofin could well inhibit virus replication (3). The auranofin was approved by FDA in clinical treatment Rheumatoid Arthritis treatments via suppress the immune cytokine activity (4). As COVID-19 virus could cause immune cytokine storm in patients, Recent reports revealed drugs inhibit immune cytokine activity could well recover severe patients (5). The auranofin combined the potential of anti-virus and the proved anti-inflammation together to recover severe COVID-19 infected patients.

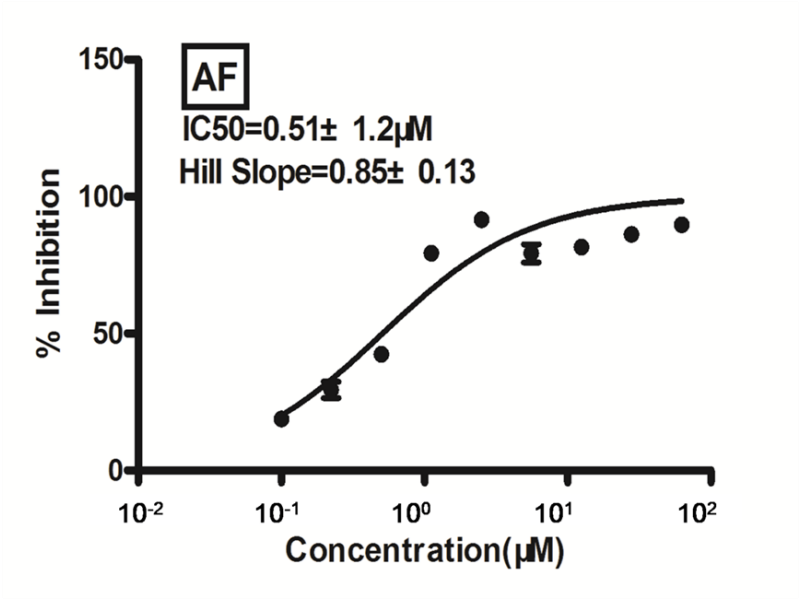

Figure 1. auranofin inhibit Mpro at dose dependent manner.

For Mpro inhibition, IC50 of Menadione (vitamin K3) is about 7.96μM, this is very valuable lead molecule for further anti-virus studies, data showed as following Figure 2. Vitamin K is widely used as dietary supplements approved by FDA (6), thus it is benefit for vitamin K3 applied for further anti-COVID-19 virus studies.

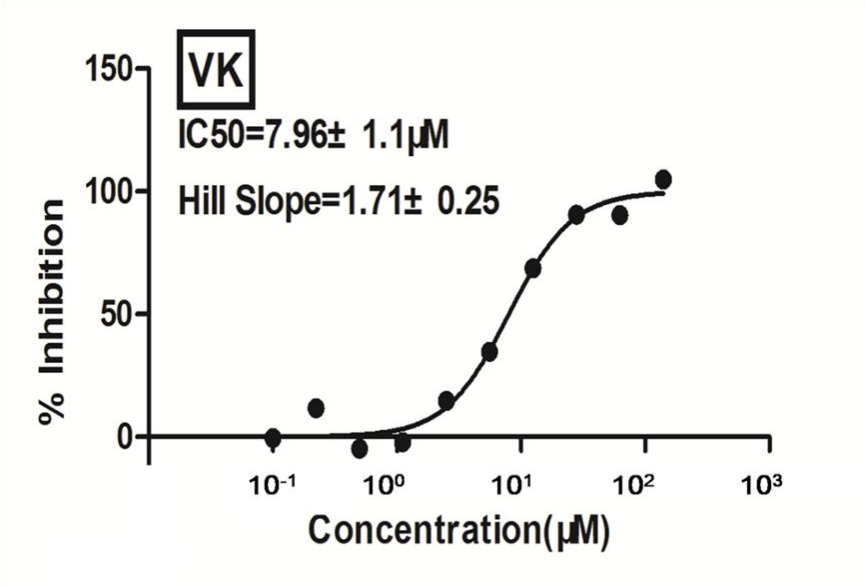

Figure 2. Vitamin K3 inhibit Mpro at dose dependent manner.

For Mpro inhibition, IC50 of phenyl isothiocyanate is about 3.68μM, data showed as following Figure 3. Phenethyl isothiocyanate is a constituent of cruciferous vegetables that has cancer preventive activity including lung, prostate, and breast cancer (7). The potential of this compound to inhibit COUID-19 virus is an interesting topic as phenethyl isothiocyanate is a good candidate to be a dietary supplement.

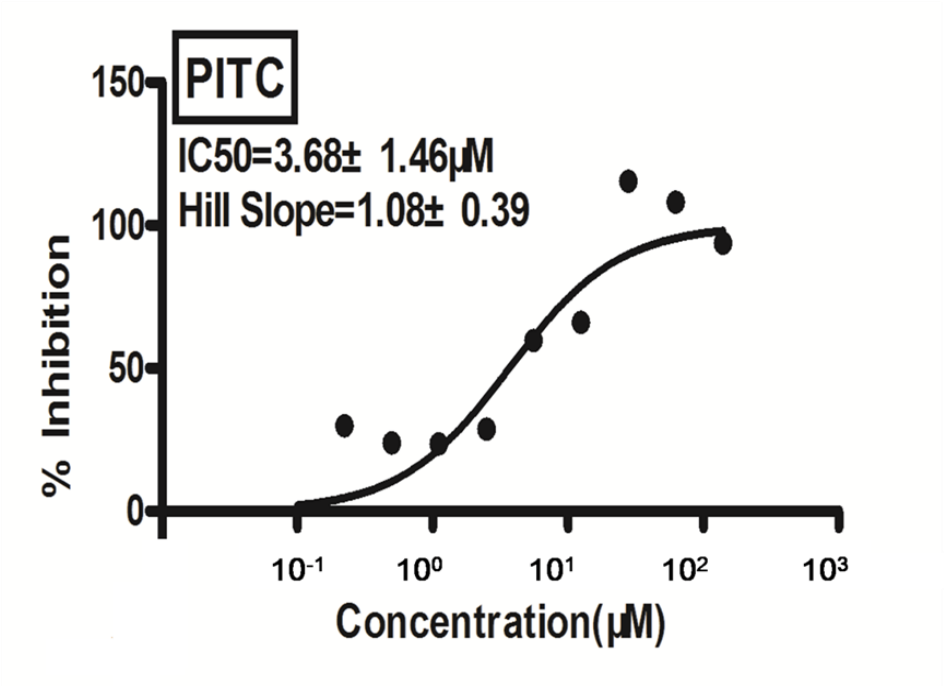

Figure 3. phenethyl isothiocyanate inhibit Mpro at dose dependent manner.

For Mpro inhibition, IC50 of glutathione gold cluster ($Au_{29}SG_{27}$) is about 1.61μM, data showed as following Figure 4. This result showed glutathione gold cluster could well inhibit Mpro in vitro. Thus it is with strong potentials to inhibit COVID-19 virus replication. Note that gold clusters are with strong anti-inflammation for

Rheumatoid Arthritis treatments in vitro/vivo, its safety index is better than FDA approved Auranofin and Methotrexate in animal studies (8, 9, 10, 11). Combined its anti-virus Mpro and clarified anti-inflammation effects, gold cluster may be with advantage in later anti-COVID-19 studies.

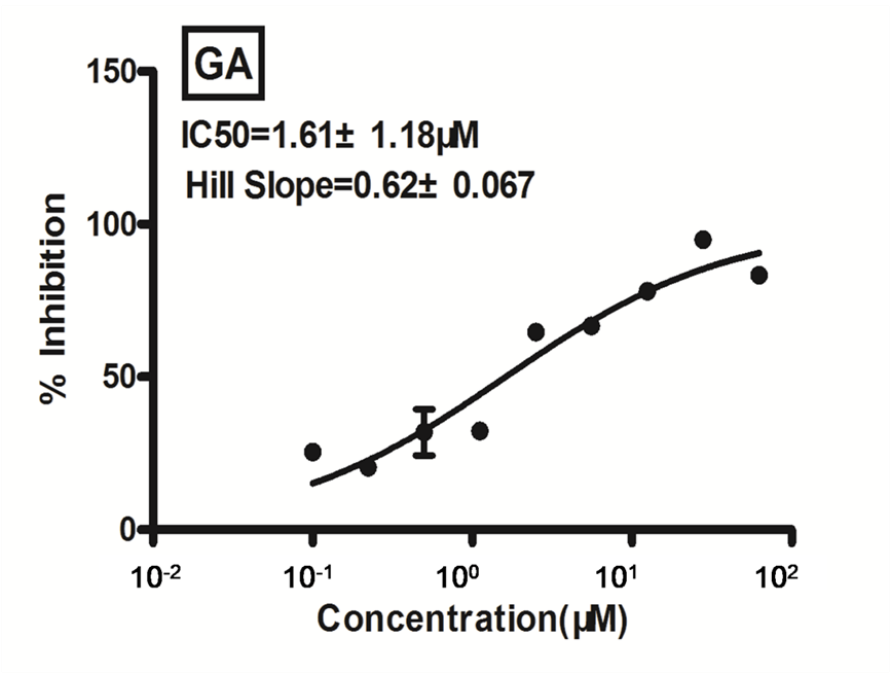

Figure 4. Gold cluster inhibit Mpro at dose dependent manner.

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