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Short Communication

Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro

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ABSTRACT

An escalating pandemic by the novel SARS-CoV-2 virus is impacting global health and effective therapeutic options are urgently needed. We evaluated the in vitro antiviral effect of compounds that were previously reported to inhibit coronavirus replication and compounds that are currently under evaluation in clinical trials for SARS-CoV-2 patients. We report the antiviral effect of remdesivir, lopinavir, homorringtonine, and emetine against SARS-CoV-2 virus in Vero E6 cells with the estimated 50% effective concentration at 23.15 μM, 26.63 μM, 2.55 μM and 0.46 μM, respectively. Ribavirin or favipiravir that are currently evaluated under clinical trials showed no inhibition at 100 μM. Synergy between remdesivir and emetine was observed, and remdesivir at 6.25 μM in combination with emetine at 0.195 μM may achieve 64.9% inhibition in viral yield. Combinational therapy may help to reduce the effective concentration of compounds below the therapeutic plasma concentrations and provide better clinical benefits.

Within three months of the first identification of SARS-CoV-2 virus in Wuhan, Hubei Province, China, the world is facing an escalating pandemic that will have significant impacts on global health systems and economy (WHO, 2019). Infection with the novel SARS-CoV-2 virus may lead to a wide range of clinical presentations from asymptomatic infection in 1% of laboratory confirmed cases to mild, severe, and critical infections in 81%, 14%, and 5% of symptomatic cases, respectively (Wu and McGoogan, 2020). The estimated symptomatic case-fatality risk (sCFR) among cases in Wuhan was 1.4%, and those aged above 59 were 5.1 times more likely to die from infection than those aged 30–59 years (Wu et al., 2020). With an estimated basic reproductive number of 2.2 (95% CI, 1.4–3.9) (Li et al., 2020), the virus will continue to spread and infect 55% of the global population over time if no effective vaccine is developed (Fine et al., 2011). There is currently no effective antiviral compound licensed for the treatment against human coronaviruses or SARS-CoV-2.

The SARS-CoV-2 virus shared 79.5% genetic homology to the SARS-CoV and both are descendants of bat coronaviruses within the *Betacoronavirus* genus (Zhou et al., 2020). Antiviral compounds previously reported to show effect against SARS-CoV or other coronaviruses may be effective against SARS-CoV-2 (Chu et al., 2004; de Wilde et al., 2014; Dyall et al., 2014; Shen et al., 2019; Cao et al., 2015). In addition, remdesivir (GS-5734), a prodrug of adenosine analog with a broad-spectrum antiviral activity against filoviruses, paramyxoviruses, and coronaviruses (Brown et al., 2019; Sheahan et al., 2017; de Wit et al., 2020), was recently confirmed to inhibit 2019-nCoV in vitro (Wang et al., 2020). According to the 7th edition of the novel coronavirus diagnosis and treatment plan issued by the National Health Commission of the People's Republic of China, options for antiviral therapy include aerosolized α-interferon, lopinavir/ritonavir, ribavirin in combination with lopinavir/ritonavir, chloroquine phosphate, or Arbidol (China National Health Commission, 2020). Ongoing clinical trials are evaluating the efficacy of remdesivir, and various HIV-protease inhibitors (lopinavir/ritonavir, ASC09/ritonavir, darunavir), reverse transcriptase inhibitor (Azvudine), anti-influenza compounds, interferon alfa-2b, or monoclonal antibody targeting PD-1 (Camrelizumab) or IL-6 (Tocilizumab) (Chinese Clinical Trial Re). We evaluated the anti-SARS-CoV-2 effect of compounds that have been under development or already approved for other clinical applications; some compounds were previously reported to inhibit coronavirus replication in vitro, and some are evaluated in clinical trials in patients with coronavirus disease (COVID-19).

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SARS-CoV-2 virus, BetaCoV/Hong Kong/VM20001061/2020, was isolated from the nasopharynx aspirate and throat swab of a confirmed COVID-19 patient in Hong Kong using Vero E6 cells (ATCC CRL-1586). Stock virus ($10^{7.25}$ $TCID_{50}$/mL) was prepared after three serial passages in Vero E6 cells in infection media (DMEM supplemented with 4.5 g/L D-glucose, 100 mg/L sodium pyruvate, 2% FBS, 100,000 U/L Penicillin-Streptomycin, and 25 mM HEPES). Compounds were sourced from MedChemExpress and Sigma-Aldrich and the stocks were prepared with DMSO (50 mM remdesivir, 100 mM favipiravir, 10 mM R-1479, 10 mM tenofovir, 10 mM fludarabine phosphate, 10 mM baloxavir, 10 mM chlorpromazine hydrochloride, 5 mM dalbavancin hydrochloride, 10 mM homoharringtonine, 10 mM lopinavir, 10 mM ritonavir) or with water (5 mM emetine dihydrochloride, 10 mM galidesivir hydrochloride, 50 mM ribavirin, 2.5 mM oritavancin diphosphate). Oseltamivir carboxylate (10 mM in water) was provided by Roche. To evaluate the effect of compounds in vitro, Vero E6 cells were pre-treated with compounds diluted in infection media for 1 h prior to infection by SARS-CoV-2 virus at MOI = 0.02. Antiviral compounds were maintained with the virus inoculum during the 2-h incubation period. The inoculum was removed after incubation, and the cells were overlaid with infection media containing diluted compounds. After 48 h incubation at 37 °C, supernatants were collected to quantify viral loads by $TCID_{50}$ assay or quantitative real-time RT-PCR (TaqMan™ Fast Virus 1-Step Master Mix) following the methods described (Chu et al., 2020). Four-parameter logistic regression (GraphPad Prism) was used to fit the dose-response curves and determined the 50% effective concentrations (EC_{50}) of the compounds that inhibit viral replication. Cytotoxicty of selected compounds was evaluated in Vero E6 cells using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

Among the 16 compounds we tested, remdesivir, lopinavir, homoharringtonine, and emetine dihydrochloride were found to inhibit SARS-CoV-2 replication in Vero E6 cells with EC_{50} under 100 μM (Table 1). Importantly, we observed that some of the compounds currently undergoing clinical trials such as ribavirin, favipiravir, oseltamivir, or baloxavir showed no apparent antiviral effect against the SARS-CoV-2 virus in vitro at concentrations under 100 μM (Table 1). Remdesivir is a 1′-cyano-substituted adenosine analogue that has been shown to inhibit human coronaviruses (hCoV-OC43 and hCoV-229E) SARS-CoV, MERS-CoV, and SARS-CoV-2 (Brown et al., 2019; Sheahan et al., 2017; de Wit et al., 2020). It is currently evaluated in phase 4 clinical trials for SARS-CoV-2. A recent study fitted viral load in linear scale (eg. the percentage of inhibition) under increasing concentrations of remdesivir reported EC_{50} against SARS-CoV-2 virus at 0.77 μM (Wang et al., 2020). We fitted viral load in logarithm scale ($log_{10}TCID_{50}$/mL and log_{10} viral RNA copies/mL) under increasing concentration of remdesivir and determined EC_{50} at 23.15 μM and 26.90 μM, respectively (Fig. 1A and Table 1). Two mutations (F476L and V553L) in the RNA-dependent RNA polymerase nsp12 of a murine hepatitis virus have been previously reported to confer resistance to remdesivir (Agostini et al., 2018). Due to insertions and deletions in nsp12, these two conserved residues are mapped at F480 and V557 in the SARS-CoV-2 isolate (GISAID# EPI_ISL_412028) used for the experiments, which should remain sensitive for remdesivir. Other adenosine analogues (galidesivir, tenofovor, or fludarabine phosphate) or nucleoside analogues (favipiravir, ribavirin, R-1479) did not inhibit viral replication under 100 μM (Table 1). However, nucleoside analogues require metabolic activation into their triphosphate forms by host cellular nucleoside kinases, which may differ among cell types. Further evaluation of the effect of nucleoside analogues in primary human airway epithelial cells would facilitate the interpretation of the results.

Lopinavir in combination with ritonavir are FDA approved HIV-1 protease inhibitors. Lopinavir was more potent in inhibiting HIV-1 than ritonavir in vitro but showed poor bioavailability in vivo. Ritonavir inhibits not only HIV-1 protease but also the host's cytochrome P450 3A4 enzyme that metabolizes lopinavir (Kempf et al., 1997). Lopinavir/ritonavir in combination prolongs bioavailability of lopinavir in vivo (Sham et al., 1998). Lopinavir but not ritonavir showed antiviral effect against SARS-CoV, MERS-CoV, and hCoV-229E in vitro, with mean EC_{50} ranged from 6.6 to 17.1 μM (de Wilde et al., 2014). Lopinavir/ritonavir in combination with ribavirin were used previously to treat SARS-CoV patients under a non-randomized clinical trial. Less SARS patients developed into ARDS or death after receiving the combination of lopinavir/ritonavir with ribavirin than historical controls who received ribavirin and corticosteroids (Chu et al., 2004). Efficacy of lopinavir/ritonavir with or without ribavirin is currently evaluated in SARS-CoV-2 patients under randomized control trials. In agreement with previous reports, we observed antiviral effect of lopinavir (EC_{50} at 26.1 μM) but not ritonavir against SARS-CoV-2 in vitro (Fig. 1B and Table 1). HIV-1 patients treated with 400 mg of lopinavir and 100 mg of ritonavir twice daily may reach the minimal lopinavir serum concentration at 9.4 μM (IQR 7.2–12.1 μM), which is below the EC_{50} against SARS-CoV-2 virus in vitro (Lopez-Cortes et al., 2013). Currently, lopinavir/ritonavir at 400mg/100 mg twice daily with or without ribavirin are part of the recommended treatment for managing COVID-19 patients in China (China National Health Commission, 2020). A recent randomized control trial reported no significant benefit of lopinavir-ritonavir in hospitalized SARS-CoV-2 patients than standard care, as the time to clinical improvement, mortality at 28 days, and viral loads at various time points were comparable between the two groups (Cao et al., 2020). Combinational therapy of lopinavir with the other effective compounds against SARS-CoV-2 virus may increase synergy and reduce the inhibitory concentration of lopinavir.

Homoharringtonine is a plant alkaloid derived from *Cephalotoxus fortunei*. It exhibits anti-tumor activity by binding to the ribosomal A site to inhibit protein translation, leading to rapid loss of short-lived proteins including Mcl-1 and c-Myc that promote the survival of leukemia cells (Dong et al., 2018; Lu and Wang, 2014). Omacetaxine, a semi-synthetic form of homoharringtonine, is approved by FDA for treatment of chronic myeloid leukemia. Homoharringtonine has also been reported to exhibit potent anti-viral activity against herpesviruses (varicella-zoster virus, herpes simplex virus-1, pseudorabies virus), coronaviruses (porcine epidemic diarrhea virus and murine hepatitis virus), rhabdoviruses (VSV and rabies virus), and other viruses (hepatitis B virus, Newcastle disease virus, and echovirus 1) (Dong et al., 2018; Andersen et al., 2019). Here, we observed homoharringtonine inhibits SARS-CoV-2 with EC_{50} at 2.10 μM (Fig. 1C and Table 1). Previous pharmacokinetic study showed that patients treated with 1.25 mg/m^2 omacetaxine every 12 h by subcutaneous injection may reach the maximal plasma concentration at 25.1 ng/mL (0.046 μM) and 36.2 ng/mL (0.066 μM) on days 1 and 11, respectively (Nemunaitis et al., 2013), which were below the EC_{50} against SARS-CoV-2 virus in vitro.

Emetine is a protein synthesis inhibitor that was used as anti-protozoan approved for treatment of ameobiasis; it also inhibits malaria by binding to the ribosomal E site of *Plasmodium falciparum* (Grollman, 1966; Wong et al., 2014). However, its potential cardiotoxicity has restricted its clinical use in the recent years. It was found to process antiviral activity against a broad range of RNA and DNA viruses, including Zika virus, Ebolavirus, Cytomegalovirus, rabies virus, HIV-1, echovirus 1, buffalo poxvirus, bovine herpesvirus 1, peste des petits ruminants virus, Newcastle disease virus, herpes simplex virus-2, metapneumovirus, Rift Valley fever virus, and influenza (Andersen et al., 2019; Chaves Valadao et al., 2015; Khandelwal et al., 2017; MacGibeny et al., 2018; Mukhopadhyay et al., 2016; Yang et al., 2018). Emetine was also identified to inhibit hCoV-OC43, hCoV-NL43, SARS-CoV, MERS-CoV, and MHV-A59 in vitro with EC_{50} reported at low micromolar range (Dyall et al., 2014; Shen et al., 2019). We observed emetine at around 0.5 μM may effectively inhibit SARS-CoV-2 virus replication (Fig. 1D and Table 1). The therapeutic plasma concentration of emetine may reach 0.075 μg/mL (0.156 μM) (Regenthal et al., 1999), which is below the EC_{50} against SARS-CoV-2 virus in vitro. The toxic plasma concentration is 0.5 μg/mL (1.04 μM) (Regenthal et al., 1999).

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Table 1
Antiviral activity of 16 compounds against SARS-CoV-2 in Vero E6 cells.

Compounds				Inhibition of SARS-CoV-2 in vitro, μM			
Name	Bioactivity	Clinical application	CAS No.	CC_{50}, μM[a]	CPE inhibition[b]	Reduction in infectious virus[c] (EC_{50})	Reduction in viral RNA copy[d] (EC_{50})
Remdesivir	adenosine analogue	Phase 4 trials for treatment of Ebola or SARS-CoV-2	1809249-37-3	> 100	25	23.15	26.90
Favipiravir	guanine analogue	Approved in Japan and China for treatment of influenza infection	259793-96-9	> 100	> 100	> 100	> 100
Ribavirin	guanosine analogue	FDA approved for treatment of chronic hepatitis C infection	36791-04-5	> 100	500	> 500	> 500
Galidesivir	adenosine analogue	Phase 2 trial for yellow fever virus infection	222631-44-9	> 100	100	> 100	> 100
R-1479	cytidine analogue	Phase 2 trial for treatment of dengue virus infection	478182-28-4	> 100	> 100	N.D.	N.D.
Tenofovor	adenosine analogue	FDA approved for treatment of HIV-1 and HBV	147127-20-6	> 100	> 100	N.D.	N.D.
Fludarabine phosphate	adenosine analogue	FDA approved for treatment of B-cell chronic lymphocytic leukemia	75607-67-9	> 100	> 100	N.D.	N.D.
Lopinavir	protease inhibitor	FDA approved for treatment of HIV-1 infection in combination with ritonavir	192725-17-0	49.75	25	26.63	26.10
Ritonavir	protease inhibitor	FDA approved for treatment of HIV-1 infection in combination with other antiretroviral agents	155213-67-5	48.91	> 100	> 100	> 100
Emetine hydrochloride	anti-protozoal	Approved in China for severe invasive amoebiasis	316-42-7	56.46	1.5625	0.46	0.50
Oritavancin diphosphate	antibiotics	FDA approved treatment for skin infection caused by Gram positive bacteria	192564-14-0	N.D.	> 100	N.D.	N.D.
Dalbavancin hydrochloride	antibiotics	FDA approved treatment for skin infection caused by Gram positive bacteria	2227366-51-8	N.D.	> 100	N.D.	N.D.
Homoharringtonine	anti-cancer	FDA approved treatment for chronic myeloid leukemia	26833-87-4	59.75	3.125	2.55	2.14
Oseltamivir carboxylate	antiviral, neuraminidase inhibitor	FDA approved treatment for influenza infection	187227-45-8	> 100	> 100	> 100	> 100
Baloxivir acid	antiviral, endonuclease inhibitor	FDA approved treatment for influenza infection	1985605-59-1	85.90	> 100	> 100	> 100
Chlorpromazine hydrochloride	antagonist for post-synaptic receptors	FDA approved treatment for schizophrenia	69-09-0	21.29	> 100	N.D.	N.D.

N.D. Not determined.

[a] CC_{50} was determined with serially-diluted compounds in Vero E6 cells at 48 h post-incubation using CellTiter-Glow Luminescent Cell Viability Assay (Promega).

[b] Compounds were serially 2-fold or 4-fold diluted from 100 μM, except ribavirin which was started at 500 μM. Cytopathic effects (CPE) of SARS-CoV-2 virus in Vero E6 cells under increasing concentration of the compounds were observed at 48 h post-infection. The lowest concentration of the compound with 100% CPE inhibition (eg. exhibiting comparable CPE of non-infected controls) was recorded.

[c] EC_{50} determined by infectious virus yield in culture supernatant at 48h post-infection ($\log_{10}$ $TCID_{50}$/mL).

[d] EC_{50} determined by viral RNA copy numbers in culture supernatant at 48h post-infection ($\log_{10}$ RNA copies/mL).

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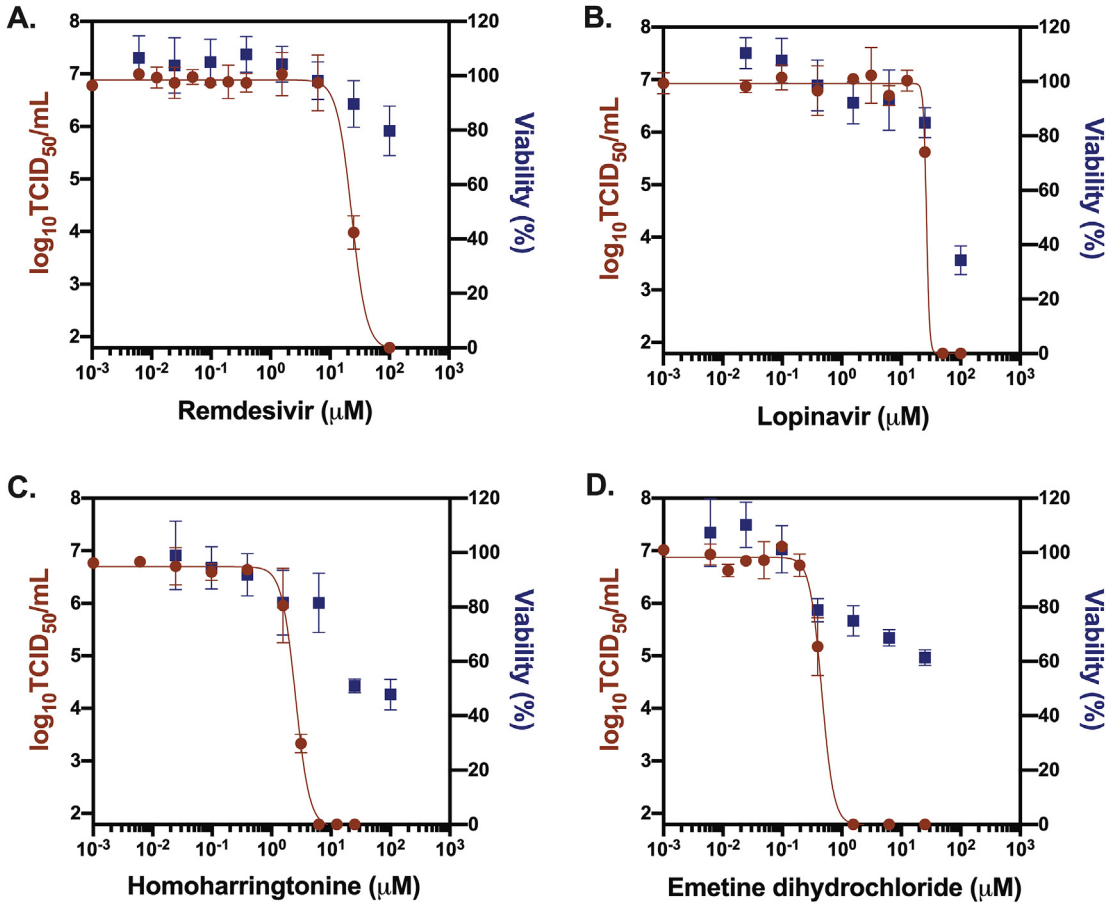

Fig. 1. Antiviral activity of remdesivir (A), lopinavir (B), homorringtonine (C) and emetine dihydrochloride (D) against SARS-CoV-2 virus in vitro. Infectious viral loads ($log_{10}TCID_{50}$/mL left Y axis) and viability (normalized to the ATP level of the Vero E6 cells incubated with infection media) under increasing concentrations of the antiviral compounds are shown.

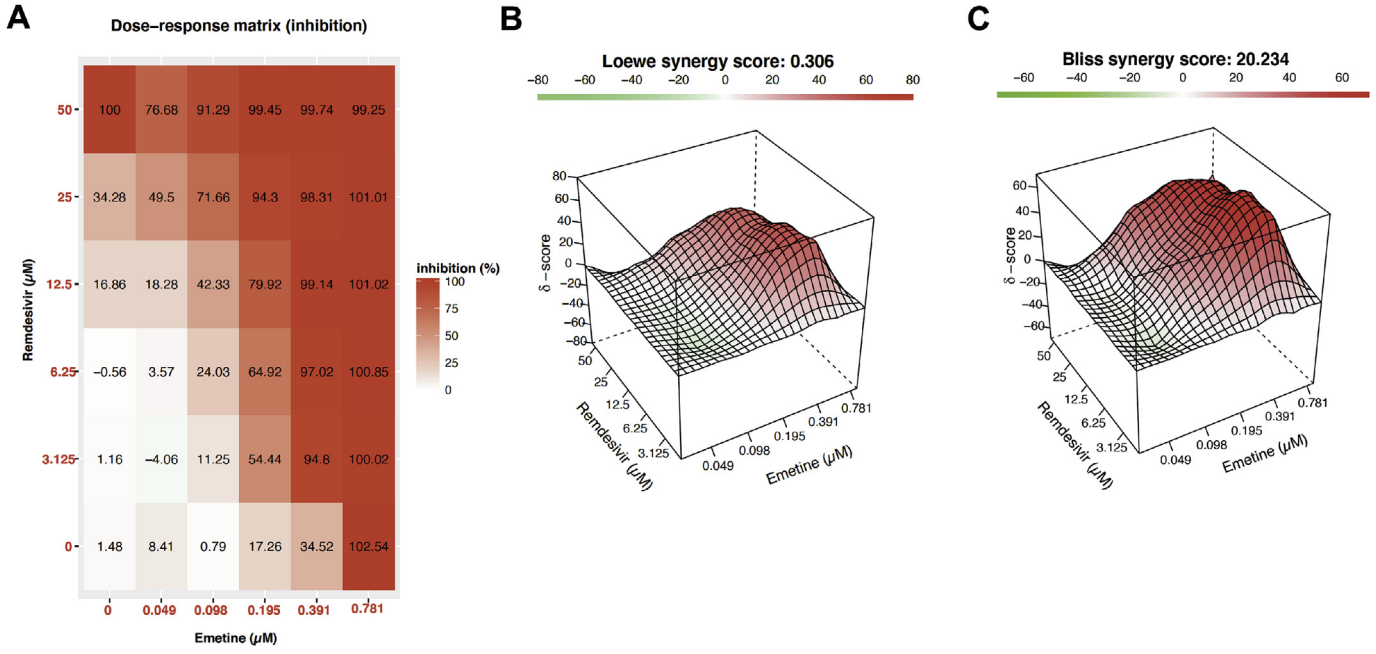

Fig. 2. Combinational effect of remdesivir and emetine dihydrochloride against SARS-CoV-2 virus in vitro. (A) Dose response matrix of serially 2-fold diluted remdesivir (0–50 μM) and emetine (0–0.781 μM) in Vero E6 cells. The percentage of viral inhibition was normalized based on viral load in logarithm scale (log_{10}RNA copies/mL), using the maximal viral RNA copies with no drug controls as 0% inhibition and the minimal RNA copies determined at 50 μM remdesivir as references. (B) The three-dimensional interaction landscapes of remdesivir and emetine were generated by SynergyFinder (Ianevski et al., 2017) based on (B) the Loewe additive model and (C) the Bliss independence model. Red colour indicates synergy while the green colour indicates antagonism of the two drugs.

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To reduce the effective concentration of individual compound below the maximal therapeutic plasma concentration, we explored the combinational effect of remdesivir and emetine in vitro. Drug interaction was evaluated using the checkerboard assay with serially 2-fold diluted remdesivir (0–50 μM) and emetine (0–0.781 μM) in combination. Remdesivir at 6.25 μM in combination with emetine at 0.195 μM may achieve 64.9% inhibition of viral yield, which can be further tested in vivo (Fig. 2A). The Loewe additive model and the Bliss independent model (Malyutina et al., 2019) were used to analyse the interaction of the two compounds using SynergyFinder (Ianevski et al., 2017). Remdesivir and emetine in combination yielded a Loewe synergy score of 0.306 (Fig. 2B) and a Bliss synergy score of 20.234 (Fig. 2C).

We confirm the antiviral activity of four compounds that have been reported to inhibit other coronavirus or SARS-CoV-2 replication in vitro. Our results suggest that combinational therapy may help to reduce the effective concentration against SARS-CoV-2 under the maximal therapeutic plasma concentration. There is an urgent research need to identify optimal dose combination of effective compounds against the SARS-CoV-2 virus for better clinical benefit.

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