Identification of Potent and Safe Antiviral Therapeutic Candidates Against SARS-CoV-2

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COVID-19 pandemic has infected millions of people with mortality exceeding >1 million. There is an urgent need to find therapeutic agents that can help clear the virus to prevent severe disease and death. Identifying effective and safer drugs can provide more options to treat COVID-19 infections either alone or in combination. Here, we performed a high throughput screening of approximately 1,700 US FDA-approved compounds to identify novel therapeutic agents that can effectively inhibit replication of coronaviruses including SARS-CoV-2. Our two-step screen first used a human coronavirus strain OC43 to identify compounds with anti-coronaviral activities. The effective compounds were then screened for their effectiveness in inhibiting SARS-CoV-2. These screens have identified 20 anti-SARS-CoV-2 drugs including previously reported compounds such as hydroxychloroquine, amlodipine besylate, arbidol hydrochloride, tilorone 2HCl, dronedarone hydrochloride, mefloquine, and thioridazine hydrochloride. Five of the newly identified drugs had a safety index (cytotoxic/effective concentration) of >600, indicating a wide therapeutic window compared to hydroxychloroquine which had a safety index of 22 in similar experiments. Mechanistically, five of the effective compounds (fendiline HCl, monensin sodium salt, vortioxetine, sertraline HCl, and salifungin) were found to block SARS-CoV-2 S protein-mediated cell fusion. These FDA-approved compounds can provide much needed therapeutic options that we urgently need during the midst of the pandemic.

Keywords: SARS-CoV-2, COVID-19, antiviral, therapy, US Food and Drug Administration-approved compounds

INTRODUCTION

Novel coronavirus (CoV)–mediated disease (COVID-19) emerged as a major pandemic and has spread across the world in such a short period since December 2019. As of October 5, 2020, more than 35.1 million confirmed infections have been reported with approximately 1 million deaths (WHO, https://covid19.who.int/). These numbers may be a vast underestimation as many of the infected patients may

remain asymptomatic and can only be detected by antibody testing (1). Similarly, many of the deaths may not be accounted for due to a lack of testing. The disease is caused by a novel CoV termed SARS-CoV-2 which belongs to the *Coronaviridae* family and is the third major CoV pandemic in the last 20 years after Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (2–7). The lack of available therapeutic options is a major limiting factor in treating these infections, leading to excessive mortality.

Currently, there is an urgent and unmet need for effective antiviral therapy that can not only decrease the disease burden in the patient but can also decrease the ability of the person to infect others. It is not practical to develop a novel drug for urgent needs such as during the current pandemic, which may take years to confirm safety and efficacy. Alternatively, it sounds like a lucrative option to repurpose the US Food and Drug Administration (FDA) approved drugs for their efficacy against SARS-CoV-2. Earlier screens have found antiviral efficacy of approved therapies such as hydroxychloroquine (8–14); however, these therapies failed to provide any beneficial effects in COVID-19 due to their toxic side effects (13–20). Finding efficacy of an approved drug against SARS-CoV-2 with minimal toxicity can provide much needed therapeutic option to treat COVID-19.

Here we screened approximately 1,700 US FDA-approved compounds to test their ability to inhibit SARS-CoV-2 replication. Here, we report 20 compounds that are highly effective in inhibiting SARS-CoV-2 replication at concentrations that were significantly lower than those having cytotoxic effects. We also investigated the possible mechanism of these compounds.

MATERIALS AND METHODS

Cells and Viruses

LLC-MK2 cells (Rhesus monkey kidney cells), which was provided by the Laboratoire des Pathogènes Emergents (LPE), Fondation Mérieux, Lyon, France, were cultured in 64% Hank's MEM and 32% Earle's MEM (Gibco, New York, USA) supplemented with 3% fetal bovine serum (FBS) (Hyclone, Utah, USA) and 1% glutamine (Thermo, Massachusetts, USA). Vero cells (African green monkey kidney cell), purchased from ATCC, were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% FBS.

Human coronavirus (HCoV) strain OC43, which is a gift from Peking Union Medical College Hospital, was propagated in LLC-MK2 cells in 0.5% FBS MEM and virus titers were determined *via* TCID50 with LLC-MK2 cells. SARS-CoV-2 virus was isolated from the respiratory samples of patients in Wuhan of Hubei Province (3). SARS-CoV-2 virus was propagated in Vero cells and used in this study. All experiments with SARS-CoV-2 virus were conducted in a BSL-3 laboratory.

HEK293T cells stably expressing recombinant human ACE2 (293T/hACE2) were maintained in Dulbecco's MEM containing 10% FBS and 100 units penicillin, and 100 μg of streptomycin per milliliter.

Antibodies

Mouse polyclonal against the OC43 N antibody was prepared in the laboratory. Rabbit polyclonal against SARS-CoV-2 N protein antibody was purchased from Sino Biological (Beijing, China). Alexa Fluor 488–conjugated goat anti-mouse IgG, Alexa Fluor 488–conjugated goat anti-rabbit IgG were purchased from Thermo.

Screening of FDA-Approved Drugs

US FDA-approved drug library which contains 1,700 compounds were purchased from TargetMol (Massachusetts, USA). LLC-MK2 cells were seeded at 2×10^4 cells per well in 96-well plates and incubated at 37°C and 5% CO_2. The next day, LLC-MK2 cells were treated with the compounds at a concentration of 10 μM. After 1 h of treatment, cells were infected with OC43 at MOI of 1. At 48 h post-infection (hpi), cells were fixed with 4% paraformaldehyde for 20 min at room temperature. Immunofluorescence staining was performed using mouse anti-OC43 NP antibody, followed by anti-mouse Alexa Fluor 488 and DAPI (Sigma, St. Louis, MO). Images were captured by Operetta (PerkinElmer, Massachusetts, USA) at the magnification of 20× objective. The infection ratios were calculated using automated image analysis software (Harmony 3.5.2, PerkinElmer). Remdesivir and DMSO were used as positive and negative controls, respectively.

The positively identified drugs from this screen were used to perform dose-response curves against OC43 on LLC-MK2 and against SARS-CoV-2 using Vero cells as described below.

IC$_{50}$ (the Half-Maximal Inhibitory Concentration), CC$_{50}$ (the Half-Maximal Cytotoxic Concentration), and SI (Selectivity Index) Determination

LLC-MK2 cells (for OC43 infection) or Vero cells (for SARS-CoV-2 infection) were seeded in 96 wells plate one day before infection at the concentration of 2×10^4 cells/well or 1.4×10^4 cells/well, respectively. For IC$_{50}$, cells were pre-treated for 1 h with each drug at concentrations 0.013, 0.041, 0.123, 0.370, 1.111, 3.333, 10, and 30 μM and then infected with the virus at MOI of 1. Cell culture media was then replaced containing same concentration of the drug to ensure presence of drug at the time of infection. At 48 hpi (OC43) or 24 hpi (SARS-CoV-2), cells were fixed with 4% paraformaldehyde for 20 min at room temperature. Immunofluorescence was conducted with mouse anti-OC43 N protein antibody or rabbit anti-SARS-CoV-2-NP antibody and followed by anti-mouse, or anti-rabbit Alexa Flour 488 and DAPI. Images were performed by Operetta with 20× objective. The IC$_{50}$ was calculated using automated image analysis software (Harmony 3.5.2, PerkinElmer).

For CC$_{50}$, cells were pre-treated with each drug at concentrations 0.013, 0.041, 0.123, 0.370, 1.111, 3.333, 10, and 30 μM, respectively. After 48 h (LLC-MK2 cells) or 24 h (Vero cells) post-treatment, cell viability was evaluated by using a CCK8 kit (Yeasen, Beijing, China) according to the manufacturer's instructions. The selectivity index was calculated using the following formula: SI = CC$_{50}$/IC$_{50}$. Graphpad Prism 7.0 was used for analyzing IC$_{50}$ and CC$_{50}$.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 20 min at room temperature, and permeabilized with 0.5% Triton X-100 for

10 min. Cells were then blocked with 5% BSA and stained with primary antibodies, followed by staining with an Alexa Fluor 488 secondary antibodies. Nuclei were counterstained with DAPI.

Quantitative RT-PCR

Vero cells were pre-treated with indicated concentrations of drugs for 1 h and incubated with SARS-CoV-2 at 0.1 MOI for 1 h. Then, cells were washed with opti-MEM for one time and incubated with indicated concentrations of drugs. At 24 hpi, supernatants were collected and viral RNA in the cell supernatants were extracted by using Direct-zol RNA MiniPrep kit (Zymo Research, CA, USA) according to the manufacturer's instructions. Viral copy numbers were measured by RT-PCR using primers and probe targeting the SARS-CoV-2 N gene. The reference standard was tenfold diluted from 1×10^9 copies to 1×10^4 copies. PCR amplification cycle was 50°C, 15 min, 95°C, 3 min; 95°C, 15 s, 60°C, 45 s+ Plate Read, 50 cycles. The amplification process, fluorescence signal detection, data storage, and analysis were all completed by quantitative fluorescence PCR and its own software (Bio-Rad CFX Manager). The copies of the virus were calculated according to the standard curve. The inhibition ratio was obtained by dividing the number of copies of the virus in the vehicle control group. The data were nonlinearly fitted by GraphPad 7.0 software to calculate the IC_{50} of each drug.
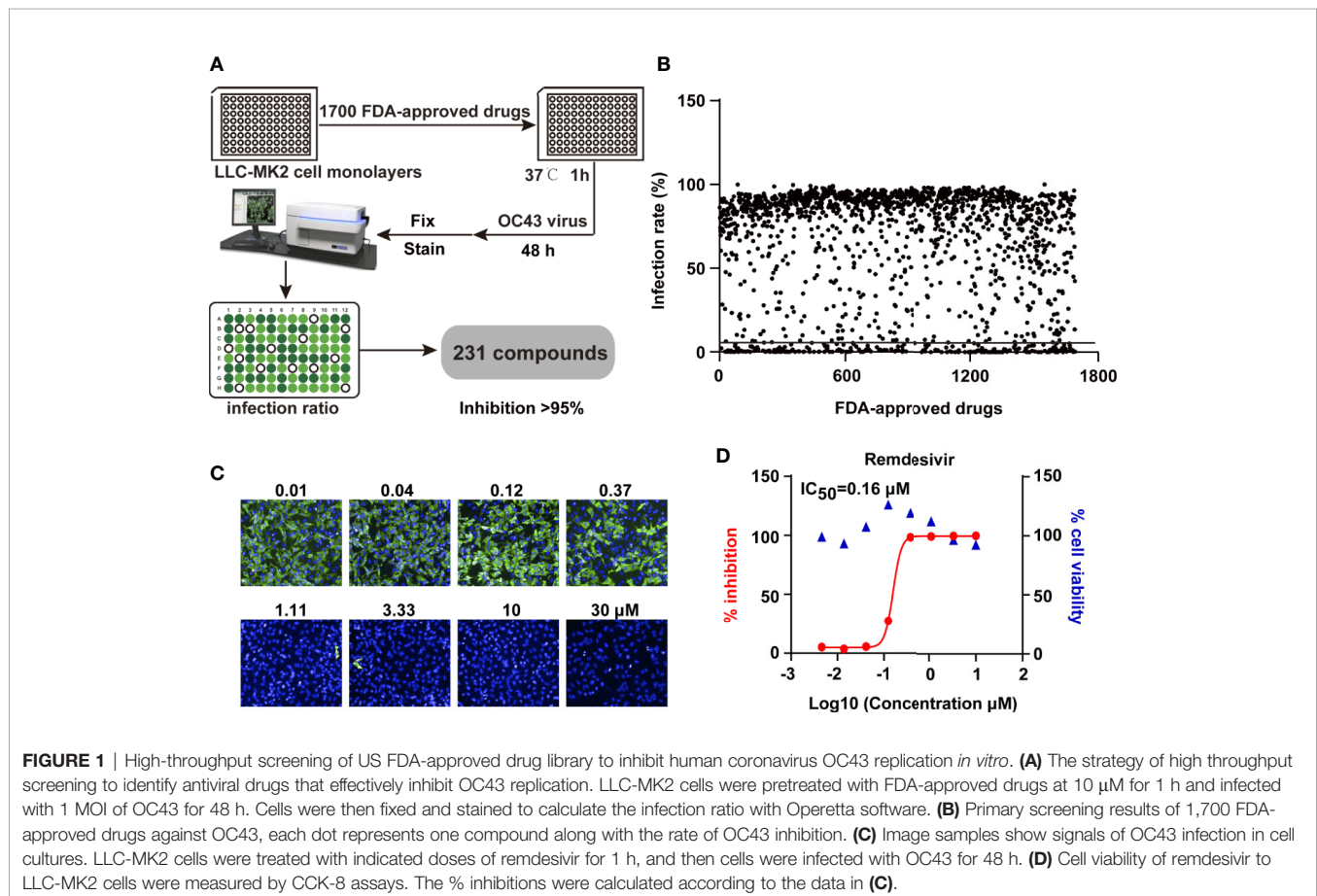
Cell-Cell Fusion Assay

Cell-cell fusion assays were performed as described previously (20). Briefly, HEK-293T cells were co-transfected with SARS-CoV-2-S glycoprotein and eGFP. At 24 h post-transfection, cells were digested with trypsin (0.25%) and overlaid on a 50% confluent monolayer of 293T-ACE2 cells at a ratio of 1:1 which were treated with candidate drugs for 1 h. After 24 h incubation, syncytial images were captured with Operetta (PerkinElmer, Massachusetts, USA).

RESULTS

Inhibitory Potential of FDA-Approved Drugs Against hCoV OC43

Initial screening was performed using HCoV-OC43 due to its low biosafety concerns. OC43 is a hCoV that usually causes mild disease in humans and cattle (21). The experimental protocol is demonstrated in **Figure 1A** using LLC-MK2 cells infected with OC43 at an MOI of 1 for 48 h in presence of the US FDA-approved compounds. The inhibitory potentials of these compounds were measured with the treatment of compounds at 10 µM for 48 h. The viral presence was detected by immunostaining for the virus and DAPI staining for the cell nuclei. The inhibitory capacity was measured using the ratio of viral fluorescence to the DAPI and is depicted in **Figure 1B**. The

FIGURE 1 | High-throughput screening of US FDA-approved drug library to inhibit human coronavirus OC43 replication *in vitro*. **(A)** The strategy of high throughput screening to identify antiviral drugs that effectively inhibit OC43 replication. LLC-MK2 cells were pretreated with FDA-approved drugs at 10 µM for 1 h and infected with 1 MOI of OC43 for 48 h. Cells were then fixed and stained to calculate the infection ratio with Operetta software. **(B)** Primary screening results of 1,700 FDA-approved drugs against OC43, each dot represents one compound along with the rate of OC43 inhibition. **(C)** Image samples show signals of OC43 infection in cell cultures. LLC-MK2 cells were treated with indicated doses of remdesivir for 1 h, and then cells were infected with OC43 for 48 h. **(D)** Cell viability of remdesivir to LLC-MK2 cells were measured by CCK-8 assays. The % inhibitions were calculated according to the data in **(C)**.

initial screen obtained 231 compounds that had the ability to inhibit OC43 replication >95%. The remdesivir was used as a positive control (**Figures 1C, D**).

Calculation of IC$_{50}$, CC$_{50}$, and SI of FDA-Approved Drugs Against OC43

Next, we sought to determine the effective concentrations of positively screened drugs in our initial approach and test the toxicity profile of these 231 drugs in relation to their viral inhibitory concentrations. Our data show that 56 of the positively screened drugs were effectively inhibited the viral replication at sub-micromolar concentrations including many of them can almost completely inhibit the viral replication at micromolar range (**Figure 2**). Surprisingly, the effective drugs against the CoV belonged to a wide range of therapeutic groups including those used for neurological diseases, hormones, enzymes, and antimicrobial agents among others (**Table 1**). The cytotoxic concentration to kill 50% of cells (CC$_{50}$) was noted for these drugs by measuring cell viability over similar concentrations. The selective index in our study was found to be >600 for 5 of the screened compounds. The SI
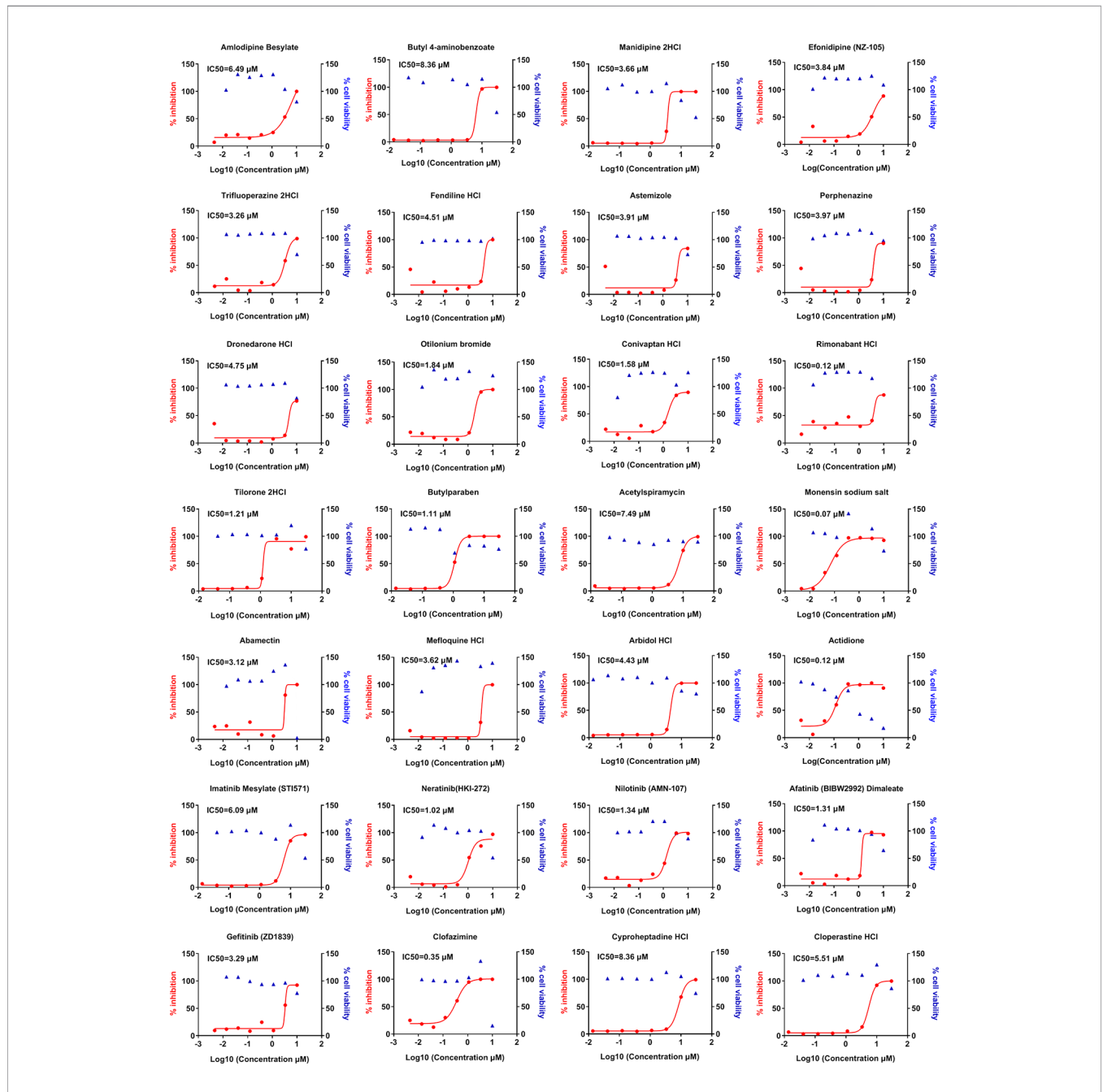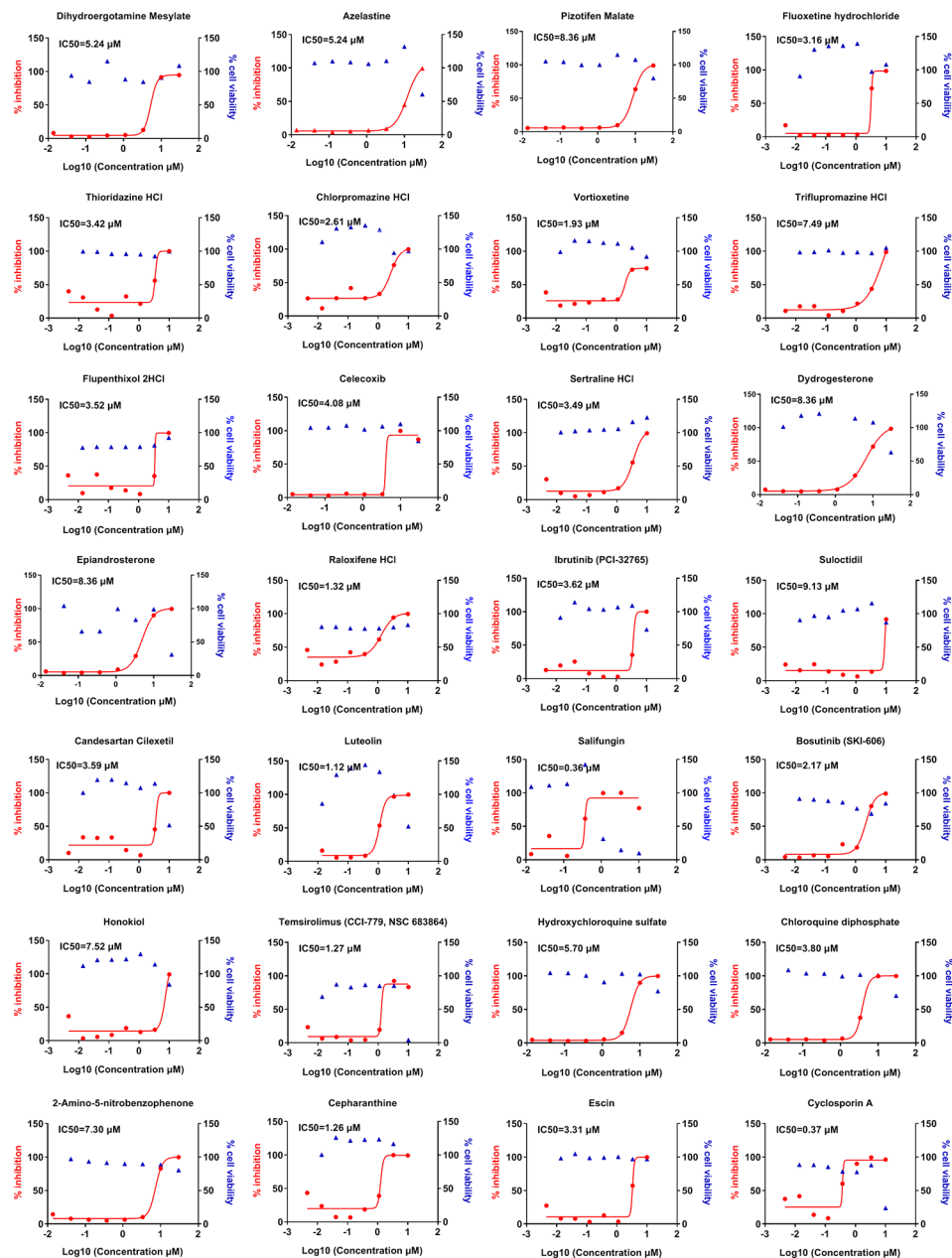

FIGURE 2 | Continued

FIGURE 2 | Dose-response curves of selected compounds from the hits against OC43 infection *in vitro*. LLC-MK2 cells were pretreated with indicated drugs at 37°C for 1 h with eight doses (0.014, 0.041, 0.123, 0.370, 1.111, 3.333, 10, 30 μM) with three-fold dilution followed by infection with OC43 at MOI of 1 for 48 h. In parallel, these compounds' effects on the cell viability in LLC-MK2 cells were measured by CCK-8 assays. The left *Y*-axis of the graphs represents % inhibition of the infection (red dots) and the right *Y*-axis of the graphs presents % cell viability (blue triangles) in the presence of the drugs.

for the hydroxychloroquine was 22 in our study, indicating increased safety of newly identified drugs compared to the hydroxychloroquine.

Testing the Antiviral Effectiveness of Positive Drugs Against SARS-CoV-2

Finally, we sought to determine whether these 56 compounds can show efficacy against SARS-CoV-2, the causative agent of COVID-19. These 56 compounds from the initial screen were

tested for their antiviral efficacy against SARS-CoV-2 in Vero cells. SARS-CoV-2 replicates within the Vero cells and causes cytopathic effects in these cells in the absence of any antiviral treatment. We generated the dose-response inhibition curves along with the cytotoxicity curves for these compounds in the presence of SARS-CoV-2 (**Figure 3**). Remdesivir was used as a positive control. Our data show that 20 of these compounds show significant efficacy in inhibiting SARS-CoV-2 replication with sub micromolar IC_{50} for many of these drugs such as

TABLE 1 | Antiviral activity of selected compounds against OC43.

Pathway	MOLENAME	Formula	Target	CC_{50} (μM)	IC_{50} (μM)	SI (CC_{50}/IC_{50})
Membrane Transporter/Ion Channel	Amlodipine Besylate	$C_{26}H_{31}ClN_2O_8S$	Carbonic anhydrase inhibitor; Calcium Channel inhibitor; PDE inhibitor	28.9	6.49	4.45
	Butyl 4-aminobenzoate	$C_{11}H_{15}NO_2$	Sodium Channel inhibitor	>30	8.36	>3.59
	Manidipine 2HCl	$C_{35}H_{40}Cl_2N_4O_6$	Calcium Channel antagonist	>30	3.66	>8.20
	Efonidipine(NZ-105)	$C_{34}H_{38}N_3O_7P$	Calcium Channel inhibitor	>10	3.84	>2.60
	Trifluoperazine 2HCl	$C_{21}H_{26}Cl_2F_3N_3S$	Dopamine Receptor; Adrenergic Receptor antagonist; Calmodulin inhibitor	>10	3.26	>3.08
	Fendiline HCl	$C_{23}H_{26}ClN$	Calcium Channel inhibitor	>10	4.51	>2.22
	Astemizole	$C_{28}H_{31}FN_4O$	Histamine Receptor; Potassium Channel inhibitor	>10	3.91	>2.52
	Perphenazine	$C_{21}H_{26}ClN_3OS$	Calmodulin inhibitor; Dopamine Receptor antagonist	>10	3.97	>2.52
	Dronedarone HCl	$C_{31}H_{44}N_2O_5S.HCl$	Potassium Channel inhibitor; Sodium Channel inhibitor; Adrenergic Receptor antagonist; Calcium Channel inhibitor	>10	4.75	>2.11
	Otilonium bromide	$C_{29}H_{43}BrN_2O_4$	Calcium Channel inhibitor; AChR antagonist	>10	1.84	>5.43
GPCR/G Protein	Conivaptan HCl	$C_{32}H_{26}N_4O_2.HCl$	Vasopressin Receptor antagonist	>10	1.58	>6.33
	Rimonabant HCl	$C_{22}H_{21}Cl_3N_4O$	Cannabinoid Receptor	>10	4.11	>2.43
Microbiology & Virology	Tilorone 2HCl	$C_{25}H_{34}N_2O_3$	Antiviral	>30	1.21	>24.79
	Butylparaben	$C_{11}H_{14}O_3$	Antibacterial	>30	1.11	>27.03
	Acetylspiramycin	$C_{45}H_{76}N_2O_{15}$	Antibiotic	>30	7.49	>4.00
	Monensin sodium salt	$C_{36}H_{61}NaO_{11}$	Antibiotic	>10	0.07	>142.86
	Abamectin	$C_{49}H_{74}O_{14}$	Antiparasitic	~10	3.12	~3.21
	Mefloquine HCl	$C_{17}H_{17}ClF_6N_2O$	Hemozoin synthesis inhibitor	12.52	3.62	3.46
	Arbidol HCl	$C_{22}H_{25}BrN_2O_3S.HCl$	Influenza Virus	8.85	4.43	2.00
	Actidione	$C_{15}H_{23}NO_4$	Antifungal	1.77	0.12	14.75
Tyrosine Kinase/ Adaptors	Imatinib Mesylate (STI571)	$C_{29}H_{31}N_7O.CH_4SO_3$	Bcr-Abl inhibitor; c-kit inhibitor; PDGFR inhibitor	>30	6.09	>4.93
	Neratinib(HKI-272)	$C_{30}H_{29}ClN_6O_3$	EGFR inhibitor; HER2 inhibitor	>10	1.02	>9.80
	Nilotinib (AMN-107)	$C_{28}H_{22}F_3N_7O$	Bcr-Abl inhibitor; Mast/stem cell GFR Kit agonist	>10	1.34	>7.46
	Afatinib (BIBW2992) Dimaleate	$C_{32}H_{33}ClFN_5O_{11}$	EGFR inhibitor; HER2 inhibitor	>10	3.62	2.76
	Gefitinib (ZD1839)	$C_{22}H_{24}ClFN_4O_3$	EGFR antagonist	>10	3.29	>3.34
Enzyme	Clofazimine	$C_{27}H_{22}Cl_2N_4$	CzcO-like inhibitor; DNA intercalation	~10	0.35	~28.57
Neuroscience	Cyproheptadine HCl	$C_{21}H_{22}ClN$	Histamine Receptor antagonist; 5-HT receptor antagonist; AChR antagonist	>30	8.36	>3.59
	Cloperastine HCl	$C_{20}H_{25}Cl_2NO$	Histamine Receptor antagonist	>30	5.51	>5.44
	Dihydroergotamine Mesylate	$C_{34}H_{41}N_5O_8S$	5-HT Receptor antagonist	>30	5.24	>5.73
	Azelastine	$C_{22}H_{25}Cl_2N_3O$	Histamine Receptor antagonist	>30	12.01	>2.50
	Pizotifen	$C_{19}H_{21}NS$	5-HT Receptor antagonist	>30	8.36	>3.59
	Fluoxetine hydrochloride	$C_{17}H_{18}F_3NO.HCl$	5-HT Receptor antagonist	>10	3.16	>3.16
	Thioridazine HCl	$C_{21}H_{27}ClN_2S_2$	5-HT Receptor antagonist; Adrenergic Receptor antagonist; Dopamine Receptor antagonist; Potassium Channel inhibitor	>10	3.42	>2.92
	Chlorpromazine HCl	$C_{17}H_{19}ClN_2S.HCl$	Histamine Receptor antagonist; Potassium Channel inhibitor; Dopamine Receptor antagonist; Adrenergic Receptor antagonist; AChR antagonist; 5-HT receptor antagonist	>10	2.61	>3.83
	Vortioxetine	$C_{18}H_{22}N_2S$	5-HT Receptor modulator	>10	1.93	>5.18
	Triflupromazine HCl	$C_{18}H_{20}ClF_3N_2S$	Dopamine Receptor antagonist; AChR antagonist; 5-HT receptor antagonist	>10	7.49	>1.34
	Flupenthixol 2HCl	$C_{23}H_{27}Cl_2F_3N_2OS$	Dopamine Receptor antagonist	>10	3.52	>2.84
	Celecoxib	$C_{17}H_{14}F_3N_3O_2$		29.83	4.08	7.31
	Sertraline HCl	$C_{17}H_{17}Cl_2N.HCl$	5-HT Receptor antagonist	>10	3.49	>2.87
Endocrinology/ Hormones	Dydrogesterone	$C_{21}H_{28}O_2$	Progesterone Receptor agonist	>30	8.36	>3.59
	Epiandrosterone	$C_{19}H_{30}O_2$	Androgen Receptor agonist	>30	8.36	>3.59
	Raloxifene HCl	$C_{28}H_{28}ClNO_4S$	Estrogen/Progestogen Receptor antagonist; SERM agonist	>10	1.32	>7.58
Angiogenesis	Ibrutinib (PCI-32765)	$C_{25}H_{24}N_6O_2$	BTK inhibitor	>10	3.62	>2.76
	Suloctidil	$C_{20}H_{35}NOS$	Antiplatelet aggregation inhibitor	>10	9.13	>1.10

(Continued)

TABLE 1 | Continued

Pathway	MOLENAME	Formula	Target	CC$_{50}$ (µM)	IC$_{50}$ (µM)	SI (CC$_{50}$/IC$_{50}$)
	Candesartan Cilexetil	$C_{33}H_{34}N_6O_6$	RAAS antagonist; ACE inhibitor	~10	3.59	~2.79
Others	Luteolin	$C_{15}H_{10}O_6$	TNF-alpha inhibitor, IL inhibitor; NF-κB inhibitor	3.5	1.12	3.13
	Salifungin	C13H9BrClNO2	Others	1.08	0.36	3.00
	Bosutinib (SKI-606)	$C_{26}H_{29}Cl_2N_5O_3$	MAPK inhibitor; Src inhibitor; Bcr-Abl inhibitor; CaMK inhibitor; CDK inhibitor	>10	2.17	>4.61
	Honokiol	$C_{18}H_{18}O_2$	Akt inhibitor; MEK inhibitor	>10	7.52	>1.33
	Temsirolimus (CCI-779, NSC 683864)	$C_{56}H_{87}NO_{16}$	mTOR inhibitor	9.3	1.27	7.32
	Hydroxychloroquine sulfate	$C_{18}H_{26}ClN_3O.H_2SO_4$	Autophagy inhibitor; TLR antagonist	>30	5.7	>5.26
	Chloroquine diphosphate	$C_{18}H_{26}ClN_3.2(H_3PO_4)$	Autophagy inhibitor; ATM/ATR activator	>30	3.8	>7.89
	2-Amino-5-nitrobenzophenone	$C_{13}H_{10}N_2O_3$	Intermediates	>30	7.3	>4.11
	Cepharanthine	$C_{37}H_{38}N_2O_6$	Others	9.1	1.26	7.22
	Escin	$C_{55}H_{86}O_{24}$	Others	>10	3.31	>3.02
	Cyclosporin A	$C_{62}H_{111}N_{11}O_{12}$	Others	~10	0.37	~27.03

nilotinib, clofazimine, and raloxifene. The effects also confirmed by immunofluorescence assay (data not shown). These compounds also belong to a wide variety of classes including cardiac glycosides, anti-malarial drug hydroxychloroquine, cyclooxygenase-2 inhibitors, and ion channel blockers. The IC$_{50}$, CC$_{50}$, and SI of these compounds are shown in **Table 2**.

Five Candidate Drugs Inhibit Cell Fusion

Finally, to determine the mechanism by which the compounds inhibit SARS-CoV-2, we investigated these candidates' effects on virus entry. First, we constructed the cell-cell fusion assays. As indicated in **Figure 4**, SARS-CoV-2 S protein expression can initiate cell fusion with ACE2-overexpressed cells, but the control vector did not. Then, we detected the effects of these indicated drugs on S protein-mediated cell fusion. Indicated drugs were added to cells at 10 µM before the co-incubation of the cells. We found that fendiline hydrochloride, monensin sodium salt, vortioxetine, sertraline hydrochloride, and salifungin inhibited the SARS-CoV-2 S protein-mediated cell fusion (**Figure 4**).

DISCUSSION

The current pandemic of COVID-19 is the third major outbreak in this century and the largest outbreak of the CoV in known human history. The three novel CoV outbreaks in such a short time are strong indicators of the potential threat posed by CoVs. While most respiratory viral infection research has been focused on influenza viruses that cause a huge burden of seasonal flu and occasionally pandemic outbreaks, CoV is likely to emerge as a similar or more severe pathogen than flu in long term.

Given the scale and devastation of the current COVID-19 outbreak and the persistent threat of CoVs in causing human disease, there is an urgent need to find effective and safe therapies to treat these patients. Currently, there are no approved therapies for CoVs including SARS-CoV-2. The experimental therapies being used with known antiviral agents either show limited efficacy (remdesivir) or have high systemic toxicity (hydroxychloroquine), limiting their usefulness (22–24). Finding new therapies that are effective and safe are urgently needed. In this study, we have identified many FDA-approved therapies that are highly effective against CoVs, including 20 of the effective agents against SARS-CoV-2. This screening confirms previous reports demonstrating anti-SARS-CoV-2 activity of hydroxychloroquine, amlodipine besylate, arbidol hydrochloride, tilorone 2HCl, dronedarone hydrochloride, mefloquine, celecoxib, and thioridazine hydrochloride (8–10, 25–27), while identifying additional 12 drugs. However, there are seven drugs have been reported when our manuscript was underreview (28–37). The underlying mechanisms of viral replication inhibition by these drugs are not clear. It is highly unlikely that these compounds will have similar antiviral mechanisms given the vast structural and pharmacological diversities of the effective antiviral compounds in our study. However, it is clear from other viral studies such as influenza or HIV, where antiviral drugs can affect various viral life cycle steps including attachment, entry, replication, assembly, and budding of viral progeny. Five drugs may inhibit S-mediated cell fusion as indicated by our data (**Figure 4**). Further studies are required to understand the precise mechanisms of each of the effective compounds found in this study.

Toxicity is one of the limiting factors in the therapeutic application of many drugs despite their known antiviral activities. Many of these drugs had SI of >600, showing promise of their usefulness at safe doses. For comparison, the SI of hydroxychloroquine was found to be 22 in our study while SI of amlodipine besylate was found to be ~3, demonstrating a much lower safety profile of this drug. Similarly, other drugs known to have low selective index such as digoxin for their approved use, also show lower SI in our screen. Five of the drugs with SI of >600
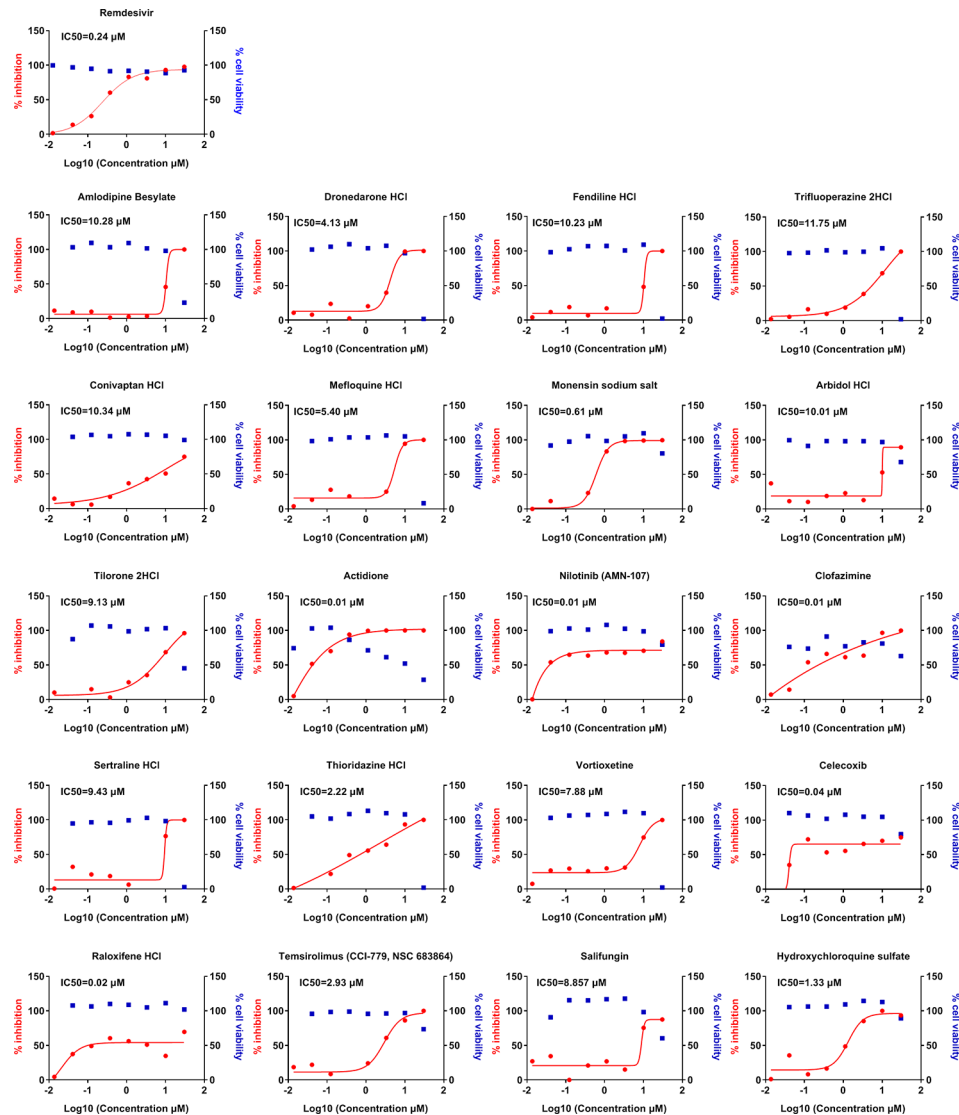
FIGURE 3 | Dose-response curves of selected compounds against SARS-CoV-2 infection. Vero cells were pretreated with indicated drugs at 37°C for 1 h with eight doses (0.014, 0.041, 0.123, 0.370, 1.111, 3.333, 10, and 30 μM) with three-fold dilutions, followed by infection with SARS-CoV-2 at an MOI of 0.1 for 24 h. The viral load in the cell supernatant was quantified by qRT-PCR. Meanwhile, cell viability in the presence of these drugs was measured in Vero cells by CCK-8 assays. The left Y-axis of the graphs represents % inhibition of viral replication (red dots) and the right Y-axis of the graphs indicates % cell viability (blue squares) in the presence of the drugs.

include tyrosine kinase inhibitor nilotinib, antibiotics such as clofazimine and actidione, and selective estrogen receptor modulators raloxifene, and non-steroidal anti-inflammatory drug celecoxib.

Betacoronaviruses have raised great public health threats to human beings, as most known HCoVs including all the three virulent HCoVs (SARS-CoV, MERS-CoV, and SARS-CoV-2) and two seasonal HCoVs (OC43 and HKU1) belong to this species (3–7, 21, 38). It is of great value to identify antivirals against a broad spectrum of HCoVs, particularly the Betacoronaviruses, to tackle such threats by pharmaceutical

interventions (39, 40). To this end, we first screened the compounds which showed apparent activity of anti-OC43, the most prevalent HCoV circulates worldwide (41). We then narrowed down the candidates by the screening on SARS-CoV-2, resulting in the identification of 20 compounds which can inhibit both OC43 and SARS-CoV-2. Our study provides a foundation for subsequent anti-HCoVs drug screening of a broad spectrum. However, further tests are warranted to verify their efficacies.

In summary, our screen identified 14 previously unknown FDA-approved compounds that are effective in inhibiting SARS-
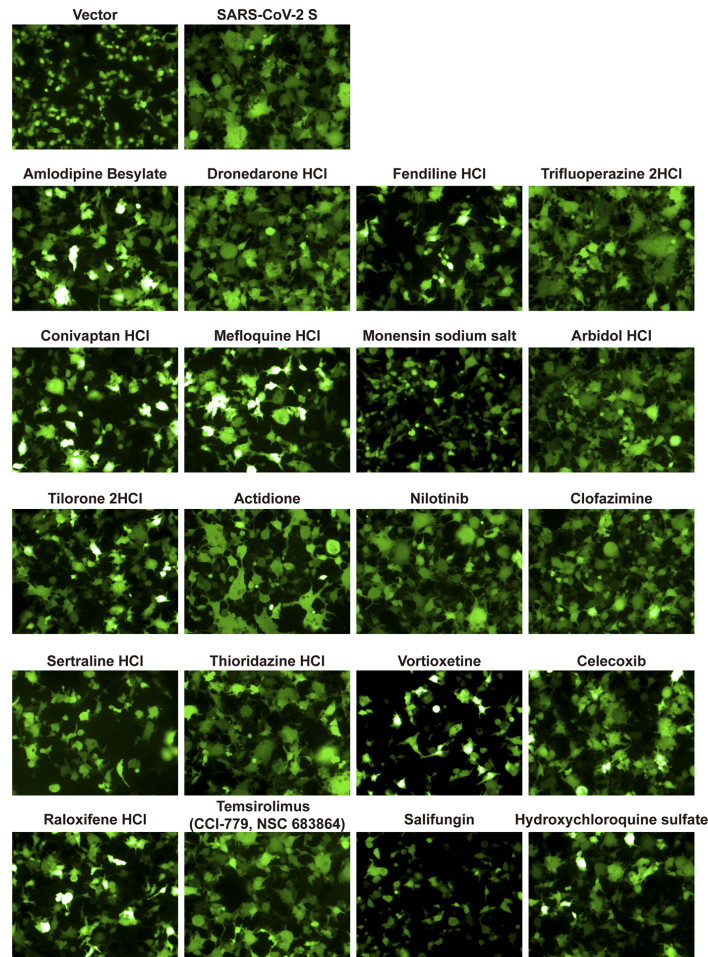
FIGURE 4 | The effect of indicated drugs on cell-cell fusion mediated by SARS-CoV-2 S protein. HEK-293T cells were co-transfected with SARS-CoV-2-S glycoprotein and eGFP. 24 h post-transfection, cells were digested with trypsin (0.25%) and overlaid on a 50% confluent monolayer of 293T-ACE2 cells at a ratio of 1:1 which were treated with candidate drugs at 10 μM for 1 h. After 24-h incubation, images of syncytia were captured with Operetta and analyzed by Harmony software.

TABLE 2 | Antiviral activity of selected compounds against SARS-CoV-2.

Pathway	MOLENAME	Formula	CC50, IC50(μM)	SI
Membrane Transporter/Ion Channel	Amlodipine Besylate	$C_{26}H_{31}ClN_2O_8S$	>30, 10.28	>2.92
	Dronedarone HCl	$C_{31}H_{44}N_2O_5S.HCl$	12.62, 4.13	3.06
	Fendiline HCl	$C_{23}H_{26}ClN$	29.24, 10.23	2.86
	Trifluoperazine 2HCl	$C_{21}H_{26}Cl_2F_3N_3S$	29.29, 11.75	2.49
GPCR/G Protein	Conivaptan HCl	$C_{32}H_{26}N_4O_2.HCl$	12.7, 10.34	1.23
Microbiology & Virology	Mefloquine HCl	$C_{17}H_{17}ClF_6N_2O$	29.13, 5.4	5.39
	Monensin sodium salt	$C_{36}H_{61}NaO_{11}$	>30, 0.6019	>49.84
	Arbidol HCl	$C_{22}H_{25}BrN_2O_3S.HCl$	22.36, 10.01	2.23
	Tilorone 2HCl	$C_{25}H_{34}N_2O_3$	28.67, 9.13	3.14
	Actidione	$C_{15}H_{23}NO_4$	6.06, 0.01	606
Tyrosine Kinase/Adaptors	Nilotinib (AMN-107)	$C_{28}H_{22}F_3N_7O$	>30, <0.01	>3000
Enzyme	Clofazimine	$C_{27}H_{22}Cl_2N_4$	>30, 0.01	>3000
Neuroscience	Sertraline HCl	$C_{17}H_{17}Cl_2N.HCl$	27.84, 9.34	2.98
	Thioridazine HCl	$C_{21}H_{27}ClN_2S_2$	27.22, 2.22	12.26
	Vortioxetine	$C_{18}H_{22}N_2S$	28.03 7.88	3.56
	Celecoxib	$C_{17}H_{14}F_3N_3O_2S$	>30, 0.04	>750
Endocrinology/Hormones	Raloxifene HCl	$C_{28}H_{28}ClNO_4S$	>30, 0.02	>1500
Others	Temsirolimus (CCI-779, NSC 683864)	$C_{56}H_{87}NO_{16}$	>30, 2.93	>10.24
	Salifungin	$C_{13}H_9BrClNO_2$	11.12, 8.86	1.26
	Hydroxychloroquine sulfate	$C_{18}H_{26}ClN_3O.H_2SO_4$	>30, 1.33	>22.56

CoV-2 besides confirming the antiviral properties of 7 previously reported compounds, validating our approach. This screen identified five new compounds highly efficacious in inhibiting the viral replication of SARS-CoV-2 with SI >600. Further studies are needed to confirm these drugs' *in vivo* efficacy in COVID-19 relevant mouse models such as those with human ACE2 transgene (42) and human clinical studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Project conception: JW, XL, and LS. Experimental design: JW, XL, DC, LS, ZZ, and LR. Experimental work: XX, CW, YW, XD, and TJ. Data analysis: JW, XL, LR, CDC, XX, and ZZ. Writing original draft: JW, XL, LS, DC, and XX. Writing review and editing: JW, XL, DC, LS, and CC. All authors contributed to the article and approved the submitted version.

FUNDING

ACKNOWLEDGMENTS

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