

COVID-19: A need for new rather than repurposed antiviral drugs.

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Abstract

Background: SARS-CoV-2 infection, the causative agent of COVID-19, has resulted in over 2,500,000 deaths to date¹. Although vaccines are becoming available, treatment options remain limited. Repurposing of compounds could reduce the time, cost, and risks associated with the development of new drugs and has been the focus of many clinical studies.

Here, we summarise available evidence on 29 FDA-approved compounds, from *in vitro* results to clinical trials, focussing on remdesivir, galidesivir and favipiravir, and test 29 antiviral compounds' activity *in vitro*.

Methods: A comprehensive search strategy was used to retrieve trials and publications related to antiviral compounds with potential efficacy to treat coronaviruses. These data were used to prioritise testing of a panel of antiviral drugs *in vitro* against patient isolates of SARS-CoV-2. An *in vitro* screen was carried out to determine the activity of 29 FDA-approved compounds.

Results: 625 clinical trials investigated 16 repurposed antiviral candidate compounds for the treatment of COVID-19. *In vitro* studies identified ten drug candidates with demonstrable anti-SARS-CoV-2 activity, including favipiravir, remdesivir, and galidesivir. To validate these findings, a drug screen was conducted using two cell lines and wildtype isolates of SARS-CoV-2 isolated from patients in the UK. While eight drugs with anti-SARS-CoV-2 activity were identified *in vitro*, activity in clinical trials has, as yet failed to demonstrate a strong effect on mortality.

Conclusions: So far, no repurposed antiviral has shown a strong effect on mortality in clinical studies. The urgent need for novel antivirals in this pandemic is clear, despite the costs and time associated with their development.

Keywords: COVID-19, SARS-CoV-2, antivirals, drug repurposing, pharmacology

Research in Context

Evidence before this study: Repurposing of existing compounds for the treatment of COVID-19 has been the focus of many *in vitro* studies and clinical trials, saving time, costs and risks associated with the research and development of new compounds.

Added value of this study: We reviewed the literature for 29 FDA-approved compounds with previously reported (or suspected) anti-SARS-CoV-2 activity and found 625 clinical trials that have been undertaken on 16 different drugs. We determined if repurposed antivirals are suitable for clinical trials based on previously published data, and conducted an additional *in vitro* screen using locally circulating strains in the UK (PHE2 and GLA1). We report the difference in IC₅₀ from published data using Wuhan1/Wash1 strains with PHE2 and GLA1, including IC₅₀ values below 100µM for galidesivir in wild-type virus. Given the limited success of repurposed compounds in the treatment of COVID-19, we comment on the urgent need for new antivirals specifically targeting SARS-CoV-2.

Implications of all the available evidence: Our data show that most prospective compounds for repurposing show no anti-SARS-CoV-2 activity, and antiviral activity *in vitro* does not always translate to clinical benefit. So far, no repurposed compound has shown a strong effect on mortality in clinical studies. Drugs, including monoclonal antibody therapies, that have been developed to target SARS-CoV-2 virus itself have shown most promise.

1 Introduction

The betacoronavirus SARS-CoV-2 is likely to be a significant public health threat to the global population in the medium to long term. This coronavirus family of RNA viruses are commonly associated with mild to moderate upper respiratory tract infections during the winter months^{2,3}. However, the emerging coronaviruses MERS-CoV⁴, SARS-CoV-1⁵ and SARS-CoV-2⁶ have high mortality rates. In addition, long-term morbidity associated with SARS-CoV-2 infection, known as ‘long COVID’⁷ is a significant concern and highlights the need for intervention with antiviral therapies. The novel coronavirus SARS-CoV-2 originated in China in late 2019 and has spread across the globe, resulting in over 2,500,000 recorded deaths to date.

Repurposing of existing compounds for the treatment of COVID-19 has been the focus of many trials and studies, despite a lack of evidence regarding their effectiveness⁸. Repurposing of compounds is made possible by shared molecular pathways across diseases, which also overcomes bottlenecks in the developmental process by cutting the time, costs, and risks associated with drug development⁹⁻¹¹. Previously developed antivirals may demonstrate activity against diverse viral families due to the presence of relatively conserved viral proteins such as DNA or RNA polymerases, integrase or viral proteases. Many viruses also require inactivation of host proteins, which could also provide targets for broad acting antivirals. Longstanding compounds have the advantage of pre-existing information regarding their pharmacokinetic and pharmacodynamic profiles and toxicity. However, repurposing drugs and compounds is not without caveats, and must occur in controlled, clinical trial settings. While a drug may be efficacious against the original target, the concentration required to combat another virus may be unachievable¹² due to toxicity or pharmacodynamic constraints in patients. Furthermore, there is a finite number of antivirals to trial, and emerging evidence is often conflicting. As a result, clinicians often make decisions in the absence of reliable data, based on preliminary reports and poorly conducted trials.

Herein, we summarise evidence on 29 FDA-approved candidate compounds, from in vitro results to clinical trials selected for potential SARS-CoV-2 antiviral activity. These potential antiviral candidates were next tested for activity against circulating SARS-CoV-2 variants in vitro.

2 Methods:

2.1 Literature review

Antiviral drugs and compounds were identified by screening literature on SARS, MERS and the emergent literature on COVID-19, coupled with the experience of the authors (see Table 1). We then undertook a comprehensive search strategy to retrieve trials and publications related to antiviral drugs and compounds potentially effective in the treatment of coronaviruses. The method was adapted from that recommended in the Cochrane Handbook for Systematic Reviews of Interventions¹³ for identifying trials. Searches for the below list of antiviral drugs and compounds were conducted between March 2020 and 28 January 2021 on ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP) and the Cochrane COVID-19 Study Register. Search terms are shown in Supplementary Table 1. In addition, we searched the Chinese Clinical Trial Register and pre-print servers medRxiv, bioRxiv, and chinaRxiv, while relevant results through our academic network were also identified.

On the Cochrane COVID-19 Study Register, free text terms were used to search for the name of each drug or compound individually; no other search terms or limits were applied. This process was repeated by searching medRxiv and bioRxiv simultaneously through bioRxiv in the title or abstract fields, and for ChinaRxiv across all fields. For ClinicalTrials.gov and the Chinese Clinical Trial Register, each drug or compound was searched in the intervention field. Results were filtered for relevance before being exported and analysed in EndNote X9. Results were also screened to include only those published in English. No date or language limits were applied to the searches.

Weekly ICTRP reports were compiled and screened for trials mentioning COVID-19 or SARS-CoV-2 in their scientific or public titles, and their details were extracted using R version 4.0.0.

Table 1 Twenty-nine compounds were identified for reports of anti-SARS-CoV-2 activity. Drugs with no oral formulations are shown in orange, original indications are shown in brackets. HBV=hepatitis B virus, HCV=hepatitis C virus, HIV = human immunodeficiency virus, EVD = Ebola virus disease, RSV= respiratory syncytial virus.

Nucleoside analogues	Protease inhibitors	Anti-influenza drugs	Others
Abacavir (HIV)	Atazanavir (HIV)	Amantadine	Emetine
Adefovir (HBV)	Danoprevir (HCV)	Baloxavir	Hydroxychloroquine
Didanosine (HIV)	Darunavir (HIV)	Favipiravir***	Sotetsuflavone
Emtricitabine (HIV, HBV)	Fosamprenavir (HIV)	Oseltamivir	
Galidesivir (HCV*)	Grazoprevir (HCV)	Umifenovir**	
Lamivudine (HIV, HBV)	Lopinavir (HIV)		
Remdesivir (EVD)	Paritaprevir (HCV)		
Ribavirin (HCV, RSV)	Ritonavir (HIV)		
Sofosbuvir (HCV)	Saquinavir (HIV)		
Telbivudine (HBV)			
Tenofovir (HIV, HBV)			
Zidovudine (HIV)			

*Galidesivir was subsequently used for filovirus infections, including Ebola¹⁴. **Umifenovir is also known as Arbidol. ***Favipiravir is a nucleoside analogue.

2.2 In vitro screen of 29 FDA-approved compounds

Cell lines

The methods of production and validation of the cell lines used in this study are fully described elsewhere¹⁵. In brief, VeroE6 (African green monkey kidney) cells obtained from Prof. Michèle Bouloy (Institut Pasteur, France) were induced to overexpress ACE2 using a modified lentiviral expression system (pLV-EF1a-IRES-Neo (Addgene, plasmid 85139)). A549 cells (human lung) expressing the NPro protein of bovine viral diarrhea virus (BVDV) were obtained from Prof. Rick Randall (University of St Andrews) and induced to overexpress ACE2 in the same manner as the VeroE6 cells.

Virus isolates and propagation

Two clinical isolates were used in the study, including the PHE2 isolate, provided by Public Health England, and a clinical isolate obtained from the MRC-University of Glasgow Centre for Virus Research, Scotland, UK (GLA1). GLA1 was obtained by inoculating a T-25 flask of VeroE6 cells with a bronchoalveolar lavage clinical sample and incubating the cells until CPE was observed. Both stocks were confirmed using metagenomic sequencing and expanded by inoculating a T-150 cm² of VeroE6 cells and incubating for 48 hours before clarifying the supernatant. A plaque assay was used to determine an accurate titre for each virus and ensure an accurate multiplicity of infection (MOI) for each isolate in subsequent experiments.

Endpoint Dilution Assay

An in vitro screen was performed to determine the activity of 29 FDA-approved compounds (see Table 1) on the ability of SARS-CoV-2 to infect and cause cytopathic effect (CPE) on the two cell lines derived from A549 and VeroE6 cells, as described above. A549-NPro-ACE2 and VeroE6-ACE2 cells were plated at a density of 1.25×10^4 cells/well in a 96-well plate in DMEM supplemented with 10% FCS the day before drug treating the cells. The media was replaced with 50ul DMEM-

GlutaMAX (ThermoFisher, cat no 10566016) supplemented with 2% FCS (ThermoFisher, cat no A4766801) containing doubling dilutions of the various compounds at the stated concentrations. The cells were incubated with the compounds for three hours before the addition of 50ul of either SARS-CoV-2 PHE2 or GLA1 isolates at a MOI of 0.05 or 0.5. The concentrations presented in the results equate to the concentration of the compounds after the addition of the virus isolates. 72 hours post infection the cells were fixed with 8% (v/v) formaldehyde in PBS for one hour, washed with PBS and then stained with Coomassie solution (2ml Coomassie Brilliant Blue (ThermoFisher cat no. 20277), 900ml Methanol, 900ml water 200ml Acetic Acid) for 30 minutes. The plates were washed in water to remove the stain and dried overnight before using a Celigo Imaging Cytometer (Nexcelom Bioscience, UK) to measure the staining intensity. Percentage cell survival was determined by comparing the intensity of the staining to an uninfected well.

Statistical packages

All graphs were produced using Prism (GraphPad, version 8) with the IC₅₀ values determined by a nonlinear regression using a sigmoidal 4PL equation.

3 Results:

3.1 Clinical trials with repurposed antivirals

The WHO's ITCRP database was reviewed for clinical trials examining the repurposing of 29 compounds for the treatment of COVID-19. Between March 2020 and January 2021, 655 such trials were registered, investigating sixteen drugs in mild to severe COVID-19 disease. For trials with registered details, median target size was 135 patients. Many trials were multi-national with multiple experimental arms. Iran had the highest number of clinical trials registered ($n=145$), followed by the US ($n=72$) (Figure 1). In Europe, Spain had the highest number of trials registered ($n=40$). 68 studies investigating drug repurposing in China, were initiated but concluded early due to a lack of patients. The number of trials remained low in Africa; however, one multicentre trial (PACTR202006537901307) investigated several antiviral compounds across 13 nations on the continent.

The most commonly repurposed inhibitor was HCQ ($n=461$, see Figure 2), a known anti-malarial and blocker of virus entry, which can be cheaply produced and dispensed around the globe. HCQ, lopinavir and ritonavir (the latter two are co-formulated) were investigated early in the pandemic (up to July 2020) but have now been removed from trials in hospitalised patients following data from the WHO's Solidarity trial in which they were shown to have no effect on mortality¹⁶⁻¹⁸.

Data on the antiviral drug remdesivir, previously tested in patients with Ebola virus disease were initially encouraging, with evidence that time to recovery was reduced in hospitalised patients¹⁹. However, these results were not replicated in the Solidarity trial^{17,20}, which also found no effect of HCQ, lopinavir/ritonavir or interferon on overall mortality, initiation of ventilation or duration of hospital stay in hospitalized patients. These conflicting results may reflect the need to initiate antiviral treatment early before the onset of pathology driven by the immune response.

Favipiravir was the subject of 73 trials. Two trials have reported a faster time to viral clearance in favipiravir treated patients compared to controls (²¹, FUJIFILM Toyama Chemical press release). Two trials are ongoing in the UK: Getafix²² and Pioneer²³. The first clinical trial assessing galidesivir in COVID-19 commenced in April 2020¹⁴ (NCT03891420) and is expected to be completed by May 2021.

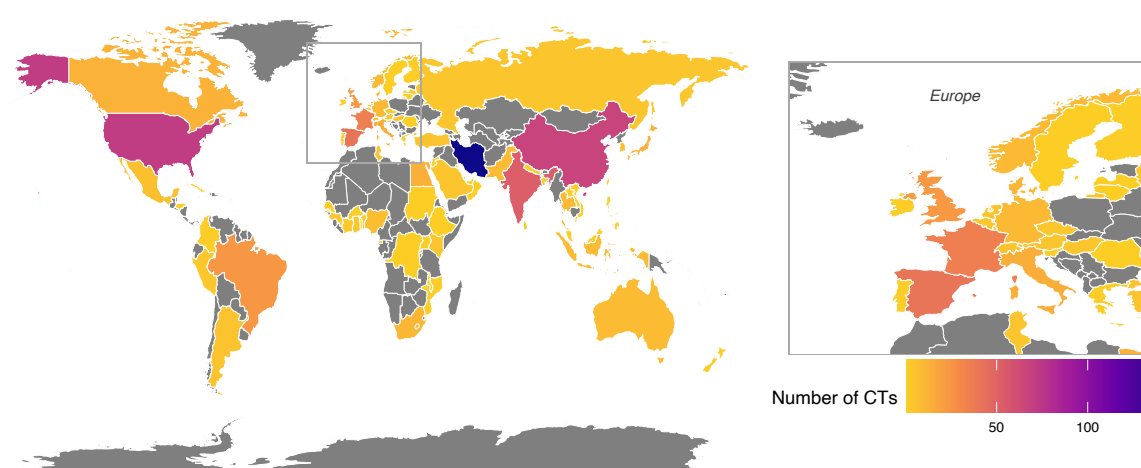


Figure 1 Number of clinical trials looking at repurposing of compounds for the treatment of COVID-19 per country. Only trials that specified countries are shown ($n=614$). Source: WHO ITCRP database, March 2020 - January 2021.

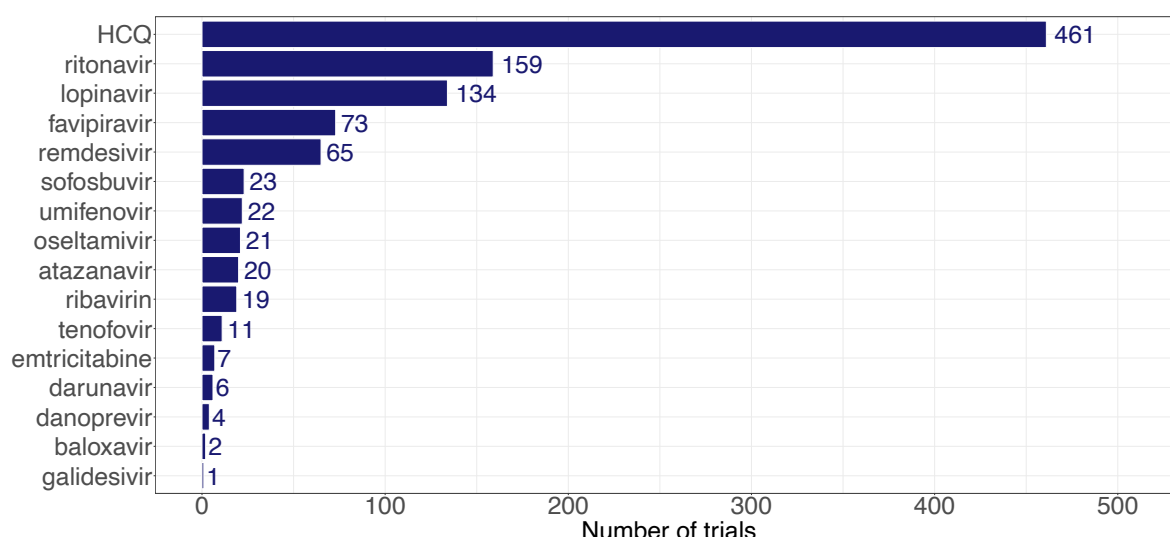


Figure 2 Number of trials looking at the repurposing of sixteen compounds as of January 2021. HCQ = hydroxychloroquine; (Although there were 655 unique trials registered, the total here adds up to 1028 as many trials investigate multiple compounds through multiple experimental arms.)

3.2 Repurposed compounds and anti-SARS-CoV-2 activity in vitro

Of 29 compounds (Table 1), ten drug candidates have repeatedly been shown to have anti-SARS-CoV-2 activity in vitro. These compounds and their EC_{50} and IC_{50} values are shown in Figure 3 (and Supp Table 1). In addition, Yamamoto et al²⁴ reported that saquinavir and atazanavir inhibited SARS-CoV-2 at 8.83 μ M and 9.36 μ M, but the C_{max}/EC_{50} and C_{max}/C_{trough} ratio was not ≥ 1 , and therefore it was considered unlikely that these drugs would be useful clinically (saquinavir $C_{max}/EC_{50} = 0.73$, $C_{trough}/EC_{50} = 0.07$, atazanavir $C_{max}/EC_{50} = 0.93$, $C_{trough}/EC_{50} = 0.19$). Remdesivir has been shown to have high antiviral activity in vitro, with consistently low EC_{50} values in VeroE6²⁵⁻²⁸, Caco-2²⁹⁻³¹, Calu-3^{32,33} and Huh7.5³⁴ cells. HCQ is active against SARS-CoV-2 in VeroE6, Huh7.5 and Calu-3 cells³⁴⁻³⁷ and is the most commonly investigated compound in clinical trials (Figure 2 and 3). Lopinavir, frequently used in combination with ritonavir, has been shown to inhibit SARS-CoV-1^{38,39} and has some activity against SARS-CoV-2^{24,25,30,32,40} in Caco-2, Calu-3 and VeroE6 cells. Several studies reported no antiviral effect associated with favipiravir under 100 μ M in VeroE6 cells^{25,40,41}, Wang et al²⁶ reported a single EC_{50} of 61.88 μ M indicating an inhibitory effect similar to that for Ebola virus. Galidesivir has been shown to have antiviral activity against SARS-CoV-1 and MERS-CoV in HeLa cells⁴² and against culture-adapted SARS-CoV-2⁴³. Ribavirin and sofosbuvir have EC_{50} values under 10 μ M in Huh7.5 and Calu-3 cells⁴⁴. Emetine, used for the treatment of amoebiasis, has been shown to inhibit MERS-CoV and SARS-CoV-2 in vitro^{25,45}. The hepatitis C virus proteases grazoprevir and danoprevir have also shown anti-SARS-CoV-2 activity with EC_{50} s below 100 μ M⁴⁶, while danoprevir is also investigated in four clinical trials. Information relevant for clinical use of these compounds are summarised in Supplementary Table 3.

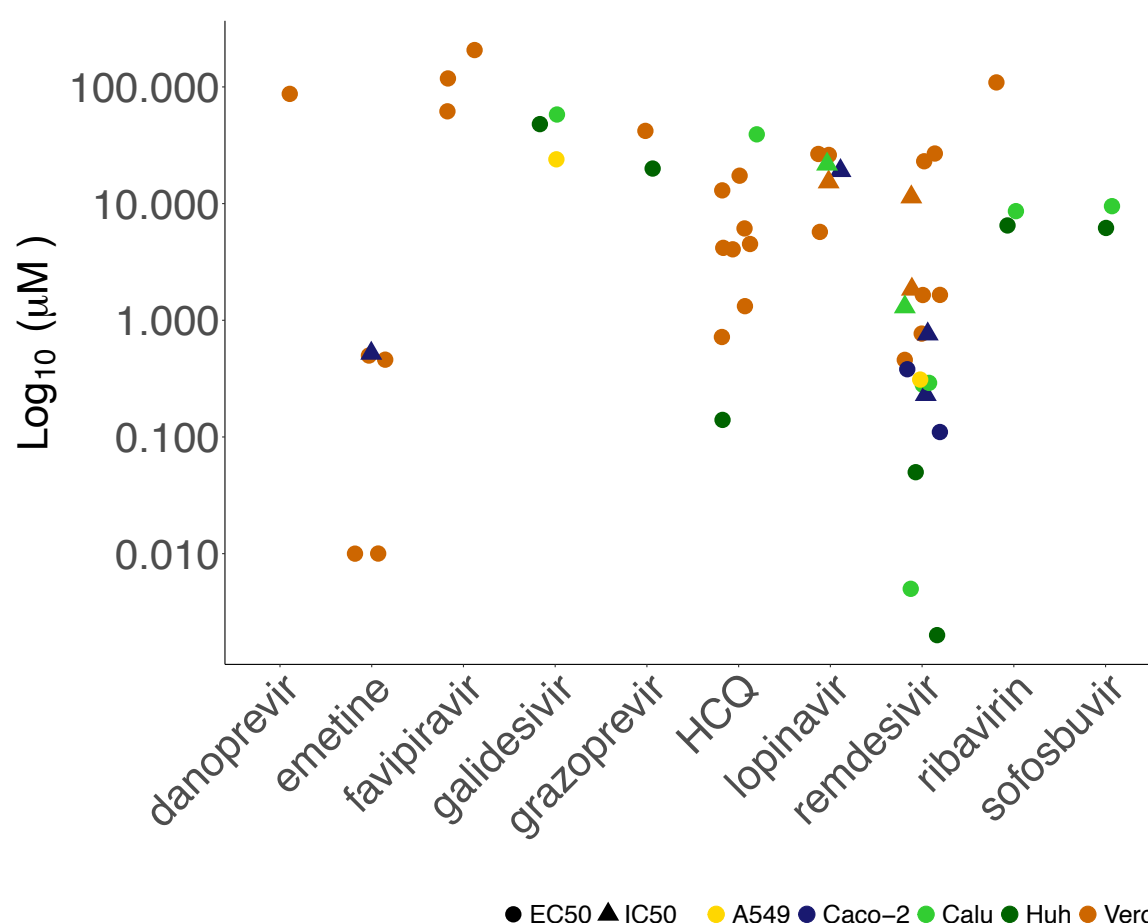


Figure 3 EC_{50} and IC_{50} (log_{10}) values of selected compounds reported in previous publications. Note that MOIs varied from 0.001 to 1; see Supplementary Table 2.

3.3 Galidesivir, remdesivir and hydroxychloroquine demonstrate high antiviral activity against SARS-CoV-2 in vitro

The antiviral activity of 29 FDA-approved compounds was tested in vitro, using VeroE6-ACE2 and A549-NPro-ACE2 cells. Of these, eight showed activity against SARS-CoV-2 PHE2 in VeroE6-ACE2 cells to varying degrees (Supp Figure 4).

Galidesivir, remdesivir and HCQ were able to increase the survival of VeroE6-ACE2 when infected with the GLA1 and the PHE2 isolates (Supp figure 2, Supp figure 3). Lopinavir, ribavirin and favipiravir reduced cell death in both cell lines to a small degree at the highest concentrations, but this coincided with a cytotoxicity, with the latter two only showing inhibition at the highest concentrations of the drugs tested against an MOI of 0.05, with little effect on the virus at an MOI of 0.5. The same effect was seen with emetine and umifenovir with both increased cell survival but at concentrations that also lead to greater cell cytotoxicity than seen in lopinavir, ribavirin and favipiravir.

The compounds were then tested on A549-NPro-ACE2 cells, which constitute a more physiologically relevant human cell line that is susceptible to SARS-CoV-2 virus infection. The results were very similar to those seen in VeroE6-ACE2 cells apart from favipiravir, which had no apparent effect (Figures 4). Again, ribavirin showed an ability to increase cell survival against the PHE2 virus but not the GLA1 isolate (Supp figure 1 and Figure 4, respectively).

IC₅₀ values were determined for the three most active compounds against the PHE2 and GLA1 viral isolates on t VeroE6-ACE2 and A549-NPro-ACE2 cells (Table 2). At an MOI of 0.5, galidesivir had an IC₅₀ of between 60.5 µM to 106.5 µM, while the IC₅₀ of HCQ ranged from 12.53 µM to 92.8 µM, depending on the viral isolate and cell line. Remdesivir demonstrated an IC₅₀ of 3.17 µM for the PHE2 and 2.62 µM for the GLA1 isolate on VeroE6-ACE2 cells. On A549-NPro-ACE2 cells the activity was even greater at 0.25 µM and 0.11 µM for the PHE2 and GLA1 cells, respectively. Overall, these results demonstrate that remdesivir and HCQ and, for the first time, galidesivir are able to significantly increase cell survival, while other compounds only show a minor activity against SARS-CoV-2 wildtype isolates, in vitro.

Table 2 IC₅₀ values (mM) of the three most active compounds against SARS-CoV-2 (PHE 2 and GLA1 isolates). HCQ = hydroxychloroquine; MOI = multiplicity of infection. R², the correlation coefficient, indicates the fit between the datapoints and fitted line (where R²=1 is a perfect fit).

	VeroE6-ACE2					A549-NPro-ACE2			
	MOI	PHE2		GLA1		PHE2		GLA1	
		0.5	0.05	0.5	0.05	0.5	0.05	0.5	0.05
Galidesivir	IC ₅₀	89.17	43.08	62.51	62.50	60.52	38.90	106.50	16.45
	R ²	1.00	0.99	0.91	0.91	0.99	0.99	0.97	0.77
Remdesivir	IC ₅₀	3.17	1.32	2.62	1.24	0.25	0.23	0.11	0.07
	R ²	0.99	0.99	0.71	0.94	0.99	0.94	0.99	0.93
HCQ	IC ₅₀	24.11	4.02	12.53	3.32	92.80	42.27	13.38	9.24
	R ²	0.99	0.99	0.83	0.73	0.99	0.99	0.94	0.79

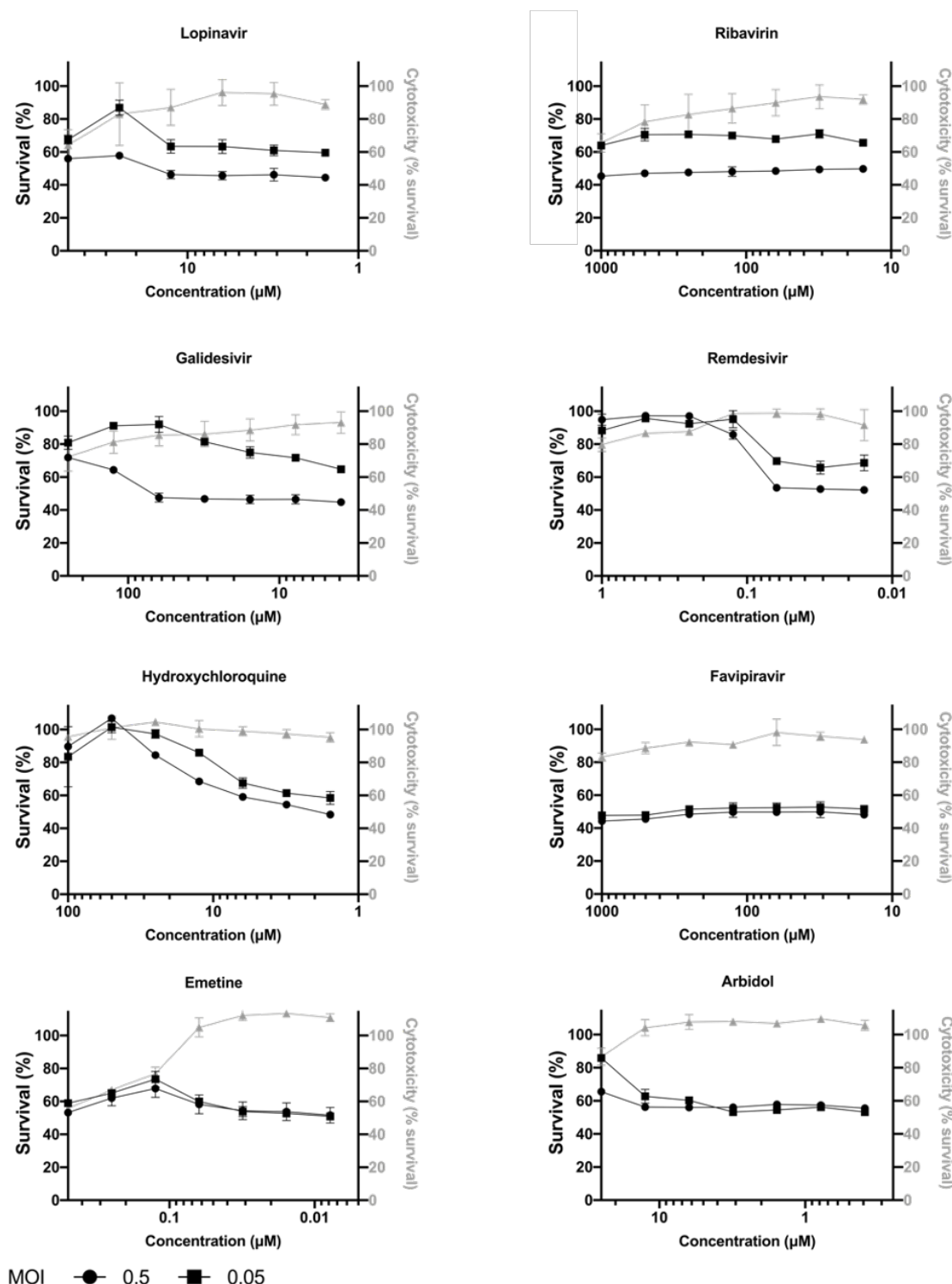


Figure 4 Assessment of SARS-CoV-2 inhibition by active FDA approved antiviral compounds. A549 cells, overexpressing ACE2 and BVDV Npro protein, were treated for three hours with varying concentrations of antiviral compounds before the addition of SARS-CoV-2 GLA1 isolate at an MOI of 0.05 and 0.5. The cells were fixed with 8% formaldehyde 72 hours post-infection and stained with Coomassie blue. Percentage cell survival was determined by comparing the intensity of the staining to uninfected and untreated wells. The data shown are representative figures of 3 independent repeats. The mean percentage cell survival of the DMSO control infected with an MOI of 0.05 and 0.5 was 50% (SD 0.502) and 43.45% (SD 0.642), respectively. The cytotoxicity of the compounds is depicted by the grey line and were determined by comparing the intensity of staining to untreated wells

4 Discussion

Drug repurposing is often attempted in preference to the development of new antiviral compounds as the timeline for initiating trials, safety risks and costs associated with research and development are reduced. They have consequently been a popular choice for clinical studies in COVID-19 since the start of the pandemic. However, despite the demonstration of antiviral activity in vitro, to date, results from clinical trials have been inconclusive or disappointing. Many of these trials were not randomised, were open-label and samples size were often small (<100 patients). The exceptions have been national and international studies such as the RECOVERY⁴⁷ and Solidarity trials¹⁷ that have provided conclusive data on both antiviral and immunomodulatory therapies. Most studies have been conducted in hospital settings, particularly in intensive care units, examining the drugs' effects in moderate to severe COVID-19 cases late on in the time course of infection, rather than during early infection. Hospitalised patients commonly present with severe late-stage disease when immunomodulatory treatments are required to reduce hyperinflammation and prevent consequent lung damage. Both dexamethasone and the IL-6 inhibitor genetic variants or virus isolates have been shown to reduce mortality in hospitalised patients⁴⁸. However repurposed antiviral compounds to date have not proved as successful.

Prior to clinical trials, the efficacy of candidate therapies is ascertained by antiviral activity in vitro. Here, 29 FDA-approved nucleoside analogues, protease inhibitors, influenza antivirals and other compounds were investigated for anti-SARS-CoV-2 activity in vitro, of which three demonstrated significant antiviral activity. Both the EC₅₀ (the concentration of a drug that gives half-maximal response) and the IC₅₀ (concentration of an inhibitor where the response is reduced by half) have been widely reported for these compounds in the literature, as indicators of potency, but do vary in their values. These values depend on the conditions of the experiment, such as MOI, cell type and lineage as well as incubation time. On review of in vitro and clinical studies, antiviral activity in cells was not found to necessarily translate to benefit in patients. For example, while HCQ and lopinavir/ritonavir showed strong SARS-CoV-2 activity in cells, they failed to lower mortality rates in hospitalised COVID-19 patients in trials and their use has been discontinued for this patient group¹⁶⁻¹⁸. Furthermore, some contraindications will require a detailed patient history and continued monitoring, which in milder cases may not be possible⁴⁹. For example, some drug candidates cause a prolonged QT interval and/or arrhythmias, or liver and kidney impairment. It is therefore crucial that such studies are carried out in robust blinded clinical trial settings to fully assess the impact of a given drug on COVID-19 outcomes. A good example of this is HCQ which showed promise in early small-scale trials but was found to cause more harm than benefit, as well as no effect on COVID-19 outcomes^{17,18} by large-scale trials. This may be related to difficulty in achieving adequate doses in vivo.

The anti-viral remdesivir have been shown to improve patient outcomes in one study, although evidence on the latter remains conflicting and no study has shown a significant effect on mortality⁵⁰. Remdesivir (GS-5734) is an adenosine nucleotide prodrug and a broad-spectrum antiviral developed by Gilead, originally used for treatment of Ebola and Marburg virus infections. As an intravenous drug that interferes with viral RNA-dependent RNA polymerase, remdesivir is most effective during early stages of infection, like most antivirals^{51,52}. In 2014, Gilead developed an interest in SARS and MERS, but no clinical trials were carried out due to inadequate patient numbers. Nevertheless, remdesivir was shown to be effective against coronaviruses in vitro^{53,54 26,31 32} and in vivo^{51,55}. Puijssers et al³³ showed that remdesivir was effective in mice infected with chimeric SARS-CoV encoding the RNA-dependent RNA polymerase of SARS-CoV-2, while a Gilead study⁵² reported its effectiveness in SARS-CoV-2 infected rhesus macaques. By the end of January 2021, there were 65 trials investigating remdesivir in COVID-19 patients registered in the WHO ICTRP database. A large, randomised trial (n=1063) funded by the NIH showed that remdesivir accelerated recovery in patients with severe COVID-19, reducing median recovery time by four days and improving survival (NCT04280705, ¹⁹). However, preliminary results from the WHO's Solidarity trial found no effect on mortality or hospital stay⁵⁶. Similarly, a smaller randomised, double-blind, placebo-controlled

multicentre trial study in China (n=237) found no benefits associated with remdesivir and reported that treatment was stopped early because of adverse events in 18 (12%) patients ([NCT04257656](#))⁵⁷.

Like remdesivir, galidesivir (BCX4430, BioCryst) is a novel synthetic adenosine analogue developed for the treatment of Ebola virus and Marburg virus disease. Galidesivir is a broad-spectrum antiviral given intravenously and has activity against several other viruses, including bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses and flaviviruses.⁴² Phase I studies found that it was generally safe and well-tolerated in healthy volunteers. Following promising results against coronaviruses in HeLa cells (MERS-CoV EC_{50} =68.5 μ M and SARS-CoV EC_{50} =57.7 μ M)⁴² and a molecular docking study showing that galidesivir can bind tightly the RdRp of SARS-CoV-2⁵⁸, a randomised, double-blind, placebo-controlled clinical trial has commenced ([NCT03891420](#)) in Brazil to consider galidesivir for treatment in COVID-19 and yellow fever. Recently, one study reported EC_{50} values under 100 μ M for galidesivir, but the experiments used cell culture-adapted SARS-CoV-2⁴³. Here, we report IC_{50} values for galidesivir against two isolates of SARS-CoV-2 demonstrating an IC_{50} of between 16.45 to 106.5 μ M, depending on the viral isolate, MOI and cell line, which is within the same activity range previous demonstrated for SARS-CoV and MERS-CoV.

The anti-influenza drug favipiravir (T-705) is also being investigated for treatment of SARS-CoV-2. Favipiravir is a pyrazinecarboxamide derivative and a pro drug that is converted to a nucleoside analogue within the cell of interest, followed by a series of phosphorylations to produce the active ribofuranosyltriphosphate T-705RTP, which selectively inhibits viral RNA-dependent RNA polymerases⁵⁹. Following a series of clinical trials, favipiravir has been licenced for severe influenza in Japan. While favipiravir has not been tested against SARS-CoV-1 and MERS-CoV, several publications have reported anti-SARS-CoV-2 in vitro, with EC_{50} values ranging from 61.9 μ M²⁶ to 207 μ M⁴¹ in VeroE6 cells. In a randomised control trial (n=240), 7-day clinical recovery was more frequent in patients taking favipiravir (71%) compared to umifenovir⁶⁰. Further trials are ongoing to evaluate the efficacy and safety of favipiravir in the treatment of COVID-19, including the Glasgow Early treatment Arm, Favipiravir (GETAFIX) in Scotland^{22,61-63}.

5 The way forward.

There is an urgent need for the development of novel antivirals to treat COVID-19 and tackle the pandemic. Since antiviral drugs are most likely to be effective early in the disease process, what are ideally required are antiviral therapies that can be taken orally and are well enough tolerated to be widely available for use in the community very soon after onset of symptoms. Currently the drug which has closest to this product profile is Favipiravir, which has had success in early clinical trials⁶⁰ but had no antiviral activity in our hands in vitro. A bespoke small molecule with a lower EC_{50} than Favipiravir might have a greater chance of success.

An alternative direction for the development of antiviral therapeutics is anti-SARS-CoV2 monoclonal antibodies (mAbs). For example, the S protein blockers casirivimab/imdevimab, also known as REGN10933/REGN10987⁶⁴ and Bamlanivimab (LY-CoV555)⁶⁵ which have both been issued with emergency use authorisation by the FDA, for use in non-hospitalised patients not requiring oxygen. Also under investigation in animal models, is CT-P59, which targets the receptor binding protein of SARS-CoV2⁶⁶. Casirivimab/imdevimab showed significant reductions in virus levels and were associated with significantly fewer medical visits⁶⁴. Bamlanivimab in combination with etesevimab decreased SARS-CoV2 log viral load by day 11 (BLAZE-1 study)⁶⁵.

The EC_{50} s for mAbs are promising (e.g. 31pM for casirivimab/imdevimab⁶⁷, however, mAb preparations have some disadvantages that will limit their use. They have to be administered intravenously, and adverse events can include serious hypersensitivity reactions and anaphylaxis. These attributes preclude the possibility of using them as community-based therapies. Further, monoclonal antibodies tend to select for escape viral variants, which may limit their useful life span. Using monoclonal antibodies in combination is an attempt to limit escape viral variants. For example, escape

variants have been documented when using either casirivimab or imdevimab individually, but so far not when used in combination ⁶⁷.

Repurposed compounds have been evaluated in hundreds of studies with limited clinical success, there remains an urgent need both for the development of novel antivirals to tackle severe disease and for a product that is safe enough and logistically practical for use in the critical early days of the disease process. It is time to think beyond the immediate emergency and fund the development of bespoke drugs for long-term solutions.

6 Acknowledgements

We would like to acknowledge the kind gift of cell lines from Prof. Michèle Bouloy (Institut Pasteur, France) and Prof. Rick Randall (University of St Andrews, UK). In addition, we would like to acknowledge the support of members of the Centre for Virus Research who provided reagents to enable the work within this study, Suzannah Rihn and Matthew Turnbull for the development of the various cell lines used, and Arthur Wickenhagen for the SARS-CoV-2 virus stocks.

Funding:

AK: UK Medical Research Council funding (MC_UU_12014/8).

DK: UK Medical Research Council MRC Precision Medicine Training Grant (MR/N013166/1-LGH/MS/MED2525).

MES and CD: Medical Research Council core award (MC_UU_1201412)

Declaration of interests:

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. JTS is a chief investigator on the GETAFIX trial (ISRCTN31062548), other authors have declared no competing interests.

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Supplementary Material

Supplementary Table 1 Terms used for searching the ICTRP database. Note that hydroxychloroquine (HCQ) and favipiravir were often misspelled, hence the more flexible terms.

	Terms used in ICTRP database search
SARS-CoV-2	"covid19", "covid-19", "sars-cov 2", "sars-cov-2", "19-ncov"
Drugs	"atazanavir", "abacavir", "amantadine", "AZT", "arbidol", "adefovir", "baloxavir", "danoprevir", "paritaprevir", "telbivudine", "sotetsuflavone", "sofosbuvir", "darunavir", "didonasine", "emetine", "emtricitabine", "favi*", "galidesivir", "gemcitabine", "hydroxychloroquine", "hydroxychloroquine", "hcq", "fosamprenavir", "lamivudine", "lopinavir", "ritonavir", "oseltamivir", "remdesivir", "ribavirin", "saquinavir", "tenofovir", "umifenovir"

Supplementary Table 2 Source and details of EC₅₀/IC₅₀ values used for Figure 3, including multiplicity of infection (MOI). Table adapted from Stanford University's Coronavirus Anti-Viral Research Database (<https://covdb.stanford.edu>). CPE = cytopathic effect.

Drug	Author (reference)*	Cell line	Value (μM)	MOI	Timepoint	Method
Remdesivir	Choy ²⁵	Vero E6	EC ₅₀ =23	0.02	48 hrs	infectious virus
		Vero E6	EC ₅₀ =26.9	0.02	48 hrs	viral RNA
	Dittmar ³⁴	Vero	EC ₅₀ =0.5	1	30 hrs	CPE
		Huh7.5	EC ₅₀ =0.002	1	30 hrs	CPE
		Calu-3	EC ₅₀ =0.005	0.5	48 hrs	CPE
	Wang ²⁶	Vero E6	EC ₅₀ =0.77	0.05	48 hrs	viral RNA
	De Meyer ³¹	Caco-2	EC ₅₀ =0.38	0.01	72 hrs	MTT method
		Caco-2	EC ₅₀ =0.11	0.01	72 hrs	CPE
	Bojkova ²⁹	Caco-2	IC ₅₀ =0.23	0.01	48 hrs	CPE
	Jeon ⁴⁰	Vero	IC ₅₀ =11.41	0.0125	24 hrs	viral N protein
	Ko ³²	Calu-3	IC ₅₀ =1.3	0.1	24 hrs	viral N protein
	Touret ³⁶	Vero E6	EC ₅₀ =1.65	0.002	48 hrs	CPE
	Pruijssers ³³	Vero E6	EC ₅₀ =1.65	0.01	48 hrs	infectious virus
		Calu-3	EC ₅₀ =0.28	0.01	72 hrs	infectious virus
	Ellinger ³⁰	Caco-2	IC ₅₀ =0.76	0.01	48 hrs	CPE
	Holwerda ²⁸	Vero E6	IC ₅₀ =1.842	0.01	24 hrs	CPE
Emetine	Ellinger ³⁰	Caco-2	IC ₅₀ =0.52	0.01	48 hrs	CPE
	Iancovski ⁴⁵	Vero E6	EC ₅₀ <0.01	0.1	72 hrs	CTG
		Vero E6	EC ₅₀ <0.01	0.1	72 hrs	Plaque assay
	Choy ²⁵	Vero E6	EC ₅₀ =0.46	0.02	48 hrs	infectious virus
		Vero E6	EC ₅₀ =0.50	0.02	48 hrs	viral RNA
Favipiravir	Wang ²⁶	Vero E6	EC ₅₀ =61.88	0.05	48 hrs	viral RNA
	Shannon ⁴¹	Vero E6	EC ₅₀ =118.3	0.002	72 hrs	CPE
		Vero E6	EC ₅₀ =207.1	0.002	72 hrs	viral replication
HCQ	Touret ³⁶	Vero E6	EC ₅₀ =4.17	0.002	48 hrs	CPE

Yao ³⁷	Vero	EC ₅₀ =6·14	0·01	24 hrs	Viral RNA
	Vero	EC ₅₀ =0·72	0·01	48 hrs	Viral RNA
Liu ³⁵	Vero E6	EC ₅₀ =4·51	0·01	48 hrs	Viral RNA
	Vero E6	EC ₅₀ =4·06	0·02	48 hrs	Viral RNA
	Vero E6	EC ₅₀ =17·31	0·2	48 hrs	Viral RNA
	Vero E6	EC ₅₀ =12·96	0·8	48 hrs	Viral RNA
Dittmar ³⁴	Vero	EC ₅₀ =1·32	1	30 hrs	CPE
	Huh7·5	EC ₅₀ =0·14	1	30 hrs	CPE
	Calu-3	EC ₅₀ =39·16	0·5	48 hrs	CPE
Lopinavir					
Ellinger ³⁰	Caco-2	IC ₅₀ =19·11	0·01	48 hrs	CPE
Choy ²⁵	Vero E6	EC ₅₀ =26·63	0·02	48 hrs	infectious virus
	Vero E6	EC ₅₀ =26·10	0·02	48 hrs	viral RNA
Yamamoto ²⁴	Vero E6	EC ₅₀ =5·73	0·01	24 hrs	viral RNA
Jeon ⁴⁰	Vero	IC ₅₀ =7·28	0·0125	24 hrs	viral N protein
Ko ³²	Calu-3	IC ₅₀ =21·7	0·1	24 hrs	viral N protein
Ribavirin					
Wang ⁵⁷	Vero E6	EC ₅₀ =109·5	0·05	48 hrs	viral RNA
Sacramento ⁴⁴	Calu-3	EC ₅₀ =8·6	0·1	48 hrs	Infectious virus
	Huh7	EC ₅₀ =6·5	0·1	48 hrs	Infectious virus
Sofosbuvir Sacramento ⁴⁴	Calu-3	EC ₅₀ =9·5	0·1	48 hrs	Infectious virus
	Huh7	EC ₅₀ =6·2	0·1	48 hrs	Infectious virus
Galidesivir Ramirez ⁴³	Huh7·5	EC ₅₀ =48	-	72 hrs	Infectious virus
	Calu-1	EC ₅₀ =58	-	72 hrs	Infectious virus
	A549	EC ₅₀ =24	-	72 hrs	Infectious virus
Grazoprevir Gammeltoft ⁴⁶	Vero	EC ₅₀ =42	0·0016	46-50 hrs	viral RNA
	Huh7·5	EC ₅₀ =20	0·0198	70-75 hrs	viral RNA
Danoprevir Gammeltoft ⁴⁶	Vero	EC ₅₀ =87	0·0016	46-50 hrs	viral RNA

* All publications are from 2020, numbers indicate full references in main text.

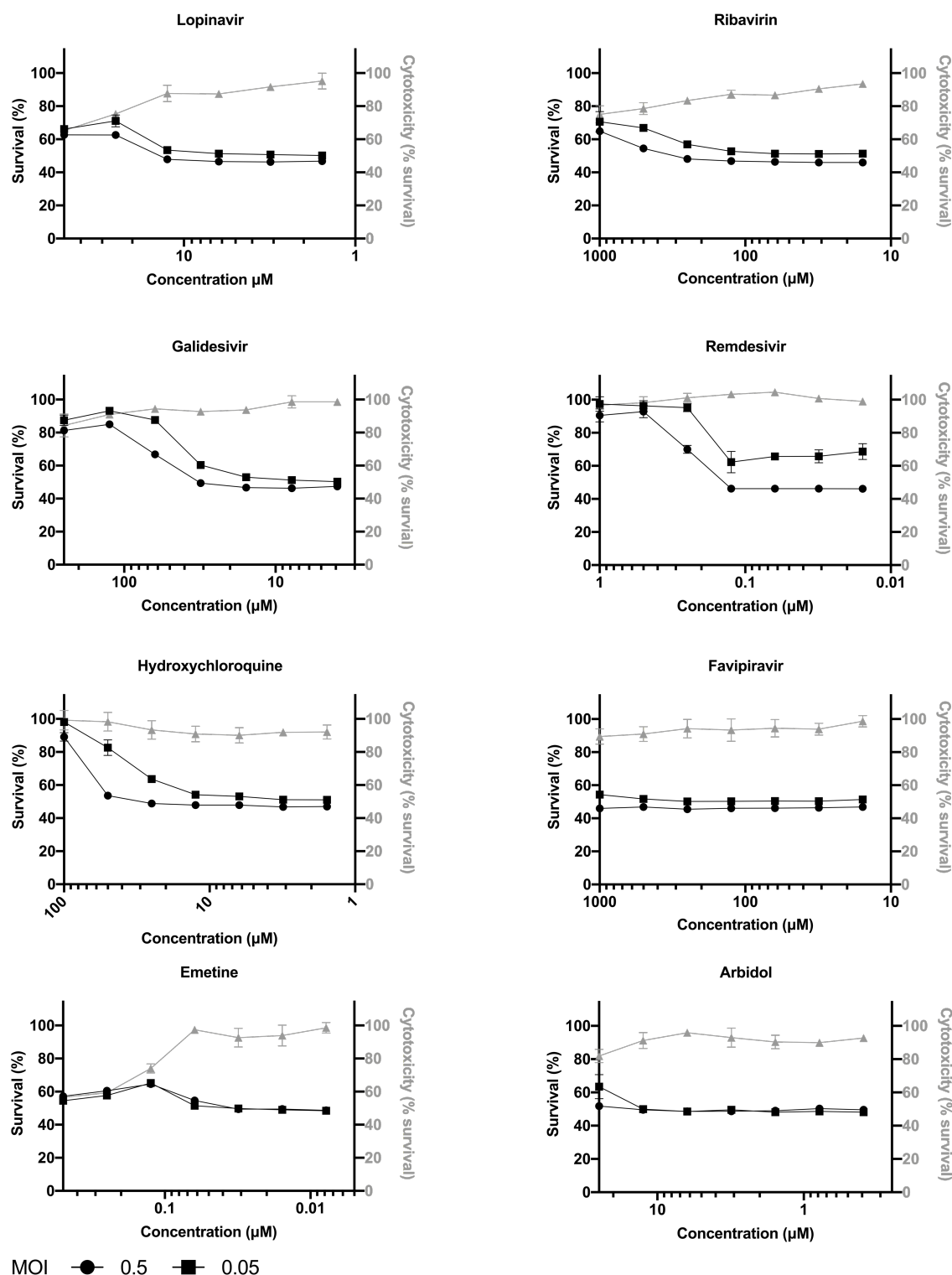
Supplementary Table 3 Contraindications and warnings associated with 29 FDA-approved repurposed compounds.

Compound	Profile	Contraindications and warnings
Abacavir	Nucleoside (guanosine) analogue. Used for treatment and prevention of HIV/AIDS in combination with other antiretrovirals.	Associated with fatal hypersensitivity reactions. HLAB5701 test required. Viiv, Ziagen, Summary of Product Characteristics
Adefovir	Nucleotide (adenosine) analogue. Used to treat chronic hepatitis B infections.	Nil. Gilead, Hepsera, Summary of Product Characteristics
Amantadine	Weak dopamine agonist. Modest antiparkinsonian effects, no longer recommended for influenza.	Individuals subject to convulsions, A history of gastric ulceration, Severe renal disease, Pregnancy and breast-feeding. Fontus, Trilasym, Summary of Product Characteristics

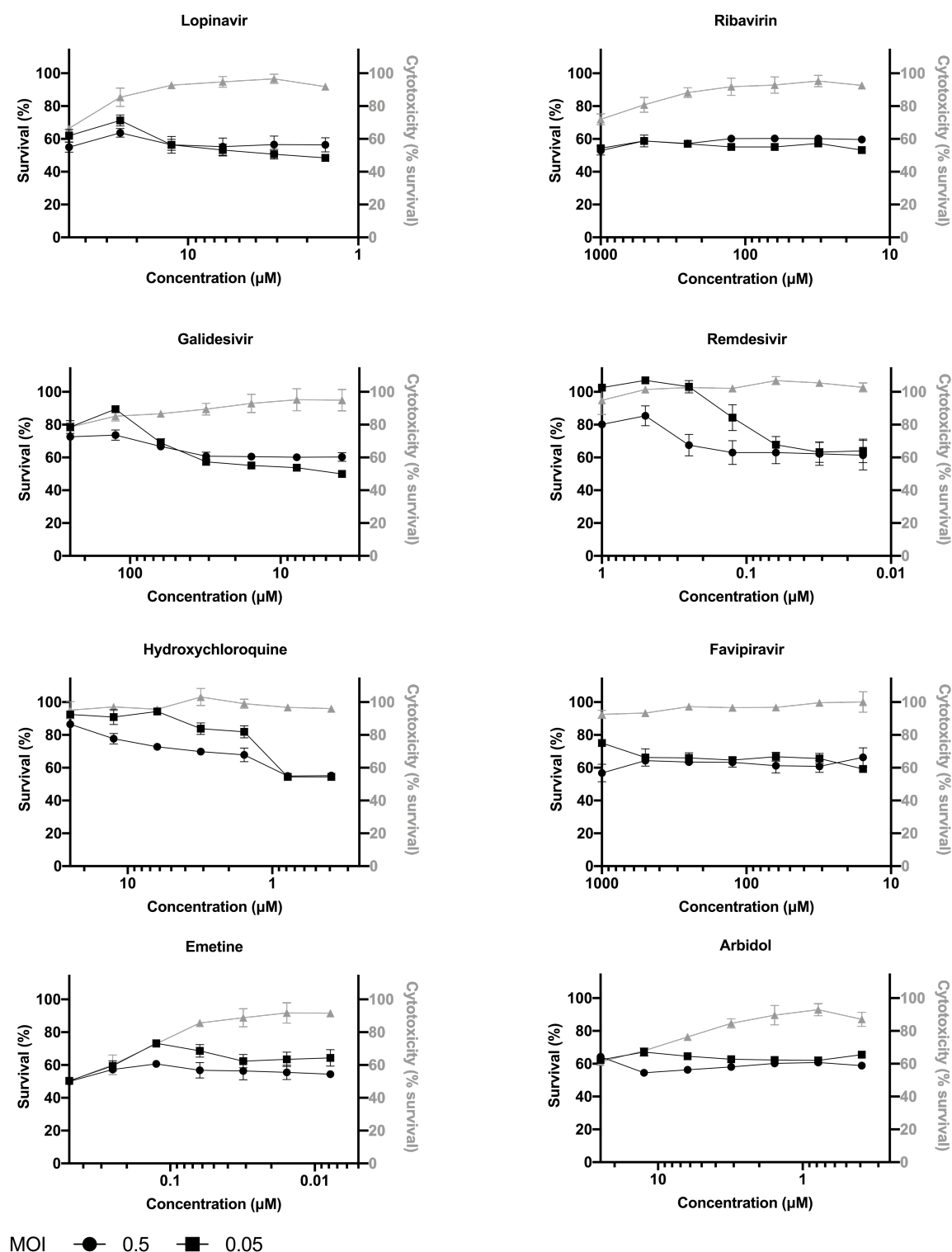
Arbidol (umifenovir)	Indole derivative. Inhibits viral entry. Used as prophylaxis and treatment in influenza A and B (China and Russia).	Contraindicated in paediatric patients under 2 years old, in first trimester of pregnancy and during breastfeeding. OTCPharm, Arbidol, manufacturer website
Atazanavir	HIV-1 protease inhibitor. Inhibits processing of Gag and Gag-Pol polypeptides, given in combination with other antiretrovirals.	Moderate to severe hepatic impairment. Avoid coadministration with alfuzosin, triazolam, orally administered midazolam, ergot derivatives, rifampin, irinotecan, lurasidone, lovastatin, simvastatin, indinavir, cisapride, pimozone, St. John's wort, nevirapine, elbasvir/grazoprevir, glecaprevir/pibrentasvir and sildenafil. Avoid co-administration with medicinal products that are substrates of the CYP3A4 isoform of cytochrome P450 and have narrow therapeutic windows. Warnings: QT prolongation: Dose related asymptomatic prolongations in PR interval with atazanavir observed have been observed in clinical studies. Caution should be used with medicinal products known to induce PR prolongations. Particular caution should be used when prescribing in association with medicinal products which have the potential to increase the QT interval and/or in patients with pre-existing risk factors (bradycardia, long congenital QT, electrolyte imbalances) BMS, Reyataz, Summary of Product Characteristics
Baloxavir marboxil	Endonuclease inhibitor. A polymerase acidic endonuclease inhibitor used for the treatment of influenza A and B.	Nil. Shionogi, Xoflusa, US Prescribing Information
Danoprevir	HCV protease inhibitor. An orally bioavailable inhibitor of hepatitis C virus non-structural protein 3/4A	Childs Pugh C, ALT >5x ULN (Unable to find Chinese license, these are exclusion criteria in studies.)
Darunavir	HIV-1 protease inhibitor. Must be co-administered with ritonavir or cobicistat and with other antiretroviral agents	Patients with severe hepatic impairment. Lopinavir/ritonavir, dabigatran, ticagrelor, strong CYP3A inducers, concomitant treatment with such medicinal products that are substrates of CYP3A4 for which elevated plasma concentrations are associated with serious and/or life-threatening events is contraindicated. These active substances include alfuzosin, amiodarone, bepridil, dronedarone, ivabradine, quinidine, ranolazine, astemizole, terfenadine, colchicine when used in patients with renal and/or hepatic impairment, ergot derivatives, elbasvir/grazoprevir, cisapride, dapoxetine, domperidone, naloxegol, lurasidone, pimozone, quetiapine, sertindole, triazolam, midazolam, sildenafil, avanafil, simvastatin, lovastatin and lomitapide. Janssen Pharmaceuticals, Prezista, Summary of Product Characteristics
Didanosine (ddI)	Nucleoside (adenosine) analogue. A nucleoside reverse transcriptase inhibitor given in combination with other antiretrovirals.	Coadministration with stavudine, allopurinol, or ribavirin is contraindicated. BMS, Videx, Summary of Product Characteristics
Emetine hydrochloride	Alkaloid of ipecacuanha. A tissue amoebicide acting mainly in the bowel wall and in the liver.	Cardiac, renal, or neuromuscular disease. Pregnancy, and in children except in severe amoebic dysentery unresponsive to other drugs. Martindale online
Emtricitabine	Nucleoside (cytidine) analogue. A nucleoside reverse transcriptase inhibitor, given in combination with other antiretrovirals.	Nil. Monitor renal function and adjust dose as per license. Gilead, Emtriva, Summary of Product Characteristics
Favipiravir	Viral RNA polymerase inhibitor. Reserved for novel or re-emerging influenza where other drugs are ineffective (Japan).	Should not be given to women who are or may become pregnant. Toyama Chemical, Avigan Tablets 200mg, Japanese License

Fosamprenavir	HIV-1 protease inhibitor. Fosamprenavir is a pro-drug of amprenavir and is given in combination with other antiretrovirals.	Narrow therapeutic window drugs that are substrates of cytochrome P450 3A4, e.g. alfuzosin, amiodarone, astemizole, bepridil, cisapride, dihydroergotamine, ergotamine, pimozone, quetiapine, quinidine, terfenadine, oral midazolam, oral triazolam, sildenafil. Medicinal products with narrow therapeutic windows that are highly dependent on CYP2D6 metabolism, e.g. flecainide and propafenone. Co-administration of lurasidone, paritaprevir, simvastatin, lovastatin, rifampicin and St Johns Wort. Viiv, Telzir, Summary of Product Characteristics.
Galidesivir	Nucleoside (adenosine) analogue. A novel RNA polymerase inhibitor, used for Ebola, Marburg, Yellow Fever and Zika.	Safe and well tolerated in Phase 1 studies. BioCryst, Galidesivir, press release
Grazoprevir	HCV protease inhibitor. Reduces viral load by inhibiting hepatitis C virus RNA replication, used with elbasvir.	Patients with moderate or severe hepatic impairment. Co-administration with inhibitors of OATP1B, such as rifampicin, atazanavir, darunavir, lopinavir, saquinavir, tipranavir, cobicistat or ciclosporin. Co-administration with inducers of cytochrome P450 3A or P-glycoprotein, such as efavirenz, phenytoin, carbamazepine, bosentan, etravirine, modafinil or St. John's wort. Merck, Zepatier, Summary of Product Characteristics
Hydroxychloroquine	Antimalarial (4-aminoquinoline). Increases pH in lysosomes, reduces signalling in inflammatory pathways.	Hypersensitivity to 4-aminoquinoline compounds. Pre-existing maculopathy of the eye. Pregnancy. Concordia Pharmaceuticals, Plaquenil, Summary of Product Characteristics
Lamivudine	Nucleoside (cytidine) analogue. Used for treatment of hepatitis B and HIV.	Nil. Renally excreted, monitor renal function and adjust dose as per license. Viiv, Epivir, Summary of Product Characteristics
Lopinavir/ ritonavir	HIV-1 protease inhibitors. An antiretroviral combination, where ritonavir boosts the effect of lopinavir.	Severe hepatic insufficiency. Should not be co-administered with medicinal products that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life-threatening events. See product license for full list of drugs. Warning: Particular caution must be used when prescribing with medicinal products known to induce QT interval prolongation. AbbVie, Kaletra, Summary of Product Characteristic
Oseltamivir	Neuraminidase inhibitor. Used to treat and prevent influenza A and B.	Nil. Roche, Tamiflu, Summary of Product Characteristics
Paritaprevir	HCV protease inhibitor. Used to treat chronic HCV in combination with ritonavir and ribavirin.	Contraindicated in patients with severe hepatic impairment. Do not co-administer with drugs that are highly dependent on CYP3A for clearance, are strong inducers of CYP3A and CYP2C8 or strong inhibitors of CYP2C8. Abbvie, Viekira Pak, US Prescribing Information

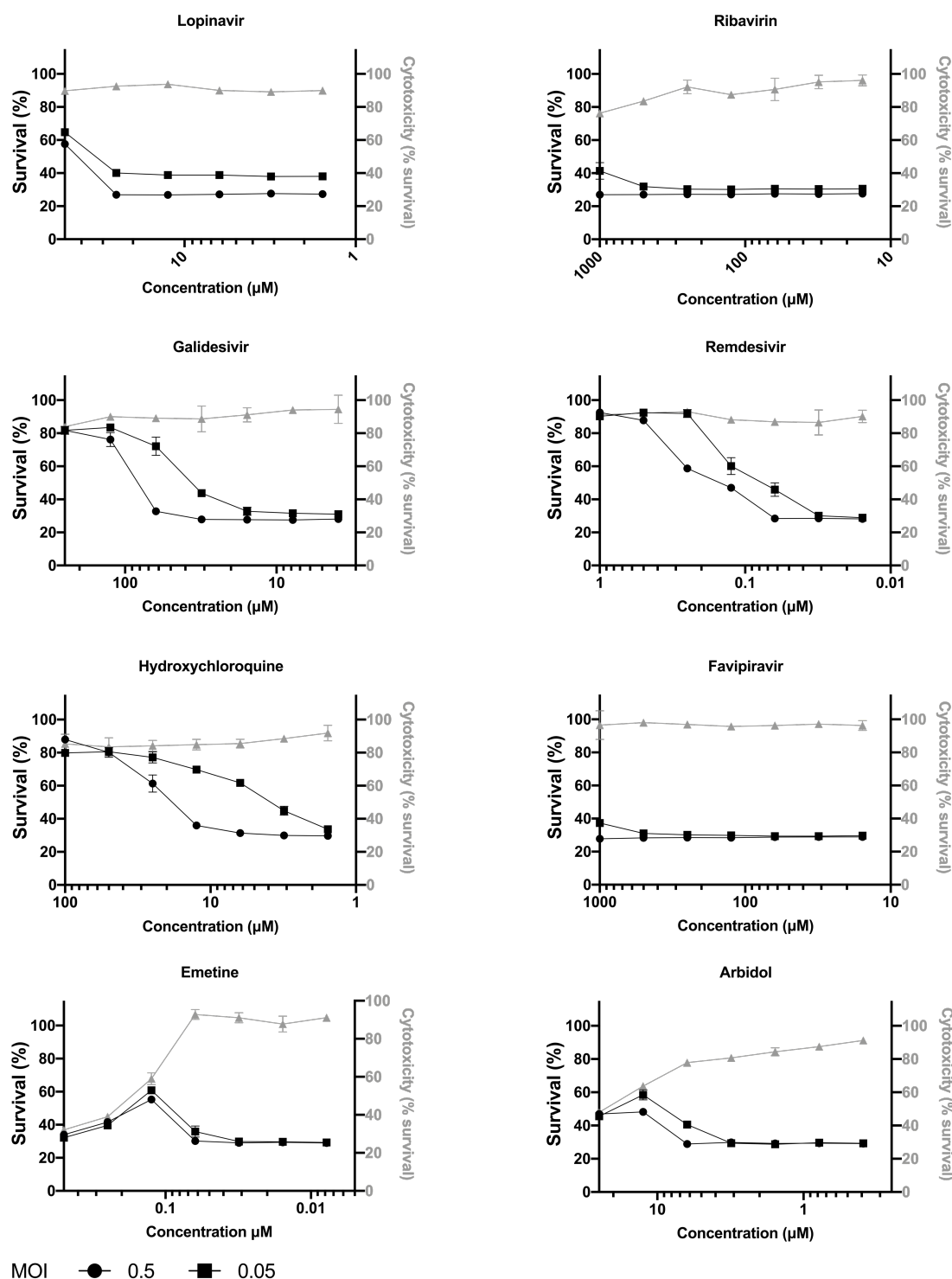
Remdesivir	Nucleotide (adenosine) analogue. A broad-spectrum antiviral that targets RdRp, developed for treatment of filovirus infections.	Nil. Gilead, Vexlury, Summary of Product Characteristics
Ribavirin	Nucleoside (guanosine) analogue. It has activity against a range of DNA and RNA viruses	Pregnant women and men whose female partners are pregnant, breast feeding, a history of severe pre-existing cardiac disease, including unstable or uncontrolled cardiac disease, in the previous six months and haemoglobinopathies. Aurobindo, ribavirin, Summary of Product Characteristics
Saquinavir	HIV protease inhibitor. Used in combination with ritonavir.	Decompensated liver disease, QT prolongation, electrolyte disturbances, particularly uncorrected hypokalaemia, clinically relevant bradycardia or heart failure with reduced left-ventricular ejection fraction, previous history of symptomatic arrhythmias, concurrent therapy with drugs that prolong the QT and/or PR interval or midazolam, triazolam, simvastatin, lovastatin, ergot alkaloids, rifampicin, quetiapine, lurasidone Roche, Invirase, Summary of Product Characteristics
Sofosbuvir	Nucleotide analogue, blocks the hepatitis C NS5B protein. Used for treatment of chronic hepatitis C.	Medicinal products that are strong P-gp inducers in the intestine (carbamazepine, phenobarbital, phenytoin, rifampicin and St. John's wort). Gilead, Sovaldi, Summary of Product Characteristics
Sotetsuflavone	DENV-NS5 RdRp inhibitor. Not yet used in patients.	N/A
Telbivudine	Nucleoside (thymidine) analogue. Impairs hepatitis B virus DNA replication.	Combination with pegylated or standard interferon alfa. Novartis, Tyzeka, Summary of Product Characteristics
Tenofovir	Nucleotide (deoxyadenosine) analogue. A selective inhibitor of viral reverse transcriptase used for the treatment of HIV and chronic hepatitis B.	Nil. Gilead, Viread, Summary of Product Characteristics
Zidovudine (AZT)	Nucleoside (thymidine) analogue. Inhibits viral reverse transcriptase, given in combination with other antiretrovirals.	Should not be given with abnormally low neutrophil counts ($<0.75 \times 10^9/\text{litre}$) or abnormally low haemoglobin levels ($<7.5 \text{ g/decilitre}$ or 4.65 mmol/litre). New born infants with hyperbilirubinaemia requiring treatment other than phototherapy, or with increased transaminase levels of over five times the upper limit of normal. Viiv, Retrovir, Summary of Product Characteristics



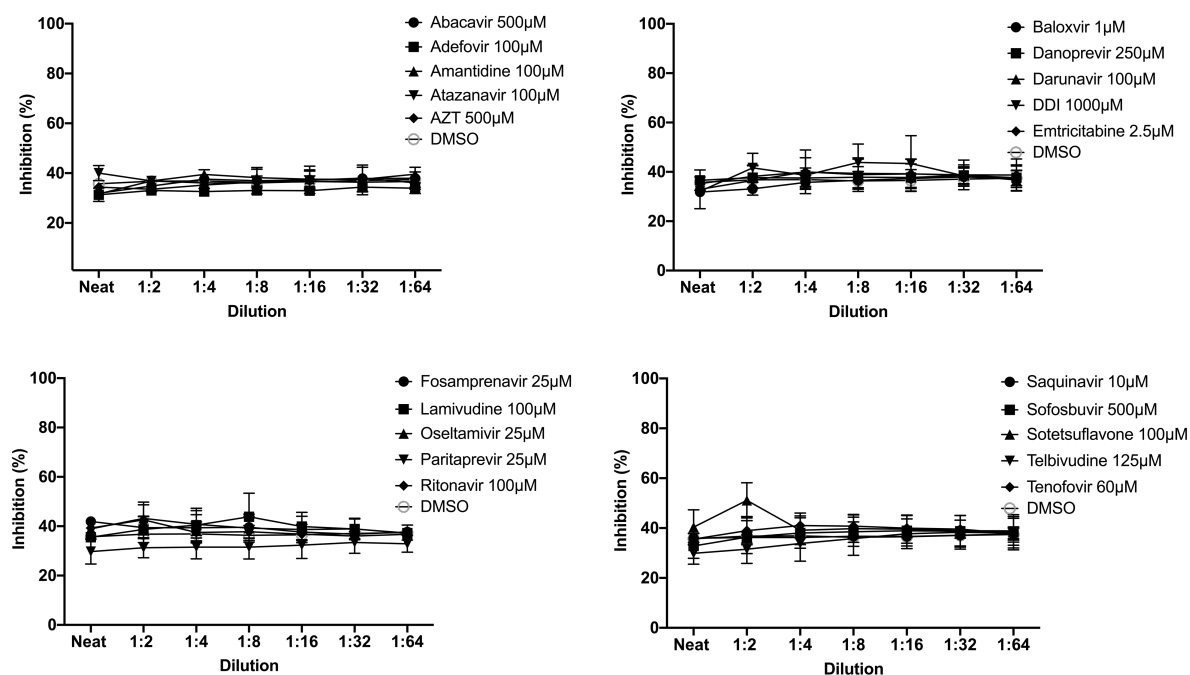
Supplementary Figure 1 Assessment of SARS-CoV-2 inhibition by active FDA approved antiviral compounds. A549 cells, overexpressing Ace2 and BVDV Npro protein, were treated for three hours with varying concentrations of antiviral compounds before the addition of SARS-CoV-2 PHE2 isolate at an MOI of 0.05 and 0.5. The cells were fixed with 8% formaldehyde 72 hours post-infection and stained with Coomassie blue. Percentage cell survival was determined by comparing the intensity of the staining to uninfected wells. The data shown are representative figures of 3 independent repeats. The mean percentage cell survival of the DMSO control infected with an MOI of 0.05 and 0.5 was 51.08% (SD 1.117) and 47.35% (SD 0.764), respectively. The cytotoxicity of the compounds is depicted by the grey line and were determined by comparing the intensity of staining to untreated wells.



Supplementary Figure 2 : Assessment of SARS-CoV-2 inhibition by active FDA approved antiviral compounds. VeroE6 cells, overexpressing Ace2, were treated for three hours with varying concentrations of antiviral compounds before the addition of SARS-CoV-2 GLA1 isolate at an MOI of 0.05 and 0.5. The cells were fixed with 8% formaldehyde 72 hours post-infection and stained with Coomassie blue. Percentage cell survival was determined by comparing the intensity of the staining to uninfected wells. The data shown are representative figures of 3 independent repeats. The mean percentage cell survival of the DMSO control infected with an MOI of 0.05 and 0.5 was 50% (SD 0.502) and 43.45% (SD 0.642), respectively. The cytotoxicity of the compounds is depicted by the grey line and were determined by comparing the intensity of staining to untreated wells



Supplementary Figure 3 Assessment of SARS-CoV-2 inhibition by active FDA approved antiviral compounds. VeroE6 cells, overexpressing Ace2, were treated for three hours with varying concentrations of antiviral compounds before the addition of SARS-CoV-2 PHE2 isolate at an MOI of 0.05 and 0.5. The cells were fixed with 8% formaldehyde 72 hours post-infection and stained with Coomassie blue. Percentage cell survival was determined by comparing the intensity of the staining to uninfected wells. The data shown are representative figures of 3 independent repeats. The mean percentage cell survival of the DMSO control infected with an MOI of 0.05 and 0.5 was 28.83% (SD 0.673) and 27.52% (SD 0.193), respectively. The cytotoxicity of the compounds is depicted by the grey line and were determined by comparing the intensity of staining to untreated wells



Supplementary Figure 4 Assessment of SARS-CoV-2 inhibition by active FDA approved antiviral compounds. A549 cells, overexpressing Ace2 and BVDV Npro protein, were treated for three hours with varying concentrations of antiviral compounds before the addition of SARS-CoV-2 PHE2 isolate at an MOI of 0.5. The cells were fixed with 8% formaldehyde 72 hours post-infection and stained with Coomassie blue. Percentage cell survival was determined by comparing the intensity of the staining to uninfected wells. The data shown are representative figures of 2 independent repeats.