

Drug Repurposing Screen for Compounds Inhibiting the Cytopathic Effect of SARS-CoV-2

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Running title: Drug repurposing screen for SARS-CoV-2

Abstract

Drug repurposing is a rapid approach to identifying therapeutics for the treatment of emerging infectious diseases such as COVID-19. To address the urgent need for treatment options, we carried out a quantitative high-throughput screen using a SARS-CoV-2 cytopathic assay with a compound collection of 8,810 approved and investigational drugs, mechanism-based bioactive compounds, and natural products. Three hundred and nineteen compounds with anti-SARS-CoV-2 activities were identified and confirmed, including 91 approved drug and 49 investigational drugs. Among these confirmed compounds, the anti-SARS-CoV-2 activities of 230 compounds, including 38 approved drugs, have not been previously reported. Chlorprothixene, methotrimeprazine, and piperacetazine were the three most potent FDA approved drugs with anti-SARS-CoV-2 activities. These three compounds have not been previously reported to have anti-SARS-CoV-2 activities, although their antiviral activities against SARS-CoV and Ebola virus have been reported. These results demonstrate that this comprehensive data set of drug repurposing screen for SARS-CoV-2 is useful for drug repurposing efforts including design of new drug combinations for clinical trials.

Keywords: COVID-19, SARS-CoV-2, cytopathic effect, drug repurposing screen

Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global health crisis. As of July 31, 2020, the global case report stands at 17.5 million, with a death toll of 677,538 (Dong et al., 2020). Only remdesivir, an investigational drug developed for Ebola virus, has been granted emergency use authorization by the US FDA (Eastman et al., 2020). Since neither an effective vaccine, nor antiviral therapy, are currently available for COVID-19, drug repurposing has received significant attention in the rapid search to fill this unmet therapeutic need.

The requirement of biosafety level 3 (BSL-3) containment laboratories for handling SARS-CoV-2 has limited the number of high throughput screening (HTS) laboratories that are capable of carrying out large scale compound screens using live SARS-CoV-2. Despite these challenges, several drug repurposing screens have been carried out using live SARS-CoV-2, showing promising results (Dittmar et al., 2020; Ellinger et al., 2020; Riva et al., 2020; Touret et al., 2020). Here we report a screening campaign against a collection of 8,810 approved and investigational drugs, mechanism-based bioactive compounds, and natural products that was carried out in quantitative HTS (qHTS) format (Inglese et al., 2006). Compounds were screened at four concentrations in a SARS-CoV-2 cytopathic effect (CPE) assay in Vero E6 cells that were selected for high ACE2 expression, with an accompanying cytotoxicity counter-assay. The primary screen yielded 319 hits with confirmed anti-SARS-CoV-2 activity. The primary screening data have been made publicly available on the NCATS OpenData Portal (<https://opendata.ncats.nih.gov/covid19/index.html>) (Brimacombe et al., 2020). We intend this as a companion to guide investigators in utilizing that data, and to present further details of qHTS with the SARS-CoV-2 CPE assay, including identification of top annotated hits.

Materials and Methods

Compounds and compound libraries

All compound libraries were assembled internally at NCATS. The NCATS pharmaceutical collection (NPC) contains 2,678 compounds, covering drugs approved by US FDA and foreign health agencies in European Union, United Kingdom, Japan, Canada, and Australia, as well as some clinical trialed experimental drugs (Huang et al., 2019). The NCATS Mechanism Interrogation Plate (MIPE) 5.0 library contains 2,480 mechanism based bioactive compounds, targeting more than 860 distinct mechanisms of action (Lin et al., 2019). The NCATS Pharmacologically Active Chemical Toolbox (NPACT) is a library of mechanistically defined molecules and natural products (5,099 compounds). Other small custom NCATS collections were also screened: anti-infective (752 compounds), kinase inhibitors (977 compounds), epigenetic modulators (335 compounds). A commercially available autophagy-focused screening library (Cayman #23537) was analyzed and 29 compounds that were not already present in our collections were purchased. All compounds were dissolved in DMSO to make 10 mM stock solutions, unless solubility was limiting, and was diluted four times at 1:5 ratio for the primary screens, and at 1:3 ratio for follow up assays at 8 concentrations.

CPE assay

A SARS-CoV-2 CPE assay was conducted in the BSL3 facilities at the contract research organization Southern Research (Birmingham, AL). Briefly, compounds were titrated in DMSO and acoustically dispensed into 384-well assay plates at 60 nL/well at NCATS, and provided to Southern Research. Cell culture media (MEM, 1% Pen/Strep/GlutaMax, 1% HEPES, 2% HI FBS) was dispensed at 5 μ L/well into assay plates and incubated at room temperature to allow for compound dissolution. Vero E6 African green monkey kidney epithelial cells (selected for high ACE2 expression) were inoculated with SARS CoV-2 (USA_WA1/2020) at multiplicity of infection (MOI) of 0.002 in media and quickly dispensed into assay plates as 25 μ L/well. The final cell density was 4000 cells/well. Assay plates were incubated for 72 h at 37C, 5% CO₂, 90% humidity. CellTiter-Glo (30 μ L/well, Promega #G7573) was dispensed into the assay plate. Plates were incubated for 10 min at room temperature. Luminescence signal was measured on Perkin Elmer Envision or BMG CLARIOstar plate readers. An ATP content cytotoxicity counter-assay

was conducted using the same protocol as the CPE assay, without the addition of SARS-CoV-2 virus.

Data analysis

Results from the primary screen and confirmation screens were processed at NCATS using a software developed in-house (Wang et al., 2010). For the CPE assay, raw plate data were normalized with DMSO-only wells as 0% CPE rescue (negative signal control) and no virus control wells as 100% CPE rescue (positive signal control). For the cytotoxicity assay, raw plate data were normalized with DMSO-only wells as 100% viability (positive signal control) and cells treated with hyamine (benzethonium chloride) control compound as 0% viability (negative signal control). The half-maximum effective values (EC_{50}) and percent efficacy were obtained by fitting the concentration-response titration data to a four-parameter Hill equation. Compounds with >55% efficacy were selected for cherry-pick confirmation. The concentration-response curves of re-tested compounds were also plotted using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA). Results in the figures are expressed as mean \pm standard deviation (SD).

Results

High throughput screening with SARS-CoV-2 CPE assay

Our aims were two-fold in initiating this program. The first was to identify active compounds that may provide opportunities for repurposing or identify mechanistic targets of interest. The second was to create a complete HTS reference dataset that can be shared openly with the scientific community. The CPE reduction assay format has been widely employed to screen for antiviral agents due to its easy of scalability for HTS (Heaton, 2017). In this assay, viral infection kills host cells and the cell viability is used as a surrogate readout for viral infection and replication. In other words, compounds with anti-viral activity rescue cells from the cytopathic effect of SARS-CoV-2 (a gain-of-signal assay).

A total of 9,952 compounds were tested in the primary screen, but due to overlap in the composition of libraries, a significant number of compounds were tested multiply. A total of 8,810 unique compounds in six compound libraries were tested in the primary screen including the NCATS Pharmaceutical Collection (NPC), NCATS Mechanism Interrogation Plate (MIPE), NCATS Pharmacologically Active Chemical Toolbox (NPACT), Epigenomic library, Autophagy library, and anti-infective library. These compounds contain 1,345 approved drugs (by FDA, EMA, DPD), 751 compounds approved outside of these countries, 1067 investigational drugs (in clinical trials), 1057 pre-clinical compounds (tested in animals) and 4,472 bioactive compounds (tool compounds) (Figure 1a). By their mechanisms of action and clinical application, these compounds are divided into diverse groups (Figure 1b).

The CPE assay performed well in the primary screen, with an average Z' factor of 0.83 over 133 plates over three batched runs (Figure 2a). Remdesivir concentration response was included as a control for each screening run and yielded consistent EC_{50} values of 4.56 μ M, 4.42 μ M and 7.28 μ M (Figure 2b). Using the criteria of >55% efficacy, 380 compounds were selected as the primary screen hits, out of which, 319 compounds were confirmed using 8-point, 1:3 titration in duplicate. Among these primary hits, 89 of 319 had previously reported activity against SARS-CoV-2, including the assays of live virus, enzymatic, or virtual screening, while 230 were novel hits from this qHTS (Table 1, S1).

91 approved drugs and 49 investigational drugs protected against cytopathic effect of SARS-CoV-2 infection

There were 56 top confirmed hits with EC_{50} values of ≤ 10 μ M and efficacy greater than 80% in the CPE assay, and greater than 10-fold selectivity index between cytotoxicity and CPE assays (Table 1, Figure 3). When grouped by mechanism of action targets, 19 compounds were GPCR modulators, 8 were host protease inhibitors, 5 were kinase modulators, and 3 were autophagy modulators (Figure 3). Interestingly, in the 56 top hits, only one, remdesivir has a known viral target as a known primary mechanism, whereas the known mechanisms of action of the other compounds are directed against host targets.

There have been several previous drug repurposing screens reported for SARS-CoV-2 in 2D cell culture infection models (Dittmar et al., 2020; Ellinger et al., 2020; Jeon et al., 2020; Riva et al., 2020; Touret et al., 2020; Weston et al., 2020). These screens had some compound overlap with our qHTS screen, particularly for the FDA approved drugs. We performed a literature search of our confirmed compounds and previous reports were noted in Table 1 and Table S1. Three of the top 56 hits were novel and FDA approved. These hits are chlorprothixene, methotrimeprazine, and piperacetazine, which showed 10 μ M potencies in the CPE assay. Four drugs approved outside of the US were also identified as novel compounds with anti-SARS-CoV-2 effects: difeterol, rescimetol, melitracen HCl, and proglumetacin. Furthermore, we identified 7 novel clinical trial drugs with anti-SARS-CoV-2 activities: N-methylpiperone HCl, Lu AE58054 HCl, balicatib, berzosertib, JTV519 hemifumarate, DMP 777, and dexanabinol. In addition to above novel hits, four drugs, approved by FDA and elsewhere, methdilazine, maprotiline HCl, deserpidine, and flunarizine, were previously reported in virtual screens against SARS-CoV-2 targets without supporting biological data. Here, we report their activities against SARS-CoV-2 infection. In addition, we have confirmed 53 approved drugs with anti-SARS-CoV-2 activity that were reported previously (Table 1 and Table S1). Together, our results demonstrate a comprehensive set of 91 approved drugs and 49 investigational drugs with anti-SARS-CoV-2 activity that can be considered for design of new clinical trials, especially drug combination therapies, to increase and improve treatment options for COVID-19.

Discussion

In contrast to the other reported drug repurposing screens for SARS-CoV-2 using a single drug concentration in the primary screens (Dittmar et al., 2020; Ellinger et al., 2020; Jeon et al., 2020; Riva et al., 2020; Touret et al., 2020; Weston et al., 2020), we have used a quantitative HTS (qHTS, concentration-response) method (Inglese et al., 2006) where four compound concentrations were used in the primary screen instead of a single compound concentration. We also assessed the cytotoxicity of each compound against Vero E6 cells (without virus infection) in parallel with the SARS-CoV-2 CPE screening. The concentration-response for each

compound used in the primary screen can improve identification of positive hits, especially these compounds with biphasic actions (bell-shaped curves) or screening errors. In addition, we have more inclusive compound collections with drugs approved by regulatory agencies outside of USA, such as Canada, Europe and Japan, that were not previously screened in SARS-CoV-2 assays. We also screened a set of investigational drugs that have human clinical data for drug properties such as the mechanism(s) of action, pharmacokinetics, and drug toxicity, which could be leveraged to speed up drug development. The other bioactive compounds screened have drug targets and mechanisms of action that may be useful for further studies of disease pathophysiology and for potential drug development.

We identified 319 compounds with activity against SARS-CoV-2 CPE from a qHTS of 8,810 unique compounds. Among the top 56 hits identified with $<10 \mu\text{M}$ EC_{50} values and $>80\%$ efficacies, the anti-SARS-CoV-2 activity of 37 of them has not been reported elsewhere. Of these novel top hits, three were FDA approved drugs with novel anti-SARS-CoV-2 activity. Chlorprothixene is a dopamine receptor antagonist, a classic antipsychotic agent approved for treatment of schizophrenia (Schrijver et al., 2016). Methotrimeprazine, also named as levomepromazine, is another tricyclic antipsychotic agent approved for psychotic disorders including schizophrenia, and manic-depressive syndromes (Sivaraman et al., 2010). Both chlorprothixene and methotrimeprazine were previously found to inhibit the SARS-CoV replication with EC_{50} s around $10 \mu\text{M}$ (Barnard et al., 2008). Piperacetazine is also an older tricyclic antipsychotic drug approved for treatment of schizophrenia (Eslami Shahrabaki et al., 2018). The antiviral effect of piperacetazine was found previously that blocked the Ebola viral entry with the EC_{50} of $9.68 \mu\text{M}$ (Kouznetsova et al., 2019).

We also confirmed the anti-SARS-CoV-2 activity of 5 compounds that were reported as virtual screening hits but had yet to be confirmed experimentally, including methdilazine by an AI prediction algorithm (Grzybowski et al., 2020), GMC 2-113 by a virtual screen of RNA dependent RNA polymerase (RdRP) (Dwivedy et al., 2020), maprotiline by a main protease docking (Chauhan, 2020), deserpidine by a NPS-16 docking (Jiang et al., 2020), and flunarizine by a spike protein docking screen (Chernyshev, 2020). Our data supports the utility of these emerging technologies and the field of AI for advancing drug development.

For *in vitro* screens of antiviral compounds, molecular target (mechanism) based assays and phenotypic assays are two major approaches. Common targets are viral enzymes such as viral protease, DNA and RNA polymerases, reverse transcriptase, and integrase. Development of assays targeting viral enzymes rely on viral enzyme expression, purification, assay development, and validation (Shyr et al., 2020). Alternatively, phenotypic assays involving live virus infections are readily executed once the viruses are isolated from patients and viral replication in appropriate host cells are established. A common live virus infection assay is the measurement of CPE in virus infected host cells. There are two possibilities (fates) for the host cells after viral infection, including cytopathic infection (i.e. death of host cells) and persistent infection (Heaton, 2017). The CPE effect can be readily measured by the ATP content cell viability assay, which is robust and amenable for HTS. Due to the nature of the CPE assay, compounds that suppress CPE can act against any part of the virus infection cycle, including the binding of virus to host cell receptor, entry into host cells, virus replication, viral assembly/budding, and virus reinfection of adjacent cells.

It is worth briefly reflecting on the limitations of the drug repurposing assay approach. A number of small molecules of interest for treating COVID19 that are currently in clinical trials were not hits in our assay. For example, the TMPRSS2 inhibitors camostat and nafamstat are protease inhibitors approved in Japan for treating pancreatitis, and known to inhibit TMPRSS2 (Shrimp et al., 2020). While TMPRSS2 is reported to be a mediator of SARS-CoV-2 cell entry, Vero E6 cells do not express TMPRSS2, so this class of compound are not active in the Vero E6 assay. The drug efflux transporter P-glycoprotein (P-gp) can reduce cellular concentrations of test agents, and as a kidney epithelial cell line, Vero E6 cells likely expresses significant P-gp concentrations, which would reduce activity of P-gp substrates (Robey et al., 2018). Remdesivir itself is a substrate of Pgp (EMA, 2020), and is weaker against SARS-CoV-2 in assays using Vero E6 cells ($EC_{50} > 1 \mu M$) compared with Calu-3 or Huh7 cell lines ($EC_{50} > 50 nM$) (2020). These examples highlight the need for careful interpretation and critical follow-up studies after initial high-throughput screening analyses.

Importantly, the comprehensive primary screen datasets of this study for approved and investigational drugs, and mechanism-based bioactive compounds have been made publicly

available in real-time on the NCATS OpenData Portal

(<https://opendata.ncats.nih.gov/covid19/index.html>) (Brimacombe et al., 2020). These datasets provide a wealth of quality live-virus data that is freely available to the research community for future studies and data mining with the aim of offering a new medicine to treat COVID-19 patients quickly and safely (Huang et al., 2020; Zhu et al., 2020a).

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Author contributions

PS, ZI, RTE prepared the assay ready plates. RB and LR conducted the CPE and cytotoxicity assays. PS, CK-T, KMW and SGM curated the compound libraries. CZC and MDH designed the experiments. CZC, BB, WZ wrote the manuscript. RH, MS, XH, HG, TZ performed data analysis and data uploads. All authors provided critical reading of the manuscript.

Figure legends

Figure 1. Compound library description. (a) By approval status: approved drug (FDA and others), clinical trial, preclinical. (b) By mechanism of action.

Figure 2. Control compounds reproducibility. Concentration response curve fittings for remdesivir in four independent runs for primary screens and hit confirmation. EC₅₀ values of 4.56 μ M, 4.42 μ M, 7.28 μ M, and 5.17 μ M of remdesivir in the CPE assay demonstrate day-to-day reproducibility of the assay.

Figure 3. Compounds concentration response curves in the CPE assay. (a) Autophagy modulators, (b) host protease inhibitors, (c) kinase modulators, (d) opioid receptor modulators, (e) serotonin receptor modulators, (f) histamine receptor modulators, and (g) dopamine and other GPCR receptor modulators. Berzosertib, VPS34-IN1, and STF-62247 showed bell-shaped concentration response due to cytotoxicity. No other compounds caused any reduction in viability in the cytotoxicity assay.

Table 1. Top confirmed anti-SARS-CoV-2 compounds

Sample ID	Sample Name	CPE EC50 (uM)	CPE % Efficacy	Cytotox CC50 (uM)	% Cytotox	Previous reports against CoVs	Approval status	MOA
Viral Target								
NCGC00686694	Remdesivir	10.0	133.1	N/A	<30	Clinical (Beigel et al., 2020)	FDA EUA	RdRP inhibitor
Autophagy Modulators								
NCGC00387732	VPS34-IN1	0.63	103.0	10.0	-76.5	None	Bioactive	Autophagy modulator
NCGC00344081	STF-62247	1.1	107.1	11.2	-56.6	None	Preclinical	Autophagy modulator; Renal cell growth inhibition
NCGC00507892	VPS34 Inhibitor 1	1.4	98.3	N/A	<30	None	Preclinical	Autophagy modulator
GPCR Modulators								
NCGC00346896	MCOPPB	3.5	85.6	N/A	<30	None	Preclinical	ORL1 (OP4, NOP) Agonists
NCGC00370950	GW 803430	3.5	93.3	N/A	<30	None	Bioactive	Melanin-concentrating hormone receptor 1 Antagonist
NCGC00017063	Amodiaquin dihydrochloride	4.0	87.2	N/A	<30	In vitro live virus (Weston et al., 2020)	FDA	Histamine receptor antagonist
NCGC00485045	N-Methylspiperone hydrochloride	4.5	80.0	N/A	<30	None	Clinical trial	Serotonin 2 (5-HT2) receptor Antagonist
NCGC00016710	Clemastine fumarate	7.9	96.0	N/A	<30	Mpro assay (Vatansever et al., 2020)	FDA	Histamine receptor antagonist
NCGC00386477	GMC 2-29	7.9	117.2	N/A	<30	None	Bioactive	5-hydroxytryptamine receptor 1D Antagonist
NCGC00378842	Lu AE58054 hydrochloride	10.0	97.2	N/A	<30	None	Clinical trial	Serotonin 6 (5-HT6) receptor Antagonist
NCGC00013683	Chlorprothixene	10.0	104.4	N/A	<30	None	FDA	Dopamine receptor antagonist
NCGC00014482	Methdilazine hydrochloride	10.0	86.4	N/A	<30	Virtual: AI prediction (Grzybowski et al., 2020)	FDA	Antihistamine
NCGC00179370	Methotrimeprazine maleate	10.0	84.6	N/A	<30	None	FDA	Antagonist for adrenergic, dopamine, histamine, cholinergic and serotonin (5-hydroxytryptamine; 5-HT) receptors
NCGC00016642	Piperacetazine	10.0	103.7	N/A	<30	None	FDA	Dopamine receptor antagonist
NCGC00181913	Difeterol	10.0	113.4	N/A	<30	None	Approved outside of US	Antihistamine
NCGC00386484	(R)-(-)-LY 426965 dihydrochloride	10.0	110.7	N/A	<30	None	Bioactive	Serotonin 2b (5-HT2b) receptor Modulator
NCGC00015608	Loperamide hydrochloride	10.0	98.6	N/A	<30	In vitro live virus (Jeon et al., 2020)	FDA	Opioid receptor agonist
NCGC00485321	Naltrindole isothiocyanate hydrochloride	10.0	114.7	N/A	<30	None	Bioactive	Delta opioid receptor Antagonist
NCGC00165726	AM1241	10.0	97.6	N/A	<30	None	Bioactive	Cannabinoid CB2 receptor Agonist
NCGC00386703	CpdD hydrochloride	10.0	96.9	N/A	<30	None	Bioactive	Ghrelin receptor Antagonist
NCGC00386219	SB 271046 hydrochloride	10.0	107.5	N/A	<30	None	Bioactive	Serotonin 6 (5-HT6) receptor Antagonist
NCGC00386479	GMC 2-113	10.0	129.7	N/A	<30	Virtual: RdRP (Dwivedy et al., 2020)	Bioactive	5-hydroxytryptamine receptor 1D Antagonist
Host Protease Inhibitors								

NCGC00386330	Z-FA-FMK	0.13	104.8	N/A	<30	Mpro assay, in vitro live virus (Zhu et al., 2020b)	Bioactive	Cathepsin L Inhibitor
NCGC00485951	VBY-825	0.14	97.8	N/A	<30	In vitro live virus (Riva et al., 2020)	Clinical trial	Cathepsin S Inhibitor
NCGC00345807	CAA-0225	0.20	99.3	N/A	<30	None	Preclinical	Cathepsin L Inhibitors
NCGC00386232	Cathepsin Inhibitor 1	0.25	114.4	N/A	<30	None	Bioactive	Cathepsin inhibitors
NCGC00163432	Calpeptin	0.50	111.7	N/A	<30	Mpro assay, in vitro live virus (Ma et al., 2020)	Preclinical	Calpain inhibitor
NCGC00485375	Z-Gly-Leu-Phe-chloromethyl ketone	1.3	87.2	N/A	<30	None	Bioactive	Granzyme B Inhibitor
NCGC00371151	Balicatib	2.0	100.3	N/A	<30	None	Clinical trial	Cruzain (Trypanosoma cruzi) Inhibitor
NCGC00161666	Calpain Inhibitor I, ALLN	2.0	111.1	N/A	<30	None	Bioactive	Calpain inhibitor
Kinase Modulators								
NCGC00263093	Apilimod	0.023	104.4	N/A	<30	Pseudovirus assay (Ou et al., 2020)	Clinical trial	IL-12 Production Inhibitor; PIKfyve inhibitor
NCGC00386313	Berzosertib	0.71	87.9	11.2	-98.5	None	Clinical trial	ATR Kinase Inhibitor
NCGC00347280	IKK-2 Inhibitor VIII	7.1	91.7	N/A	<30	None	Preclinical	IKK-2 (IKK-beta) Inhibitor
NCGC00387166	NSC 33994	8.9	107.6	N/A	<30	None	Bioactive	Jak2 Inhibitor
NCGC00159456	Imatinib	10.0	119.0	N/A	<30	Clinical (Morales-Ortega et al., 2020)	FDA	Bcr-Abl kinase inhibitor; KIT inhibitor; PDGFR tyrosine kinase receptor inhibitor
Others								
NCGC00178090	Pristimerin	0.11	87.4	1.1	-93.2	SARS Mpro assay (Ryu et al., 2010)	Preclinical	Monoacylglycerol lipase (MGL) inhibitor
NCGC00385252	alpha-L-Arabinopyranose	2.4	104.0	N/A	<30	None	Bioactive	Induces Pbad promoter expression in E. coli
NCGC00351072	ML414	3.2	79.6	N/A	<30	None	Bioactive	Oligosaccharyltransferase inhibitor
NCGC00379165	IT1t dihydrochloride	3.5	96.3	N/A	<30	None	Bioactive	CXCR4 inhibitor
NCGC00485648	S-15176 difumarate salt	3.8	127.4	N/A	<30	None	Bioactive	Oxidative Stress Inhibitor
NCGC00384450	JTV519 Hemifumarate	5.5	85.7	N/A	<30	None	Clinical trial	Ryanodine receptor (RyR) inhibitor
NCGC00253604	Rescimetol	8.9	81.8	N/A	<30	None	Approved outside of US	Antihypertensive agent
NCGC00164559	Duloxetine hydrochloride	10.0	90.0	N/A	<30	Mpro assay (Vatansever et al., 2020)	FDA	Norepinephrine reuptake inhibitor; Serotonin-norepinephrine reuptake inhibitor (SNRI)
NCGC00181168	Trifluomeprazine 2-butenedioate	10.0	90.2	N/A	<30	None	Bioactive	Antipsychotic Agents
NCGC00169804	Asteriscunolide D	10.0	93.3	N/A	<30	None	Bioactive	Natural product
NCGC00485925	Genz-123346 (free base)	10.0	99.4	N/A	<30	In vitro live virus (Vitner et al., 2020)	Bioactive	Ceramide glucosyltransferase Inhibitor
NCGC00015708	Maprotiline hydrochloride	10.0	103.7	N/A	<30	Virtual: Mpro docking (Chauhan, 2020)	FDA	Norepinephrine reuptake inhibitor; Tricyclic antidepressant
NCGC00168786	Deserpidine	10.0	84.7	N/A	<30	Virtual: NSP16 docking (Jiang et al., 2020)	FDA	Angiotensin converting enzyme inhibitor

NCGC00015096	Amiodarone hydrochloride	10.0	100.5	N/A	<30	Clinical (Castaldo et al., 2020)	FDA	Potassium channel blocker
NCGC00181088	Melitracen hydrochloride	10.0	97.1	N/A	<30	None	Approved outside of US	Antidepressive Agents, Tricyclic;
NCGC00015428	(+/-) - Fluoxetine	10.0	115.8	N/A	<30	In vitro live virus (Zimniak et al., 2020)	FDA	Selective serotonin reuptake inhibitor (SSRI)
NCGC00018102	Flunarizine	10.0	94.1	N/A	<30	Virtual: Spike docking (Chernyshev, 2020)	Approved outside of US	Calcium channel blocker
NCGC00183024	Proglumetacin	10.0	87.6	N/A	<30	None	Approved outside of US	Cyclooxygenase inhibitor
NCGC00378760	DMP 777	10.0	92.5	N/A	<30	None	Clinical trial	Leukocyte elastase Inhibitor
NCGC00476094	Dexanabinol	10.0	110.8	N/A	<30	None	Clinical trial	NMDA antagonist

References

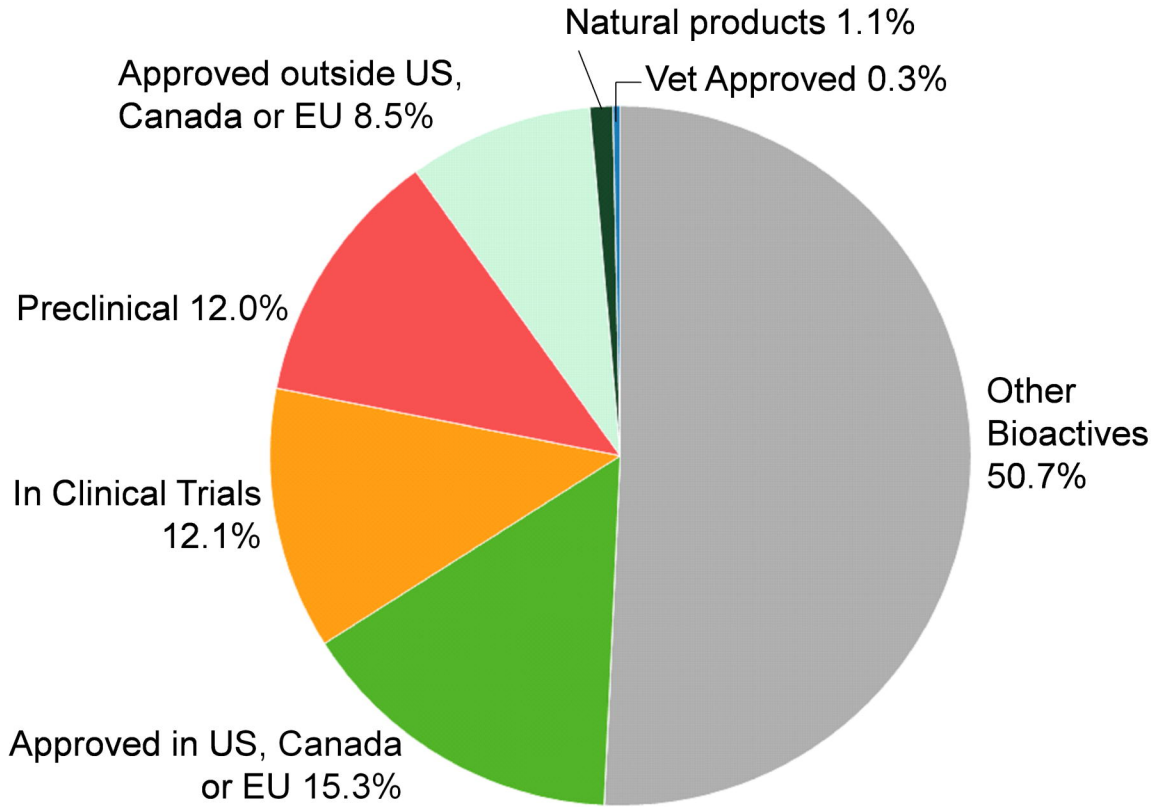
- (2020). *Coronavirus Antiviral Research Database* [Online]. Stanford University. Available: <https://covdb.stanford.edu/search/?compound=Remdesivir&target=Polymerase> [Accessed 5 Aug 2020].
- Barnard, D.L., Day, C.W., Bailey, K., Heiner, M., Montgomery, R., Lauridsen, L., et al. (2008). Is the anti-psychotic, 10-(3-(dimethylamino)propyl)phenothiazine (promazine), a potential drug with which to treat SARS infections? *Antiviral Research* 79(2), 105-113. doi: 10.1016/j.antiviral.2007.12.005.
- Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., Zingman, B.S., Kalil, A.C., et al. (2020). Remdesivir for the Treatment of Covid-19 - Preliminary Report. *N Engl J Med*. doi: 10.1056/NEJMoa2007764.
- Brimacombe, K.R., Zhao, T., Eastman, R.T., Hu, X., Wang, K., Backus, M., et al. (2020). An OpenData portal to share COVID-19 drug repurposing data in real time. *bioRxiv*. doi: 10.1101/2020.06.04.135046.
- Castaldo, N., Aimo, A., Castiglione, V., Padalino, C., Emdin, M., and Tascini, C. (2020). Safety and Efficacy of Amiodarone in a Patient With COVID-19. *JACC: Case Reports* 2(9), 1307-1310. doi: 10.1016/j.jaccas.2020.04.053.
- Chauhan, N. (2020). Possible Drug Candidates for COVID-19. *ChemRxiv*. doi: 10.26434/chemrxiv.11985231.v1.
- Chernyshev, A. (2020). Pharmaceutical Targeting the Envelope Protein of SARS-CoV-2: the Screening for Inhibitors in Approved Drugs. *ChemRxiv*. doi: 10.26434/chemrxiv.12286421.v1.
- Dittmar, M., Lee, J.S., Whig, K., Segrist, E., Li, M., Jurado, K., et al. (2020). Drug repurposing screens reveal FDA approved drugs active against SARS-Cov-2. *bioRxiv*. doi: 10.1101/2020.06.19.161042.
- Dong, E., Du, H., and Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 20(5), 533-534. doi: 10.1016/S1473-3099(20)30120-1.
- Dwivedy, A., Mariadasse, R., Ahmed, M., Kar, D., Jeyakanthan, J., and Biswal, B.K. (2020). In silico characterization of the NiRAN domain of RNA-dependent RNA polymerase provides insights into a potential therapeutic target against SARS-CoV2. *OSF Preprints*. doi: 10.31219/osf.io/wd6zu.
- Eastman, R.T., Roth, J.S., Brimacombe, K.R., Simeonov, A., Shen, M., Patnaik, S., et al. (2020). Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent Sci* 6(5), 672-683. doi: 10.1021/acscentsci.0c00489.
- Ellinger, B., Bojkova, D., Zaliani, A., Cinatl, J., Claussen, C., Westhaus, S., et al. (2020). Identification of inhibitors of SARS-CoV-2 in-vitro cellular toxicity in human (Caco-2) cells using a large scale drug repurposing collection. *Research Square*. doi: 10.21203/rs.3.rs-23951/v1.
- EMA (2020). "Summary on Compassionate Use: Remdesivir Gilead", (ed.) H.M. Division.).
- Eslami Shahrbabaki, M., Dehnavieh, R., Vali, L., and Sharafkhani, R. (2018). Chlorpromazine versus piperacetazine for schizophrenia. *Cochrane Database of Systematic Reviews*. doi: 10.1002/14651858.CD011709.pub2.
- Grzybowski, B., Gryniewicz, G., Szymkuć, S., Molga, K., Wołos, A., Gajewska, E.P., et al. (2020). Suggestions for second-pass anti-COVID-19 drugs based on the Artificial Intelligence measures of molecular similarity, shape and pharmacophore distribution. *ChemRxiv*. doi: 10.26434/chemrxiv.12084690.v2.
- Heaton, N.S. (2017). Revisiting the concept of a cytopathic viral infection. *PLoS Pathog* 13(7), e1006409. doi: 10.1371/journal.ppat.1006409.

- Huang, R., Xu, M., Zhu, H., Chen, C.Z., Lee, E.M., He, S., et al. (2020). Massive-scale biological activity-based modeling identifies novel antiviral leads against SARS-CoV-2. *bioRxiv*. doi: 10.1101/2020.07.27.223578.
- Huang, R., Zhu, H., Shinn, P., Ngan, D., Ye, L., Thakur, A., et al. (2019). The NCATS Pharmaceutical Collection: a 10-year update. *Drug Discov Today* 24(12), 2341-2349. doi: 10.1016/j.drudis.2019.09.019.
- Inglese, J., Auld, D.S., Jadhav, A., Johnson, R.L., Simeonov, A., Yasgar, A., et al. (2006). Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc Natl Acad Sci U S A* 103(31), 11473-11478. doi: 10.1073/pnas.0604348103.
- Jeon, S., Ko, M., Lee, J., Choi, I., Byun, S.Y., Park, S., et al. (2020). Identification of Antiviral Drug Candidates against SARS-CoV-2 from FDA-Approved Drugs. *Antimicrob Agents Chemother* 64(7). doi: 10.1128/AAC.00819-20.
- Jiang, Y., Liu, L., Manning, M., Bonahoom, M., Lotvola, A., and Yang, Z.-Q. (2020). Repurposing Therapeutics to Identify Novel Inhibitors Targeting 2'-O-Ribose Methyltransferase Nsp16 of SARS-CoV-2. *ChemRxiv*. doi: 10.26434/chemrxiv.12252965.v1.
- Kouznetsova, J., Sun, W., Martínez-Romero, C., Tawa, G., Shinn, P., Chen, C.Z., et al. (2019). Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerging Microbes & Infections* 3(1), 1-7. doi: 10.1038/emi.2014.88.
- Lin, G.L., Wilson, K.M., Ceribelli, M., Stanton, B.Z., Woo, P.J., Kreimer, S., et al. (2019). Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening. *Sci Transl Med* 11(519). doi: 10.1126/scitranslmed.aaw0064.
- Ma, C., Sacco, M.D., Hurst, B., Townsend, J.A., Hu, Y., Szeto, T., et al. (2020). Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. *bioRxiv*. doi: 10.1101/2020.04.20.051581.
- Morales-Ortega, A., Bernal-Bello, D., Llerena-Barroso, C., Frutos-Pérez, B., Duarte-Millán, M.Á., García de Viedma-García, V., et al. (2020). Imatinib for COVID-19: A case report. *Clinical Immunology* 218. doi: 10.1016/j.clim.2020.108518.
- Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., et al. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 11(1), 1620. doi: 10.1038/s41467-020-15562-9.
- Riva, L., Yuan, S., Yin, X., Martin-Sancho, L., Matsunaga, N., Pache, L., et al. (2020). Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature*. doi: 10.1038/s41586-020-2577-1.
- Robey, R.W., Pluchino, K.M., Hall, M.D., Fojo, A.T., Bates, S.E., and Gottesman, M.M. (2018). Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* 18(7), 452-464. doi: 10.1038/s41568-018-0005-8.
- Ryu, Y.B., Park, S.-J., Kim, Y.M., Lee, J.-Y., Seo, W.D., Chang, J.S., et al. (2010). SARS-CoV 3CLpro inhibitory effects of quinone-methide triterpenes from *Tripterygium regelii*. *Bioorganic & Medicinal Chemistry Letters* 20(6), 1873-1876. doi: 10.1016/j.bmcl.2010.01.152.
- Schrijver, E.J.M., de Graaf, K., de Vries, O.J., Maier, A.B., and Nanayakkara, P.W.B. (2016). Efficacy and safety of haloperidol for in-hospital delirium prevention and treatment: A systematic review of current evidence. *European Journal of Internal Medicine* 27, 14-23. doi: 10.1016/j.ejim.2015.10.012.
- Shrimp, J.H., Kales, S.C., Sanderson, P.E., Simeonov, A., Shen, M., and Hall, M.D. (2020). An Enzymatic TMPRSS2 Assay for Assessment of Clinical Candidates and Discovery of Inhibitors as Potential Treatment of COVID-19. *bioRxiv*. doi: 10.1101/2020.06.23.167544.

- Shyr, Z.A., Gorshkov, K., Chen, C.Z., and Zheng, W. (2020). Drug discovery strategies for SARS-CoV-2. *J Pharmacol Exp Ther*. doi: 10.1124/jpet.120.000123.
- Sivaraman, P., Rattehalli, R.D., and Jayaram, M.B. (2010). Levomepromazine for schizophrenia. *Cochrane Database of Systematic Reviews*. doi: 10.1002/14651858.CD007779.pub2.
- Touret, F., Gilles, M., Barral, K., Nougairède, A., Decroly, E., de Lamballerie, X., et al. (2020). In vitro screening of a FDA approved chemical library reveals potential inhibitors 1 of SARS-CoV-2 replication. *bioRxiv*. doi: 10.1101/2020.04.03.023846.
- Vatansever, E.C., Yang, K., Kratch, K.C., Drelich, A., Cho, C.-C., Mellot, D.M., et al. (2020). Targeting the SARS-CoV-2 Main Protease to Repurpose Drugs for COVID-19. *bioRxiv*. doi: 10.1101/2020.05.23.112235.
- Vitner, E.B., Avraham, R., Achdout, H., Tamir, H., Agami, A., Cherry, L., et al. (2020). Antiviral activity of Glucosylceramide synthase inhibitors against SARS-CoV-2 and other RNA virus infections. *bioRxiv*. doi: 10.1101/2020.05.18.103283.
- Wang, Y., Jadhav, A., Southal, N., Huang, R., and Nguyen, D.T. (2010). A grid algorithm for high throughput fitting of dose-response curve data. *Curr Chem Genomics* 4, 57-66. doi: 10.2174/1875397301004010057.
- Weston, S., Coleman, C.M., Haupt, R., Logue, J., Matthews, K., and Frieman, M.B. (2020). Broad anti-coronaviral activity of FDA approved drugs against SARS-CoV-2 in vitro and SARS-CoV in vivo. *bioRxiv*. doi: 10.1101/2020.03.25.008482.
- Zhu, H., Chen, C.Z., Sakamuru, S., Simeonov, A., Hall, M.D., Xia, M., et al. (2020a). Mining of high throughput screening database reveals AP-1 and autophagy pathways as potential targets for COVID-19 therapeutics. *arXiv* 2007.12242v1.
- Zhu, W., Xu, M., Chen, C.Z., Guo, H., Shen, M., Hu, X., et al. (2020b). Identification of SARS-CoV-2 3CL Protease Inhibitors by a Quantitative High-throughput Screening. *bioRxiv*. doi: 10.1101/2020.07.17.207019.
- Zimniak, M., Kirschner, L., Hilpert, H., Seibel, J., and Bodem, J. (2020). The serotonin reuptake inhibitor Fluoxetine inhibits SARS-CoV-2. *bioRxiv*. doi: 10.1101/2020.06.14.150490.

Figure 1

A



B

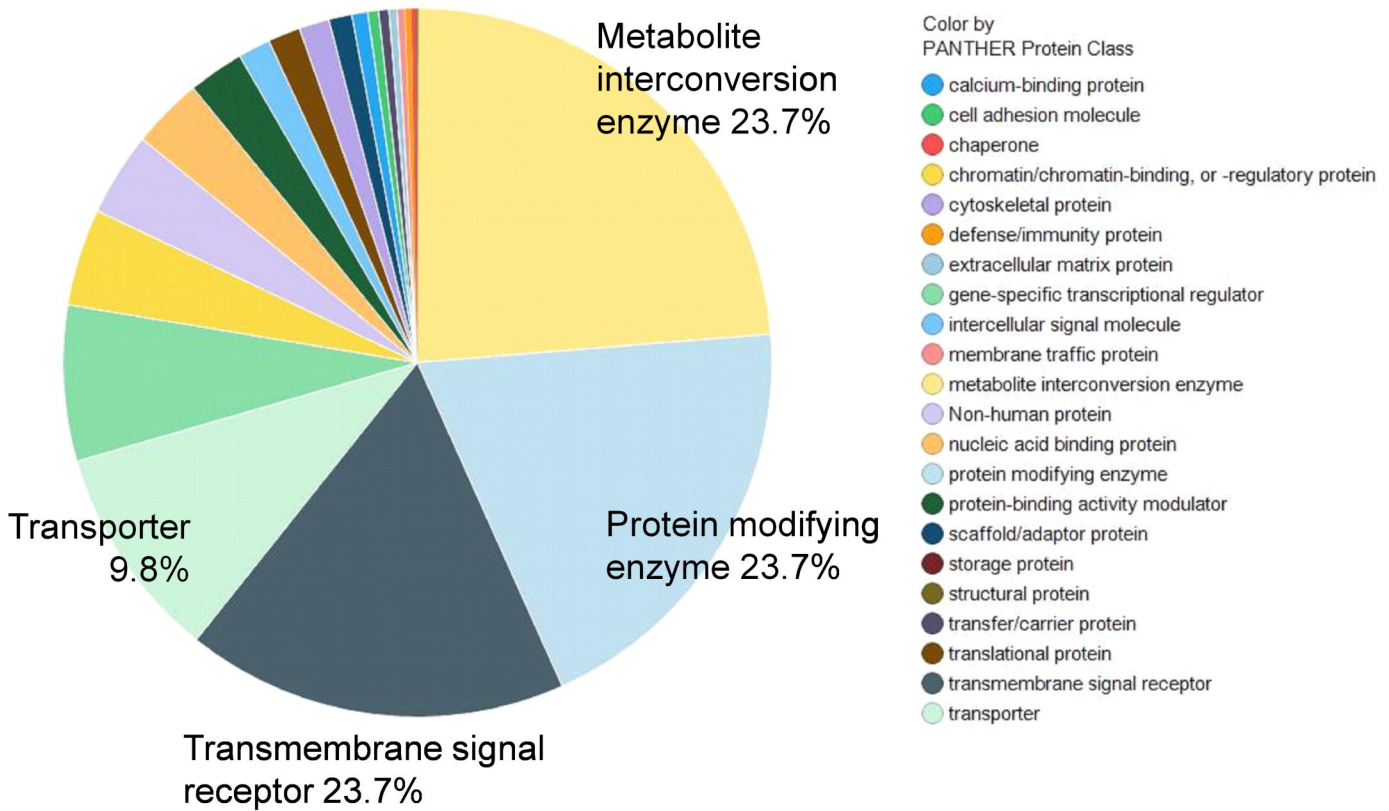
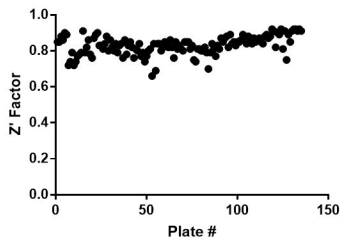


Figure 2

a. Assay plate statistics



b. Control compound reproducibility

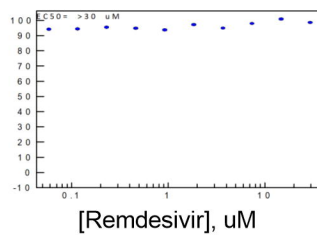
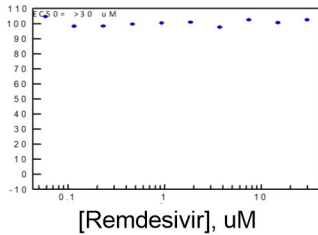
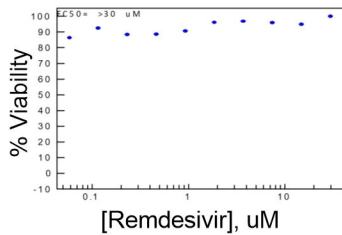
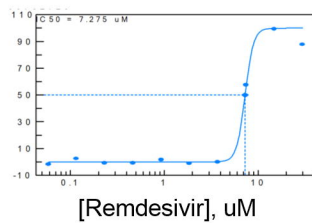
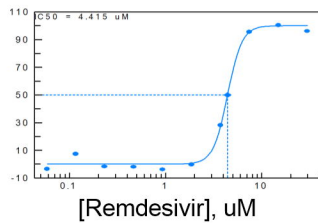
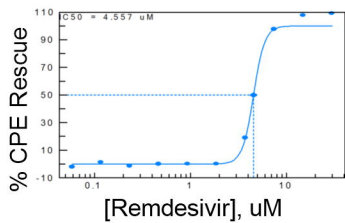
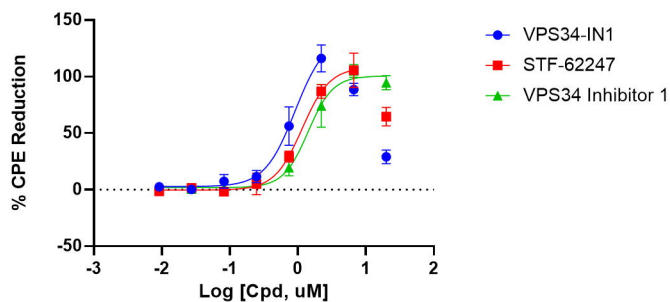
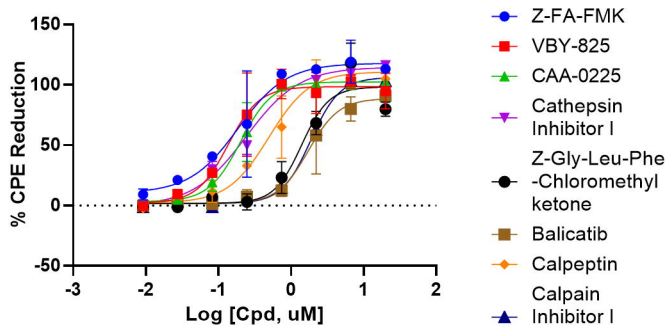


Figure 3

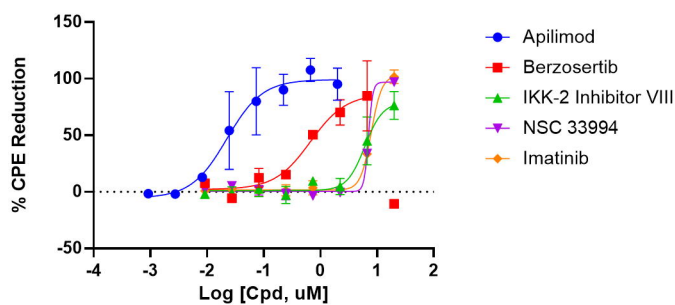
a. Autophagy modulators



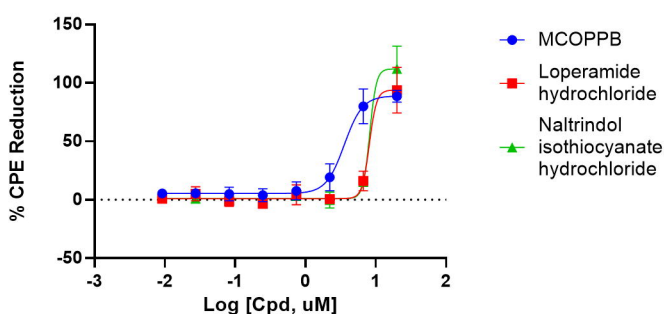
b. Host protease inhibitors



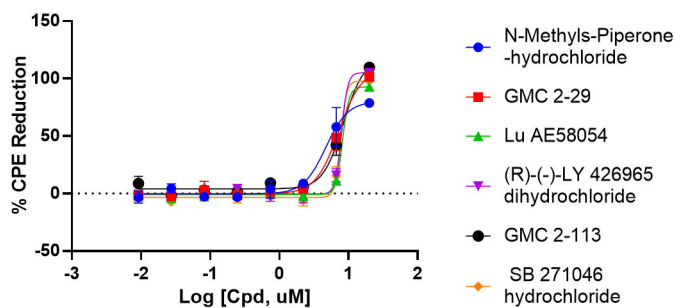
c. Kinase modulators



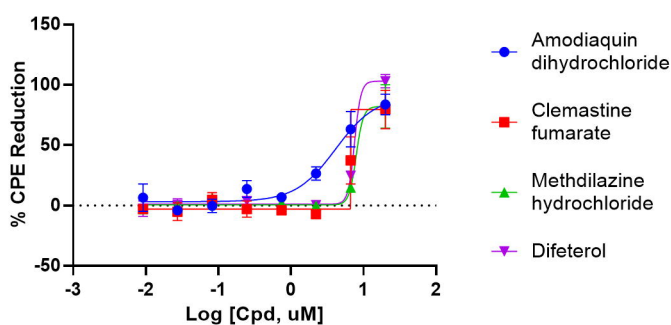
d. GPCR modulators: Opioid receptors



e. GPCR modulators: Serotonin receptors



f. GPCR modulators: Histamine receptors



g. GPCR modulators: Dopamine receptor and others

