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Repurposing drugs as COVID-19 therapies: A toxicity evaluation

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Drug repurposing is an appealing method to address the Coronavirus 2019 (COVID-19) pandemic because of the low cost and efficiency. We analyzed our in-house database of approved drug screens and compared their activity profiles with results from a severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) cytopathic effect (CPE) assay. The activity profiles of the human ether-à-go-go-related gene (hERG), phospholipidosis (PLD), and many cytotoxicity screens were found significantly correlated with anti-SARS-CoV-2 activity. hERG inhibition is a nonspecific off-target effect that has contributed to promiscuous drug interactions, whereas drug-induced PLD is an undesirable effect linked to hERG blockers. Thus, this study identifies preferred drug candidates as well as chemical structures that should be avoided because of their potential to induce toxicity. Lastly, we highlight the hERG liability of anti-SARS-CoV-2 drugs currently enrolled in clinical trials.

Keywords: Drug repurposing; SARS-CoV-2; COVID-19; hERG; Phospholipidosis; Autophagy; Cytotoxicity; High-throughput screening; In vitro assay

Introduction

Since the emergence of the new coronavirus SARS-CoV-2 in December 2019 in Wuhan, China, there has been a grave need for the development of a treatment for COVID-19. As of January 2022, the WHO estimated the number of confirmed cases of COVID-19 to be ~54 million in the USA and 290 million globally, and this number continues to rise. There is currently only one US Food and Drug Administration (FDA)-approved treatment (remdesivir) for SARS-CoV-2 infection. Many additional potential treatments are being studied; however, they must pass crucial toxicity and efficacy hurdles in clinical trials before becoming available on the consumer market as treatment. Therapeutic development is a costly and time-consuming process and, thus, researchers are turning to a different approach to address the SARS-CoV-2 pandemic. Given that many existing drugs and drug candidates can be repurposed for use in a new disease indication, this approach is a more feasible option to treat patients with COVID-19 because these therapeutics have already cleared several key hurdles along the drug development pipeline. By repurposing drugs, scientists can meet the demands of new treatments in a timely manner and accelerate the clinical translation from basic research to therapeutic interventions.

The NCATS Pharmaceutical Collection (NPC) is a collection of nearly 3000 small-molecule drugs that have been approved for clinical use or investigational purposes by the FDA, European (European Medicines Agency; EMA), Japanese (Pharmaceuticals and Medical Devices Agency; PMDA), Australian, and Canadian authorities.[1,2] The NPC library was specifically created to enable drug repurposing and has been screened against nearly 1000 *in vitro* assays in a quantitative high-throughput screening (qHTS) format, encompassing a wide range of targets and pathways related to diseases and/or toxicity.[3–6] In an effort to identify repurposed antiviral drugs, a phenotypic assay was used to measure the CPE of SARS-CoV-2 on Vero E6 cells infected for 72 h.[7] The primary screening of the NPC with this SARS-CoV-2 CPE assay revealed 319 hits[7] with confirmed anti-SARS-CoV-2 activ-

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1359-6446/Published by Elsevier Ltd.
https://doi.org/10.1016/j.drudis.2022.04.001

ity; the related primary screening data are available from the NCATS OpenData Portal (https://opendata.ncats.nih.gov/-covid19/index.html).[8]

COVID-19 is often presented as an acute respiratory infection; however, the disease can also cause damage to multiple organ systems, including heart, lung, and blood. Thus, serious safety issues regarding heart rhythm problems as well as blood and lymph system disorders, kidney injuries, and liver complications must be considered when developing treatments for this coronavirus. To assess the potential toxic side effects of anti-SARS-CoV-2 compounds and better understand their antiviral mechanisms, we compared the SARS-CoV-2 CPE screening data with the NPC qHTS data against other targets and pathways via a correlation analysis to identify assays with activity patterns similar to that of the SARS-CoV-2 CPE assay.[9] Here, we review the activities of the anti-SARS-CoV-2 compounds identified from both the SARS-CoV-2 CPE assay and literature in a panel of other assays that were found to be significantly correlated with the CPE assay, including several toxicity-related assays. The goals of this study were to: assess the toxicity potential of the compounds using the results from the aforementioned assays; characterize the structural features of compounds with different activity profiles; and identify repurposing candidates with minimal toxicity liabilities. We evaluated the effectiveness of these drugs against SARS-CoV-2 and examined the pathways and mechanisms of action that can be further explored when developing new treatments.

Mining qHTS data reveals toxicity concerns for anti-SARS-CoV-2 compounds

For activity profile analysis, compound activity was represented by 'curve rank',[10,11] a numeric measure between –9 and 9 based on potency, efficacy, and the quality of the concentration–response curve, such that a large positive number indicates a strong activator, a large negative number indicates a strong inhibitor, and 0 means inactive. Activity profile similarity between the SARS-CoV-2 CPE assay and an NPC screen against another target or pathway was measured by the Pearson correlation coefficient (r) with a P-value calculated for the significance of correlation.[9] Several toxicity-related assays were found to be significantly correlated with the CPE assay, including a hERG assay ($r = 0.36$, $P < 1 \times 10^{-20}$),[12,13] a PLD assay ($r = 0.34$, $P = 1.0\ 2 \times 10^{-14}$),[12–15] and 39 cell viability assays ($P < 0.01$; Table S1 in the supplemental information online).[16–19] Drug-induced blockage of the hERG channel can lead to QT interval prolongation and torsades de pointes (TdP), a potentially lethal ventricular tachyarrhythmia, and these adversities have been obstacles in drug development.[20] Therefore, it is crucial to highlight the hERG liability of compounds early on during the preclinical phase of drug discovery. Drug-induced PLD is a lysosomal storage disorder characterized by the accumulation of phospholipids.[21] It is primarily the result of an inhibition of lysosomal phospholipase activity by the drug.[22] Although there is still no clear evidence that PLD has significant toxicological effects in animals or humans, several research groups have found that the PLD

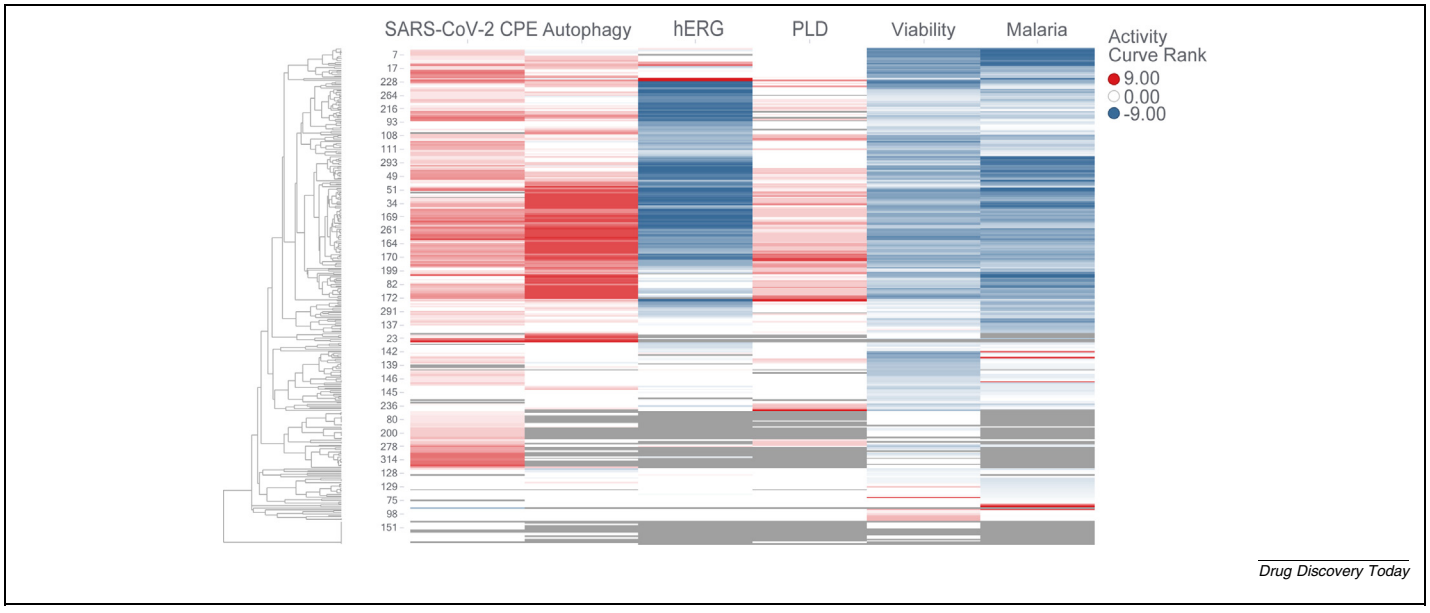

FIG. 1

Activity profiles of the 331 potential anti-severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) compounds in the NCATS Pharmaceutical Collection (NPC). The activity profile of the compounds in the CPE assay was found to correlate significantly with the compound activity profiles in a panel of assays, including autophagy, human ether-à-go-go-related gene (hERG), phospholipidosis (PLD), cell viability (39 assays), and malaria (30 parasite strains) assays. In the heat map, each row is a compound and each column is an assay. The heat map is colored by 'curve rank',[10,11] a numeric measure (between –9 and 9) of compound activity based on potency, efficacy, and the quality of the concentration–response curve, such that a large positive number indicates a strong activator (red), a large negative number indicates a strong inhibitor (blue), and 0 means inactive (light gray). Dark gray indicates missing data. The cell viability assay column shows the average curve rank from 39 assays with different assay conditions and/or cell types, and the malaria assay column shows the average curve rank from 30 assays using different parasite strains. Compound clustering was performed in TIBCO® Spotfire® Analyst 7.11.1.

inducer, gentamicin, causes renal tubular toxicity in the affected tissue.[23,24] Another PLD inducer, amiodarone, was reported to induce liver cirrhosis accompanying PLD in a patient with long-term administration of a daily low dose of amiodarone. Amiodarone-induced pulmonary toxicity in rats might be linked to the induction of PLD, suggesting that drug-induced PLD has potential adverse effects.[25] Although the toxicological significance of PLD is still under investigation, additional information on the PLD potential of drugs is often requested by the FDA during the drug development process.[26] Cell viability is a readout that assesses the integrity of the cells in which a loss in signal is attributed to cell death or cytotoxicity. The cell viability assay is often conducted as a counter-screen to flag potential cytotoxic compounds.[10] Thus, the significant correlations observed between the CPE assay and hERG, PLD, and many cell viability assays raise concerns regarding the toxicity potentials of the anti-SARS-CoV-2 drugs. The activity profiles of the 331 potential anti-SARS-CoV-2 compounds across the CPE and other related assays are shown in Fig. 1, with details provided in Table S2 in the supplemental information online. To further analyze the structure–activity relationship of these 331 compounds, they were clustered based on structural similarity (ECFP4 fingerprints) resulting in a total of 50 clusters and 25 singletons (Fig. 2). A representative structure for each cluster was selected and can be observed in Table S3 in the supplemental information online.

Anti-SARS-CoV-2 compounds exhibit different levels of hERG liability.

hERG inhibition has become the single most-frequent cause for drug withdrawals; thus, this liability is a crucial concern for potential drug candidates, especially anti-COVID-19 treatments, because of the cardiac complications frequently observed in patients with COVID-19. The NPC contains old drugs (approved before 2000) that might have inhibitory activity against the hERG channel, although the FDA started to regulate hERG channel toxicity in drug development during the early 2000s.[27,28] To assess the hERG liability of the potential anti-SARS-CoV-2 com-

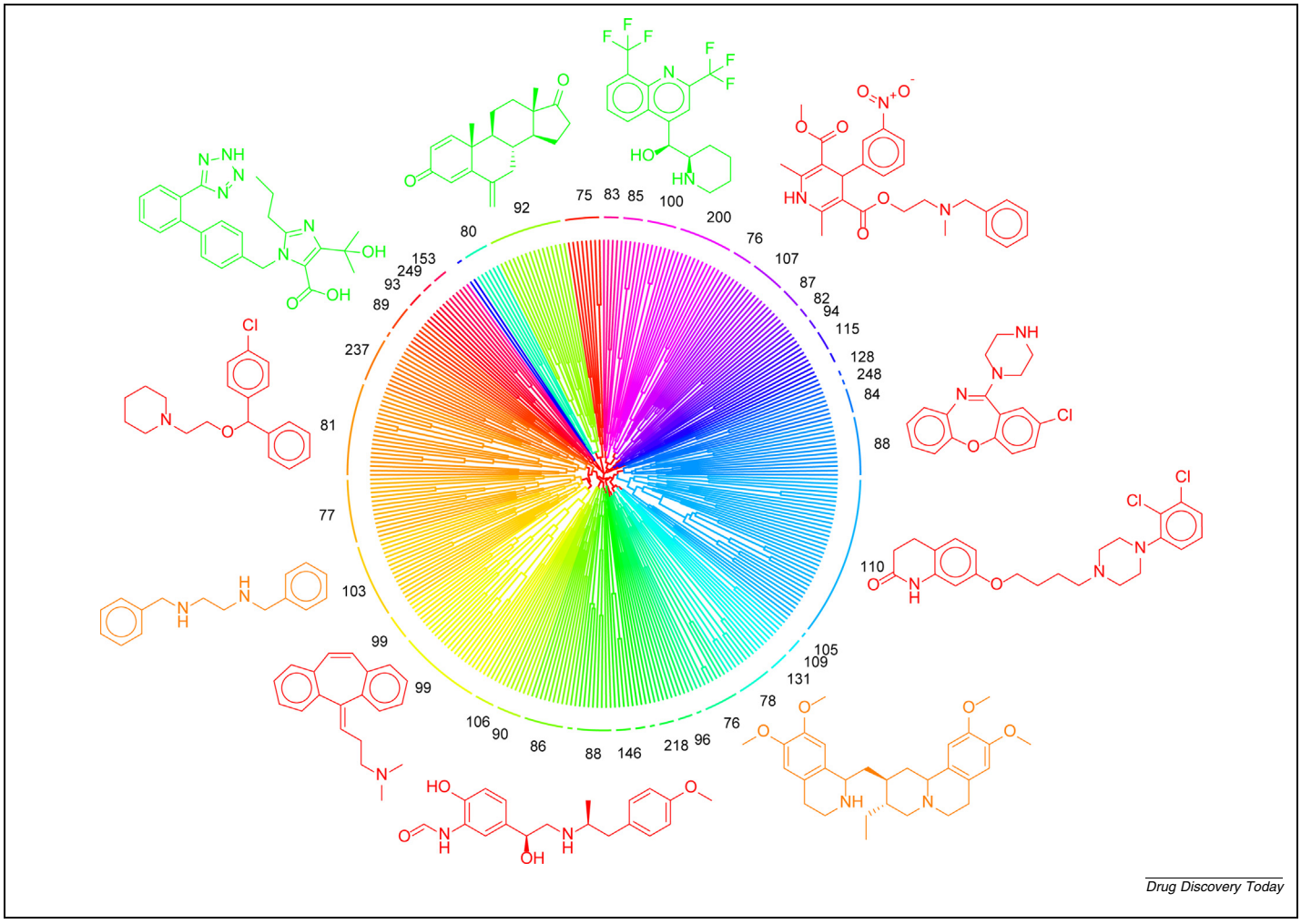

FIG. 2

Clustering of the 331 potential anti-severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) compounds based on structure similarity. Compound structures were converted to ECFP4 fingerprints (1024-bit). Hierarchical clustering was conducted using the R base stats package with binary distance and the complete linkage method. Visualization of the results was performed using the ggplot2 package in R. In the dendrogram, different clusters are represented by different colors. Representative structures are also shown for example clusters. Structures are colored by the level of hERG liability, with red indicating high liability, orange indicating moderate liability, and green indicating low liability.

pounds, we grouped the compounds into three tiers of hERG liability based on their activity observed in the hERG inhibition assay[12,29] (Table S2 in the supplemental information online): low (curve rank < 1.0), medium (curve rank between 1.0 and 4.0), and high (curve rank > 4.0), and evaluated the compounds that corresponded to each category. We observed a range of drugs (118 compounds from ten clusters) that exhibited low hERG liability, including those that are intended to treat high blood pressure, Alzheimer's disease, viral infection, and depression/nerve pain. These drugs act by inhibiting selected targets to provide therapeutic relief for its indented areas. Drugs with moderate hERG liability (33 compounds from 12 clusters) were found to overlap with the categories previously listed and also included antiparasitic and antihistaminic drugs. High hERG liability compounds (107 compounds from 25 clusters) tended to focus on old-generation antihistamines and antidepressants, which inhibited serotonin, norepinephrine, and dopamine reuptake. To further examine the hERG liability of compounds from different structure classes, curve ranks from the hERG assay were averaged for each compound cluster to produce a hERG liability score.

Ten out of the 50 clusters were considered to have high hERG liability (curve rank > 4.0), including clusters 74, 77, 81, 86, 88, 99, 107, 110, 124, and 200 (Fig. 2). Compounds that exhibited high hERG liability were found mostly to comprise old-generation antihistamine, antipsychotic, antidepressant, and antihypertensive drugs with well-established cardiovascular toxicities. For example, drugs in clusters 88, 99, and 110 are used to treat psychotic behavior, including schizophrenia (e.g., olanzapine). Cluster 200 contains various calcium channel blockers (e.g., cilnidipine) that are intended to treat cardiovascular conditions. Although many calcium channel blockers exhibit inhibitory activity against hERG channels, they usually do not have clinical cardiac symptoms of QT prolongation, as explained by the inhibition of the L-calcium channel that reduces intracellular calcium and, thus, compensates for hERG channel inhibitory activity *in vivo*.[30,31] Given that the long-term cardiovascular effects of calcium channel blockers *in vivo* remain unknown, caution must be exercised with administration of these drugs.[32] Twelve out of the 50 clusters were found to have moderate hERG liability (curve rank between 1.0 and 4.0), including clusters 78, 85, 96, 100, 103, 104, 109, 122, 172, 184, 218, and 248 (Fig. 2). Most compounds that demonstrated moderate hERG liability exhibited antipsychotic and antihypertensive properties and are intended to treat malaria, Alzheimer's disease, and fungal infections. For example, clusters 85 and 184 mainly contain antiviral drugs that disrupt DNA replication by inhibiting key proteins essential for viral infection (e.g., chloroquine). Cluster 78 contains drugs with a range of physiological effects, including antiviral, anti-inflammatory, and antihypertensive properties (e.g., tetrandrine). Clusters 100, 109, and 218 comprise antifungal drugs that have additional antibacterial and anti-inflammatory activities (e.g., ciclopirox). Twenty-five out of the 50 clusters exhibited low hERG liability (curve rank < 1.0) (75, 76, 79, 80, 82–84, 87, 89, 90, 92–94, 105, 106, 114, 115, 128, 131, 135, 145, 146, 153, 237, and 249) (Fig. 2). Most of the compounds with low hERG liability treat bacterial and viral infections, cardiovascular conditions, and cancer. A prior study suggested that some antibiotic compounds exhibit hERG channel blockage and can lead to cardiac toxicity.[33] Clusters 80, 84, 90, and 153 comprised antineoplastic agents that are used to treat various types of cancer (e.g., lung, skin, and breast) by inhibiting the proliferation of cancer cells (e.g., masitinib). Drugs in clusters 75, 82, 89, 106, and 135 include antibacterial agents that are used to treat a variety of bacterial, fungal, and parasitic infections for both human and veterinary uses (e.g., azithromycin). Clusters 93, 94, 105, and 115 contain antiviral agents used to treat viral infections, with some agents exhibiting antiretroviral and antimalarial properties that can be used in combination with other drugs to treat HIV infection and malaria, respectively (e.g., efavirenz).

Minimizing toxicity in anti-COVID-19 drug development

The COVID-19 pandemic has presented an urgent need to pursue rapid development of new and repurposed drugs for treatment. Since the initial outbreak of SARS-CoV-2 in late 2019, repurposing existing and clinical investigation drugs has become an appealing method to combat this virus because there have been hundreds of clinical trials for COVID-19 treatment using repurposed drugs.[34] This approach uses known safety profiles of drugs and allows for the rapid transition into the drug development pipeline, because approved drugs have already passed the safety and efficacy tests required by the FDA approval process. In an effort to address these urgent health needs, we explored the activity of 331 compounds from the NPC that showed anti-SARS-CoV-2 activities and evaluated their safety profiles in regard to hERG inhibition, PLD induction, and cytotoxicity. Here, we further summarize drug clusters to avoid and make recommendations on other drugs that are desirable candidates for COVID-19 therapies. Table 1 provides a comprehensive list of drug clusters, drug classes, and drug examples categorized by hERG liability (high, moderate, and low). The analysis presented here can lead to more in-depth insights into drug properties related to mechanisms of action, pharmacokinetics, and toxicity.

In an effort to better identify compounds that are most and least likely to cause cardiotoxicity via hERG channel blockage, we analyzed the hERG inhibition activity of 331 potential anti-SARS-CoV-2 compounds identified from drug repurposing screens based on their structural class and grouped the classes into three categories: high, moderate, and low hERG liability. Drugs that exhibited high hERG liability were commonly found to be antihistamines, antipsychotics, and antihypertensive drugs based on our analysis. Several antihistamines (astemizole, cluster 81; diphenhydramine, cluster 107) and antipsychotics drugs (mesoridazine and chlorpromazine, cluster 99; haloperidol, cluster 110) have known TdP risks; thus, it is plausible that these compounds were classified as high hERG liability based on our analysis.[35,36] In fact, astemizole was found to cause fatal arrythmias in high doses or in combination with certain other common drugs, and it was withdrawn from the market in several countries during the late 1990s for this very reason.[37] PLD-inducing drugs share similar molecular properties found in cationic amphiphilic drugs (CADs). They have been identified in clin-

ical applications, including antidepressants, antipsychotics, antibiotics, antihistaminic, antiarrhythmics, and antimalarial drugs.[38] Both drug-induced PLD and hERG blockage are adverse effects of drugs. We further examined the PLD induction potential of the high hERG-liability drug clusters and found that most of the drugs in these clusters also showed various levels of PLD induction (Table S2 in the supplemental information online), consistent with previous findings that many hERG inhibitors are also PLD inducers.[12,13] For these reasons, caution must be exercised when considering such drug candidates as potential COVID-19 treatments.

Compounds that showed moderate hERG liability comprised drugs used to treat malaria, Alzheimer's disease, and fungal infections that also exhibit antipsychotic and antihypertensive properties. Although several antimalarial drugs are known to induce cardiotoxicity associated with QT prolongation, there is no clear relationship between the potency of a hERG inhibitor and the likelihood of prolonging the QT interval and inducing TdP; thus, it is generally accepted to consider a moderate drug-induced hERG liability as a risk factor.[39] Approximately 50% of the moderate hERG liability compound clusters showed a certain level of PLD induction, although lower PLD activity was observed compared with high hERG liability compound clusters.

Drugs that exhibited low hERG liability tend to be those that treat bacterial and viral infections as well as cardiovascular conditions, such as arrhythmias and high blood pressure. Additionally, this group includes antineoplastic agents used to treat various types of cancer. Changes in the expression of voltage-sensitive channels, such as hERG, have been reported in cancer; thus, inhibition of hERG channels could serve as an important target in the interference of cancer progression.[40,41] Overall, ~20% of drugs with low hERG liability also showed low PLD activity. Given that hERG liability decreases among the drug clusters, PLD activity also tends to decrease, suggesting a correlation between hERG inhibition and PLD induction. Thus, the drug classes that showed low hERG liability, which also exhibited low PLD induction potential, should be prioritized for further analysis as potential COVID-19 therapies.

The role of autophagy in the antiviral mechanism and toxicity potential of anti-SAR-CoV-2 compounds

We previously found a significant correlation between the activity profiles of the SARS-CoV-2 CPE assay and an autophagy assay.[9] That is, compounds (57%; 127 out of 221) that were active in the CPE assay were likely to exhibit their anti-SARS-CoV-2 activity through autophagy (Fig. 1), a cellular stress response that is responsible for the removal of cellular waste material by lysosomes.[42] Autophagy is a catabolic pathway that is commonly involved in the antiviral response during viral infection. As a result, autophagy has a central role in the immune response at multiple levels. Chloroquine and hydroxychloroquine are antimalarial drugs that are enrolled in clinical trials as potential anti-COVID-19 treatments.[43,44] These drugs also inhibit autophagy by preventing the fusion of the autophagosome with lysosome and deacidifying the lysosome.[45] Malaria is an infectious disease that is often treated with drugs that target the autophagy mechanism. The NPC was previously screened against over 60 different malarial parasites.[46] Interestingly, the activity profiles from 30 of these parasite strains were found to be significantly correlated with the NPC activity profile of the SARS-CoV-2 assay ($P < 10^{-20}$) (Fig. 1; Table S1 in the supplemental information online). In fact, 68% (226 out of 331) of the anti-SARS-CoV-2 compounds showed inhibition in at least one of the 30 malaria assays (Table S1 in the supplemental information online). As such, other antimalarial drugs could also be repurposed as anti-SARS-CoV-2 treatments via the common autophagy mechanism.

The correlations between the activity profiles of the SARS-CoV-2 CPE assay and the autophagy, hERG, and PLD assays indicate that autophagy modulators are also likely to be hERG inhibitors and/or PLD inducers and might further result in cytotoxicity. An examination of the 153 autophagy modulators in the set of anti-SARS-CoV-2 compounds revealed that 77% inhibited hERG, 59% induced PLD, and 93% reduced cell viability (Fig. 1; Table S2 in the supplemental information online). Linkages between hERG and PLD have been reported previously through shared pharmacophores.[12,13] Many CADs with a shared cationic amphiphilic structure[14] have the capacity to induce PLD accumulation and also tend to block hERG.[47] In our study, compounds with such chemotypes could also act as autophagy modulators. Moreover, autophagy and hERG inhibition appear to be linked biologically according to previous findings.[48] Autophagy is a crucial cellular mechanism that supports the replenishment of primary biomolecules, but it can also activate a cell death pathway. In melanoma cell lines, the stimulation of the hERG channel was found to induce autophagy via activation of an AMPK-dependent signaling pathway.[48]

Taken together, autophagy appears to be a double-edged sword: it is a common mechanism for anti-infective drugs and can be exploited for anti-COVID-19 and antimalarial treatments, while, at the same time, compounds that target autophagy could result in hERG inhibition and/or PLD induction, leading to adverse effects (Fig. 3). Thus, for safer drug development, it is important to select autophagy modulators without the undesirable hERG/PLD features or identify compounds that inhibit SARS-CoV-2 through other targets or mechanisms. The clinical application of potential anti-SARS-CoV-2 drugs relies on a more in-depth understanding of the interplay between the autophagy mechanism and hERG and PLD interference.

Toxicity potential of anti-SARS-CoV-2 drugs in clinical trials

Several of the drugs investigated in this study are enrolled in worldwide clinical trials as possible treatments for SARS-CoV-2 (Table 2). Remdesivir, an antiviral drug initially used to treat the Ebola virus, and hydroxychloroquine (moderate hERG liability, cluster 85) or chloroquine (moderate hERG liability, cluster 85), immunosuppressant and antiparasitic drugs, respectively, are being tested among other compounds as a treatment and have been reported to result in significant effects in the control of SARS-CoV-2 infection.[49] However, the risks and benefits of these compounds in patients with varying levels of COVID-19 symptoms remain uncertain and several clinical trials (e.g., NCT04349410, Phase II/III) are underway to thoroughly evaluate

the toxicity potential of these drugs.[50] Ivermectin (moderate hERG liability; cluster 75) is an antiparasitic drug used to treat intestinal strongyloidiasis and onchocerciasis, two conditions caused by parasitic worms.[51] Recent research has shown ivermectin to reduce the replication of SARS-CoV-2 *in vitro*; however, these findings warrant further investigation to better predict the clinical efficacy and utility of ivermectin in patients with SARS-CoV-2.[52,53] Several clinical trials (e.g., NCT04374019, Phase II) are underway to look at ivermectin as a complementary drug along with other interventions in patients with COVID-19. Famotidine (low hERG liability; singleton) is an antihistamine and antacid that is used to treat a variety of gastrointestinal disorders, including gastric or duodenal ulcer, gastroesophageal reflux disease, and pathological hypersecretory conditions. The use of famotidine has been associated with improved clinical outcomes in patients hospitalized with COVID-19.[54] These findings are observational and suggest that randomized controlled trials (e.g., NCT04370262, Phase III) be carried out to reach a definitive conclusion on the potential of this drug to treat COVID-19.

Furthermore, imatinib (low hERG liability; cluster 84) is a tyrosine kinase inhibitor and antineoplastic agent that is used to treat certain types of cancer. Imatinib has shown *in vitro* antiviral activity against SARS-CoV-1 and Middle East respiratory syn-

TABLE 1

Drug clusters, classes, and examples categorized by hERG liability (high, moderate, and low).[a]

hERG liability	Cluster no.	Drug class	Drug example
High	74	Antimalarial, antianginal	Mefloquine hydrochloride, perhexiline
	77	Antiarrhythmic, antidepressant	Propafenone hydrochloride, bifemelane
	81	Antihistamine	Astemizole
	86	Vasopressor	Dopamine hydrochloride
	88	Antipsychotic	Olanzapine
	99	Antipsychotic, antihistamine	Desipramine hydrochloride, promethazine
	107	Vasodilator, antihistamine	Nafronyl oxalate, diphenhydramine hydrochloride
	110	Antipsychotic	Opipramol dihydrochloride
	124	Antifungal, gastrointestinal	Omoconazole, sofalcone
	200	Calcium channel blocker	Cilnidipine
Moderate	78	Antihypertensive	Reserpine
	85	Antimalarial	Chloroquine
	96	Iron chelator	Deferasirox
	100	Antifungal, antineoplastic	Lufenuron, lapatinib
	103	Antihepatitic, anesthetic	N,N′-Dibenzylethane-1,2-diamine dihydrochloride, oxethazaine
	104	Dopamine promoter	Pergolide methanesulfonate, cabergoline
	109	Antifungal	Terconazole
	122	Anesthetic	Dyclonine hydrochloride
	172	Cholinesterase inhibitor, antipsychotic	Tacrine hydrochloride, blonanserin
	184	Antiviral	Darunavir
	218	Antifungal	Ciclopirox
	248	Mucolytic	Ambroxol hydrochloride
Low	75	Antibacterial	Azithromycin
	76	Antispasmodic, anticholinergic	Oxybutynin chloride
	79	Diuretic, antiparasitic, antineoplastic	Triamterene, pyrimethamine, 6-thioguanine
	80	Antineoplastic, antifungal	Parthenolide, siccanin
	82	Coccidiostat	Diclazuril
	83	Antiarrhythmic	Protionamide
	84	Antineoplastic	Masitinib
	87	Antisecretory	Omeprazole
	89	Antibacterial	Enoxacin
	90	Laxative	Oxyphenisatin
	92	Steroid	Pregnenolone
	93	Immunosuppressive	Mycophenolic acid
	94	Antiviral	Ribavirin
	105	HIV antiviral	Efavirenz
	106	Antiparasitic	Nitazoxanide
	114	Antihypertensive	Fenoldopam
	115	Antimalarial	Sulfadoxine
	128	Antidepressants	Idazoxan hydrochloride
	131	Analgesic	Meptazinol hydrochloride
	135	Antibacterial	Cefamandole sodium
	146	Antibacterial	Chloroxine
	145	Anti-inflammatory	Anthralin
	153	Antineoplastic	Bexarotene
	237	Antihypertensive	Losartan
	249	Retinoid	Isotretinoin

[a] Scores > 4 were designated as high hERG liability, scores between 1 and 4 were assigned as moderate hERG liability, and scores < 1 were considered to be low hERG liability.

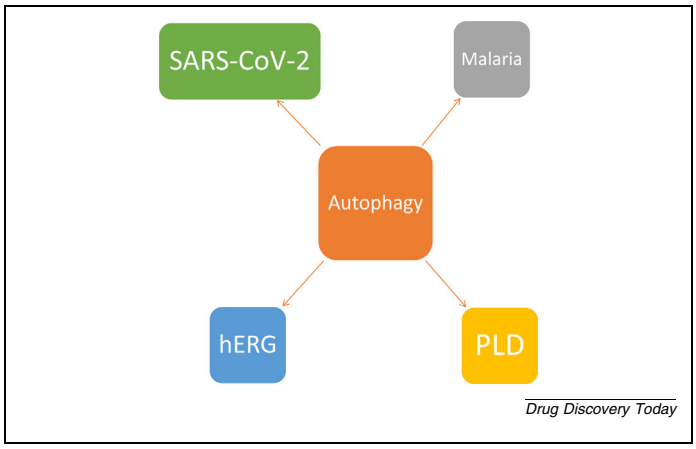

FIG. 3

The role of autophagy in antiviral mechanisms and potential toxic effects. The compound activity profile of autophagy was found to be significantly correlated with those of anti-severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), malaria, human ether-à-go-go-related gene (hERG), and phospholipidosis (PLD). These findings suggest that autophagy has a role in the antiviral mechanisms of anti-SARS-CoV-2 and antimalarial compounds, and that antiviral compounds that act through the autophagy mechanism could result in toxicities via hERG inhibition and/or PLD induction.

drome (MERS)-CoV, which is related to SARS-CoV-2; thus, it has been postulated that this drug could be an unexplored treatment for SARS-CoV-2 infection.[55,56] Several clinical trials (e.g., NCT04394416, Phase III) are underway to evaluate the safety and efficacy of imatinib for the treatment of SARS-CoV-2. Similarly, chlorpromazine (high hERG liability, cluster 99) has been shown to inhibit *in vitro* viral replication of SARS-CoV-1 and MERS-CoV.[57] Several clinical trials (e.g., NCT04366739, Phase III) have begun to assess the antiviral action of chlorpromazine on patients with SARS-CoV-2. Favipiravir (low hERG liability, cluster 94) is an RNA-dependent RNA polymerase (RdRp) inhibitor that is active against a range of RNA-based viruses and is currently only approved in Japan for the treatment of the emerging influenza. Favipiravir was not tested in our hERG or PLD assays and it exhibited no activity in the SARS-CoV-2 CPE assay. This suggests that the compound acts via mechanisms not captured by the CPE assay because this assay is limited by only measuring cell-killing effects as the endpoint. Rather, favipiravir exerts antiviral effects through a unique mechanism of action that inhibits viral gene replication within infected cells to prevent further propagation.[58] Clinical trials (e.g., NCT04346628, Phase II) have been conducted to evaluate the efficacy and safety of favipiravir in patients with COVID-19. The antiviral drugs niclosamide (low hERG liability; cluster 106) and nitazoxanide (low hERG liability; cluster 106) are enrolled in clinical trials (e.g., NCT04603924, Phase II/III; NCT04486313, Phase III) to evaluate their potential to be repurposed as treatment for COVID-19. Niclosamide exhibited moderate activity in the autophagy assay, whereas nitazoxanide showed no activity against autophagy. Both drugs were active in the CPE assay, with niclosamide exhibiting low activity and nitazoxanide exhibiting moderate activity. Based on our screening data, these compounds show promising results as good candidates with low hERG and PLD liabilities for COVID-19 therapies.

Azithromycin (low hERG liability; cluster 75) is a well-tolerated, broad-spectrum antibiotic that is prominently used in the USA to treat a variety of bacterial infections.[59] The antiviral effects of azithromycin result from the stimulation of type I and II interferon production as well as genes involved in virus recognition, such as *MDA5* and *RIGI*.[60] These immune responses are universally involved in the innate response against infectious agents and potentially against SARS-CoV-2. Azithromycin has been proposed in conjunction with chloroquine or hydroxychloroquine to treat bacterial infections in patients with COVID-19.[44] In our analysis, azithromycin showed no hERG inhibition, suggesting that this drug has a low risk in causing hERG-related cardiotoxicity for patients with COVID-19 and should be further explored to increase the efficacy of novel SARS-CoV-2 therapies. Azithromycin is involved in multiple clinical trials (e.g., NCT04370782, Phase IV) to assess its efficacy and safety in the treatment of COVID-19. Table 2 provides a comprehensive list of the 11 compounds mentioned above that are enrolled in clinical trials as potential treatments for SARS-CoV-2.

Application of *in vitro* assays in drug repurposing

The use of *in vitro* assays to prescreen drug candidates for their potential toxicity has become an essential step in the drug-repurposing process. These cost-effective *in vitro* assays that cover multiple toxicological targets and pathways can efficiently test many compounds/drugs in a very short amount of time, providing important information on repurposed drugs with their toxicological profiles. The combination of *in silico* models[12,61,62] for drug design to limit the risk/toxicity of drugs based on chemical structure and use of a battery of *in vitro* assays as a prescreen can serve as a model to evaluate the activity–safety profile of a candidate molecule. Drug-repurposing screens using *in vitro* assays against new targets typically identify many active compounds. However, the activities of most of these identified compounds against new targets are weak. The EC_{50} or IC_{50} values of the approved drugs (i.e., their activities against the new targets) are often greater than human plasma drug concentrations. This indicates that a compound that showed activity *in vitro* is not likely to be active in humans because the human plasma drug concentration is lower than the required compound concentration *in vivo*. To resolve this issue, the drug combination therapy approach has been proposed. Two or more drugs used in combination acting through different mechanisms could lead to synergistic effects. The use of drugs in combination against new targets can subsequently reduce individual drug concentrations through this synergistic effect. Thus, we can use *in vitro* assays to screen for synergistic drug combinations for potential clinical applications.[35] Additionally, *in vitro* assays can be used to predict potential toxicity, which is usually available for single drugs but not drug combinations.

One of the limitations of our research is the clinical application of the identified approved drugs to patients. Currently, there are no standards in drug repurposing in clinical practice. Safety is still the number-one issue for drug-repurposing applications in a clinical setting. The best example of drug repurposing in clinical practice are treatments for bacterial infections. Once an approved antibiotic agent is identified by the *in vitro* antibiotic susceptibil-

TABLE 2

Potential drug candidates to treat SARS-CoV-2.[a]

Drug	SARS-CoV-2 CPE EC_{50} (μM)	hERG IC_{50} (μM)	PLD EC_{50} (μM)	US status[b]	Original indication	MOA	Target
Chloroquine	5.8	29	11	Approved; enrolled in Phase III clinical trial	Antimalarial	Inhibits heme polymerase and terminal glycosylation of ACE2	Glutathione *S*-transferase A2/Mu 1, TNF, Toll-like receptor 9, high mobility group protein B1, ACE2
Hydroxychloroquine sulfate	Inactive	Inactive	N/A	Approved; enrolled in Phase IV clinical trial	Antimalarial	Exact unknown; might be based on ability to bind to, and alter, DNA	Toll-like receptors 7/9
Ivermectin	1.3	9.7	17	Approved; recruiting	Antiparasitic	Binds selectively and with high affinity to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells of microfilaria	*Onchocerca volvulus*, glutamate-gated chloride channel, GABA-A receptor, glycine receptor subunit α1/3, *Caenorhabditis elegans*
Famotidine	Inactive	Inactive	N/A	Approved; recruiting and completed	Antihistamine, antacid	Binds competitively to H2-receptors located on basolateral membrane of parietal cell, blocking histamine affects	Histamine H2 receptor, solute carrier family 22 member 3
Imatinib	10	Inactive	N/A	Approved; recruiting	Antineoplastic agent	Inhibits BCR-ABL tyrosine kinase	Platelet-derived growth factor receptor α, Bcr/Abl fusion protein, stem cell growth factor receptor, platelet-derived growth factor receptor beta
Chlorpromazine	11	6.3	10	Approved; not yet recruiting	Antipsychotic	Acts as antagonist on different postsynaptic receptors on dopaminergic, serotonergic, histaminergic, α1/α2, and muscarinic M1/M2 receptors	Dopamine D1/D2 receptor, 5-hydroxytryptamine receptor 1A/2A, α-1A/1B adrenergic receptor, histamine H1 receptor, potassium voltage-gated channel subfamily H member 2, D(1) dopamine receptor, Dopamine D3–D5 receptor, 5-hydroxytryptamine 2 receptor, α-1/2 adrenergic receptors, muscarinic acetylcholine receptor M1/M3, sphingomyelin phosphodiesterase, calmodulin, α1-acid glycoprotein, 5-hydroxytryptamine receptor 6/7, histamine H4 receptor
Remdesivir	9.0	N/A	N/A	Approved; enrolled in Phase III clinical trial	Antiviral	Competes with ATP for incorporation into newly synthesized viral RNA by corresponding RdRp complex	Replicase polyprotein 1ab, RNA-directed RNA polymerase L
Favipiravir	Inactive	N/A	N/A	Approved; clinical trial completed	Antiviral	Selectively inhibits RNA polymerase and prevents replication of viral genome	RNA-directed RNA polymerase catalytic subunit
Niclosamide	0.23	Inactive	1.0	Withdrawn; recruiting for clinical trial	Anthelmintic	Uncouples oxidative phosphorylation or stimulates ATPase activity in adult worms	DNA

TABLE 2 (CONTINUED)

Drug	SARS-CoV-2 CPE EC_{50} (μM)	hERG IC_{50} (μM)	PLD EC_{50} (μM)	US status[b]	Original indication	MOA	Target
Nitazoxanide	6.3	Inactive	N/A	Approved; enrolled in Phase III clinical trial	Antiprotozoal	Disrupts energy metabolism in anaerobic microbes by inhibiting PFOR cycle	Pyruvate-flavodoxin oxidoreductase
Azithromycin	11	Inactive	N/A	Approved; enrolled in Phase IV clinical trial	Antimicrobial	Inhibits bacterial protein synthesis by binding to 50S ribosomal subunit of bacterial 70S ribosome	23S ribosomal RNA, protein-arginine deiminase type 4

[a] Potency values are listed for each compound when tested against SARS-CoV-2 CPE, hERG, and PLD assays.
[b] US status: intended use; treatment for COVID-19.

ity test using the isolated bacteria from a patient sample, the physician might decide to use the drug to treat a patient even though the approved clinical indication does not include this pathogen.[63,64] For the other clinical applications of approved drugs, clinical trials with regulatory approval are usually needed, although the length of preclinical studies might be shorter if the dosages, ages, and administration routes of the repurposed drugs are the same as their approved indications.

Concluding remarks

In summary, we evaluated 331 anti-SARS-CoV-2 compounds identified from drug-repurposing efforts for their toxicity potential, especially in terms of cardiotoxicity via hERG inhibition. Compounds in the approved drug library that showed high hERG liability tend to commonly be antihistamine, antipsychotic, and antihypertensive drugs. Alternatively, compounds that had low hERG liability generally comprised antibacterial, antiarrhythmic, and antineoplastic drugs. These drugs pose a reduced risk for inducing cardiovascular abnormalities and their repurposed potential as COVID-19 treatments should be further explored. In addition, our study revealed that the SARS-CoV-2 CPE assay captured many autophagy modulators, and significant correlations were found between autophagy modulation, hERG inhibition, and PLD induction. These results suggest that caution needs to be taken when selecting drugs that target autophagy for anti-COVID-19 drug development, because they pose a higher risk of toxicity via hERG inhibition and/or PLD induction.

Acknowledgments

We thank the teams at NCATS/DPI for generating and making publicly available the COVID-19 screening data used in this study. This work was supported by the Intramural Research Programs of the National Center for Advancing Translational Sciences, National Institutes of Health.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.drudis.2022.04.001.

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