

## **Screening of FDA-approved drugs using a MERS-CoV clinical isolate from South Korea identifies potential therapeutic options for COVID-19**

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## **Abstract**

In 2015, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) reached the Republic of Korea through nosocomial transmission and was the largest epidemic outside of the Arabian Peninsula. To date, despite various strategies to identify CoV interventions, only limited therapeutic options are available. To address these unmet medical needs, we used a South Korean MERS-CoV clinical isolate and screened 5,406 compounds, including United States Food and Drug Administration (FDA)-approved drugs and bioactive molecules, for their activity against the isolate. The primary assay confirmed 221 hits by dose-response curve analysis and identified 54 hits with a therapeutic index (TI) greater than 6. Time-of-addition studies with 12 FDA-approved drugs demonstrated that 8 and 4 therapeutics act on the early and late stages of the viral life cycle, respectively. Among the drugs were e.g., three cardiotoxic agents (ouabain, digitoxin, digoxin) with a TI greater than 100, an anti-malaria drug (atovaquone; TI >34), an inhalable corticosteroid (ciclesonide; TI >6), etc. Together, our results identify potential therapeutic options for treating MERS-CoV infections and could provide a basis for agents against a wider range of coronavirus-related illnesses, including the currently emerging Coronavirus Disease 2019 (COVID-19) outbreak.

**Keywords:** Middle East Respiratory Syndrome Coronavirus; coronavirus disease; clinical isolate; high-throughput screening; FDA-approved drugs; drug repurposing; COVID-19

**Abbreviations:** MERS-CoV, Middle East Respiratory Syndrome Coronavirus; COVID, Coronavirus Disease; HCS, high-content screening; DRC, dose-response curve; TI, therapeutic index

Coronaviruses (CoV) are enveloped, positive-sense, single-stranded RNA viruses in the *Coronaviridae* family of the *Nidovirales* order. CoV usually cause mild to severe respiratory tract infections (Perlman and Netland, 2009). Only two known types of human coronaviruses were described before 2003, coronavirus 229E and OC43, which caused mild, cold-like symptoms (Hamre and Procknow, 1966; McIntosh et al., 1967). However, an outbreak of Severe Acute Respiratory Syndrome (SARS) in 2003, which occurred mainly in Southeast Asia, was attributed to a SARS-related coronavirus. The 2003 SARS outbreak resulted in 8,096 confirmed cases and 774 deaths (fatality rate of 9.6%) (WHO, 2003). Following the SARS epidemic, the novel coronavirus Human Coronavirus-Erasmus Medical Center (HCoV-EMC) was isolated in 2012 from a patient in Saudi Arabia who developed pneumonia and renal failure (AM, Zaki, van Boheemen S, Bestebroer TM, Osterhaus AD, 2012). From the first outbreak in 2012 to January 2019, the HCoV-EMC epidemic resulted in 2,449 laboratory-confirmed cases and at least 845 deaths (fatality rate of 34%), mainly in the Arabian Peninsula. Thus, HCoV-EMC was renamed Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (WHO, 2019).

Although most cases of MERS-CoV infection were reported in the Arabian Peninsula, another major outbreak occurred in South Korea in 2015 (Korea Centers for Disease Control and Prevention, 2015; Yang et al., 2015). Notably, aside from the index case of MERS-CoV, the majority of viral transmissions in South Korea was nosocomial, with 186 confirmed cases across 16 clinics (Cho, 2015; Korea Centers for Disease Control and Prevention, 2015). Furthermore, continuous waves of MERS outbreaks in the Middle East have been reported by the World Health Organization (WHO), although they have been smaller than the major 2014 outbreak (WHO, 2019). Due to the severity of infection and urgent medical need for effective MERS treatment, several approaches for therapeutic development have been attempted (Zumla et al., 2016). In clinical studies, a combination of ribavirin and interferon-alpha (IFN- $\alpha$ ) therapy improved survival rates when administered early after the onset of infection. However, ribavirin and IFN- $\alpha$  treatments had no significant effect on patients in the late

stage of infection (Omrani et al., 2014; Shalhoub et al., 2014; Spanakis et al., 2014). These results suggest that broad-spectrum antiviral administration to MERS patients is effective at some points during infection, although a specific MERS-CoV treatment may be required full antiviral activity.

With the current pandemic of Coronavirus Disease 2019 (COVID-19), which emerged from Wuhan, China in late 2019, countries worldwide are working to control the spread of this devastating virus. The ongoing COVID-19 pandemic has already caused many human casualties and significant socio-economic losses globally. More than 219,000 confirmed COVID-19 cases and over 9,000 related fatalities worldwide have been reported (March 19, 2020). Unfortunately, CoV-specific United States Food and Drug Administration (FDA)-approved drugs are still not available in clinics.

To address urgent unmet medical needs and facilitate development of effective treatments for CoV patients, we implemented a high-content screening (HCS) strategy with the goal of repurposing newly identified MERS-CoV inhibitors for a wider range of CoV, including COVID-19. Utilizing a Korean MERS-CoV patient isolate, we screened 5,406 compounds, including FDA-approved drugs, bioactives, kinase inhibitors, and natural products. Our library included 60% of all FDA-approved drugs (1,247 out of 2,069 total) (Fig. 1A). Compounds were tested for anti-MERS-CoV activity by determining the expression levels of viral spike (S) protein in infected Vero cells using immunofluorescence analysis (IFA). Two independent screens were conducted (screen 1 and screen 2) using chloroquine as a reference inhibitor at 100  $\mu$ M for the maximum inhibitory concentration ( $IC_{90} = 93 \mu$ M) (De Wilde et al., 2014). The calculated Z'-factor of  $>0.78$  indicated a good separation of infected cells treated with the dimethyl sulfoxide (DMSO) control and chloroquine (Fig. 1B). Two independent HCS analyses were further conducted to select for hits demonstrating a high degree of correlation between the two replicates ( $R^2 = 0.91$ ) (Fig. 1C). Primary hits were identified by selecting compounds that demonstrated  $>70\%$  MERS-CoV inhibition and  $>70\%$  cell viability, thereby identifying 256 compound hits (Fig. 1D). These hits were then confirmed using 10-point dose-response curve (DRC) analysis. From the DRC assays,  $IC_{50}$  and 50% cytotoxicity concentrations

(CC<sub>50</sub>) were determined for each compound (Fig. 1D). A representative 10-point DRC analysis is shown in Supplementary Fig. 1. Based on the IC<sub>50</sub> and CC<sub>50</sub> analyses, we calculated therapeutic index (TI) by the ratio of CC<sub>50</sub>/IC<sub>50</sub>. From the 256 initial compound hits, 35 compounds were denoted as inactive [therapeutic index (TI ratio of CC<sub>50</sub>/IC<sub>50</sub>) values <1] and eliminated from the list of confirmed hits. Of the resulting 221 confirmed hits, 54 final compounds with an *in vitro* TI >6 were selected for further testing (Fig. 1D).

Our approach aimed to identify FDA-approved drugs and bioactives that could be promptly repurposed or developed, respectively, to treat MERS-CoV and potentially COVID-19-infected patients. In previously reported studies, small molecule libraries including approximately 300 drugs with FDA-approval or in clinical development were screened against MERS-CoV (De Wilde et al., 2014; Dyllal et al., 2014). Here, our screening included 1,247 FDA-approved drugs, which covers approximately 60% of all FDA-approved compounds. As a result, we identified drugs not found in previous studies, indicating that opportunities for identifying novel FDA-approved drugs and bioactives by screening larger compound libraries still exist. Moreover, we corroborated four previously identified hits, including emetine dihydrochloride, ouabain, cycloheximide, and nelfinavir mesylate. These data strongly suggest that, despite the use of different viral isolates, our HCS assays and previously published screens reproducibly identified drugs that could be repurposed as potential therapeutics options for patients suffering CoV infections (Dyllal et al., 2014).

Next, we classified the entire compound library, including the final hit compounds, into 43 categories of distinct pharmacological actions using publicly available drug databases. The results are shown in Fig. 2, with the distribution of the entire library shown as gray bars and the final hits indicated in black. Notably, the cardiovascular agents group contained 14 compounds with TI >6 (26% of final hits). From this analysis, we found that the majority of final hits are cardiovascular agents (14 out of 54) and belong to a class of cardiac glycosides. These compounds are naturally derived agents used for treating cardiac abnormalities and modulate sodium-potassium pump action (Prassas

and Diamandis, 2008). However, glycosides have also been reported to exhibit antiviral activity against herpes simplex virus and human cytomegalovirus (Bertol et al., 2011; Kapoor et al., 2012). Consistent with these previous studies, our data also indicate that the cardiac glycosides ouabain, digitoxin, and digoxin efficiently inhibit MERS-CoV infection. Specifically, ouabain has been found to block the entry stage of CoV, such as MERS-CoV, through Src kinase signaling (Burkard et al., 2015). Based on these data, we speculate that cardiac glycosides may act as anti-MERS-CoV agents through blockage of viral entry. However, more experimental work will be required to elucidate the exact mechanism by which this occurs.

Repurposing FDA-approved drugs and inhibitors with known biological functions may facilitate faster drug development due to their known pharmacological activities and safety profiles. Therefore, we prioritized 12 FDA-approved drugs and six bioactives not yet reported to have anti-CoV activities and summarized their information in Tables 1 and 2, respectively. An additional 26 inhibitors identified by HCS include bioactive molecules and drugs that have been in clinical trials. These inhibitors were analyzed by selectivity index (SI) based on the ratio of  $CC_{50}$  to  $IC_{50}$  ( $CC_{50}/IC_{50}$ ). Inhibitors with SI values between six and >156 are shown in Supplementary Table 1.

To investigate whether the FDA-approved drugs act on the early or late stages (pre- or post-entry) of the viral life cycle, we conducted time-of-addition studies. Vero cells were treated with each drug at a concentration higher than its  $IC_{90}$  at 1 h prior to infection or at 0, 1, 2, 3, and 4 h post-infection (hpi). Viral infection was then quantified by IFA as previously described. Infected cells treated with 0.5% DMSO were normalized to 100% infection. Chloroquine was used as an early-stage inhibitor control and inhibited MERS-CoV infection up to 30% until 3 hpi. However, chloroquine had no significant effect when administered at 4 hpi (Fig. 3). A similar effect was observed for treatment with ouabain, digitoxin, digoxin, niclosamide, regorafenib, nelfinavir mesylate, ciclesonide, and benidipine hydrochloride, which all inhibited MERS-CoV infection only when administered before 4 hpi (Fig. 3, Supplementary Fig. 2). These data are consistent with

previous reports indicating that ouabain and other cardiotonic steroids effectively block clathrin-mediated CoV endocytosis (Burkard et al., 2015; Zumla et al., 2016). In contrast, atovaquone, lercanidipine hydrochloride, permethrin, and octocrylene had only minor inhibitory effects throughout the time-course assay, indicating that these drugs likely act on later stages of the viral life cycle (Supplementary Fig. 2). Notably, our results indicate that the dihydropyridine calcium channel blockers lercanidipine hydrochloride and benidipine hydrochloride, display different patterns of viral inhibition (Epstein, 2001; Yao et al., 2006). This observation could be explained by the differing types of calcium channels that each drug affects: benidipine hydrochloride blocks triple voltage-gated calcium channels, whereas lercanidipine hydrochloride blocks single voltage-gated channels (Klein and Köppel H, 1999; Ozawa et al., 2006; Wirtz and Herzig, 2004).

In summary, we identified 12 FDA-approved drugs that could be repurposed for MERS-CoV or COVID-19 therapy. In particular, the cardiotonic drugs ouabain, digitoxin, and digoxin (TI >100 in monotherapy) are of interest that, when combined with remdesivir, a drug currently in clinical trials for treating COVID-19, or other therapeutics might yield drug synergism. Important to note, ciclesonide, used to treat asthma and allergic rhinitis by suppressing the immune system, was recently shown to inhibit MERS-CoV (Matsuyama et al., 2020) and reported by Japanese medical doctors to have improved pneumonia symptoms in three COVID-19 patients. However, further *in vitro* and *in vivo* studies are required to investigate their exact antiviral mechanisms, determine potential synergistic effects, and confirm their antiviral efficacy to prioritize and select drugs for potential use in patients affected by the ongoing COVID-19 pandemic.

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**Table 1. Hit profiling and anti-MERS-CoV efficacies of FDA-approved drugs in Vero cells<sup>1</sup>.**

Drug Name	Trade Name	Putative Drug Target	Pharmaceutical Action	IC <sub>50</sub> <sup>2</sup> (μM)	SD <sup>3</sup> (±)	CC <sub>50</sub> <sup>4</sup> (μM)	TI <sup>5</sup>
Ouabain <sup>#</sup>	Strodival	Na, K-exchanging ATPase pump	Cardiotonic agent	0.08	0.0066	>25	>312.5
Digitoxin <sup>#</sup>	Digitaline	Ca, Na-exchanging ATPase pump	Cardiotonic agent	0.16	0.0003	>25	>156.3
Digoxin <sup>#</sup>	Lanoxin	Ca, Na-exchanging ATPase pump	Cardiotonic agent	0.17	0.0084	>25	>147.1
Niclosamide <sup>#</sup>	Niclocide, others	ATP synthase	Agrochemical	0.55	0.0363	>25	>45.5
Atovaquone <sup>*</sup>	Mepron	unknown (lipophilic)	Anti-infective agent	0.72	0.0585	>25	>34.7
Regorafenib <sup>#</sup> (Bay 73-4506)	Stivarga	Multiple kinases	Anti-neoplastic agent	2.31	0.0834	>25	>10.8
Lercanidipine hydrochloride <sup>*</sup>	Zanidip	Calcium channel blocker	Cardiovascular agent	2.36	0.1654	>25	>10.6
Permethrin <sup>*</sup>	Elimite, others	Na channel	Agrochemical	3.60	0.7573	>25	>6.9
Octocrylene <sup>*</sup>	Non	Skin	Additive in sunscreen	3.62	0.6435	>25	>6.9
Nelfinavir mesylate <sup>#</sup>	Viracept	HIV-1 protease	Antiviral agent	3.62	0.0177	>25	>6.9
Ciclesonide <sup>#</sup>	Alvesco, others	Glucocorticoid ligand	Pharmaceutical and therapeutic agents	4.07	0.4907	>25	>6.1
Benidipine hydrochloride <sup>#</sup>	Coniel	Calcium channel blocker	Cardiovascular agent	4.07	0.7234	>25	>6.1

<sup>#</sup>Drug acting on the early stage of the viral life cycle (according to time-of-addition study).

<sup>\*</sup>Drug acting on the late stage of the viral life cycle (according to time-of-addition study).

<sup>1</sup>DrugBank database (version 5.0) was used for investigating FDA-approved drugs.

<sup>2</sup>50% inhibitory concentration (IC<sub>50</sub>).

<sup>3</sup>Standard deviation (SD) of replicated IC<sub>50</sub> values.

<sup>4</sup>50% cytotoxicity concentrations (CC<sub>50</sub>).

<sup>5</sup>Therapeutic Index (TI): ratio of CC<sub>50</sub>/IC<sub>50</sub>.

**Table 2. Hit profiling and anti-MERS-CoV efficacies of selected bioactives in Vero cells<sup>1</sup>.**

<b>Inhibitor Name</b>	<b>Pharmaceutical Action</b>	<b>IC<sub>50</sub><sup>2</sup> (μM)</b>	<b>SD<sup>3</sup> (±)</b>	<b>CC<sub>50</sub><sup>4</sup> (μM)</b>	<b>SI<sup>5</sup></b>
Emetine dihydrochloride	Anti-neoplastic agent	0.08	0.0054	>25	>312.5
Oxyclozanide	Antiparasitic agent	0.07	0.0060	20.92	298.9
Cycloheximide	Protein synthesis inhibitor	0.16	0.0140	>25	>156.3
Lanatoside C	Cardiotonic agent	0.19	0.0103	>25	>131.6
Calcimycin	Antibacterial agent	0.20	0.0165	18.10	90.5
Digitoxigenin	Cardiotonic agent	0.29	0.0220	>25	>86.2

<sup>1</sup>DrugBank database (version 5.0) was used for investigating bioactives.

<sup>2</sup>50% inhibitory concentration (IC<sub>50</sub>).

<sup>3</sup>Standard deviation (SD) of replicated IC<sub>50</sub> values.

<sup>4</sup>50% cytotoxicity concentrations (CC<sub>50</sub>).

<sup>5</sup>Selectivity Index (SI): ratio of CC<sub>50</sub>/IC<sub>50</sub>.

## Figure Legends

**Fig. 1. Overview of small-molecule compound library composition and hit triage.** (A) Our small-molecule compound library primarily comprised bioactives and FDA-approved drugs, with a small proportion of natural products and kinase inhibitors. (B) High-content screening (HCS) of 5,406 compounds in two batches in duplicate and calculation of Z'-factors between high (MERS-CoV infection, black) and low (mock, green) values. (C) Correlation of duplicate screening sets. Compounds with MERS-CoV inhibition and cell viability >70% were regarded as primary hits. Scatter plot showing MERS-CoV inhibition ratios overlaid with cell viability ratios. (D) Flowchart of HCS hit selection and confirmation of final hit selection. Compounds (cpds).

**Fig. 2. Pharmacological action profiling of all library compounds and confirmed hits.** The 54 final hits were sorted into 43 pharmacological action categories. Gray and black bars indicate the distribution of all screened compounds and confirmed hits with a therapeutic index (TI) >6. The vertical axis displays counts of each compound on a log scale with +1 added to the count to prevent negative values.

**Fig. 3. Time-of-addition study with selected FDA-approved drugs.** Five FDA-approved drugs were analyzed by time-course experiments to determine the stage of MERS-CoV life cycle inhibition. Vero cells were infected at a multiplicity of infection (MOI) of 5 with MERS-CoV, and FDA-approved drugs were administered at 6 time points pre- or post-infection as indicated. Drug concentrations were higher than the 90% inhibitory concentration (IC<sub>90</sub>) values of the drugs, and chloroquine was used as an early-stage control inhibitor.

## **Supplementary Figure Legends**

**Supplementary Fig. 1. Example images of MERS-CoV inhibition in Vero cells.** (A) The dose-response curve (DRC) for lanatoside C is a representative DRC illustrating the inhibition of MERS-CoV in Vero cells. (B) HCS was performed using an image-based assay, and compound efficacy was measured by MERS-CoV S protein inhibition. Images depict 0%, 50%, and 100% inhibition as indicated in the DRC. Scale bar = 100  $\mu\text{m}$ .

**Supplementary Fig. 2. Time-of-addition study with additional FDA-approved drugs.** Seven FDA-approved drugs not shown in Fig. 3 were analyzed by time-of-addition assay experiments as described in that figure.

## Supplementary Table 1

Inhibitor Name	IC <sub>50</sub> <sup>1</sup>	CC <sub>50</sub> <sup>2</sup>	SI <sup>3</sup>
Cycloheximide	0.16	>25	>156.3
Convallatoxin	0.31	>25	>80.6
Gitoxigenin diacetate	0.48	>25	>52.1
Antimycin A	0.36	>25	>69.4
Strophanthidinic acid lantone acetate	0.56	>25	>44.6
Strophanthidin	0.56	>25	>44.6
IMD0354	0.25	8.74	35.0
Digoxigenin	1.13	>25	>22.1
Leoidin	1.26	>25	>19.8
Deguelin(-)	1.47	>25	>17.0
Dihydrorotenone	1.52	>25	>16.4
Amuvatinib (MP-470)	1.60	>25	>15.6
Raf265 derivative	1.86	>25	>13.4
MK-886	1.91	>25	>13.1
Proscillaridin	2.05	>25	>12.1
Torin 1	2.08	>25	>12.0
Mundulone	1.21	14.58	12.0
7,8-Dihydroxyflavone	2.11	>25	>11.8
XL765-Voxtalisisib	2.17	>25	>11.5
Thapsigargin	0.49	5.55	11.3
Torin 2	2.44	>25	>10.2
STF-62247	2.54	>25	>9.8
WAY-600	2.58	>25	>9.7
Isorotenone	2.90	>25	>8.6
Cyclopiazonic acid	3.17	>25	>7.9
AS-252424	1.78	14.14	7.9
AM 580	3.32	>25	>7.5
CI-1040	3.50	>25	>7.1
Fenretinide	2.80	19.85	7.1
Gedunin	3.59	>25	>7.0
cx-4945 (Silmitasertib)	3.66	>25	>6.8
VU 0155069	3.69	>25	>6.8
Dihydro-munduletone	3.72	>25	>6.7
Cypermethrin	3.77	>25	>6.6
(Z)-pregna-4,17(20)-diene-3,16-dione	3.85	>25	>6.5
Brivanib (BMS-540215)	4.13	>25	>6.1

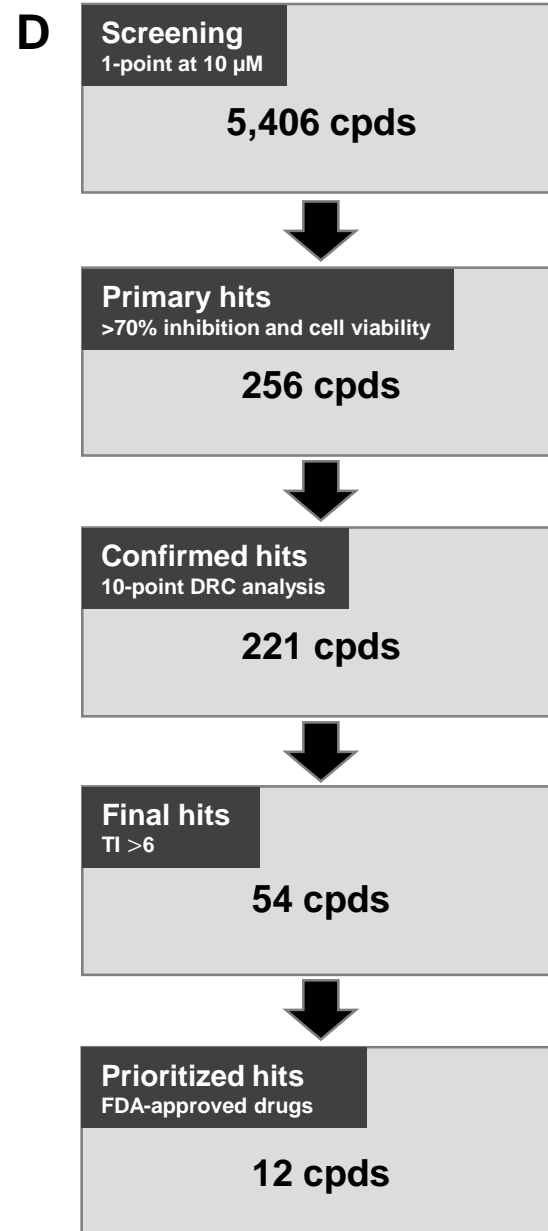
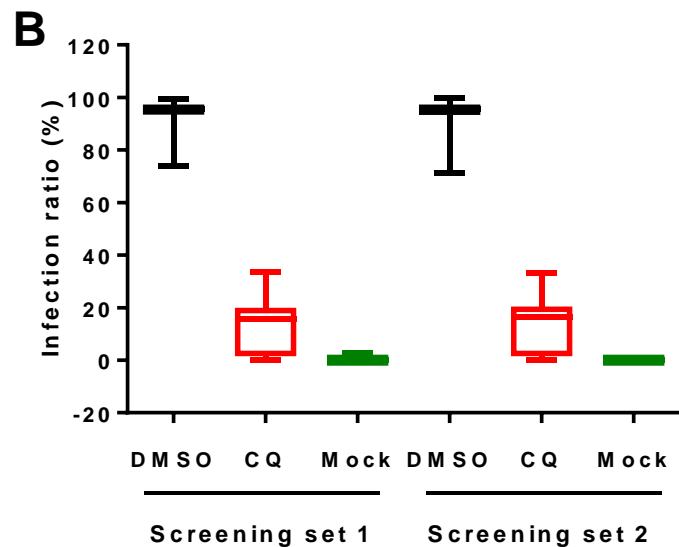
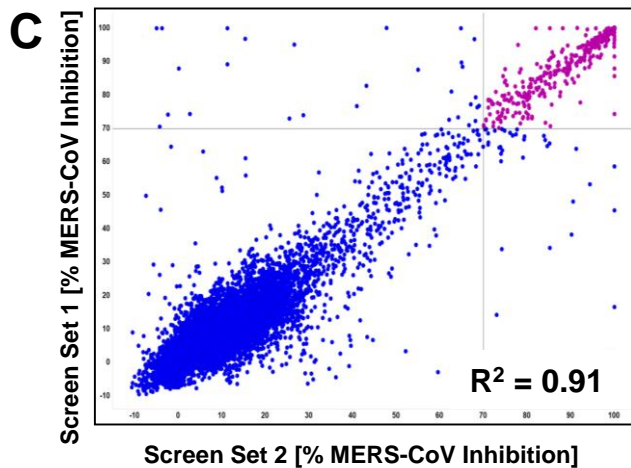
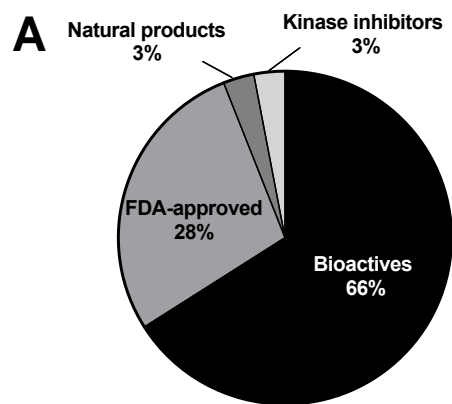
<sup>1</sup>50% inhibitory concentration (IC<sub>50</sub>).

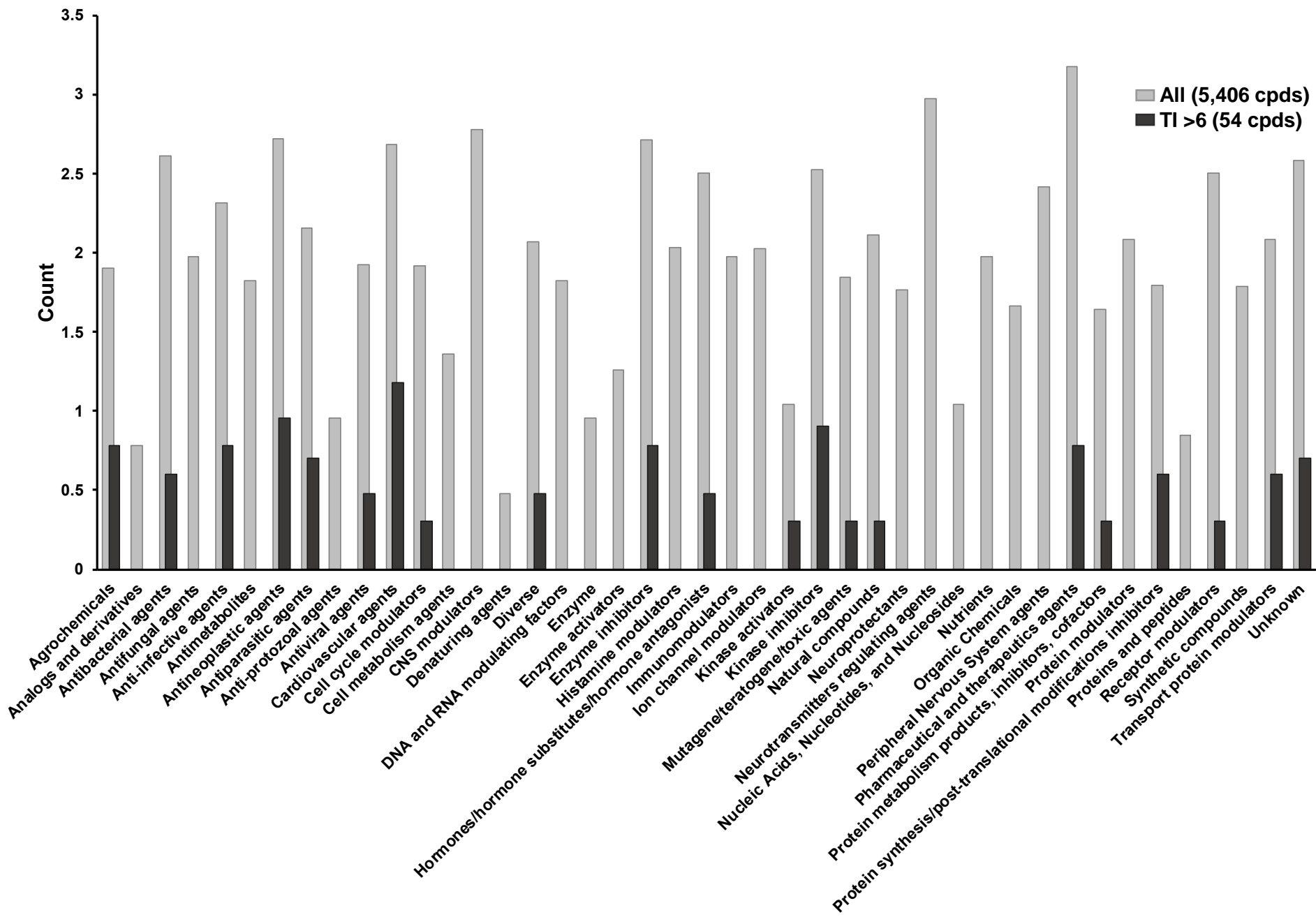
<sup>2</sup>50% cytotoxicity concentrations (CC<sub>50</sub>).

<sup>3</sup>Selectivity Index (SI): ratio of CC<sub>50</sub>/IC<sub>50</sub>.



**Fig. 1.**



**Fig. 2.**

**Fig. 3.**

