

EDITORIAL

Convergent evidence for the antiviral effects of several FDA-approved phenothiazine antipsychotics against SARS-CoV-2 and other coronaviruses

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There is an urgent need to identify rapidly accessible and effective medications to treat coronavirus disease 2019 (COVID-19). At present, this is undoubtedly the most important global priority in clinical research. The most obvious drugs to repurpose against the new coronavirus have been those agents effective against other viruses. However, to date, these agents have not shown conclusive evidence of effectiveness. Thus, the systematic screening and validation of alternative repurposed drugs for COVID-19, using *in vitro* and *in vivo* models, may provide evidence to rapidly identify new candidates with potential antiviral efficacy against SARS-CoV-2 for further testing.

A previous screen evaluating thousands of FDA-approved drugs for antiviral activity against two other highly pathogenic human coronaviruses, SARS-CoV and MERS-CoV, was performed years ago. This study found 27 drugs that inhibited the replication of SARS-CoV and MERS-CoV *in vitro*,¹ belonging to 13 different classes of pharmaceuticals.

Twenty FDA-approved candidate drugs showing antiviral effects against SARS-CoV and MERS-CoV in the initial study¹ were retested *in vitro* against the new SARS-CoV-2.² Of these, 17 also displayed significant antiviral activity, inhibiting SARS-CoV-2 at a safe (non-cytotoxic) range of IC50 values,² i.e., these 17 drugs targeted, in a convergent manner, more than one human coronavirus type.

From these previous studies, we observed that, out of the 17 drugs showing inhibition of SARS-CoV-2 viral activity, four (23.5%) agents (promethazine, fluphenazine, chlorpromazine, and thiethylperazine) belong to the phenothiazine class, which is widely used in psychiatry and other areas of medicine. This observation was not reported in the

original study. Among these four phenothiazines, only chlorpromazine was further studied, with additional positive findings related to inhibition of cytopathic effect (CPE), mRNA synthesis, and SARS-CoV-2 viral particle production.²

Recently, the National Center for Advancing Translational Research (NCATS), affiliated with the National Institutes of Health (NIH), made available the COVID-19 OpenData Portal to openly and quickly share drug repurposing data testing for antiviral activity against SARS-CoV-2.³ Nearly 3,000 approved drugs have been tested and data obtained based on NCATS-validated SARS-CoV-2 assays (latest update in September 2020).

In the present report, we provide the first convergent evidence from two validated datasets evaluating antiviral effects against different human coronavirus types, with a particular focus on SARS-CoV-2. We reviewed the NCATS approved drug collection (NPC) (2,677 drugs total, any formulation). We selected as criteria the presence of one or more parameters with high-quality evidence as well as at least 3 (out of 4) key parameters with any level of evidence (Table 1). We hypothesized that antipsychotic drugs sharing the three-ring structure of the phenothiazine class, with different side chains, would display a unique pharmacological profile to convergently inhibit SARS-CoV-2.

Parameters included the surface ACE2 receptor protein, which has been shown to be the primary host factor recognized and targeted by SARS-CoV-2 virions. Disrupting the spike protein-ACE2 interaction may limit the ability of SARS-CoV-2 virions to infect the host. Two specific ACE2-related parameters were evaluated: spike-ACE2 protein-protein interaction (AlphaLISA-1) and spike-ACE2 protein-protein interaction (TruHit Counter-screen-2).⁴ In terms of live virus infectivity, two other parameters were included: SARS-CoV-2 CPE, a live

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Table 1 Effects of multiple phenothiazines matching criteria for antiviral effects against SARS-CoV-2

Drug	Antiviral criteria*	Viral entry		Live virus infectivity	
		Spike-ACE2 protein-protein interaction (AlphaLISA)	Spike-ACE2 protein-protein interaction (TruHit Counterscreen)	Cytopathic effect (CPE)	Host tox counterscreen
Fluphenazine	Yes	++	++	+	+
Chlorpromazine	Yes	+	++	+	+
Thiethylperazine	Yes	++	++		+
Promethazine	Yes	++	+	+	
Perphenazine	Yes	++	++	+	
Thiopropazine	Yes	++	++	+	
Periciazine	Yes	+	++	+	
Trifluoperazine	Yes	+	++	+	+
Triflupromazine	Yes	+	++	+	+
Thioridazine	Yes	++	++		+

Any evidence: yes or no; if yes, quality of evidence: + low; ++ high.
 Bold type indicates phenothiazines that also showed significant antiviral effects against other coronaviruses in previous datasets.
 * Selected drug has at least high-quality evidence for one parameter and any level of evidence for at least three or four parameters.

SARS-CoV-2 virus assay, measured the ability of compounds to reverse the viral-induced CPE in Vero E6 host cells; a similar parameter was the *host tox counter screen*, a cell-based assay measuring host cell ATP content as a readout for cytotoxicity in Vero E6 cells selected for high ACE2 expression.⁵

The results are presented in Table 1 (findings reflect the NCATS dataset available in September 2020). We found a total of 137 FDA-approved drugs matching the predefined criteria for antiviral effects against SARS-CoV-2. We also evaluated whether any specific pharmacological class would contain a cluster of agents displaying significant antiviral activity. It was observed that only the phenothiazine class contained at least 10 drugs matching criteria for antiviral effects against SARS-CoV-2. Exactly 10 phenothiazines (7.3% of the total, see Table 1) matched the criteria for anti-SARS-CoV-2 effects.

This list includes chlorpromazine, which had been recently highlighted based on preliminary positive results.^{2,6} Fluphenazine also showed antiviral effects against SARS-CoV, MERS-CoV, and SARS-CoV-2 in previous studies,^{1,2} which was confirmed in the present study (showing slight superiority over chlorpromazine). Thiethylperazine and promethazine, the two other drugs (out of 17) that had shown antiviral effects against SARS-CoV and MERS-CoV *in vitro*,^{1,2} also matched criteria for antiviral effects against SARS-CoV-2 in our study (Table 1).

Other phenothiazines matching the criteria were thioridazine, perphenazine, thiopropazine, periciazine, trifluoperazine, and triflupromazine. Strikingly, no other pharmacological class showed similar results in terms of the absolute or relative number of agents with such activity.

This is the first description of phenothiazines as a class with potential utility in the treatment of COVID-19 based on *in vitro* studies. The present report provides evidence supporting convergent *in vitro* antiviral effects against SARS-CoV-2 of a cluster of drugs belonging to the same pharmacological class (with a phenothiazine skeleton). Interestingly, besides their efficacy as antipsychotics (and antiemetics in certain cases), this class

of agents has also shown antiviral, antibacterial, and anti-prion activities in previous *in vitro* studies (reviewed in Varga et al.⁷).

Phenothiazines have good overall distribution throughout the body, including the lungs, and cross the blood-brain barrier, which could eventually help to prevent psychiatric and neurological consequences of COVID-19. The potential antiviral effects of phenothiazines have been associated with their ability to inhibit viral binding to plasma membrane receptors, thus blocking endocytosis and inhibiting the required DNA replication. The few early studies on the role of phenothiazines only focused on the antiviral activity of chlorpromazine,⁷ while other similar agents described herein were not studied further regarding their potential antiviral effects against human coronaviruses.

We conclude that several typical antipsychotics sharing the same phenothiazine skeleton may have antiviral effects against SARS-CoV-2. Further confirmatory *in vivo* and epidemiological studies are warranted.

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