RESEARCH ARTICLE

Antiviral Potency of Antipsychotics: A New Therapeutic Strength

Zeidan H A[1]*, Amer S K[2]

[1] *College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport, Alexandria, Egypt, , 9 Abou Qear, Abu Qir Al Gharbeyah, Montaza 2, Alexandria Governorate 5528341*

[2] *Department of Pharmaceutics, Division of Pharmaceutical Sciences, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport, Alexandria, Egypt, 9 Abou Qear, Abu Qir Al Gharbeyah, Montaza 2, Alexandria Governorate 5528341*

Received: 12th Sep 2024; Revised: 23rd Oct, 2024; Accepted: 15thNov, 2024; Available Online: 25th Dec, 2024

ABSTRACT

The rationale behind alternative medicine is to repurpose existing medicines to meet unmet medical needs. Antipsychotics, originally developed to treat mental disorders such as schizophrenia and bipolar disorder, are now being evaluated for broader therapeutic potential. From safety data and known mechanisms of action, scientists are testing the effects of antipsychotics (APs) on viruses as a consequence to the lack of successful antivirals available. For example, some APs inhibit viral replication, prevent viral entry into host cells, or modulate immune responses. However, pre-experimental and clinical studies are needed to validate these findings. Drug repurposing has been a promising strategy, specifically after the devastating COVID-19 pandemic. We created this systematic review aiming to gather reliable scientific evidence to help examine the success of repurposing antipsychotics as possible candidates for numerous viral infections, particularly SARS-CoV2 infection. As of August 2024, we carried out random effect meta-analysis on many clinical studies where most studies provided data supporting the beneficial effect of repurposing APs as antivirals for COVID-19 treatment. On the contrary, one study provided data showing that APs increased the risk of severe COVID-19 and mortality; even though, their use should be examined case by case and psychiatric patients should not be encouraged to discontinue their psychiatric treatment. Based on numerous studies, we observed that AP drugs of the phenothiazine class (promethazine, fluphenazine, chlorpromazine, and thiethylperazine) display a unique pharmacological profile to convergently inhibit SARS-CoV-2. Interestingly, only the phenothiazine class contained at least 10 drugs matching criteria for antiviral effects against SARS-CoV-2. In addition to 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. Therefore, drug repurposing of APs could be a great chance to combat several viral infections while bypassing long-term drug development and providing rapid solutions to emerging health challenges.

Keywords: Drug repurposing, Antipsychotics, Antivirals, COVID-19, Phenothiazines, Drug development

How to cite this article: Zeidan H A, Amer S K. Antiviral Potency of Antipsychotics: A New Theraputic Strength. International Journal of Pharmaceutical Quality Assurance. 2024;15(4):2176-83. doi: 10.25258/ijpqa.15.4.12

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Viral infections have been a terrorizing global health threat ever since the end of the 19th century when tobacco plants showed mosaic-like patterns of damage which was attributed to the first ever discovered virus, the Tobacco Mosaic virus.[1] Along the centuries to premodern and modern times, colonization, wars and slavery have contributed to countless infectious diseases with increasing prevalence and destructive sequelae. Fortunately, within the prior two decades, improvements within the medical field, well-provided health care and advanced hygiene and sanitation managed to minimize morbidity and mortality rates induced by infectious diseases, particularly viral infections.[2]

Prevalence, which can be expressed either as the number of cases per population unit or as a percentage, is a measure of the disease frequentness within a population. While the density of individuals possessing immunoglobulins to a specific virus in a population is defined as seroprevalence.[3] In 2024, a seroprevalence report, also known as a serosurvey, for Parvovirus B19 (B19V) virus among healthy blood doners in Iran was constructed. Results showed that 8 out of 400 individuals (2 %) showed positive results for anti-B19V IgM, and 306 out of 400 individuals (76.5 %) displayed positive results for anti-B19 IgG. Therefore, a single donation from a donor possessing anti-IgM antibodies was thereafter established as B19V DNA-positive through Real-Time PCR.[4]

Moreover, a recent and growing concern was raised regarding dengue, a mosquito-borne viral infection transmitted via vector Aedes albopictus, imposing a severe health hazard to all attendees of the 2024 Olympic Games. As in 2022 and 2023, France reported 65 and 43 cases of dengue, respectively, inclusive of cases near Paris.[5]

Emerging antivirals have succeeded to treat some fatal viral infections, for example Sofosbuvir for Hepatitis C Virus (HCV), which was a world-wide health menace specifically in Egypt. The introduction of Sofosbuvir (Sovaldi) in addition to the implementation of "100 million Healthy Lives" precipitated in great prevalence reduction of HCV in

*Author for Correspondence: hania.ahmedzeidan02@gmail.com

Table 1: Studies showing the Anti-viral effect of Chlorpromazine

Year	Chlorpromazine Results
2020	At GHU PARIS Psychiatrie & Neurosciences (Sainte-Anne hospital, Paris, France) an observation was made regarding the low incidence rate of symptomatic forms of COVID-19 among patients on CPZ than among the clinical staff.
2021	Studies on CPZ showed positive findings related to inhibition of cytopathic effect (CPE), mRNA synthesis, and SARS-CoV-2 viral particle production.
	HEK293T-ACE2-TMPRSS2 cells were exposed to pseudotyped viruses displaying the SARS-CoV-2 spike protein, and antipsychotic drugs (chlorpromazine, flupentixol, and pimozide) were tested for their antiviral activity. After 24 hours of incubation, all tested compounds significantly inhibited viral infection, with minimal reductions in cell viability.

Table 2: Studies showing the Anti-viral effect of Lithium

Year	Lithium Results
1993	Lithium γ-linolenate (Li-GLA) was tested for its activity in selectively killing H9 cells chronically infected with HIV-1_{RF}. After 4 days incubation with Li-GLA approximately 90% of the $H9_{RF}$ cells were non-viable compared to 20% of uninfected H9 cells.
2011	Different concentrations of lithium chloride (LiCl) were examined for their effect on the infection by Porcine epidemic diarrhea virus (PEDV) in Vero cells and by Bovine rotavirus (BRV) in MA104 cells. The maximum non-toxic concentrations of LiCl in these cells are 50 mM and 25 mM, respectively, and at such concentrations, the infection was inhibited by more than 95%.
2022	Clinical studies showed that lithium possesses more potent antiviral activity against DNA viruses, specifically Herpes viruses, compared to its activity against RNA viruses, including Coronaviruses.

Egypt.[6] On the other hand, antivirals have numerous drawbacks commencing from their synthesis up until their market cost price. Firstly, the period of time required for the virus-specific drug development process, with an average of a decade or more between research and drug approval, is a major obstacle.[7] Secondly, manufacturing of to-be-approved antivirals necessitates extremely high costs which limit the number of manufacturers who can commit to such costs.

Recently, due to the lack of efficient antivirals available to combat the countless viral infections circulating the globe, a new therapeutic strategy was introduced shedding the light on many conventional drugs, for instance AP drugs. Known to be a serendipitous process, drug repositioning is the use of an existing medication, with established safety to humans and animals, for a novel indication. Consequently, the tedious and time-consuming process of drug development is bypassed leading directly to preclinical and clinical trials thus depleting the time and costs.[8]

Phenothiazines, including chlorpromazine (CPZ), fluphenazine, perphenazine, prochlorperazine and thioridazine, are common antipsychotics used in the treatment of psychosis, schizophrenia and bipolar disorders, but they also show antiviral, cancer, bacterial, fungal, protozoal and prion actions. Their antiviral activity is attributed either to their ability to disturb the binding process of the virus to the host plasma membrane thus impede the entry of the virus or preventing the DNA replication by inserting DNA bases.[9]

The aim of this study is to explore the potential of repositioning AP drugs as antiviral agents. While APs have demonstrated significant effects in disputing various viral infections, this approach remains as auspicious strategy for addressing a wide range of viral diseases. The study seeks to contribute to the growing body of evidence supporting the dual functionality of AP medications in the treatment of viral infections.

METHODOLOGY

A comprehensive literature search was conducted using various search engines to collect research articles and papers related to repositioning of antipsychotics from 1993 to 2024. The drugs of interest included Chlorpromazine, Lithium, Thioridazine, Clozapine, Fluphenazine, Trifluoperazine, Haloperidol, Perphenazine and Prochlorperazine, totaling 9 drugs. In whole, twenty-five papers were included in the review, consisting of four review articles, twenty-one laboratory-based experimental research studies and a prevalence report. The articles were sourced from nine academic publishing platforms, with seven papers obtained from PMC, five from Frontiers, three from Viruses, one from each of Nature, Russian Journal of Bioorganic Chemistry, European Journal of Pharmacology, International Journal of Antimicrobial agents, L'encephale, Brazilian Journal of Psychiatry, Journal of Integrative Science, Biosafety and Health, Psychiatry Research and Antiviral Research.

RESULTS

Chlorpromazine

Chlorpromazine (CPZ) has shown significant potential in inhibiting various viral activities, including Measles virus germination, HIV infection, Hepatitis B virus DNA replication, and the replication of both SARS and Human

Table 3: Table 1: Studies showing the Anti-viral effect of Thioridazine

Thioridazine	
Year	Results
2020	Antiviral activity of thioridazine against Ebola virus (EBOV) was analyzed. The concentration of the drug which caused 50% inhibition of host viability (without virus) were 6.24 ± 0.79 and 2.06 ± 0.12 μM for Vero E6 and HepG2 host cells, respectively.Also, thioridazine concentration of 10 μM strongly (>90%) inhibits EBOV-VLP entry into SNB19 cells. Accordingly, thioridazine possesses anti-EBOV activity with EC_{50} = 1.45 ± 0.26 μM.
2022	HEK293T-ACE2 cells were incubated for 1 h with DMSO as a control or the indicated compounds at two concentrations (2 μM, 5 μM). Then cells were infected with the pseudovirus harboring spike protein from the original strain (SARS-CoV-2-WH01) and a luciferase reporter gene. After 48 h, luciferase assays were used to examine pseudovirus infectivity. Trifluoperazine 2HCl, monensin sodium salt, tilorone 2HCl, actidione, clofazimine, sertraline HCl, thioridazine HCl, vortioxetine, and salifungin significantly inhibited SARS-CoV-2-WH01 pseudovirus infection at both doses, by more than 50%, suggesting that these nine drugs can impede the cellular entry of SARS-CoV-2.

Table 4: Studies showing the Anti-viral effect of Clozapine

Clozapine	
Year	Results
2019	• A clinical study was carried out testing the effect of varying concentrations of clozapine on the reactivation of EBV into the lytic cycle. Clozapine at 2, 10, or 50 μM did not induce BZLF1 viral gene expression in the HH514-16 Burkitt lymphoma cells compared to untreated cells. When added with butyrate, lower concentrations of clozapine (i.e., 2 or 10 μM) inhibited BZLF1 expression by ~40%–50% compared to the level reached in cells treated with only NaB. Clozapine at 50 μM inhibited lytic reactivation by NaB by >95% compared to the reactivation seen with NaB alone.

Coronavirus 229E, all within low micromolar concentrations. Additionally, in vitro studies have demonstrated CPZ's antiviral efficacy against a range of viruses, such as Influenza, Alphaviruses, John Cunningham virus, Japanese encephalitis virus, Bronchitis virus, MHV-2, Zika virus, and Dengue virus. CPZ operates primarily by antagonizing clathrin-mediated endocytosis, through the translocation of clathrin and AP2 from the cell surface to intracellular endosomes, a mechanism that is often utilized to study viral entry into host cells.[10]

During the 2020 COVID-19 pandemic, with limited antivirals available for SARS-CoV-2, repositioning existing psychotropic drugs, particularly antipsychotics, emerged as a cost-efficient and time-saving alternative to the traditional drug development. A study conducted at GHU PARIS Psychiatrie & Neurosciences (Sainte-Anne hospital, Paris, France) suggested that CPZ might offer protective effects against COVID-19, potentially through its antagonism of the ACE2 signaling pathway due to the observation noticed when lower incidence rates of symptomatic forms of COVID-19 occurred among patients on antipsychotics particularly CPZ than among the clinical staff.[11] Furthermore, another study was carried out on twenty FDA-approved drugs showing antiviral effects against SARS-CoV and MERS-CoV in a previous study.

These drugs were retested in vitro against the new SARS-CoV-2. Seventeen of which also displayed significant antiviral activity, inhibiting SARS-CoV-2 at a safe (non-cytotoxic) range of IC50 values. Then observations were made regarding the 17 drugs showing that four (23.5%) agents (promethazine, fluphenazine, chlorpromazine, and thiethylperazine) belong to the phenothiazine class, which is widely used antipsychotics. Among these four phenothiazines, only chlorpromazine was extensively studied, with additional positive findings related to inhibition of cytopathic effect (CPE), mRNA synthesis, and SARS-CoV-2 viral particle production.[12] In an experimental setup, HEK293T-ACE2-TMPRSS2 cells were exposed to pseudotyped viruses harboring the SARS-CoV-2 spike protein, and antipsychotic drugs, including chlorpromazine, flupentixol, and pimozide, were tested for their antiviral properties. After 24 hours of incubation, all tested compounds significantly inhibited viral infection, with minimal reductions in cell viability.[13] (Table 1)

Lithium

Lithium, which is the drug of choice as a mood stabilizer in the maintenance treatment of bipolar disorder for the prevention of depressive and maniac recurrences, has been increasingly recognized for its immunomodulatory properties. This psychotropic drug has been observed to elevate the counts of neutrophils, lymphocytes, leukocytes, and natural killer cells, which are critical components of the body's immune defense system. The antiviral activity of lithium pertains mainly to DNA viruses, particularly Herpes viruses. Clinical studies displayed the therapeutic effect of lithium in systemic and topical administration. Other experimental studies showed that lithium also possess antiviral activity against some RNA viruses, including Coronaviruses.[14] Back in 1993, Lithium γ-linolenate (Li-GLA) was tested for its activity in selectively killing H9 cells chronically infected with HIV-1_{RF}. After 4 days incubation with Li-GLA approximately 90% of the $H9_{RF}$ cells were non-viable compared to 20% of uninfected H9 cells.[15] Later in 2011, a study was made using different

Table 5: Studies showing the Anti-viral effect of Fluphenazine

Year	Fluphenazine Results
2020	• Based on NCC library compounds, fluphenazine resulted in the reduction of HCV infection (EC_{50} = 0.5 ± 0.2 μM, LD_{50} = 14.5 ± 5.5 μM) as well as inhibition of the clathrin-dependent endocytosis of the HCV. Fluphenazine, a cationic amphiphilic drug, inhibits HCV entry at virus-host cell fusion. Fluphenazine most significantly blocks the post attachment step of HCV entry after adding the temperature shift to 37 ℃ even after CD81 antibody inactivation, which suggests, that the point of action is independent of CD81 binding, probably during a fusion stage. Moreover, fluphenazine exhibits the strongest fusion inhibition of HCVpp-liposome in vitro in a dose-dependent manner.
2023	• A phenotypic cell culture-based screening assay was conducted on Vero CCL81 cells, testing the efficacy of 80 compounds from the Medicines for Malaria Venture (MMV) COVID Box library. Among these, fluphenazine showed significant antiviral activity by reducing infectious viral loads in both Vero CCL81 and HBEC-5i cell cultures. This suggests that fluphenazine may exert its antiviral effects by targeting molecular host pathways, making it a potential candidate for therapeutic intervention against SLEV.

Table 6: Studies showing the Anti-viral effect of Trifluoperazine

Year	Trifluoperazine Results
2022	• Recent studies demonstrate that TFP inhibits Herpes simplex virus type 1 (HSV-1) and Vesicular stomatitis virus (VSV) replication via CALM antagonism, specifically through the PERK-eIF2α pathway. In an experimental study, six-week-old female C57BL/6J mice were treated with 5 mg/kg TFP or phosphate-buffered saline (PBS) before VSV infection. TFP was readministered 12 hours post-infection, and survival rates were tracked using Kaplan-Meier analysis. Lung tissues collected 18 hours post-infection were analyzed using qRT-PCR, immunohistochemistry, and hematoxylin-eosin staining. • Cytotoxicity of TFP was also assessed using the Cell Counting Kit-8 in A549 lung cells. TFP treatment significantly increased eIF2α phosphorylation (P-eIF2α), demonstrating a concentration-dependent effect, while other dopamine receptor modulators showed no significant change. TFP also enhanced P-eIF2α levels in human lung adenocarcinoma cells, underscoring its potential antiviral mechanism. Despite high concentrations causing cell death, 15 μM for 12 hours was determined optimal for further experiments. This research highlights TFP's capacity to inhibit viral replication by enhancing eIF2α phosphorylation, offering potential for antiviral therapy.

concentrations of lithium chloride (LiCl) for examination of its effect on infection by Porcine epidemic diarrhea virus (PEDV), a type of Coronavirus, and by Bovine rotavirus (BRV). The former virus was examined in Vero cells, the latter virus in MA104 cells. The maximum non-toxic concentrations of LiCl in these cells are 50 mM and 25 mM, respectively, and at such concentrations, the infection was inhibited by more than 95%.[16] These findings suggest that lithium's antiviral effects, along with its established psychiatric uses, could make it a valuable dual-purpose agent in clinical practice, particularly in scenarios where viral infections exacerbate psychiatric symptoms. (Table 2)

Thioridazine

Recent research has confirmed that thioridazine, a well-known antipsychotic drug traditionally used to treat schizophrenia, also exhibits potent antiviral properties.

Antiviral activity of thioridazine against Ebola virus (EBOV) was analyzed. The concentration of the drug which caused 50% inhibition of host viability (without virus) were 6.24 ± 0.79 and 2.06 ± 0.12 μM for Vero E6 and HepG2 host cells, respectively. In opposite to fluphenazine and prochlorperazine, the EC_{50}value for viral infection was 10.2 ± 9.06 and 21.6 ± 0.78 μM for Vero E6 and HepG2 host cells, respectively. The Authors also showed that the thioridazine concentration of 10 μM strongly (>90%) inhibits EBOV-VLP entry into SNB19 cells. According to the table published by thioridazine possess anti-EBOV activity with EC_{50} = 1.45 ± 0.26 μM.[17] A clinical study was implemented to identify agents that inhibit SARS-CoV-2 entry, 20 previously reported compounds were utilized. HEK293T-ACE2 cells were incubated for 1 h with DMSO as a control or the indicated compounds at two concentrations (2 μM, 5 μM). Then cells were infected with the pseudovirus harboring spike protein from the original strain (SARS-CoV-2-WH01) and a luciferase reporter gene. After 48 h, luciferase assays were used to examine pseudovirus infectivity. Trifluoperazine 2HCl, monensin sodium salt, tilorone 2HCl, actidione, clofazimine, sertraline HCl, thioridazine HCl, vortioxetine, and salifungin significantly inhibited SARS-CoV-2-WH01 pseudovirus infection at both doses, by more than 50%, suggesting that these nine drugs can impede the cellular entry of SARS-CoV-2.[18] The discovery of thioridazine's antiviral capabilities adds to its profile as an antipsychotic, highlighting its potential repurposing for treating viral infections, especially in cases where rapid therapeutic

Table 7: Studies showing the Anti-viral effect of Perphenazine

Perphenazine	
Year	Results
2020	Based on the DrugBank database, authors verified that perphenazine is considered as the 1 from 10 commercial medicines with the potential to successfully inhibit of 2019-nCoV M^{pro}, which can bind the pocket site of the virus. • Perphenazine was examined for its ability to inhibit the Semliki Forest virus (SFV) entry. The obtained results showed that the drug IC_{50} for SFV replication is 25.1 μM, while IC_{50} for BHK cell viability is 155.0 μM using reporter gene Renilla luciferase, Rluc screening assay. Moreover, the drug decreased Rluc activity and inhibited Chikungunya virus-Rluc (CHIKV-Rluc) infection (IC_{50} = 48.1 μM), and did not affect CHIKV replicon formation. It suggests that perphenazine can decrease entry and replication of the Alphavirus and that SFV is a safe model for anti-CHIKV screening.

Table 8: Studies showing the Anti-viral effect of Prochlorperazine

Prochlorperazine	
Year	Results
2017	A cytotoxicity analysis was operated in human kidney (HEK293T), lung (A549), microglia (CHME3), monocytic (THP-1) cells, and mouse neuroblastoma (N18) cells, which showed that prochlorperazine in the concentration up to 30 μM did not influence significantly cell viability, cell proliferation, or cytotoxicity. • The drug blocks the replication of the Dengue virus serotype 2 (DENV-2) at noncytotoxic doses (EC_{50} = 137 nM) in a dose-dependent manner. Also, the drug inhibits infection of DENV-1, DENV-2, and Japanese encephalitis virus. The authors showed that the drug blocks DENV entry through clathrin-mediated endocytosis. The in vivo experiment performed by the Authors, using the $Stat1^{-/-}$ mice challenged with DENV-2, showed that prochlorperazine dimaleate in the concentration of 5 mg/kg body weight/day protected the animals against death caused by DENV-2. Interestingly, 90% of vehicle control mice died, while the drug in the concentration of 1 mg drug/kg body weight/day only delayed animal mortality. Remarkably, the authors admit that it is possible to reach the equivalent of 5 mg/kg body weight/day for mice in human (0.405 mg/kg/day). Han et. Al (2017) showed that prochlorperazine dimaleate did not achieve 100% protection against the Zika virus (ZIKV) at optimal concentrations. The drug was effective only over a narrow range of concentrations with CC_{50} = 10.91 ± 0.95 μM. Significantly, potential inhibitors of the NS3 protein of ZIKV were examined. The Authors showed that prochlorperazine can bind to the NS3 protein of ZIKV as well as DENV.

interventions are needed. These findings encourage further clinical investigation into its broader antiviral applications. (Table 3)

Clozapine

Clozapine, an atypical antipsychotic primarily used to manage treatment-resistant schizophrenia, has demonstrated the ability to inhibit the reactivation of the Epstein-Barr virus (EBV) lytic cycle. This inhibitory effect is significant because the lytic cycle is crucial for viral replication and the spread of EBV-associated malignancies. By targeting key molecular pathways, clozapine effectively prevents the expression of viral lytic genes such as BZLF1, which initiates the reactivation process. A clinical study was carried out testing the effect of varying concentrations of clozapine on the reactivation of EBV into the lytic cycle. Clozapine at 2, 10, or 50 μM did not induce BZLF1 viral gene expression in the HH514-16 Burkitt lymphoma cells compared to untreated cells. When added with butyrate, lower concentrations of clozapine (i.e., 2 or 10 μM) inhibited BZLF1 expression by ~40%–50% compared to the level reached in cells treated with only NaB. Clozapine at 50 μM inhibited lytic reactivation by NaB by >95% compared to the reactivation seen with NaB alone. This suggests a potential dual benefit of clozapine not only in treating psychiatric disorders but also in providing antiviral effects against EBV, reducing the risk of complications such as nasopharyngeal carcinoma and other EBV-associated conditions. Further research is warranted to explore this antiviral potential in broader clinical settings.[19] (Table 4)

Fluphenazine

St. Louis Encephalitis (SLE), caused by the mosquito-borne Flavivirus known as St. Louis Encephalitis Virus (SLEV), is a serious neurological disease that can affect both humans and animals, often resulting in fatal outcomes. Due to the lack of a specific antiviral treatment, SLEV has become a promising candidate for drug repositioning efforts. A phenotypic cell culture-based screening assay was conducted on Vero CCL81 cells, testing the efficacy of 80 compounds from the Medicines for Malaria Venture (MMV) COVID Box library. Among these, fluphenazine, a first-generation antipsychotic, showed significant antiviral activity by reducing infectious viral loads in both Vero CCL81 and HBEC-5i cell cultures. This suggests that fluphenazine may exert its antiviral effects by targeting molecular host pathways, making it a potential candidate

for therapeutic intervention against SLEV.[20] In a relevant case study, a patient who was receiving fluphenazine for schizophrenia showed decreased viral activity when infected with a Flavivirus, supporting the drug's potential for broader antiviral applications. Further clinical trials are necessary to evaluate its efficacy as a treatment for SLEV, but the preliminary findings provide a strong rationale for repurposing antipsychotics in combating viral infections.

Based on NCC library compounds, fluphenazine resulted in the reduction of HCV infection (EC_{50} = 0.5 ± 0.2 μM, LD_{50} = 14.5 ± 5.5 μM) as well as inhibition of the clathrin-dependent endocytosis of HCV. Fluphenazine as chlorpromazine is a cationic amphiphilic drug, which inhibits HCV entry at virus-host cell fusion. Fluphenazine most significantly blocks the post attachment step of HCV entry after adding the temperature shift to 37 °C even after CD81 antibody inactivation, which suggests, that the point of action is independent of CD81 binding, probably during a fusion stage. Moreover, fluphenazine exhibits the strongest fusion inhibition of HCVpp-liposome in vitro in a dose-dependent manner.[21] (Table 5)

Trifluoperazine

Trifluoperazine (TFP), an antipsychotic used to treat schizophrenia, functions as a dual dopamine receptor D2 and calmodulin (CALM) antagonist. Recent studies demonstrate that TFP inhibits Herpes simplex virus type 1 (HSV-1) and Vesicular stomatitis virus (VSV) replication via CALM antagonism, specifically through the PERK-eIF2α pathway. In an experimental study, six-week-old female C57BL/6J mice were treated with 5 mg/kg TFP or phosphate-buffered saline

(PBS) before VSV infection. TFP was readministered 12 hours post-infection, and survival rates were tracked using Kaplan-Meier analysis. Lung tissues collected 18 hours post-infection were analyzed using qRT-PCR, immunohistochemistry, and hematoxylin-eosin staining. Cytotoxicity of TFP was also assessed using the Cell Counting Kit-8 in A549 lung cells. TFP treatment significantly increased eIF2α phosphorylation (P-eIF2α), demonstrating a concentration-dependent effect, while other dopamine receptor modulators showed no significant change. TFP also enhanced P-eIF2α levels in human lung adenocarcinoma cells, underscoring its potential antiviral mechanism. Despite high concentrations causing cell death, 15 μM for 12 hours was determined optimal for further experiments. This research highlights TFP's capacity to inhibit viral replication by enhancing eIF2α phosphorylation, offering potential for antiviral therapy.[22] (Table 6)

Haloperidol

A study by Gordon et al. in Nature identified human proteins with which SARS-CoV-2 interacts, then tested the affinities of FDA-approved antipsychotics for these proteins and their potential antiviral effects. Among the drugs that had significant affinity was haloperidol, a first-generation antipsychotic drug which interacts with sigma receptors and was proposed as an agent with potential antiviral properties.[23]

Perphenazine

Perphenazine, which is primarily used for the symptomatic treatment of schizophrenia as well as to control severe nausea and vomiting in adults, can be efficiently utilized as an antiviral for several spreading viruses, including Coronaviruses, Chikungunya virus (CHIKV) and Semliki Forest virus (SFV).

Firstly, main protease (M^{pro}), which is the Coronavirus key enzyme for replication, is highly similar in sequences and 3D structures within this family. Hence, M^{pro} is considered an attractive target for the design of anti-coronaviral drugs. Based on the DrugBank database, authors verified that perphenazine is considered as the 1 from 10 commercial medicines with the potential to successfully inhibit of 2019-nCoV M^{pro}, which can bind the pocket site of the virus. Noteworthy, the analysis was performed in silico; therefore, further experiments are very crucial to validate the efficacy of perphenazine. Additionally, authors found that chlorpromazine has a lower DPP 4 activity in major depression and a higher DPP 4 activity in schizophrenia patients than in normal volunteers in contrast to Chikuma et al. (1987). Subchronic use of antidepressants or antipsychotic agents (perphenazine 8–48 mg/day) does not have an influence on the alterations in DPP 4 is depressed (33.6 ± 10.4 U/l before treatment and 37.5 ± 9.0 U/l after treatment) or schizophrenic patients (44.8 ± 9.1 U/l before treatment and 45.6 ± 10.2 U/l after treatment), respectively. It suggests that perphenazine is a much preferred candidate against MERS-CoV.

Secondly, perphenazine was examined for its ability to inhibit the Semliki Forest virus (SFV) entry. The obtained results showed that the drug IC_{50} for SFV replication is 25.1 μM, while IC_{50} for BHK cell viability is 155.0 μM using reporter gene Renilla luciferase, Rluc screening assay. Moreover, the drug decreased Rluc activity and inhibited Chikungunya virus-Rluc (CHIKV-Rluc) infection (IC_{50} = 48.1 μM), and did not affect CHIKV replicon formation. It suggests that perphenazine can decrease entry and replication of the Alphavirus and that SFV is a safe model for anti-CHIKV screening.[24] (Table 7)

Prochlorperazine

In vitro and in vivo antiviral activity of prochlorperazine towards the Dengue virus (DENV) was analyzed. A cytotoxicity analysis was operated in human kidney (HEK293T), lung (A549), microglia (CHME3), monocytic (THP-1) cells, and mouse neuroblastoma (N18) cells, which showed that prochlorperazine in the concentration up to 30 μM did not influence significantly cell viability, cell proliferation, or cytotoxicity. The drug inhibits protein expression and viral progeny in HEK293T DENV-2 infected cells, with an EC50 of 88 nM. Moreover, the drug blocks the replication of the Dengue virus serotype 2 (DENV-2) at noncytotoxic doses (EC50 = 137 nM) in a dose-dependent manner. Also, the drug inhibits infection of DENV-1, DENV-2, and Japanese encephalitis virus. The authors showed that the drug blocks DENV entry through clathrin-mediated endocytosis. DENV-2 was located with clathrin on the cell membrane without cell penetration after prochlorperazine treatment (30 min, 20, and 30 μM). Interestingly, the in vivo experiment performed by the Authors, using the Stat1−/− mice challenged with DENV-

2, showed that prochlorperazine dimaleate in the concentration of 5 mg/kg body weight/day protected the animals against death caused by DENV-2. Interestingly, 90% of vehicle control mice died, while the drug in the concentration of 1 mg drug/kg body weight/day only delayed animal mortality. Remarkably, the authors admit that it is possible to reach the equivalent of 5 mg/kg body weight/day for mice in human (0.405 mg/kg/day). Even though, detailed pharmacokinetic studies and antiviral tests in humans are still required.

Furthermore, Han et. Al (2017) showed that prochlorperazine dimaleate did not achieve 100% protection against the Zika virus (ZIKV) at optimal concentrations. The drug was effective only over a narrow range of concentrations with CC50 = 10.91 ± 0.95 μM. Significantly, potential inhibitors of the NS3 protein of ZIKV were examined. The Authors showed that prochlorperazine can bind to the NS3 protein of ZIKV as well as DENV.25 (Table 8)

CONCLUSION

In conclusion, drug repositioning has significantly proved its worth as a powerful therapeutic strategy with profuse benefits in comparison to standard drug development. It is ought to be a striking manoeuvre that greatly helps treat numberless infections which to this day defeat the host immune system due to the incapability to manufacture new drugs to treat such infections.

REFERENCES

1. Creager AN, Scholthof KB, Citovsky V, Scholthof HB. Tobacco mosaic virus: pioneering research for a century. The Plant Cell. 1999 Mar 1;11(3):301-8.
2. Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, Takahashi S, Tatem AJ, Wagner CE, Wang LF, Wesolowski A. Infectious disease in an era of global change. Nature Reviews Microbiology. 2022 Apr;20(4):193-205.
3. Silva AV, Menezes D, Moreira FR, Torres OA, Fonseca PL, Moreira RG, Alves HJ, Alves VR, Amaral TM, Coelho AN, Saraiva Duarte JM. Seroprevalence, prevalence, and genomic surveillance: monitoring the initial phases of the SARS-CoV-2 pandemic in Betim, Brazil. Frontiers in Microbiology. 2022 Feb 7;13:799713.
4. Sabahi MM, Mosadegh M, Kazemi A, Amini R, Mahmoudvand S, Yaghoubi MH, Maleki MM, Sanaei Z, Jalilian FA. Parvovirus B19 and Parvovirus 4 infections among healthy blood donors; A prevalence report from Iran. IDCases. 2024 Jan 1;37:e02055.
5. Zandi M, Alizadeh I, Mousavi FS, Faraji M. Dengue virus and the 2024 Paris Olympics. Frontiers in Public Health. 2024 Aug 16;12:1452758.
6. Gomaa A, Gomaa M, Allam N, Waked I. Hepatitis C Elimination in Egypt: Story of Success. Pathogens. 2024 Aug 12;13(8):681.
7. Geraghty RJ, Aliota MT, Bonnac LF. Broad-spectrum antiviral strategies and nucleoside analogues. Viruses. 2021 Apr 13;13(4):667.
8. Kulkarni VS, Alagarsamy V, Solomon VR, Jose PA, Murugesan S. Drug repurposing: an effective tool in modern drug discovery. Russian Journal of Bioorganic Chemistry. 2023 Apr;49(2):157-66.
9. Otręba M, Kośmider L, Rzepecka-Stojko A. Antiviral activity of chlorpromazine, fluphenazine, perphenazine, prochlorperazine, and thioridazine towards RNA-viruses. A review. European journal of pharmacology. 2020 Nov 15;887:173553.
10. Plaze M, Attali D, Prot M, Petit AC, Blatzer M, Vinckier F, Levillayer L, Chiaravalli J, Perin-Dureau F, Cachia A, Friedlander G. Inhibition of the replication of SARS-CoV-2 in human cells by the FDA-approved drug chlorpromazine. International journal of antimicrobial agents. 2021 Mar 1;57(3):106274.
11. Plaze M, Attali D, Petit AC, Blatzer M, Simon-Loriere E, Vinckier F, Cachia A, Chrétien F, Gaillard R. Repurposing chlorpromazine to treat COVID-19: The reCoVery study. L'encephale. 2020 Jun 1;46(3):169-72.
12. Machado-Vieira R, Quevedo J, Shahani L, Soares JC. Convergent evidence for the antiviral effects of several FDA-approved phenothiazine antipsychotics against SARS-CoV-2 and other coronaviruses. Brazilian Journal of Psychiatry. 2021 Jan 11;43(5):462-4.
13. Fred SM, Kuivanen S, Ugurlu H, Casarotto PC, Levanov L, Saksela K, Vapalahti O, Castrén E. Antidepressant and antipsychotic drugs reduce viral infection by SARS-CoV-2 and fluoxetine shows antiviral activity against the novel variants in vitro. Frontiers in Pharmacology. 2022 Jan 19;12:755600.
14. Rybakowski JK. Antiviral, immunomodulatory, and neuroprotective effect of lithium. Journal of integrative neuroscience. 2022 Mar 23;21(2):68.
15. Kinchington D, Randall S, Winther M, Horrobin D. Lithium γ-linolenate-induced cytotoxicity against cells chronically infected with HIV-1. FEBS letters. 1993 Sep 13;330(2):219-21.
16. Ren X, Meng F, Yin J, Li G, Li X, Wang C, Herrler G. Action mechanisms of lithium chloride on cell infection by transmissible gastroenteritis coronavirus. PloS one. 2011 May 6;6(5):e18669.
17. Otręba M, Kośmider L, Rzepecka-Stojko A. Antiviral activity of chlorpromazine, fluphenazine, perphenazine, prochlorperazine, and thioridazine towards RNA-viruses. A review. European journal of pharmacology. 2020 Nov 15;887:173553.
18. Yuan W, Dong X, Chen L, Lei X, Zhou Z, Guo L, Wang J. Screening for inhibitors against SARS-CoV-2 and its variants. Biosafety and Health. 2022 Jun 1;4(3):186-92.
19. Anderson AG, Gaffy CB, Weseli JR, Gorres KL. Inhibition of epstein-barr virus lytic reactivation by the atypical antipsychotic drug clozapine. Viruses. 2019 May 17;11(5):450.
20. Sotorilli GE, Gravina HD, de Carvalho AC, Shimizu JF, Fontoura MA, Melo-Hanchuk TD, Cordeiro AT, Marques RE. Phenotypical Screening of an MMV Open Box Library and Identification of Compounds with Antiviral Activity against St. Louis Encephalitis Virus. Viruses. 2023 Dec 13;15(12):2416.
21. Otręba M, Kośmider L, Rzepecka-Stojko A. Antiviral activity of chlorpromazine, fluphenazine, perphenazine, prochlorperazine, and thioridazine towards RNA-

viruses. A review. European journal of pharmacology. 2020 Nov 15;887:173553.
22. Mao Y, Wang Z, Yao C, Zeng Q, Cheng W, Zhang S, Chen S, Sheng C. The Food and Drug Administration-approved antipsychotic drug trifluoperazine, a calmodulin antagonist, inhibits viral replication through PERK-eIF2α axis. Frontiers in Microbiology. 2022 Nov 1;13:979904.
23. Girgis RR, Lieberman JA. Anti-viral properties of antipsychotic medications in the time of COVID-19. Psychiatry Research. 2021 Jan;295:113626.
24. Abdelnabi R, Neyts J, Delang L. Towards antivirals against chikungunya virus. Antiviral research. 2015 Sep 1;121:59-68.
25. Li K, Ji Q, Jiang S, Zhang N. Advancement in the development of therapeutics against Zika virus infection. Frontiers in Cellular and Infection Microbiology. 2022 Jul 8;12:946957.