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Evaluating the Potential of Repurposing Commercially Available Drugs for the Treatment of Viral Infections

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Evaluating the Potential of Repurposing Commercially Available Drugs for the Treatment of Viral Infections

by

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Abstract:

Viral infections that are often overlooked as common seasonal illnesses such as influenza can rapidly become a public threat. They threaten society as new, more dangerous strains of these common viruses emerge and as strains develop resistance to current vaccines and antiviral treatments (Kochanek, Murphy, Xu, & Tejada-Vera, 2014). To combat this, the development of antiviral treatments with novel mechanisms of action is essential. Repurposing drugs instead of developing new drugs can save years of development time and hundreds of millions of dollars (DiMasi, Hansen, & Grabowski, 2003). To support the effort to discover drugs with unique mechanisms of action, a library of commercially available compounds was screened for antiviral activity. The compounds were tested against influenza A virus, enterovirus D68 (EV-D68), respiratory syncytial virus (RSV), and parainfluenza virus (PIV). In addition, fluoxetine hydrochloride (Prozac®) was tested for activity against EV-D68 as a proof-of-concept to verify our methodology and to support the idea that repurposed drugs may have antiviral activity with potential to function as effective treatments.

Antiviral activity was tested using in vitro antiviral assays that measure virus-induced cytopathic effect (CPE) in the presence of test compounds. CPE was measured by neutral red (NR) staining. Partial antiviral activity was observed for several compounds against influenza, and that activity was confirmed using a direct virus yield reduction assay (VYR) for multiple drug concentrations. However, no antiviral activity was observed for any of the compounds evaluated against EV-D68, RSV, or PIV. The drugs with anti-influenza activity may have potential for further development into effective antiviral treatments.

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Abstract:

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Antiviral activity was tested using *in vitro* antiviral assays that measure virus-induced cytopathic effect (CPE) in the presence of test compounds. CPE was measured by neutral red (NR) staining. Partial antiviral activity was observed for several compounds against influenza, and that activity was confirmed using a direct virus yield reduction assay (VYR) for multiple drug concentrations. However, no antiviral activity was observed for any of the compounds evaluated against EV-D68, RSV, or PIV. The drugs with anti-influenza activity may have potential for further development into effective antiviral treatments.

Introduction:

New viruses are constantly emerging as public health threats, and known viruses are constantly evolving resistance to current antiviral drugs. It is important to continue developing and testing antiviral drugs with new mechanisms of action to keep up with ever-changing viruses. This study focused on two dangerous viruses that are often overlooked: influenza and enterovirus D68 (EV-D68) (Messacar et al., 2016). Influenza is currently among the top causes of death in America (Kochanek, Murphy, Xu, & Tejada-Vera, 2014). Historical and current strains of the virus have been known to have very high mortality and transmission rates (Dawood et al., 2012; Reid, Taubenberger, & Fanning, 2004). The H5N1 strain, often known as the "bird flu," is a recently emerged strain that causes mortality in more than half of those infected (Beigel et al., 2005).

There are several vaccines and antiviral treatments available to prevent and treat influenza infections. However, influenza virus is subject to both antigenic shift and drift which help the virus evade immune responses, limiting the effectiveness of vaccines. In addition, influenza virus is able to develop resistance to antiviral therapies that are

widely used through the same mechanisms. An important example is amantadine, an antiviral first reported in 1963 designed for use against influenza (Maugh, 1976). Circulating influenza strains have since become so resistant to amantadine that the CDC currently recommends against its use (Fiore et al., 2011). H1N1 strains of influenza resistant to oseltamivir, a modern flu drug, have already been observed to be circulating at high frequency (Dharan et al., 2009).

The other focus virus of this study, EV-D68, is a recently emerging virus belonging to the Picornaviridae family, which also includes polio and rhinoviruses. EV-D68 causes respiratory disease and is mainly symptomatic only in children (Oberste et al., 2004). It was not a subject of public concern until 2014, when there was an outbreak of EV-D68 with over 1000 reported cases of severe respiratory disease. In addition, in the U.S. alone, there were at least 100 reported cases of acute flaccid paralysis associated with EV-D68 with symptoms similar to paralytic poliomyelitis. Since this outbreak, additional cases have been reported worldwide. The CDC estimates that millions more were infected but did not develop symptoms severe enough to warrant testing for the virus. Currently there are no FDA-approved treatments or vaccines available for prevention or treatment of EV-D68 (Messacar et al., 2016). Several antivirals have demonstrated activity *in vitro*, but many of these compounds are still in the early stages of investigation (Smee, Evans, Nicolaou, Tarbet, & Day, 2016). Due to the stringent approval process of the FDA, it is important to continue to evaluate new compounds for activity against EV-D68 as none of these compounds are guaranteed to be approved for use in humans.

To contribute to the development of new antiviral treatments for these viruses, test drugs were selected and tested in the course of this study under the rational of repurposing drugs for antiviral treatment. Developing new drugs is a costly in both money and time. Current estimates place those costs close to one billion dollars and 15 years of development time to secure FDA approval for each new drug that reaches the market (DiMasi et al., 2003; DiMasi, Hansen, Grabowski, & Lasagna, 1991). Repurposing already approved drugs can be completed in about two years and saves approximately 40% of the cost of developing new drugs (Chong & Sullivan, 2007). Our prediction was that by testing a commercially available library of drugs, we might be able to find compounds with antiviral activity suitable for clinical treatment of viral infections or future development of more potent antiviral treatments.

Beyond the cost-savings of repurposing drugs, another benefit of this strategy is that it allows the discovery of drugs with novel mechanisms of antiviral action to which viruses have not developed resistance. For example, a new anti-flu drug, Baloxavir marboxil (trade name Xofluza®), was approved in October 2018 (Hayden et al., 2018). Baloxivir functions by inhibiting the cap-snatching mechanism of the flu polymerase which is a novel mechanism of action among FDA-approved anti-flu therapies (Noshi et al., 2018). This compound is an excellent example of how compounds with novel mechanisms of action can be useful in fighting viral infections.

Methods

To find compounds with potential to be repurposed to treat influenza and EV-D68 infections, a commercially available chemical library containing many FDA-approved drugs was used as a starting point (TargetMol Fluorochemical Library, Catalog No. L5100). Due to a lack of resources, we were not able to screen all 586 compounds in the library. Therefore, the potential for antiviral activity of each compound was estimated based upon searches using Google Scholar and PubMed to find any data for each compound. From the library, a total of 33 compounds were selected for testing against influenza and EV-D68. According to compound availability, 14 compounds were also tested against respiratory syncytial virus (RSV) and parainfluenza virus (PIV). The full list of compounds and the rational for testing each one is displayed in Supplementary Table 1.

Compounds were selected for testing because they previously demonstrated activity against other viruses or belonged to classes of compounds known to occasionally demonstrate antiviral activity, which could bring mechanisms of action previously unused in influenza and EV-D68 treatment to light. One class of compounds broadly selected for testing was protease inhibitors. When appropriately targeted to viral proteins, protease inhibitors block viral replication. Another broad class of drug tested in this experiment were drugs targeting membrane transporters or ion channels Though many of these compounds have known uses in neurology, they have also occasionally demonstrated antiviral activity.

In addition to testing the library of drugs mentioned above, to validate our assays and the concept of repurposing drugs for use as antiviral treatments, the well-known antidepressant fluoxetine hydrochloride, better known as Prozac®, was evaluated for antiviral activity against EV-D68 and coxsackievirus B3 (COXV). The rationale for this was based on previous studies that demonstrated the *in vitro* activity of fluoxetine against EV-D68 and coxsackievirus B3 (Rhoden, Zhang, Nix, & Oberste, 2015a; Ulferts et al., 2013). Replicating these positive results also lends support to the idea that repurposed drugs can have antiviral activity. However, it is important to note that despite the effectiveness of fluoxetine *in vitro*, fluoxetine was not effective in animal models of EV-D68 infection (Hixon, Clarke, & Tyler, 2017).

All viruses used in this study except for PIV were obtained from ATCC (Manassas, VA) and propagated in cell culture. The strains were influenza A H1N1 (A/California/07/2009, ATCC VR-1894), enterovirus D68 (US/KY/14-18953, ATCC VR-1825), influenza A PR8 (A/PR/8/34 TC Adapted, ATCC VR-1825), respiratory syncytial virus strain A2 (ATCC VR-1540), and coxsackievirus B3 (ATCC VR-688). Parainfluenza virus type 3 strain 14702 was acquired from a clinical isolate (J. Bouvin, Hosp. St. Justine, Montreal, Canada). These viruses were propagated in cell culture to create virus stocks. The cell lines used in this study were MDCK (ATTC CCL-34), RD (ATTC CCL-136), MA-104 (ATTC CRL-2378.1), and Vero 76 (ATCC CRL-1587).

All drugs were tested using a primary cytopathic effect (CPE) reduction assay as described by Smee et al., 2016. Briefly, testing was completed by evaluating 4 log-dilution concentrations of each compound in 96-well plates. At each concentration, 3 wells of near-confluent cell cultures seeded the day previously were infected with virus and exposed to the test compound to measure the protection from virus-induced CPE offered by the compound. Further details of cell seeding and viral dose for each virus can be found in Table 1. Two uninfected wells were also tested at each drug concentration to determine the cytotoxicity of the compounds. Untreated cell controls and virus controls were also included on each plate. Positive control compounds with known antiviral activity were included with each test.

Following viral incubation, neutral red was added to each well and the plates were incubated for two hours at 37°C. Neutral red is absorbed only by living cells. Following incubation, the wells were aspirated to remove neutral red and rinsed with PBS to ensure all unincorporated dye was removed, and an extraction buffer was added to solubilize the neutral red. The percentage of living cells in each well was estimated by comparing absorbance as measured at 540 nm by a spectrophotometer to that of untreated cell control wells. Virus-induced CPE was normalized to untreated virus control wells. The 50% effective (EC50, virus-inhibitory) concentrations and 50% cytotoxic (CC50, cell-inhibitory) concentrations were determined through linear regression on a Microsoft Excel spreadsheet. Dividing the CC50 values by the EC50 values gives a selectivity index (SI) value, which is used to determine the antiviral efficacy of a compound. A higher SI value is indicative of increased antiviral activity and decreased cellular cytotoxicity.

Additionally, the compounds that demonstrated activity against influenza were also tested by virus yield reduction (VYR) to determine the concentration at which 90% of active virus production was inhibited by the compound (EC90). For this test, compounds were tested at 8 half-log concentrations. Prior to neutral red staining, samples of supernatant were harvested from each drug concentration. These samples were evaluated for viral titer using an end-point dilution method and were calculated using the Reed-Muench method (Reed & Muench, 1938). The titers from each concentration of the compound were compared to the untreated virus control to determine viral load reduction, and EC90s were calculated by linear regression.

Results:

The rationale of this project was partially encouraged by the previous successful *in vitro* testing of fluoxetine against EV-D68 and coxsackievirus (Rhoden et al., 2015a; Ulferts et al., 2013; Zuo et al., 2012). As shown in Table 2, our results matched closely with the published results, which validated our *in vitro* testing methods.

When tested against influenza A, several of the drugs demonstrated modest antiviral activity, as shown in Table 3. JNJ-42041935 appeared to have the most activity

as measured by EC90, but its activity was still modest. Many of these drugs were also partially active against influenza PR8, as shown in Table 4.

All 33 compounds were evaluated against EV-D68, but no antiviral activity was observed. All anti-EV-D68 SI values were less than 2. Of those 33 compounds, 14 were tested against RSV and PIV, and none demonstrated activity against either virus (data in Supplementary Table 1).

Discussion and Conclusions:

The close agreement regarding the antiviral activity of fluoxetine between the published data and our measured results validated our ability to accurately measure antiviral activity in repurposed pharmaceuticals (Rhoden, Zhang, Nix, & Oberste, 2015b; Ulferts et al., 2013; Zuo et al., 2012). The confirmed evidence of fluoxetine's activity against EV-D68 supports the idea that pharmaceuticals designed for other purposes can have potential to be repurposed as antiviral treatments.

Most of the compounds found in this study were not active against the viruses tested. By offering this information for publication, we hope to help other researchers avoid needlessly re-testing these compounds for antiviral activity. Many publications about new antiviral compounds often focus on a specific compound or set of compounds that are highly active. However, they occasionally include lists of other inactive compounds or strains of virus that were resistant to the focus compound to avoid repetition (Kumaki et al., 2018; Smee, Evans, Nicolaou, Tarbet, & Day, 2016; Smee, Hurst, Day, & Geiben-Lynn, 2014).

The compounds with the highest partial activity found in this study shown in Tables 2 and 3 are not active enough to be considered for immediate development as antiviral treatments. However, they may indicate a good starting point for the investigation and synthesis of new types of compounds. Normally SI values of greater than 10 are desirable to indicate noteworthy activity, but SI values of 4-9 can suggest partial activity. Determining the exact mechanism by which these compounds partially inhibit influenza may lead to development of stronger compounds with similar modes of action. The best way to continue this research would be to investigate the molecular mechanisms of the partially active drugs found in this study. For example, 5α-reductase inhibitors, a family of chemicals which include Dutasteride, one of the most active anti-flu compounds in this study, were shown to be highly active against influenza in a previous study. (Al-Mohizea, Al-Omar, Abdalla, & Amr, 2012). By discovering their specific molecular mechanisms of action, some of the other compounds in this study could be developed into chemical families with similarly potent results.

Another avenue to further pursue this research would be to continue to screen other compound libraries for FDA-approved drugs with potential to be repurposed as antiviral treatments. The major limitation of this study was that due to funding reasons, we were forced to screen a relatively narrow selection of drugs. Future studies could expand on our work by testing a greater variety of compounds for antiviral activity.

Table 1: Virus testing parameters.

Virus	Cells	Cells per Well	$CCID_{50}$ per Well	Days Incubated
H1N1 Cali	MDCK	60,000	63	3
H1N1 PR8	MDCK	60,000	63	3
EV-D68	RD	60,000	160	3
RSV	MA-104	40,000	41	4
PIV	MA-104	40,000	31	7

H1N1 (Cali) refers to influenza A/California/07/2009, and H1N1 (PR8) refers to influenza A/PR/8/34 TC adapted. RSV and PIV refer to respiratory syncytial virus and parainfluenza virus, respectively. $CCID_{50}$ is the 50% cell culture infectious dose- a dose of virions that will infect 50% of cell cultures on average. The $CCID_{50}$ number for each virus is how many infectious doses each infected well received to ensure infection.

Legend for Tables 2-4: EC50: 50% effective concentration; concentration at which 50% of viral-induced cytopathic effect was prevented. CC50: 50% cytotoxic concentration; concentration where compound toxicity caused 50% cell death. SI: selective index; SI is found by dividing EC50 by CC50. Higher SI values indicate increased antiviral activity and decreased compound toxicity.

Table 2: Comparing published fluoxetine antiviral activity with study measures.

	Measured Values µM			Published Values µM		
Virus	**EC50**	**CC50**	**SI**	**EC50**	**CC50**	**SI**
EV-D68	1.21±0.38	13.01±3.26	10.8	0.34-1.05	n/a	n/a
COXV	0.98	9.3	9.5	3.36±.47	28	8.3

The published EV-D68 values are from Rhoden et al., 2015, and represent the range of EC50 values measured for 4 different strains of EV-D68. The Coxsackievirus values were obtained from Zuo et al., 2012.

Table 3: Antiviral activity against Influenza A/California/07/2009 (H1N1).

	EC50	CC50	SI	EC90	SI BY VYR
Dutasteride	26 ± 27	>125 ± 0	>4.8	69 ± 79	>1.8
P22077	17 ± 9.2	52 ± 15	3.2	31 ± 16	1.7
JNJ-42041935	40 ± 21	120 ± 12	2.9	22 ± 6.4	5.3
AEBSF HCl	32 ± 25	>125 ± 0	>3.9	86 ± 55	>1.4
Ribavirin	38 ± 30	>4100	>109	170 ± 8.6	>265

EC50 and CC50 values are in µM. Ribavirin served as a positive control. EC50 and CC50 values were tested in four replicates. EC90 was tested in duplicate.

Table 4: Antiviral activity against TC-adapted Influenza A/PR/8/34

	EC50	CC50	SI	EC90	SI BY VYR
Dutasteride	31 ± 9.9	>125 ± 0	>4.0	28 ± 14	>4.5
P22077	14 ± 7.3	29 ± 14	2.0	18 ± 4.9	1.6
JNJ-42041935	120 ± 17	>125 ± 0	>1.1	37 ± 4.7	>3.4
AEBSF HCl	27 ± 4.2	>125 ± 0	>4.6	34 ± 15	>3.7
Ribavirin	13 ± 5.2	>4100	281	15 ± 1.8	>283

EC50 and CC50 values are in μM. Ribavirin served as a positive control. EC50 and CC50 values were tested in three replicates. EC90 was tested in duplicate.

Supplementary Table 1: Antiviral activity of multiple FDA-approved or bioactive compounds against influenza virus (H1N1), respiratory syncytial virus (RSV), and parainfluenza virus (PIV). SI values are the 50% cytotoxic concentration divided by the 50% effective concentration. Higher SI values indicate higher antiviral activity and lowered cellular toxicity.

Compound Name and CAS Number	H1N1 (Cali) SI	H1N1 (PR8) SI	EV-D68 SI	RSV SI	PIV SI	FDA Approval	Target	Testing Rationale
Dutasteride *164656-23-9*	4.8‡	4†	0*	0	0	Approved	Reductase inhibitor	Similar compounds have high antiviral activity. (Al-Mohizea et al., 2012)
AEBSF hydrochloride *30827-99-7*	3.9‡	4.6†	0*	0	0	N/A	Serine Protease inhibitor	Reported activity against RSV. (Van der Gucht et al., 2017)
P22077 *1247819-59-5*	3.2‡	2†	1.2	0	0	N/A	DUB inhibitor	Active against HIV-1. (Setz et al., 2017)
Ketanserin *74050-98-9*	3	0	0	0	0	Approved	5-HT Receptor antagonist	Active against JCV. (Nukuzuma, Nakamichi, Nukuzuma, & Takegami, 2009)
JNJ-42041935 *1193383-09-3*	2.9‡	1.1†	0.8*	0	0	N/A	HIF/HIF Prolyl-Hydroxylase	Protease inhibitor.
Odanacatib (MK-0822) *603139-19-1*	2.7*	*nt*	0	*nt*	*nt*	Approved	Cysteine Protease inhibitor	Protease inhibitor.
Trelagliptin *865759-25-7*	2.4*	*nt*	0	*nt*	*nt*	Approved	DPP-4 inhibitor	Protease inhibitor.
PMSF *329-98-6*	2.4*	*nt*	0	*nt*	*nt*	N/A	Multiple Protease inhibitor	Protease inhibitor.
YO-01027 (Dibenzazepine) *209984-56-5*	2.3*	*nt*	0	*nt*	*nt*	N/A	Gamma-secretase inhibitor	Protease inhibitor.

Compound Name and CAS Number	H1N1 (Cali) SI	H1N1 (PR8) SI	EV-D68 SI	RSV SI	PIV SI	FDA Approval	Target	Testing Rationale
MK3102 *1226781-44-7*	2.2*	*nt*	0	*nt*	*nt*	Approved	DPP-4 inhibitor	Protease inhibitor.
Dolutegravir sodium *1051375-19-9*	2*	*nt*	0	*nt*	*nt*	Approved	Integrase inhibitor	Protease inhibitor.
Paliperidone *144598-75-4*	1.9*	*nt*	0	*nt*	*nt*	Approved	Neurogenic antagonist	In silico evidence of antiviral activity. (Patel & Kukol, 2017)
Alvelestat (AZD9668) *848141-11-7*	1.7†	0	0*	0	0	Clinical Trials	Serine Protease inhibitor	Protease inhibitor.
IU1 *314245-33-5*	1.5*	*nt*	0	*nt*	*nt*	N/A	DUB inhibitor	Active against Dengue virus and against other flaviviruses. (Nag & Finley, 2012)
PD 151746 *179461-52-0*	1.5*	*nt*	0	*nt*	*nt*	N/A	Cysteine protease inhibitor	In silico evidence of antiviral activity. (Byler, Collins, Ogungbe, & Setzer, 2016)
Z-FA-FMK *197855-65-5*	1.4*	*nt*	0	*nt*	*nt*	N/A	Cysteine Protease inhibitor	Active against reoviruses. (Kim et al., 2010)
Flufenamic acid *530-78-9*	1.4	4	0	0	0	Approved	COX inhibitor	Active against influenza and encephalomyocarditis. (Chan et al., 2013; Inglot, 1969)
Danoprevir (ITMN-191) *8508-76-88-9*	1.3*	*nt*	0	*nt*	*nt*	Clinical Trials	HCV Protease inhibitor	Protease inhibitor
Sitagliptin *486460-32-6*	1.2*	*nt*	0	*nt*	*nt*	Approved	DPP-4 inhibitor	Protease inhibitor
Trelagliptin succinate *1029877-94-8*	0†	0	0*	0	0	Approved	DPP-4 inhibitor	Protease inhibitor

Compound Name and CAS Number	H1N1 (Cali) SI	H1N1 (PR8) SI	EV-D68 SI	RSV SI	PIV SI	FDA Approval	Target	Testing Rationale
Elvitegravir (GS-9137, JTK-303) *697761-98-1*	0	*nt*	0	*nt*	*nt*	Approved	Integrase inhibitor	Protease inhibitor
Tiplaxtinin (PAI-039) *393105-53-8*	0*	*nt*	0	*nt*	*nt*	Clinical Trials	PAI inhibitor	Active against rhabdoviruses. (Estepa & Coll, 2015)
DAPT (GSI-IX) *208255-80-5*	0*	*nt*	0	*nt*	*nt*	Clinical Trials	Gamma-secretase inhibitor	Protease inhibitor
LY411575 *209984-57-6*	0*	*nt*	0	*nt*	*nt*	N/A	Gamma-secretase inhibitor	Active against Hepatitis C. (Otoguro, Tanaka, Kasai, Yamashita, & Moriishi, 2016)
Lomerizine hydrochloride *101477-54-7*	0*	*nt*	1.1	*nt*	*nt*	Approved	Calcium Channel inhibitor	Active against JEV and Hepatitis B. (van de Klundert, Zaaijer, & Kootstra, 2016; Wang et al., 2017)
Teriflunomide *108605-62-5*	0*	*nt*	0	*nt*	*nt*	Approved	Dehydrogenase inhibitor	Active against TMEV. (Gilli, Li, Royce, DiSano, & Pachner, 2017)
Mosapride Citrate *112885-42-4*	0*	*nt*	0	*nt*	*nt*	Approved	5-HT Receptor agonist	Neurological drug.
Leflunomide *75706-12-6*	0	0	1.7	2.1	0	Approved	Protein-tyrosine kinase 2 antagonist; AhR agonist; Dehydrogenase inhibitor	Active against EV-71, CMV, and HPV. (Hung, Shih, Chang, Fang, & Hsu, 2014; Waldman et al., 1999)

Compound Name and CAS Number	H1N1 (Cali) SI	H1N1 (PR8) SI	EV-D68 SI	RSV SI	PIV SI	FDA Approval	Target	Testing Rationale
Trifluoperazine dihydrochloride *440-17-5*	0	0	0	0	0	Approved	Neurological targets.	Active against Influenza and Epstein-Barr. (Nemerow & Cooper, 1984; Ochiai, Kurokawa, & Niwayama, 1991)
Mefloquine hydrochloride *51773-92-3*	0	0	0	0	0	Approved	Hemozoin synthesis inhibitor	Active against multiple viruses. (Brickelmaier et al., 2009)
Flupenthixol dihydrochloride *51529-01-2*	0	0	0	0	0	Approved	Dopamine Receptor antagonist	Active against HSV-22. (Kristiansen, Andersen, Vestergaard, & Hvidberg, 1991)
Spiperone *749-02-0*	0	0	0	0	0	Approved	Dopamine Receptor antagonist	Active against several polyamaviruses.(Goodwin, Atwood, & DiMaio, 2009)
Trifluprомazine hydrochloride *1098-60-8*	0	0	0	0	0	Approved	Neurogenic antagonist	Active against MERS, SARS, and HSV-1. (Dyall et al., 2014; Purohit et al., 2012)

H1N1 (Cali) refers to influenza A/California/07/2009, and H1N1 (PR8) refers to influenza A/PR/8/34 TC adapted. Compounds were tested with different numbers of replicates against each virus according to compound availability and according to the activity observed in first and second replicate activity of each compound. *nt:* Not tested. *2 replicates. †3 replicates. ‡4 replicates. Numbers with no notation had 1 replicate assay.

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Reflective Writing

After dozens of hours spent using pipettors, microscopes, spreadsheets and databases, I finally found the end results of all my hard work: that none of the compounds I had spent so much time and effort researching, selecting, and testing were active enough to make a real difference in the world or even to be published in a professional journal. However, I also found something else: that I genuinely enjoy research and that I feel that asking and answering questions has intrinsic value even when the results are unremarkable. To me personally, that result was worth all the effort.

This research project has been an incredible experience for me as a biology student and taught me many important lessons about what a career in research might be like. This is especially important to me as pursuing a research career remains my current plan for the future. The most important things that I've gained from this project have been learning about my own motivations, increasing and understanding my work ethic, and an opportunity for growth as a scientist, none of which are things I could have learned in a classroom.

Regarding my motivations, I've discovered more about what kind of questions I'm interested in answering as a scientist. I've found that the more specific the question, the more interested I am in it. I'm interested in deeply looking into small details rather than trying to find shallow answers for many questions. Unfortunately, I've made that discovery because of the relatively shallow nature of this project. I've taken many compounds with minimal evidence of possible antiviral activity and tested them against a panel of viruses to see if they worked. As the project has progressed, I've become more interested in finding out *why* the compounds that have showed some measure of activity have worked. What are they binding to in the virus or the cell that is inhibiting viral replication? Is it possible to refine the chemical structure of these compounds to have a stronger antiviral effect? Could the compound work in animals or even humans? In the future, I'd consider large-scale screening like what I've done for this project as more of a preliminary step to more specific and interesting questions.

Even though I'd prefer to work with more specific questions, another important thing I've learned through this project is that I can push through stalls, delays, and mistakes in the course of a project. This has been by far the largest project I've ever worked on, and has required an incredible amount of work to complete. Oftentimes the parts that required the most grit and determination were parts of the project that I didn't think would be very difficult or take a long time, only to see them balloon into formidable challenges. Taking this project to completion taught me what it feels like to approach a difficult problem, and step by step whittle it into manageable chunks until the whole things is done. I've also learned a lot about what it's like to work with a committee and professors, who though concerned and caring, are very busy. I've found that this type of committee work is not like turning in a test or an assignment in a normal class – it

requires a lot more coordination and teamwork with them to make sure things get done on a reasonable schedule.

Finally, this project taught me a lot of specific lessons about what it will take to become a full-blown research scientist. One thing that I quickly realized that I did not know as much about as I thought I did was the importance and power of organization. Simple things like writing everything down and keeping all your files and information in as few places as possible that now seem obvious were not part of my workflow when I started this project. Though I'm sure I had been taught those things in lab classes and in other places, the tough teacher of experience was what really convinced me that these things are actually important. This project has also taught me a lot about the power of planning experiments well. As a biologist, many of our experiments are dependent on the growth and progress of organisms at a time scale we can't control and can only plan around. The longer this project went on, the better I got at planning individual experiments to work on a timeframe that worked well for me. Also, I learned that the more time spent time planning and preparing for an experiment, the more smoothly it would go. For example, I found that if I spent time the day before an experiment labeling all of the sample containers and plates and rounding up supplies, things would go ten times more smoothly than if I had tried to do that all on the same day as when I actually ran the experiment.

Another important lesson this project taught me about being a scientist was how to deal with mediocre results through no fault of your own. Beyond dealing with disappointment and waning motivation to continue testing in a project where the preliminary results weren't too exciting, it also showed me how to adapt to the results you get. For example, if I was to continue in this project, I'd be enthusiastic to investigate the moderately active anti-flu compounds that I found to see why they work, which would likely be critical if this was a doctoral-level thesis or a major grant project.

Beyond teaching me skills I could use as a future scientist, this project also has directly helped me with my future career as a scientist. I recently accepted an invitation to study in Dartmouth College's Molecular and Cellular Biology doctoral program. I have no doubt that being able to talk about how I largely independently planned, executed, and reported a large project like this helped to demonstrate my work ethic and growing skills as a scientist.

Though the compounds I found in this study aren't ready to be used to treat viral infections, I hope that what I learned in the processes of testing them can make a difference in the world. My motivation to become a research biologist is a genuine hope that I can use my talents to help out people. Everyone you and I know has had their life touched by vaccines, pharmaceuticals, and medical science. My hope is that I too can learn to ask and answer the kinds of questions that led to these discoveries that will improve people's lives across the world.

Author Biography

Brennan will be completing his Honors degree in Biology with an emphasis in Cellular and Molecular Biology in Spring 2019 after four years of study at Utah State University. As a student, he had the chance to work as a lab technician and undergraduate researcher at the Institute for Antiviral Research for more than two and a half years. Immediately following graduation, he plans to live in China for three months over the summer as a way of cementing his minor in Mandarin Chinese. On his return to America, he will begin doctoral studies in Molecular and Cellular Biology in Dartmouth College in New Hampshire. Though he has not decided on a specific focus within Molecular Biology, he is enthusiastic to begin lab rotations to find a sub-topic to continue pursuing as a professional researcher.