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COVID-19 Infection Prevention through Natural Product Molecules

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ABSTRACT

This project evaluates natural product molecules with the potential to prevent 2019-nCOV infection. The molecules theoretically work by blocking the ACE2 protein active site in human airways. Previous work focused on modeling candidate natural compounds, but this work examined baicalin, hesperetin, glycyrrhizin, and scutellarin in experimental in vitro studies, which included recombinant protein inhibition assays, cell culture virus inhibition assays, and cytotoxicity assays. The project delivered selectivity indices (ratio that measures the window between cytotoxicity and antiviral activity) of the four natural compounds that will help guide the direction of SARS-CoV-2 therapeutic development.

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1. INTRODUCTION

The COVID-19 pandemic requires urgent countermeasures, and naturally occurring product molecules are attractive for therapeutic or prophylactic usage. A fairly recent preprint article (H. Chen; Q. Du. "Potential natural compounds for preventing 2019-nCoV infection", 01/20/2020, not yet peer-reviewed) focuses on the modeling of five different natural product molecules for blocking the ACE2 protein active site in human airways, the select site where COVID-19 enters cells and replicates. Putative binding poses were generated using the AutoDock Vina software package with the default scoring function (Trott O, Olson AJ, 2010). Their modeling shows that all five compounds could potentially have inhibition effects, but no actual experimental work had been performed. This high-risk project sought to investigate the utility of four of these compounds (scutellarin, glycyrrhizin, baicalin, hesperetin) through three experimental assays:

1) Determine the *in vitro* toxicity of these compounds in several SARS-CoV-2 permissive tissue culture lines

2) Determine the inhibitory ability of these compounds in an ACE2::SARS-CoV-2 spike protein ELISA assay

3) Determine the effectiveness of these compounds at stopping SARS-CoV-2 infection *in vitro* cell experiments.

Overall, compounds exhibited no toxicity until extreme dosages and times were reached. However, neither the ELISA assay nor cell assay using pseudotyped SARS-CoV-2 showed any inhibition of infection.

2. EXPERIMENTS

Experiment 1: Compound Cytotoxicity Assays

Three cell types were assayed (VERO, kidney epithelial type; A549, lung epithelial type; and HepG2, liver hepatocyte) at 24/48/72 hours of compound treatment. Blasticidin, an antibiotic, was used as a control.

Results confirmed that the natural product molecules are non-toxic, though at 72 hours of treatment some compounds showed toxicity at extremely high dosages (right side of each graph). These compounds were hesperetin, a flavonoid found in citrus fruits, and baicalin, a flavone used in herbal supplements.

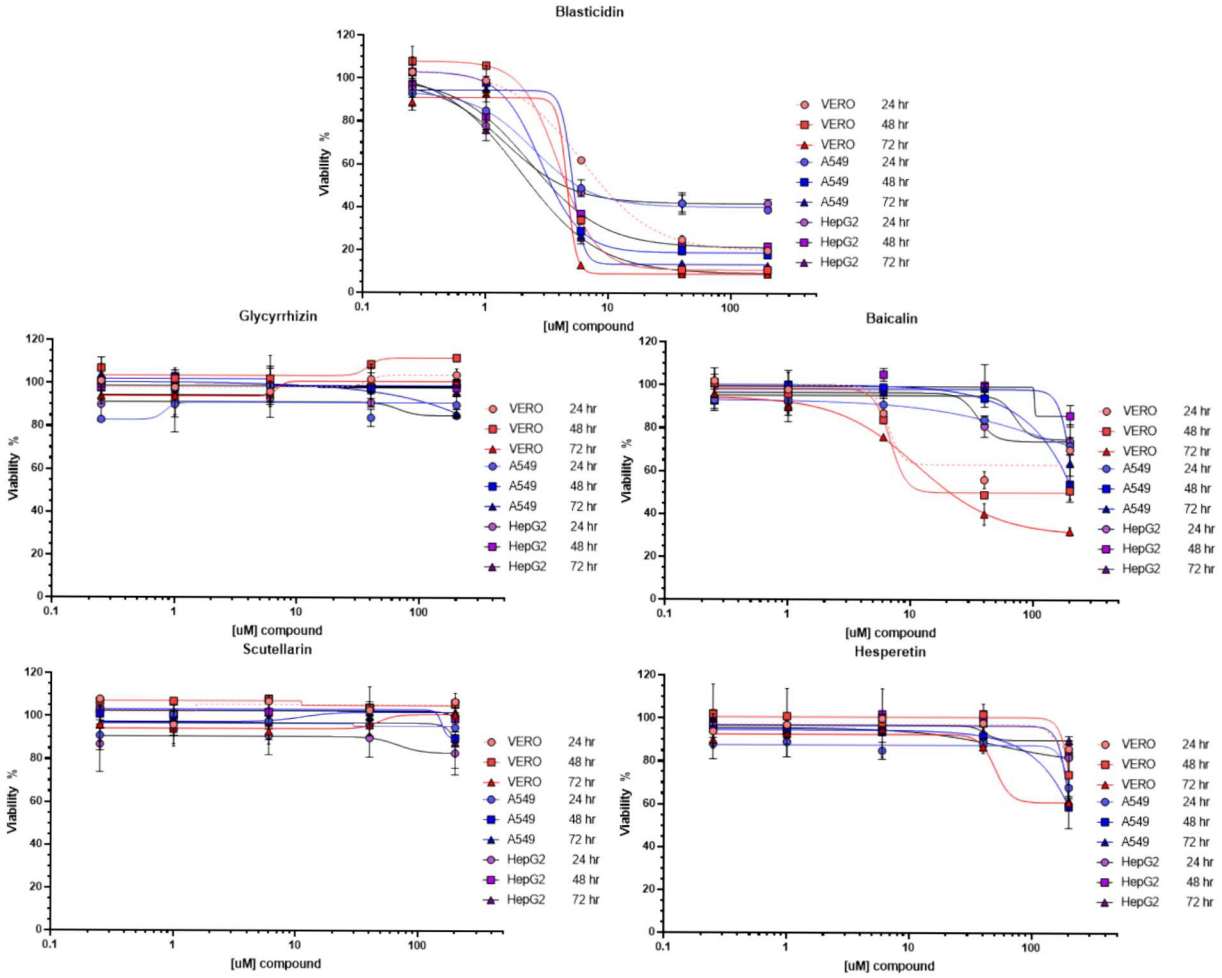

Figure 1. Cell viability graphs of VERO, A549, and HepG2 cell lines with the four natural product molecules of interest. Error bars are standard deviations of triplicate assay runs.

Experiment 2: ACE2 Binding to SARS-CoV-2 Spike Protein via ELISA Inhibition Assay

ELISA assay was done with ACE2-Fc at 20 ng/mL treatment (approximately 150-170 picomolar). Compound treatment was used at up to 150 uM.

Results from the ELISA assay reveal that none of the natural product molecules inhibit the binding of ACE2-Fc to SARS-CoV-2 viral spike protein. However, this assay was done using recombinant spike-protein that is surface-bound, which may have resulted in steric differences that are not physiologically representative. Without a known positive-control inhibitor to utilize in this assay, we chose to proceed to the final cell-based assay using live virus.

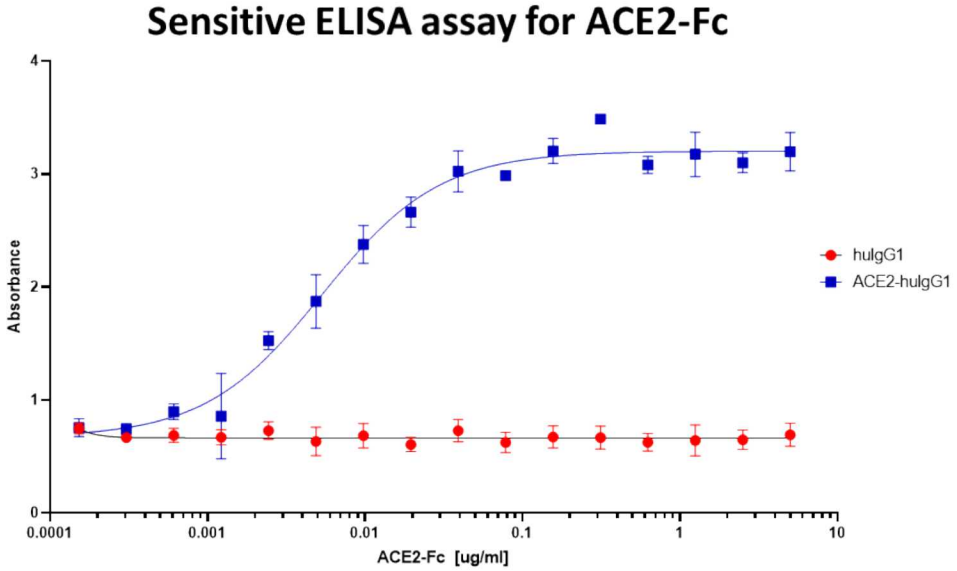

Figure 2. ELISA assay for ACE-2-Fc. Error bars are standard deviations of triplicate assay runs.

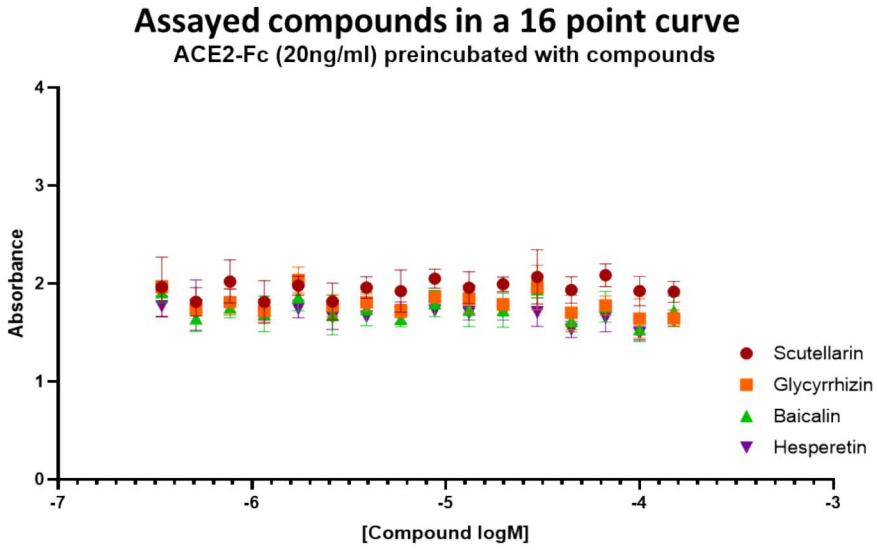

Figure 3: ELISA inhibition assay with the four natural product molecules of interest. Compounds do not inhibit ACE2-Fc binding to viral spike. Error bars are standard deviations of triplicate assay runs.

Experiment 3: ACE2 Inhibitor Screen using a Cell-Based Assay

The experiment began by pre-treating virus-permissive cells (A549, lung epithelial type) with the compounds for one hour, and then infecting them with a SARS-CoV-2-rLuc pseudotyped virus. After one hour of incubation, the media was changed. Virus-infection levels were assayed 16 hours later by addition of the substrate for luciferase and evaluated by mean chemiluminescent values per well. The compound Imatinib was used as a positive control and showed near complete reduction of pseudotyped-virus infection.

The natural molecules showed similar luciferase values as the untreated virus-only control, indicating no reduction in virus-infection in the cell-based assay up to 10 μM.

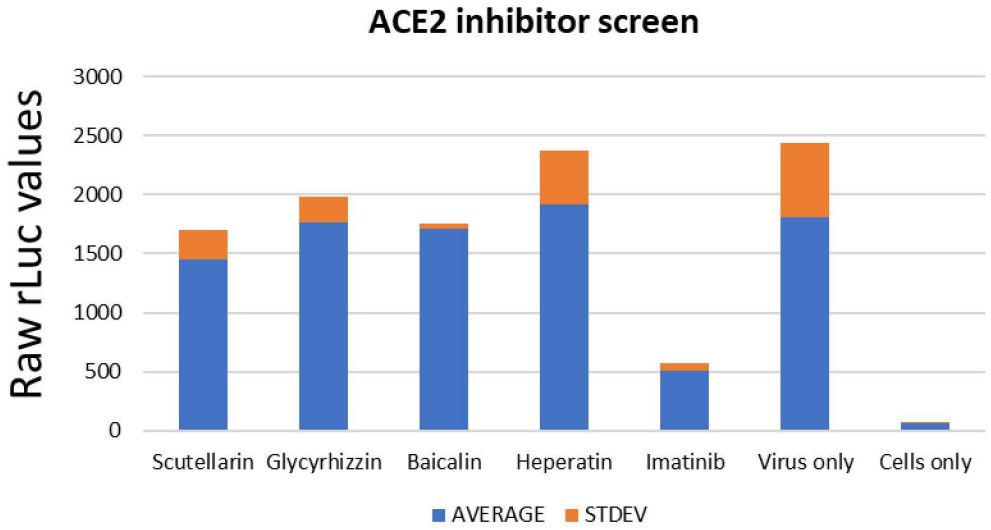

Figure 4. Luciferase values for the four natural product molecules of interest with SARS-CoV-2 pseudotyped virus. Concentration of compounds = 10 μM.

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