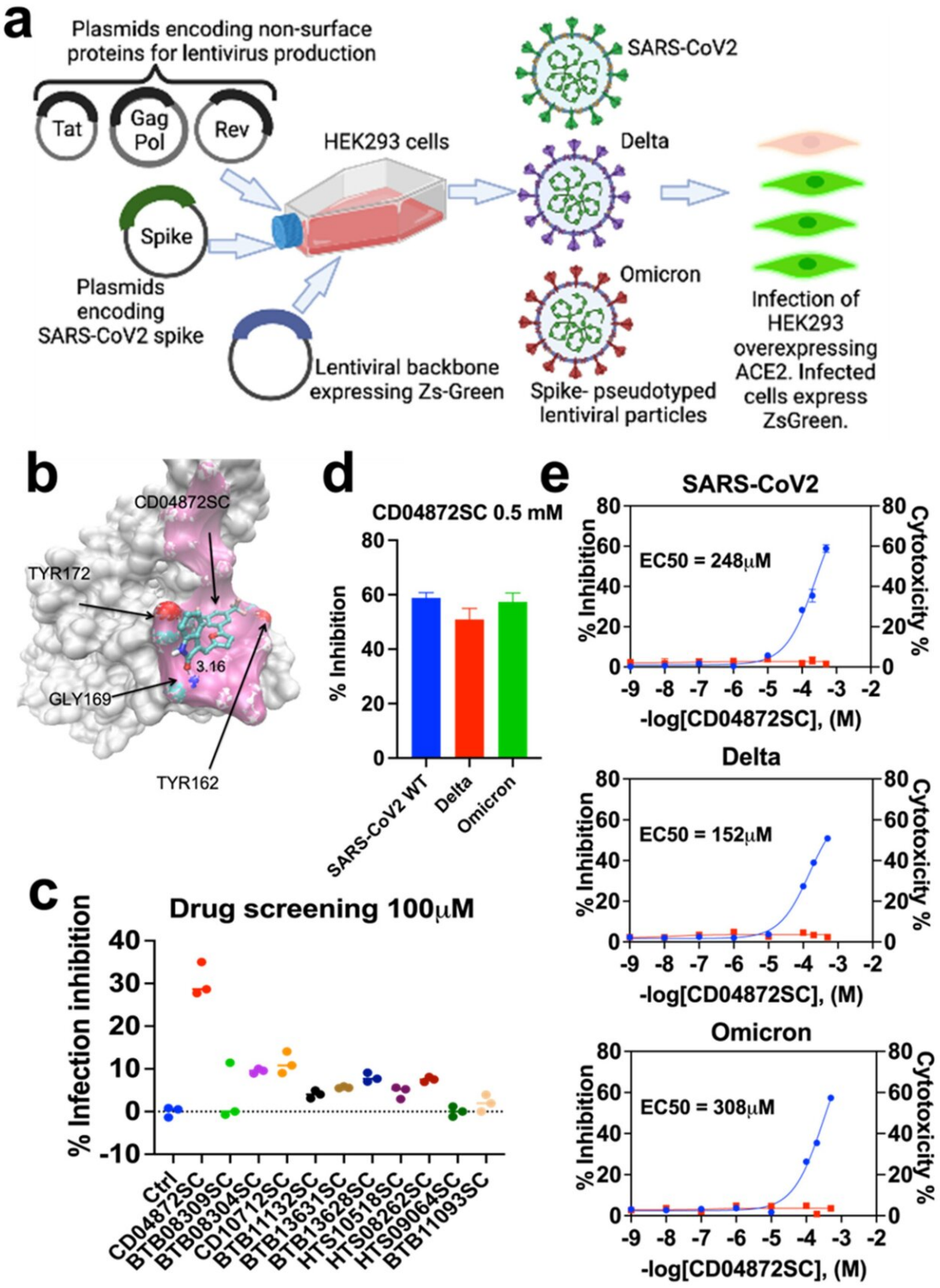
Discovery of drug candidate that neutralizes SARS-CoV-2 could reduce length of infection upon exposure

March 28 2023, by Laurie Fickman


a
Plasmids encoding non-surface proteins for lentivirus production
Tat
Gag Pol
Rev
Spike
Plasmids encoding SARS-CoV2 spike
HEK293 cells
Lentiviral backbone expressing Zs-Green
SARS-CoV2
Delta
Omicron
Spike- pseudotyped lentiviral particles
Infection of HEK293 overexpressing ACE2. Infected cells express ZsGreen.
b
CD04872SC
TYR172
3.16
GLY169
TYR162
d
CD04872SC 0.5 mM
% Inhibition
SARS-CoV2 WT
Delta
Omicron
e
SARS-CoV2
EC50 = 248μM
% Inhibition
Cytotoxicity %
-log[CD04872SC], (M)
Delta
EC50 = 152μM
Omicron
EC50 = 308μM
c
Drug screening 100μM
% Infection inhibition
Ctrl
CD04872SC
BTB08309SC
BTB08304SC
CD10712SC
BTB11132SC
BTB13631SC
BTB13628SC
HTS10518SC
HTS08262SC
HTS09064SC
BTB11093SC

Lentiviral system approach and antiviral activities of the test drugs against SARS-CoV-2 and its variants delta and omicron in vitro. (a) HEK293 cells were transfected with a plasmid encoding a lentiviral backbone (genome) expressing a marker protein, a plasmid expressing spike proteins of SARS-CoV-2 and its variants delta and omicron, and plasmids expressing the other proteins needed for virion formation (Tat, Gag-Pol, and Rev). The transfected cells produced lentiviral particles with SARS-CoV-2 spikes on their surface. These viral particles can infect cells that overexpress the ACE2 receptor. The readout to measure the grade of viral infection is the green fluorescence of ZsGreen1 from infected cells. (b) The molecular structure shows the interface of the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (white surface) with the human ACE2 receptor (pink surface). CD04872SC is predicted to bind on the face leading to the potential disruption of the complex. (c) Drug screening of the selected top compounds (each at a concentration of 100 μM) was evaluated for their antiviral activity against SARS-CoV-2, as defined by their percent infection inhibition. (d) The maximum antiviral activity against SARS-CoV-2, and its two major variants delta and omicron, for lead drug candidate CD04872SC. (e) The mean percent inhibition of viral infection and percent cell cytotoxicity of lead drug candidate CD04872SC in HEK293 cells expressing the ACE2 receptor. In brief, HEK293 cells overexpressing the ACE2 receptor were pre-treated with the lead drug candidate CD04872SC for 1 h. Next, 10 μL of the lentiviral suspensions expressing the spike protein was then added to the culture plates and incubated for 48 h. After the incubation period, the green fluorescence of ZsGreen1 protein was measured with the plate reader Synergy 2 (BioTek, Winooski, VT, USA) using the FITC channel. The mean percent inhibition and percent cytotoxicity is represented on each of the graph's left and right y-axis, respectively. For each of the three graphs, blue curves indicate the percent of infection inhibition and red curves represent the percent of cytotoxicity. The results are expressed as mean ± s.e.m. of three experiments performed in triplicate; each triplicate was averaged before calculating the s.e.m. Credit: *Biomedicines* (2023). DOI: 10.3390/biomedicines11030916

The discovery of a small molecule in the research lab of Bradley McConnell, professor of pharmacology at the University of Houston

College of Pharmacy, may well be the genesis of new medication which could shorten the course of the SARS-CoV-2 virus. Unlike Pfizer's antiviral treatment Paxlovid, which is only useful during the first three days of showing symptoms, this possible new medication could reduce the course of the virus upon exposure.

"Neutralizing small molecules may provide immediate protection against viral infection, and thus be suitable for people of all ages, and may be particularly suitable for high-risk populations and immunocompromised individuals who typically do not generate sufficient antibodies after vaccination," said McConnell in *Biomedicines*. "This exciting new small molecule drug candidate has the potential to be developed into an alternative drug treatment for COVID-19."

The molecule's discovery began during the height of the pandemic when students in the McConnell lab were working from home and an idea hatched that perhaps a tiny molecule could impact the virus. The team screened 1,509,984 feature-rich compounds in the UH Research Computing Data Core, home of the Hewlett Packard Enterprise Data Science Institute.

The team searched for a molecule that could interrupt the interaction between the spike protein, located on the outside of the coronavirus, and its human target to enter into human cells, the ACE2 protein.

"Our team is thrilled with the discovery of a small molecule therapeutic that inhibits the interaction between the spike protein of the COVID-19 virus and the ACE2 receptor of the infected individual," said McConnell. "We are grateful that the University had the high-performance computing power available on campus to advance our work so effectively. This is a discovery that could ultimately impact many lives."

McConnell's team includes Arfaxad Reyes-Alcaraz, Hanan Qasim, Elizabeth Merlinsky, Tasneem Islam, and Bryan Medina from the UH College of Pharmacy; Robert J. Schwartz, John W. Craft, Jr. from the UH Department of Biology and Biochemistry; and Glenn Fox, Rogers State University in Oklahoma.

During the experimentation phase, the team selected the top 15 molecules that disrupted the interaction between the spike protein and the ACE2 receptor. Molecular dynamic simulations revealed that some of the compounds from these libraries had favorable interactions with the spike protein's ACE receptor binding domain interface, leading to a potential neutralization of the SARS-CoV-2 infection, and one particular molecule rose to the top: CD04872SC, which formed the closest association.

"We were able to experimentally observe that CD04872SC also inhibited the infection of the SARS-CoV-2 variants delta and omicron," said Reyes-Alcaraz, the study's first author.

"To demonstrate the binding between CD04872SC and the spike proteins of each variant, we performed a thermal shift assay which measures changes in the thermal denaturation temperature, serving as an indicator of the stability of a protein under varying conditions such as when bound by a drug, pH, ionic strength, or sequence mutation," said Craft, associate professor in the Department of Biology and Biochemistry.

It's a medical discovery whose time has come.

The SARS-CoV-2 virus and variants delta and omicron are still a major threat to patients of all ages. The variants demonstrate how easily the virus can accommodate antigenic changes in its spike protein without the loss of fitness.

"The omicron variant has particularly stressed health care systems around the world. Therefore, identifying effective antiviral agents to combat this infectious disease is urgently needed," said McConnell.

For the future, McConnell suggests further development of molecule derivatives and preclinical trials.

"This promising drug candidate lead should be developed into a family of derivatives that could be further refined, possibly leading to a more efficacious and cost-effective alternative to expensive neutralizing treatments," said McConnell.

More information: Arfaxad Reyes-Alcaraz et al, A Small Molecule That In Vitro Neutralizes Infection of SARS-CoV-2 and Its Most Infectious Variants, Delta, and Omicron, *Biomedicines* (2023). DOI: 10.3390/biomedicines11030916

Provided by University of Houston