Supplementary Material

Enhanced anti-infective activities of sinapic acid through nebulization of lyophilized protransferosomes

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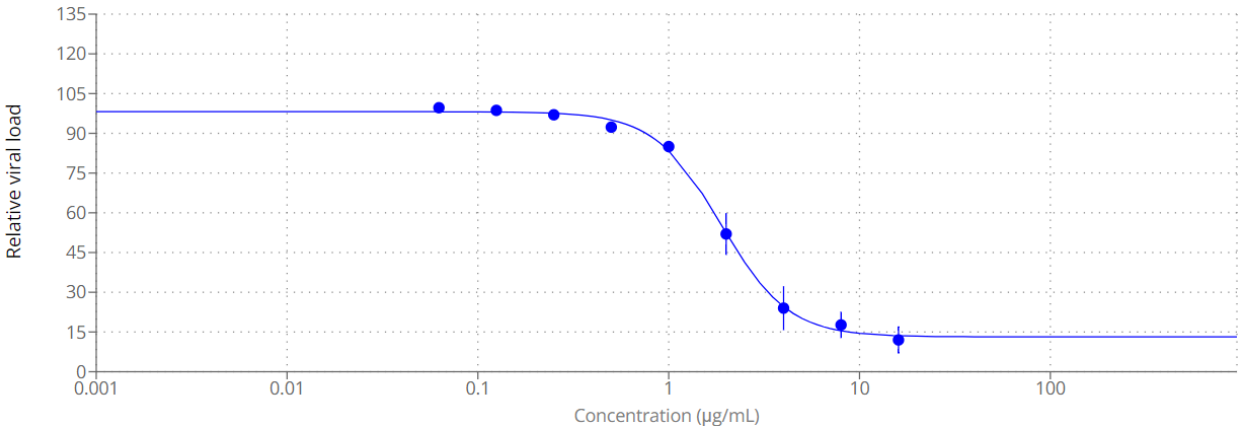

Figure S1. SA inhibitory activity against SARS CoV-2 replication (IC_{50} = 2.29 ± 0.065 µg/mL)

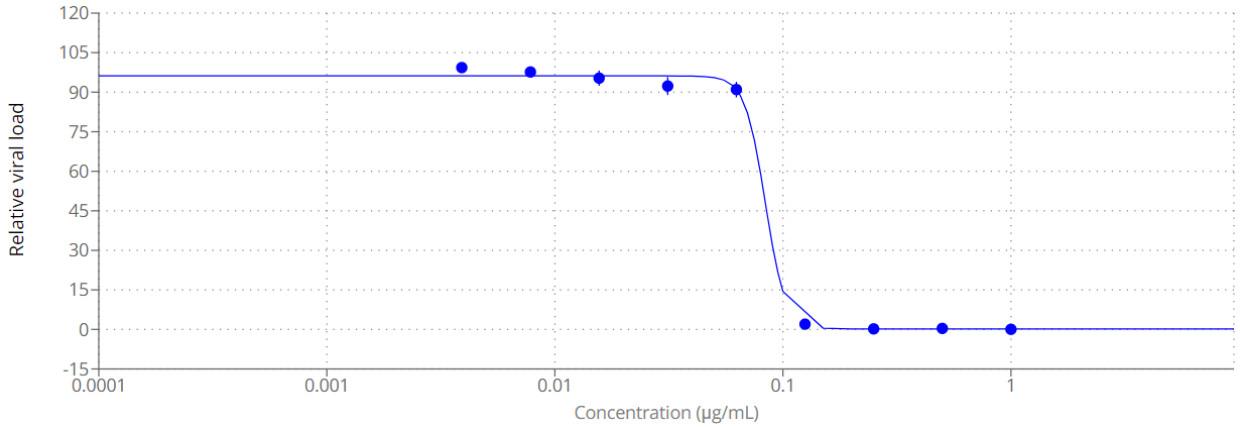

Figure S2. Remdesivir inhibitory activity against SARS CoV-2 replication (IC_{50} = 0.0837 ± 0.002 µg/mL)

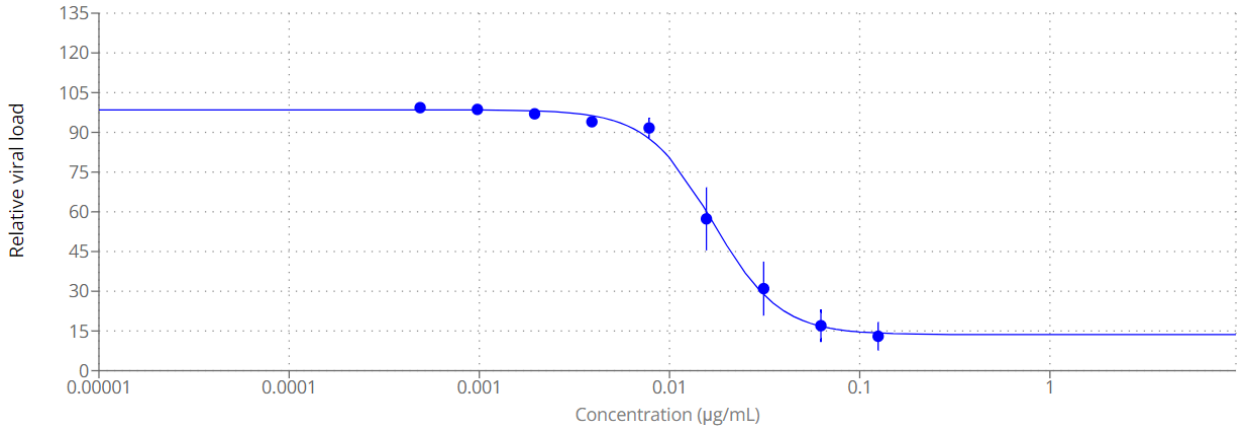

Figure S3. Lyophilized SA formula inhibitory activity against SARS CoV-2 replication (IC_{50} = 0.0179 ± 0.0089 µg/mL).

Table S1. Antibacterial activity

	Enterococcus faecalis	Streptococcus mutans	Enterobacter sp.	Escherichia coli 0157 ATCC 700728	Pseudomonas aeruginosa ATCC 90902	Salmonella typhimurium ATCC 14028	Staphylococcus aureus ATCC 25923	Mycobacterium sp.
SA	**68.532±0.215**	**53.649±1.25**	**68.55±1.66**	**51.205±0.35**	**80.163±1.12**	**73.235±1.36**	**65.051±0.35**	**36.505±1.62**
Nano-SA	**88.255±1.56**	**79.52±0.23**	**83.25±1.79**	**74.332±0.62**	**93.597±0.55**	**87.308±1.27**	**79.052±0.15**	**41.251±1.72**
Ciprofloxacin (5µg/mL)	**97.28±1.03**	**97.28±0.98**	**-**	**98.20±0.52**	**96.56±1.13**	**98.01±2.11**	**98.25±0.35**	**-**

Table S2. Antifungal activity

	Candida albicans	*Aspergillus niger*
SA	82.223± 0.25	84.233± 0.15
Nano-SA	94.522± 0.11	96.481± 0.18
Nystatin (10ug/mL)	97.225	98.356

Antiviral assay

MTT cytotoxicity assay

To determine the IC_{50} for initial antiviral screening, stock solutions of test compounds in 10% DMSO and ddH_2O were diluted to working solutions with DMEM. The cytotoxic effect of the test compounds was evaluated in Vero-E6 cells using a modified version of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). After placing the cells in 96-well plates (100 µL/well at a density of 3105 cells/mL) at 37 °C and 5% CO_2, they were incubated for 24 hours. After 24 hours, cells were exposed to different concentrations of the tested agents in triplicate. After an additional 24 hours, the supernatant was discarded, and the cell monolayers were washed three times with sterile 1 PBS. Each well received 20 µL of the 5 mg/mL MTT stock solution before being incubated at 37 °C for four hours. The particles of formed formazan were dissolved in 200 µL of acidified isopropyl alcohol (0.04 µM HCl in 100% isopropanol ¼ 0.073 mL HCL in 50 mL isopropanol). The absorbance of formazan solutions was then calculated using a microplate reader at a wavelength of 540 nm. The percentage of cytotoxicity in comparison to untreated cells was determined using the formula below:

$$\%\text{Cytotoxicity} = \frac{(Absorbance\ of\ cells\ without\ treatment - absorbance\ of\ cells\ with\ treatment) x\ 100}{Absorbance\ of\ cells\ without\ treatment}$$

Viral replication assay

Vero cells were seeded at a density of 4×10^4 cells per well in a 24-well plate. After a duration of twenty-four hours, the cells were subjected to infection using the SARS CoV-2 strain (SARS-CoV-2/human/SAU/85791C/2020 isolate), with a multiplicity of infection (MOI) of 0.5. After a duration of 1 hour, the inoculum was extracted and substituted with a fresh medium containing varying amounts of the test chemical. The quantification of viral RNA quantity in the supernatant was performed using real-time PCR methodology during the exponential development phase of the viruses (i.e., after 2 days). For RNA preparation, a simple and inexpensive method (Boom et al., 1990) was adopted. A 140-µL aliquot of supernatant was mixed with 560 µL chaotropic lysis buffer (Qiagen) and incubated at room temperature for 15 min. The lysate was added to 100 mg diatomaceous silica (Sigma–Aldrich) suspended in 560 µL ethanol and incubated with agitation for 30 min at room temperature. The diatomaceous silica was pelleted by centrifugation and the pellet was washed with 500 µL AW1 buffer (Qiagen), subsequently with 500 µL AW2 buffer (Qiagen), and finally with 400 µL acetone. The pellet was dried at 56 °C and the RNA eluted with 100 µL water. Quantitative real-time PCR assays were performed with the purified RNA based on previously published protocols (Drosten et al., 2003; Asper et al., 2004). The PCR reaction conditions were as the following: 25-µL reaction based on Superscript II RT/Platinum Taq polymerase one-step RT-PCR kit (Invitrogen): 5µL RNA, 1× buffer, 3.6 mM additional $MgSO_4$, 0.6 µL enzyme mixture, 240 nM probe BNITMSARP (FAM-TCG TGC GTG GAT TGG CTT TGA TGT-TAMRA), 200 nM primer BNITMSARS1 (TTA TCA CCC GCG AAG AAG CT), and 200 nM primer BNITMSARAs2 (CTC TAG TTG CAT GAC AGC CCT C). The PCR cycling condition: 7000 SDS machine (Applied Biosystems): 15 min at 45 °C; 3 min at 95 °C; 40 cycles with 15 s at 95 °C and 30 s at 58 °C with fluorescence measured at 58 °C. The concentrations required to inhibit virus replication by 50% (IC_{50}) were calculated by fitting a sigmoidal curve to the data following logarithmic transformation of the drug concentration (Figures 1S-3S).

References

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