The iminosugars celgosivir, castanospermine and UV-4 inhibit SARS-CoV-2 replication

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic poses an unprecedented challenge for healthcare and the global economy. Repurposing drugs that have shown promise in inhibiting other viral infections could allow for more rapid dispensation of urgently needed therapeutics. The Spike protein of SARS-CoV-2 is extensively glycosylated with 22 occupied N glycan sites and is required for viral entry. In other glycosylated viral proteins, glycosylation is required for interaction with calnexin and chaperone-mediated folding in the endoplasmic reticulum, and prevention of this interaction leads to unfolded viral proteins and thus inhibits viral replication. As such, we investigated two iminosugars, celgosivir, a pro-drug of castanospermine, and UV-4, or N-(9-methoxynonyl)-1-deoxynojirimycin, a deoxynojirimycin derivative. Iminosugars are known inhibitors of the α-glucosidase I and II enzymes and were effective at inhibiting authentic SARS-CoV-2 viral replication in a cell culture system. Celgosivir prevented SARS-CoV-2-induced cell death and reduced viral replication and Spike protein levels in a dose-dependent manner in culture with Vero E6 cells. Castanospermine, the active form of celgosivir, was also able to inhibit SARS-CoV-2, confirming the canonical castanospermine mechanism of action of celgosivir. The monocyclic UV-4 also prevented SARS-CoV-2-induced death and reduced viral replication after 24 hours of treatment, although the reduction in viral copies was lost after 48 hours. Our findings suggest that iminosugars should be urgently investigated as potential SARS-CoV-2 inhibitors.

Introduction

The emergence of a novel coronavirus, SARS-CoV-2, that causes a sometimes-fatal respiratory disease (COVID-19), has led to a pandemic infecting millions of people and overwhelming health care facilities across the globe. The unprecedented challenge of the pandemic has led to intensive investigation of
therapeutics for SARS-CoV-2 and a particular interest in repurposed drugs that could be used to inhibit the viral replication in host cells. Repurposed drugs have the advantages of being well-characterized and safety tested in humans, providing the potential to move more rapidly for use as a therapeutic if their efficacy can be confirmed.

Among pre-approved drugs are iminosugars, a class of sugar mimetic compounds in which the cyclic oxygen of the sugar is replaced with a nitrogen. Iminosugars are structurally analogous to sugar substrates and inhibit the ER α-glucosidases I and II (α-glu I and α-glu II), which are required for the folding of many glycoproteins. The iminosugar N-butyl-deoxynojirimycin (NB-DNJ or Miglustat), is FDA-approved in humans for treatment of Gaucher’s disease, a metabolic lysosomal storage disease. Additionally, NB-DNJ has mild antiviral effects at higher concentrations against a range of enveloped viruses including dengue virus (DENV) (Miller et al., 2018). The antiviral mechanism of action is believed to be mediated by inducing misfolding of viral N-linked glycoproteins through inhibition of host endoplasmic reticulum-resident α-glucosidase enzymes (Alonzi et al., 2017). This leads to reduced glycoprotein production, as shown in DENV (Perry et al., 2013) and hence lower production of virions, both in vitro and in vivo (Tyrrell et al., 2017; Warfield et al., 2020).

Iminosugars include monocyclic derivatives of the deoxynojirimycin (DNJ), such as N-(9-methoxynonyl)-1-deoxynojirimycin or UV-4, referred to as UV-4 hereafter. UV-4 is an inhibitor of filoviruses in vitro (Warfield et al., 2017) and has antiviral effects in vivo against dengue virus (Perry et al., 2013) and influenza virus (Tyrrell et al., 2017; Warfield et al., 2020). The bicyclic celgosivir, a castanospermine prodrug, has been extensively studied as an inhibitor of dengue virus, where it is a more potent inhibitor than castanospermine itself (reviewed in (Miller et al., 2018)). Celgosivir has been shown to be safe in humans Phase 1b trials (Low et al., 2014), with an in vivo efficacy in dengue virus mouse models dependent on timing, dosage and other factors (Watanabe et al., 2016).
It is widely accepted that a key mechanism by which iminosugars act as antivirals is their ability to disrupt glycoprotein folding (Alonzi et al., 2017). The first enzymes in the N-linked glycosylation pathway are the ER glucosidase I and II enzymes. All nascent N-linked glycans have three terminal glucose residues at the terminus when added to a protein, which are removed sequentially with α-glu I removing the first glucose residue and α-glu II removing the next two residues (Compain & Martin, 2007). The ER-chaperone proteins calnexin and calreticulin recognize mono-glucosylated glycans for folding. However, inhibition of the α-glu I and α-glu II enzymes prevents this process, resulting in misfolded proteins (Figure 1). Viruses that depend on the calnexin/calreticulin cycle are therefore likely to be sensitive to inhibition of α-glu I and α-glu II by iminosugars.

The Spike protein (S) of SARS-CoV-2 is the viral protein responsible for viral entry (Hoffmann et al., 2020). The S protein is highly glycosylated with N-glycans and has 22 putative N-glycan sites on the ectodomain, all of which are occupied when produced in 293FT cell culture (Watanabe et al., 2020). We hypothesized that treatment with iminosugars would inhibit SARS-CoV-2 viral production in cell culture, as they do for other enveloped viruses with heavily glycosylated proteins (Alonzi et al., 2017). Despite iminosugar mechanisms of action being similar, some iminosugars are more or less effective than others against particular viruses, for example against dengue virus (Miller et al., 2018). The urgency in finding effective SARS-CoV-2 therapeutics has led to speculation about the efficacy of iminosugar inhibitors, including a review of potential mechanisms (Williams & Goddard-Borger, 2020), but thus far there is limited experimental data testing this hypothesis. Groups performing large-scale drug screens have included certain iminosugars, showing that 1-deoxynojirimycin (DNJ), a first generation iminosugar, does not reduce SARS-CoV-2 replication in cell culture (Gordon et al., 2020). However, our results indicate the UV-4, a monocyclic derivative of DNJ is able to inhibit SARS-CoV-2 in vitro. Further, celgosivir, the pro-drug of castanospermine, and castanospermine itself, are able to inhibit viral replication of SARS-CoV-2 in vitro. Since both monocyclic and bicyclic iminosugars with the same putative mechanism of action are
able to inhibit the virus, iminosugars and inhibitors of α-glu I and α-glu II should be further investigated in *in vivo* models as potential SARS-CoV-2 therapeutics.

**Methods**

**Virus.** SARS-Related Coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH (catalog number NR-52281). Virus was expanded and titered in Vero E6 cells. All experiments were conducted in a Biosafety Level 3 lab with approved protocols.

**Cell death inhibition assay.** Iminosugar inhibition of SARS-CoV-2 was measured using a cell death inhibition assay. Relative cell numbers were measured by assessment of cellular metabolic activity using the XTT colorimetric assay (ThermoFisher). Vero-E6 cells were seeded and incubated until at least 90% confluent. Cells in 96-well culture plates were treated with celgosivir (Millipore Sigma, SML2314), castanospermine (MedChemExpress, HY-N2022) or N-(9-methoxynonyl)-1-deoxyojirimycin/SP187/UV-4 (MedChemExpress, HY-U00160) at final concentrations of 1μM, 10μM or 100μM for 1 hour and then infected with a multiplicity of infection (MOI) of 0.05 in triplicate for each condition and incubated for 48 hours (with compounds in the media). XTT reagent was added for 4 hours and absorbance was read at 450 nM as per the manufacturer’s protocol.

**Measurement of viral genomes by quantitative-PCR (qPCR).** Cells treated with celgosivir, castanospermine or UV-4 as above were infected at an MOI of 0.05 for 24 or 48 hours in triplicate, at which point supernatants were harvested and placed in AVL buffer (Qiagen) before removal from the BSL3. RNA was extracted using Qiagen viral RNA mini kit, following the manufacturer’s protocol. To quantify the level of virus, qPCR was performed using the TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystems). Primers against the N gene of SARS-CoV-2 from the 2019-nCoV CDC EUA Kit (Integrated DNA technologies) were used for detection of SARS-CoV-2. Standard curves were
constructed using the 2019-nCoV_N_Positive Control (integrated DNA technologies) and were used to determine copies/mL of SARS-CoV-2 N. Each condition (in triplicate) was assayed in duplicate. Statistical analysis was performed using GraphPad (GraphPad Prism 7.0).

**Western blots.** Supernatants were removed from a 96-well plate of Vero E6 cells and the cells were added to 150μL cell lysis buffer (10 mM Tris, 150 mM NaCl, 0.02% NaN3, 1% Sodium deoxycholate, 1% Triton X-100, 0.1% SDS) with 1x protease/phosphatase inhibitor cocktail (FisherScientific). The denatured protein lysates were separated using 10% SDS-PAGE gels, and transferred using iBlot 2 NC mini stacks (ThermoFisher). After transfer, blots were blocked with 5% milk in PBST for 1 hour at RT and probed with SARS-CoV-2/SARS-CoV Spike Antibody (ProSci, 1:500) (overnight at 4°C). After washing, 3 times with 0.01% Tween in PBS, Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP (Thermodisher, 1:5000 and β-Actin (D6A8) Rabbit mAb (HRP Conjugate) (Cell Signaling Technology, 1:3000, 1 hr at RT), were added for 1 hour at RT. After washing 3 times with 0.01% Tween in PBS, the Pierce ECL Western Blotting Substrate (Thermofisher) was used for development. Images were taken using a Bio-rad chemidoc and analyzed with ImageJ.

**Results**

Iminosugar treatment inhibits SARS-CoV-2-mediated cytopathic effect

The antiviral properties of the iminosugars celgosivir, castanospermine and UV-4 were first assessed using a cell death inhibition assay with Vero E6 cells, an African green monkey kidney cell line with defects in interferon production that is commonly used for viral infections. Infection of Vero E6 cells with a low MOI of SARS-CoV-2 results in widespread cell death 48 hours post-infection. Antiviral compounds that mitigate virus-induced cell death result in a higher absorbance signal (O.D.) than control infected cells. Since SARS-CoV-2 is a cytopathic virus, cell death can be used as a measure of viral infection. The range of 1 μM to 100 μM was chosen based on celgosivir EC50s against different
strains of dengue virus in Vero E6 cells ranging from 2.4 μM to 51.0 μM (Watanabe et al., 2016). Vero cells were infected at a MOI of 0.05. Treatment with 100 μM of celgosivir and 100 μM of UV-4 produced a 2-fold increase in the relative number of live cells, with and 10 μM of each also producing a 1.5 fold increase in the number of live cells, demonstrating a dose-dependent significant increase in the number of live cells compared to untreated after 48 hours (Figure 2). Castanospermine, the conversion product of celgosivir, has a shorter half-life in vivo (Sung et al., 2016). Our data shows that castanospermine also had less potent inhibition of SARS-CoV-2 induced cell-death. At 100 μM of castanospermine there was a significant increase in live cells compared to untreated infected cells, but no significant difference at either 10 μM or 1 μM of castanospermine (Figure 2). The higher potency of celgosivir than castanospermine has been seen with other viral pathogens (Bhushan et al., 2020; Liu et al., 1990; Miller et al., 2018) and is attributed to celgosivir having 30-50 times higher absorption into cells (Whitby et al., 2004).

UV-4, a DNJ derivative, decreases SARS-CoV-2 viral replication at 24 hours post-infection

Viral replication was measured in supernatants taken from Vero E6 cells at 24 and 48 hours post-infection by using primers against the SARS-CoV-2 nucleocapsid (N) protein. We examined UV-4 treatments at 24 and 48 hours post-infection to determine if doses of 100 μM to 1 μM could inhibit viral replication. In both repeats at 24 hours post-infection, both 100 μM and 10 μM significantly reduced the number of copies of virus compared to the untreated group (Figure 3A). However, although there is a downward trend at 48 hours, there are no significant differences between the treated UV-4 samples and the untreated samples, suggesting that the iminosugar may be cleared and the viral replication recovers after 48 hours (Figure 3B). We also found that N-methyl-1-DNJ, a first generation DNJ derivative, did not inhibit either virus-induced cell death or viral replication at 100, 10, or 1 μM (data not shown); however, these concentrations are much lower than the EC50 for DNJ against dengue (308 μM (Sayce et al.,
and so it is likely the concentrations tested were not optimal to see inhibition with N-methyl-1-DNJ.

_Celgosivir decreases SARS-CoV-2 viral replication in a dose-dependent manner_

At 24 hours, in two separate experiments, there was a dose dependent reduction in viral replication in response to celgosivir treatment (Figure 4A). The differences between the untreated, 100 μM and 10 μM were significant at both 24 hours and 48 hours. The 1 μM celgosivir treatment did not reduce viral genomes to a level lower than the untreated, showing again that the celgosivir action is dose-dependent and that the lowest dose is not sufficient to inhibit the virus robustly (Figure 4A and 4B). Castanospermine treatment only significantly decreases viral copies at the highest dose of 100 μM, again suggesting it has lower potency than the pro-drug form (Figure 4A and 4B).

_Celgosivir treatment decreases SARS-CoV-2 Spike protein levels in infected cells_

As iminosugars inhibit a component of the glycosylation pathway, we then looked at levels of the Spike protein, the most extensively glycosylated SARS-CoV-2 protein. As the celgosivir produced the most potent inhibition of the virus by both examining viral copies and cell survival, we investigated the impact of celgosivir treatment on S protein levels. Lysates were taken from infected cells treated with celgosivir after 24 hours, and blots were probed for the S protein (Figure 4C and D). The celgosivir treated lysates had lower levels of S protein compared to the actin loading control, with the lowest quantity of S protein in the 100 μM group. Blots showed that the S protein in all lanes is at the same molecular weights (two bands at ~160 kDa and ~130kDa), suggesting the S protein is not being produced with impaired glycosylation. However, there may be changes in glycan structure (such as high mannose glycans in place of complex glycans) that do not change the molecular weight to a level that is distinguishable in our immunoblot assays, and this should be investigated further.
Discussion

Antiviral therapies against SARS-CoV-2 are urgently required and iminosugar inhibitors provide a rich area for investigation. While the variable efficacies of different iminosugars are not well-characterized in viral infections, the activity of nine different iminosugars (the panel did not include UV-4 or celgosivir) were recently investigated in uninfected cells, revealing some that specifically target α-glucosidase I, and others α-glucosidase II (O’Keefe et al., 2019). Better characterization of the action of different iminosugars may also come from molecular modeling of iminosugar-ER α-glucosidase interactions, and this may provide insights into the most efficacious iminosugar structures. Our data shows that celgosivir, castanospermine and UV-4 treatments are all able to inhibit SARS-CoV-2 replication at varying potencies. Individually, the analyzed iminosugars may have effects separate from ER α-glucosidase inhibition caused by their other degradation products, but the inhibition from the different drugs in the same family suggests the common mechanism of ER α-glucosidase inhibition. The mechanism by which iminosugars inhibit SARS-CoV-2 replication, and the proteins in the virus they affect, are currently unknown, but further investigation into these mechanisms should be analyzed. In the more-studied SARS-CoV-1, a tertiary hydroxyl DNJ iminosugar, IHVR-19029, disrupts the interaction of SARS Spike protein and calnexin, and produces SARS virions with significantly lower infectivity than without the inhibitors (Fukushi et al., 2012), suggesting an antiviral iminosugar may have a similar mechanism in SARS-CoV-2. While it is likely that the heavily glycosylated Spike protein would be impacted by inhibition of α-glucosidases I and/or II, it is also possible that iminosugars could impact the ACE2 receptor, which is required for viral entry. ACE2 is glycosylated, and ACE2-mediated transduction of lentiviral pseudotyped SARS-CoV-1 may be impaired by treatment with iminosugars (Zhao et al., 2015). Repurposed drugs have the advantage of having some safety data available, and celgosivir has been shown previously to be safe in humans, with a maximally tolerated dose (MTD) of 400 mg qd (once a day) for 12 weeks (Sung et al.,
We believe iminosugars should be urgently investigated as a potential therapeutic for SARS-CoV-2.

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References

Alonzi, D. S., Scott, K. A., Dwek, R. A., & Zitzmann, N. (2017). Iminosugar antivirals: The therapeutic sweet spot. *Biochemical Society Transactions, 45*(2), 571–582. https://doi.org/10.1042/BST20160182

Bhushan, G., Lim, L., Bird, I., Chotohe, S. K., Nissly, R. H., & Kuchipudi, S. V. (2020). Iminosugars With Endoplasmic Reticulum α-Glucosidase Inhibitor Activity Inhibit ZIKV Replication and Reverse Cytopathogenicity in vitro. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.00531

Compain, P., & Martin, O. R. (Eds.). (2007). *Iminosugars*. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470517437

Fukushi, M., Yoshinaka, Y., Matsuoka, Y., Hatakeyama, S., Ishizaka, Y., Kirikae, T., Sasazuki, T., & Miyoshi-Akiyama, T. (2012). Monitoring of S Protein Maturation in the Endoplasmic Reticulum by Calnexin Is Important for the Infectivity of Severe Acute Respiratory Syndrome Coronavirus. *Journal of Virology, 86*(21), 11745–11753. https://doi.org/10.1128/JVI.01250-12

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., O'Meara, M. J., Rezelj, V. V., Guo, J. Z., Swaney, D. L., Tummino, T. A., Huettenhain, R., Kaake, R. M., Richards, A. L., Tutuncuoglu, B., Foussard, H., Batra, J., Haas, K., Modak, M., … Krogan, N. J. (2020). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*. https://doi.org/10.1038/s41586-020-2286-9

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N.-H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 andTMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell, 181*(2), 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052
Liu, P. S., Hoekstra, W. J., & Kinga, C.-H. R. (1990). Synthesis of potent anti-HIV agents: Esters of castanospermine. *Tetrahedron Letters, 31*(20), 2829–2832. https://doi.org/10.1016/0040-4039(90)80159-J

Low, J. G., Sung, C., Wijaya, L., Wei, Y., Rathore, A. P. S., Watanabe, S., Tan, B. H., Toh, L., Chua, L. T., Hou, Y., Chow, A., Howe, S., Chan, W. K., Tan, K. H., Chung, J. S., Cherung, B. P., Lye, D. C., Tambayah, P. A., Ng, L. C., ... Vasudevan, S. G. (2014). Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): A phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. *The Lancet Infectious Diseases, 14*(8), 706–715. https://doi.org/10.1016/S1473-3099(14)70730-3

Miller, J. L., Tyrrell, B. E., & Zitzmann, N. (2018). Mechanisms of Antiviral Activity of Iminosugars Against Dengue Virus. *Advances in Experimental Medicine and Biology, 1062*, 277–301. https://doi.org/10.1007/978-981-10-8727-1_20

Perry, S. T., Buck, M. D., Plummer, E. M., Penmasta, R. A., Batra, H., Stavale, E. J., Warfield, K. L., Dwek, R. A., Butters, T. D., Alonzi, D. S., Lada, S. M., King, K., Klose, B., Ramstedt, U., & Shresta, S. (2013). An iminosugar with potent inhibition of dengue virus infection in vivo. *Antiviral Research, 98*(1), 35–43. https://doi.org/10.1016/j.antiviral.2013.01.004

Sayce, A. C., Alonzi, D. S., Killingbeck, S. S., Tyrrell, B. E., Hill, M. L., Caputo, A. T., Iwaki, R., Kinami, K., Ide, D., Kiappes, J. L., Beatty, P. R., Kato, A., Harris, E., Dwek, R. A., Miller, J. L., & Zitzmann, N. (2016). Iminosugars Inhibit Dengue Virus Production via Inhibition of ER Alpha-Glucosidases—Not Glycolipid Processing Enzymes. *PLoS Neglected Tropical Diseases, 10*(3), e0004524. https://doi.org/10.1371/journal.pntd.0004524

Sung, C., Wei, Y., Watanabe, S., Lee, H. S., Khoo, Y. M., Fan, L., Rathore, A. P. S., Chan, K. W.-K., Choy, M. M., Kamaraj, U. S., Sessions, O. M., Aw, P., Sessions, P. F. de, Lee, B., Connolly, J. E., Hibberd, M. L., Vijaykrishna, D., Wijaya, L., Ooi, E. E., ... Vasudevan, S. G. (2016). Extended Evaluation of
Virological, Immunological and Pharmacokinetic Endpoints of CELADEN: A Randomized, Placebo-Controlled Trial of Celgosivir in Dengue Fever Patients. *PLOS Neglected Tropical Diseases*, 10(8), e0004851. https://doi.org/10.1371/journal.pntd.0004851

Tyrrell, B. E., Sayce, A. C., Warfield, K. L., Miller, J. L., & Zitzmann, N. (2017). Iminosugars: Promising therapeutics for influenza infection. *Critical Reviews in Microbiology*, 43(5), 521–545. https://doi.org/10.1080/1040841X.2016.1242868

Warfield, K. L., Alonzi, D. S., Hill, J. C., Caputo, A. T., Roversi, P., Kiappes, J. L., Sheets, N., Duchars, M., Dwek, R. A., Biggins, J., Barnard, D., Shresta, S., Treston, A. M., & Zitzmann, N. (2020). Targeting Endoplasmic Reticulum α-Glucosidase I with a Single-Dose Iminosugar Treatment Protects against Lethal Influenza and Dengue Virus Infections. *Journal of Medicinal Chemistry*, 63(8), 4205–4214. https://doi.org/10.1021/acs.jmedchem.0c00067

Watanabe, S., Chan, K. W.-K., Dow, G., Ooi, E. E., Low, J. G., & Vasudevan, S. G. (2016). Optimizing celgosivir therapy in mouse models of dengue virus infection of serotypes 1 and 2: The search for a window for potential therapeutic efficacy. *Antiviral Research*, 127, 10–19. https://doi.org/10.1016/j.antiviral.2015.12.008

Whitby, K., Taylor, D., Patel, D., Ahmed, P., & Tyms, A. S. (2004). Action of celgosivir (6 O-butanoyl castanospermine) against the pestivirus BVDV: Implications for the treatment of hepatitis C. *Antiviral Chemistry & Chemotherapy*, 15(3), 141–151. https://doi.org/10.1177/095632020401500304
**Figure legends**

Figure 1: Schematic of putative action of iminosugars celgosivir, castanospermine (CST) and UV-4 on SARS-CoV-2 Spike folding. Nascent glycoproteins are tri-glucosylated, and have their glucose residues sequentially trimmed by ER α-glucosidase I and II. Mono-glucosylated nascent proteins are bound by lectin-like chaperones Calnexin and Calreticulin (CNX and CRT) to mediate correct folding, at which point α-glucosidase II removes the final glucose and the protein is released for further processing. If the protein is still unfolded, enzyme UGGT1 re-adds the single glucose as a signal for chaperone mediated binding. Inhibition of the α-glucosidases prevents this process, and so can result in improper folding of glycosylated proteins. Schematic created with Biorender.com using SARS-CoV-2 structure from the protein data bank (PDB 6VXX).
Figure 2: Cell death inhibition with celgosivir, castanospermine (A) and UV-4 (B) treatment in Vero E6 cells. Vero E6 cells were treated with the indicated amounts of celgosivir, castanospermine, or UV-4 prior to and during infection with SARS-CoV-2. At 48 hours post-infection, live Vero E6 cells were determined by absorbance in an XTT assay. Statistical significance was determined by one-way ANOVA and Tukey’s multiple comparisons. Significance is *= P ≤ 0.05; **= P ≤ 0.01; ***= P ≤ 0.001. Average cell survival percentages in the right-hand panel were determined by setting uninfected to 100% and untreated infected to 0%; average cell survival for each treatment as a percentage of uninfected is recorded.
Figure 3: UV-4 treatment reduces SARS-CoV-2 replication in cell culture at 24 hrs. (A, B) qPCR analysis of SARS-CoV-2 viral genomes present at 24 (A) and 48 (B) hours post-infection with UV-4 treatment (A, B) with statistical significance determined by one-way ANOVA and Tukey’s multiple comparisons. Significance is *= P ≤ 0.05; **= P ≤ 0.01; ***= P ≤ 0.001.
Figure 4: Celgosivir and castanospermine treatment reduces SARS-CoV-2 replication in cell culture. (A, B) qPCR analysis of SARS-CoV-2 viral genomes present at 24 (A) and 48 (B) hours post-infection with cegosivir and castanospermine treatment, with combined qPCR analysis from two independent experiments (A, B) with statistical significance determined by one-way ANOVA and Tukey’s multiple comparisons. Significance is *= P ≤ 0.05; **= P ≤ 0.01; ***= P ≤ 0.001. (C) Western blot analysis of SARS-CoV-2 Spike protein after treatment with cegosivir, compared to actin (D) Quantification of western blot SARS-CoV-2 Spike/Actin ratio and analyzed with ImageJ.
