Evaluation of SARS-CoV-2 3C-like protease inhibitors using self-assembled monolayer desorption ionization mass spectrometry

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Abstract # AAVGV0025

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the COVID-19 pandemic that began in 2019. The coronavirus 3-chymotrypsin-like cysteine protease (3CLpro) controls replication and is therefore considered a major target for antiviral discovery. Developing robust and sensitive in vitro screening assays is key to rapidly identify the most promising inhibitors of SARS-CoV-2 3CLpro.

Methods: SARS-CoV-2 3CLpro inhibitors were evaluated in a novel self-assembled monolayer desorption ionization mass spectrometry (SAMDI-MS) in parallel with a standard FRET enzymatic assay, with and without reducing agent. Antiviral potency of control compounds was also determined in cells infected with OC-43 or SARS-CoV-2. Potential for compound cytotoxicity was conducted in parallel with antiviral assessment (1).

The label-free SAMDI-MS assay was optimized and validated with known inhibitors of coronavirus 3CLpro such as GC376 (IC50 = 0.060 µM), calpain inhibitors II and XII (IC50 ~20-25 µM). The FDA-approved drugs shikonin, disulfiram, and ebselen did not inhibit SARS-CoV-2 3CLpro activity in the SAMDI-MS assay under physiologically relevant reducing conditions. The three drugs did not directly inhibit human β-coronavirus OC-43 or SARS-CoV-2 in vitro, but instead induced cell death. Compared with a traditional FRET readout, the SAMDI-MS assay offers greater sensitivity and eliminates false positive inhibition from compound interference with the optical signal. In conclusion, the SAMDI-MS 3CLpro assay, combined with antiviral and cytotoxic assessment, provides a robust platform to evaluate antiviral agents directed against SARS-CoV-2.

Buffer optimization of SAMDI-MS assay

Comparison of 3CLpro inhibition between FRET and SAMDI-MS

Robustness of the SAMDI-MS assay

Results and Conclusions

The label-free SAMDI-MS assay was optimized and validated with known inhibitors of coronavirus 3CLpro such as GC376 (IC50 = 0.060 µM), calpain inhibitors II and XII (IC50 ~20-25 µM). The FDA-approved drugs shikonin, disulfiram, and ebselen did not inhibit SARS-CoV-2 3CLpro activity in the SAMDI-MS assay under physiologically relevant reducing conditions. The three drugs did not directly inhibit human β-coronavirus OC-43 or SARS-CoV-2 in vitro, but instead induced cell death. Compared with a traditional FRET readout, the SAMDI-MS assay offers greater sensitivity and eliminates false positive inhibition from compound interference with the optical signal. In conclusion, the SAMDI-MS 3CLpro assay, combined with antiviral and cytotoxic assessment, provides a robust platform to evaluate antiviral agents directed against SARS-CoV-2.

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Effect of reducing conditions on inhibition potency

IC50 measurements of 6 reported 3CLpro inhibitors measured by SAMDI-MS in the absence of reducing agent (grey), in the presence of 1 mM DTT (red), and in the presence of 1 mM glutathione (blue). Experiments were performed in duplicate and error bars represent standard deviation.

Summary of antiviral activity against OC43-CoV and SARS-CoV-2

Test Article OCV-43 Hela Cells OCV-43 MRC-5 Cells SARS-CoV-2 VeroE6 Cells EC50 (µM) CC50 (µM) EC50 (µM) CC50 (µM) EC50 (µM) CC50 (µM) Remdesivir 0.114±0.009 >1 0.21 ≥50 n.d. n.d. GS-441524 n.d. n.d. n.d. n.d. <0.8 55±18 GC376 0.42±0.033 >10 0.83 >50 10±4.2 >100 Calpain Inhibitor II 20.7±3.3 >100 20.95 >100 27±1.4 >100 Calpain Inhibitor XII 15.2±2.4 60.3±8.3 6.93 >50 1.3±0.57 27±0.0 Ebselen >100 15.9±2.4 >100 8.23 >100 37.5±9.2 Disulfiram >100 15.9±2.4 >100 8.23 >100 37.5±9.2 Shikonin >100 1.4±0.001 >100 0.32 >100 1.5±0.07

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