IDENTIFICATION OF BROAD-SPECTRUM ANTIVIRAL SMALL-MOLECULES IN THE POST PANDEMIC ERA

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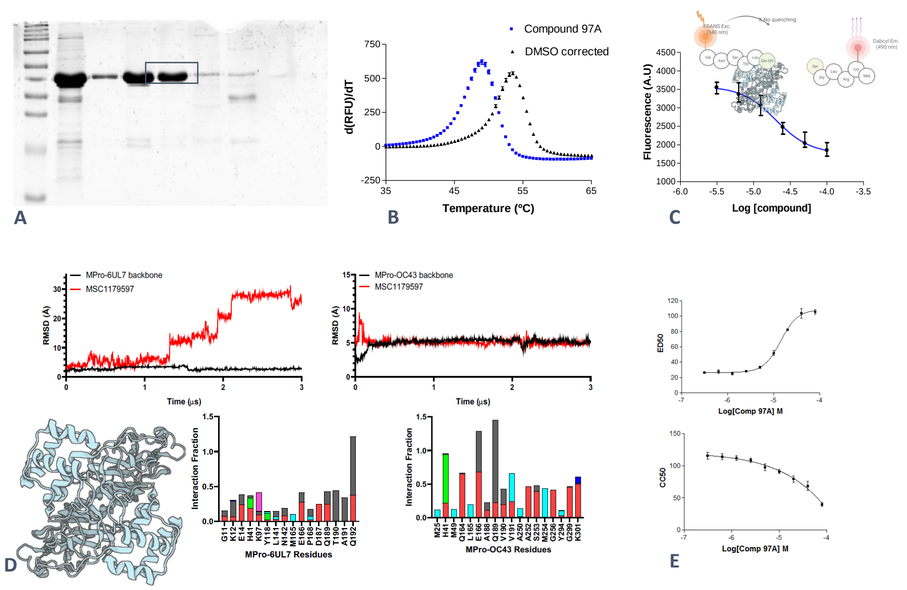
Abstract

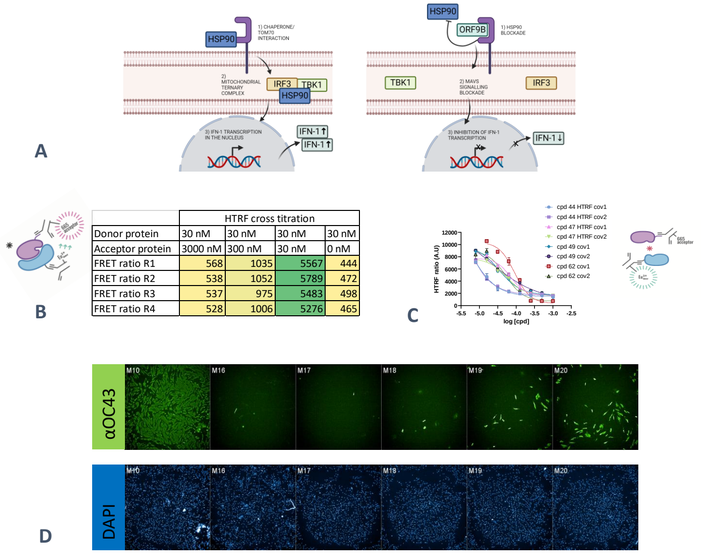
To date, severe acute respiratory syndrome coronaviruses continue to represent a global health issue for being highly transmissible, airborne pathogens. An **important coronaviral drug target** is the **main protease (Mpro)**, very conserved among the whole Coronaviridae family (α-, β-, and γ-coronaviruses), and whose clinically validated inhibitor Nirmatrelvir is expected to lose effectiveness over time because of the emergence of Mpro mutants in key residues for Nirmatrelvir effective blockade [1]. We have setup an automated thermal shift assay against Mpro (Z´-factor of 0.8) to identify alternatives to Nirmatrelvir. We have confirmed the activity of Cpd97A, a novel Mpro destabilizer [2], in a functional FRET enzymatic assay. In silico experiments with Cpd97A against the Mpro target from **both SARS-COV-2 and OC43** support the broad-spectrum antiviral activity of such compound, which has been biologically confirmed in a beta coronavirus model. **Another COVID-related issue** that remains unsolved is **how to prevent the severe symptoms caused by the transcriptional blockade of interferon-1** production that takes place in the early stages of the infection. Molecularly, it is known that Orf9b is a unique accessory protein of SARS-COV1 and 2 that is implicated in such immune evasion by targeting mitochondrial receptor TOM70. Neither **TOM70/ORF9b** inhibitors have been reported so far, nor high throughput screening (HTS) assays to identify them [3]. We present the first HTS assay for the identification of TOM70/ORF9b inhibitors that is based on the HTRF technology (Z´-factor 0.7), which has identified a set of 4 small-molecules. To our knowledge can be considered first-in-class inhibitors, of which Cpd62 is the most active in a beta coronavirus functional model.

Identification of broad-spectrum antivirals against Mpro-3CL



A. Successful purification of recombinant Mpro-3CL of SARS-COV-2. **B.** Thermal shift assay validation of Cpd97A (ΔTm = 4.5 ± 0.1 µM). **C.** Enzymatic FRET assay validation of Cpd97A (IC50 = 14.4± 4.1 µM). **D.** In silico validation of Cpd97A against Mpro3CL of SARS-COV-2 and OC43. **E.** Antiviral CPE assay on OC43 beta coronavirus (ED50 = 14.14 ± 3.78 µM).

Identification of first-in-class antivirals for hTom70



A. Biology of the TOM70/Orf9b interaction. **B.** Homogeneous Time-Resolved Fluorescence (HTRF) setup with the two recombinant proteins. **C.** HTRF confirmation of selected hits from the primary screen. **D.** Titration of the antiviral activity of Cpd62 on a OC43 beta coronavirus assay monitored by fluorescence microscopy in the High Content Screening format (green, fluorescence immunodetection of OC43´s nucleocapsid; blue, DAPI nuclear staining). From left to right, concentrations of Cpd62 treatments are 0 (vehicle alone), 40, 20, 10, 5 and 2.5 µM respectively.

Conclusions

- ✓ Our new **recombinant protein production** platform has led to the discovery of novel antiviral natural products, adding relevant **capabilities** to our portfolio.
- ✓ In silico experiments with **Cpd97A** suggest a **broad-spectrum activity against Mpro** that is further demonstrated in a **beta coronavirus functional model.**
- ✓ We present the **first High Throughput Screening (HTS)** assay for the identification of **TOM70/ORF9b inhibitors** which has identified a set of 4 small-molecules that can be considered **first-in-class**.
- ✓ The **scope** of the mitochondrial receptor TOM70 as a broad-spectrum antiviral target needs further studies to determine **how the TOM70 interactome contributes to the life cycle of other coronaviruses beyond SARS-COV1/2.**

References

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