High-content screening for the discovery of new antivirals.

Blanca Martínez-Arribas[1], Frederick Annang[2], Rosario Díaz-González[1], María C. Ramos[2], Francisco Castillo[2], Fernando Reyes[2], Olga Genilloud[2], Luis Miguel Ruiz-Pérez[1], Francisca Vicente[2], Dolores González-Pacanowska[1].

[1]Instituto de Parasitología y Biomedicina López-Neyra. Consejo Superior de Investigaciones Científicas. Granada, Spain.

[2]Fundación MEDINA, Parque Tecnológico de Ciencias de la Salud, Granada, Spain.

Coronaviruses (CoVs) have the ability to propagate and generate new species that cause epidemic diseases. The impact of the SARS-CoV-2 pandemic has revealed the urgent need for broad-spectrum antivirals to prevent future viral pandemics of unknown origin. Human coronavirus OC43 (HCoV-OC43) is a betacoronavirus that exhibits several common features with SARS-CoV-2 regarding structure and biology, which makes it a well-matched surrogate for SARS-CoV-2. We have successfully developed different methodologies for the identification of novel antivirals including inhibition of the cytopathic effect (CPE), estimation of antiviral activity by immunofluorescence-based high-content screening and determination of viral load by quantitative RT-PCR in 96 and 384-well formats. We have performed a pilot screen of the MEDINA collection of microbial extracts and other available natural product compounds using these methodologies. A primary screening with 1280 microbial extracts was accomplished by evaluating the inhibition of the CPE in MRC-5 cells 5 days post-infection with HCoV-OC43. Extracts that showed an inhibition of the CPE higher than 40% were selected as hits. Cherry picking and dose response analysis together with low resolution HPLC-MS deconvolution identified a total of 14 extracts of high interest for further analysis. A series of natural product components and several novel compounds with potential antiviral properties were identified in the HPLC-MS analysis. Further analysis of extract composition using high-resolution technologies and identification of active components may lead to the discovery of novel natural products with therapeutic potential in the treatment of viral infections. We also evaluated the antiviral activity of natural compounds that exhibit carbohydrate binding properties. These compounds derive from distinct species in nature and have the ability to bind to carbohydrates present in the viral envelope. Several active compounds emerged from this analysis.