

## Title

### **Assessing the antiviral activity of the natural product MM 46115 against respiratory viruses**

## Abstract

The influenza A virus is a major cause of severe respiratory tract infection, which results in 200,000 hospitalisations per year, in the UK. Vaccines have been developed to control the spread of infection, but vaccine hesitancy and antigenic drift limit their effects. Patients hospitalised with severe infection are given antiviral treatments, however, the virus has managed to develop resistant mutations against all classes of existing antivirals, rendering many of them ineffective. Therefore, there is a crucial need for new, effective treatments.

We aimed to investigate the antiviral activity of a tetronic acid macrolide called MM 46115 against the influenza virus. The natural product is produced by the soil bacterial species *Actinomadura pelletieri* and has previously been demonstrated to have antiviral activity against multiple respiratory viruses, including influenza A, parainfluenza and respiratory syncytial virus.

We confirmed these early findings using plaque assays with the influenza A virus strain A/WSN/33, which demonstrated an  $IC_{50}$  of 1.25  $\mu$ g (2.804  $\mu$ M). Further plaque assay and quantitative reverse transcription-PCR data from infections performed over a period of 2 – 24 hours showed treatment with MM 46115 produced lower virus titres for up to 6 hours, compared to without treatment. Cell viability assays were also performed using Madin-Darby canine kidney (MDCK) cells and human lung adenocarcinoma cells (A549) to investigate the cytotoxicity levels of MM 46115. Addition of MM 46115 (12.5 – 0.0125  $\mu$ g/mL) did not significantly reduce cell viability compared to the untreated group, however, a higher concentration of 125  $\mu$ g reduced cell viability to 80%. Furthermore, haemagglutinin inhibition (HAI) assays showed MM 46115 does not interfere with viral agglutination of red blood cells, thereby confirming the molecule does not bind to viral surface haemagglutinin to cause its anti-influenza activity.

Therefore, our data indicates that MM 46115 (12.5 – 0.125  $\mu$ g/mL) possesses antiviral activity against influenza A without significant toxicity, and its mechanism of action does not involve haemagglutinin inhibition.