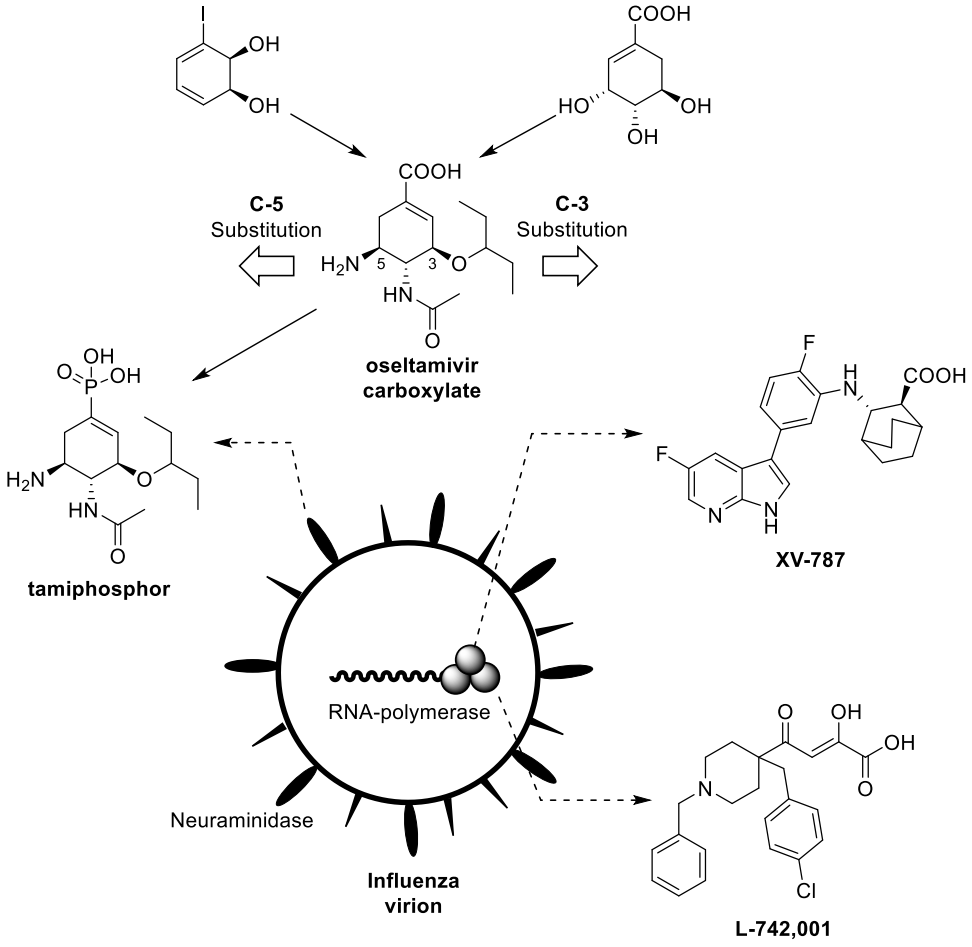
Abstract

Influenza viruses cause respiratory illnesses which can vary in severity depending on the strain of the virus, as well as the age and health condition of the host. Influenza remains a major threat to public health due to its nature prone to suffer mutations. As a result, vaccines have to be reformulated annually and new strains may cause sporadic global pandemics. Furthermore, the recent emergence of resistant strains of the virus against the current standard of care (oseltamivir and zanamivir) underlines the need of novel anti-influenza therapeutics.

The aim of this dissertation work is to contribute to the discovery of new anti-influenza inhibitors either by rational drug-design and optimization of oseltamivir structure, or by developing screening assays suitable for the discovery of novel inhibitors of the enzymes neuraminidase or RNA-polymerase.



Scheme 1. Overview of the strategy used for the development of new anti-influenza therapeutics. The dashed arrows indicate the inhibitors that were converted into probes and their corresponding target enzymes

Two main modification points were explored for the improvement of oseltamivir properties (Scheme 1); modifications at carbon C-3 aimed to overcome oseltamivir resistance caused by common mutations like H274Y, meanwhile modifications at carbon C-5 have been used to explore the binding mode of the inhibitors to a cavity adjacent to the catalytic site known as the "150-cavity".

A second strategy was used in the pursuit of new anti-influenza inhibitors which involved the development of novel assays suitable for the screening of novel neuraminidase and RNA-polymerase inhibitors. Several detection probes based on the structure of known inhibitors (tamiphosphor, XV-787 and L-742,001) of the respective proteins were designed, prepared, optimized and successfully applied for the development of screening assays based on either DIANA or AlphaScreen technology. Two new inhibitors of the PA subunit of RNA-polymerase with IC_{50} in the low micromolar range were found during the first screening campaign using the developed AlphaScreen assay.

Keywords: *Influenza, oseltamivir, inhibitors, drug design, neuraminidase, RNA-polymerase, detection probes, DIANA assay, AlphaScreen, screening assays*