Abstract

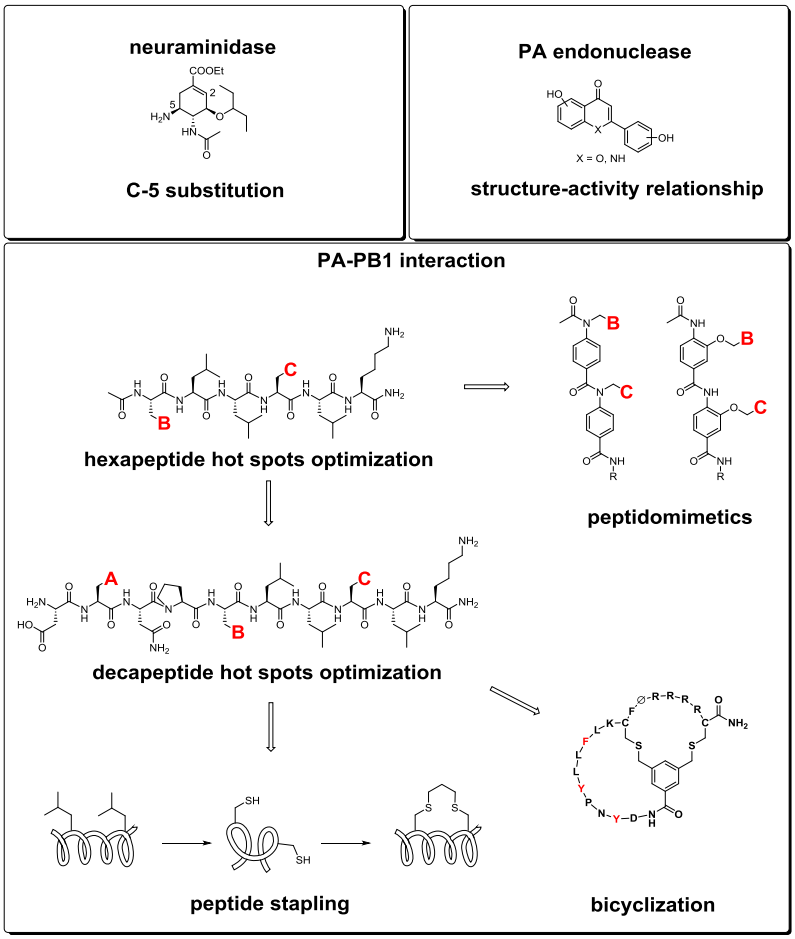
Influenza is an infectious disease caused by the influenza virus. This virus causes a severe viral infection that spreads easily from person to person in yearly pandemics. Vaccination is the most effective way to prevent the infection, however, due to the high rate in mutations of the virus, the vaccine needs to be often reformulated. Another option to combat influenza is based on administration of antiviral drugs. Clinical studies of isolated influenza strains („avian flu“ H5N1, 2004; „swine flu“ H1N1, 2009) revealed resistance towards known influenza neuraminidase inhibitors (zanamivir, oseltamivir). The resistance is caused by structural changes close to the enzymatic site. This calls for the development of new neuraminidase inhibitors as well for development of inhibitors targeting different influenza enzymes.

This Thesis is focused on design and synthesis of new inhibitors of influenza neuraminidase and RNA-dependent RNA polymerase, namely PA subunit and the assembly of PA-PB1 heterodimer enzymes (Scheme 1).

Influenza neuraminidase inhibitors were prepared by C-5 derivatization of oseltamivir followed by subsequent extension of its structure with binders of 150-cavity. Binding potencies of new oseltamivir derivatives against two influenza strains were determined.

The next part contributed to the elucidation of the anti-influenza effect of quercetin and related compounds as RNA-dependent RNA polymerase inhibitors. The mechanism of action of flavonoids as PA endonuclease inhibitors was discussed. This work features the extensive structure-activity relationship study accomplished on flavonoids as PA endonuclease inhibitors. Submicromolar inhibitors of PA endonuclease were successfully prepared and the Thesis covers numerous common synthetic approaches toward flavonoids.

The last part of the Thesis deals with preparation of PA-PB1 protein-protein interaction inhibitors. The crystal structure of PA C-terminal domain with a truncated decapeptide sequence derived from PB1 N-terminal subunit was used to design peptidomimetics based on the oligobenzamide scaffold. Phenylalanine and two tyrosine residues were identified to be crucial for the nanomolar activity of the PB1-derived decapeptide. In the subsequent work, the original hot spots were optimized using solid-phase peptide synthesis of hexapeptide inhibitors.



Scheme 1. Outline of three different topics of this thesis and the different strategies used.

The aim of this optimization was to increase the solubility of compounds while maintaining or even improving the inhibitory activities. The knowledge gathered was then applied in the design of new decapeptide inhibitors. Another crystal structure of optimized decapeptide with PA C-terminal domain was solved. Gained structural information was used to design stapled decapeptides with the intention to increase binding efficiency. Since the peptide inhibitors are inefficient in entering the cells, the peptide inhibitors were equipped with cell-penetrating sequence. These combined peptides were than cyclized around a central fragment to increase the proteolytic stability and cell-penetrating ability. Upon entering the infected cell, these bicyclic peptides should become linear and inhibit formation of PA-PB1 heterodimer.

Keywords: *neuraminidase, polymerase, enzyme, inhibition, synthesis, design*