ABSTRACT

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Title of the master thesis: Beta-secretase: production and *in vitro* testing of inhibitors.

Beta-secretase 1 plays a major role in the amyloid hypothesis in Alzheimer´s disease pathogenesis. Non-physiologically β-secretase together with γ-secretase cleaves amyloid precursor protein and is responsible for the production of amyloid beta peptides ($A\beta_{1-42}$), which accumulates in extracellular space and creates amyloid plaques. These are recognized by immune systems as antigens. Inflammatory response, which appear, can lead into neurodegeneration. Alzheimer´s disease is a complex multifactorial progressive disease which cause cognitive decline. Due to fatal consequences a patient depends on the care of other family members.

This master thesis aims to own expression, purification, and validation of recombinant β-secretase 1. It´s purification was made using Ni-NTA agarose. Concentration of protein was defined for characterization. For identification, a western blot method was used.

Recombinant β-secretase was used for experimental *in vitro* inhibition efficiency determination of 19 compounds by fluorescence spectrophotometric method. For the most effective inhibitor K1142 was determined IC_{50} value, which was compared to standard inhibitor Verubecestat. Unfortunately, K1142 is not a better inhibitor than standard Verubecestat. In conclusion, a pKa value was determined and used for the calculation of BBB score, which predicts transport into the brain.

Keywords: inhibitors, *in vitro* determination, beta-secretase, Alzheimer´s disease, production of recombinant proteins