Summary of PhD thesis of Teresa Łepek

"Design and synthesis of PACE4 peptide inhibitors as potential anticancer compounds"

The aim of the doctoral thesis was the synthesis of peptide inhibitors of PACE4, an enzyme that belongs to the family of the proprotein convertases (PCs). It plays a crucial role in the progression and proliferation of prostate cancer (PCa). The research group from the University of Sherbrooke (Canada), led by Professor Robert Day, designed and developed a highly potent and selective PACE4 inhibitor, known as Multi-Leu peptide (ML) with the sequence: Ac-LLLLRVKR-NH_2. Its pharmacological profile was further improved by the incorporation of arginine mimetic (4-amidinobenzylamide, Amba) and an unnatural amino acid residue (*D*-Leu) at its *C*- and *N*-terminus, respectively. As a result of those modifications they obtained the compound C23 with significantly increased *ex vivo* plasma stability due to the inhibition of the activity of exoproteases, such as amino- and carboxypeptidases. However, the core of the peptide is still susceptible to the activity of endoproteases, especially the peptide bond between the arginine and leucine residues at the P4 and P5 positions of the ML peptide.

The main goal of the research was to enhance the stability of the PACE4 inhibitor by using two different approaches. The first one, was the introduction of the Arg and Leu mimetics in the critical positions that are susceptible to the enzymatic degradation. The second procedure was to use various types of the ML peptide cyclization (head-to-tail, head-side chain cyclization and the introduction of a disulfide bridge into the molecule).

All peptides were synthesized by standard solid phase peptide synthesis (SPPS), using an amide resin (ML inhibitor analogs, Fmoc/tBu strategy) and 2-chlorotrityl-chloride resin (modifications of C23, Fmoc/tBu strategy combined with the synthesis in solution), manually or using an automatic synthesizer. After the removal of compounds from the resin, crude peptides were purified (by the preparative HPLC) and identified (MS, analytical HPLC). All analogs were obtained in good yield with the purity exceeded 98%.

The biological activity of all peptides were achieved by determination of the inhibitory effect toward PACE4 and furin (inhibition constants, K_i's) *via* competitive kinetic assays and antiproliferative tests (IC_{50}). The stability profile of the most potent analogs were evaluated using the mouse plasma and human serum and additional kinetic studies with PC5/6 and PC7 were conducted to determine the selectivity index toward other PCs.

The present work led us to determine the structure-activity relationship (SAR) between the designed potential peptide inhibitors and enzymes (PACE4 and furin). Moreover, strategies that we have applied enabled us to indicate the beneficial modifications of Multi-Leu and C23 peptides on the inhibitory properties toward PCs and anti-proliferative effect on PCa cell lines, allowing us to design and develop a pharmaceutical with anticancer properties in the future.