Wioletta Rut

Abstract of the PhD thesis „Natural and unnatural amino acids in design of active and selective activity-based probes for cysteine and threonine proteases”

Ubiquitin-proteasome system plays a pivotal role in maintaining the intracellular protein homeostasis. Disturbances in the functioning of this system can lead to several pathological events including tumor progression and neurodegenerative diseases. There are several therapeutic strategies to treat these diseases and one of them is proteasome inhibition. Research conducted by many scientific groups is focused on developing selective chemical tools for proteasome investigation, which will allow for better understanding of its biological functions.

The purpose of the first part of the dissertation was to obtain selective chemical compounds for monitoring the activity of three catalytic proteasome β subunits. In the first stage of the study substrate specificity profile of proteasome β subunits was determined using hybrid combinatorial substrate libraries (HyCoSuL) containing natural and unnatural amino acids. Then, based on obtained substrate specificity profiles, new, selective substrates for each catalytic proteasome subunit were designed and synthesized. In the last stage of the study activity based probes containing selective peptide sequences were synthesized. The selectivity of these compounds was confirmed in cell lysate studies.

The second part of the dissertation was focused on cysteine proteases exhibiting deubiquitinating activity, that are an alternative therapeutic target for ubiquitin-proteasome system. Deubiquitinating enzymes (DUBs) are responsible for removing ubiquitin from its peptide conjugates. The purpose of this part of the work was to design and synthesize chemical tools that would enable DUBs activity monitoring. As deubiquitinating enzyme, SARS PLpro was chosen because it is an important target in antiviral therapy. The implementation of this goal included synthesis of combinatorial substrate library containing natural and unnatural amino acids, determination of SARS PLpro substrate specificity in P4 and P3 positions, and kinetic studies of designed fluorogenic substrates. New SARS PLpro active substrates were obtained, as well as specific peptide sequences that were used in the synthesis of selective SARS PLpro inhibitors.

Proteasom and deubiquitinating enzymes are important therapeutic targets for the treatment of viral, neurodegenerative and cancer diseases. The lack of specific chemical tools for the exploration of these enzymes, significantly impairs determining their role in normal and pathological conditions. Using combinatorial substrate library containing a wide variety

of unnatural amino acids allowed to design selective peptide sequences for each catalytic proteasome subunit and SARS PLpro. The results obtained in the dissertation (selective substrates, inhibitors and activity based probes) serve as excellent tools for determining 20S proteasome and SARS PLpro biological functions, monitoring their activity in the cells. These selective chemical compounds may be useful for better understanding the role of these enzymes in pathological states.