Summary of the doctoral dissertation of Przemysław Karpowicz, M.Sc. tilted: „Searching of an allosteric modulators of the proteasome`s activity". The dissertation was prepared under the supervision of Dr. hab. Elżbieta Jankowska and Dr. Maria Gaczynska (University of Texas Health Science Center at San Antonio, USA)

The proteasome (20S) is a multicatalytic and multisubunit protease complex responsible for most of cellular protein turnover. Proteasomes are localized in both cytoplasm and nucleus where they degrade misfolded or oxidatively damaged proteins. Most of these substrates are marked beforehand by attachment of multiple chains consisting of small protein called ubiquitin (Ub). The substrates tagged with Ub are recognized and degraded by proteasomal complex called 26S, on the proteasome-ubiquitin pathway (UPS). The catalytic functions of all assemblies under "proteasome" name are performed by the 20S proteasome. The 20S proteasome is built from four stacked heptameric rings arranged in a specific order α/β/β/α. The two outer rings form a gate guarding hidden inside catalytic centers residing in β subunits. Six catalytic centers inside the proteasome "barrel" cleave peptide bonds preferentially on the carboxyl sides of specific types of amino acid residues: hydrophobic (chymotrypsin-like; ChT-L) activity, basic (trypsin-like; T-L) activity, and post-glutamyl peptide hydrolyzing (PGPH; post-acidic/caspase-like) activity. Coordinated actions of the three types of active sites assure that the enzyme cleave efficiently a variety of cellular substrates. The top surface of the outer α rings called "alpha face" provides binding sites for different allosteric regulators e.g. 19S cap which together with 20S form 26S proteasome. When allosteric regulators bind to outer - α rings, allosteric interactions are transferred between the catalytic centers and the regulatory particles. Probably this process involves a long-distance transfer of structural signals, which is an example of the allosteric mechanism.

Since the proteasome takes part in literally every cellular process and in etiology of many diseases, like: cachexia, cancer, cardiac dysfunction, it does not seem to be a good target for any kind of specific pharmacological intervention. Fortunately, the clinical success of bortezomib (Velcade®) proved this impression wrong. However, bortezomib is not an ideal therapeutics because of high cytotoxicity and high relapse rate. Bortezomib blocks the ChT-L active site, indiscriminately halts most of the proteasome-mediated proteolysis, and triggers apoptosis. As opposed to active site inhibition, precise regulation of the proteasome activities is possible via allosteric interactions. Allosteric control of proteasome activity may give a chance for a future widespread use of this ubiquitous protease as a pharmaceutical target.

The substrate-specific inhibition of the proteasome based on allosteric interactions is possible, as proved by the actions of the viral protein HIV-1 Tat.

As established, short 12-residue fragment of this protein called Tat1 peptide is able to modulate 20S proteasome activity in a way similar to the whole protein. Tat peptides are able not only to inhibit the human 20S proteasome but also to compete with some regulators in binding to the α face. Unfortunately, we still do not know enough about the nature of allosteric regulation and structure/activity relationship to efficiently control the phenomena and rationalize the design of more effective compounds/pharmaceutics in the future.

The presented doctoral dissertation describes 37 compounds - potential inhibitors of the human 20S proteasome with the mechanism of action based most probably on allosteric regulation. In this dissertation I report the design and biological effects of twelve-residue long peptides derived from the viral HIV-1 Tat protein (Tat peptides). Ten of the synthesized compounds have incorporated unnatural modifications which are potential β-turn inducers. I have tested the modulatory effect of all compounds on the isolated human 20S proteasome with fluorogenic model substrates. At the same time I have checked if compounds are not degraded by the enzyme. I also used the α-synucleinas a protein model substrate to test if and how new derivatives alter the proteasomal degradation pattern. For this purpose I used electrophoretic techniques and mass spectrometry. Finally, Ihave employed circular dichroism, ^{1}HNMR spectroscopy combined with molecular modeling and microscale thermophoresis to determine the structure-activity relationship and influence of the modifications on their abilities to form a complex with the 20S proteasome. As a preliminary study I have tested the cytotoxicity of some compounds on cancer cells (HeLa).

The study of single and multiple alanine-walking analogs of Tat1 allowed to identify structural groups of “hot spots” (pharmacophores). The localization of these "hot spots" correlates very well with the turns predicted by the molecular modeling. Ihave found that introduction of a β-turn moiety into pharmacophore region improves inhibitory potency of the resulted Tat1analogs, as compared with the canonical Tat1 peptide. The best compound from this group seems to be the peptide with the Tic-D-Oic moiety in the 8-9 position. Conformational study showed that Tat1 peptide and its alanine-scan analogs tend to have unstructured or PPII conformation. However, the peptide with Tic-D-Oic moiety incorporated in the position 8-9 has probably a β-turn conformation, as determined by means of circular dichroism spectroscopy. My study shows that capability of the compounds to inhibit the proteasome depends on the presence of a β-turn conformation, but not on the type of the turn inducer is important, but the length of the basic sequence flanking it on both sides. Some of the synthesized compounds with a β-turn moiety have quite low IC_{50} (about 70 nM) for ChT-L and PGPH activity of the human 20S proteasome, which makes them about 3,5 times more potent inhibitors than Tat1 peptide. Those compounds are not only the most potent inhibitors among the synthesized Tat1 analogs but probably also among the low-mass allosteric inhibitors of the 20S proteasome published so far. The study of Tat1 peptides showed that allosteric phenomenon of their action on the 20S proteasome depends not only on the accumulation of basic amino acid residues but rather on the special arrangement of these residues. Similar conclusions can be made based on the results of microscale thermophoresis. Importantly, the introduction of a β-turn renders the Tat1 derivative highly cytotoxic for human cultured cancer cells (HeLa.S3), in a sharp contrast to the non-toxic Tat1 peptide.