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Design, chemical synthesis and kinetic studies of peptide non-covalent and covalent proteasome inhibitors

Degradation of proteins in eukaryotic cells is carried out in two ways: either *via* lysosomal proteolysis or *via* the ubiquitin-proteasome pathway. The 26S proteasome is a protein complex composed of a 20S particle (which is responsible for proteolysis) and two 19S regulatory subunits. In mammalian cells, the 20S proteasome can predominately exist in three forms, such as: constitutive proteasome, immunoproteasome and thymoproteasome. The constitutive proteasome is present in all eukaryotic cells, whereas the immunoproteasome is abundantly expressed in cells of hematopoietic origin. The third isoform – thymoproteasome – is found exclusively in cortical thymic epithelial cells. All these proteasomes are responsible for ensuring regular cell cycle progression, immune surveillance and biological homeostasis. However, due to their prominent role in various essential cellular processes, they are also involved in pathogenesis of different disease conditions, such as various cancers and inflammatory-related diseases.

The proteasome is considered as a very important target for anticancer therapy. It was shown that the proliferating cells of malignant tumors are more sensitive to proteasome inhibition than normal cells. Therefore, research of constitutive proteasome and immunoproteasome inhibitors have been conducted for many years in numerous academic and pharmaceutical centers.

Two groups series of inhibitors of the human 20S proteasome were designed and synthesized as part of this doctoral dissertation. Peptide aldehydes, composed of 4 proteinogenic amino acid residues were the first group of compounds. Those inhibitors were synthesized and analyzed *in vitro* against human constitutive proteasome 20S and immunoproteasome 20S. Their primary structures were designed based on the structures of novel peptide substrates, which were obtained *via* combinatorial approach.

Peptide aldehydes with the primary sequence IPMD-al, having at their *N*-terminus acyl groups with a long hydrophilic (O2Octa-IPMD-al) or hydrophobic (Ahx-IPMD-al) chain terminated with an amino group, showed the highest activity against the β1c subunit. Among

the tested compounds, most peptide aldehydes having the primary sequence VVMR-al belong to the strongest β2c inhibitors. Peptide O1Pent-VVMR-al turned out to be the most potent one. Inhibitors O2Octa-VVFF-al, O2Octa-VLFF-al and O2Octa-hF-LFF-al showed the highest activity against the β5c and β5i subunit. According to the IC_{50} values, peptide aldehydes O2Octa-VLSF-al and O2Octa-VNSF-al are 2.7-times stronger inhibitors of the β5i subunit than β5c.

The second group of compounds were novel analogues of sunflower trypsin inhibitor (SFTI-1). [Arg^5,$Lys^{7,8}$]SFTI-1 was used as a starting structure and its primary structure was subjected for further modification. Among these peptides were acyclic, monocyclic (either with disulfide bridge or with cyclic backbone) and bicyclic (containing both, side-chain and backbone cyclization), *N*-terminal acylated peptides having either hydrophobic or hydrophilic moieties, truncated analogues without *N*-terminal Gly^1 and/or Arg^2 residues, Gly^1 and/or Arg^2 substituted peptides and the double-sequence analogues with one or two disulfide bridges. Inhibitory activities of these analogues were studied *in vitro* against human 20S constitutive proteasome.

In general, all inhibitors which had two *N*-terminal residues containing guanidyl moieties, at the positions 1 and 2, were apparently stronger than their counterparts having only one such residue. The cyclization via disulfide bridge and/or backbone seemed to be important and desirable but did not play the crucial role in the interactions with the 20S proteasome. The double-sequence analogues were among the strongest proteasome inhibitors.