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ABSTRACT OF PHD THESIS

Design and synthesis inhibitors of protein-protein interaction for systems: menin-MLL and p53-MDM2

Protein-protein interactions play an important role in biological processes and play a key role in the development of many diseases, including various types of cancer. Unfortunately, the discovery of new synthetic protein-protein interactions inhibitors is a very difficult task. It is often problematic to develop low-molecular-weight compounds which can reach small area of the proteins. The presented work was focused on design and synthesis of the peptide inhibitors of menin-MLL and p53-MDM2. Peptides are not preferred candidates for drug because of their low proteolytic stability. However, due to the introduced peptide modifications of the backbone and side chains, molecules with desirable properties can be obtained, especially with high proteolytic stability, which is very important in therapeutic applications.

This work seeks out the new high-activity peptide inhibitors of p53-MDM2 and menin-MLL interactions. In both cases, inhibitors may be used as anticancer drugs. The main design strategy consisted of stiffening the peptide structure in the active conformation by introducing β-amino acid residues or cyclization. The aim of these modifications was to increase inhibitory activity and decrease proteolysis.

The starting point of the study was designing of peptide protein-protein inhibitors of menin-MLL and p53-MDM2 interactions, using previously published crystal structures fragments of proteins interaction with the application of computerized methods. The next stage of work was scientific description a synthesis method for obtaining planned structures, which contained synthesis-suitable building blocks, peptide synthesis on solid support and modification of side chains. Afterwards received peptides were tested for biological activity in relation to selected protein-protein systems. The obtained results allowed for optimizations which purpose was to gain the most active peptides. In the final stage of research, for selected peptides, crystal structures of the protein-peptide complex were obtained and proteolysis susceptibility was designated.

The scan of β-amino acid of peptide inhibitor of p53-MDM2 interaction (24 peptides were synthesized and tested) were obtained, which indicated possible places to substitutions of β-amino acids residues. As a result 11 peptides which contains of 3 or 4 stiffened β-amino acids residues were designed and obtained (*cis*- or *trans*-2-aminocyclopentanecarboxylic acid). The circular dichroism confirmed the preference of all obtained peptides in the direction of to helical conformation. The most active peptide inhibitors of p53-MDM2 interaction exhibited 2 row higher inhibitor activity compared to α-peptide PMI. The most active peptide received K_i =0.4 nM for MDM2 and K_i = 23 nM for MDMX.

The linear and cyclic peptide inhibitors of menin-MLL interactions were designed. Subsequently effective method for synthesis of cyclic peptides using a metathesis reaction (Grubbs 2 generation catalyst) and peptide hydrogenation reaction on a solid (Wilkinson catalyst) was developed. The structure optimization was realized by taking into account the length of the linker, the N- and C- terminus, the Phe9 and Ala11 residues (26 peptides were obtained). Peptide inhibitors of menin-MLL interaction were obtained with inhibitory activity in the nanomolar range, which the most active peptide exhibited IC_{50} = 19 nM.