

BIFUNCTIONAL INHIBITORS OF PROTEIN KINASES FOR INTRACELLULAR APPLICATIONS

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Although a limited number of protein kinase (PK) inhibitors have reached drug market, protein kinases (PK) are the second largest group of drug targets after G-protein-coupled receptors, and they account for 20–30% of the drug discovery programs of many companies [1]. Despite serious selectivity problems, the main efforts of pharmaceutical companies have concentrated in the direction of the development of ATP competitive inhibitors.

Several strategies to design bisubstrate analog inhibitors for PK have been described [2]. Bisubstrate (bifunctional) inhibitors structurally mimic both substrates of a kinase and simultaneously interact with the respective binding domains of the enzyme. Pharmacological potential of this logical and promising approach of inhibitor design has been limited by low bioavailability of compounds comprising peptide moieties and charged phosphates.

The bifunctional inhibitors of basophilic PK designed by us comprise moieties targeted at the ATP binding site, adenosine-5'-carboxylic acid (Adc), and the protein/peptide substrate binding site-directed oligoarginine [3]. These motifs were connected *via* a linker chain whose structure was optimized in QSAR studies. Additionally, this approach enables the application of the knowledge about the effect of the structure of nucleotides on kinetics of phosphoryl transfer reactions [4]. Later we demonstrated [5] that adenosine-oligoarginine conjugates (ARC) were cell-permeable, which pointed to the potential applicability of the bisubstrate analogue approach for rational design of potent and selective inhibitors for the regulation of the intracellular protein phosphorylation equilibrium.

ARC-s as peptide-type compounds are prone to proteolytic degradation, therefore enhancement of their stability, in order to prolong their *in vivo* and intracellular half-lives, is of high importance. Moreover, the applicability of the compounds *in vitro* and *in vivo* experiments demands regulation of their cellular uptake and intracellular targeting. The results of our studies in this field will be presented.

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