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BISUBSTRATE PROBES IN FLUORESCENCE POLARIZATION-BASED BINDING/DISPLACEMENT ASSAYS FOR HTS OF PROTEIN KINASE INHIBITORS

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Protein kinases (PKs) play a key role in the regulation of protein functions in living cells. Over 400 human diseases (including cancer, diabetes, Alzheimer, malaria, hypertension) have been linked to aberrant PK signaling, which has made PKs important drug targets [1]. This has caused an increasing need for the elaboration and improvement of analytical methods for high-throughput screening (HTS) and characterization of new compounds.

We have previously developed inhibitors for basophilic protein serine/threonine kinases with affinities in the subnanomolar region [2,3].These ARC-type inhibitors comprise analogues of both substrates, an adenosine mimic targeted to the ATP-binding site and an arginine-rich peptide targeted to the binding domain for the protein to be phosphorylated.

ARCs possess high affinity (K_d < 0.1 nM) that is preserved during labeling of ARCs with fluorescent dyes or other entities. The selectivity of ARCs towards the different basophilic protein kinases can be achieved by utilization of different nucleosidic fragments, linkers, and peptide moieties [4].

The bisubstrate character of the fluorescently marked ARC-type probes enables their application in the binding/displacement assay with fluorescence polarization/anisotropy detection (in the 384-well microtiter plate format on a fluorescence plate-reader) for the determination of affinities for both ATP- and protein substrate-competitive inhibitors of PKs (e.g., PKA, PKB, PKC, PKG, ROCK, MSK1, etc.) [5]. The probes can also be used for the determination of the active concentration of kinases, and as a cAMP sensors.

The features of the assay including homogeneity, single-step, quickness (less than 1 h), and no need for special substrates and capricious antibodies, support the use of the assay for HTS.

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