The Development of Biligand Inhibitors and Non-Radioactive Inhibition Assay for Protein Kinase Aurora B

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The goal of given project was to synthesize and biochemically characterize a new class of compounds that could report and block the abnormally elevated activity of Aurora B. The compounds represented biligand class of inhibitors, incorporating an ATP-analogue (previously reported inhibitor MLN8054) and a fragment of endogenous activator of Aurora B (protein INCENP). Between two fragments, a linker was added which consisted of a chiral element (either L- or D-lysine) and a longer alkyl chain (aminohexanoic acid). For characterization of efficiency and selectivity of the obtained compounds towards Aurora B and a closely related protein kinase Aurora A, a new fluorometric non-radioactive inhibition assay was developed. The latter used fluorescently labeled TAMRA-Kemptide as a substrate and took advantages of thin-layer chromatography for separation of the phosphorylated and non-phosphorylated substrate forms. The compounds showed 50% inhibition of Aurora B and Aurora B/INCENP at 1 μM concentration of compounds and upon further modification, could take full advantage of their biligand nature. New assay can be applicable for further screening of selective activators or inhibitors (including potential drug candidates) targeting Aurora B.