Biochemical context drives conformational-selectivity of inhibitors bound to Aurora A Kinase

ABSTRACT

Activation of protein kinases is driven by large-scale conformational rearrangements induced by regulatory factors, such as phosphorylation or endogenous protein-partner binding. Many small-molecule kinase inhibitors are known to bind preferentially to either the active DFG-in state or the inactive DFG-out state, yet the interactions between inhibitor-induced and regulatory-induced structural changes is not well understood. While crystallography has provided a wealth of structural information to aid in the rational design of conformationally-selective inhibitors, static crystal structures fail to capture the level of dynamics and conformational ensembles that the kinase domain exists in. Using a solution-based high-throughput time-resolved fluorescence methodology, which was developed in our lab and can also be applied to the study of other kinases, we have profiled a panel of Aurora inhibitors against the activation states of the mitotic kinase Aurora A, to quantify the changes in inhibitor-induced conformational shifts. We found that all inhibitors in the panel promote either the active DFG-in state or the inactive DFG-out state to remarkably differing extents that provide insight into inhibitor selectivity patterns for different activation states of Aurora A. The results show that DFG-out inhibitors preferentially favor binding to the dynamic pool of Aurora A that is activated by phosphorylation on the activation loop, whereas DFG-in inhibitors bind better to the more static form of Aurora A that is shifted to the DFG-in state by the allosteric activator Tpx2. Further, these results indicate that many of the inhibitors in clinical development may selectively target different pools of Aurora that vary in the level of their conformational dynamics. These results provide a wealth of structure-activity information that have broader implications for the rational design of inhibitors that exploit differences in conformational dynamics to gain increased selectivity.