From chemistry to cell activity: Identification of cell-active small-molecule inhibitors of AP-2-Associated Protein Kinase 1 (AAK1)

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Introduction: AP-2-Associated Protein Kinase 1 (AAK1) is a serine/threonine kinase that can regulate clathrin-mediated endocytosis. This process is important both in cell physiology and in the pathogenesis of viruses such as HCV and Dengue, so there may be therapeutic value in modulating AAK1 activity. However, AAK1 has not been explored as a drug target; specific chemical inhibitors would be the best tools to investigate this possibility, yet no such inhibitors are known.
Objective: Dr. Wilson's group at SGC-UNC has identified compounds that bind tightly to AAK1A (IC50 < 50 nM) in vitro. Our objective was to verify whether those compounds bind and inhibit AAK1 inside human cells.
Material and Methods: We employed three experimental methods to test cellular activity. First, the Cellular Thermal Shift assay, whereby binding of the ligand to AAK1 in cells is detected by its ability to stabilize the protein against thermal denaturation. Second, inhibition of phosphorylation of an AAK1 target site, Thr162 of AP-2. Third, the effect of the inhibitor on cellular viability.
Results and Discussion: The vehicle and negative control displayed similar temperatures of melting, 51.3°C and 52.2°C, respectively. The Tm for three AAK1 inhibitors tested was 54.20°C, 55.4°C and 53.5°C. The three positive compounds, but not the negative control, can decrease AP-2 phosphorylation and are cytotoxic for HEK293 cells. The compounds can reach and inhibit AAK1 inside the cells. Further experiments are needed to characterize those compounds as probes, including EC50 measurements in cells and the mechanism of cellular toxicity.
Conclusion: We successfully found compounds that bind to and inhibit AAK1 activity inside the cells. These molecules have now been distributed to collaborators who can test the biological consequences of AAK1 inhibition, which are crucial for evaluation of AAK1 as a potential drug target, and the utility of the compounds as starting points for drug design.

Keywords: Protein kinase, AAK1, small-molecule inhibitors,

Acknowledgement: Dr. Wilson, University of North Carolina,USA

Supported by FAPESP