

A High-Content Subtractive Screen for Selecting Small Molecules Affecting Internalization of GPCRs

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ABSTRACT

G-protein-coupled receptors (GPCRs) are pivotal in cellular responses to the environment and are common drug targets. Identification of selective small molecules acting on single GPCRs is complicated by the shared machinery coupling signal transduction to physiology. Here, the authors demonstrate a high-content screen using a panel of GPCR assays to identify receptor selective molecules acting within the kinase/phosphatase inhibitor family. A collection of 88 kinase and phosphatase inhibitors was screened against seven agonist-induced GPCR internalization cell models as well as transferrin uptake in human embryonic kidney cells. Molecules acting on a single receptor were identified through excluding pan-specific compounds affecting housekeeping endocytosis or disrupting internalization of multiple receptors. They identified compounds acting on a sole GPCR from activities in a broad range of chemical structures that could not be easily sorted by conventional means. Selective analysis can therefore rapidly select compounds selectively affecting GPCR activity with specificity to one receptor class through high-content screening.