

## Abstract

The ubiquitin proteasome system is attracting much interest as a source of potential drug targets. The ubiquitination process requires the transfer of one or more ubiquitin adducts to a substrate protein, thus modulating the substrate's stability, function or subcellular location. Intervention in ubiquitination by small compounds may correct cell pathologies or provide the mechanism for host cell directed antiviral drugs. The RING finger protein POSH is a host-cell ubiquitin ligase essential for HIV budding. We have performed extensive HTRF assay development and screening of small molecule compound libraries for POSH inhibitors and further established filtering and differential assays. Potent and selective compounds have been identified and optimized. Validated POSH inhibitors have demonstrated activity by inhibiting ubiquitination of POSH and a cellular POSH substrate, and more importantly, have demonstrated antiviral effects on HIV-1 and HIV-2. Two other screening campaigns for E3 inhibitors have also been conducted using HTRF methods to monitor poly-ubiquitin chain formation or E3-substrate binding in two distinct ubiquitination systems. These campaigns have also produced validated hits, thus providing a proof-of-concept for E3 targeted drug screening. Design of specificity and selectivity assays will be discussed, as well as a comparison of HTRF with traditional and alternative ubiquitination methodologies.