Developing small molecule inhibitors requires a robust screening cascade which can efficiently identify compounds which engage the target protein, determine selectivity over undesirable related proteins and demonstrate activity in downstream functional assays.

Here we present data on the screening cascade we have successfully developed for a client project to identify small molecule inhibitors of a DNA damage response protein. Utilising the Promega NanoBRET™ Target Engagement Assay has enabled us to distinguish molecules which bind to the target protein in a cellular context using full length protein constructs whilst also showcasing differences in affinity to a related but off-target protein. CellTiter-Glo® has been effectively used to identify compound-induced toxicity and establish effective non-toxic concentration ranges for downstream functional assays. To demonstrate on-target activity we developed two plate-based flow cytometry assays to interrogate the mechanism of action of the protein of interest. A TUNEL assay examines the ability of test compounds to inhibit DNA single strand break formation whilst hits are confirmed in an orthogonal cell cycle assay using Bromodeoxyuridine (BrdU) and 7-Aminoactinomycin D (7-AAD) staining to categorise the position of cells within the cell cycle. Overall, this has resulted in a robust screening cascade which has been effectively utilised to screen test compounds and identify inhibitors of the protein target.

If you would like to discuss our work on this project or your own drug discovery needs then please join us at the poster exhibition or at the exhibitor stand B20.