Identification and Characterization of Potential BRDT Inhibitors by Fragment-Based Screening Using Differential Scanning Fluorimetry, PrOF-NMR, and Protein Crystallography

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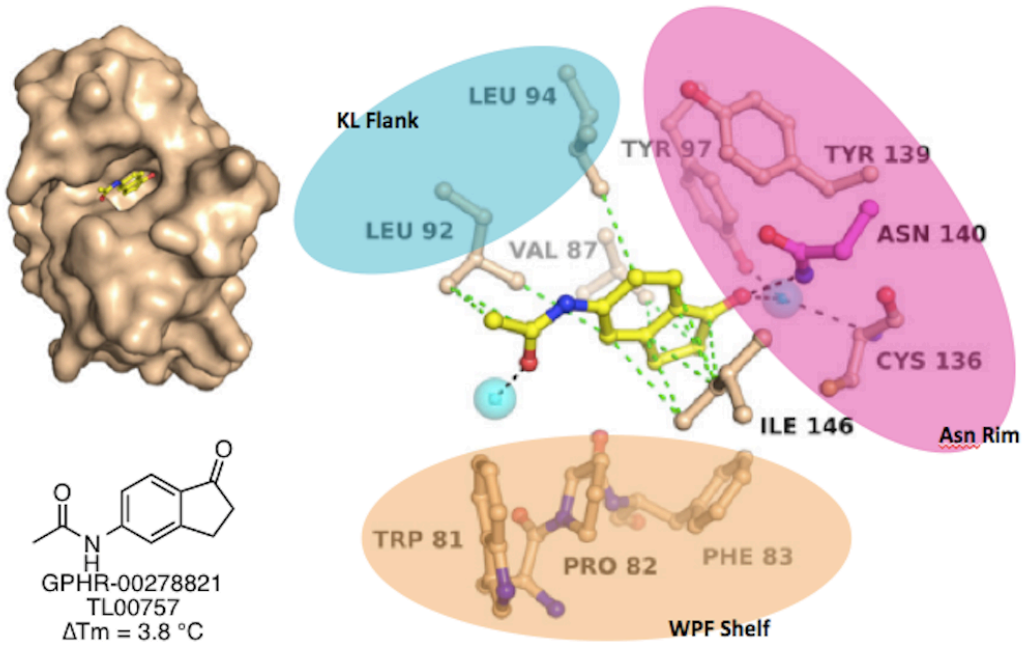
Bromodomain (BRD) containing proteins are essential for acetylated lysine (Kac) recognition on histone proteins during transcriptional activation. BRDT is a testis-specific BRD protein required for male germ cell differentiation has been validated as a target for non-hormonal male contraception.

Differential scanning fluorimetry (DSF), a thermal shift assay, was used to detect protein binding in a fragment library screen, with a hit identified as a fragment increasing the transition temperature (T_m) of the protein ≥ 0.5 °C as compared to the DMSO control. From this screen, 22 compounds were confirmed as BRDT stabilizers upon repurchasing and dose response testing. A protein-observed ^{19}F-NMR (PrOF-NMR) assay with ^{19}F-labeled BRDT was used to confirm protein binding. Fluorescence polarization was also used to determine K_i and IC_{50} values.

The sixteen PrOF-NMR confirmed hits were then taken into crystallographic studies to provide data on binding interactions. A number of crystal structures have been solved with BRD4 and BRDT, allowing for the prioritization of potential lead compounds. Structure-activity relationships will be established for selected hits.

Fragment TL00757 is currently in hit-to-lead optimization to identify potent and selective BRDT inhibitors. A ligand growth strategy is underway to increase BRDT affinity and selectivity over the closely related protein BRD4 by increasing molecular interactions in the ZA channel and particularly with Arg154.



Crystal structure of DSF hit TL00757 (GPHR-00278821) with BRD4.