Dual screening using protein-observed fluorine NMR uncovers the first selective inhibitor for BPTF

Andrew K. Urick

We describe a ^{19}F NMR dual screening method for detecting bromodomain-ligand interactions using fluorine-labeled aromatic amino acids on two different bromodomains. Bromodomains are integral domains of proteins in chromatin biology, acting as epigenetic regulatory proteins that control the accessibility of DNA for transcription. Overexpression of bromodomain containing proteins has been linked to disease states such as cancer and cardiac hypertrophy, so the ability to selectively inhibit bromodomain interactions could result in new therapeutic treatment options. Due to the excellent chemical shift dispersion of ^{19}F resonances within fluorine-labeled proteins, two fluorinated bromodomains were analyzed simultaneously with a single NMR experiment. Over 200 small molecules were screened against the first bromodomain of Brd4 and the bromodomain of BPTF. We report the first small molecule binder selective for BPTF over Brd4, in addition to two new classes of molecules that bind to the first bromodomain of Brd4. These hits were validated in a complementary differential scanning fluorimetry assay, and potency determined via fluorescence polarization for Brd4 and isothermal titration calorimetry for BPTF. The speed, ease of interpretation, and low concentration of protein needed for protein-observed fluorine NMR binding experiments affords an excellent method to discover and characterize new and selective ligands for bromodomain-containing proteins.