Discovery of Selective Inhibitors and Fluorescence Tools for Testis-Specific Bromodomain (BRDT)

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Testis-specific bromodomain (BRDT) is a member of the bromodomain and extra-terminal (BET) protein family. BRDT interacts with acetylated lysine residues of histones and is important for the process of spermatogenesis. BRDT is expressed only in the testis and BRDT-1 knockout mice are infertile. Therefore, the inhibition of BRDT is a promising strategy for male contraception, however no selective BRDT inhibitors have been discovered yet. High sequence homology between BRDT and bromodomain-containing protein 4 (BRD4) hinders the discovery of selective BRDT inhibitors. In our study, 2,4-disubstitued pyrimidine derivatives of SG3-179, a potent inhibitor of BRDT and BRD4, were designed on the basis of the "arginine hypothesis." Arg54 of BRDT is only present in BRDT and is not a conserved residue in other BET family proteins. We therefore hypothesize that interaction between Arg54 and the designed compound may lead to BRDT selective inhibition. In order to address selectivity, novel bivalent inhibitors were designed and synthesized. In an alpha screen assay, the monovalent compound showed similar potency against BRD4 (IC_{50} = 98 nM) and BRDT ((IC_{50} = 45 nM). However, the bivalent inhibitor GXH-II-052 exhibited 30-fold selectivity for BRDT (IC_{50} = 8 nM) over BRD4 (IC_{50} = 229 nM). Validation of this data with orthogonal assays as well as investigation into the possible mechanism of action is underway. In addition to inhibitor design, novel probes were prepared for use in a fluorescence polarization assay. A series of SG3-179 analogs linked to the Bodipy fluorophore was synthesized and photo-physical properties were measured. Optimization of the fluorescence polarization assay has begun.