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Discovery of glycosyltransferase inhibitors of GALNT3 using the assay optimization and a transcreener UDP2 assay and orthogonal pooled screening

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Glycosyltransferase enzymes participate in diverse metabolic and regulatory roles by catalyzing the transfer of sugars to protein, lipid and carbohydrate acceptors as well as to other endogenous and xenobiotic molecules. Of more than 200 human glycosyltransferases (GTs), there are over 20 distinct polypeptide N-acetylgalactosaminyltransferases (GALNTs) that catalyze the initial step of O-glycosylation by transferring GalNac to Thr or Ser residues on multiple targets including mucins. Abnormal post-translational glycosylation of mucin is a driver of cancer-associated changes diversely affecting growth and survival of cancer cells and their ability to invade and metastasize. GALNT3 overexpression and dysregulation has been directly linked to multiple cancers including gastric, pancreatic, ovarian, lung and others making it a compelling target for drug discovery. Development of an HTS workflow for GALNT3 is described here. Recombinant First, GALNT3 enzyme activity robustness was first evaluated tested and optimized in the Trasncreener UDP2 Assay with the donor and acceptor substrates UDP-GalNac and Mucin 10 (153-165) EA2 peptide. Second, in order to perform a pilot screen, optimal GALNT3 concentration and Km values for both substrates were determined. Thirdly, A pilot screen was run using the TR-FRET-based assay with a with a diverse, pre-screened filtered 8,000 compound OPS orthogonally pooled compound set library from LCGC from the Lankenau Institute for Medical Research (LIMR) Chemical Genomics Center which allowed screening of 8,000 compounds in duplicate in just five 384-well plates. Hits were confirmed followed by dose-response curves of all potential hits measurements with the primary screening assay and then further validated with a FP based Transcreener UDP2 Assay second assay format. Finally, two confirmed hits were further evaluated for the longevity of target engagement by doing jump dilution performing rapid dilution experiments to assess measure dissociation rates residence time and reversibility.

Biography

Tom Zielinski has worked at Bellbrook Labs for over 8 years where he is the Manager of Biochemical assays. Over 20 years, he has been an R&D Scientist who has helped develop numerous cellular and biochemical assays for enzymes in diverse classes such as GPCRs, kinases, methyltransferases and glyosyltransferases.

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