Development and Optimization of AlphaScreen Assay for Discovery of Galectin-1/-3 Inhibitors

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HYPOTHESIS

An assay format for the discovery of new inhibitors of galectin-1/-3 (Gal-1/-3) using the amplified luminescent proximity homogenous (AlphaScreen) technology[1] in a competitive binding configuration will be developed and optimized.

BACKGROUND

Gal-1 and Gal-3 has been extensively studied in cancer research due to their role in tumor progression and metastasis, and tumor immunology.[2] The discovery of gal-1/3 carbohydrate ligand inhibitors is crucial to block their cancer-promoting roles *in vivo*. However, the development of high-throughput screening (HTS) assays with increased sensitivity for the identification of potent and selective inhibitors of galectins has been hampered by the weak binding affinities between galectins and their carbohydrate ligands. We have recently developed AlphaScreen assay in a competitive binding configuration for discovery of inhibitors of glycan-lectin interactions.[3]

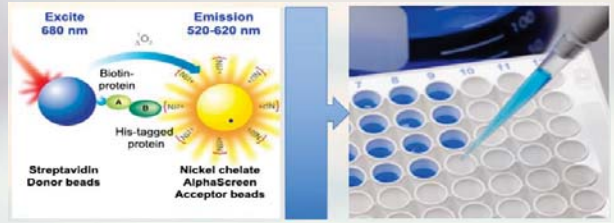

Figure 1. AlphaScreen assay

AlphaScreen assay is a bead-based proximity assay (Figure 1). The excitation of the donor beads (680 nm) provokes the release of the singlet oxygen, that is transferred to the acceptor beads, resulting in a light emission (520-620 nm).[2]

METHODS

The AlphaScreen competitive assay was optimized for use in white 384-well Opti plates :

1. Biotin-ASF (200 µM) and His-tagged gal-3 (5 µM) were added to the wells.
2. The inhibitor was added at different concentrations (0 to 10 mM) and plate was incubated for 1h at 37 °C.
3. Donor and acceptor beads were added to the wells and the plate was incubated for another hour.
4. Assay plate was read using Software Gen5 2.07.
5. Concentration-response curves were plotted in GraphPad Prims software in addition to determination of IC_{50} (Figure 2).

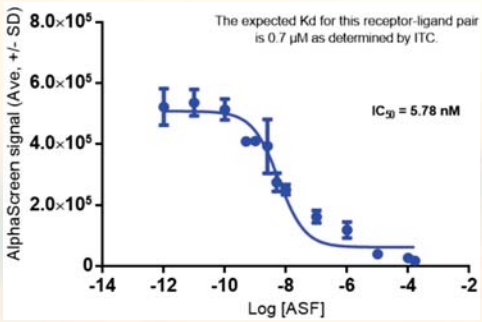

Figure 2. Assay optimization with unlabeled ASF.

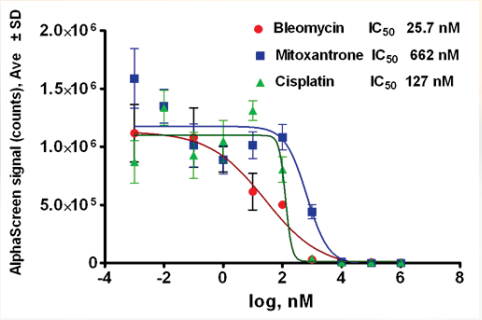

Figure 3. Pilot screen with the FDA approved oncology drug library (n = 101) available from Developmental Therapeutics Program NCI/NIH.

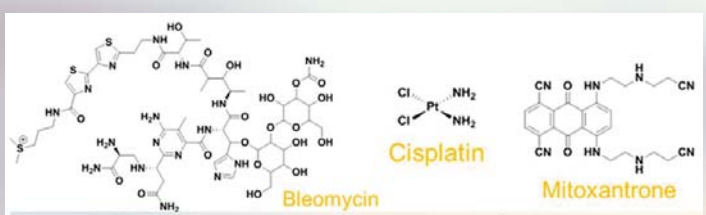

Figure 4. Molecular structure of bleomycin, cisplatin, mitoxantrone.

SUPPORTING DATA

The optimization and development of AlphaScreen for identification of gal-1 inhibitors was hampered by weak binding affinities of gal-1 for its binding ligands (µM range). We have optimized the AlphaScreen assay for gal-3, and screened an FDA approved oncology drug library (n = 101) from the NIH. We identified three compounds with positive activity in dose response experiments: : bleomycin (IC50 = 25.7 nM), cisplatin (IC50 = 127 snM), and mitoxantrone HCl (IC50 = 662 nM) (Figures 3 and 4).

CONCLUSION

The study represents an effort for development and optimization of the HTS assay for the identification of potent and selective inhibitors of galectins.

The AlphaScreen assay could not have been developed for gal-1 at the level that would satisfy the guidelines from the National Chemical Genomics Center. However, AlphaScreen assay for gal-3 inhibitors were identified three compound: bleomycin, cisplatin, and mitoxantrone.

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