Identification of G9a inhibitors by AlphaLisa™ technology and hit confirmation using MT-Glo™

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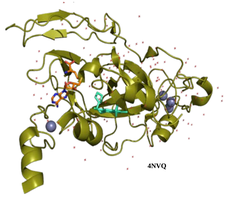
Abstract # 99

BACKGROUND

- G9a (EHMT2) is a HKMT that contains a SET domain and localizes in euchromatin regions where it catalyzes the mono- and di-methylation of H3K9
- Overexpressed in several tumors such as lung, CRC, HCC, and bladder
- It represses tumor suppressor gene expression (p21, RunX3), and silencing of G9a leads to decreased proliferation of colon and prostate cancer cell lines
- To identify novel G9a inhibitors, we screened Inventiva's proprietary compound library (IVALib) using an AlphaLisa assay. In order to optimally screen the full library, the AlphaLisa assay, utilizing H3-derived peptides and a specific antibody against H3K9me2, was developed in a 384 well format with robust Z-factor (0.84) and signal to noise ratios of around 800



- Despite the advantages offered by the AlphaLisa technology, such as high S/N ratios and ease of automation, it has also been reported that this technology can generate a number of false positives. To overcome this problem, we switched to a different technology during the hit confirmation process by using a methyltransferase Glo (MT-Glo) assay which measures the reaction product SAH by a luminescence read-out

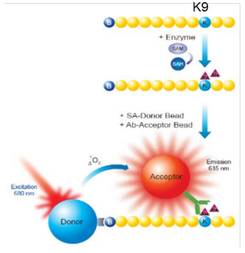
IVALib

- 240,000 Compounds
- IVALib has been designed and optimized over years for drug discovery programs
- More than 70% of the compounds are original when compared to Zinc library
- Compounds are available as liquid solutions and 70% as powders, and all the library is stored in controlled environment
- Regular quality controls are performed and a collection enrichment to maintain diversity and originality is in place
- Good hit rate on internal screening programs achieved
- Library available for external drug discovery partnerships

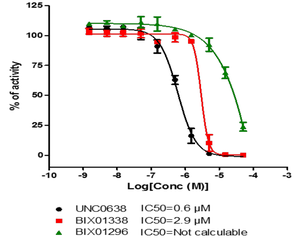
PRIMARY SCREENING ASSAY

We internally optimized an AlphaLisa® assay using G9a enzyme (BPS Bioscience), H3 biotinylated-peptide (Anaspec) and a specific Ab against H3K9me2 (Perkin-Elmer)

The assay gives excellent reproducibility (Z=0.84) in 384w format

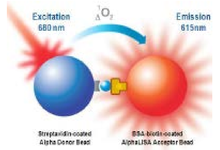


Activities of reference compounds were assayed

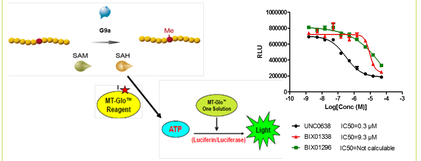


COUNTER SCREENING

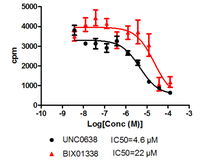
TruHits assay: streptavidin-coated donor and biotin-coated acceptor beads



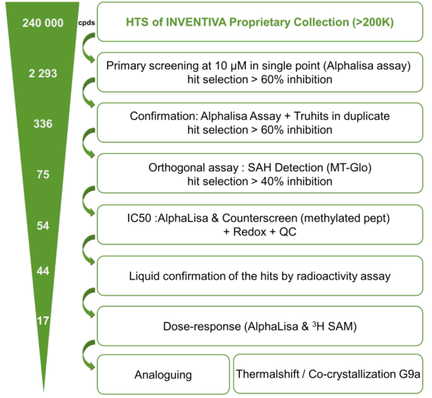
MT-Glo: G9a reaction product SAH is converted into ATP (luminescence readout)



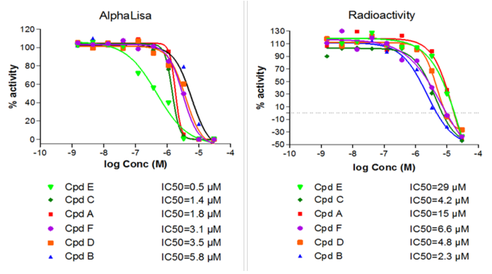
FlashPlate radioactivity assay using G9a, H3K9 peptide and 3H-SAM



FROM HTS TO VALIDATED HITS



Example of hits showing activity in the µM range



Thermal Shift Assay

- Selected series exhibit ΔT_{G9a} = 1.6-2.4°C

CONCLUSIONS

- Inventiva's proprietary compound collection screen allowed the identification of G9a hit inhibitor compounds
  - False positive compounds were rejected using orthogonal counter-screens assays
  - The biomolecular interaction of our hits with G9a was confirmed using a thermal shift assay
  - G9a crystallography is ongoing
- We have identified chemical matters to enter into H2L phase to develop selective inhibitors of G9a
- G9a program is available for setting-up a drug discovery partnership.
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