IDENTIFICATION OF G9a INHIBITORS BY ALPHALISA™ AND HIT CONFIRMATION USING MT-Glo™

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1-INTRODUCTION

Epigenetic control of gene expression is a complex process involving several enzymes and proteins which modify the acetylation and methylation status of the chromatin components, DNA and histones. These epigenetic processes play a major role in cell proliferation, survival and differentiation. Dysregulation of epigenetic control mechanisms is implicated in many diseases such as cancer, and thus these mechanisms are recognized as validated drug targets

Inventiva has several research programs in the epigenetic area, with a specific focus on histone lysine methyltransferases (HKMTs). Emerging evidence suggests that G9a, a HKMT, is able to mono and dimethylate H3K9 leading to the inactivation of tumor suppressor genes, and to play a predominant role in lung and ovarian cancers. 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 (IVALib), the AlphaLisa assay, utilizing H3-derived peptides and specific antibody against H3K9me2, was developed in 384 well format with robust Z-factor (0.84) and signal to noise ratios around 800.

Despite the advantages offered by the AlphaLisa technology, such as high S/N ratios and easiness of automation, it has also been reported that this technology can generate a number of false positive[1-3].

To overcome this problem, we switched to a different technology during the hit confirmation process by using in collaboration with Promega, a methyltransferase Glo (MT-Glo) assay which measures the reaction product SAH by a luminescence read-out.

2-IVALib

240,0 00 Compounds,

IVALib has been designed over years for drug discovery programs

More than 70% of the compounds are original when compared to Zinc library[4]

Compounds are available as liquid solutions and 70% as powders

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

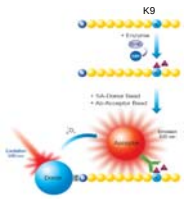
Good hit rate on internal screening programs achieved

Library available for external drug discovery partnerships

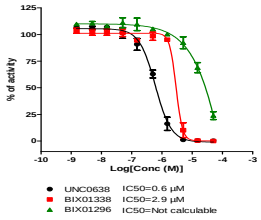
3- PRIMARY SCREENING ASSAY

We internally optimized an AlphaLisa® assay (PerkinElmer) using a G9a enzyme (BPS Bioscience), an 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

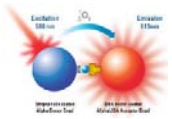


4- COUNTER SCREENING ASSAYs

Despite the advantages of AlphaLisa technology such as high S/N ratios, high sensitivity and easy automation, it has been reported that this technology can generate false positive. To overcome this problem a number of counter screen assays have been implemented in the hit confirmation process.

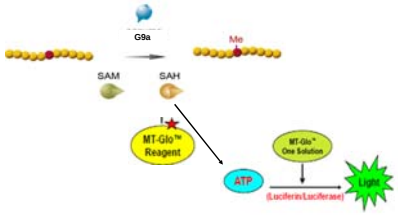
1. TruHits assay

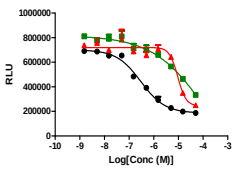
This assay used Streptavidin-coated donor beads and biotin-coated acceptor beads



3. MT-Glo

The G9a reaction product SAH is converted to ATP which is then measured by a luminescence readout

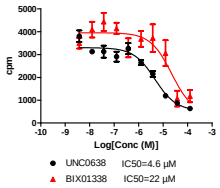




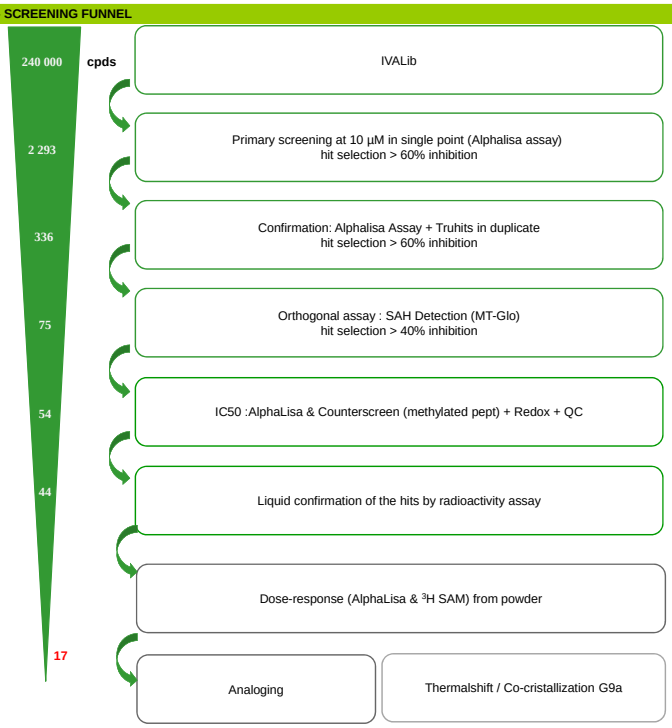
4. AlphaLisa using already methylated peptide

5. Radioactivity assay using ^{3}H-SAM

A FlashPlate assay using G9a, H3K9 peptide and ^{3}H-SAM was set-up



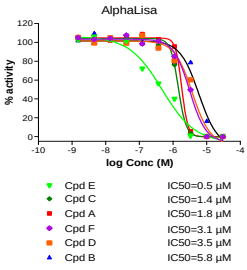
5- SCREENING FUNNEL

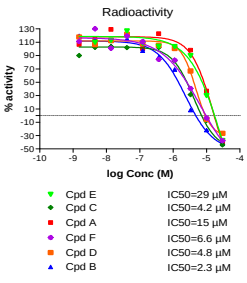


6- RESULTS

Example of 6 hits showing activity in the micromolar range both in AlphaLisa and radioactivity assays

Several families and singletons were identified





7 - CONCLUSIONS

- The 240 000 compound IVALib library was screened on G9a using AlphaLisa technology.
- Additional counter screening assays (MT-Glo, radioactivity) in the hit confirmation process allowed to eliminating false positives, with 44 compounds remaining. Further validation was performed leading to hit status.
- Among the 17 confirmed hits, several chemical families and singletons have been identified and are currently subjected to further analysis (TSA, Xtallisation) before launching HTL program.
- G9a program is available for setting-up a drug discovery partnership.
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- References

1. Baell *et al.* 2010, J Med Chem, 53:2719.
2. Bocker *et al.* 2011, J Biomol Screen 16:765.
3. Schorpp *et al.* 2013, J Biomol Screen 19:715.
4. Irwin *et al* J. 2012, Chem Inf Model 52:1757.