A rational approach for the discovery of inhibitors of NSD2 for the treatment of cancer

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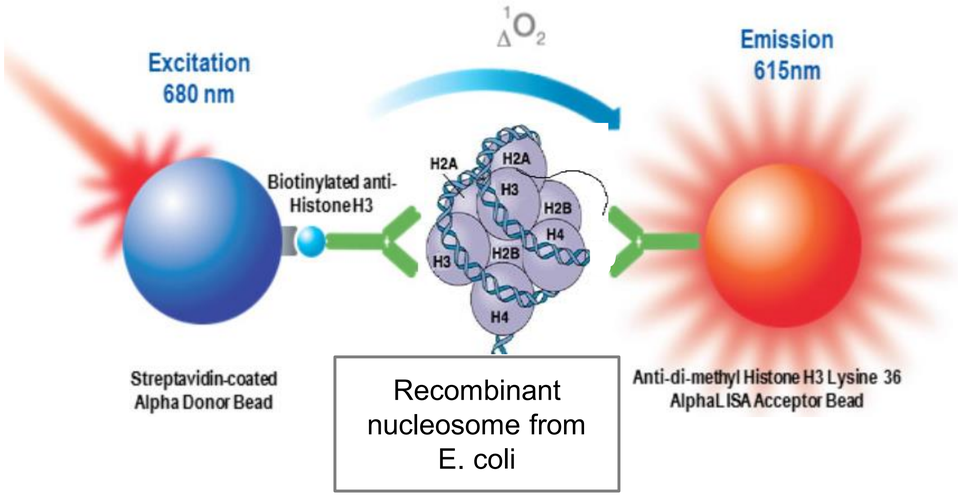
1-INTRODUCTION

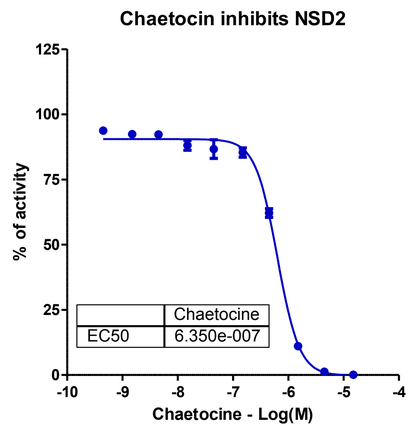
Multiple myeloma (MM) is a plasma cell malignancy which accounts for approximately 10% of hematologic malignancies. Despite the introduction of new therapeutic agents, MM remains incurable and nearly all patients ultimately relapse. About 20% of MM are due to a chromosomal translocation t(4;14) leading to overexpression of the NSD2 histone methyltransferase. NSD2 catalyzes dimethylation of lysine 36 on histone H3 (H3K36me2) and is associated with transcriptionally active regions. Several studies have shown that in MM harboring the translocation t(4;14), oncogenic programming is dependent on the methyltransferase activity of NSD2. Thus, NSD2 is a potential therapeutic target for MM for which no curative treatment is available to date. In addition, the NSD2 overactivity is also observed in prostate and lung cancers. To address these medical needs, Inventiva has started a drug discovery program on NSD2 inhibitors.

2- ASSAY PRINCIPLE

The assay is based on AlphaLISA technology and relies on the detection of H3K36me2 marks on nucleosome by a specific antibody.



3- VALIDATION OF THE ASSAY WITH REFERENCE COMPOUNDS



Chaetocin, a non specific inhibitor, is able to block NSD2 activity in a dose dependent manner, with an IC50 of 635 nM, similar to the ones described in the literature.

This result validates the assay and the automation

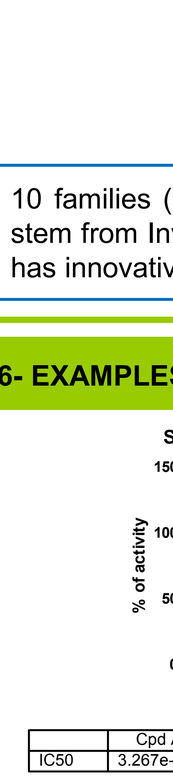
4- INVENTIVA LIBRARY: IVALib

- **240,000** Compounds
- **2/3rd Original** compared to the Zink library[1]
- Designed over years for drug discovery programs
- Available as liquid solutions and 70% as powders
- Stored in controlled environment
- Regular quality control
- Collection enrichment for improving diversity and maintaining originality
- **Good hit rate** on internal screening programs

Library available for external partnerships

1) I. Sterling et al *J. Chem. Inf. Model. 2012*

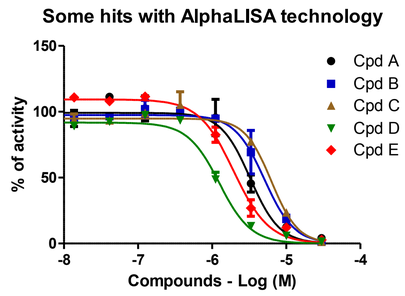
5- SCREENING AND HIT SORTING



- Inventiva library
- Primary screening at 10 µM in single point (Alphalisa assay)
  hit selection > 60% inhibition
- Confirmation: Alphalisa Assay + Truhit in duplicate
  hit selection > 70% inhibition
- DR IC50 :AlphaLisa & Counterscreen (Truhit) + Redox + QC
- Orthogonal confirmation by a radioactivity assay using ^{3}H SAM
  Ongoing
- Confirmation with powders in DR (AlphaLisa)
- Binding assay: TSA
- Analoging
- Selection of two-three series for hit to lead

10 families (≥ 3 members) have been identified. Interestingly, 9 clusters are stem from Inventiva's exclusive compound collection, suggesting that Inventiva has innovative inhibitor chemotypes for NSD2.

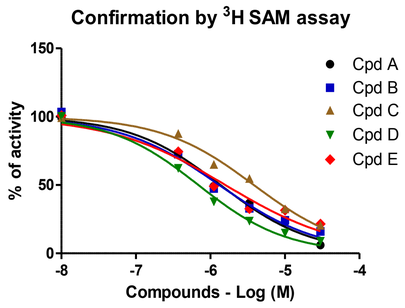
6- EXAMPLES OF HITS



	Cpd A	Cpd B	Cpd C	Cpd D	Cpd E
IC50	3.267e-006	5.081e-006	6.284e-006	1.249e-006	1.968e-006

Five examples of hits that were confirmed by radioactivity assay. Values of IC50 are similar between the two technologies.

Confirmation by ^{3}H SAM assay



	Cpd A	Cpd B	Cpd C	Cpd D	Cpd E
IC50	1.365e-006	1.317e-006	3.832e-006	6.896e-007	1.590e-006

In parallel to the biochemical screening, we are developing secondary cellular assays based on the H3K36me2 methylation and proliferation to further confirm hit activity.

7 - CONCLUSIONS

To our knowledge, no NSD2 inhibitor have been identified to date despite several screening effort performed by other groups. Our library has already produced new chemical starting points for other KMTs, and we believe that our hits could be promising starting points to generate potent and selective NSD2 inhibitors.