A rational approach for the discovery of NSD2 inhibitors for the treatment of multiple myeloma

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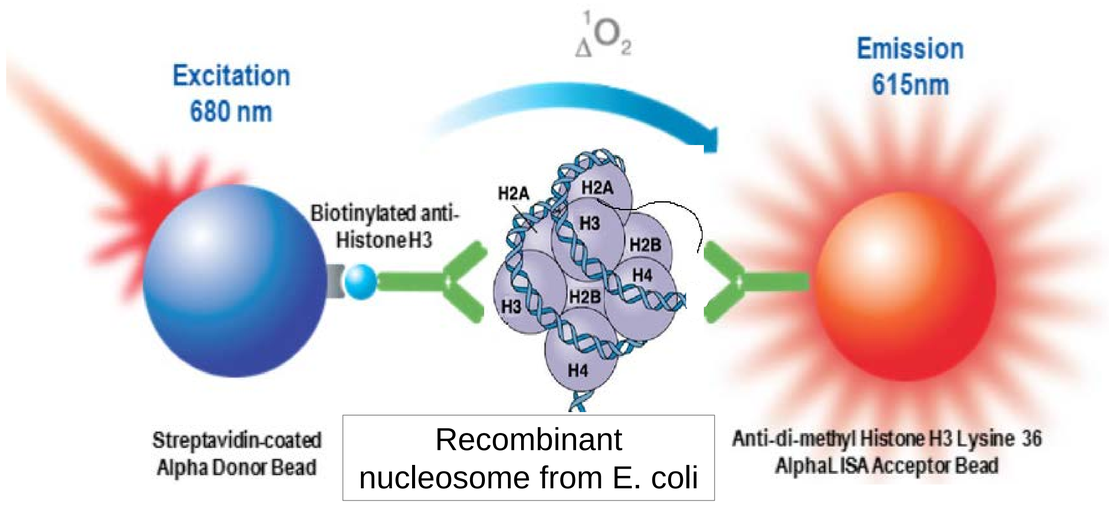
1- INTRODUCTION

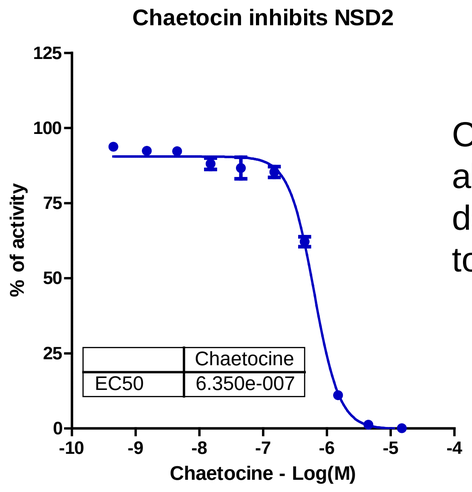
Multiple myeloma (MM) is a plasma cell malignancy which accounts for approximately 10% of hematologic malignancies. Despite the introduction of new therapeutic agents, MM remains incurable and nearly all patients ultimately relapse. About 20% of MM are due to a chromosomal translocation t(4;14) leading 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 target for MM, for which no selective drug is available to date. 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 hNSD2 activity in a dose dependent manner, with IC50 similar to the ones described in the literature.

This result validates the assay and the automation

4- INVENTIVA LIBRARY

- **240,000** Compounds, Ro5 compliant
- **2/3rd Original** compared to Zinc library
- Designed over years for drug discovery programs
- Available as liquid solutions and 70% as powders
- Stored in controlled environment
- Regular quality control
- Collection enrichment maintaining diversity and originality
- **Good hit rate** on internal screening programs

Library available for external partnerships

5- SCREENING AND HIT SORTING

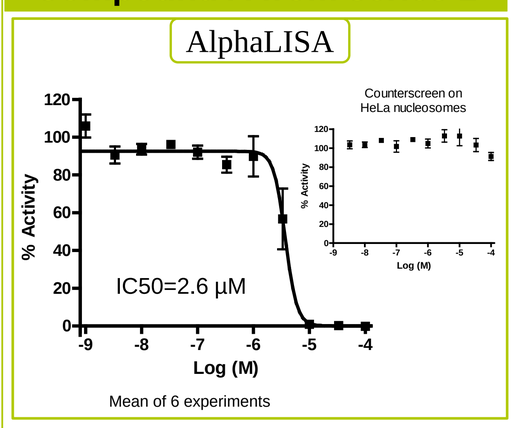
Inventiva library: IVALib (240,000 compounds)

1424	HTS: 10 µM in single point (AlphaLISA assay) hit selection > 60% inhibition
277	Confirmation: AlphaLISA Assay + Truhit in duplicate hit selection > 70% inhibition
209	DR IC50: AlphaLISA & Counter screen; QC Redox assay
120	Orthogonal confirmation by a radioactivity assay using ^{3}H SAM and full length hNSD2
8	Resynthesis of metal free batches
4	Binding confirmation by SPR, MST, TSA and/or NMR
2	SAR on confirmed hits on going

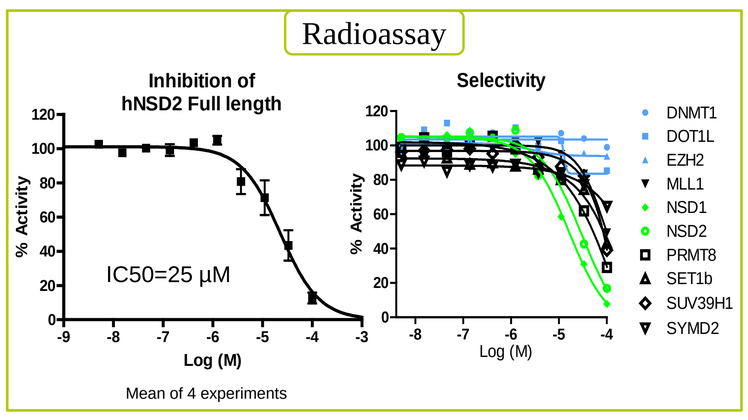
Identification of 2 validated novel chemical series

6- EXAMPLES OF HITS

Compound A from series 1



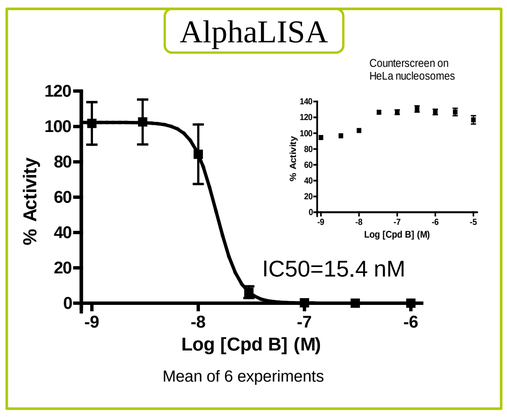
Mean of 6 experiments



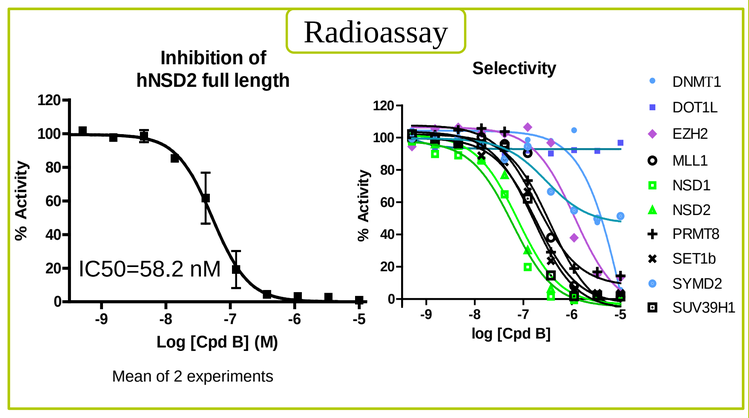
Mean of 4 experiments

Biophysic binding	RMN / STD on NSD2	SPR on NSD2	TSA on NSD1
Cpd A	STD positive with epitopes	Binder	ΔT= -8.5°c

Compound B from series 2



Mean of 6 experiments



Mean of 2 experiments

Biophysic binding	RMN / STD on NSD2	SPR on NSD2	TSA on NSD1
Cpd B	STD positive	Binder	ΔT= -11.6°c

Both series show binding to NSD2

7- CONCLUSIONS

- Using the AlphaLISA technology followed by a several orthogonal counter-screens, we have identified and selected 2 chemical matters to enter into H2L phase
- The biomolecular interaction of our hits with NSD2 has been confirmed by several biophysic technics such as SPR, MST, TSA and NMR
- NSD2 program is available for setting-up a drug discovery partnership