Identification of novel EZH2 inhibitor scaffolds

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BACKGROUND

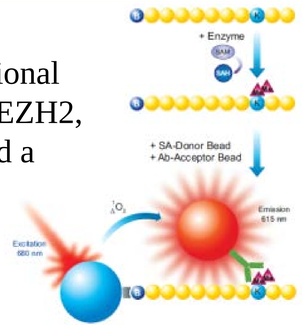
The histone-lysine N-methyltransferase (HKMT) EZH2 is often over activated by gain-of-function mutations or over expressed in cancer. This leads to an increase in H3K27 methylation in tumors, resulting in tumor suppressor gene silencing. Regarding the therapeutic potential of EZH2 inhibitors, several groups have initiated drug discovery programs leading to the identification of potent inhibitors. Surprisingly, most EZH2 inhibitors identified harbour the same pyridone pharmacophore. We have screened our proprietary library and identified new classes of EZH2 inhibitors

PROPRIETARY LIBRARY COLLECTION

- **218,000** Compounds
- **Med. Chem. focused** (designed by med chemists for med chem programs)
- No unwanted groups and focused on desirable calculated properties
- **Original: 71%** from internal design
- Quality control and Collection enrichment maintaining diversity and originality
- **Good hit rate** on internal screening programs

PRIMARY SCREENING ASSAY

We internally optimized an AlphaLISA® assay (PerkinElmer) in a 384-well plate format using a functional EZH2 complex (PRC2 pentameric complex including EZH2, EED, AEBP2, SUZ12 and RbAp48), an H3 peptide and a specific Ab against H3K27me1/2



The assay gives excellent reproducibility (Z=0.77) and a dynamic range superior to radioactive test

SCREENING AND VALIDATION STRATEGY

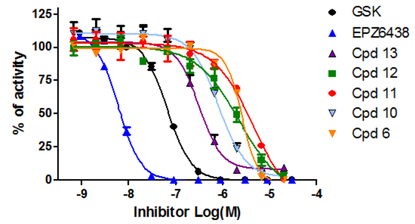
HTS on Proprietary Library Delivered Novel Hits
after Thorough Counterscreen Verification and in Depth Selection

	Inventiva proprietary library	218.000 cpds
Hit Finding	• Primary screening at 10 µM in single point (AlphaLISA® assay) with Hit selection > 50% of inhibition • Confirmation and counter screen for AlphaLISA® technology	211 cpds
Hit Selection	• Dose response (AlphaLISA® assay) • QC, chemical matter • Redox sorting	44 cpds
Hit Confirmation	• Hit sorting: inhibitor in Orthogonal assays (chemiluminescence assay and/or ^{3}H-SAM) • Comparison of inhibitory activity in EZH2 mutant isoforms (EZH2 wt, A677G, A687V, Y641F and Y641N mutants) • SAM competition assay	13 cpds

RESULTS

Hits display good starting inhibitory properties against EZH2 with IC50 down to 314 nM

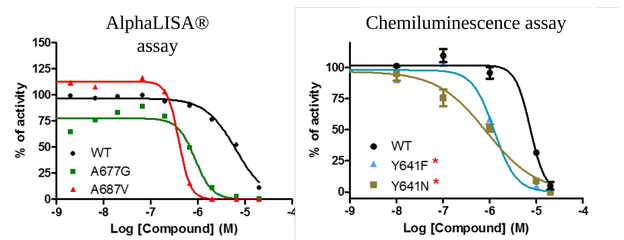
Several potent inhibitors were identified with AlphaLISA® assay and validated by other technologies such as chemiluminescence and radioactive methyl donor



	GSK	EPZ	Cpd 6	Cpd 10	Cpd 11	Cpd 12	Cpd 13
IC50 (nM)	66	6	2571	816	4624	2206	314

Hits display differential activities against different EZH2 isoforms

Some compounds show better inhibitory activity against the mutant isoforms



	WT	A677G	A687V
IC50 (nM)	6298	873	395

	WT	Y641F	Y641N
IC50 (nM)	7404	1257	849

*: trimethylation readout

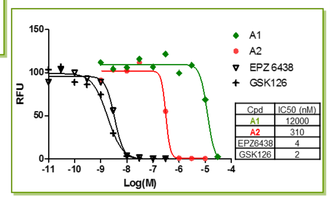
Hits have been clustered in 4 chemical series and optimization is ongoing

Example of rapid progress for one of the four chemical series with already 50 fold improvement in enzymatic assay and better eADME parameters

Coumpound A1

IC_{50} EZH2wt : 12 µM
Ligand Efficiency : 0.43
SAM competition : Yes

Perm Caco2(AB) : 24.10^{-6} cm/s
Clint (µL/min/mg prot) : >1000
Solubility : 51 µM



Compound A2

IC_{50} EZH2wt : 310 nM
Ligand Efficiency : 0.59
SAM competition : Yes

Perm Caco2(AB) : 27.10^{-6} cm/s
Clint (µL/min/mg prot) : 31
Solubility : 25 µM

Preliminary SAR : Yes
Original Structure: Yes

CONCLUSIONS

- Novel EZH2 Inhibitors with Differentiating Features to the Competitor Compounds
- Successful Optimization Will Be Performed Based on:
  - Critical choice of starting Hit series, including those with a different MOA
  - Focus on cell permeability and other ADME properties to improve bioavailability