Identification of novel EZH2 inhibitor scaffolds

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1-INTRODUCTION

The histone-lysine N-methyltransferase (HKMT) EZH2 is often over activated by gain-of-function mutations in lymphomas or over expressed in solid tumors. This over activation is associated with increase in H3K27 methylation in tumors, leading to decreased tumor suppressor gene expression and poor prognosis. 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 to date harbour the same pyridone pharmacophore. We decided to screen our proprietary compound library to identify new EZH2 inhibitors. Primary screening campaign was successful, since we have been able to discover new classes of EZH2 inhibitors.

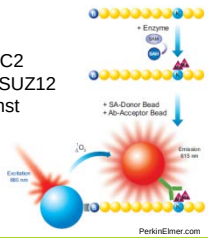
2-PROPRIETARY LIBRARY COLLECTION

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

3- PRIMARY SCREENING ASSAY

We internally optimized an AlphaScreen® assay (PerkinElmer) 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



4- SCREENING AND VALIDATION STRATEGY

Primary screening (AlphaScreen® assay)
Inventiva proprietary library (200 K compounds)

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Counter screen (false positives removal)

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Dose response (Alphascreen assay)

↓

Hit mining, QC, chemical matter

↓

Second validation (chemiluminescence assay)

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Comparison of inhibitory activity
EZH2 wt, A687, A677, Y641F and Y641N mutants

↓

SAM competition assay

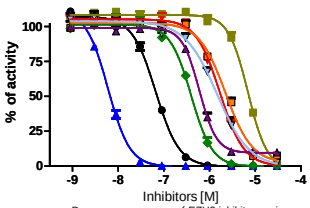
↓

Validated hits / New scaffolds inhibitors
- **12 SAM non competitor hits**
- **17 SAM competitor hits**

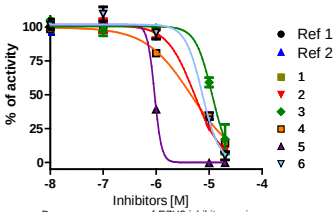
5- RESULTS

Hits display good starting inhibitory properties against EZH2 with IC50 down to 400 nM

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



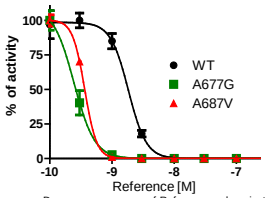
Dose curve response of EZH2 inhibitors using AlphaScreen® assay



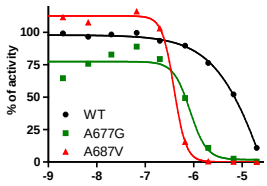
Dose curve response of EZH2 inhibitors using cheminuminescent assay

Hits display differential activities against different EZH2 isoforms

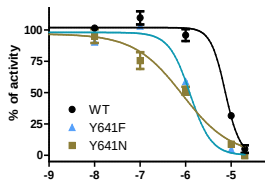
Compounds were tested against four EZH2 mutants, Y641F, Y641N, A677G and A687V, some compounds show better inhibitory activity on the mutant isoforms than on the WT isoform



Dose curve response of Ref compound against EZH2 variants



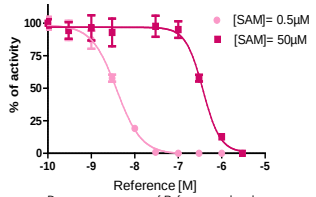
Dose curve response of EZH2 inhibitors (AlphaScreen®) against EZH2 variants



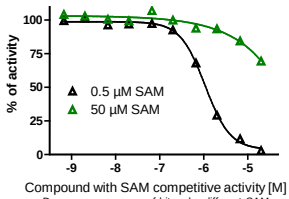
Dose curve response of EZH2 inhibitors (AlphaScreen®) against EZH2 variants

Several hits did not behave as SAM competitors

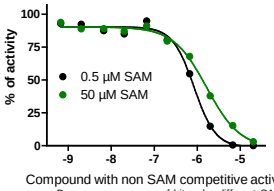
Compounds were tested against increasing concentration of SAM. Different patterns of inhibition were observed with compounds, some of them retaining full inhibitory activity whatever the SAM concentration used



Dose curve response of Ref compound under different SAM concentration (AlphaScreen®)



Dose curve response of hit under different SAM concentration (AlphaScreen®)



Dose curve response of hit under different SAM concentration (AlphaScreen®)

CONCLUSIONS

Screening of our proprietary compound collection allowed the identification of new EZH2 inhibitors. Identified compounds are from different chemical families and none of them harbor the pyridone pharmacophore usually seen in EZH2 inhibitors. Most of the hits comply with Lipinski rules, with molecular weight below 350 kD and have good potential for novel IP. Hits were validated for their ability to inhibit different EZH2 isoforms and some of them were found to behave as non SAM competitors. This screening campaign demonstrates the possibility to identify new scaffold of EZH2 inhibitors with potential new pharmacological properties. Hits are currently optimized for further validation in *in vitro* and *in vivo* disease relevant models