Dual-Platform Screening of Compound Libraries to Identify Novel Inhibitors of Histone Lysine Demethylase PHF8

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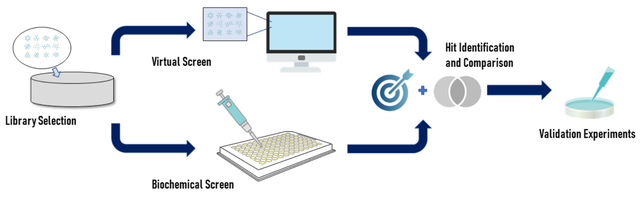
Introduction

- Plant homeodomain finger protein 8, or PHF8, is a Jumonji C domain containing histone demethylase primarily responsible for the erasure of methyl (-CH3) group post translational modifications on histone tails – specifically those of H3K9me1/2, H3K27me1/2 and H4K20me1 (histone modification shorthand where H3/H4 = histone type, K = lysine, number = lysine position, and me1/2 = mono- or di-methylation)
- The PHF8 mediated removal of these marks on histone proteins modulate the expression of genes related to an array of cellular process including, but not exclusive to: cell cycle progression, inflammatory responses, and hypoxic response
- PHF8 dysregulation has been implicated in tumor proliferation, metastases, and therapeutic resistance in various human cancer types making it a promising target to attenuate cancer progression
- However, there has been little work done in identifying viable small molecule inhibitors of PHF8 that may have potential to be implemented in chemotherapeutic therapies

Materials & Methods

Dual Platform Screen Schematic:



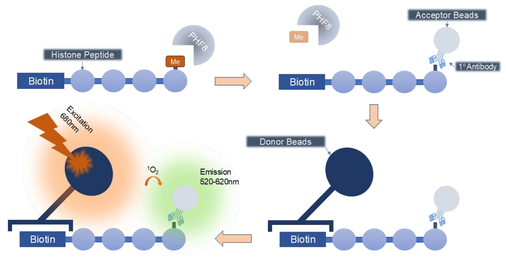
Virtual Screen:

- We performed the structure-based virtual screening using AutoDock Vina using the PHF8 structure (PDB: 3K3N) and docked each small molecule from our small molecule library to assess their potential to bind to the PHF8 active site
- After docking experiments were completed, docking scores (-ΔG) for each molecule were filtered based on three efficiency metrics: ligand efficiency (LE), lipophilic ligand efficiency (LLE), fit quality (FQ)
- Molecules that scored in the top 20% in 2/3 of the selected efficiency metrics were classified as a hit in our virtual screen – **this resulted in the identification of 121 small molecule hits for further analysis**

Biochemical Screen:

- We utilized the luminescence-based assay AlphaScreen as the biochemical assay of choice to identify small molecule inhibitors for PHF8
- Alpha signal counts were collected from a Varioskan Lux Multimode Microplate Reader and percent inhibition values were based on the signal reduction compared to the positive control (PHF8 + histone peptide + no small molecule inhibitors)
- **Molecules that showed a >30 percent inhibition were classified as hits**

AlphaScreen Schematic:



Pharmacologic Analysis:

- The SwissADME web-tool was utilized to predict drug-likeness and pharmacologic properties of small molecule hits across both screening platforms
- We specifically focused on the oral bioavailability and absorptive properties of our hits to assess whether our identified hits are bioavailable in-vivo
- ToxTree-3.0 was utilized in order to predict in-vivo toxicity across different physiological systems and CYP mediated metabolic activation of selected hits

Figure 1: Percent Inhibitions

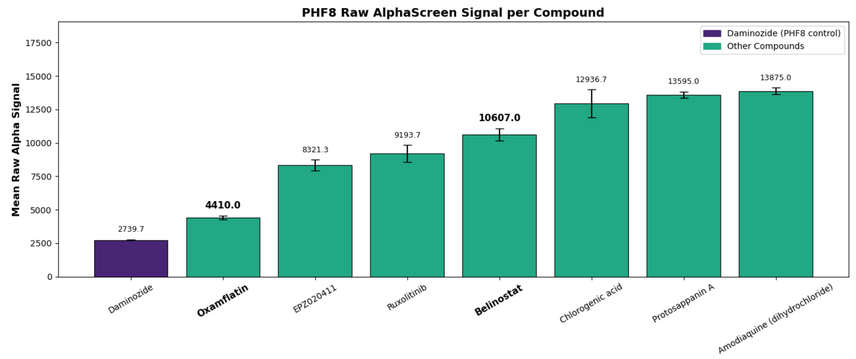


Figure 1: AlphaScreen signal and percent inhibition values show Oxamflatin and Belinostat (bolded) were identified as hits in the biochemical screen and virtual screen. The screen above quantified inhibition for 23 small molecules and had a Z'=0.668 and S/N=111.6.

Figure 2: Enrichment Analysis

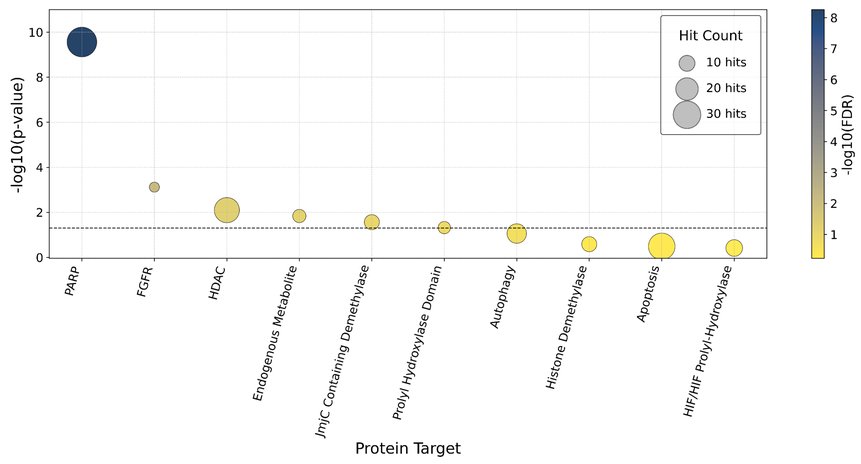


Figure 2: Enrichment analysis of the virtual screen shows that small molecules targeting biologically significant proteins including JmjC Containing Demethylases, Prolyl Hydroxylase Domain. PARP and HDAC inhibitors also show significant enrichment. Oxamflatin and Belinostat are known HDAC inhibitors that were previously identified as hits in the biochemical screen. Further analysis of PARP inhibitors and FGFR inhibitors will be assessed in the future.

Figure 3: Bioavailability

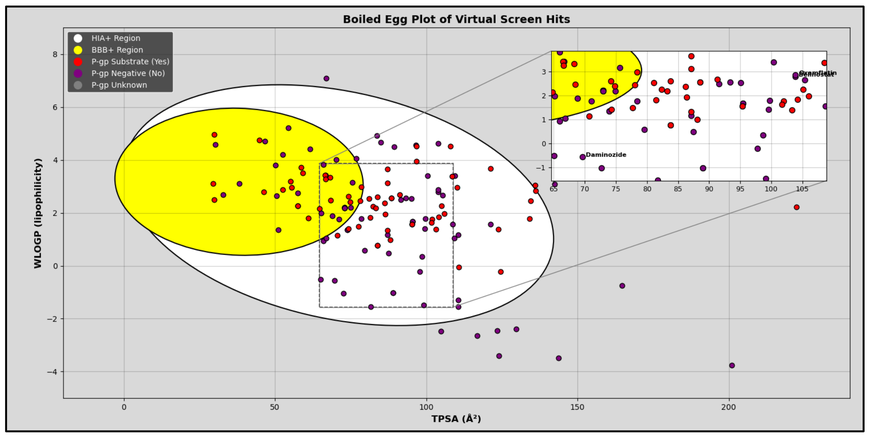


Figure 3: Bioavailability analysis was conducted based on all virtual screen hits. We further found that find that oral bioavailability of preliminary hits, Oxamflatin and Belinostat, have predicted intestinal absorption and are not P-gp substrates.

Figure 4: Toxicity Profiling

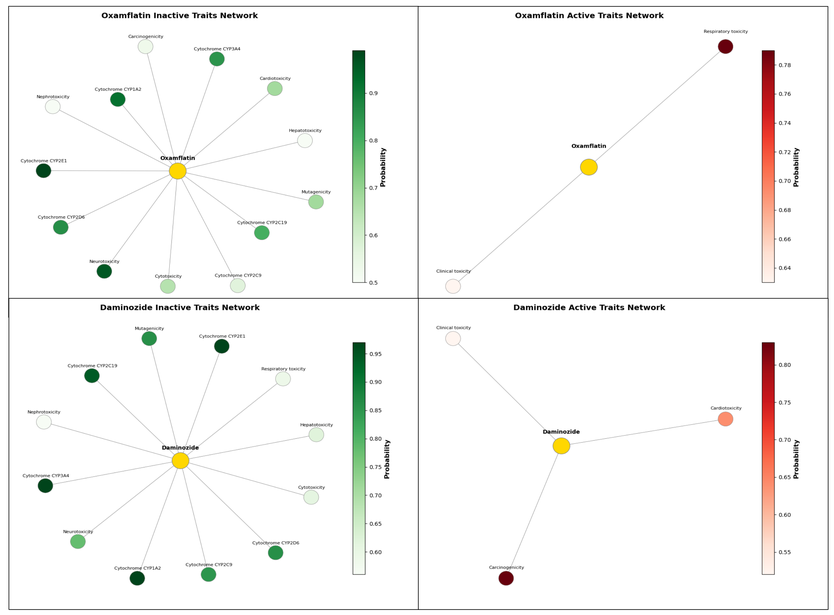


Figure 4:Clinical toxicity and CYP metabolism profiles of Oxamflatin and Daminozide. Respiratory toxicity is a potential contraindication of utilizing Oxamflatin in a clinical setting

Conclusions

- When assessing the validity of our virtual screening approach we found enrichment for small molecules that target Jumonji C containing histone demethylases and Prolyl Hydroxylase Domain containing proteins
  - Important to consider that both of these domains are structurally similar and utilize a His-Asp/Glu triad
- Enrichment for these domains indicate the virtual screen is able to identify candidate inhibitors for PHF8
- AlphaScreen is reliable in determining PHF8 small molecule inhibition in a quantifiable manner
- There is parallel agreement between both platforms of screening for certain compounds (thus far: Oxamflatin and Belinostat), indicating that these compounds may be true inhibitors of PHF8
- Pharmacologic analysis of bioavailability integrated in the workflow allows filtering of small molecule inhibitors that have drug like potential
- Toxicity based analyses further allow for identification of small molecules that may have clinical efficacy
- Overall, our dual-platform method shows promise in identifying potential small molecule inhibitors with parallel agreement among both platforms

Future Directions

- Further complete AlphaScreen assay on entire library and analyze the degree to which all hits (>30% inhibition) map back onto virtual screen hits
- Counter-screen hits to rule out false positives of within the hits identified by AlphaScreen
- Conduct dose-dependent assays to observe whether candidate small molecule inhibitors work in a dose dependent manner
- Shift to cell-based assays once compounds with the most potent inhibition are identified and selected
  - Validate growth inhibition, and protein/gene expression profiles in order to assess PHF8 inhibition in comparison to PHF8 knockout systems
- In-vivo experiments of tumor growth inhibition once inhibitors are validated
- Assess selected inhibitors' pharmacologic properties to verify in-silico data to further strengthen the candidacy for using repurposed inhibitors in PHF8 inhibition

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