Identification of novel p300 molecular glue degraders using MOPED™ Emerald

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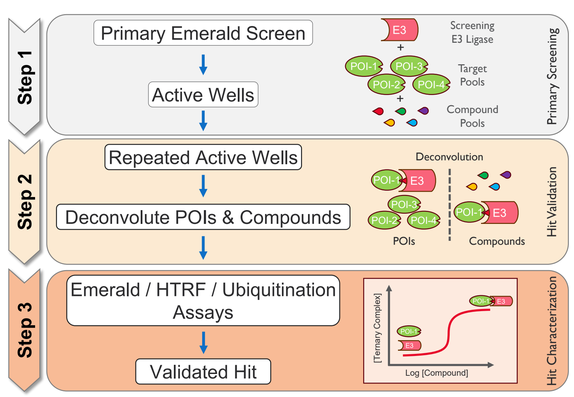
Key Findings

- The MOPED™ Emerald high-throughput screen can identify compounds that induce TC formation between a POI and an E3 ubiquitin ligase.
- 15 p300 BRD TC hits identified from two E3s
- Hit optimization resulted in 200-fold improvement in potency and 3-fold increase in amount of TC formed.
- 1 optimized compound resulted in a p300 protein decrease of 35% that was dependent on the E1, the proteasome and p300- binding.
- This protein decrease resulted in an equivalent decrease in H3K27 acetylation in CBP KO cells.
- The MOPED™ Emerald platform was able to identify compounds that induced a productive POI-E3 ternary complex.

Background / Introduction

- MOPED™ Emerald is a high-throughput multiplexed screening platform that can identify compounds that induce ternary complex (TC) formation between a protein of interest (POI) and an E3 ubiquitin ligase.
- Emerald assay has greater sensitivity for detecting TC formation compared to traditional assays.
- p300 (EP300) is a drug target in CBP loss of function cancers, identified through synthetic lethality screens.
- Multiple companies, including SK Life Science Labs, have an interest in developing p300 inhibitors or heterobifunctional degraders.
- A p300 molecular glue degrader may solve potential challenges of heterobifunctional degraders.

A **MOPED™ Emerald hit progression model**



(A) Hit progression model and workflow for primary Emerald screening supports an efficient method for high-throughput molecular glue screening.

Figure 1. Active wells identified in p300 BRD Emerald Assay screen

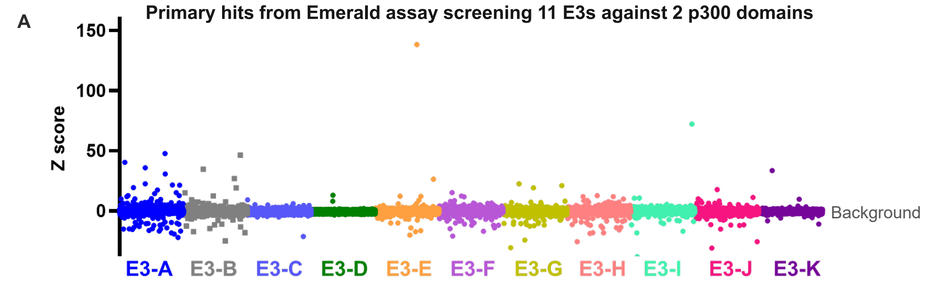


Figure 1. (A) MOPED™ Emerald primary screen to identify pools of compounds that induce ternary complex formation between p300 domains (BRD or BRD-HAT) and a panel of 11 E3 ubiquitin ligases.

Figure 2. Confirmation of Emerald hits using orthogonal assays

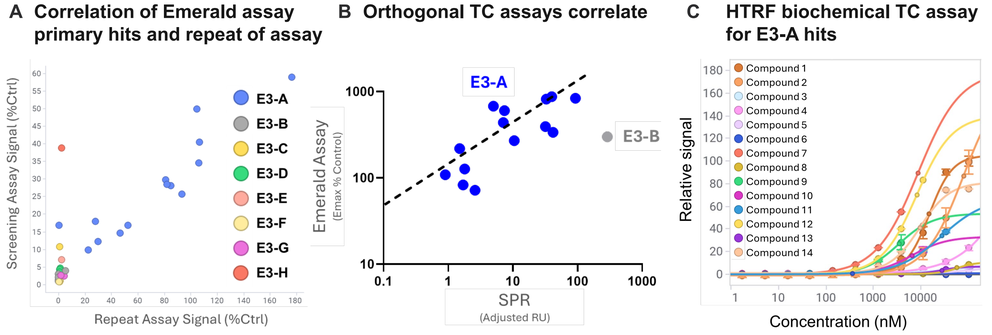


Figure 2. (A) Correlation plot of primary screen signal results versus signal from repeat testing of primary active wells to identify hits. **(B)** Correlation of orthogonal SPR assay to Emerald assay results for confirmed hits for E3-A (14 hits) and E3-B (1 hit). **(C)** HTRF biochemical TC assay for the 14 E3-A hits.

Figure 3. Hit optimization of ternary complex formation

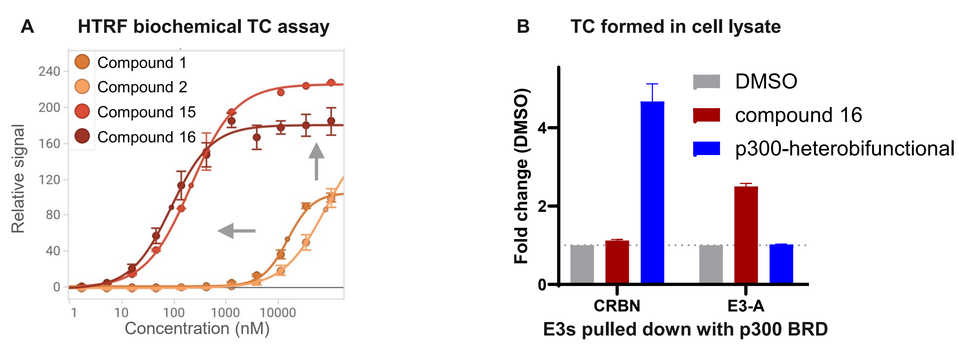


Figure 3. (A) HTRF TC assay dose response curves comparing two of the initial hits and two optimized compounds showing ~200-fold improvement in EC50 and ~3-fold increase in Emax. **(B)** Mass spectrometry results measuring amount of recombinant E3-A or CRBN pulled out of cellular lysates containing 100 nM recombinant p300 BRD in the presence of DMSO, compound 16 or a p300-heterobifunctional compound.

Figure 4. Formation of productive ternary complex

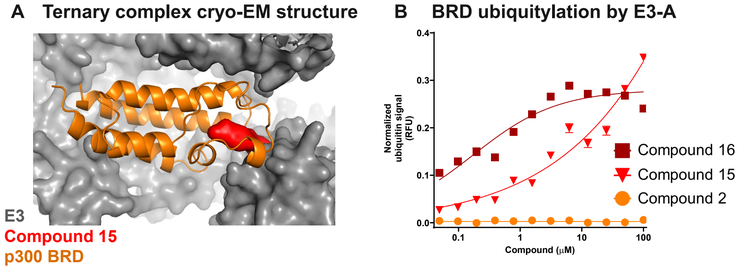


Figure 4. (A) We solved a 3.6 Å resolution cryo-EM TC structure with compound 15 (red) the p300 BRD (orange) and E3-A (gray). **(B)** In vitro ubiquitylation assay demonstrates that compound 15 (red triangles) and compound 16 (maroon squares) form a productive ternary complex that can mediate ubiquitylation of the p300 BRD.

Figure 5. Degradation of p300 and reduction in H3K27ac in cells

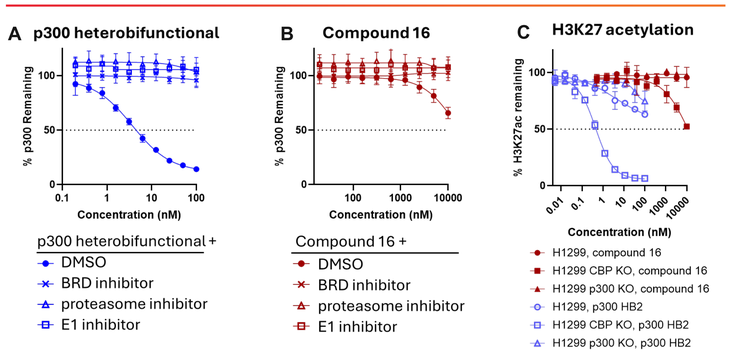


Figure 5. p300 protein levels were monitored using cells CRISPR engineered to contain a HiBiT tag at the p300 locus. p300 HiBiT assay was read at 6 hours post treatment with **(A)** a p300 heterobifunctional degrader or **(B)** p300 molecular glue compound 16. BRD inhibitor, 1 uM CCS1477; proteasome inhibitor, 4 uM MG-132; E1 inhibitor, 10 uM TAK-243. **(C)** H1299, H1299-CBP-KO, H1299-P300-KO cells were treated with a p300-heterobifunctional degrader (blue) or compound 16 (maroon).

Conclusions

- Identified 15 p300 BRD hits with 2 different E3s from a library of ~500k compounds using our MOPED Emerald platform
- Hits formed ternary complexes (TC) and demonstrated productive TC through in-vitro ubiquitylation activity
- Hit optimization resulted in a 200-fold improvement in TC EC50 and 3-fold increase in TC Emax.
- One optimized compound resulted in cellular destruction in an E1-, proteasome- and p300 binding-dependent manner
- Our MOPED™ Emerald multiplexed high-throughput screening platform is able to identify compounds that induced a productive POI-E3 ternary complex