European Lead Factory

Using a library of Pan-Assay Interference (PAINS) small molecules to understand and improve HTS outcomes

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What is the European Lead Factory?

The European Lead Factory enables European academics and SMEs to screen their novel molecular drug targets against the Joint European Compound Library (JECL), a collection of up to 500,000 diverse structures. The main deliverable from the ELF is a high quality list of up to 50 structures to which the academic or SME programme owner gains development rights.

The importance of understanding assay/target liabilities

A well designed screening cascade is the difference between identifying high quality, optimisable hits and wasting significant resources chasing compounds or sample contaminants that act via undesirable mechanisms (i.e. protein/compound aggregation, protein oxidation, chelation of essential ions or interference of assay readouts and technologies). To understand how susceptible targets are to such PAINS inhibition we use a library of literature derived molecules during assay development/optimisation.

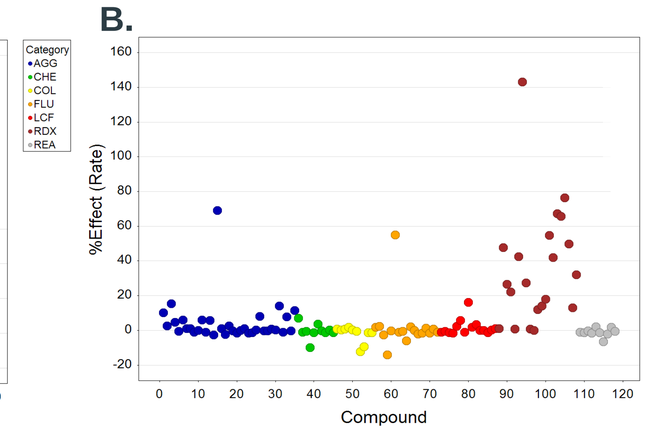


Figure 1 – A biochemical assay of target enzyme function was developed at ESC Newhouse. The assay is monitored as a loss of absorbance at 340nm as the reaction progresses. Data are mean +/- standard deviation, n = 15 wells on one 384 well plate.

Table 1 - 118 compounds were identified in the literature as PAINS/frequent hitter molecules and prepared as 10mM screening solutions, plated and binned by class.

# Compounds	Description	Acronym
35	Aggregators	AGG
10	Chelating	CHE
10	Coloured	COL
17	Fluorescent	FLU
21	Redox cycling	RDX
15	Luciferase	LCF
10	Reactive	REA

Figure 2 – Compounds were screened at 10µM n = 3 randomly distributed across a 384 well plate. **A.** Mean % effect was calculated relative to control of the 40 minute endpoint read. **B.** Mean % effect was calculated relative to control for the reaction rate across the 40 minute time course.

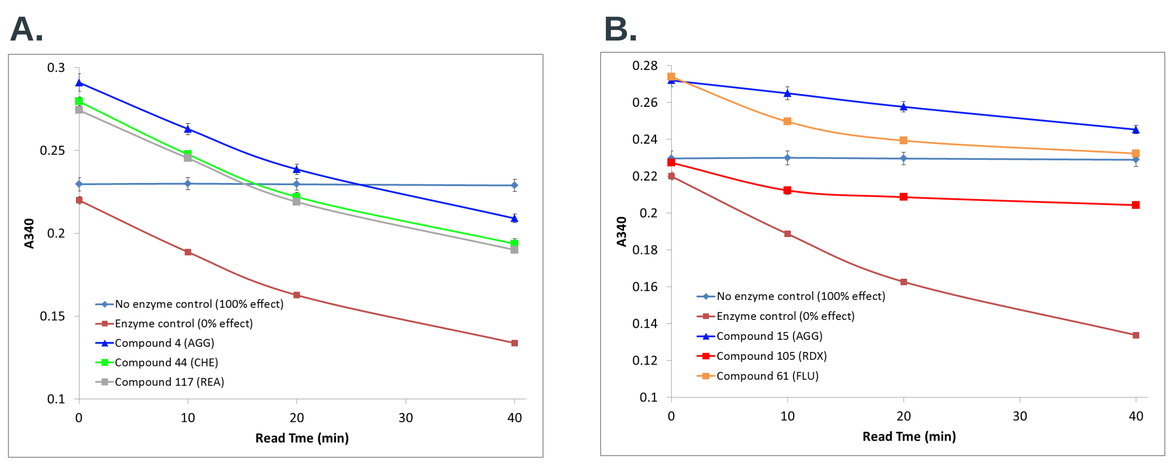
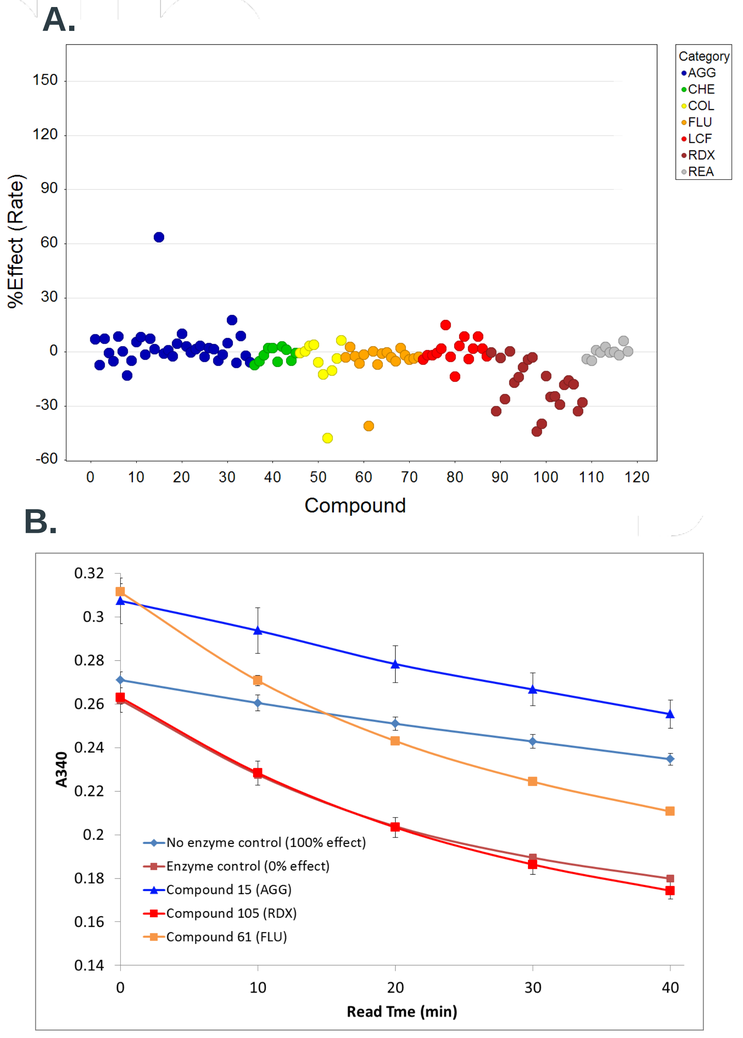


Figure 3 – Compound absorbance likely accounts for the high hit rate when analysing the endpoint data. **A.** Reaction progress curves for hit compounds that were active when calculating % effect from the 40 minutes endpoint but inactive when using reaction rate. **B.** Reaction progress curves for hit compounds that were active when calculated as an endpoint or as a rate.

Figure 4 – Addition of catalase to the assay abrogates inhibition by redox cycling compounds. **A.** Mean % effect relative to control for the reaction rate across a 40 minute time course. **B.** Reaction progress curves in the presence of catalase for the compounds shown in Fig. 3B. The aggregator compound 15 still inhibits the reaction but compounds 105 and 61 are no longer active. Compound 61 was later shown active in a chemical redox assay.



Summary

The use of a PAINS library showed an ELF target to have a significant liability towards redox cycling compounds. This knowledge informed design of a screening cascade whereby hit compounds will be tested in the presence of catalase and compound effect will be gauged from the reaction rate and not simply as an endpoint. This will facilitate triage of a manageable number of compounds to progress to orthogonal biophysical platforms.

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