From Target to Degrader - Accelerating PROTAC® Drug Discovery with Direct-to-Biology

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1. INTRODUCTION

Reducing the time and cost of developing PROTAC degraders is a major challenge in the pharmaceutical industry. A PROTAC consists of an E3 ligase ligand (E3-L), a protein of interest ligand (POI-L), and a linker. The efficiency of PROTACs depends on forming a ternary complex with E3 ligase and the POI, and linker optimization plays a critical role in enhancing potency, cooperativity, and physicochemical properties. However, the large size and complex design of PROTACs make optimization time-consuming, especially when relying on cell-based assays.

In this study, we carried out an initial virtual screen and medicinal chemistry-driven optimisation of novel BRD4 binders and identified suitable exist vectors for PROTAC design using computational modelling techniques. We subsequently applied a Direct-to-Biology (D2B) approach, combining automated chemistry with screening of crude reaction mixtures to enable the rapid exploration of our BRD4 PROTAC chemical space, providing a time- and cost-effective route to PROTAC identification.

2. IDENTIFYING NOVEL INHIBITORS OF BRD4

Following a virtual screening of Enamine's drug-like library for BRD4 BD1 hits (*Figure 1A*), we identified 50 virtual hits. These compounds were first evaluated for affinity to BRD4 by SPR (*Figure 1B*), with subsequent validation in a cell-based assay, displayed as an LLE plot (*Figure 1C*) and by Fluorescence Polarization (data not shown). By docking our novel compound into the crystal structure of the BRD4 active site, we identified two exit vectors to guide the development of our PROTACs (*Figure 1D*).

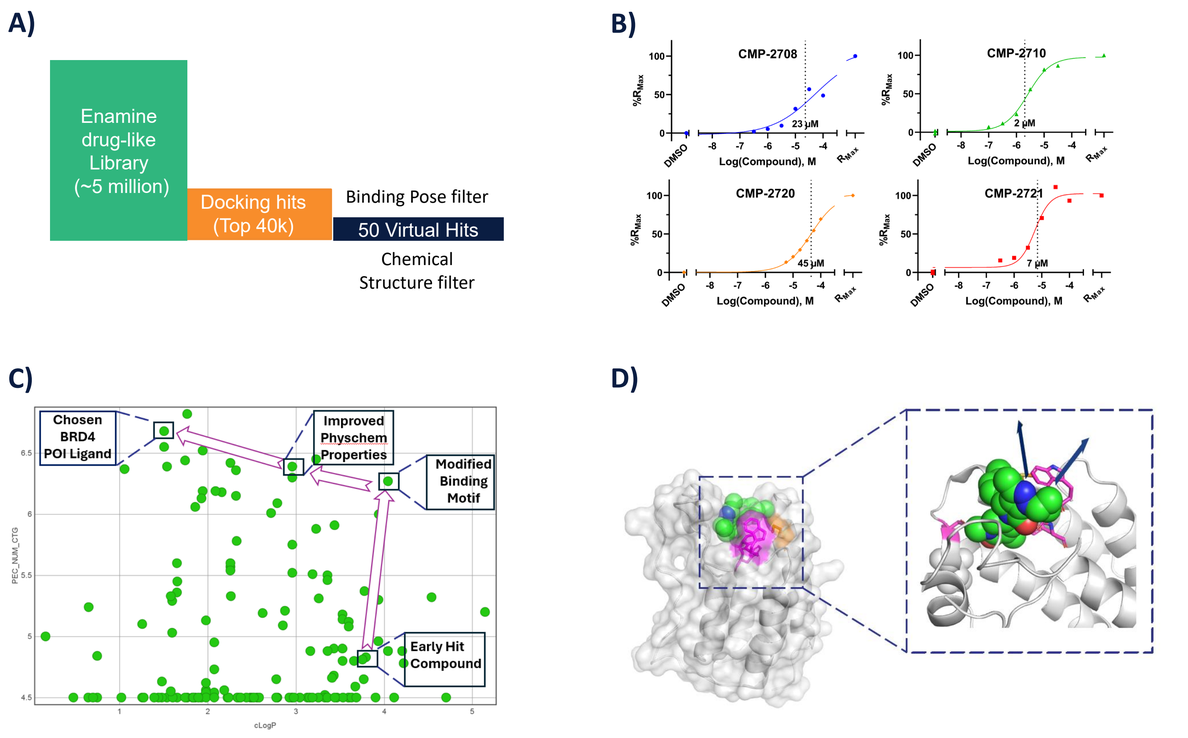


Figure 1. A) High level overview of virtual screening. B) SPR data for hit compounds. C) LLE plot and SAR development using a BRD4 dependant MCF7 cell line. D) Docked image of our hit molecule using pdb: 6ZCI, exemplifying two potential exit vectors.

3. CONCEPT'S PROTAC DIRECT-TO-BIOLOGY PLATFORM

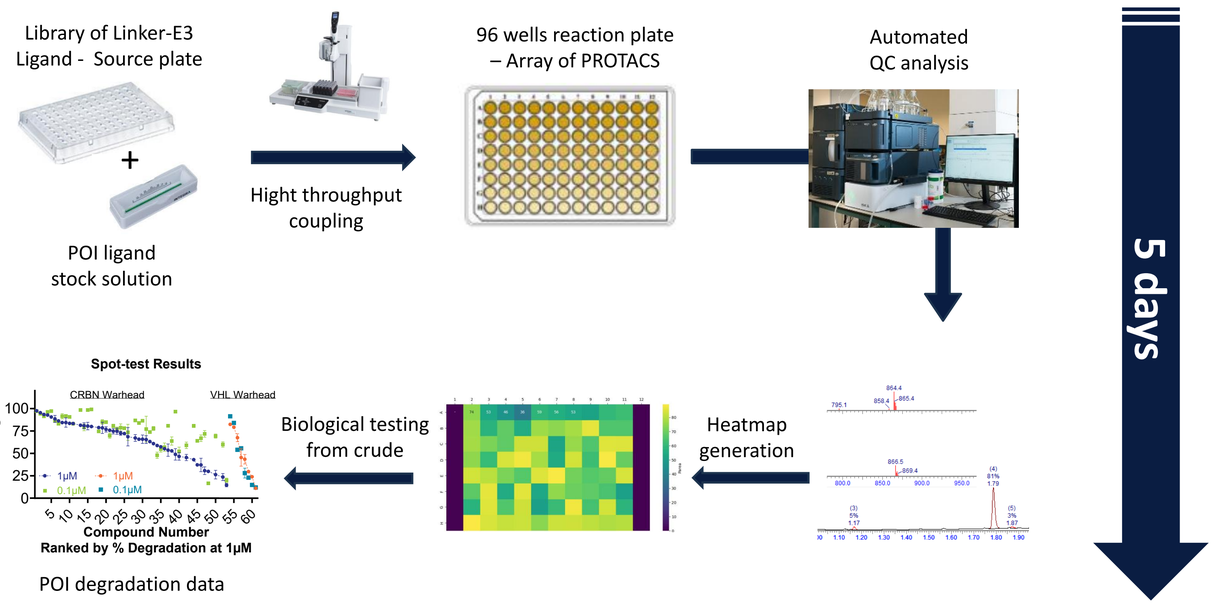


Figure 2. Overview of the automated D2B platform allowing high-throughput PROTAC hit finding and rapid SAR generation.

D2B is a semi-automated process offering the possibility for rapid biological evaluation of the crude reaction mixtures within a plate format, reducing the cycle of PROTAC optimization to only 5 days (*Figure 2*). We offer a range of reactions types to generate the PROTAC library, including amidation, reductive amination, S_NAr and cross-coupling.

REFERENCES

1) *ACS Med. Chem. Lett.* 2022, 13, 7, 1182–1190 2) *J. Med. Chem.* 2023, 66, 22, 15437–15452 3) *ACS Med. Chem. Lett.* 2023, 14, 12, 1882–1890. PROTAC is a registered trademark of Arvinas Operations Inc.

4. PLATE-BASED PROTAC SYNTHESIS

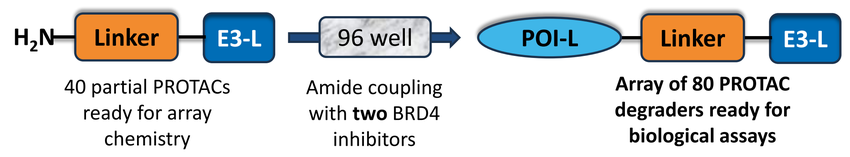
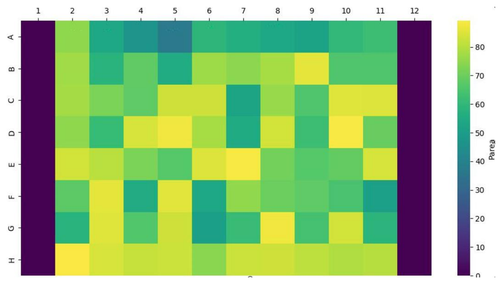


Figure 3. Generating 80 BRD4 PROTAC candidates in a single Direct-to-Biology campaign.

Two novel BRD4 inhibitors identified within our laboratories were synthesized in bulk and coupled to an array of E3-Ligand-Linker using amide coupling reaction (*Figure 3*). Amide coupling is a high yielding reaction as shown by the heatmap generated using PyParse (*Figure 4A*). *Figure 4B* shows the benefits of using a D2B approach compared to the conventional process which is extremely time and cost consuming.

A)

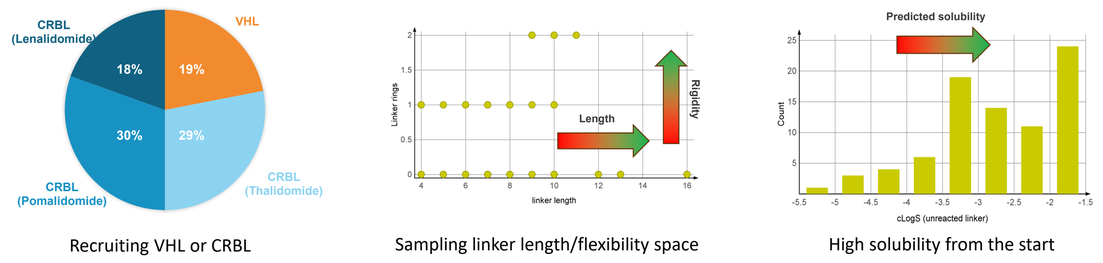


B)

80 PROTACS	Conventional	D2B
Scale	20 µmol	2 µmol
POI-Ls	1-1.3 g	60-80 mg
80 x E3-L- Linker intermediates	∑ 1.3 g	∑ 80 mg
Solvent	DMF (300 µL per vial)	DMSO (100 µL per vial)
Purification	40 L of MeCN	---------
Time	5-7 weeks	5 days

Figure 4. A) Heatmap for reaction conversion generated using PyParse B) Comparison of conventional synthesis (purified) and D2B (non-purified) workflow for a theoretical 80 PROTACS library.

A continuously expanding PROTAC D2B library



- ✓ > 80 E3 Ligase-linker reactants
- ✓ VHL and CRBL-based
- ✓ Ready to react (on 96-wells)
- ✓ Flexible and semi-rigid
- ✓ 4-16 bond length

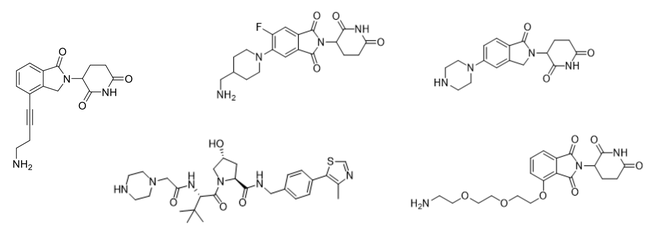
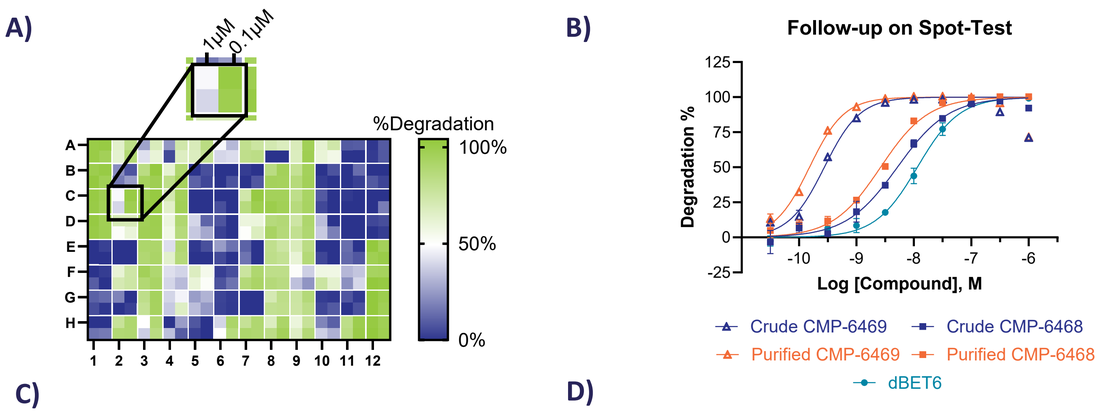


Figure 5. We are continuously expanding our PROTAC D2B library with new generation E3 ligase binders and linkers. Our library focuses on the recruitment of VHL and CRBL, samples a wide range or linker length and flexibility and high solubility degraders.

5.BIOLOGICAL RESULTS

Crude compound mixtures were screened in a BRD4-HiBiT degradation assay. From a 96-well plate stock, liquid handlers diluted the crude compound mixture to 1 µM and 0.1 µM in 384-well cell plates (*Figure 6A*), minimizing false negatives from the hook effect, a common observation with PROTACs. Compounds of interest were advanced, and a full dose-response assay was performed to profile degradation potential in parallel with purified batches (*Figure 6B*). No significant potency differences were observed between crude and purified compounds, as confirmed by BRD4-HiBiT degradation (*Figure 6B*) and JESS Western Blot when probed with a BRD4 antibody (*Figure 6C*). From initial screening, PROTACs with picomolar DC_{50} values were identified, and further ADME profiling was conducted by our DMPK team to assess key physicochemical properties (*Figure 6D*).



	CMP-6469	CMP-6468
MW	799.2	877.4
ChromLogD	5.62	4.42
Ksol	< 6.4 µM	47 µM
DC_{50}	0.28 nM	2.63 nM
D_{max}	98.5%	100%

Figure 6. A) Heat map of %degradation data B) Full Dose-response of some of the PROTAC Hits identified. C) CMP-6469, assayed through JESS WB D) Table showing some of the PhysChem properties of the PROTACs screened.