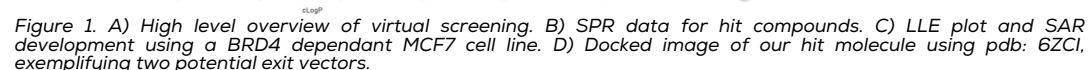


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Reducing the time and cost of developing PROTAC degraders remains a significant challenge in the pharmaceutical industry. PROTACs consist of an E3 ligase ligand (E3-L), a protein of interest ligand (POI-L), and a linker. Their efficiency depends on the formation of a ternary complex between the E3 ligase and the POI, with linker optimization playing a key role in enhancing potency, cooperativity, and physicochemical properties. However, due to their large size and complex design, PROTAC optimization is time-consuming, particularly when relying on cell-based assays. In this study, we employed a direct-to-biology (D2B) approach, which combines automated chemistry with the screening of crude reaction mixtures. This high-throughput method allows for rapid exploration of linker intermediates, offering a time- and cost-efficient pathway for PROTAC optimization.

Following a virtual screening of Enamine's drug-like library for BRD4 BD1 hits (Figure 1A), we identified 30 candidate compounds for further profiling. These compounds were initially evaluated for activity using an SPR assay (Figure 1B), followed by validation in a cell-based assay, displayed as an LLE plot (Figure 1C), and an FP assay (data not shown). Docking one of our novel compounds into the crystal structure of the BRD4 active site revealed two exit vectors, which we used to guide the development of our PROTACs (Figure 1D).



BocHN-Linker-E3-L → Boc Deprotection → H₂N-Linker-E3-L → 96 Well → POI-L-Linker-E3-L

Library of custom made or commercial Boc protected partial PROTACs

40 partial PROTACs ready for array chemistry

Amide coupling with two BRD4 inhibitors

Array of 80 PROTAC degraders ready for biological assays

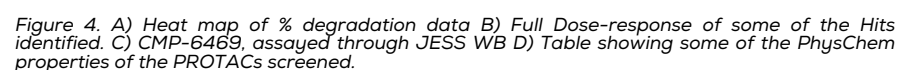
Figure 2. Our approach for high-throughput plate-based chemistry for direct biological assays.

Using the identified exit vectors of our molecules (Section 1), we coupled our novel compounds to a range of E3-ligand-linker constructs via amide coupling (Figure 2), generating multiple PROTAC variants, as exemplified in Figure 3A. As shown in Figure 3B, the D2B approach significantly reduces both time and cost compared to conventional methods. Key factors such as the shape, length, and attachment point of the linker, as well as its physicochemical properties, are essential for designing potent and selective PROTAC degraders. The high efficiency of the amide coupling reaction is visualized through a PyParse-generated heatmap (Figure 3C). Table 1 summarizes the systematic design and development of our linker intermediates library.



Table 1. Different categories of E3-L-Linker intermediates within our library

Crude compound mixtures were screened using a BRD4-HiBiT degradation assay. Starting from a 96-well plate stock, liquid handlers diluted the crude mixtures to 1 μ M and 0.1 μ M in 384-well cell plates (Figure 4A), effectively minimizing false negatives caused by the hook effect, a known phenomenon with PROTACs. Compounds of interest were then advanced to a full dose-response assay, profiling degradation potential in parallel with purified batches (Figure 4B). No significant differences in potency were observed between crude and purified compounds, as demonstrated by BRD4-HiBiT degradation (Figure 4B) and JESS Western Blot analysis with a BRD4 antibody (Figure 4C). From the initial screening, we identified PROTACs with picomolar DC₅₀ values, and these were further characterized by our DMPK team through ADME profiling to assess key physicochemical properties (Figure 4D).



Physchem properties assessment and linker design

Library of Linker-E3 Ligand
- source plate

POI ligand stock solution

High throughput amide coupling using integra ViaFlo pipetting robot

96 wells reaction plate - array of PROTACS

Automated QC analysis

5 days

Spot-test Results

Direct biological assays - rapid screening

Heatmap generation

POI degradation data - hits validation

Figure 5. Overview of the automated D2B platform developed at Charnwood Discovery 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 reducing the cycle of PROTAC optimization to only 5 days.

In this work, we demonstrate how the D2B approach accelerates PROTAC discovery, reducing the hit-finding timeline from several months to just 5 days. We highlight our computational design capabilities at Charnwood Discovery by identifying new, highly potent BRD4 inhibitors. Additionally, we validate our D2B platform through plate chemistry and high-throughput cell assay validation. Our in-house library is readily available for reaction with your POI-ligand, supporting integrated drug discovery projects.

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



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