Integrated PROTAC Discovery Platform: From Design To BRD4 Degradation

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Introduction

Targeted protein degradation has emerged as a transformative approach in drug discovery, enabling selective removal of proteins previously considered undruggable. PROTACs (PROteolysis TArgeting Chimeras) are leading this field [1], but their discovery requires the integration of diverse scientific disciplines — from structural design and linker chemistry to ternary complex formation and cell-based validation. To meet this need, we offer a fully integrated PROTAC discovery platform that supports pharmaceutical and biotech partners from early idea to validated degrader compounds.

Aim

The aim of this study was to establish a fully integrated platform for the rapid and efficient discovery of PROTACs. Designed as a modular and partner-focused solution, the platform enables seamless progression from ligand identification and virtual library design to synthesis, biological profiling, and degrader validation. As a proof of concept, we applied the workflow to the identification of BRD4-targeting PROTACs recruiting Cereblon (CRBN) as the E3 ligase. The project was used to demonstrate the platform's ability to efficiently deliver active degraders with validated cellular activity, confirming its suitability for supporting early-stage drug discovery programs in a streamlined and collaborative format.

Research Background

This study was initiated to demonstrate the capabilities of our integrated PROTAC discovery platform, which enables rapid progression from concept to validated degraders. As a model system, we selected BRD4 as the target protein [4] and Cereblon (CRBN) as the E3 ligase [3]. The platform workflow included ligand identification, virtual library construction, in silico filtering, high-throughput synthesis, and in vitro evaluation. The virtual library was designed by systematically combining BRD4 and CRBN binders with chemically diverse linkers. Predictive tools such as QSAR models, machine learning, and docking-based ternary complex modeling were applied to prioritize candidates. This structured approach enabled efficient down-selection from over a thousand virtual designs to a focused set of compounds for synthesis and biological validation. The resulting data serve to benchmark the performance, speed, and flexibility of the platform — and demonstrate its suitability for application across different target-ligase combinations.

Methods

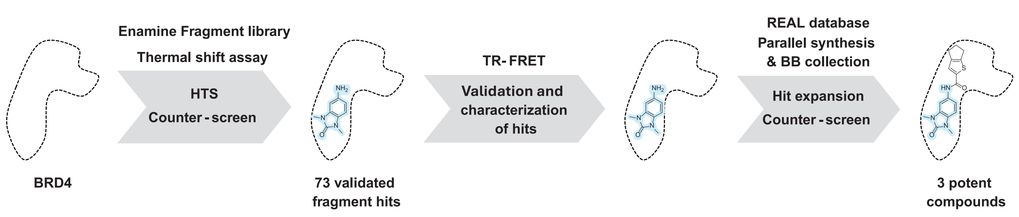
A focused virtual PROTAC library was generated by combining selected BRD4 and CRBN ligands with diverse linkers. The library was filtered using 2D QSAR, machine learning, and ternary complex modeling to prioritize candidates.
135 compounds were synthesized via high-throughput parallel chemistry and verified by LCMS.
In vitro evaluation was performed in four stages:
1. TR-FRET to assess CRBN–PROTAC binary complex formation
2. SPR to evaluate BRD4–PROTAC binding
3. TR-FRET for ternary complex formation (CRBN:PROTAC:BRD4)
4. Cell-based TR-FRET to measure BRD4 degradation in HEK293T cells

Tagged constructs (FLAG-CRBN/DDB1 and Biotin-Avi-BRD4(BD1)) were used in Stages 1–3 to assess complex formation.

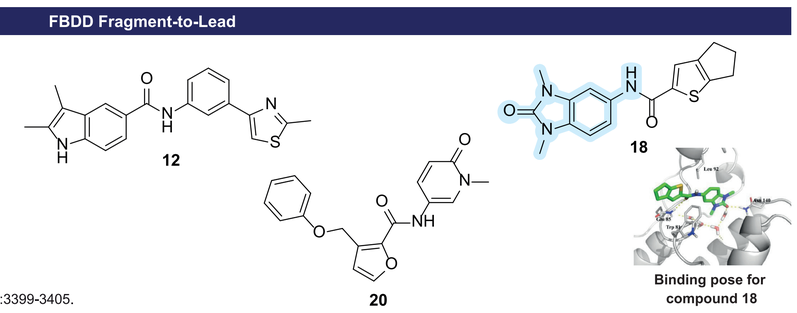
Results

Ligand identification

FBDD Hit identification



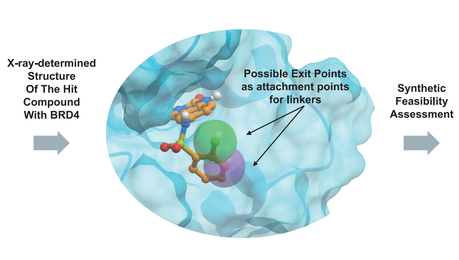
FBDD Fragment-to-Lead



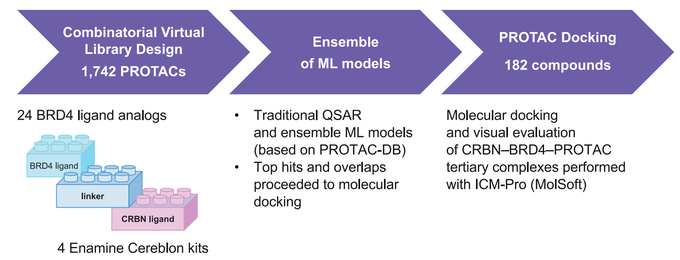
Borysko P. *et al.* Bioorg Med Chem. 2018;26(12):3399-3405.

PROTAC® design and synthesis

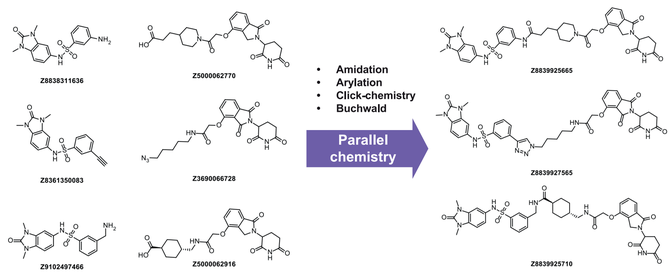
PROTAC modeling-Exit vector selection



PROTAC Library design (2 weeks)



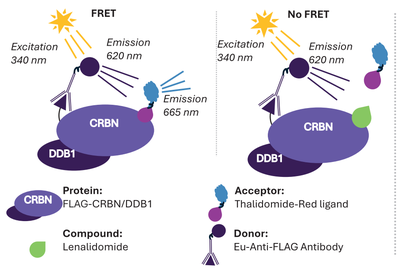
PROTAC synthesis (3 weeks)



In vitro evaluation

Binary complex formation with CRBN TR-FRET

Binary complex formation with Cereblon (1 week)



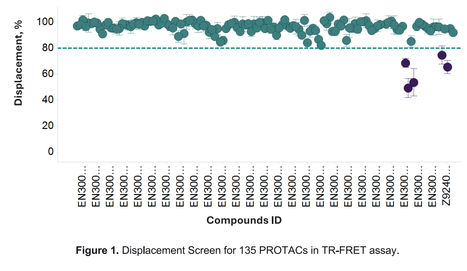
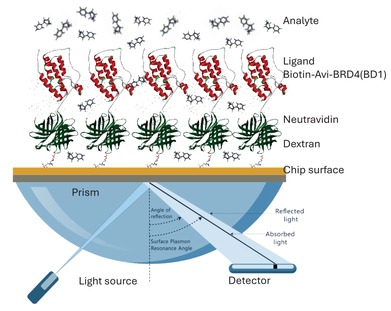


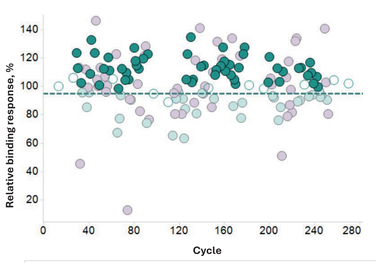
Figure 1. Displacement Screen for 135 PROTACs in TR-FRET assay.

Out of the 135 PROTACs tested at a single concentration (11 μM), 129 exhibited displacement greater than 80% under the tested conditions, confirming the formation of CRBN-PROTAC complexes (Figure 1).
Consequently, these 129 compounds were selected for further assessment in Stage 2.

Binary complex formation with BRD4 SPR assay

Binary complex formation with BRD4 (1 week)





Screening of 129 PROTACs at a single concentration (50 μM) identified 56 promising candidates that exhibited binding activity greater than 95% of the response of the reference ligand JQ-1 at a saturated concentration. These compounds also demonstrated good binding behavior, such as absence of non-specific binding to the reference flow cell and no stickiness (Figure 2).
Identified 56 compounds were selected for further assessment in Stage 3.

Figure 2.
Binding Screen for 129 PROTACs in SPR assay. The relative binding response is presented after blank subtraction, molecular weight adjustment, and normalization to the JQ-1 response at saturated concentration.

Ternary complex formation TR-FRET assay

Protein degradation Cell-based TR-FRET BRD4 degradation assay

Ternary complex formation (1 week)

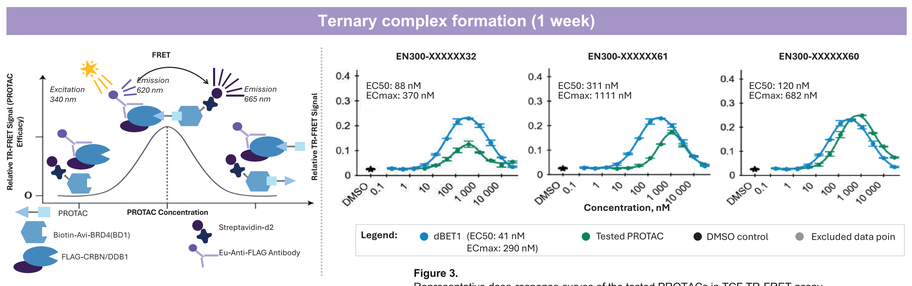


Figure 3.
Representative dose-response curves of the tested PROTACs in TCF TR-FRET assay.
Analysis parameters: Nonlinear regression, bell-shaped dose-response model.

The 56 selected PROTACs were tested at 12 concentrations using a 3-fold dilution series starting from 30 μM. TCF was initiated by 47 PROTACs, with 17 exhibiting ECmax values below 500 nM (Fig.3). All 47 PROTACs with confirmed ternary complex formation were selected for further assessment in Stage 4.

Protein degradation (1 week)

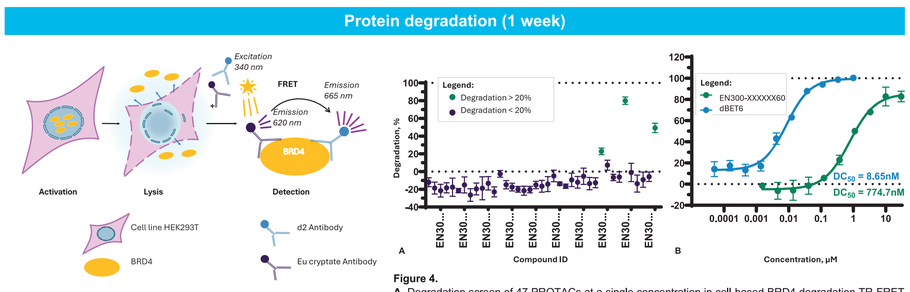


Figure 4.
A. Degradation screen of 47 PROTACs at a single concentration in cell-based BRD4 degradation TR-FRET assay. **B.** Representative DRC of the tested PROTAC (EN300-XXXXXX60) in cell-based BRD4 degradation TR-FRET assay. Analysis parameters: Nonlinear regression, Sigmoidal 4PL model.

Screening of 47 PROTACs at a single concentration (30 μM) showed degradation activity by three PROTACs, with BRD4 degradation levels of 80%, 53%, and 23% under tested conditions (Fig.4A).
These three compounds were further assessed in a dose-response format (3-fold dilution series starting from 30 μM, 10 points) where DC50 value was determined for one PROTAC (775 nM) (Fig.4B).

Conclusions

Our integrated PROTAC discovery platform enabled rapid progression from ligand identification and virtual library design to synthesis, biological profiling, and degrader validation. This led to the identification of three active BRD4 degraders, with up to 80% cellular degradation and a lead compound showing a DC_{50} of 775 nM. Completed in 16 weeks, this case highlights the platform's speed, scalability, and adaptability to diverse targets, with timelines adjustable depending on project complexity.

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