

REAL approach to developing CRBN molecular glue libraries and E3 ligand-linker conjugate kits

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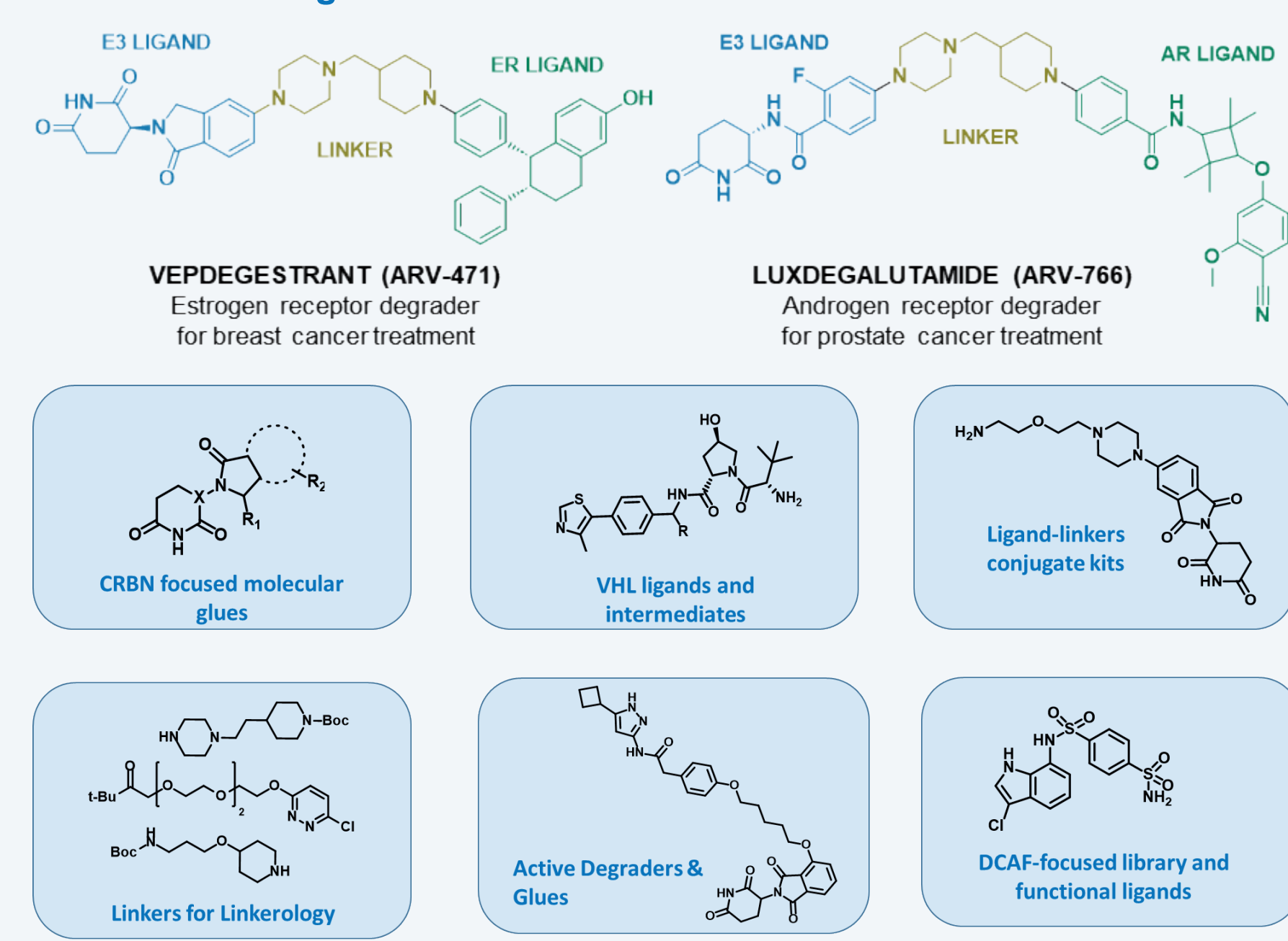
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Introduction and Highlights

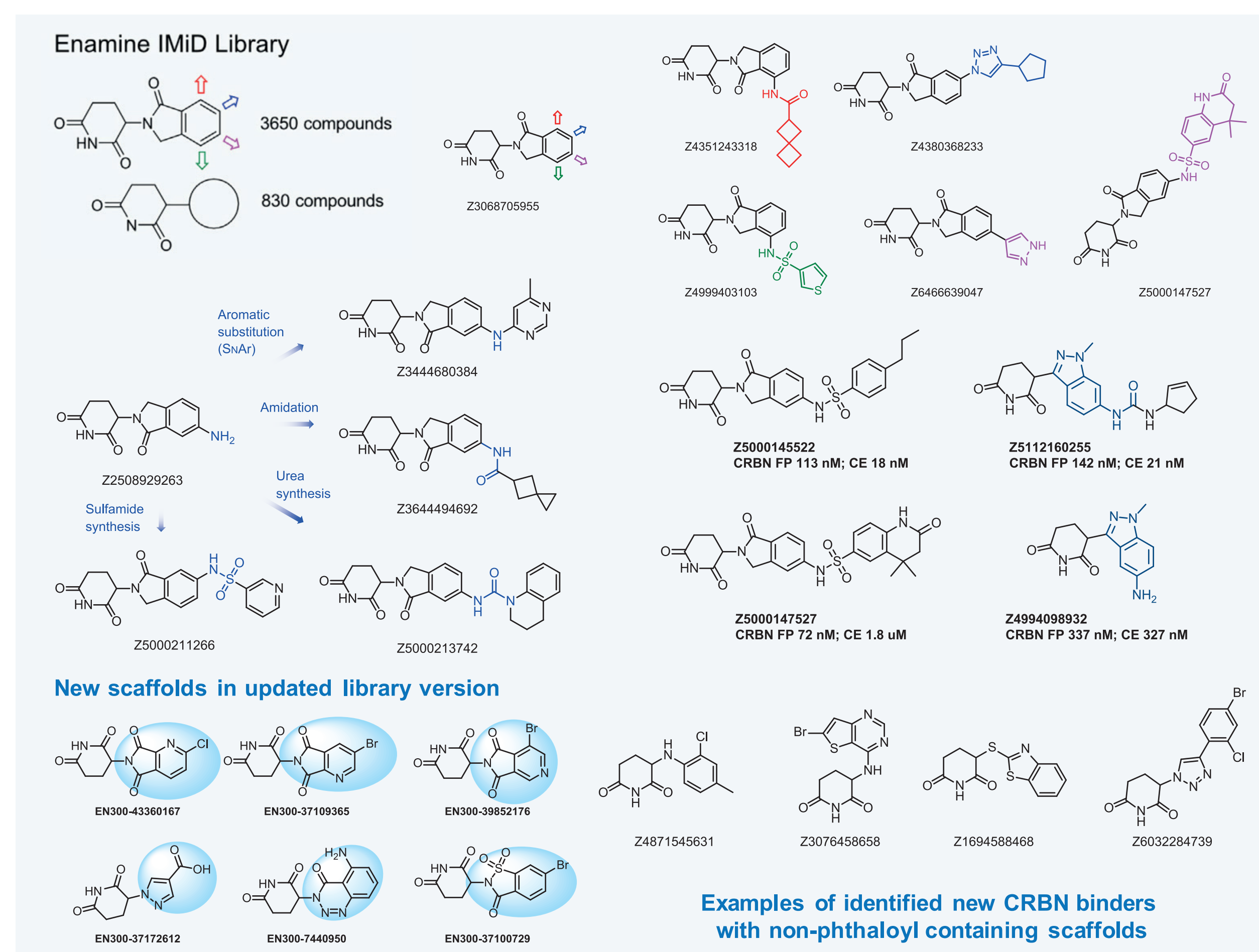
- CRBN-based PROTACs and molecular glues proved to be the most efficient approach in developing new effective oral degraders with a significant prevalence in clinical trials. Recent efforts have led to the exciting discovery of next-generation IMiD scaffolds, which eliminate undesirable off-target degradation profile¹.
- Here, we describe the design, synthesis, and biological evaluation of the first edition of the IMiD library. Key scaffolds and their decoration approaches to achieve high diversity and flexibility in structure modification.
- Design and synthesis of E3 ligand-linker conjugate kits for further one-step coupling with POI ligands and probes or reporters.
- Developed engineered *h*CRBN construct, CRBN_ΔHBD, expressed in *E. coli* revealed application of HTS methods for searching new CRBN binders; 4480-membered IMiD Library was screened and followed with SAR analysis².
- Design and enumeration of REAL CRBN focused space for fast hit follow-up and straightforward lead generation relying on active core scaffolds.

More than 20 degraders in clinical trials



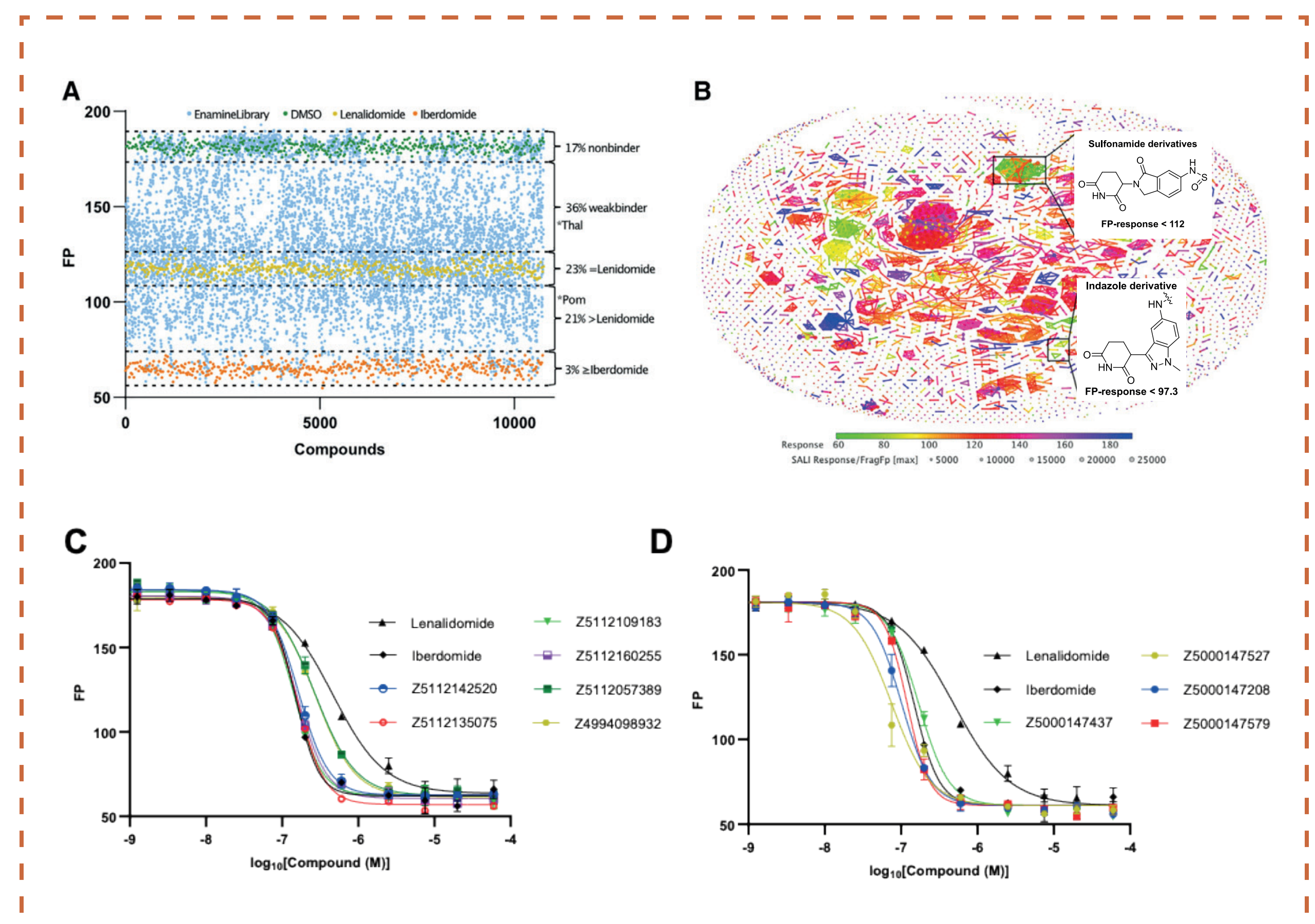
IMiD Library design

The library was specially designed for the CRBN screening campaigns and investigation of new CRBN-based molecular glues: 3650 compounds contained lenalidomide core with various substitution in phthaloyl ring and additional 830 compounds contained scaffold variation with close similarity to typical IMiD structures. Synthesis was performed starting from available glutarimide-containing intermediates.



Binary SAR screening results

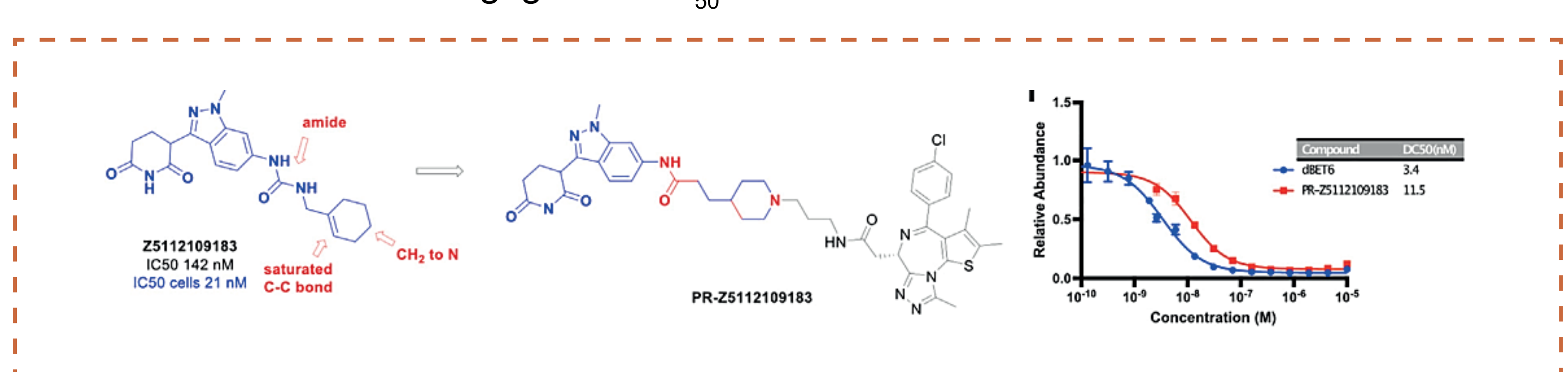
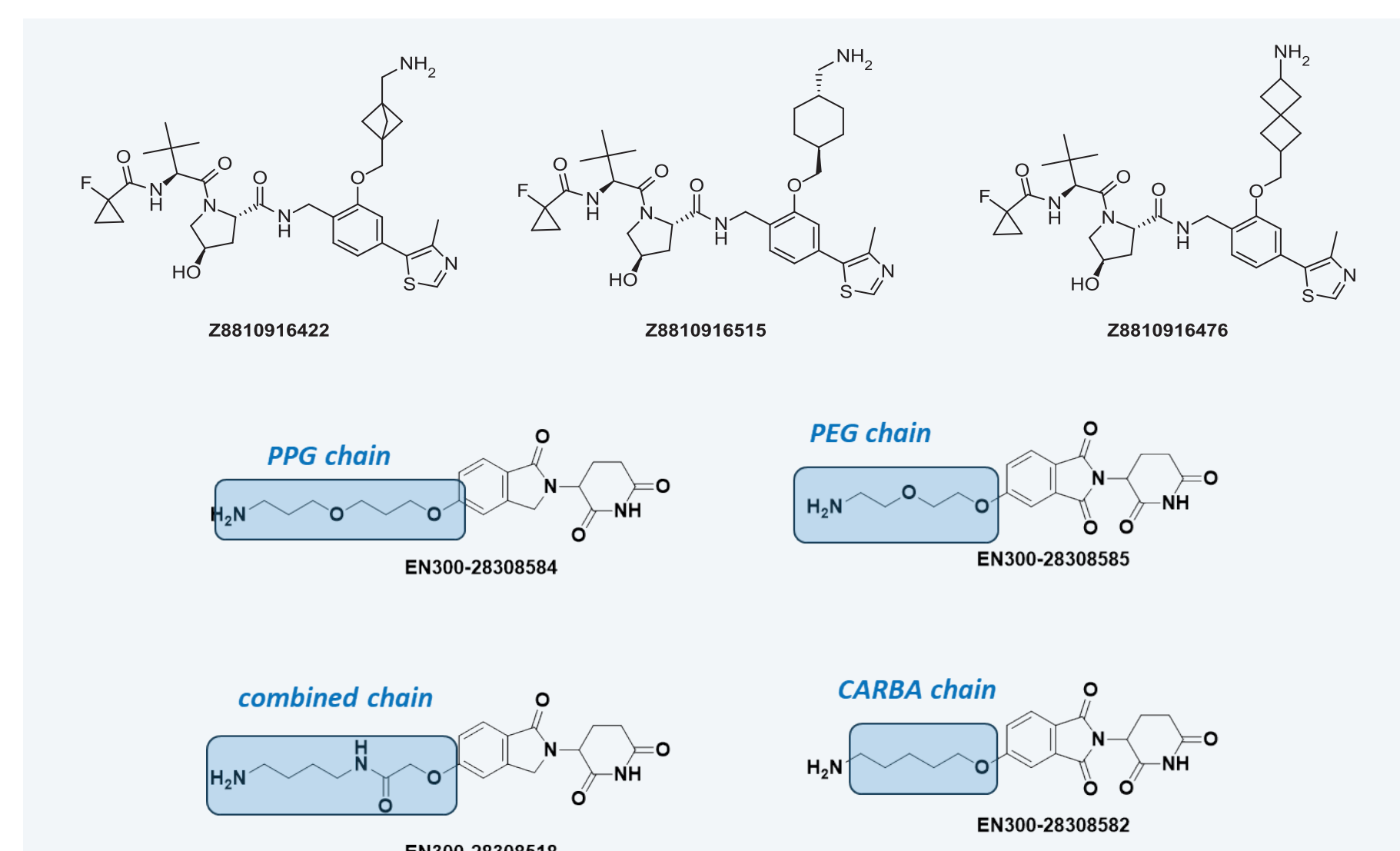
The newly engineered CRBN_ΔHBD construct was established as relevant and allows the study of binary and ternary complex formation using tool compounds. Next, it was optimized for high-throughput screening assays to search for the next generation of scaffolds that may overcome the intrinsic issues with thalidomide. The FP assay was then used for binary SAR screening of the IMiD library as the first step in searching for promising binders².



Ligand-linkers conjugate kits and fast PROTAC synthesis

To accelerate new PROTAC synthesis, we designed small sets with CRBN and VHL binders as anchors with variable ligand length and construction (PEG-, Carba- and rigidified). Six ready-to-couple sets were developed for quickly access to PROTAC construction.

Identified indazole-based compounds are suitable for the development of bifunctional degraders. Design of PROTAC molecule PR-Z5112109183 starting from indazole containing hit compound Z5112109183. Optimized for fast synthesis PROTAC was tested for BRD4 degradation activity in endogenous HiBiT BRD4 reporter cells, revealing efficient degradation with half-maximal degradation concentration (DC_{50}), after 5 h, in the low nanomolar range. The *in vitro* IC_{50} as well as cellular CRBN engagement IC_{50} is indicated.



An engineered cereblon optimized for high-throughput screening and molecular glue discovery
Bailey, Henry J. et al. *Cell Chemical Biology*, Volume 32, Issue 2, 363 - 376.e10, Feb 20, 2025

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