Challenging the Paradigm: PROTACs and Chimeric Degraders as a Novel Strategy for New Antibiotic Development Against Antimicrobial Resistance

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Introduction

- The pharmaceutical industry faces an urgent need for novel antibacterial strategies as antimicrobial resistance (AMR) continues to erode the efficacy of traditional antibiotics. Proteolysis-targeting chimeras (PROTACs) and other chimeric targeted protein degraders offer a disruptive modality that challenges conventional drug design by inducing selective degradation of disease-relevant proteins rather than inhibiting their function.

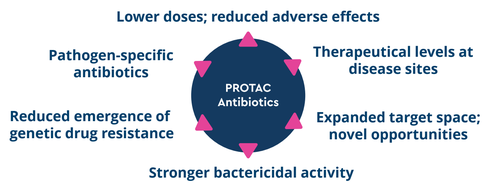
- While PROTACs have gained traction in oncology or immunology, their application in antibacterial drug discovery remains largely unexplored, due to the fact that PROTAC technology was so far restricted to the ubiquitin tagging system of eukaryotes, and has yet to be transferred to degradation pathways in bacteria (as some bacteria utilize a similar system for targeted protein degradation, such as phosphoarginine as degradation tag for the ClpC-ClpP protease). Such degraders could circumvent common resistance mechanisms, including target mutation and efflux, by acting catalytically and engaging previously "undruggable" targets.

Objectives

- We are discussing recent advances in the design of PROTACs, their pharmacology and ADME challenges, and key considerations for translation of this modality to bacterial systems. Early proof-of-concept studies suggest that chimeric degraders could expand the antibacterial target landscape and offer a new class of precision antibiotics with reduced resistance liability.

- Selvita, within the integrated drug discovery platform, is well-positioned and experienced to support the development of such first-in-class antibacterial degraders, where rapid iteration and cross-functional collaboration are essential, which includes:
  - Structure-based drug design and medicinal chemistry to optimize bifunctional molecules
  - Biophysical/biochemical assays to validate ternary complex formation and degradation efficiency
  - Cell-based assays to confirm degradation and downstream biological effects
  - ADME-Tox profiling, including PBPK modeling, specific for degraders
  - *In vitro*, translational, and *in vivo* pharmacology

Opportunity for Antibacterial Target Landscape



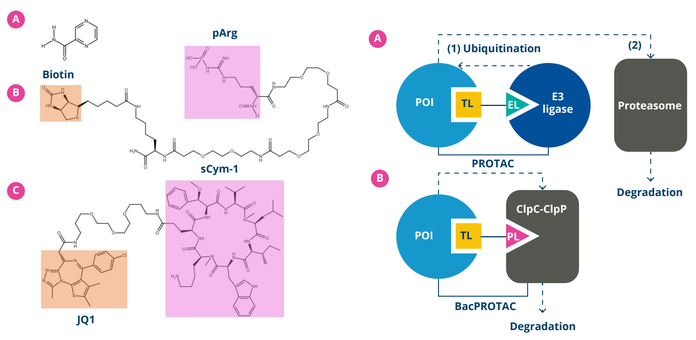


Figure 5. Left. Structures of (A) pyrazinamide (target is aspartate decarboxylase), (B) and (C) BacPROTACs. Critical moieties in the bifunctional BacPROTACs are highlighted. B, BacPROTAC-1: POI-targeting biotin moiety (model POI monomeric streptavidin) linked to a phosphorylated arginine moiety (pArg). C, BacPROTAC-3: POI-targeting JQ1 moiety (for bromodomain testis) linked to a cyclomarin A-like cyclic peptide (sCym-1). **Right.** Mediation of target degradation by antibacterial BacPROTACs versus human PROTACs. Exposed degradation tag is a substrate of the bacterial caseinolytic protease complex (ClpC-ClpP).

DMPK approach for PROTACs at Selvita

- Selvita is participating in a joint effort along with 5 big pharmaceutical companies to investigate the translation of PK or PROTACs to human (publication will soon be released)
- We also have recently given a webinar showing how PBPK can help PROTACs drug discovery

SCAN THE CODE TO WATCH OUR WEBINAR

We generate information – integrate – identify liabilities – optimize assay conditions – improve data quality – build relevant cascades → we can do it using Model Informed Approaches

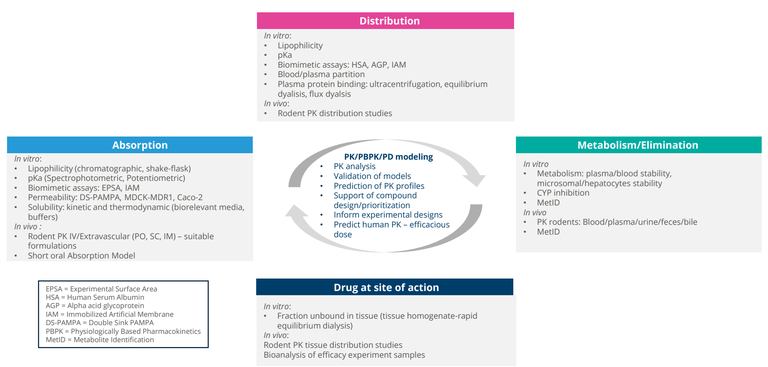


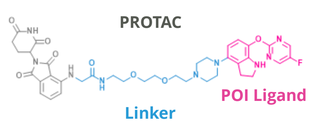
Figure 7. Comprehensive DMPK approach for PROTACs

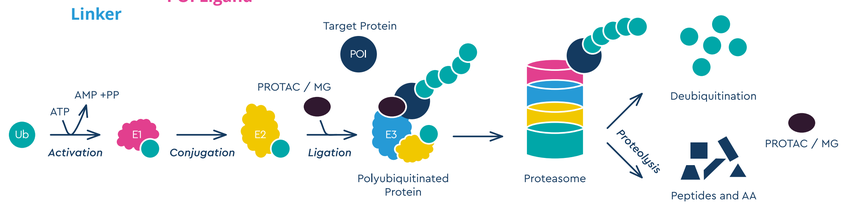
Proteolysis Pathway and PROTACs vs. Molecular Glues

- Proteolysis maintains cell homeostasis and is implicated in numerous pathological conditions
- Regulated proteolysis is the enzymatic breakdown of proteins into peptides and amino acids for future cell protein synthesis
- The major protein degradation pathway is the Ubiquitin Proteasome System (UPS) which selectively targets intracellular proteins for degradation by ubiquitin (Ub) tagging
- Ubiquitination occurs through the sequential action of ubiquitin-activating enzymes (E1), ubiquitin conjugating enzymes (E2), and ubiquitin ligases (E3)
- Polyubiquitinated proteins are recognized and degraded by the proteasome
- Proteolysis targeting chimeras (PROTACs) and molecular glues (MG) facilitate a proximity-induced mechanism to selectively target and degrade disease-causing proteins of interest (POI)

KEY ADVANTAGES
- Catalytic mechanism-of-action
- Improved selectivity and efficacy
- Potential to target 'undruggable'

Descriptor	PROTACs	Molecular Glues
Category	Bivalent	Monovalent
Linker	Yes	No
Molecular weight	700-1500 Da	<500 Da
Lipinski's rule of 5	Defy (F% challenging)	Within (F% feasible)
Target	Predictable	To be determined
Binding pocket	Required	Not required
Binding attribute	E3 ligase and POI ligands crosslinked	Promote protein-protein interaction (E3 ligase + POI)
Advantage over occupancy driven pharmacology	Catalytic mechanism of action	Event-driven catalytic mechanism of action





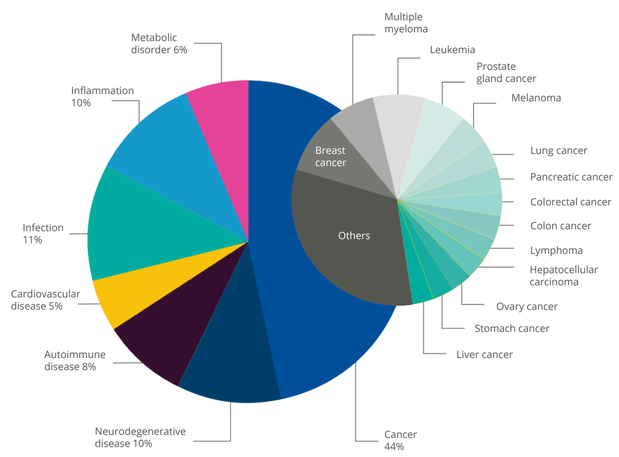


Figure 1. Proteolysis Pathway and Merits of Targeted Protein Degradation (TPD), including Disease Area Landscape

SelvitaDegraders Platform, Experience, and Stages of PROTAC Design Workflow

- SelvitaDegraders platform is fully integrated
- All-inclusive understanding of biochemical, cell-based, biophysical, and DMPK assessment – along with chemistry, CADD, structural biology, and translational support to facilitate the discovery and development of protein degraders
- 12 Targeted Protein Degradation (TPD) projects have been conducted, many of them fully integrated
- 2 large integrated projects are ongoing (MedChem / CADD-AI / ADME-PK / Pharmacology support)

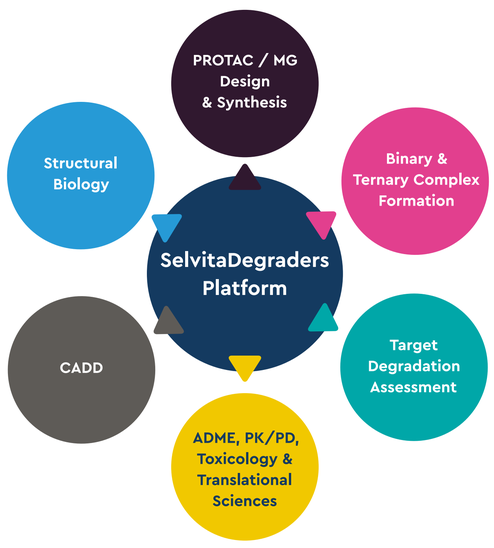


Figure 2. Integrated SelvitaDegraders platform

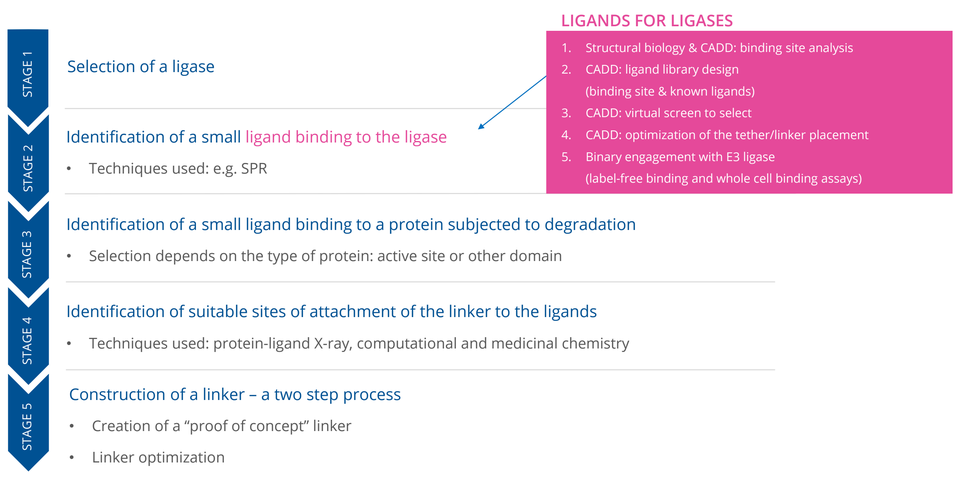


Figure 3. Stages of PROTAC Design Workflow

Selvita's MedChem / CADD-AI Capabilities and Experience with PROTACs

- MedChem design of PROTACS to meet TCP, followed by synthetic design and production
- CADD/AI/Modelling
  - Workflow for ternary complex prediction that won a worldwide competition
  - Linker design: (in-house Linkerer program), PROTAC DB screening
- Synthesis/optimization of POI ligand (binding modes and exit vector)
- Selection of warhead suitable for E3 ligase (Cereblon, VHL, IAP, MDM2, +others) including non-IMID-based CRBN binders to avoid neo-substrates
- Validation/optimization of linker (composition, rigidity, length) and attachment sites with appropriate reactive functional groups
- SAR optimization to improve solubility and permeability, and reduce metabolism
- Robust coupling chemistry (amines, ethers, amides, click chemistry, etc.)
- Scale-up of intermediates and final products

SelvitaDegraders Track Record
- **> 200** discovery chemists
- **> 5 years** synthetic experience with linkers, E3 ligase and POI ligands
- **> 10 clients/collaborations**
- **> 1000 final PROTACs synthesized**
- **> 200 diverse linkers available**
- **> 500 E3 ligase ligands prepared**

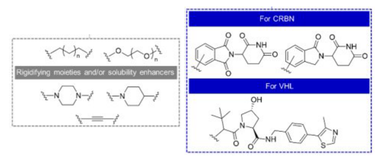


Figure 6. Construction of Linkers and E3 Ligase Ligands

Selvita's Pharmacology Capabilities / Experience Specific for PROTACs

- Selection of E3 ligase (Cereblon, VHL, IAP, mdm2, others) and identification of ligand with affinity for POI (e.g. SPR, CADD/AI & Structural biology)
- Measurement (incl. kinetic) of protein degradation in PROTAC-treated cells - TARGET-HiBiT / LgBit by Western blot / JESS or phenotypic degradation
- PROTAC target engagement and permeability measurement in cells - NanoBRET
- Monitoring formation of binary or ternary E3-PROTAC-POI complexes by ITC, SPR, TRIC, FRET, HTRF, FP, Lumit, AlphaLISA, or native MS
- Proteasomal recruitment (NanoBRET proteasome assay)
- Target ubiquitinylation (Wes, NanoBRET)
- Cereblon binding assay and neo-substrate screening assays
- Serum shift assay
- Protein half-life assay (target turnover time) – CHX (cycloheximide) chase assay

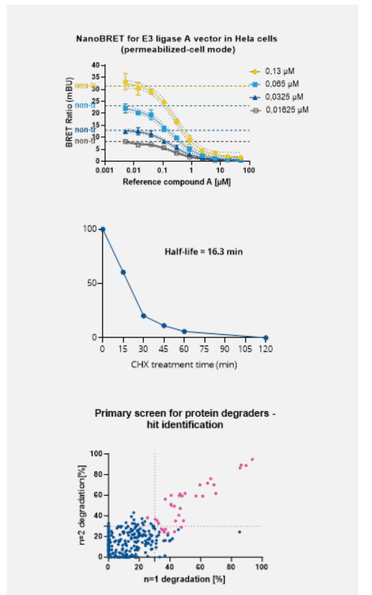


Figure 8. Selected results from PROTAC-related assays

Conclusions

- For pharmaceutical R&D, this approach represents a paradigm shift - one that aligns with the industry's push toward modality diversification and mechanism-based innovation.

- Degrader-based antibiotics such as BacPROTACs, which successfully bypass the human E3 ligase, providing an entry strategy for the generation of antibacterial PROTACs and could become a cornerstone of next-generation anti-infective pipelines, offering both therapeutic novelty and strategic differentiation in a crowded and high-risk development space

- Selvita, through its experience working with PROTACs, is well-positioned to support the development of such first-in-class antibacterial degraders, where rapid iteration and cross-functional collaboration are essential

Literature

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