PhD Project Proposal

Funder details

Studentship funded by:	MRC iCASE award (AstraZeneca)

Project details

Project title:	**Project title:** Developing Direct-to-Biology for Molecular Glue Discovery

Supervisory team

Primary Supervisor:	Professor Zoran Rankovic (ICR)
Associate Supervisor(s):	Dr. Niall Anderson (AstraZeneca) Dr. John Caldwell (ICR)
Secondary Supervisor:	Dr. Gary Newton (ICR)

Divisional affiliation

Primary Division:	CCDD
Primary Team:	TPD
Site:	Sutton

Project background

Targeted protein degradation (TPD) is a novel and rapidly emerging drug discovery paradigm that provides opportunities for tackling currently intractable oncoproteins and to deliver breakthrough therapies. The TPD paradigm includes two main approaches focusing on molecules with a similar proteasome-dependent mechanism of action, namely, proteolysis targeting chimeras (PROTACs)[1], and molecular glue degraders (MGDs).[2] MGDs are small molecules capable of binding to an E3 ligase and altering its surface and specificity, leading to the recruitment, ubiquitination, and subsequent degradation of substrates that are normally not targeted by the ligase alone (neosubstrates). Therefore, this approach offers an unprecedented opportunity to degrade currently intractable and undruggable targets.

However, in contrast to PROTACs, designing MGD is exceedingly challenging, and their discovery and optimization relies mostly on serendipity or, through screening focused libraries. We anticipated an opportunity to accelerate this process through the combination of high-throughput chemistry (HTC) with direct-to-biology (D2B) screening. A high-throughput miniaturised D2B approach, which streamlines the target discovery pipeline by obviating the need for separation and purification, can be used to accelerate the MGD discovery by reducing the cost, time and increasing throughput of the design-synthesis-test cycle iterations. However, the success of the D2B process is dependent on high yielding reactions and extensive experimental design to limit the noise and interference of crude reaction mixtures with biological assays. Consequently, the scope of D2B-compatible chemical reactions reported in the literature is rather limited, including mostly peptide coupling and recently reported multi-component reactions.

Here we propose to expand the scope of D2B approaches to other frequently utilised synthetic reactions in drug discovery, including Suzuki–Miyaura, Buchwald–Hartwig, and SNAr reactions.[3]

Project aims

Aim 1: Reaction Optimization. Commonly utilized synthetic reactions such as Suzuki–Miyaura, Buchwald–Hartwig, and SNAr reactions will be optimised for the D2B format.

Aim 2: Library design and synthesis. A cereblon-focused library will be designed and synthesized using the reactions developed in Aim 1.

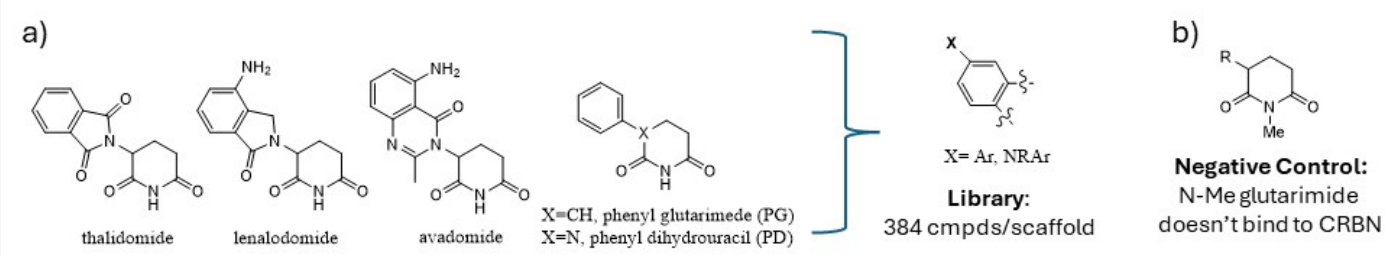
Aim 3: Library screening. The library will be screened in biochemical (CRBN-FP assays), and cancer cell viability assay (MV4-11, CTG) to identify novel neosubstrates.

Research proposal

Aim 1: Reaction Optimization.

To expand the current D2B chemistry toolbox, we selected some of the most frequently used reaction in drug discovery, including Suzuki–Miyaura, Buchwald–Hartwig, and SNAr. These reactions will be employed to diversify cereblon ligands such as thalidomide, lenalidomide, avadomide, phenyl glutarimide (PG), and phenyl dihydrouracil (PD) cores (Figure 1a). The reactions will be caried out in 384-well plates, while iDOT or Echo will be used for liquid handling. Reagent scavengers will be used to remove unwanted reaction components. Importantly, the reactions will be optimised: (1) for high conversion with minimal impurities; and (2) ensuring the reagents and generated impurities don't interfere with the intended assays systems. Advanced experimental design methods and machine learning will be employed to optimize the reactions and select building blocks/reagents.[4] For each scaffold a negative control containing N-methylated glutarimide ring that prevents binding to cereblon, will be synthesized to assess potential assay interference. Activity of negative controls detected in biological assays would suggest potential non-specific reagent interference. The library production (Aim 2) will be initiated only after a robust confirmation of negative controls' inactivity in the intended biological assays.

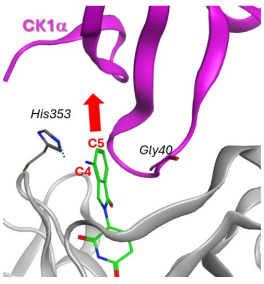
Figure 1. (a) Immunomodulatory IMiD Drugs (IMiDs); (b) Diversity is to be introduced in the IMiD aromatic group.



Library Design and Synthesis (Aim 2)

Reactions optimised in Aim 1 will be used to synthesize a library of cereblon ligands based on the IMiD cores (Figure 1), designed to form interactions with classical IMiD G-loop degron. Publicly available crystal structures of IMiDs in complex with CRBN-DBB1 reveal the IMiDs binding site as a shallow hydrophobic pocket on the substrate recognition surface. The core feature of the IMiDs binding to the CRBN are the conserved glutarimide ring interactions with three tryptophan residues and two hydrogen bonds to His378 and Trp380, while the rest of the molecule is exposed on the surface of the protein, and available for interactions with degron motif present in C2H2 zinc fingers such as IKZF1 (Figure 2).[5] Since neo-substrate selectivity is influenced by alterations of the Cereblon surface upon molecular glue binding, we hypothesize that structurally novel Cereblon ligands binding to the thalidomide pocket would result in recruitment of novel neosubstrates of potential therapeutic interest. The library will be designed so that the diversity introduced by reactions optimised in Aim 1 would project towards the degron loop (Figure 2). A total of 384 analogues will be synthesized around each of the 6 cores shown in Figure 1a. The building blocks will be selected based on their (1) availability, (2) diversity and (3) predicted physicochemical properties. For each scaffold a negative control containing N-methylated glutarimide will

Figure 2. The structure of lenalidomide co-crystalised in ternary complex with CK1a and CRBN/DDB1 (PDB: 5FQD). The red arrow shows the vector by which the newly introduced groups will project.



be synthesized within the library to evaluate potential assay interference. We will also confirm that the final products are not commercially available.

Library Screening and Hit confirmation (Aim 3)

The library synthesized in Aim 2 will be tested in CRBN-FP assay to confirm cereblon binding. This will be followed up by testing the library in several haematological and solid tumour cell lines, including MV4-11 (AML) and HB03 (medulloblastoma) for their effect on cell viability by CellTiter-Glo® (CTG) luminescent assay. The screening will be carried out in a 384-well plate format over 72 hours incubation, and resulting luminescence will be measured by plate reader. All negative controls synthesized withing the library should be inactive (<20% cell death). Hits exerting >30% cell death will be resynthesized, purified and their full dose-response determined, followed by testing in lenalidomide competition assay to confirm CRBN dependency. Most potent compounds (10-15 hits) will be then progressed to proteomics to establish their mechanism of action.[6]

Literature references

1. Békés, M., Langley, D. R. & Crews, C. M. PROTAC targeted protein degraders: the past is prologue. *Nat. Rev. Drug Discov.* (2022) doi:10.1038/s41573-021-00371-6.

2. Geiger, T. M., Schäfer, S. C., Dreizler, J. K., Walz, M. & Hausch, F. Clues to molecular glues. *Curr. Res. Chem. Biol.* **2**, 100018 (2022).

3. Brown, D. G. & Boström, J. Analysis of Past and Present Synthetic Methodologies on Medicinal Chemistry: Where Have All the New Reactions Gone?: Miniperspective. *J. Med. Chem.* **59**, 4443–4458 (2016).

4. McCorkindale, W., Filep, M., London, N., Lee, A. A. & King-Smith, E. Deconvoluting low yield from weak potency in direct-to-biology workflows with machine learning. *RSC Med. Chem.* **15**, 1015–1021 (2024).

5. Nishiguchi, G. *et al.* Selective CK1α degraders exert antiproliferative activity against a broad range of human cancer cell lines. *Nat. Commun.* **15**, 482 (2024).

6. Wang, Z. *et al.* Direct-to-biology, automated, nano-scale synthesis, and phenotypic screening-enabled E3 ligase modulator discovery. *Nat. Commun.* **14**, 8437 (2023).

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants: Organic Chemistry

Intended learning outcomes:

- Expand organic chemistry knowledge and experimental skills
- Learn to use modern automation and liquid handling systems
- Develop chemical biology knowledge skills, e.g. perform screening by routine biological assays such as fluorescence polarization (FP), and CellTiter-Glo (CTG) assays.
- Develop writing and oral reporting skills
- Develop knowledge of Targeted Protein Degradation (TPD)

Advertising details

Project suitable for a student with a background in: ☐ Biological Sciences

- [ ] Physics or Engineering
- [x] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science