The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	**Design and synthesis of libraries of bifunctional degraders for the discovery of new cancer targets**

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Swen Hoelder
Other supervisory team members:	Dr Benjamin Bellenie Dr Olivia Rossanese Professor Julian Blagg

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Medicinal Chemistry 4
Other Division (if applicable):	Cancer Biology
Other Team (if applicable)):	Proteomics and Metabolomics Facility

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

The success of targeted cancer drug discovery is critically dependent on the identification of protein targets that can be modulated by small molecules to give a specific anti-cancer effect. The aim of this project is to apply synthetic organic chemistry and medicinal chemistry to the design and synthesis of a library of compounds which are able to promote protein degradation ("bifunctional degraders"), and use these to identify novel protein targets for cancer drug discovery.

Bifunctional degraders [1] are a recently discovered class of molecules in which an E3 ubiquitin ligase binding compound (E3B) is linked to a warhead that binds a therapeutic target (figure 1A). Unlike conventional small molecule drugs which bind to, and block the action of a protein target, degraders catalyse the formation of a complex between an E3 ligase and target protein, enabling ubiquitination and hence the rapid and irreversible degradation of the target (figure 1B)[1,2].

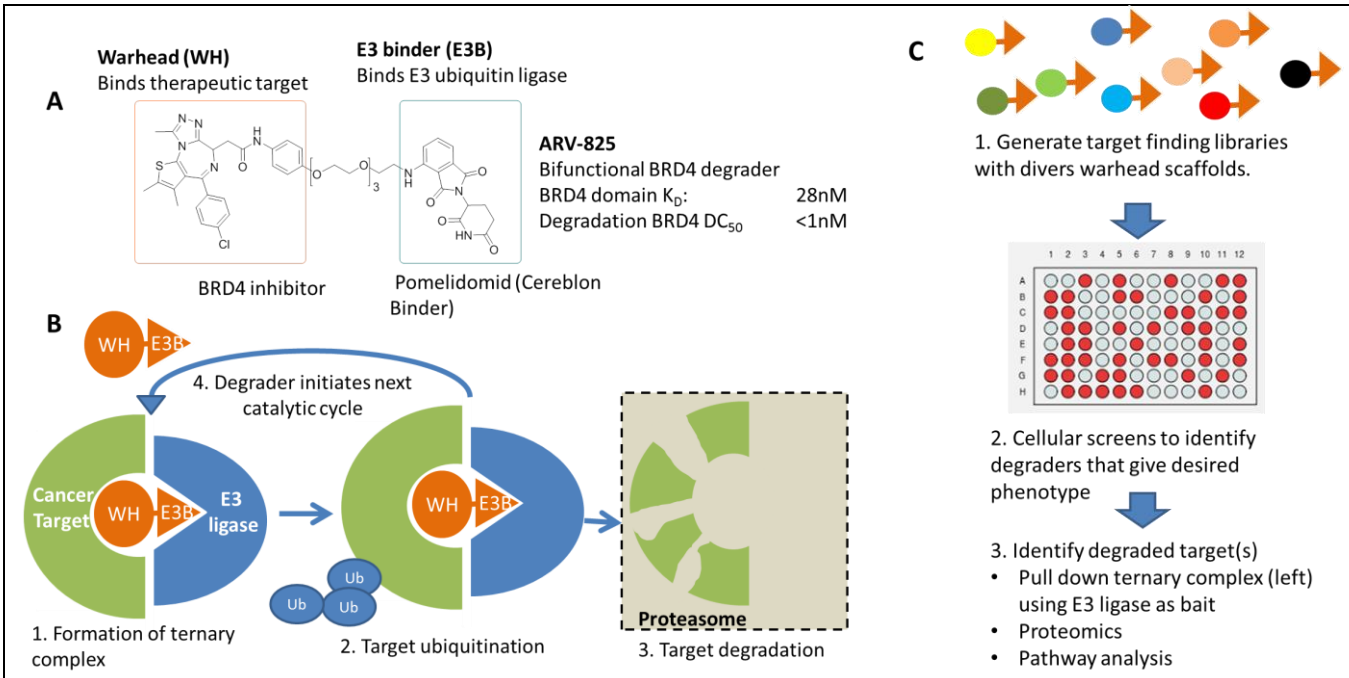

Figure 1: bifunctional degraders, structure (A), catalytic cycle (B) and target finding approach (C)

We will apply this concept to target finding, by designing and synthesising bifunctional degrader libraries, and screening them in cellular assays. Chemoproteomics will be used to identify which targets are degraded (figure 1C). Key advantages of this target identification approach are that identified targets are druggable (are known to bind to small molecules) and hit compounds have the potential to represent cellular probes and starting points for chemistry.

The student will be supported by a supervisory team with both academic and industrial expertise in synthetic and medicinal chemistry, cancer biology and chemoproteomics. The student will primarily be placed in the Medicinal Chemistry 4 team, working with Dr Swen Hoelder and Dr Ben Bellenie within the Division of Cancer Therapeutics at the ICR's Sutton, London research site. The successful candidate will also have the opportunity to spend time in the Target Evaluation and Molecular Therapeutics team, supervised by Dr Olivia Rossanese, and in the proteomics facility, working with Dr Jyoti Choudhary. This project is fully funded for four years.

PROJECT AIMS

Overall aim:

- **Establish a new target identification approach that explores libraries of bifunctional degraders and demonstrate proof of concept.**

Required steps:

1. Discovering and developing appropriate synthetic routes to enable the preparation of bifunctional degraders.
2. Using physicochemical properties and medicinal chemistry expertise to enable the design of degraders which can cross the cell membrane.
3. Synthesis of validation sets of compounds, followed by preparation of a 200-compound library of degraders.
4. Testing of compounds in a range of cellular assays, and development and application of chemoproteomic methods to identify targets.

RESEARCH PROPOSAL

1. Development of synthetic routes to libraries of bifunctional degraders

In the initial phase of the project, the student will apply synthetic routes known in the literature, combined with in house experience to prepare degraders to be used in initial validation studies. An example of a published synthetic route is shown in Figure 2. Development of new or modified synthetic routes will then be undertaken, in order to enable the parallel synthesis of larger sets of compounds. This will enable the successful candidate to build on their existing theoretical and practical knowledge of organic synthesis, and develop skills in parallel synthesis and purification, supported by experienced scientists within the Medicinal Chemistry 4 team and the chemistry department. The department also hosts a series of external speakers, and runs chemistry problem sessions to enable a well-rounded training in organic chemistry.

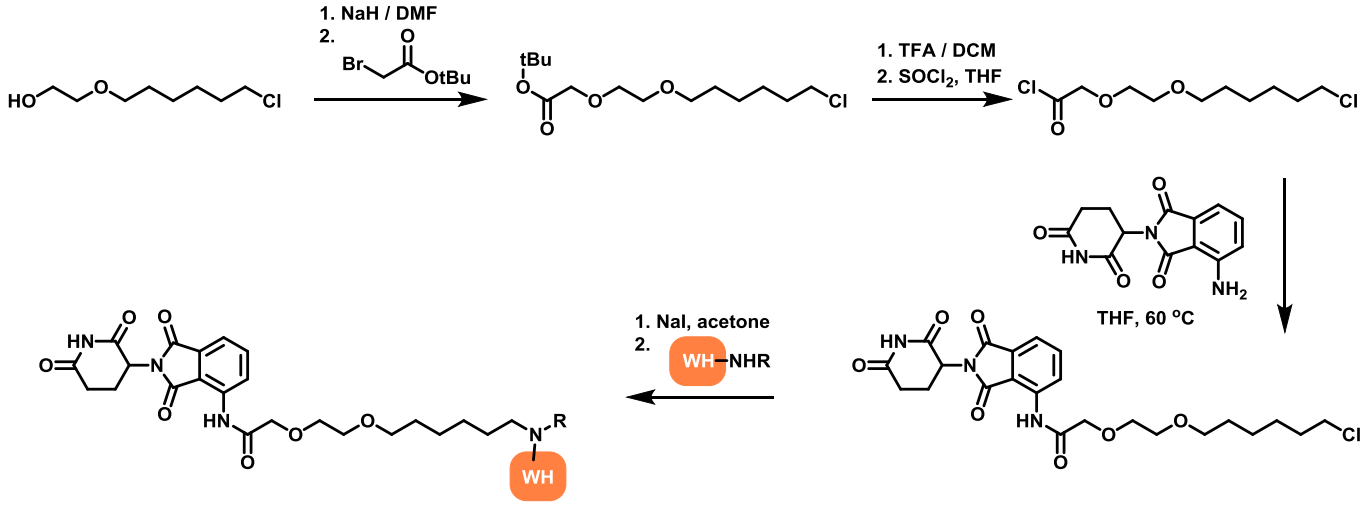

Figure 2: Example of synthetic route for synthesis of bifunctional degraders, based on [3]

2. Application of medicinal chemistry principles to the design of cell-permeable compounds

Compounds must be membrane-permeable in order to show activity in cellular assays. The student will use calculated and measured physicochemical properties and permeability data to build an improved understanding of factors affecting permeability for this class of molecules, and use this to design compounds with appropriate properties. The successful candidate will have the opportunity to develop their knowledge of medicinal chemistry principles by participating in workshops and seminar sessions, and to apply this knowledge in the design of new compounds with improved properties.

3. Design and synthesis of validation sets

In order to show degrading activity, designed compounds must be able to bind to their protein targets. The student will design and synthesise a validation set based on a degraders known from the literature which target kinases. By systematically reducing the size and complexity of these molecules, we will build an understanding of the relationship between size and complexity, warhead binding affinity and ability to degrade the target, and kinase selectivity (see below).

4. Using chemoproteomics to understand selectivity

Whole kinome proteomics using Kinobeads [4] will be established to compare selectivity as observed by kinome proteomics with biochemical selectivity of the warheads. These experiments will provide critical insights into how biochemical selectivity translates into selective degradation, in particular to which extent the design of the linker, E3B and warhead affect selectivity. The student will have the opportunity to work

with Dr. Jyoti Choudhary in the proteomics facility to develop methods and test compounds. Prior experience in this area is not required as full training will be provided.

5. Design, synthesis and testing of compound library

Using methods developed in the first part of the project, and design principles developed through synthesis and testing of validation sets, the student will design and prepare a library of novel compounds. It is anticipated that parallel synthesis will enable preparation of a 200-compound set, and this will be supported by the availability of automated purification and analysis equipment. The student will have the opportunity to work with the Target Evaluation and Molecular Therapeutics team, led by Dr. Olivia Rossanese, to test these compounds in cellular assays. Hit compounds will be further followed up by proteomics to determine their mechanism of action. Further optimisation of hit compounds towards chemical probes may be carried out using medicinal chemistry design principles.

Outcomes

Specific outcomes of this project are a new and validated approach for target discovery, target finding libraries and design criteria for the synthesis of more extensive libraries. The project also has the potential to deliver chemical probes and discover new cancer targets. The novelty of this work and relevance for cancer therapy will enable publications in quality journals.

The student will gain extensive experience of synthetic organic chemistry, develop capabilities in medicinal chemistry, and have the opportunity to expand their skill set into other aspects of cancer drug discovery including phenotypic screening and chemoproteomics.

LITERATURE REFERENCES

1. Ottis, P.; Crews, C. M. (2017) Proteolysis-Targeting Chimeras: Induced Protein Degradation as a Therapeutic Strategy. *ACS Chem. Biol.* 12 (4), 892-898.
2. Lu, J.; Qian, Y.; Altieri, M.; Dong, H.; Wang, J.; Raina, K.; Hines, J.; Winkler, J. D.; Crew, A. P.; Coleman, K.; Crews, C. M. (2015) Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem Biol* 22 (6), 755-63.
3. Lai, A. C.; Toure, M.; Hellerschmied, D.; Salami, J.; Jaime-Figueroa, S.; Ko, E.; Hines, J.; Crews, C. M., Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL (2016) *Angew. Chem. Int. Ed.* 55 (2), 807-810.
4. Daub, H.; Olsen, J. V.; Bairlein, M.; Gnad, F.; Oppermann, F. S.; Körner, R.; Greff, Z.; Kéri, G.; Stemmann, O.; Mann, M. (2008) Kinase-Selective Enrichment Enables Quantitative Phosphoproteomics of the Kinome across the Cell Cycle. *Molecular Cell* 31 (3), 438-448.

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: e.g. BSc or equivalent in specific subject area(s)	MSc or equivalent in chemistry, medicinal chemistry or a related subject
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you	• Modern methods for organic synthesis and purification • Parallel synthesis • Medicinal chemistry

expect the student to have attained on completion of the project.	• Hands on experience in proteomics • Hands on experience in using cellular assays and phenotypic screening