The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

FUNDER DETAILS	
Studentship funded by:	Medical Research Council - Doctoral Training Partnership (MRC DTP)
Funder specific requirements:	All MRC DTP students will attend taught courses one day a week for the first nine months of the PhD. This training will cover computational and thematic science training as well as core and transferable skills. Students will spend the remainder of the four years on their PhD project full time with monthly cohort activities.
PROJECT DETAILS	
Project Title:	**Identifying Small Molecules that Bind to the Cbl-b E3-Ligase**
SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Gary Newton
Associate Supervisor(s):	Dr Matthew Cheeseman Dr Esther Arwert
Secondary Supervisor:	Professor Swen Holder
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Medicinal Chemistry Team 3

SHORT ABSTRACT

The Casitas B-lineage E3 ligases are a family of closely related RING-type, mono-subunit E3 ligases (c-Cbl, Cbl-b, Cbl-3) which play important roles in the regulation of tyrosine kinases. Cbl-b has attracted particular interest as it has been shown to be a key check-point regulator of both the innate and adaptive immune system and mice lacking Cbl-b have demonstrated enhanced T-cell killing and spontaneous rejection of tumours.[1] Small molecules that bind to Cbl-b may therefore act as novel immune checkpoint regulators. Furthermore, as Cbl-b is an E3 ligase they could also serve as the basis for generating novel, alternative PROTACs.

BACKGROUND TO THE PROJECT

The N-terminal region of Cbl-b consists of a tyrosine kinase binding domain (TKB), which binds to specific phospho-tyrosine peptide sequences within target proteins, a flexible linker region, containing a tyrosine residue, whose phosphorylation releases the auto-inhibition of the third component, the Ring domain that binds to the E2-ligase.[2] Phosphotyrosine containing pentamers with specific amino acid sequences can bind with low micromolar affinity to the TKB domain of Cbl-b and related family members.[3] Furthermore, a number of crystal structures have been published showing how Cbl TKB domains bind to specific peptides.[2,4] Using these as starting points we propose to take a petidomimetic approach to generate compounds that bind to Cbl-b with improved affinity and properties, enabling them to be used as cellular probes that could be developed into novel inhibitors and/or PROTACs.

The TKB peptide binding domain contains a number of regions that contribute to the binding affinity. In addition to the phosphotyrosine binding site there is a secondary binding site surrounded by a number of tyrosine and phenylalanine residues. This site would appear to be under-utilised by known peptide binders and therefore opportunities exist to enhance affinity by targeting π-stacking interactions with truncated inhibitors. At the bottom of the phosphotyrosine binding pocket there is a cysteine residue (Cys-289) which could also be targeted utilising the experience within our team working on covalent inhibitors,[5,6] to generate covalent reversible and/or irreversible inhibitors.

Although there is interest in pursuing Cbl-b as an immuno-oncology target, to date, there have been only a limited number of publications describing Cbl-b inhibitors. These have largely been in the form of a series of patents from Nurix.[7] These compounds would appear to target the interaction between Cbl-b and the E2 ligase rather than the substrate binding site as we propose, hence would likely have a different specificity profile and would unlikely to be suitable for generating PROTACs.

PROJECT AIMS

- To identify small molecules that bind to the peptide binding grove of the Cbl-b TKB domain
- Establish assays for compound testing
- Evaluate potential to target Cys-289 to generate covalent binders
- Use peptidomimetic approach to replace phosphotyrosine residue and better target the secondary binding site
- Develop novel Cbl-b cellular probes and/or utilise as the basis for generating novel PROTACs

RESEARCH PROPOSAL

It is envisaged that the research proposal will take place in several phases.

<u>Phase 1</u>

- Synthesis of fluorescence polarisation (FP) probe Flu-pYTPEP-NH2
- Synthesis of literature standards for testing in the assay
- Limited exploration of SAR typically peptides (3-5 amino acids) to expand knowledge and evaluate which positions may tolerate and/or benefit from increased lipophilic interactions

- Set up FP assay using commercially available Cbl-b TKB domain and/or synthesise protein according to literature methods

Phase 2

- Evaluate whether non-hydrolysable phosphotyrosine mimetics are tolerated to increase stability
- Evaluate whether it is possible to increase the binding affinity and at the same time reduce the number of hydrogen bond donors and acceptors to enhance cellular activity by better targeting the secondary binding site with truncated analogues
- Generate improved FP probe

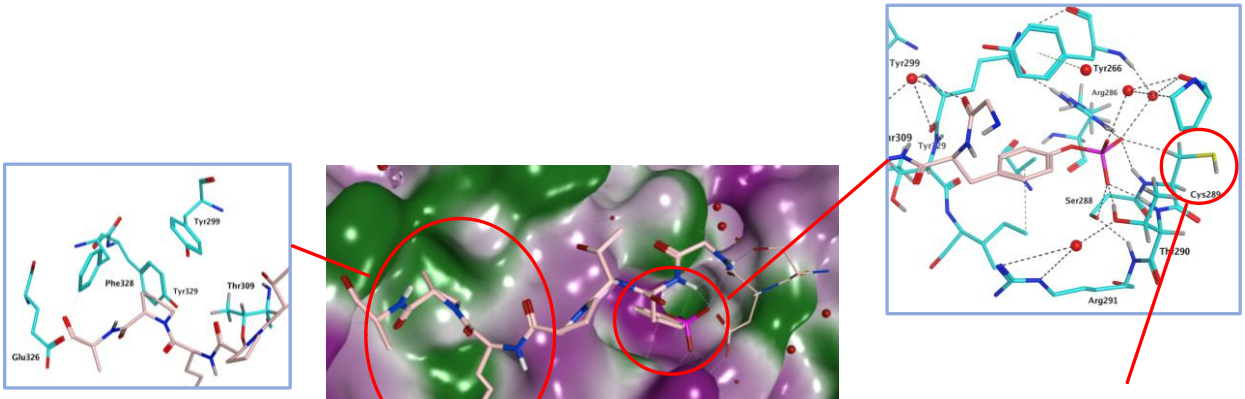
Phase 3

- Evaluate a more extensive set of phosphotyrosine mimetics to improve cellular permeability
- Evaluate potential for covalently binding to Cys-289 using both time dependant FP assay and mass spectrometry with the aim of removing the need for a polar phosphotyrosine mimetic
- Combine different approaches to generate improved compounds

Phase 4

- Depending upon progress the compounds will be evaluated in immune cells to look at the activation of T or NK cells and/or utilised to generate PROTACs
- Cell permeability will additionally be evaluated using in-house assays such as Caco-2; depending upon success in achieving cell permeable ligands prodrug strategies and/or the addition of cellular targeting peptides will be considered

Further details on the proposed approaches are shown in Fig 1 and 2 below



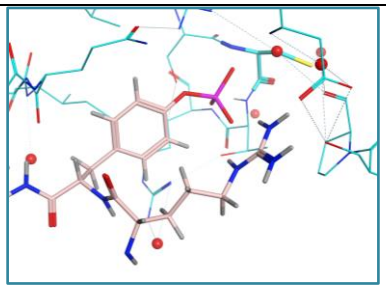
Look to replace to better target secondary binding Site

ZAP-70 phosphopeptide bound to Cbl-b (PDB: 3ZNI)

Cys that could be targeted

Fig. 1: Shows ZAP-70 phosphopeptide bound to Cbl-b highlighting several areas that could be targeted to improve potency and properties

Fig 2. Shows EGFR phosphopeptide bound to Cbl-b TKB domain (PDB: 3PFV). Phosphotyrosine residues could potentially be replaced with phosphotyrosine mimetics including novel macrocycles that mimic pseudo macrocycle made by adjacent Arg or Asn with phosphotyrosine group



Alignment with Research Themes

This project aligns with the Advanced Therapeutics Theme. It will be multi-disciplinary in nature, with interfaces between medicinal chemistry, structural chemistry, biology and structural biology and will be carried out in the multi-disciplinary Cancer Therapeutics Unit. In alignment with the objectives for this theme it will focus on novel immune checkpoint inhibitors and aim to discover novel molecular probes that could be used to explore the biology and/or the further development of novel therapeutics. Furthermore, Cbl-b binders could be used to generate novel degradors using the PROTAC approach and thereby provide alternatives to the commonly used cereblon or VHL approaches.

LITERATURE REFERENCES

[1] Jafari D. *et al.*, E3 ubiquitin ligase Casitas B lineage lymphoma-b and its potential therapeutic implications for immunotherapy, *Clinical and Experimental Immunology*, **204**, 14-31 (2020)

[2] Dou H. *et al.*, Essentiality of a non-RING element in priming donor ubiquitin for catalysis if a monomeric E3, *Nature Structure & Molecular Biology*, **20** (8), 982-982 (2013)

[3] Hu L-B. *et al*., An affinity prediction approach for the ligand of E3 ligase Cbl-b and an insight into substrate binding pattern, *Biorg. Med. Chem*., **38**, 116130 (2021)

[4] Dou H. et al., Structural basis for autoinhibition and phosphorylation dependant activation of c-Cbl, *Nature Structure & Molecular Biology*, **19** (2), 184-193 (2012)

[5] Newton G. *et al*. The discovery of potent, selective and reversible inhibitors of the house dust mite peptidase allergen Der p 1: An innovative approach to the treatment of allergic asthma, *J. Med. Chem*, **94**, 9447-9462

[6] Pettinger *et al*. Kinetic optimisation of lysine-targeting covalent inhibitors of HSP72, *J. Med. Chem.*, **62** 11383-11398 (2019)

[7] WO201948005, WO2020210508, WO2020236654, WO2021021761

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:	MSc or MChem in Chemistry or Medicinal Chemistry, incorporating laboratory-based training in synthetic chemistry

Intended learning outcomes:	• Expertise in synthetic and medicinal chemistry • Structure-based drug design • Expertise in developing covalent inhibitors • Experience in setting up and running assays • Knowledge of protein synthesis
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☐ Biological Sciences ☐ Physics or Engineering ☒ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	1. Medicinal Chemistry
	2. Synthetic Chemistry
	3. Structure Based Drug Design
	4. Assay Development
	5. Immuno-oncology
	6. PhD London