The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Activating and directing post-translational modification by the design and synthesis of heterobifunctional small molecules
Short Project Title:	Small molecule activators of post-translational modification

SUPERVISORY TEAM	
Primary Supervisor(s):	Swen Hoelder
Associate Supervisor(s):	Ben Bellenie Jason Kettle (AstraZeneca) Amine Sadok
Backup Supervisor: (must have IRS status)	Keith Jones Raj Chopra
IRS Partner : (only required where Primary is a CDF, Associate Honorary Faculty or an ICR Fellow – ***see above***)	
Lead contact person for the project:	Ben Bellenie

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Medicinal Chemistry 4
Other Division (if applicable):	
Other Team (if applicable)):	Translational Cancer Discovery

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Summary

In this project, we will design and synthesise heterobifunctional ligands which mediate the post-translational modification of a target protein, such as inducing phosphorylation of a protein kinase. Such ligands would have application as chemical tools to investigate signalling pathways and explore novel biology, and could be optimised to develop potential therapeutics with novel modes of action. This project is in collaboration with AstraZeneca as part of the MRC Industrial Collaborative Awards in Science and Engineering (iCASE) studentship scheme, and is fully funded for four years.

Small molecules which inhibit post-translational modification are valuable tools in understanding biological pathways. In addition, many drug molecules work in this way - for example kinase inhibitors prevent phosphorylation of a substrate, switching off the relevant signal transduction pathways. Small molecules which selectively activate post-translational

modification are no less valuable, but significantly more challenging to design.[1] We propose an alternative approach to enable selective activation of signalling pathways: to design and synthesise heterobifunctional molecules which can hijack and redirect the cell's own methods for activating a target, such as phosphorylation.

Precedent for the feasibility of this approach is provided by recent work on heterobifunctional degraders ("PROTACs", Figure 1a) which have emerged as a new class of chemical tools and potential therapeutics [2]. By bringing together two proteins to form a ternary complex with the ligand, target protein and an E3 ligase, PROTACs enable ubiquitin transfer to the target protein, triggering degradation and hence resulting in an overall loss of function. In a similar manner, our proposed "heterobifunctional activators" would mediate formation of a complex between a kinase and a target protein whose activity depends on phosphorylation. Resulting phosphate transfer would then lead to activation of the target and its downstream signalling pathways (Figure 1b).

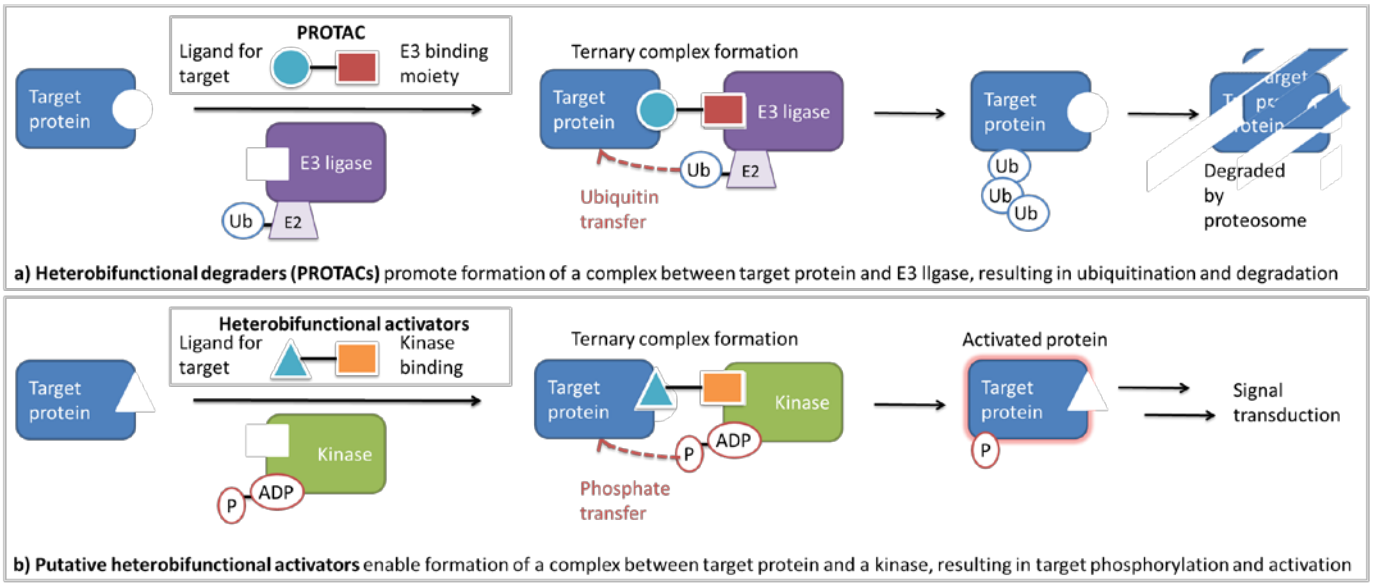

Figure 1: Mechanism of action of heterobifunctional degraders and proposed activators

This approach would:

1. Provide new chemical tools to enable selective switch-on of post-translational modification, enabling investigation of the biological function of components of a signalling pathway.
2. Enable alternative signal transduction pathways by changing the native substrate preference of a kinase, leading to "rewiring" of signalling pathways and hence potentially novel biology.
3. Open up a new method for therapeutic exploitation – the targeted modulation of post-translational modification.

PROJECT AIMS

Overall aim:

- **Demonstrate that heterobifunctional ligands can enable selective post-translational modification of a target protein by bringing together two protein components.**

Required steps:

- **Design and synthesis of heterobifunctional ligands**
- **Test for binding to individual proteins; check cell permeability; refine design as necessary**
- **Check for phosphorylation of target protein**

- **Confirm activation by looking at downstream or phenotypic effects**

RESEARCH PROPOSAL

Demonstrating proof of concept – mediating kinase phosphorylation of a non-native substrate

Phosphorylation by a kinase is a common and important mechanism of signal transduction. The specificity of phosphorylation is mediated by the nature of the residue being phosphorylated and its neighbouring residues, and by recognition of the protein surface by the kinase [3]. It is hypothesised that a heterobifunctional ligand could modify substrate recognition by mediating formation of a complex between a kinase and a neo-substrate (a protein that the kinase does not normally bind to, but that can be activated by phosphorylation). This would enable phosphorylation of the neo-substrate, activating an alternative signalling pathway.

We will therefore design and prepare a set of heterobifunctional ligands aiming to promote this ternary complex formation and hence mediate phosphorylation. The design of the ligand must incorporate a kinase binding component and a substrate binding component, connected by a linker. The design of each of these components is discussed below.

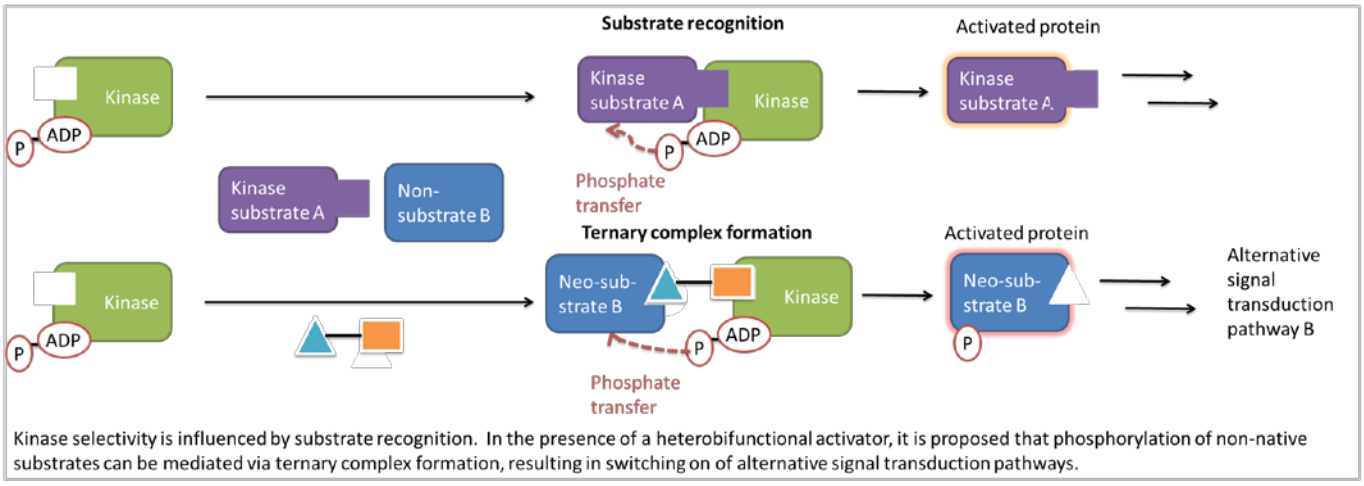

Figure 2: Kinase-targeting heterobifunctional activators are proposed to activate alternative signal transduction pathways

a) Design and testing of kinase binding component

In order to avoid inhibition of the kinase activity, a non-ATP competitive allosteric binder will be required, which must be activating or functionally neutral. It must bind at a site appropriately positioned in space to allow a linker to point towards potential substrates. In the initial part of this project, the student will review allosteric kinase ligands both from the literature and from in-house ICR and AZ experience, in order to select appropriate compounds. Published X-ray co-crystal structures will be used to identify which positions on the molecule are solvent-facing, and hence are suitable for attaching a linker. Compounds with "dummy" linkers will be prepared [Figure 3], and tested to ensure that addition of the linker has not removed the kinase binding affinity. These compounds will also serve as controls in later experiments as described below.

b) Design and testing of substrate binding components

In order to demonstrate proof of concept – that the heterobifunctional ligand can mediate phosphorylation – we will initially focus on well-understood substrate classes in areas of existing in house ICR expertise, where known

ligands are available. Possible targets include HSP90 and tyrosine kinases.

i) HSP90

Tyrosine phosphorylation has been shown to regulate HSP90 - for example, by enhancing its interaction with certain client proteins.[4] Tool compounds which are able to mediate selective phosphorylation would be of value in understanding how this important molecular chaperone is regulated. Multiple classes of HSP90 inhibitors are known, including AUY922, discovered at the ICR and Vernalis, and now in clinical trials. [5]

ii) Tyrosine kinases

Tyrosine kinases are key signal transduction nodes, and the ability to control their activation would be of value in understanding biological pathways and potentially in the development of new therapeutic paradigms. In addition, the existence of a wide range of molecular biological tools, such as phospho-specific antibodies to detect phosphorylation, and a number of known small-molecule binders, makes the targeting of this class by heterobifunctional molecules a viable option.

The student will review and select appropriate small molecule binders, identify appropriate linker positions based on X-ray and known SAR, and prepare test compounds with "dummy" linkers as described previously.

c) Linker design and development of synthetic routes

Work on PROTACs suggests the length of the linker is an important consideration for design, in order to enable formation of a ternary complex [2], and this is likely to be the case here also. Computational design work will be undertaken to determine the minimum linker length required to enable complex formation. Based on in house experience and literature reports, synthetic routes will be developed and optimised to enable parallel synthesis of a set of compounds with different linker lengths. An example synthetic route is shown in Figure 3.

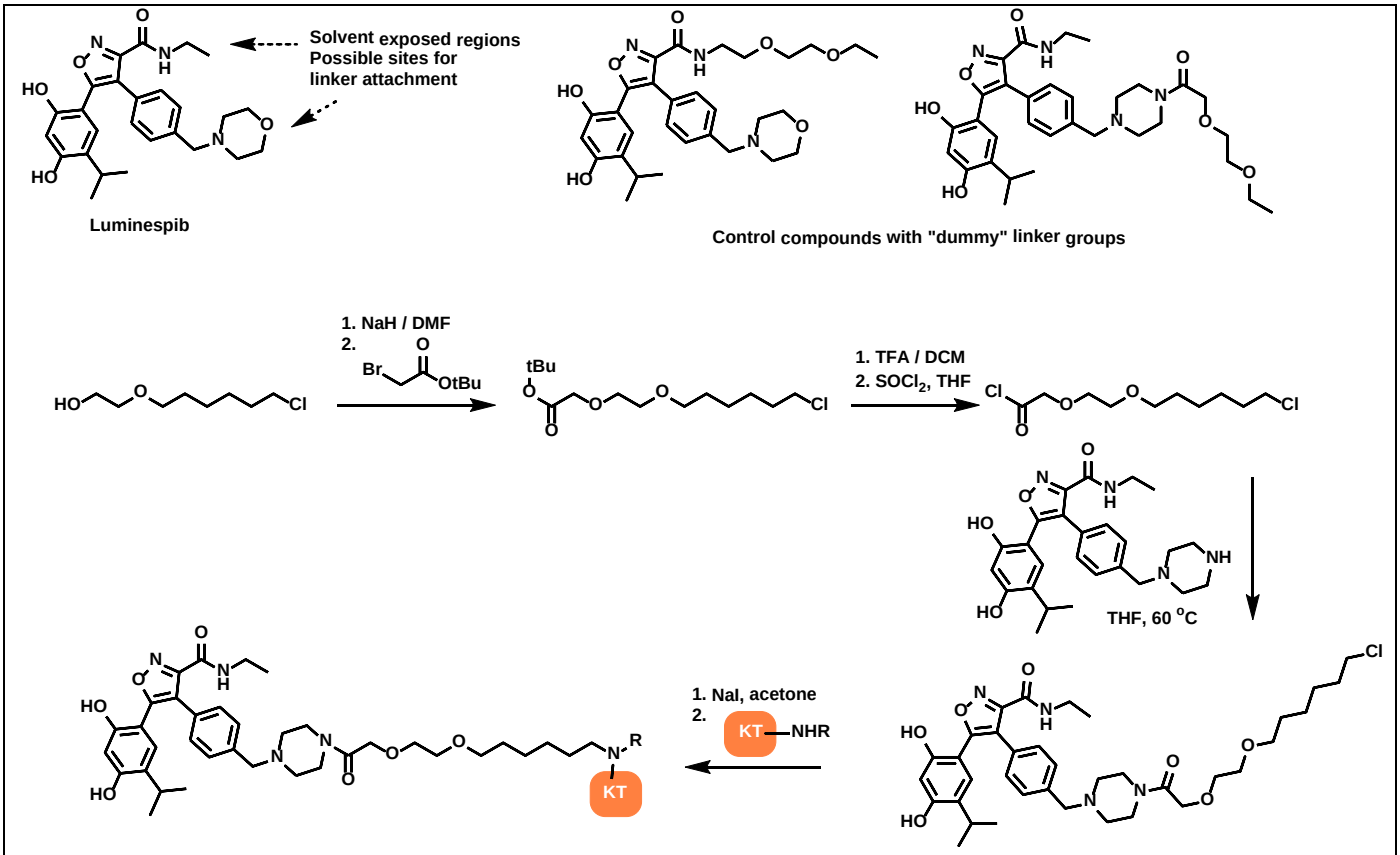

Figure 3: Example synthetic route to proposed kinase targeted (KT) activator of HSP90 phosphorylation

d) 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.

e) Design, synthesis and testing of heterobifunctional activator library

Based on chemical route development, binding validation studies and understanding of physicochemical properties, an initial library of potential heterobifunctional activators will be designed and synthesised incorporating selected substrate and kinase binding groups as described above. The student will work with the Translational Cancer Discovery team at ICR in order to demonstrate initial proof of concept – confirming phosphorylation of the target, for example by Western blotting using phospho-specific antibodies. Results will be compared to control compounds - the monomers containing "dummy" linkers – individually or in combination to confirm that the effect is specific to the heterobifunctional ligands.

Further libraries may be designed in order to optimise the observed effect, or to target other substrates of interest to ICR or AZ.

Outcomes

Demonstration of phosphorylation leading to a downstream effect would provide proof-of-concept for this novel

approach to design small molecules which can mediate targeted post-translational modification. This would not only enable design and synthesis of specific tools for probing basic biology, but also a means for exploration of novel biology resulting from the redirection of existing, or switching on of novel non-natural signalling pathways. The novelty of this work and broad relevance in building understanding of biological pathways, and possible applications as therapeutics will enable publications in high quality journals. Successful proof of concept would also support future grant applications to expand the application of this concept.

Expertise and support

The student will be supported by a supervisory team with both academic and industrial expertise in synthetic and medicinal chemistry, and cancer biology. The student will work in the CRUK Cancer Therapeutics Unit - one of the largest and most successful academic drug discovery groups in the world – at the ICR's Sutton, London campus, and will be supervised by Dr Ben Bellenie and Dr Swen Hoelder within the Medicinal Chemistry 4 team, and by Dr Amine Sadok and Prof Raj Chopra within the Translational Cancer Discovery team. The student will also spend at least three months working alongside industry scientists at AstraZeneca's Cambridge site. The student will gain extensive experience of synthetic organic chemistry, develop capabilities in medicinal chemistry (both practically and by attendance of ICR and AstraZeneca seminars and workshops), and have the opportunity to expand their skill set into related areas, including molecular cell biology.

LITERATURE REFERENCES (Please use the Harvard system of referencing and provide up to 10 key references)

1. Bishop, A.C. and Chen, V.L. (2009) Brought to life: targeted activation of enzyme function with small molecules. *J. Chem. Biol*. 2, 1-9.
2. Ottis, P.; Crews, C. M. (2017) Proteolysis-Targeting Chimeras: Induced Protein Degradation as a Therapeutic Strategy. *ACS Chem. Biol.* 12 (4), 892-898.
3. Kobe, B; Kampmann, T.; Forwood, J.K.; Listwan, P.; Brinkworth, R. I. (2005) Substrate specificity of protein kinases and computational prediction of substrates. *Biochim. Biophys. Acta* 1754 (1-2), 200-209.
4. Mollapour, M and Neckers, L (2012) Post-translational modifications of Hsp90 and their contributions to chaperone regulation. *Biochim. Biophys. Acta* 1823 (3), 648-655
5. Jhaveri, K; Taldone, T; Modi, S and Chiosis, G (2012) Advances in the clinical development of heat shock protein 90 inhibitors in cancers. *Biochim. Biophys. Acta* 1823 (3), 742-755

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 expect the student to have attained on completion of the project.	• Modern methods for organic synthesis and purification • Medicinal chemistry • Parallel synthesis • Build understanding of disease-relevant biology • Opportunity to develop new skills in molecular cell biology.

ADVERTISING DETAILS

Project suitable for a student with a background	☐ Biological Sciences

in: (Please tick all categories that apply – your project will be advertised under all selected categories)	☐ Physics or Engineering ☒ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science
Keywords: Please provide 4-6 words/short phrases that potential students may type into search engines (e.g. Google) to search for PhDs similar to yours – e.g. 'cancer predisposition genes', 'physics PhD London' etc.	**1. Organic synthesis**
	2. Medicinal chemistry
	3. Chemical biology
	4. Chemistry PhD
	5.
	6.