ENABLING RESEARCH THROUGH PROVISION OF CHEMICAL TOOLS

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Tocris aim to develop and commercialize innovative new tools to enable researchers in all aspects of the life sciences. Currently, there are over 4000 products in the Tocris catalog spanning chemical probes, photoactivatable 'caged' compounds, tools for chemogenetics, bioactive compound libraries and fluorescent dyes and probes. In recent years, we have started to look for ways to use the skill set of the Tocris chemistry team to further elaborate on initial chemical probes and compounds developed in academia and industry, to generate new chemical tools to answer biological questions. Two case studies in different research areas are presented below, exemplifying the range of projects that Tocris support.

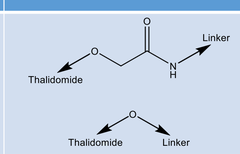
CASE STUDY 1: PROTACs – Building Chemical Libraries to support PROTAC R&D

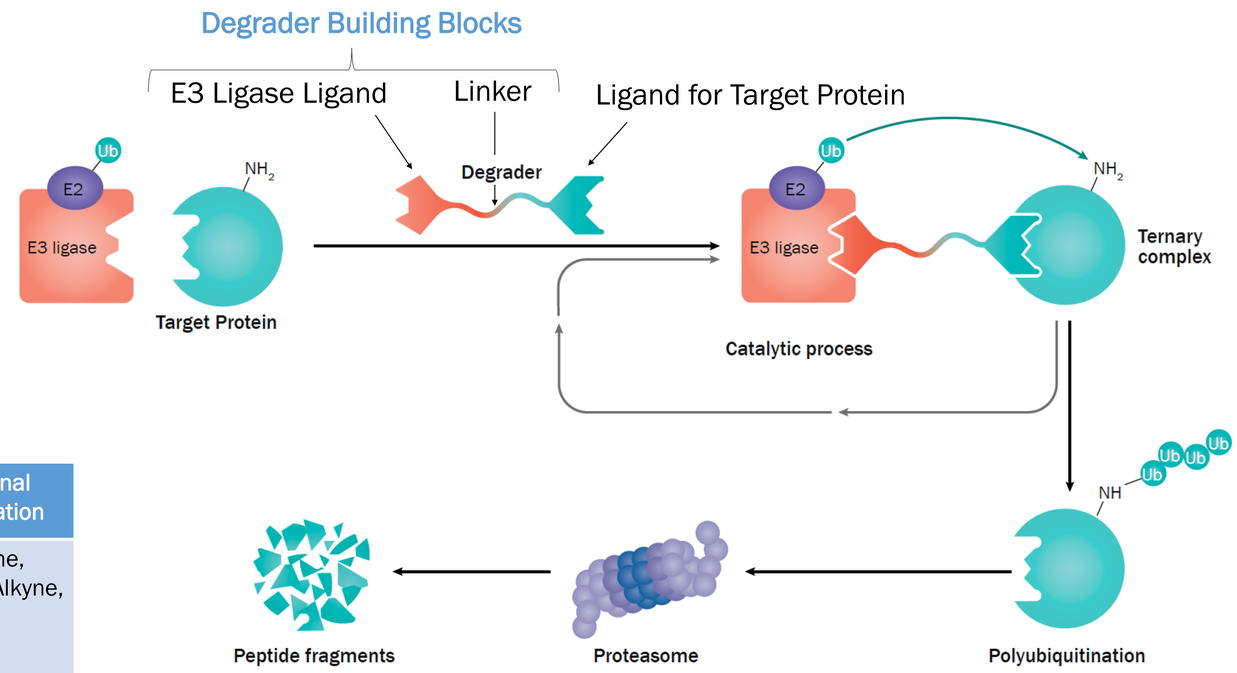
Degraders (e.g. PROTACs™, SNIPERs etc) are bifunctional small molecules that harness the Ubiquitin Proteasome System (UPS) to selectively degrade target proteins within cells. They represent an exciting new modality, repurposing small molecule ligands to achieve selective degradation (knock-down) of target proteins.

Currently, predictions regarding the optimal nature of each component cannot be done *a priori* and empirical effort is required to guide the development process.[1]

DEGRADER BUILDING BLOCKS

We are building a library of Degrader Building Blocks to support new researchers entering this field. The Building Blocks consist of different E3 Ligase Ligands and Linkers, ready to conjugate to a chosen Target Ligand.

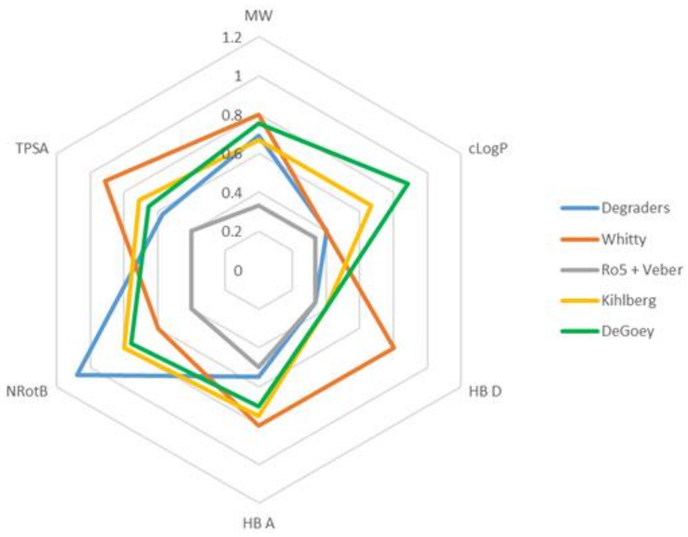
E3 Ligase	E3 Ligase Ligand	E3 Ligase Exit Vector	Linker Type	Linker Length	Chemical functional group for conjugation
Examples: VHL, CRBN, cIAP/XIAP, MDM2	E.g. for CRBN: Thalidomide, Lenalidomide, Pomalidomide		PEG / Alkyl	Number of repeats of Linker monomer	e.g. Primary amine, Carboxylic acid, Alkyne, Azide



The Degrader Building Blocks range now contains ~50 combinations that are enabling researchers' early stage Degrader discovery projects. To further aid researchers entering this field, we are building a set of medicinal chemistry principles to help guide the design of new Degraders in this new area of chemical space:

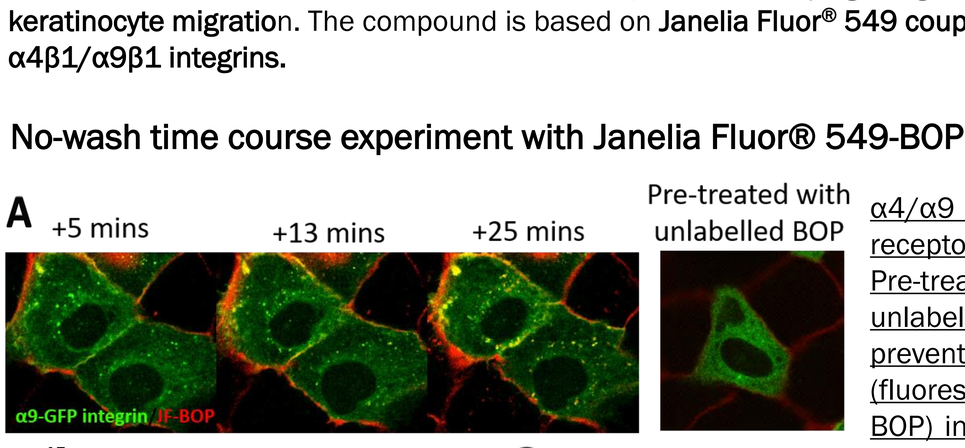
Beyond the Rule of 5 – Degrader Chemical Space

Degraders occupy a new area of beyond rule of 5 (bRo5) chemical space. To understand the properties required for a cell permeable Degrader and inform further development, we have compiled a database of >400 Degrader structures from the peer reviewed literature. All Degraders were classified according to their effectiveness ('Degrader Score' – a single measure of efficacy taking into account DC_{50}, D_{max} and Degrader incubation time) and physicochemical properties.



Degraders occupy a distinct area of bRo5 chemical space (Figure 1). Properties such as hydrogen bond donor/acceptor count and TPSA are noticeably reduced compared to other bRo5 compound classes with similar MW.

Figure 1. Radar diagram illustrating outer limits of chemical space occupied by compound classes from different published analyses: "Ro5 and Veber"[2, 3]; orally absorbed drugs and clinical candidates with MW >500Da, "Kihlberg"[4]; orally available preclinical compounds breaking >1 of Lipinski's rules in Abbvie's preclinical DMPK database, "DeGoey"[5] orally available macrocyclic drugs, "Whitty"[6], and Degrader molecules, "Degraders", this study.



To probe any correlation between Degrader Score and physicochemical properties, data was subject to PCA. The correlation loadings for PC1 versus PC2 are shown in Figure 2 (left). For both predominant classes of Degraders (CRBN- and VHL-based), increased Degrader Score is associated with increasing cLogP and decreasing TPSA and HBD count.

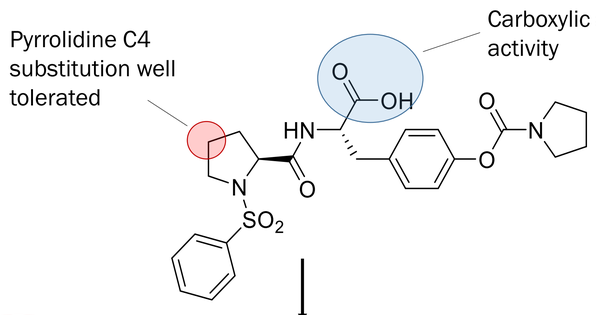
Figure 2. PCA analysis of VHL-based Degraders (A) and CRBN-based Degraders (B). Each variable is shown as a vector. Angles between vectors indicate their degree of correlation. Positively correlated variables are grouped together (≈ 0° angle). Negatively correlated ones are positioned on opposite sides of the plot origin (≈ 180° degrees).

CASE STUDY 2: Optical Tools for Integrin Biology

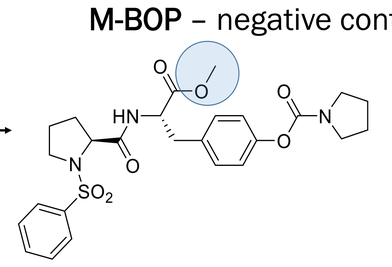
The α4β1 and α9β1 receptors share some common ECM ligands, and have been shown to promote both cell migration in a number of cell types *in vitro* and wound healing *in vivo*. However, their relative contribution in maintaining epidermal homeostasis and potential contributions to pathological processes in the skin remain unclear. To further elucidate the role of these integrins in epithelial cell migration, we are developing chemical tools to probe their function.

We report the development of a **new fluorescent probe for studying integrin-dependent control of keratinocyte migration**. The compound is based on **Janelia Fluor® 549 coupled to a dual inhibitor of α4β1/α9β1 integrins.**

BOP – Tool compound - Dual α4β1/α9β1 integrin inhibitor

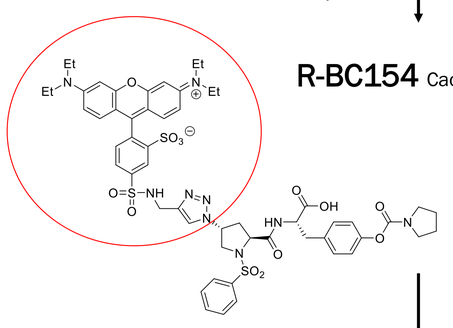


M-BOP – negative control



Addition of a single methyl group is sufficient to 'cage' activity of BOP. Further work is underway to add a photocaging group at this position to enable 2-photon uncaging of BOP.

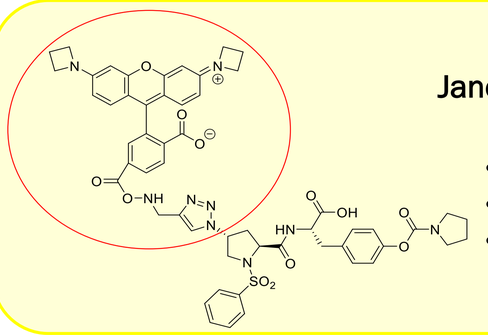
R-BC154 Cao, B., et *al. Org. Biomol. Chem.* **12**, 965 (2014)



Published fluorescent version of BOP

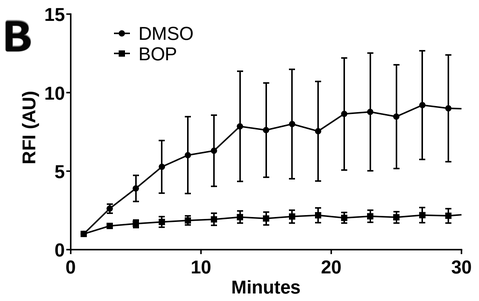
- Wash out required due to background signal
- Photobleaching problematic for time-course experiments

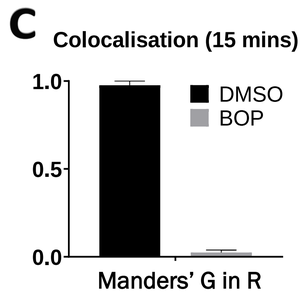
Janelia Fluor® 549-BOP



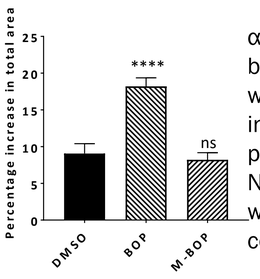
- Much brighter than R-BC154
- Photostable
- Does not require wash out

No-wash time course experiment with Janelia Fluor® 549-BOP





α4/α9 inhibition drives receptor internalisation. Pre-treating colonies with unlabelled BOP for 1H prevents JF-BOP (fluorescently labelled BOP) internalisation. N=1 +/- SEM A. Example images. B. Internal red (JF-BOP) internal signal over time. C. Co-localisation of internal integrin (green) and JF-BOP (red) after 15 mins JF-BOP treatment.



α4β1/α9β1 blockade with BOP increases protusions. No effect with M-BOP control.

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1. Fisher, S. L. *et al. Curr. Opin. Chem. Biol.* **44**, 47-55 (2018).
2.Lipinski, C. A., *Adv. Drug. Del. Rev.* **23**, 3-25 (1997)
3. Veber, D. F. Y., *J. Med. Chem.* **45**, 2615-2623 (2002)
4. Doak, B. C., *Chem. & Biol.* **21**, 1115-1142 (2014)
5. DeGoey, D. A. *et al. J. Med. Chem.* **61**, 2636-2651 (2017)
6. Villar, E. A. *et al. Nat. Chem. Biol.* **10**, 723-731 (2014)

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