# Charles River Laboratories Degrader Discovery Toolbox: Facilitating the Rapid and Efficient Development of PROTACs®

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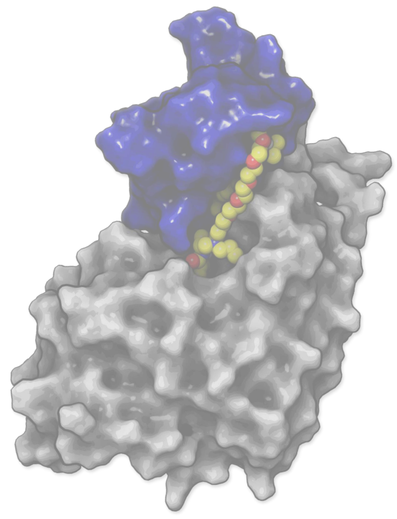
## 1 Introduction

Proteolysis targeting chimeras (PROTACs®) are heterobifunctional molecules that exploit the body's endogenous ubiquitin proteasome system (UPS) to induce targeted protein degradation. PROTACs® consist of two ligands, which bind to the target protein of interest (POI) and an E3 ubiquitin ligase, connected by a linker. The formation of a productive POI-PROTAC®-E3 ligase ternary complex (TC) facilitates the (poly)ubiquitination of the target protein, marking it for degradation by the 26S proteasome.

The length and composition of the linker has a significant impact on the physicochemical properties and bioactivity of the PROTAC®. Nevertheless, linker optimisation can be a time-consuming, iterative process that must be carried out for each ligand pair.[1] A recent statistical analysis of 422 reported degraders highlighted that 64% of PROTACs® employed simple (poly)ethylene glycol (PEG) or alkyl linkers.[2]

We set out to design a degrader toolbox, comprising of linkers attached to E3 ligase ligands, that would enable us to shorten the time required to achieve the following goals in partnership with our collaborators:

- Establish target degradability.
- Investigate how linker affects ternary complex formation.
- Provide a set of degraders covering a range of physicochemical property space.



## 2 Linkerology

Optimisation of physicochemical parameters is essential to develop successful degraders, and the linker has a significant impact on these properties.

- Formation of intramolecular hydrogen bonds near cell membranes has been identified as an important factor in degrader permeability. This 'Chameleonicity' is influenced directly by the length, flexibility, and heteroatomic composition of the linker.[3]
- Compound acidity/basicity has a significant impact on physicochemical properties. Simple changes within the linker, for example changing an amide attachment vector to an amine, or switching a piperazine for a piperidine ring, can have a significant impact on PROTAC solubility and permeability.

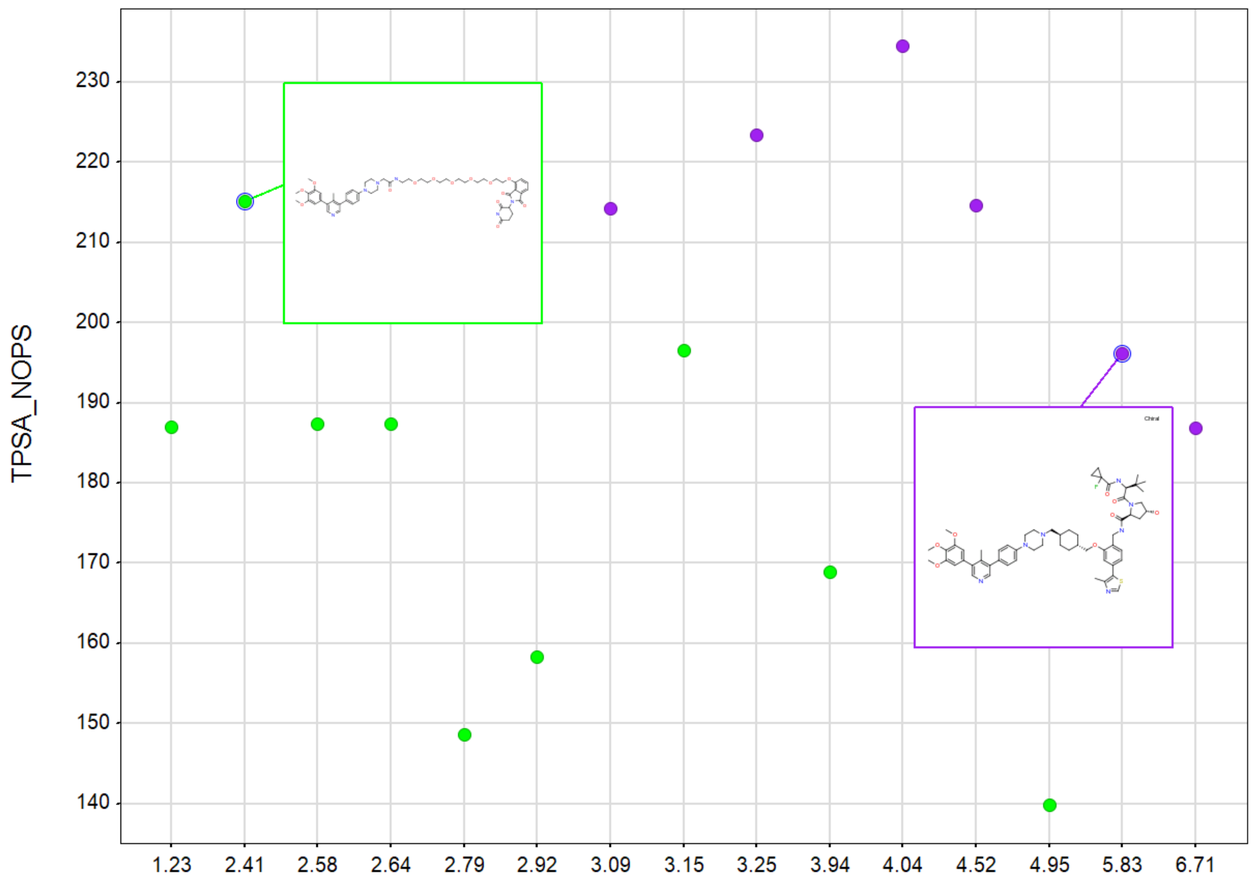
## 3 Methodology

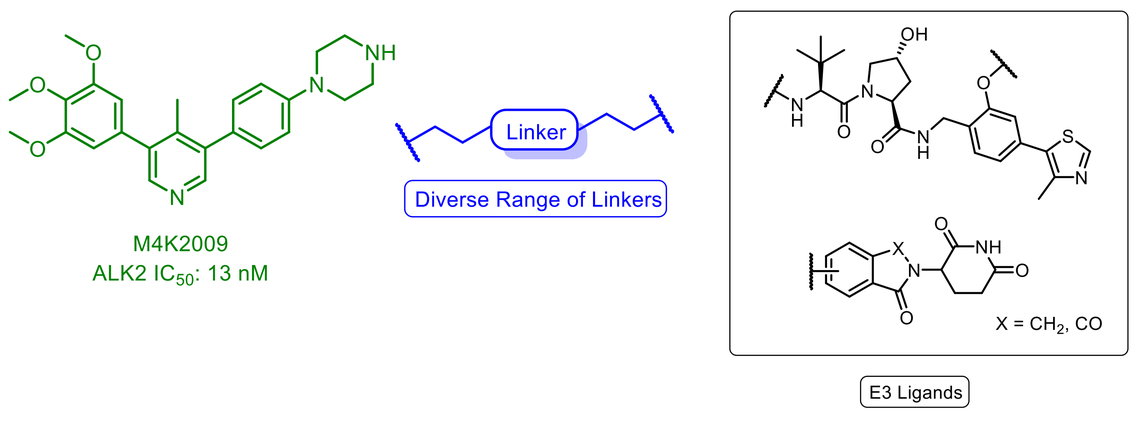
To achieve our objectives, a degrader toolbox of late-stage, functionalised building blocks was assembled.

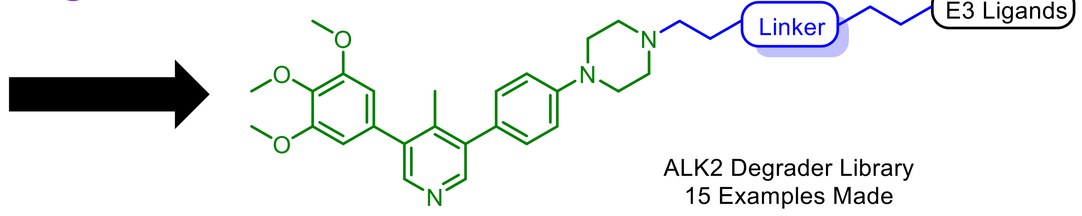
- A selection of E3 ligase binders were conjugated to a diverse range of linkers.
- A variety of linker attachment points were employed, requiring minimal alteration to attach a POI ligand.
- Linker design was inspired by reported orally bioavailable degraders to improve the likelihood of achieving good permeability and solubility.

## 4 ALK2 Degrader Case Study

- Activin Receptor-like Kinase 2 (**ALK2**) is a protein implicated in conditions including Fibrodysplasia Ossificans Progresiva (**FOP**) disorder and Diffuse Intrinsic Pontine Glioma (**DIPG**).
- **M4K2009** is a potent and selective **ALK2** inhibitor developed as a potential therapy for DIPG.[4,5]
- **ALK2** degradation was explored as a complementary approach to inhibition.
- The CRL Degrader Discovery Toolbox was developed to contain a variety of highly functionalised intermediates (E3 ligands + Linkers), enabling the rapid and efficient synthesis of degraders from a POI ligand.
- We used intermediates from the toolbox to synthesise a set of 15 **ALK2** degraders, which were used to establish target degradability.
- The degraders were analysed *in silico*, to demonstrate the range of chemical space accessible from a single ligand, using our toolbox.




**Target Engaged → Target Degraded → ADME Investigated → Further Optimisation Planned**

## 5 Conclusion

- The CRL Degrader Discovery Toolbox is a powerful instrument for the rapid and efficient synthesis of degrader sets to establish target degradability.
- A continually evolving linker library covering a variety of chemical space has been established.
- This approach operates in synergy with our other drug discovery offerings to enable efficient investigation of protein degradation as an alternative modality to small molecule inhibition.

## 6 References

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