Design and synthesis of fluorescent coumarin based Click-Formed PROteolysis Targeting Chimeras for Bromodomain-containing protein 4

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Abstract

Target protein degradation has emerged as a powerful strategy for developing research tools and therapeutics. PROTACs' unique mode of action offers advantages over small-molecule inhibitors, but it also imposes significant challenges in their development. PROTACs comprise three components: a ligand for the protein of interest (POI), an E3 ligase ligand, and a linker that each require careful optimization. This is a time and resource consuming process that often follows an empirical methodology. Gaining a deeper understanding of PROTACs' mode of action in live cells would provide better guidance for their rational design. To enable live-cell monitoring of degradation, we explored the incorporation of a fluorescent tag into PROTACs. Previous studies have shown that protein degradation can be tracked in vitro, when a fluorescent ligand for the target protein (POI) is used as recruiting ligand in a PROTAC. However, this design limits the ability to optimise either fluorescence or degradation activity, as structural modifications to the POI-recruiting ligand may affect one property at the expense of the other. To overcome these issues, we designed fluorescent Click-Formed PROteolysis TArgeting Chimeras (CLIPTACs) featuring the fluorophore embedded within the linker. This strategy offers greater flexibility in fluorophore selection, as the fluorophore is introduced as an independent moiety separate from the E3 ligase and POI binders. It also helps minimise the overall increase in molecular weight of the PROTAC molecule, preserving its efficiency and drug-like properties. The modular architecture of CLIPTACs enabled the incorporation of a coumarin fluorophore directly within the linker region. To maintain synthetic accessibility and facilitate further derivatization, the linker– fluorophore unit incorporates both azide and alkyne functionalities. These groups are commonly used in turn-on fluorophores and compatible with click chemistry, enabling the construction of fluorescent CLIPTACs capable of in situ activation. While in vitro studies are underway, preliminary results suggest that this strategy is a viable route to developing active fluorescent degraders.

Biography

Marisa Santibanez Moran completed her Bachelor of Science degree in Biological and Pharmaceutical Chemistry at the National Autonomous University of Mexico in 2020. She conducted her research project under the supervision of Dr Jose Luis Medina-Franco working on chemoinformatic tools for Data Mining, with an emphasis on Drug Discovery. Later, in 2021, Marisa completed her Master of Science in Drug Chemistry at Newcastle University, UK, where she received the prize for best overall student. Her MSc. research project with Dr Kate Madden and Dr Agnieszka Bronowska focused on the design and synthesis of anti-inflammatory drugs targeting the complement component C5. She is currently in the final year of her PhD in Dr Manuela Jörg's group at the Monash Institute of Pharmaceutical Sciences. Her research focuses on the synthesis and evaluation of multifunctional PROTACs and CLIPTACs as chemical probes.