CeTPD Journal Club

Targeted protein degradation, medicinal chemistry, chemical structural biology & cell biology

Journal Club

October 2023

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors

This month's editors are (from left to right): Mark McNeil, Mokhitli Morake and Valentina Spiteri

"The JC is a great way to see PROTAC development and research being applied to a wide range of different fields and interests. As the majority is heavily chemistry focussed, it's great to add papers specifically looking at cell biology, using PROTAC's as a tool for research."

Mark obtained his BSc in Immunology at the University of Edinburgh, before completing his PhD at the University of St Andrews. Mark studied the interactions of the immune system and viruses, specifically myeloid cells and human cytomegalovirus. Mark joined the Almirall team in May 2023, with a keen interest in academic/industry blends.

"JC offers an opportunity to keep up with recent developments in the ever-evolving Targeted Protein Degradation field. It is also a great reference for a medicinal chemist working in the field as it highlights the most recent trends in PROTACs synthesis and physicochem optimization strategies."

Mokhitli obtained his PhD from the University of Cape Town working on organic synthesis and medicinal chemistry project towards identification of antimalarial leads. He joined CeTPD in January 2023 as part of the ACBI medicinal chemistry team.

"Journal Club is an oppurtnity of researchers to highlight relevant literature month-to-month in an easy to read format. I have been a keen reader of the Journal Club even before I joined the group and I now relish the opportunity to contribute to this resource."

Valentina completed her undergraduate degree in biochemistry at the University of Surrey followed by a PhD in Molecular and Structural Biology at University college London. In August 2021 she joined the ACBI team as a structural biologist/biophysicist and earlier this year moved to the Eisai collaboration.

Targeted Protein Degradation

Cell Biology

Contributor: Mark

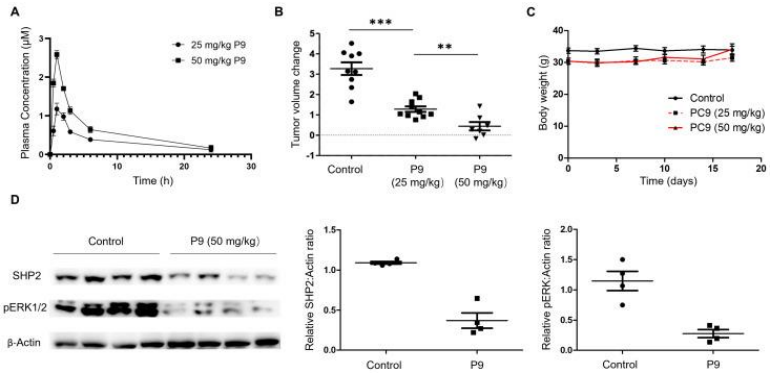
Discovery of a SHP2 degrader with *in vivo* anti-tumor activity.

Jinmin Miao[§], …, Zhong-Yin Zhang*.
Molecules **2023**, *28*, 6947

The authors designed a series of PROTACS targeting SHP2 for use as an anti-tumour drug.

The design and construction of SHP2 PROTAC's is discussed and presented along with the exploration of the E3 ligands and linkers. The efficacy and speed of the PROTAC P9 was shown to be very efficient and a fast degrader of SHP2. P9 was then shown to significantly reduce the growth of tumour cells both *in vivo* and *in vitro*.

This is a great paper telling their story from start to finish from developing a PROTAC to testing it in mice and showing its potency against tumour cells.



Cell Biology

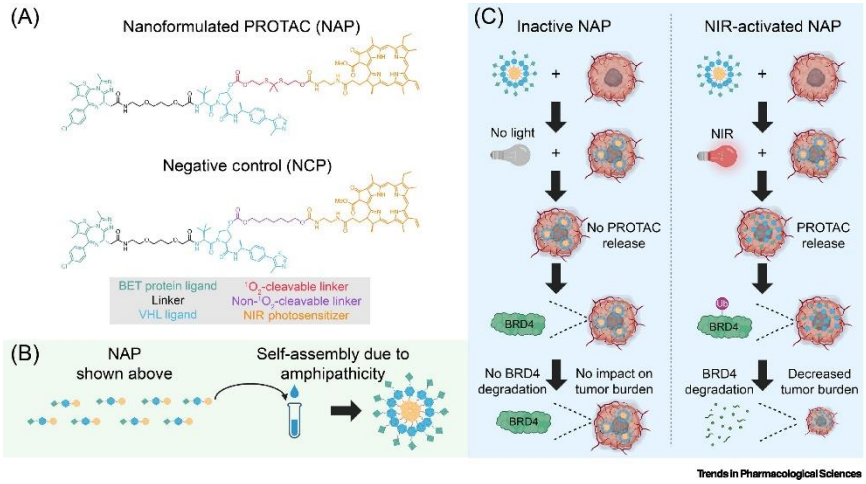
Contributor: Mark

Lighting the way to tumor destruction.

Christina C. Kuismi[§] & Behnam Nabet*
Trends Pharmacol. Sci., **2023**, *44*, 750

A great spotlight article highlighting solutions for tissue toxicity from systematic off-target protein degradation through PROTAC treatment. Novel techniques are highlighted and described, potentially allowing PROTACs to only be activated in isolated tissues.

The use of PROTACs which are conditionally activated by NIR irradiation in deep tissue to induce destruction of target proteins (BRD2/3/4/T) in tumours is discussed. NIR irradiation activated PROTAC action which resulted in reduced tumour burden, but without toxicity.



These advancements could further increase the successful application of PROTACs as cancer therapies by targeting a specific protein, in a specific tissue, reducing side-effects. This principle could potentially be applied to many small molecules.

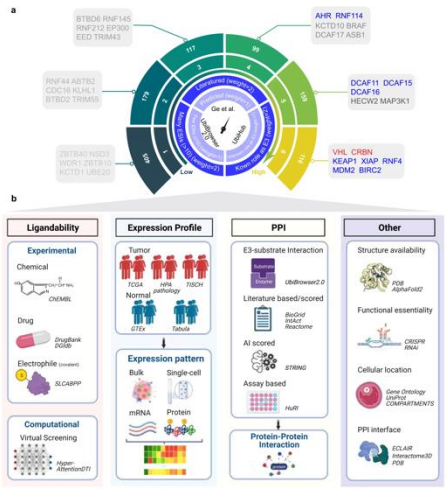
Cell Biology | Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Valentina

Expanding PROTACtable genome universe of E3 ligases

Yuan Liu[§], …, Leng Han*

Nature Communications **2023**, *14:6509*



Less than 2% of the reported human E3 ligases have so far been exploited for TPD. In this work E3 ligases are systematically characterised for several attributes including chemical ligandability, expression patterns, protein-protein interactions (PPIs), structure availability, functional essentiality, cellular localisation, and PPI interface. The study draws on 30 large scale data sets, including ligase lists, ligand sources, expression landscapes, PPI database, structure data bases, essentiality screens, cellular localisation, and PPI interfaces. The authors found that 76 E3 ligases could potential be recruited for TPD. The authors assigned a confidence score 1-6 with 6 being the best, based on how much data was available for each ligase. Ligandability was accessed using databases including ChEMBL, DrugBank DGldb, SLCABPP. They used transcriptomics and proteomics data to map expression landscape and found 363 ligases that are highly expressed in over 30 cancer types, highlighting the pan-cancer potential of these E3 ligases. To understand the expression at the single-cell resolution, five single-cell RNA-seq tumor datasets spanning different cancers and curated in Tumor Immune Single-cell hub, and expression patterns within tumors and identified several novel E3 ligases that were primarily expressed in malignant cells. From the PDB they found that 414 of E3 ligases have experimental structures determined, including 9 E3 ligases that are already co-opted for PROATC development and 405 that are potentially novel.

The authors developed a web portal that can be used by the community to find promising E3 ligases that allows you to view the E3 ligase portal, perform a general E3 ligase search and to search by target. The portal allows user to curate the data they view by turning on different parameters to narrow down a search.

The authors highlight a need for large-scale singe-cell proteomics data and the importance of post-translational modifications and understanding the role they play in regulating the activity and abundance of E3 ligases. This web portal provides an easy-to-use tool to help researchers make decisions and potentially better match E3 ligases to specific targets for specific diseases.

Cell Biology | Chemistry

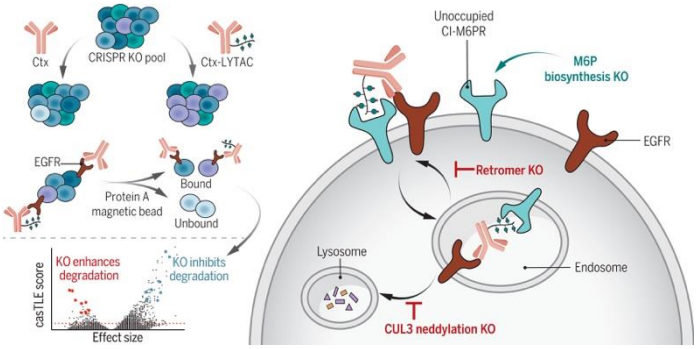
Contributor: Valentina

Elucidating the cellular determinants of targeted membrane protein degradation by lysosome-targeting chimeras

Green Anh[§], …, Steven M. Banik* and Carolyn R. Bertozzi*

Science **2023**, 382

The cellular characteristics that impact the ability to hijack lysosomal trafficking remains elusive. LYTACs widen the scope of TPD to include secreted and plasma membrane targets. LYTACs traffic proteins between the plasma membrane and lysosomes by receptors such as cation-independent mannose-6-phosphate receptor (CI-M6PR).



The authors deploy a genome-wide CRISPR knockout screening using magnetic cell sorting that was complemented by proteomics to identify and characterise components that modulate LYTAC-mediated membrane protein degradation. The study characterises three systems that impact LYTAC efficiency. Firstly, the retromer complexes recycles LYTAC-CI-M6PR complexes from endosomes to the cell surface before dissociation of LYTAC from the receptor. When the retromer complex is knocked out it reduced recycling of LYTAC-target complex enhancing degradation. Secondly, the genes responsible for neddylation of CUL3 were disrupted and the activation of its E3 activity was found to be crucial for the trafficking of LYTAC-target complexes to lysosomes. Finally, that LYTAC activity was enhanced by the disruption of mannose-6-phosphate (M6P) biosynthesis due to an increase in the number of unoccupied receptors. These receptors were found to be occupied by M6P-modified lysosomal glycoproteins.

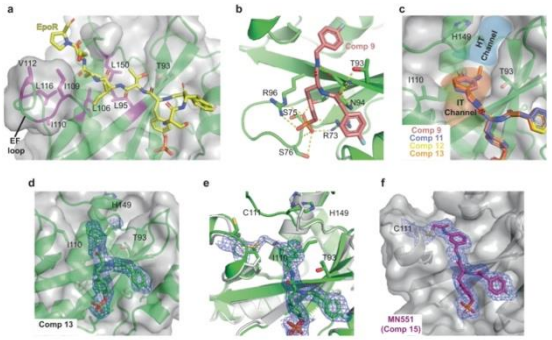
Unpacking the mechanisms that underpin extracellular degraders' mode of action will no doubt help to expedite the development of these degraders and as the authors predict will impact other therapeutic modalities that harness trafficking from the cell surface to the lysosome.

Cell Biology | **Chemistry** | **Modelling/Simulation** | **Structural Biology/Biophysics**

Contributor: Valentina

Structure-based design of a phosphotyrosine-masked covalent ligand targeting the E3 ligase SOCS2

Sarath Ramachanran[§], Nikolai Makukhin[§], …, Alessio Ciulli*
Nature Communications **2023**, *14*



Despite being a promising target drug discovery effort, targeting SH2 domains stalled in the early 2000s due to the poor drug-like of their phosphate-containing natural substrate and mimetics that deamed these domains "undruggable". The team serendipitously discovered that a covalent modification of CYS111 in a co-crystal structure which was then leveraged for rational design of a cysteine-directed electrophilic covalent inhibitor MN551 which was then modified to contain a pivaloyloxymethyl (POM) protecting group, MN714, which made the molecule sufficiently cell permeable as evidenced by the in cell ^{19}F NMR, which also demonstrated that once in the cell the molecule is sufficiently unmasked. Targeting an E3 ligase using a covalent handle is only feasible if the E3 ligase has a sufficient half-life, cycloheximide treatment (to block protein resynthesis in the cell) demonstrates that SOCS2 has a half-life longer than 24 hours.

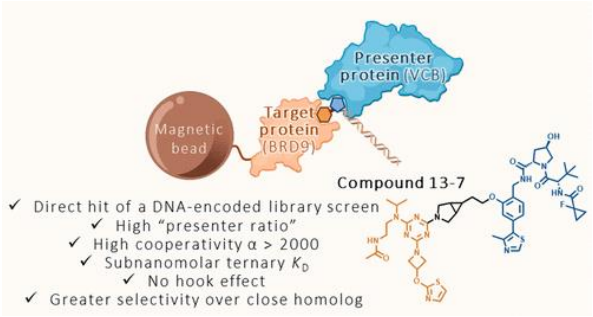
MN551/M714 could be leveraged as tools for understanding the impact of cellular inhibition of SOCS2 and as probes to study JAK/STAT signalling. Additionally, given the over reliance of the TPD field on VHL and Cereblon as E3 ligases of choice for PROTAC development, the discovery of SOCS2 binders opens the door for further innovation in this space.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Ollie

Rational Screening for Cooperativity in Small-Molecule Inducers of Protein–Protein Associations

Shuang Liu[§], …, Stuart L. Schreiber*
JACS, **2023**, 145, 23281



This paper from Liu and colleagues from Stuart Schreiber's lab in collaboration with Novartis, presents a cell-free approach to targeted screening for molecular glues. By employing a DNA-encoded library (DEL) screen, in which the target protein, BRD9, is immobilized on magnetic beads and incubated with the DEL library in the presence or absence of the 'presenter' protein VCB (pVHL-elongin C-elongin B complex). In essence, hits were categorized using the ternary complex enrichment compared to binary enrichment in the absence of VCB, which the authors term the 'presenter ratio'. A higher ratio is shown to be representative of higher cooperativity and thus better gluing between the target and the E3 ligase.

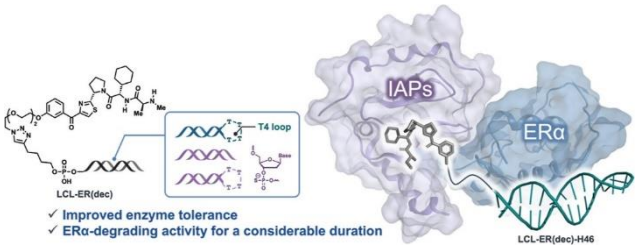
This methodology enables the rapid and high throughput generation of compound libraries that can glue the target and E3 pair of choice. The authors also provide evidence supportive of the consensus that strong positive cooperativity (α) correlates well with proteasomal degradation in cells. Indeed, the more cooperative compounds do not display any hooking. Interestingly, their lead compound **13-7** has low binding to BRD9 alone, but showed the strongest degree of ternary complex formation, highlighting the fact that with molecular glues, weak binary affinity to the target or E3 are not necessarily a cause for concern at the onset of a focussed glue discovery programme or academic project!

Cell Biology | Chemistry

Contributor: Mokhitli

Structural Optimization of Decoy Oligonucleotide-Based PROTAC That Degrades the Estrogen Receptor

Miyako Naganuma[§],…,Yosuke Demizu*
Bioconjugate Chem. **2023**, *34*, 1780



The study follows up on the previous work which successfully developed decoy oligonucleotide-based LCLER(dec) PROTACs which target the estrogen receptor α (ERα). In the previous report, designed PROTACs had potential for intracellular nucleases degradation and the current study addresses this by incorporation of the phosphorothioate group into the oligonucleotides and the T4 hairpin loop at the end of the decoy. These provided stability against the nucleases and identified a stable lead decoy oligonucleotide dec-PROTAC **LCL-ER(dec)-H46** with improved ERα binding affinity.

Key cellular proteins such as transcription factors are difficult to target and there is paucity of ligands available to target them. As such, a novel approach conjugating the decoy oligonucleotides binding ERα to LCL161 warhead recruiting the inhibitor of apoptosis protein [IAP] E3 ligase, herein reported, successfully uncovered PROTACs which induce ERα degradation. A significant highlight is in the use of chemically stable phosphorothioate in the oligonucleotide backbone which also increased permeability for these PROTACs. Although difficult targets such as the ERα could be engaged through this novel PROTAC design approach, clinical success may be limited by the PK issues and requirement for special administration as current methods may lead to instability of the decoy PROTACs. Prospects for successful clinical application will thus depend on developing feasible delivery routes of these PROTACs as authors point out.

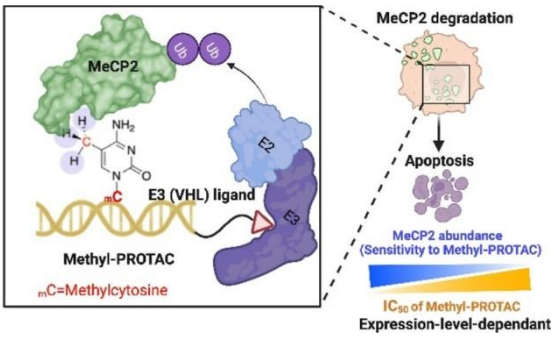
Cell Biology | Chemistry

Contributor: Mokhitli

Methylated Nucleotide-Based Proteolysis-Targeting Chimera Enables Targeted Degradation of Methyl-CpG-Binding Protein 2

Zhen Wang[§], …, Wenyi Wei*

J. Am. Chem. Soc. **2023**, *145*, 21871



The study reports on novel Methylated Nucleotide-Based PROTACs (methyl-PROTACs) which are designed by linking the VHL ligand to an oligodeoxynucleotide methylated at the C5 position of the cytosine that recruits methyl-CpG-binding protein 2 (MeCP2) for degradation. These methyl-PROTACs showed high specificity for MeCP2, selectively targeted cancerous cells over normal cells, triggered apoptosis and have IC_{50} values as high as 18 nM against selected cancer cell lines.

The work expands on the significant role of the DNA-based PROTACs approach to enable the degradation of proteins like MeCP2 which currently do not have specific inhibitors. The C5-cytosine methylation increased specificity of the PROTACs to the target protein highlighting the importance of understanding sites on either the protein or PROTAC that are required for modification in order to yield active compounds. As MeCP2 is also an important target for neurological diseases, the concept of methylated cytosine may be applied in that area to identify active PROTACs. In general, the paper highlights the power of the protein degradation platform that enables cellular depletion of disease-causing proteins previously deemed undruggable.

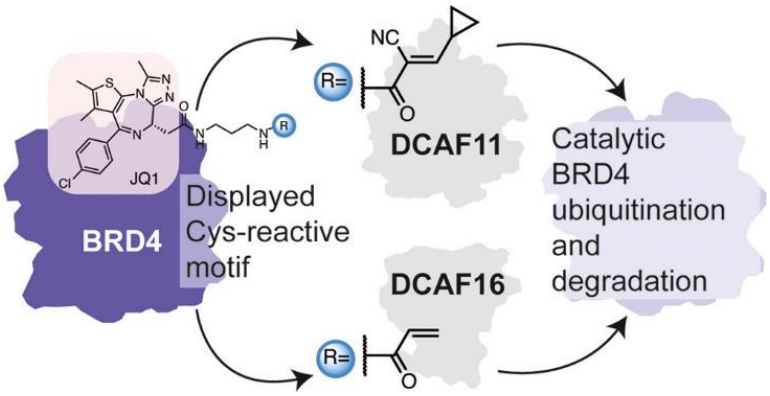
Cell Biology | Chemistry

Contributor: Mokhitli

Chemical Specification of E3 Ubiquitin Ligase Engagement by Cysteine-Reactive Chemistry

Roman C. Sarott[§],…, Nathanael S. Gray*

J. Am. Chem. Soc. **2023**, *145*, 21937



The authors describe a molecular glue approach with specificity for DCAF11 over DCAF16 for degradation of BRD4. This specificity is influenced by the cysteine-reactive motif appended to the JQ1, known BRD4 ligand. This work finds that substitution of the JQ1 derivatives with acrylamide group replacing the tert-butyl group of the parent compound induce BRD4 degradation and highest specificity is established with the cyanoacrylamide compounds. The latter engaged DCAF11 with highest specificity and resulted in highest degradation although there was also DCAF16 engagement albeit with lesser effect.

Attachment of cysteine-reactive warheads to existing protein ligands offers opportunity for a wide variety of E3 ligases to be engaged without the requirement for a specific E3 ligase ligand. This approach depends only on the identification of cysteine residues in E3 ligases that can react with the warhead. The potential of this concept is targeting numerous E3 ligases from slightly modified compounds and thus engaging multiple ubiquitination pathways. This work is the first to identify that DCAF11 attaches to the cynoacrylamide warhead and its thus its relevance for BRD4 degradation. An application of this reactive group to other protein ligands may increase their specificity to the targets, enable multiple

ubiquitin pathways to be engaged and dissuade the need for specific E3 ligase ligands. Thus, cysteine reactive moieties offer great potential to an evolving field of molecular glues degraders.

Other Paper Highlights

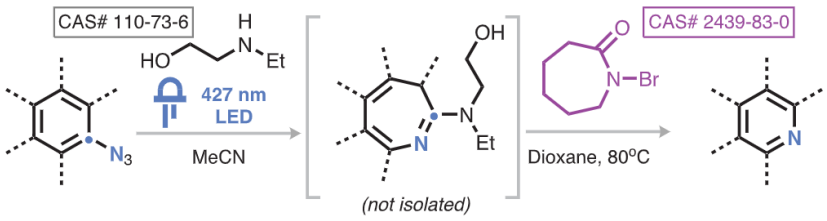
Chemistry

Contributor: Calum

Aromatic nitrogen scanning by *ipso*-selective nitrene internalization

Tyler J. Pearson[§], …, Mark D. Levin*
Science **2023**, *381*, 1474

The concept of "nitrogen scanning" – systematic modification of benzenoid fragments to pyridyl rings – is a commonly utilised concept in drug discovery for the modulation of physicochemical properties (introduction of hydrogen-bond acceptors). However, accessing each pyridine isomer requires a time-consuming parallel, bottom-up synthesis approach.



As part of their programme of skeletal editing, in this work Levin and co-workers report the direct, regioselective conversion of aryl azides to pyridines *via* a carbon-to-nitrogen replacement reaction. The two-step procedure involves (i) the photochemical conversion of aryl azides to 2-amino-3*H*-azepines, followed by (ii) oxidative C2-selective carbon extrusion to afford the pyridine products. Key to the success of this methodology is the use of an amine bearing a pendant alcohol: the generated azepine can subsequently undergo spirocyclisation and concomitant carbene elimination through a reduction of angle strain. The authors demonstrate the applicability of the protocol through the streamlined synthesis of azasteroid from estrone.

Whilst the method is not without limitations (installation of the azide functionality is first required, which can require multiple steps) this exciting protocol enables the site-specific replacement of a carbon atom with nitrogen and will surely prove extremely useful by enabling quick access to pyridyl derivatives of lead compounds.

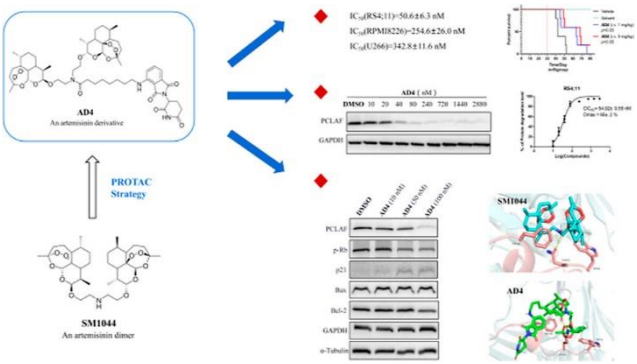
Cell Biology | Chemistry | Computational Chemistry

Contributor: Mokhitli

Facilitated Drug Repurposing with Artemisinin-Derived PROTACs: Unveiling PCLAF as a Therapeutic Target

Yan Li,…, Bin He*
J. Med. Chem. **2023**, *66*, 11335

Artemisinin and its derivatives (artemisinins, ARTs) are important first-line drugs used for the treatment of malaria. The authors of the current work repurpose ARTs for the development of anticancer compounds through targeted protein degradation strategy resulting in ARTs-based PROTACs. They find that these PROTACs indeed possess anticancer properties (IC_{50} = 50.6 nM for a highly active PROTAC named **AD4**) resultant from desired protein degradation profile. They further highlight the target protein of these PROTACs as PCLAF protein in RS4;11 cells.



The drug repurposing strategy applied in this work is advantageous from development perspective in that compounds explored already possess required human PK properties and safety profile. Furthermore, it opens an avenue for the use of natural products such as artemisinin in PROTACs development. While ARTs are effective antimalarials, they have not yet shown desirable anticancer activity. ARTs-based PROTACs reported displayed high anticancer activity and are found to induce PCLAF cellular depletion thus implicating it as the target of ARTs. This is a significant finding in that

the exact target of ARTs is still speculative even against *Plasmodium*. These findings will potentially impact areas beyond oncology and thus spur interest for the application of PROTACs platforms in discovery programs of endemic diseases such as malaria and the use of natural products as ligands.

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