Targeted protein degradation, medicinal chemistry & chemical structural biology literature highlights

Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

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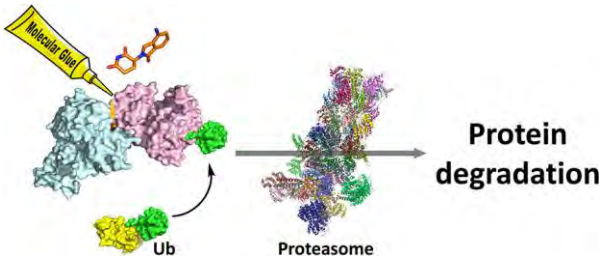
Targeted Protein Degradation

Contributor: Ross

Molecular Glues for Targeted Protein Degradation: From Serendipity to Rational Discovery

Guoqiang Dong[§], Yu Ding[§], Shipeng He[§], Chunquan Sheng*

J. Med. Chem. **2021**. DOI: 10.1021/acs.jmedchem.1c00895



This review highlights the advances of molecular glues in the TPD field. Following a brief introduction of molecular glues with notable examples of the early examples, the authors move to comparing glues vs. PROTACs indicating the promiscuity of these molecules. A few case studies highlight the distinct types of glues in the literature and clinical trials and include IMiDs, aryl sulfonamides and multi-electrophilic site polyketides and explain the mechanism of actions where applicable. Finally, the current strategies for rational discovery of molecular glues are covered. High throughput screening, the use of multicovalent natural products and chemo genetic screening have all been used to discover degraders. Three separate chemo genetic screening strategies were found in the discovery of cyclin K degraders: data mining [(*R*)-CR8]; scalable chemical profiling [dCeMM1/2/3/4]; and chemical genetics [HQ-461]. Machine learning is also proposed to help with the vast data made available from HTS, multiomics and PPI network data.

The authors note that only one compound has been rationally designed and made it to clinical trials, while methods are well established in the PROTAC field.

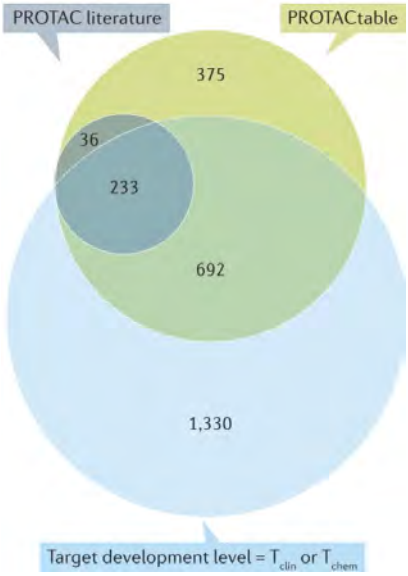
A useful publication on the molecular glue topic. While the benefits of glues are clear (permeability, PK/PD profiles, etc.) there are also challenges e.g. potential off target effects and we are still far from being able to rationally design these types of drug molecules. Undoubtedly molecular glues and PROTACs, albeit fundamentally working the same way, provide highly complementary chemical strategies in degrader drug discovery.

Contributor: Ross

The PROTACtable genome

Melanie Schneider[§], Chris J. Radoux[§], Andrew Hercules[§], …, Andrew R. Leach*

Nat. Rev. Drug Discov. **2021** DOI: 10.1038/s41573-021-00245-x



An interesting overview and explanation of a database that suggests (accompanied by other evidence) if your target is 'PROTACtable'. Building on the 'druggable genome' this publication elegantly describes an approach to determine whether a certain target would be worth putting into a PROTAC campaign.

This first describes how the various literature proteins are 'bucketed' (8 in total; condensed into clinical evidence, literature evidence, discovery opportunity and incomplete evidence) and scored by location in the cell - higher scores for cytoplasm, cytosol, or nucleus. By using their well set out rules, the authors note that there are >1000 targets in the discovery opportunity category and further conclude that 31% of PROTACtable targets lie outside the ITG classification of T_{clin}, T_{chem} and would be of interest for further understanding of the biological roles and disease relevance. When constraining further to targets with drugs or drugs in clinical trials, 199 out of 269 do not have an approved drug, and 145 have neither drug nor compound in clinical trials. Caveats are also included at the end of the publication, indicating an appreciation that the framework is not a finished article.

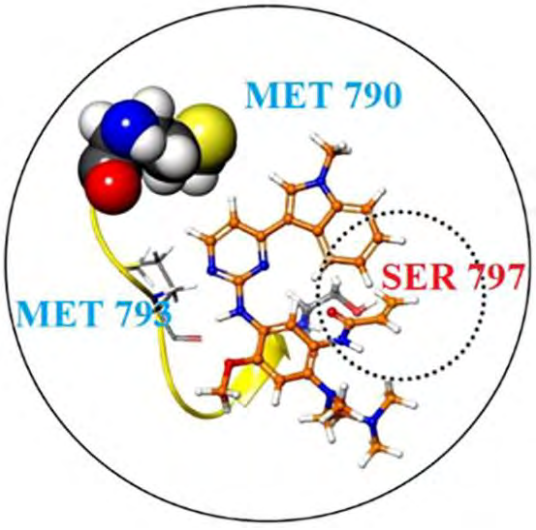
This seems to be a database that will be of huge benefit to the TPD field and will likely have multiple iterations and versions, possibly even separate to the variety of degraders.

Contributor: Ross

Emerging Approaches to Overcome Acquired Drug Resistance Obstacles to Osimertinib in Non-Small-Cell Lung Cancer

Matin Shaikh[§], Yashodeep Shinde[§], Rahul Pawara[§], …, Iqrar Ahmad*, Harun Patel*

J. Med. Chem. **2021** DOI: 10.1021/acs.jmedchem.1c00876



This is a complete and comprehensive overview of Osimertinib resistance in NSCLC, and the options available to pharma. The mechanisms of Osimertinib resistance are analysed in detail, of which 71% of resistance is attributed to EGFR-dependant resistance through C797S (10-26% of patients), L718Q, L792H, G796R/S/D, G724S mutations or T790M loss/reduction/disappearance. Thirteen separate EGFR-independent resistance mechanisms are also described. A multitude of combination therapies are shown to have promise to recover the resistance, though none have been approved by the FDA. PROTAC technology is also discussed, highlighting works by He (410 nM DC_{50}; 68% D_{max}) and Zhang (45 nM [CRBN]; 35 nM [VHL] DC_{50}) who both show potent degradation of EGFR suggesting a definite avenue for addressing the resistance.

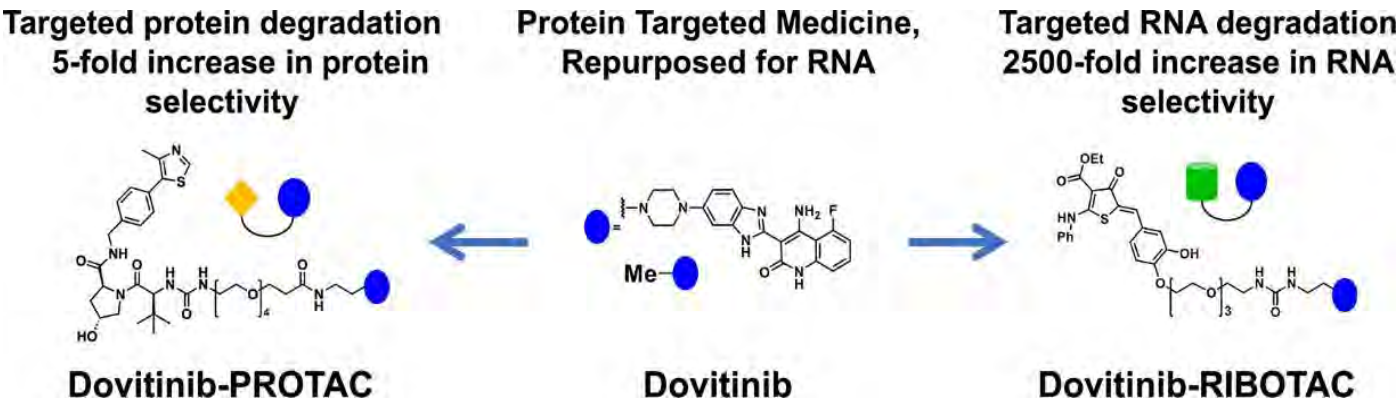
While many options remain open, all requiring further optimisation, PROTACs are again at the forefront of drug discovery options and appear to remain there for the foreseeable future.

Contributor: Jack

Reprogramming of Protein-Targeted Small-Molecule Medicines to RNA by Ribonuclease Recruitment

Peiyuan Zhang[§], Xiaohui Liuhinde[§], …, Mattew. D. Disney*

J. Am. Chem. Soc. **2021** *143*, 13044



The work in this paper aims to repurpose small molecules previously used as inhibitors or in protein degraders to target RNA degradation (RIBOTACs). Reengineering compounds to target different disease pathways, including RNA, would significantly reduce the time and effort required to bring new medicines to the clinic.

The authors identify four drugs, that are currently used to treat multiple conditions, that bind to specific RNA sequences (Dovitinib, Delparantag, Piorocsntrone and Metiazinic acid). Of these four, Dovitinib a tyrosine kinase inhibitor, was shown to bind to the pre-miR-21 sequence with a K_d of 3 μM. Pre-miR-21 has previously been associated with both cancer and kidney disease.

Repurposing Dovitinib, a RIBOTAC and PROTAC were synthesised to target both pre-miR-21 and its kinase target RTK FLT3. The Dovitinib PROTAC was able to successfully degrade RTK FLT3 with low nanomolar activity and the equivalent RIBOTAC reduced pre-miR-21 levels, again with nanomolar activity. It was further demonstrated that the RIBOTAC had over 2500-fold selectivity for its RNA target than the kinase that Dovitinib was originally designed for. With these results the authors demonstrate the RIBOTAC can inhibit breast cancer metastasis in a xenograft mouse model.

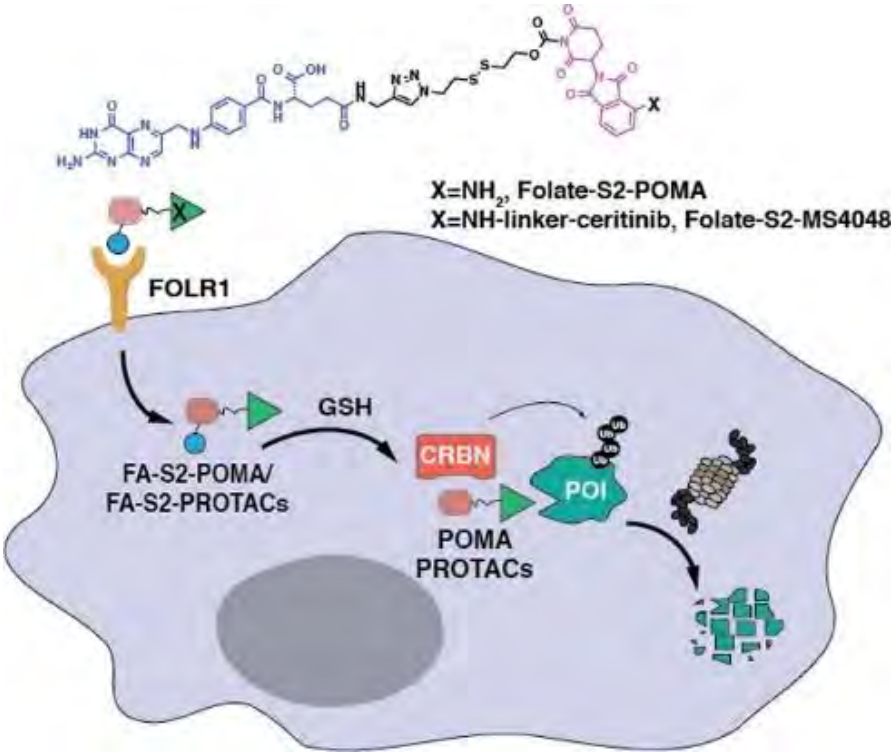
The work in this paper displays how repurposing traditional small molecule inhibitors towards targeted degradation can lead to rapid development of new medicines. Furthermore, RIBOTACs can offer a significant advantage over oligonucleotide therapies due to greater cell permeability and pharmacokinetics.

Contributor: Jack

Folate-Guided Protein Degradation by Immunomodulatory Imide Drug-Based Molecular Glues and Proteolysis Targeting Chimeras

He Chen[§], Jing Liu[§], …, Wenyi Wei*, Jian Jin*

J. Med. Chem. **2021** DOI: 10.1021/acs.jmedchem.1c00901



One of the major drawbacks of PROTAC mediated proteolysis in the treatment of cancer is selectivity for disease over healthy cells. The work in this paper focuses on developing a general strategy for targeting cancer cells through folate receptor *α* (FOLR1). Specifically, immunomodulatory imide drug (IMiD) based molecular glues had not previously been explored for targeted cancer cell delivery.

Using previous work from the group and to test their hypothesis, folate-caged pomalidomide IMiD molecular glue and anaplastic lymphoma kinase PROTAC were both synthesised with reducible disulphide linkers (FA-S2-POMA and FA-S2-MS4048). Utilising disulphide linkers allowed efficient release of degraders into the cytosol of relevant cell lines and both compounds were able to degrade their target proteins in a concentration and time-dependant manner.

Importantly, the work highlights that only cell lines expressing FOLR1 suffer from protein degradation and low FOLR1 expressing cell lines display only negligible changes in protein levels. Furthermore, knockdown of FOLR1 completely abolished both compounds ability to degrade target proteins indicating that FA-S2-MS4048 degrades in a FOLR1 dependant manner.

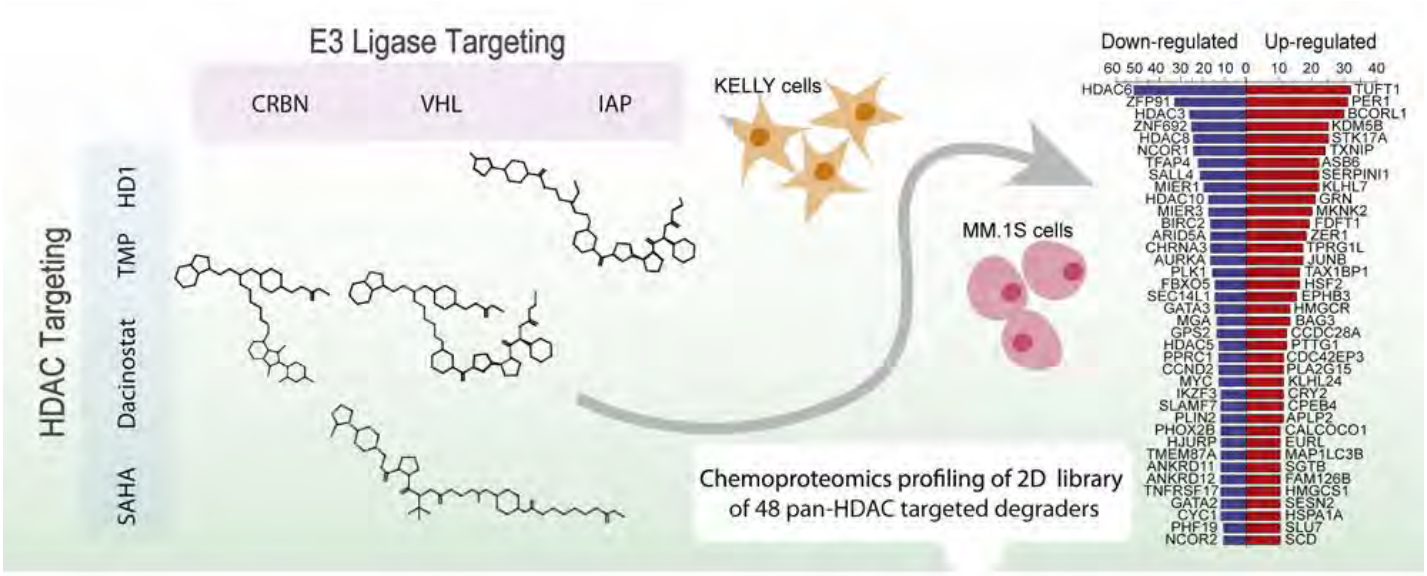
The findings in this paper solidify the folate-based approach for targeting cancer and provides a general platform for further cancer cell targeted therapies using a folate-based strategy. In addition, this is the first reported approach used to target IMiD molecular glues to cancer cells to prevent non-specific cell death.

Contributor: Jack

Chemo-proteomics exploration of HDAC degradability by small molecule degraders

Yuan Xiong [§], Katherine A. Donovan [§], Nicholas A. Eleuter[§], …, Eric. S. Fischer*

Cell. Chem. Biol. **2021**, DOI: 10.1016/j.chembiol.2021.07.002 Click or tap here to enter text.



Abnormal expression of histone deacetylases (HDACs) has been identified to play key roles in cancer cell progression and survival, making them ideal targets for protein degradation technologies. To date many HDAC inhibitors suffer from isoform selectivity and as such this work seeks to screen the accessibility and degradability of HDACs.

A series of 48 multi-HDAC-targeting degraders were synthesised based on cereblon, VHL and IAP E3 ligase binders. Using quantitative proteomics HDAC6, HDAC8 and HDAC3 were identified as the most degradable. Further proteomic analysis identified that HDAC selectivity is attainable, with one compound screened showing complete selectivity for degradation of the HDAC6 isoform over a 16 h time period. Additional investigation highlighted that a celebron based degrader had a preference towards degradation of HDAC6 and HDAC8 whereas the analogous VHL based degraders showed preference towards degradation of HDAC3. These observations further highlight the significant differences in ternary complex formation with different protein isoforms.

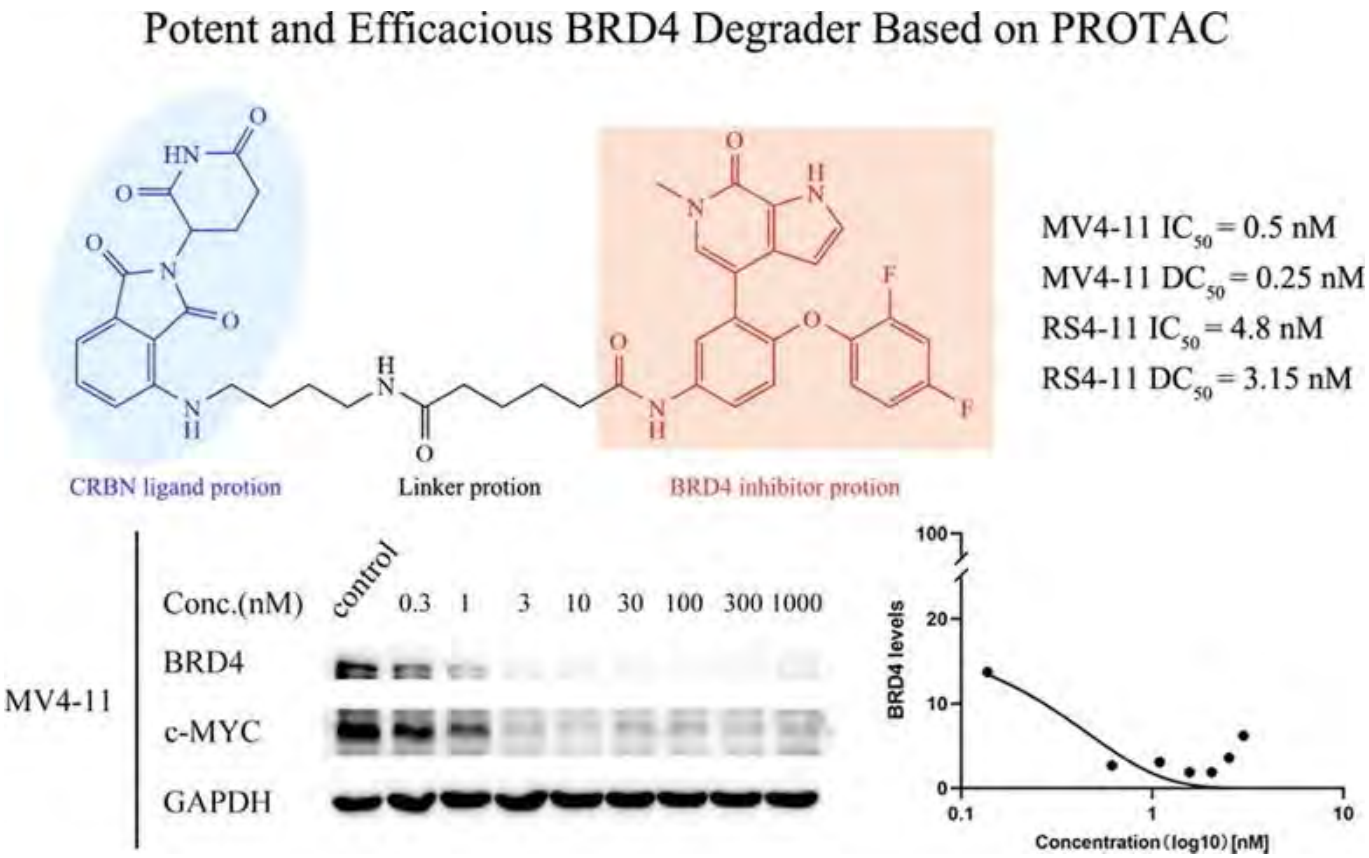
The ability to rationally design new protein degraders is of great importance and the work carried out in this paper significantly advances the SAR profiles of potential HDAC degrading molecules. Finally, the findings from this work appreciably add to the stockpile of information surrounding the biological role of HDAC proteins.

Contributor: Jack

Structure-guided discovery of novel potent and efficacious proteolysis targeting chimera (PROTAC) degrader of BRD4

Wang Xiang [§], Qiwei Wang[§], …, Yaojie Shi *, Luoting Yu*

Biorg. Chem. **2021**, DOI: 10.1016/j.bioorg.2021.105238



Bromodomain-containing protein 4 (BRD4) is an established anti-cancer target for both inhibitors and protein degraders. The authors from this work aim to further develop BRD4 degraders with an improved solubility and bioavailability profile. The poor solubility and bioavailability of the current degraders can be partly attributed to their high molecular weight so in this paper the strategy is to design a novel BRD4 degrader with lower molecular weight. ABBV-075 is a pan-BET inhibitor that has entered phase I clinical trials and thus explored in the low molecular weight PROTAC design.

The use of pomalidomide as a cereblon binding domain was deemed preferential to VHL based systems due to its lower molecular weight and subsequently a series of pomalidomide based cereblon binders were synthesised using ABBV-075 derivatives. The combination of ABBV-075 linked to pomalidomide delivered the most potent compound (**15**) carrying an IC_{50} = 0.5 nM and 4.8 nM in MV4-11 and RS4-11 cell lines respectively, and a reduced molecular weight of 821.8. Degradation experiments in MV4-11 and RS4-11 cell lines identified **15** as a potent protein degrader with DC_{50} = 0.25 nM and 3.13 nM respectively.

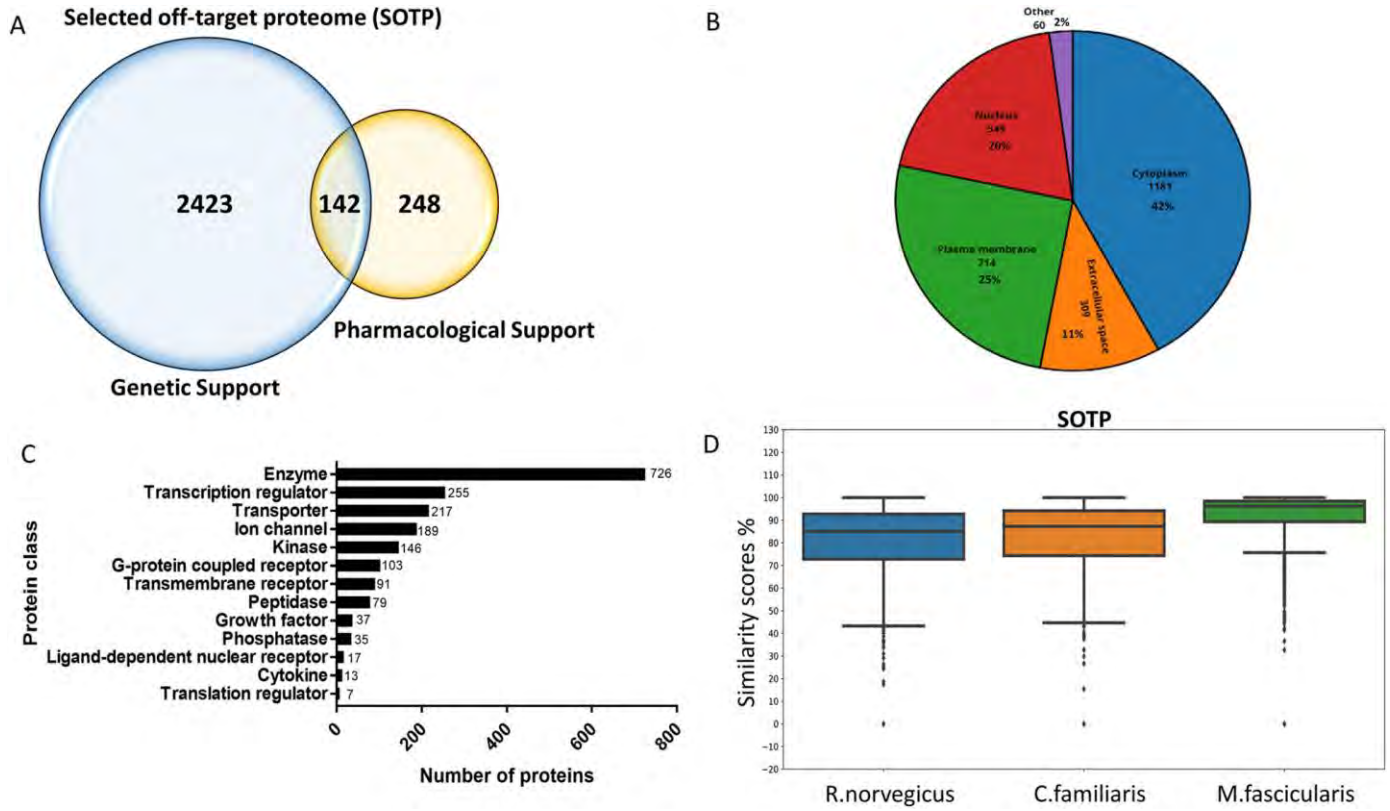
The work presented further broadens the area of BRD4 degradation, although the bioavailability and solubility of **15** is not discussed this could be worthy of further research to assess its pharmacokinetic properties.

Contributor: Zoe

A proteomic platform to identify off-target proteins associated with therapeutic modalities that induce protein degradation or gene silencing

Xin Liu[§], …, Fan Fan*

Sci Rep **2021**, *11*, 15856



The identification of off-target proteins is a vital step in the evaluation of adverse drug reactions during the clinical development of novel drugs. Existing in vitro assays for the assessment of secondary pharmacology effects are only applicable for peptides and small molecules and do not satisfy the consideration of protein abundance changes observed with off-target activities of PROTACs and RNAi therapeutics. In this study, a new proteomics-based platform was developed for the biologically relevant screening of off-target proteins caused by treatments with PROTACs and RNAi drugs.

A total of 2813 proteins involved in major organ systems were chosen as the selected off-target proteome (SOTP), 2423 were identified based on the analysis of genetic evidence, 248 were chosen based on pharmacology evidence whilst 142 were chosen as a result of both. In order to enable good coverage of the SOTP in a manageable number of cell lines, the authors created an iterative algorithm using transcriptomic data from 932 cell lines to identify 4 in which 80 % of the SOTP were collectively expressed. In the final stage, the intracellular and extracellular proteins from the selected cell lines were quantified by mass spectrometry which revealed 1828 were part of the SOTP.

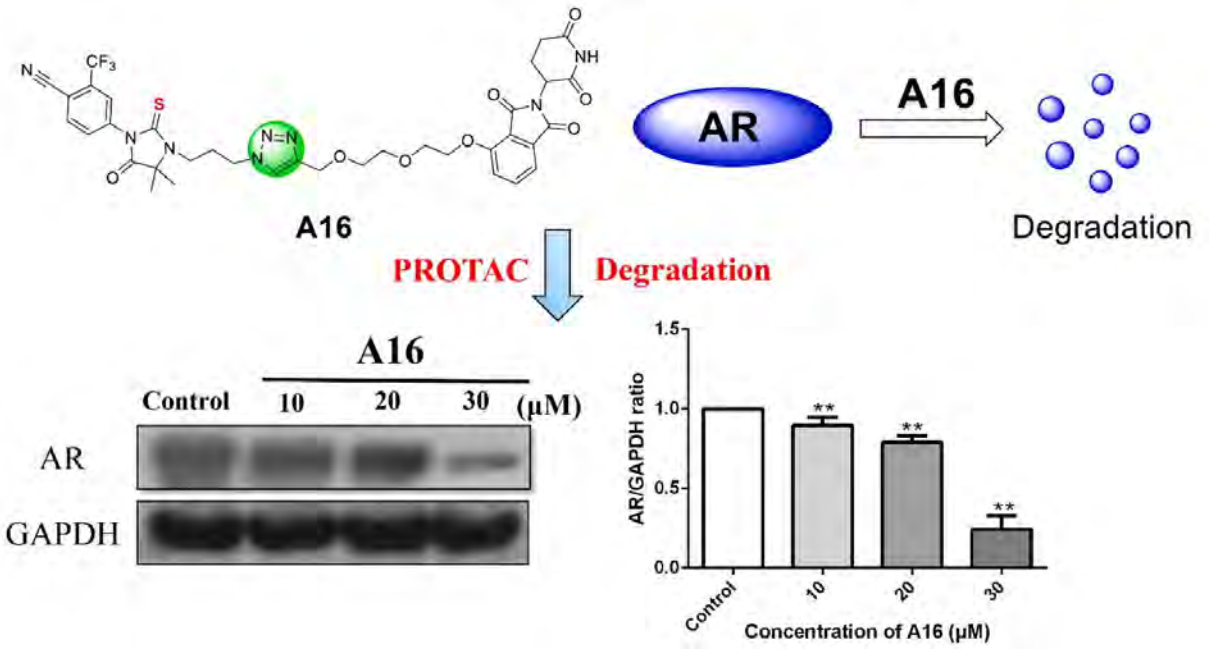
This is a highly customisable platform that could be utilised in the identification of off targets associated with therapeutic modalities that induce protein degradation or gene silencing. This system also has the potential to be used for additional applications such as in drug repurposing for drugs that achieve efficacy via off-targets.

Contributor: Zoe

Designed, synthesized and biological evaluation of proteolysis targeting chimeras (PROTACS) as AR degraders for prostate cancer treatment

Jian-Jia Liang[§], Hang Xie[§]…, Hong-Min Liu*, Li-Hong Shan*

Bioorg. Med. Chem **2021**, *45*, 116331



Prostate cancer is proving to be a significant health problem in men, being the second most frequently diagnosed cancer and with no current cure for the metastatic castration-resistant form (CRPC). The androgen receptor (AR) is overexpressed in CRPC and several PROTAC molecules targeting the AR have already been reported. Through a series of experiments the authors of this paper designed, synthesised and evaluated a group of phthalimide based PROTACs against the AR.

The group designed heterobifunctional compounds based on the high-affinity AR agonist RU59063. Derivatives were connected to a CRBN ligand via a 1,2,3-triazole linker. Fluorescence polarisation (FP) assays highlighted that most PROTACs in the series had a good relative binding affinity (RBA) compared to testosterone with compound A16 displaying the best RBA. The authors also demonstrated there was an optimal length of the linker for affinity. Western blotting showed that of all the compounds, A16 exhibited the best degradation profile in LNCaP cells, however it was only modest at 32 %.

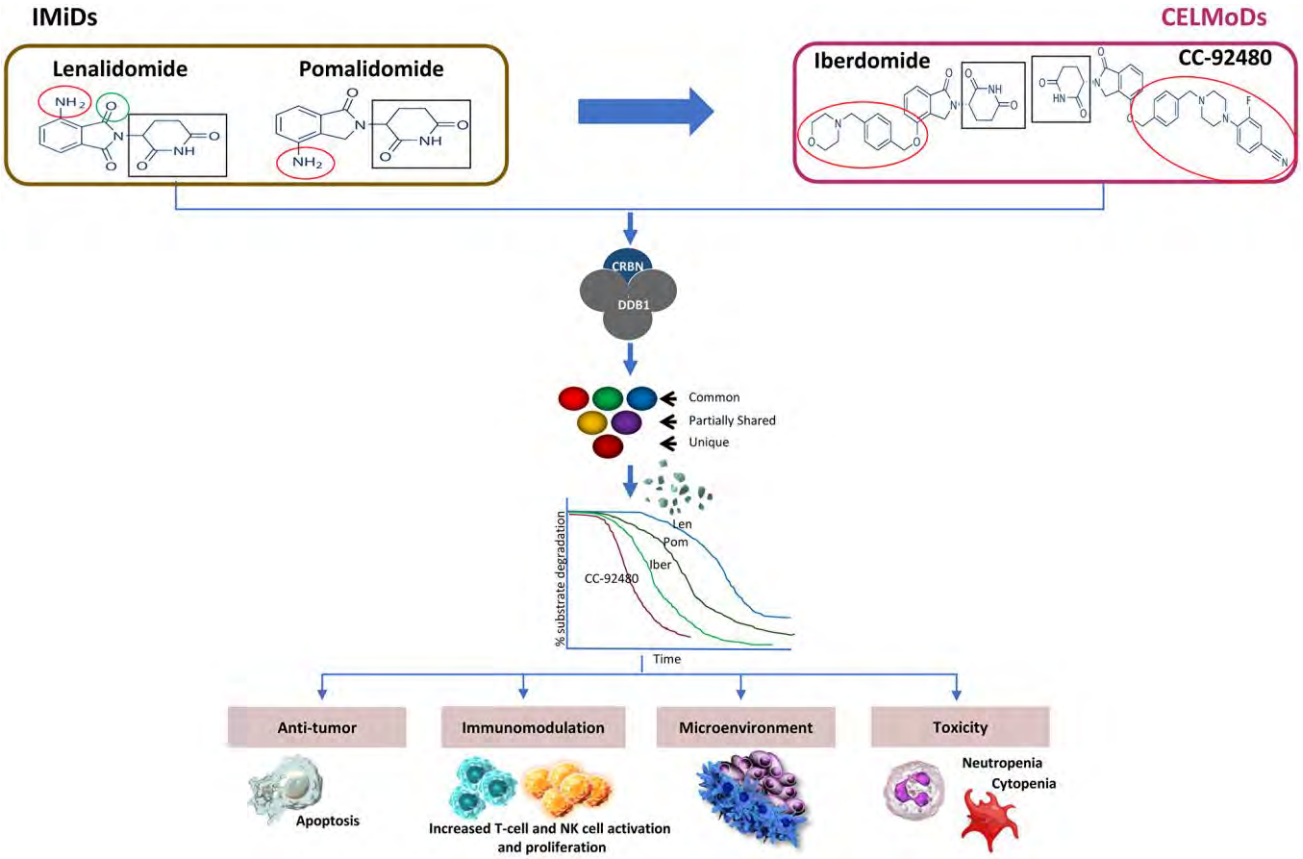
This study provides promising preliminary results of AR degradation through a PROTAC based on a well-known AR agonist. Although the group explored molecular docking to understand the mechanism of A16 degradation, more experiments are needed, potentially guided through ternary complex structural data, to more rationally design highly potent AR degraders.

Contributor: Zoe

Developing next generation immunomodulatory drugs and their combinations in multiple myeloma

Anjan Thakurta[§]*, William E. Pierceall, Michael D. Amatangelo, Erin Flynt, Amit Agarwal

Oncotarget **2021**, *12*, 1555



Cereblon-targeting immunomodulatory agents (IMiDs) play a major role in the combination therapy for treating the currently incurable malignancy, multiple myeloma (MM). In this study, the authors take learnings from clinical studies and compare what is known about the molecular mechanisms of IMiDs and next generation cereblon E3 ligase modulators (CELMoDs) to suggest a strategic framework to optimise efficacy and safety of combinations using these drugs.

The group analysed the main findings from nonclinical research that showed the CELMoDs contain additional phenyl and morpholino moieties for enhanced interactions with cereblon and substrates, CELMoDs IBER and CC-92480 bind to cereblon with a 10-20-fold higher affinity than traditional IMiDs and they are more potent degraders. In addition, clinical studies provided information about the efficacy, toxicity and combination treatments with IMiDs and CELMoDs and a particular case showed there were increased cereblon aberrations in relapsed/refractory MM (RRMM) patients relapsing on IMiD-based treatments. The authors hypothesised that CELMoD therapies could overcome this clinical resistance based on the in vitro studies. It was concluded that combination therapies with CELMoDs should be based on overlapping mechanisms of each contributing therapeutic modality that subsequently counterbalance to provide the most effective therapy with the least undesirable side effects.

CELMoDs are looking to be a promising alternative to IMiDs to treat certain forms of MM. There is still knowledge to be gained on the molecular mechanisms of CELMoDs that would enhance the strategic framework outlined in this study.

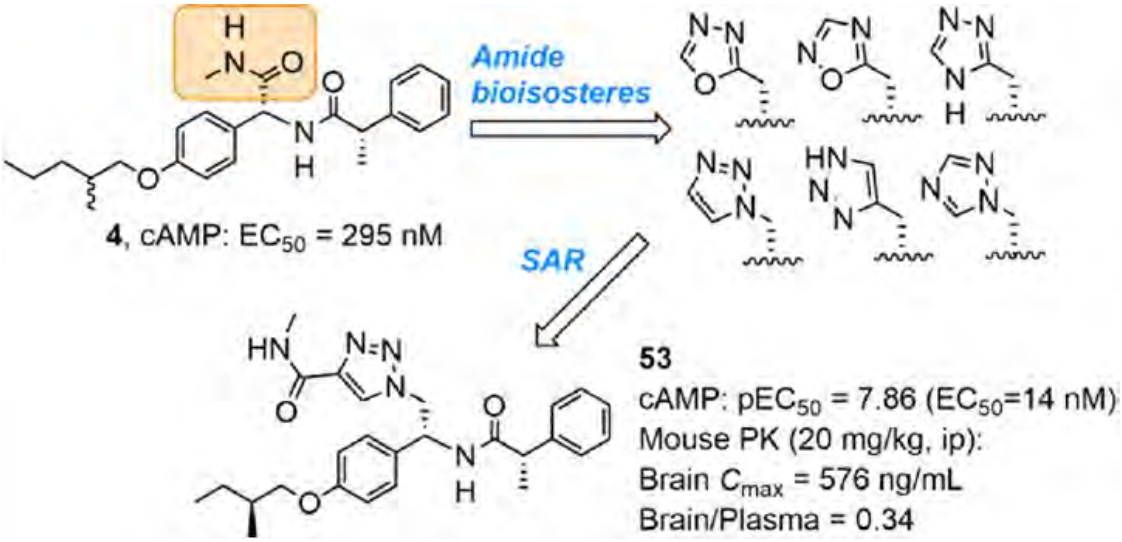
Other Paper Highlights

Contributor: Will

Evaluation of Amide Bioisosteres Leading to 1,2,3-Triazole Containing Compounds as GPR88 Agonists: Design, Synthesis, and Structure–Activity Relationship Studies

Md Toufiqur Rahman[§], …, Chungyang Jin*

J Med Chem **2021**, DOI: 10.1021/acs.jmedchem.1c01075



GPR88 is a class A GPCR for which no endogenous ligand has been identified. Knock-out studies in rodents and transcriptional profiling have implicated GPR88 to be involved in a series of disease states of the nervous system including Parkinsons Disease, Schizophrenia and drug addiction. The authors of this manuscript have previously described a small molecule activator of GPR88. In this study they demonstrate optimisation of a second chemical series based on a so-called 2-AMPP scaffold. They identify a molecule (**53**) capable of potent and specific binding to GPR88 in striatal membrane preparations, as demonstrated by comparing [35S]GTPγS binding affinity in samples from WT vs GPR88 K.O. mice. Activation of GPR88 would be expected to increase GTPγS binding affinity. Of particular interest in this study is a survey of 5-membered heterocyclic amide isosteres. Whilst the authors find that in their case a 1,4-disubstituted 1H-1,2,3-triazole is optimal, a range of other heterocycles are used, and robust chemistries provided to access them.

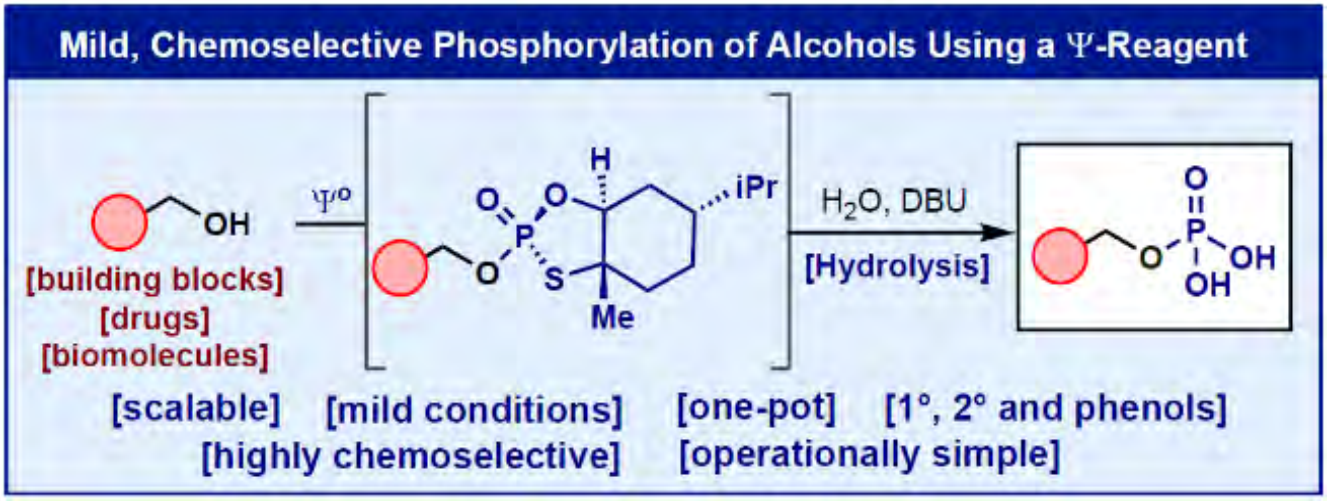
The medicinal chemistry approach used here serves as a great resource for those wishing to investigate amide bioisosteres. Whilst there isn't any chemistry that is fundamentally new, this is a very clear application of a range of robust methods for accessing 5-membered heterocycles. It would have been of interest to see discussion of brain concentrations versus IC50s and brain penetration in the context of estimated free brain concentrations and unbound plasma:brain ratios.

Contributor: Tasuku

Mild and Chemoselective Phosphorylation of Alcohols Using a Ψ-Reagent

Michał Ociepa[§], …, Phil S. Baran*

ChemRxiv **2021**, DOI: 10.33774/chemrxiv-2021-4ksng



Phosphate groups are known to be contained in not only active pharmaceutical ingredients but also agricultural chemicals, and efficient synthetic methods for introducing phosphate groups are critical in these fields. However, the current methods have problems such as low yield, low selectivity, and harsh reaction conditions. The development of a more efficient and versatile method has been desired. In 2018, the author's group reported an efficient method for the construction of phosphothioate using the newly developed Ψ^0 reagent, and in this paper, they extended the method to the phosphorylation of alcohols. It was found that the reaction proceeded under mild conditions using a small excess of Ψ^0 reagent and DBU, and the desired phosphate derivatives were obtained in good yields. This reaction is applied to a broad range of substrates with the reaction proceeding selectively onto primary alcohols even in the presence of amines. Surprisingly, when the reaction was applied to peptides, the phosphorylation only proceeded with the hydroxyl group of tyrosine and did not affect glutamine, asparagine, or histidine residues. This new method is expected to be very useful in the synthesis of various biologically active substances.

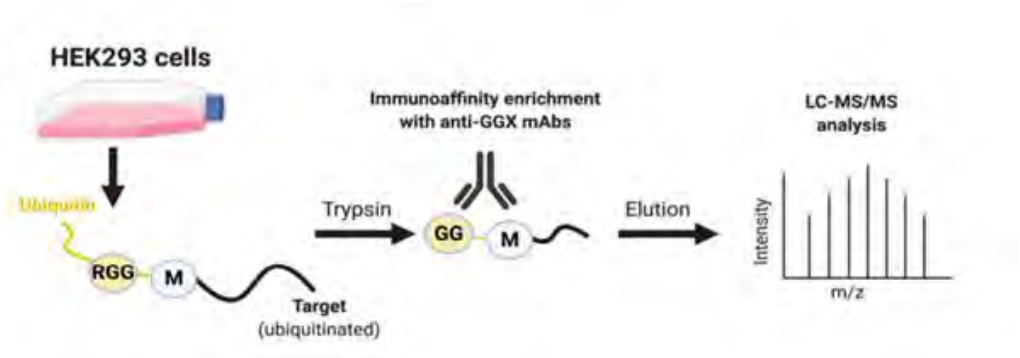
It's also worth checking out their great paper that reported a novel and efficient method of synthesizing chiral phosphothio ester by using chiral Ψ-reagents. See https://science.sciencemag.org/content/361/6408/1234

Contributor: Sarah

Antibody toolkit reveals N-terminally ubiquitinated substrates of UBE2W

Christopher W. Davies [§], James T. Koerber*

Nat. Commun. **2021**, DOI: https://doi.org/10.1038/s41467-021-24669-6



The ubiquitin conjugating enzyme (E2), Ube2W, is unique from other E2s as it catalyses the transfer of ubiquitin to the N-termini of proteins. The endogenous substrates of Ube2W are unknown, as is the role of N-terminal ubiquitination. Here, the authors discovered four monoclonal antibodies (mAbs) that selectively recognise tryptic peptides containing a diglycine sequence at their N-termini (anti-GGX), corresponding to N-terminal ubiquitination. Notably, the anti-GGX mAbs showed no cross-reactivity with isopeptide-linked diglycine-modified lysine containing peptides that correspond to canonical ubiquitination. The anti-GGX mAbs were used for immunoaffinity enrichment of N-terminally modified proteins from a doxycycline inducible *Ube2W* HEK293 cell line. Subsequent proteomic analysis revealed 73 cellular N-terminally modified substrates of Ube2W. Intriguingly, the peptide abundance for most of these substrates remained unaltered by the addition of a proteasome inhibitor. Suggesting that in this system N-terminal ubiquitination functions independently of proteasomal degradation.

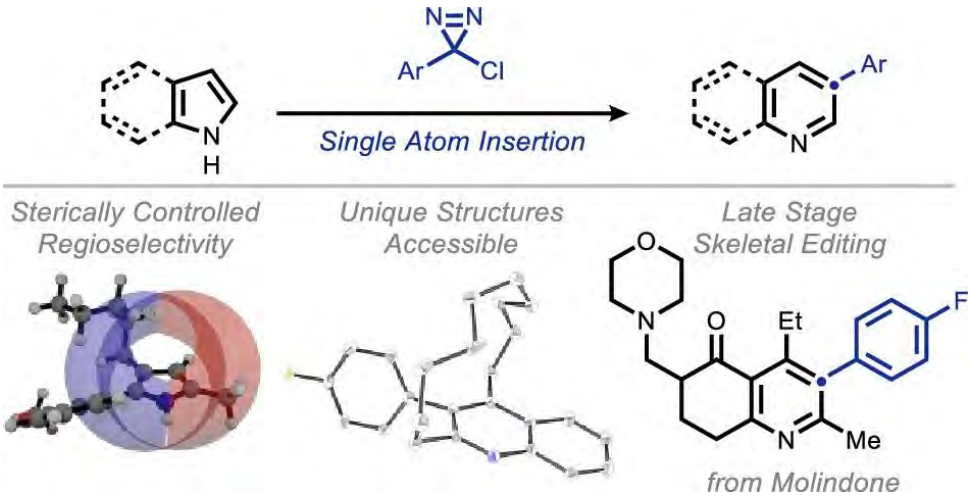
The antibody toolkit described in this paper is a great advancement for research into N-terminal ubiquitination. The authors demonstrate its utility by revealing the elusive Ube2W substrates from a doxycycline inducible *Ube2W* HEK293 cell line. Whether these substrates would also be identified in a cell line expressing endogenous levels of Ubew2 remains to be determined.

Contributor: Claudia

Carbon Atom Insertion into Pyrroles and Indoles Promoted by Chlorodiazirines

Balu D. Dherange [§], Patrick Q. Kelly, Jordan P. Liles, Matthew S. Sigman, Mark D. Levin*

J. Am. Chem. Soc. **2021**, *143*, 11337



This skeletal editing method by the Levin group targets the formation of polysubstituted pyridines and quinolines – desirable motifs for medicinal compounds – by ring expansion through single-atom insertion into pyrrole and indole skeletons. Direct generation of 3-(hetero)aryl pyridines and quinolines was achieved by insertion of arylchlorocarbenes using α-chloroaryldiazirines as thermal carbene precursors. The α-chloro(hetero)aryldiazirine reagents can be conveniently prepared by oxidation of commercially available amidine precursors in a single step, allowing easy access of a rich library of diverse α-chloroarylcarbene precursors. A broad set of polyfunctionalized indoles and pyrroles were suitable substrates for the ring expansion procedure, provided that the substrate's 2-position was functionalized. Regioselectivity in asymmetrically functionalized pyrroles is governed by steric factors and can be easily predicted. The ring expansion methodology proceeds smoothly with various aryldiazirines, including *ortho-*, *meta-* and *para-*substituted arenes and heteroarenes. Alkyldiazirines and electron-rich aryldiazirines, however, favour alternative modes of reactivity and do not lead to ring expansion.

Although not without limitations, this method is a promising novel tool for synthetic organic and medicinal chemists to access challenging 3-substituted pyridine and quinoline motifs. The protocol is operationally simple and based on widely available reagents and therefore is especially attractive for late-stage functionalisation of medicinal compounds. This ring expansion method is highly advantageous for cases where common cross-coupling chemistry faces limitations, such as heteroaryl-heteroaryl couplings or introduction of 2-fluorinated or polyfluorinated arenes and allows introduction of halogenated sites for further down-stream diversification.

University of Dundee

Ciulli Laboratory
School of Life Sciences
Dow Street, Dundee,
DD1 5EH
United Kingdom

lifesci.dundee.ac.uk/groups/alessio-ciulli/publications/journal-club

@alessiociulli @CharlCrowe

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