ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Versatile One-Pot Synthesis of Hydrophobic Tags by Multicomponent Reactions

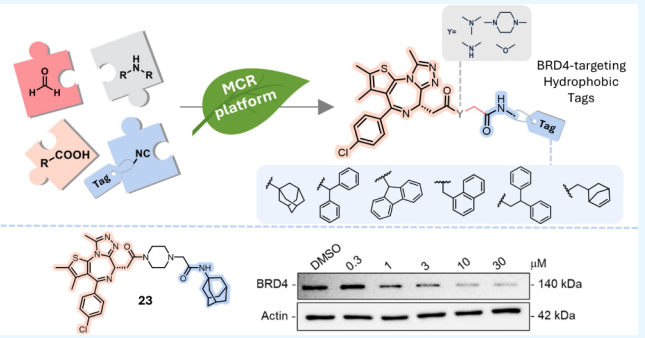
Federica Carolina Balestrero,[#] Laura Gioiello,[#] Georgia Goutsiou,[#] Sabina Sangaletti, Rita Maria Concetta Di Martino,[*] Fabrizio Condorelli, Ambra A. Grolla,[#] and Tracey Pirali[#]

Cite This: *ACS Omega* 2025, 10, 4745−4753

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Among the various strategies being developed in the field of protein degraders, HyTags remain relatively underexplored, despite their advantages over PROTACs. Their synthesis typically involves multistep procedures, including the use of coupling reagents and protection/deprotection steps. To develop a more sustainable and streamlined approach, we designed a versatile multicomponent platform that generates HyTags with diverse linkers and hydrophobic moieties in high yields. Using (+)-JQ1 as the POI ligand, we synthesized a series of BRD4-targeting HyTags and discovered that compound **23** induces degradation of BRD4 *via* the autophagy-lysosome pathway through ER stress. This finding further supports the valuable application of this synthetic methodology in the search for effective degraders.



1. INTRODUCTION

With the advancement of scientific research, more and more mechanisms underlying various pathologies are being progressively elucidated, leading to the identification of new potential pharmacological targets. Yet, some of these targets, including transcription factors, phosphatases, scaffold and epigenetic proteins, are deemed "undruggable". This is primarily attributed to the absence of well-defined ligand-binding pockets, particularly when the objective is to disrupt protein−DNA or protein−protein interactions (PPIs). Additionally, the high mutation rate of the protein's active site or the limited structural differences between target isoforms may pose obstacles to the development of selective inhibitors.[1]

Targeted protein degradation (TPD) is an emerging strategy to overcome these limitations in drug discovery. TPD triggers the depletion of the protein of interest (POI) through intracellular mechanisms, such as the ubiquitin-proteasome system (UPS) or autophagy-lysosome system (ALS).[2,3] Over the years, different types of degraders have been conceived, serving both as therapeutic modalities and chemical tools, including Proteolysis Targeting Chimeras (PROTACs).[4,5] PROTACs are heterobifunctional molecules composed of two pharmacophores: a ligand for the POI—the so-called *warhead*—and an *anchor* able to recruit an E3 ligase, connected by a linker. By inducing PPI between the target and the E3 ligase, PROTACs can foster the degradation of the POI *via* the ubiquitin-dependent proteasome 26S.[6] This approach has led to the development of countless PROTAC degraders, several of which are currently advancing in clinical trials (e.g., estrogen receptor degrader ARV-471 in phase III).[7,8]

A relatively underexplored technology that is gaining more interest in the field of drug discovery is the hydrophobic tag (HyTag)-based protein degradation.[9] HyTags are composed of a POI ligand, a linker, and a highly hydrophobic functionality that mimics a partially denatured protein state, thus hijacking the cellular unfolded protein response (UPR) that eventually triggers the degradation of the desired target.[10]

After Crews's pioneering work on the first HyTag, which, due to its adamantane moiety, was able to degrade HaloTag fusion proteins *in vivo*,[11] further exploration led to the discovery of other tags, namely *tert*-butylcarbamate-protected arginine (Boc_3Arg),[12] pyrene,[13] fluorene,[14] carborane,[15] menthoxyacetyl,[16] norbornene,[17] and *tert*-butylcarbamate-protected lysine (Boc_2Lys).[18] Some examples of HyTag-based degraders reported in the literature are depicted in Figure 1.

Compared to PROTACs, whose mechanism relies solely on the UPS, HyTags induce the degradation of the POI through multiple pathways that depend on the type of tag carried (Figure 2). For example, while HyTags bearing a fluorene moiety activate the UPR by triggering endoplasmic reticulum (ER)

Received: October 25, 2024
Revised: December 4, 2024
Accepted: December 19, 2024
Published: January 30, 2025

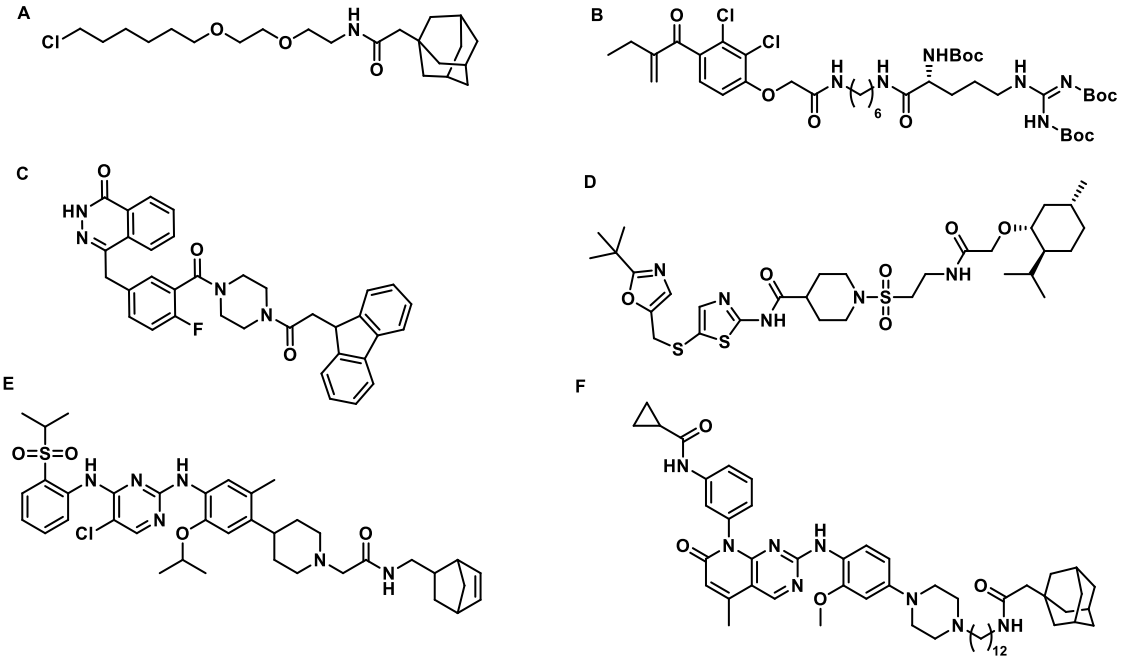


Figure 1. Representative HyTags reported in the literature: (A) HaloTag fused protein degrader;[11] (B) glutathione S-transferase (GST)-α1 degrader;[12] (C) poly[ADP-ribose] polymerase (PARP)1 degrader;[14] (D) cyclin-dependent kinase (CDK) 9 degrader;[16] (E) anaplastic lymphoma kinase (ALK) degrader;[17] (F) protein kinase B (AKT3) degrader.[19]

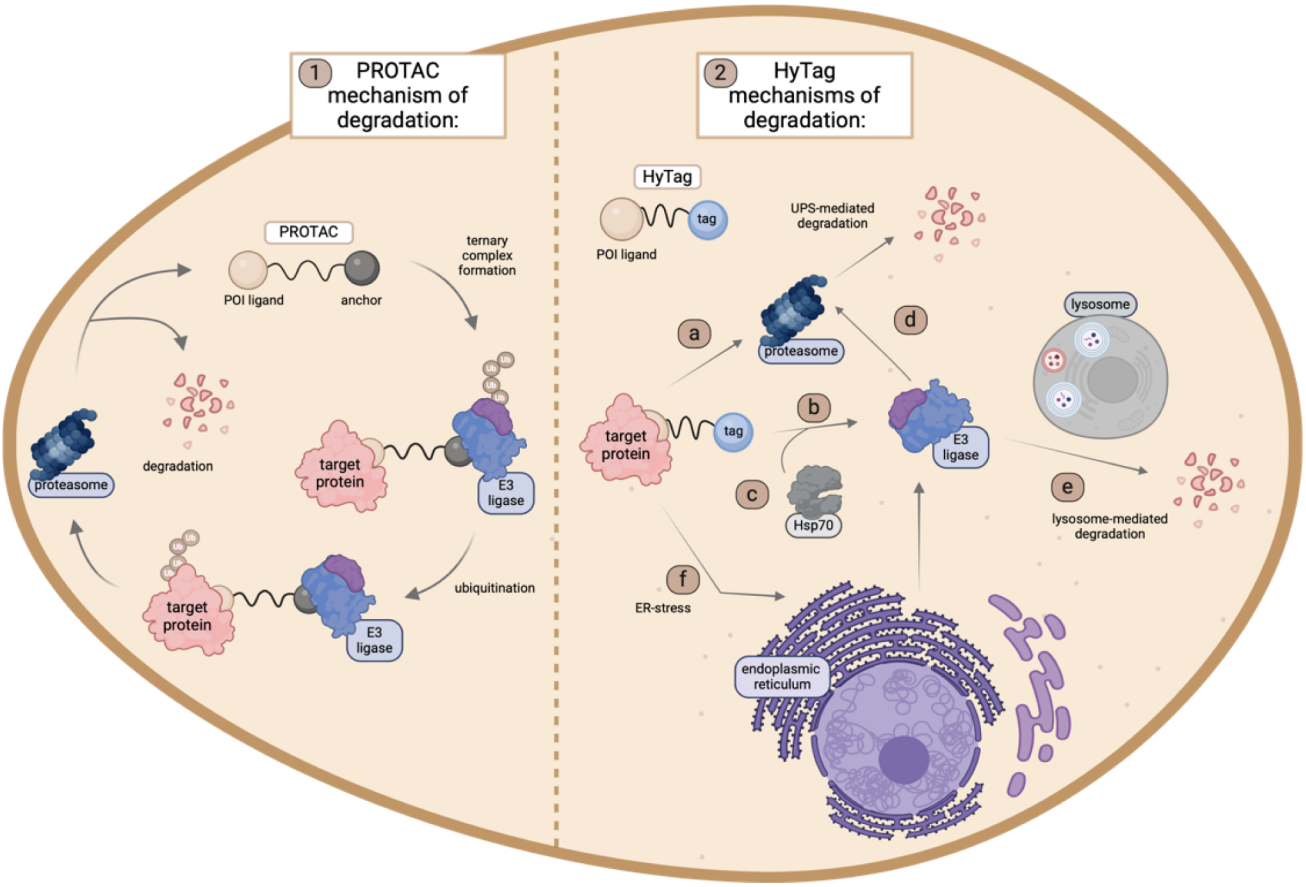


Figure 2. (1) Degradation cycle of PROTACs. (2) Mechanisms of degradation of HyTags: the hydrophobic moiety can directly recruit (a) the 20S proteasome in the absence of protein ubiquitination; (b) an E3 ligase; and (c) the chaperonin Hsp70 that mediates the interaction between the HyTag and the E3 ligase. The E3 ligase allows the degradation of the target protein by the (d) 26S proteasome or the lysosome. HyTags can also induce ER stress, which eventually activates the UPR-mediated protein degradation (f).

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

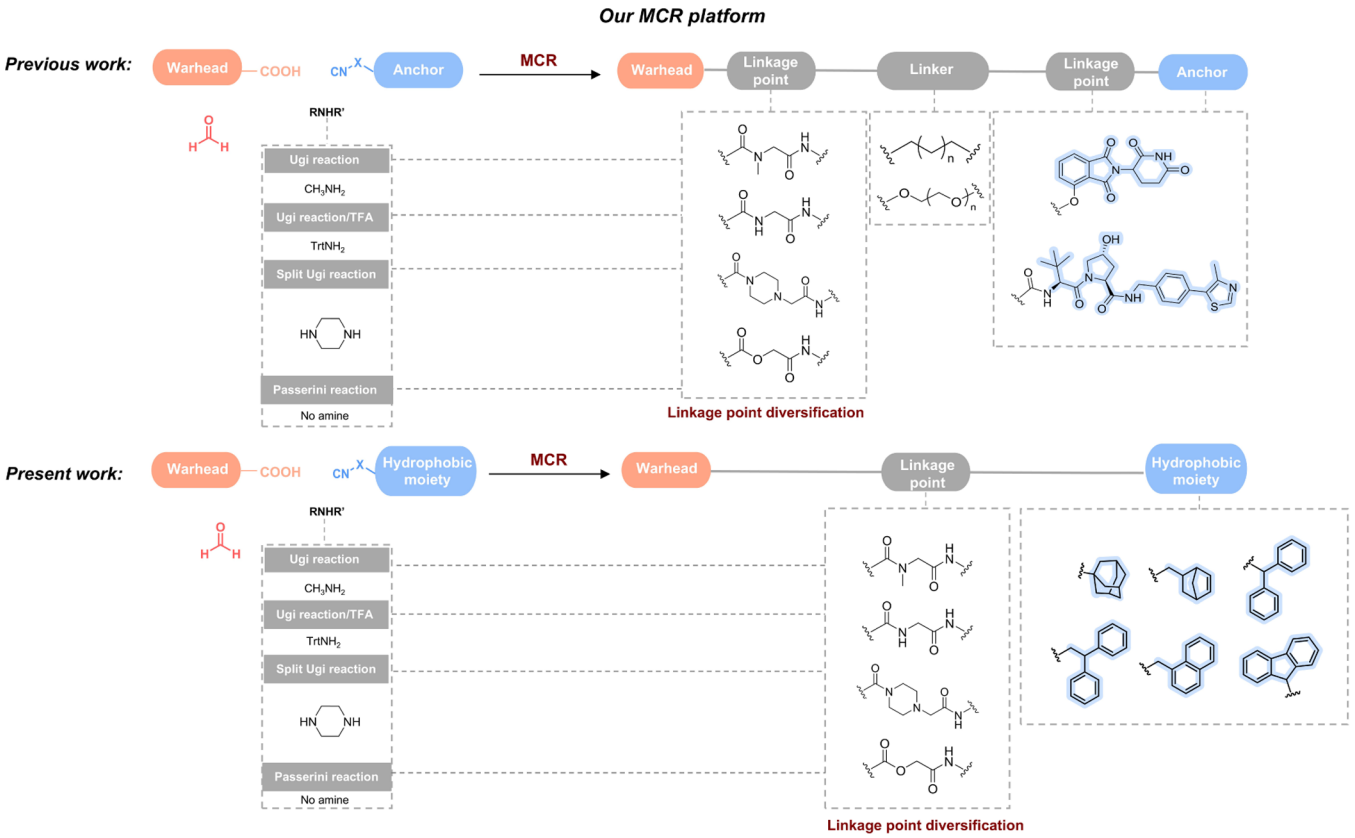


Figure 3. Our MCR-based platform for the synthesis of PROTACs (previous work) and HyTags (present work).

stress,[14] compounds endowed with Boc_3Arg can directly bind the protein to the 20S core proteasome in a ubiquitin-independent manner.[20] On the other hand, adamantyl induces the degradation of the POI mainly through UPS, either with or without the direct recruitment of the chaperonin Hsp70.[19,21] However, further research is required to elucidate precisely how HyTags promote degradation.

Although no HyTag-based degraders have progressed into clinical stages so far, they hold significant promise in drug discovery due to several advantages over PROTACs. Specifically, HyTags have a simpler chemical structure with a lower molecular weight and a reduced number of hydrogen bond donors/acceptors. These features confer upon them a more favorable drug-likeness profile and bioavailability, as well as the potential for targeting proteins related to central nervous system diseases.[22,23] Moreover, contrary to PROTACs, they show a better capability of inducing the degradation of proteins involved in PPIs or protein complex formation.[16,24,25] Lastly, HyTags do not exhibit the "hook effect" when tested at high concentrations, and, owing to the synergistic effect of their multiple degradation mechanisms, their degradation activity may be augmented and can be observed even at shorter incubation times compared to the corresponding PROTAC degraders.[18]

Since they lack the ligand for an E3 ligase, the molecular design of HyTags might be expected to be straightforward. Nevertheless, just like PROTACs, most HyTags reported in the scientific literature were discovered either by serendipity or by testing libraries of compounds, rather than by rational design.[12,16,26] Therefore, to identify the most promising HyTag, a huge structure−activity relationship study (SAR) is necessary. This study should be carried out around the hydrophobic moiety, as well as around the linker, by varying its length, chemical composition, and linkage point to the POI ligand. Such an exploration often demands massive synthetic and screening efforts.

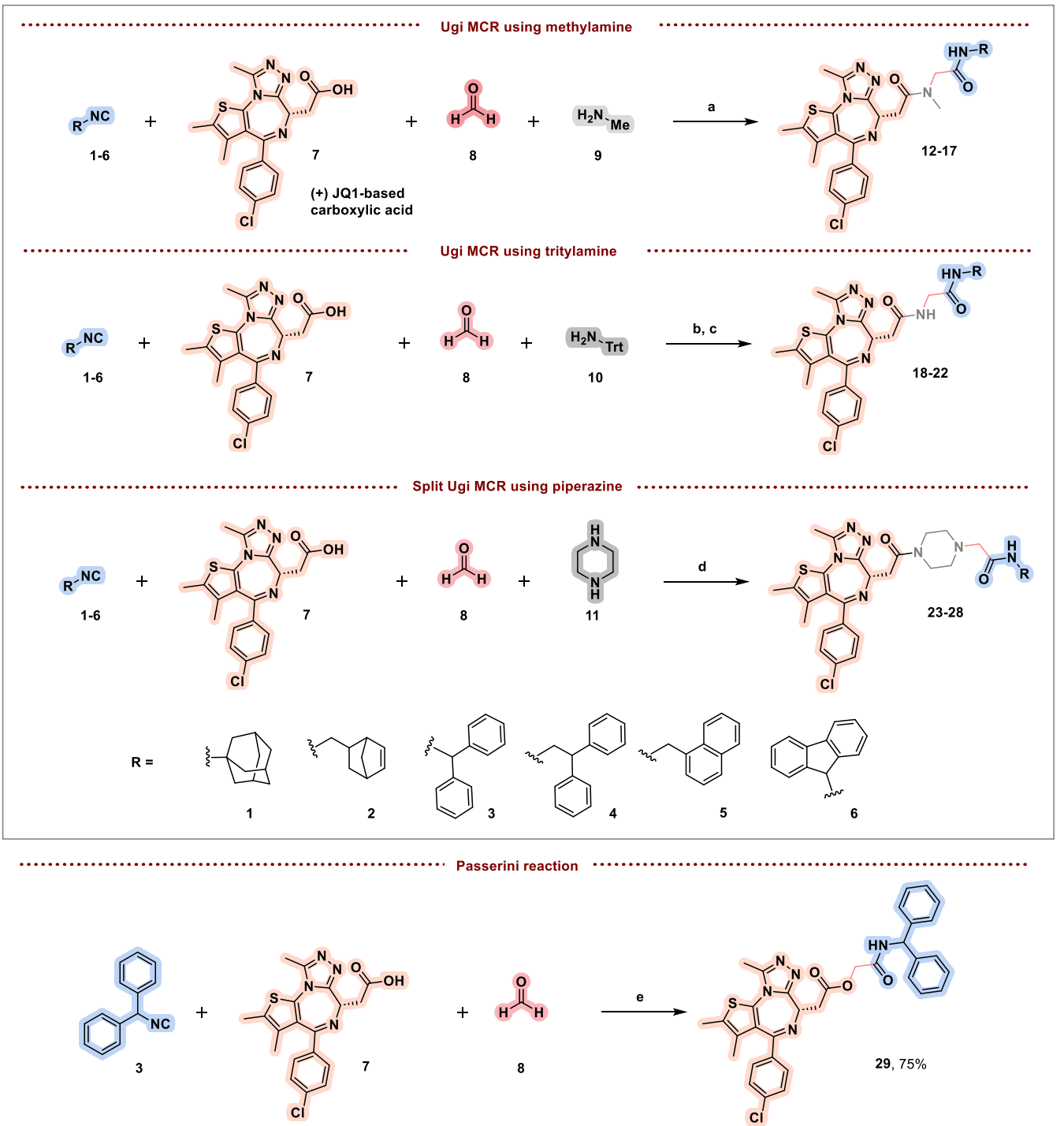
Recently, with the aim of providing a new chemical toolkit for simplified access to PROTACs, we conceived a synthetic platform based on multicomponent reactions (MCRs),[27] namely the Ugi reaction, two of its variants, and the Passerini reaction (Figure 3).[28]

By capitalizing on the modularity and efficiency of MCRs, this platform enabled the *one-pot* assembly of highly decorated PROTACs for the selective targeting of bromodomain-containing protein 4 (BRD4), a protein involved in epigenetics and used as a probe in many studies of drug discovery regarding protein degraders.[29] Our BRD4-targeting PROTACs were readily obtained with good yields, avoiding cumbersome multistep synthetic routes usually needed for the asymmetric diversification of both sides of the linker.[30] Notably, most of these reactions are far from the principles of *green chemistry*, which, in response to the growing environmental pollution, aims at mitigating the impact of chemical processes on human health and the Earth. In contrast, by cutting down waste, by reducing the use of hazardous substances and, in turn, by adopting straightforward procedures, our MCRs platform perfectly aligns with the concept of *green chemistry*.[31]

To further study the scope of this methodology and demonstrate its efficiency and versatility, in this work we describe the application of the MCR platform to the synthesis of novel HyTags, once again using BRD4 as a proof-of-concept.

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

Scheme 1. Multicomponent Synthesis of HyTags Targeting BRD4[i]



[i]Reagents and conditions: (a) MeOH, 0 °C, 4 h; (b) MeOH, 40 °C, 1 h, then overnight at rt; (c) TFA, CH_2Cl_2, 0 °C, 30 min, then rt, 3 h; (d) MeOH, reflux, 4 h; (e) CH_2Cl_2, 40 °C, 3 h.

2. RESULTS AND DISCUSSION

For the synthesis of HyTags targeting BRD4, we used the following building blocks (Scheme 1):

(i) Six different isocyanides bearing those hydrophobic groups described in the already reported HyTags **1**–**6**;

(ii) The (+)-JQ1-based carboxylic acid **7** as a POI ligand;

(iii) Paraformaldehyde or formalin as the carbonyl source **8**;

(iv) In the four-component Ugi reactions, three different amines **9**–**11** were used: methylamine **9** as an unhindered primary amine, tritylamine **10** as a surrogate of ammonia,[32] and piperazine **11** as a bis-secondary diamine.[33] The last two methodologies originated from previous research conducted by our laboratory.

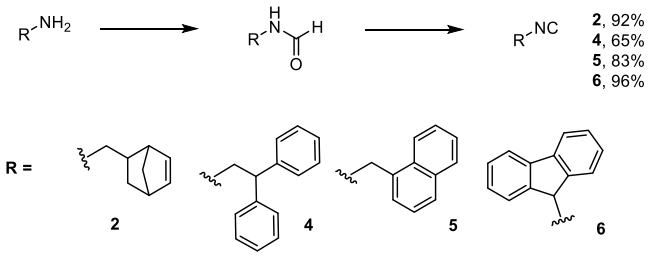
The selected tags comprised fragments with different degrees of lipophilicity, dimensions, and flexibility. Specifically, they included: (i) adamantane (as in **1**), the most frequently reported substructure in the field of HyTags, which to date has been exploited to efficiently degrade targets related to various

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

diseases, ranging from cancer to neurodegenerative disorders;[10] (ii) norbornene (as in **2**), a cage-hydrocarbon which, among the reported tags, exhibits the smallest molecular weight, thereby conferring better pharmacokinetic properties;[17] (iii) diphenylmethane (as in **3**), methyldiphenylmethane (as in **4**), naphthalene (as in **5**), and fluorene (as in **6**), a group of aromatic hydrocarbons endowed with high lipophilicity and remarkable steric hindrance.

While the (+)-JQ1-based carboxylic acid **7**, formaldehyde, and all the selected amines were commercially available, some isocyanides needed to be prepared by using a two-step protocol as depicted in Scheme 2. First, the primary amine bearing the

Scheme 2. Preparation of Isocyanides[i]



[i]Reagents and conditions: (a) ethyl formate, Et_3N, reflux, 7 h; (b) $POCl_3$, Et_3N, CH_2Cl_2, 0 °C for 15 min, then rt for 2 h. Yields refer to the two-step sequence.

HyTag moiety was treated with ethyl formate at reflux in a formylation reaction to give the corresponding *N*-formamide. After removal of the formylating agent, the crude products were converted to the corresponding isocyanides by a dehydration reaction using $POCl_3$ and Et_3N in CH_2Cl_2. The desired isocyanides **2** and **4**−**6** were afforded in good to excellent yields (65−96%) and were further used in the MCRs.

When using methylamine **9**, the four components were reacted in MeOH at 0 °C for 4 h to afford HyTags **12**−**17** in moderate to excellent yields (50−78%). In the case of tritylamine **10**, upon completion of the MCR, the trityl group was cleaved by treating the crude compounds with TFA at 0 °C in CH_2Cl_2, affording HyTags **18**−**22** in yields ranging from 62% to 71%. During the deprotection step, all HyTags were stable except for the norbornene-bearing one, which decomposed. In the split Ugi reaction, all components were combined in MeOH and heated at reflux to afford the desired HyTags **23**−**28**. Of note, besides affording excellent yields (67−96%), the use of piperazine as the amine component allows for the easy introduction in the linker of this heterocycle, which is known to enhance both degradation efficiency and ADME properties of degraders.

Following previous studies reporting that the substitution of an amide group with an ester moiety at the linkage point between the POI ligand and the linker might improve cellular permeability without decreasing metabolic stability,[34] the isocyanide **3** was reacted in a Passerini MCR with the (+)-JQ1-based carboxylic acid **7** in the presence of formaldehyde **8** in CH_2Cl_2 at 40 °C to afford the HyTag **29** in 75% yield. All of the final synthesized HyTags are shown in Figure 4.

As a proof-of-concept, the platform was applied to degrade BRD4 in the triple-negative breast cancer cell line, 4T1. This cell line was chosen based on its ability to express high levels of BRD4. As a result, eight out of 18 compounds exhibited good degradation activity. Indeed, as shown in Figure 5A, eight HyTags compounds were able to partially degrade BRD4 at the concentration of 30 μM, after 8 h of incubation. As the degradation induced by **23** (bearing the adamantane-based tag) and **26** (bearing methyldiphenylmethane) was significant under these conditions, these two compounds were selected for further analysis. We performed a dose−response curve (0.3−30 μM) for 8 h that allowed us to demonstrate the dose-dependency of both compounds in inducing such degradation (Figure 5B). Notably, compound **26** was more potent as it caused a more marked degradation of BRD4 (DC_{50} of 10.2 μM and D_{max} of 83.3%), compared to compound **23** (DC_{50} of 24.7 μM and D_{max} of 57.4%).

To further characterize the mechanism of action of these two compounds, we investigated whether BRD4 degradation was based on proteasome or lysosome activity. Therefore, BRD4 levels were assessed in 4T1 cells preincubated (30 min) with pharmacologically active concentrations of the proteasome inhibitor bortezomib, or the lysosome inhibitor chloroquine, just before being exposed to compound **26** or compound **23**. As shown in Figure 6A, neither inhibitor affected the **26**-induced degradation of BRD4. In contrast, only chloroquine counteracted the effects of **23** on BRD4 protein levels, suggesting that this specific HyTag acts *via* the lysosomal pathway of degradation.

As mentioned above, the literature on HyTags has documented that UPR, resulting from ER stress, may be responsible, at least in part, for POI degradation. Three ER membrane-associated sensors are responsible for the activation of the UPR signaling pathway: activating transcription factor-6 (ATF6), double-stranded RNA-dependent protein kinase (PKR)-like eukaryotic initiation factor 2α (eIF2α) kinase (PERK), and inositol-requiring transmembrane kinase/endoribonuclease 1 (IRE1). To investigate if this pathway is involved in the effects of **23** and **26** on BRD4 protein levels, we evaluated the expression of *Atf4*, *Atf6,* and *Xbp1s* genes, as each of these transcription factors participates in one of the three branching pathways of the UPR activation.[35] As shown in Figure 6B, both compounds could activate UPR, although through a mechanism different from that of thapsigargin (TG), a known ER stress inducer. In fact, while all 3 genes were induced by TG treatment, only *Atf4* increased (up to 5-fold compared to controls) upon exposure to compound **23** or compound **26** (Figure 6B).

BRD4 is widely recognized for regulating the expression of oncogenes in cancer cells. Therefore, we evaluated whether targeted inhibition of BRD4, following exposure to HyTags, could induce growth arrest or apoptosis of the 4T1 breast cancer cell line.[36] As summarized in the graphs depicted in Figure 6C, both HyTags **23** and **26** caused a dose-dependent cytotoxicity, with an IC_{50} of 20.6 ± 3.2 μM and 15.9 ± 7.3 μM, respectively. Conversely, both compounds were less efficient and potent in reducing healthy fibroblast viability compared with what was observed by testing 4T1 cells. Indeed, IC_{50} was not reached even at the concentration of 30 μM (see Figure S23).

3. CONCLUSIONS

In this follow-up work, we expanded our recently published MCR-based platform to synthesize a variety of HyTags for protein degradation. Our synthetic strategy works in one-pot under mild conditions, is versatile, avoids the use of peptide coupling reagents, and does not require protecting groups (except for the trityl group in one of the Ugi variants). By modifying the tag moiety, we were able to rapidly generate 18 different HyTags compounds, exploiting three variants of the

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

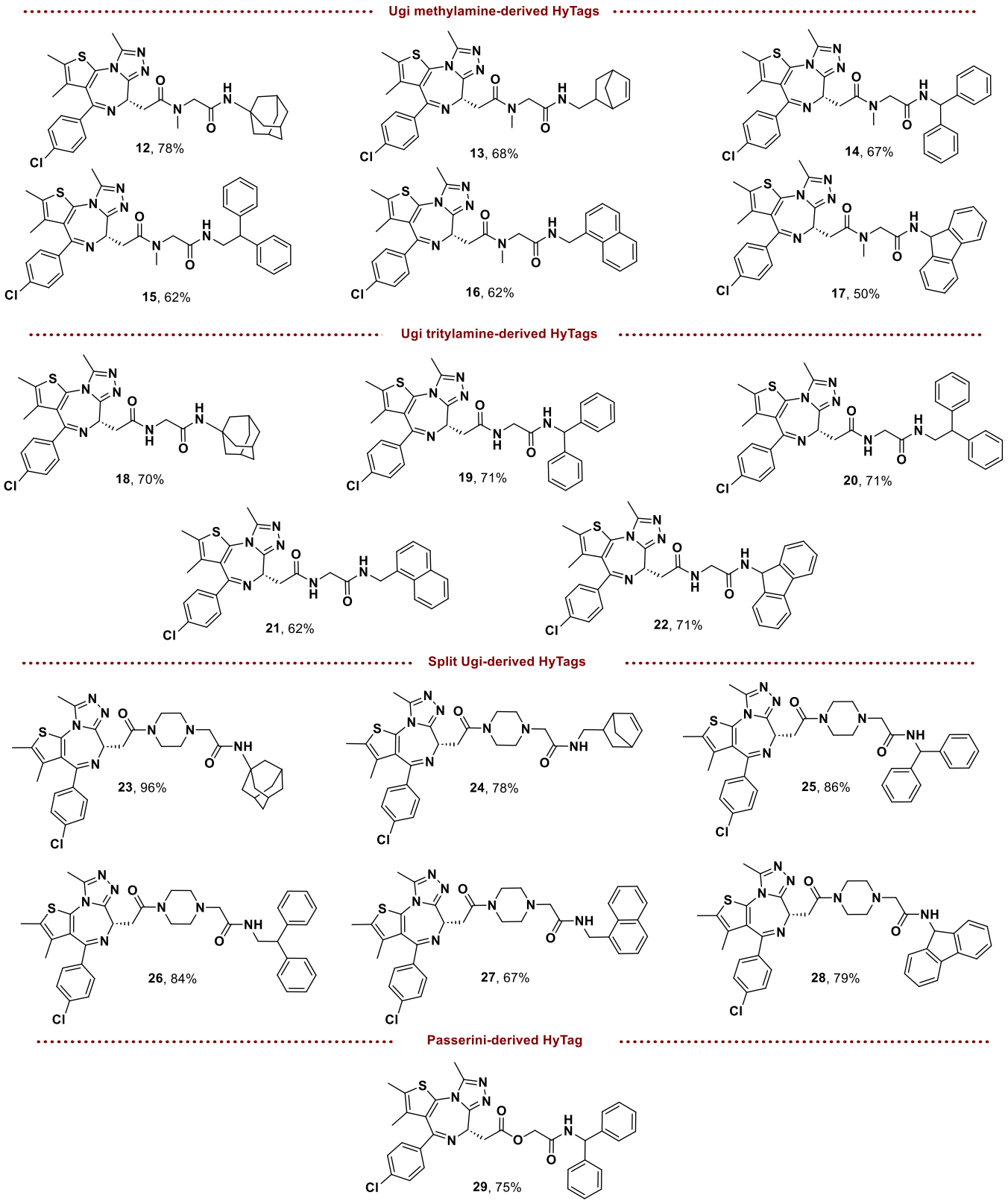


Figure 4. HyTags were synthesized using our MCR platform.

Ugi reaction as well as the Passerini one and varying the attachment point between the POI ligand and the lipophilic tag. Of note, the split Ugi reaction affords the highest yields and allows for the easy introduction of piperazine, a privileged substructure in protein degraders. Within this series, **23**, bearing (+)-JQ1 as the POI ligand and adamantane as the lipophilic tag, induces BRD4 degradation through the autophagy-lysosome system *via* ER stress.

In conclusion, our results corroborate the potential of HyTags as a new approach for targeted protein degradation and highlight

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

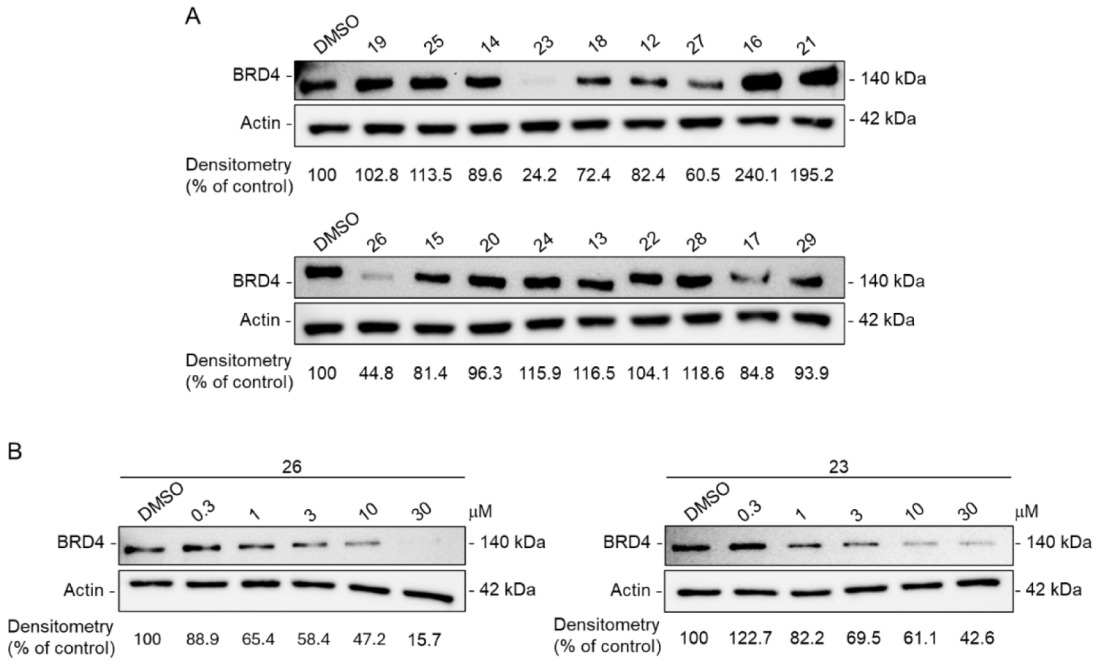


Figure 5. BRD4 degradation after treatment with HyTags. (A) Western blot analysis of BRD4 degradation onto 4T1 cells lysates after 8 h exposure to BRD4-targeting HyTags (30 μM). Actin was used as an internal control for equal loading. (B) Dose−response experiments of BRD4 degradation after 8 h treatments with DMSO (vehicle), **26** or **23** (0.3−30 μM). Immunoblots are representative of three independent experiments.

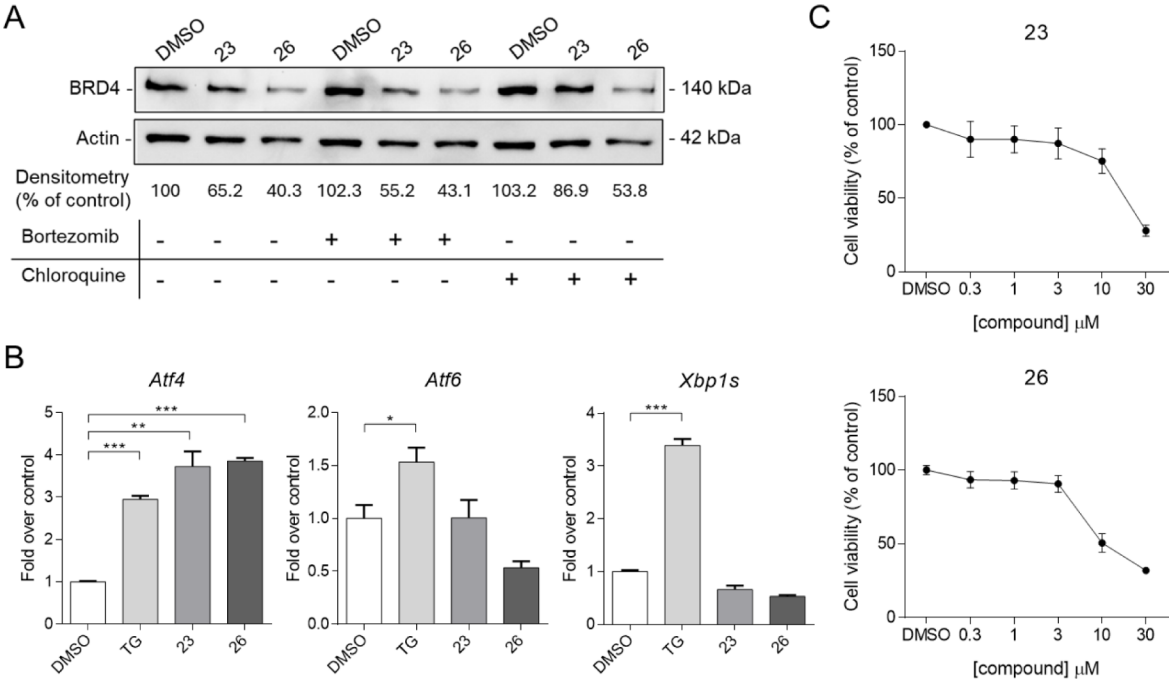


Figure 6. Characterization of HyTags mechanism of action. (A) Western blot analysis of BRD4 protein levels after 8-h treatments with DMSO (vehicle), compound **26** or compound **23** (30 μM) alone, or in combination with bortezomib (5 nM) or chloroquine (100 μM). Immunoblots are representative of three independent experiments. (B) RT-PCR analysis of transcripts for the UPR-mediated degradation markers *Atf4*, *Atf6*, and *Xbp1s* (see text for an explanation) after 4-h treatments with vehicle (DMSO), or 30 μM concentration of either **26** or **23**. Thapsigargin (TG, 1 μM) was used as a positive control to activate the UPR by inducing ER stress. Results are expressed as mean ± SEM of three independent experiments. (C) MTT assays to evaluate 4T1 cell viability after 24-h treatments with increasing concentration (0.3−30 μM) of compound **23** or compound **26**. Results are expressed as the mean ± SEM of three independent experiments.

our multicomponent platform as an efficient method for making protein degraders easily accessible to chemists with minimal effort.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c09726.

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

Chemistry experimental details, including ^{1}H and ^{13}C NMR spectra of all synthesized HyTags, and additional experimental information about biological assays (PDF)

AUTHOR INFORMATION

Corresponding Author

Rita Maria Concetta Di Martino − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy;* orcid.org/0000-0003-2287-3331; Email: rita.dimartino@uniupo.it

Authors

Federica Carolina Balestrero − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy*

Laura Gioiello − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy; Molecular Immunology Unit, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan 20133, Italy;* orcid.org/0009-0005-4197-5343

Georgia Goutsiou − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy*

Sabina Sangaletti − *Molecular Immunology Unit, Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan 20133, Italy*

Fabrizio Condorelli − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy*

Ambra A. Grolla − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy*

Tracey Pirali − *Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale, Novara 28100, Italy;* orcid.org/0000-0003-3936-4787

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c09726

Author Contributions

#F.C.B., L.G., G.G., A.A.G., and T.P. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is part of the project NODES that has received funding from the MUR−M4C2 1.5 of PNRR with grant agreement no. ECS00000036 (to R.M.C.D.M. and T.P.), AIRC under IG 2023 - ID. 29452 project (to T.P.), PRIN 2022 20228YKCF2 and Fondazione Cariplo Rif. 2020-3598 (to A.A.G.), AIRC ID. IG 29261 project (to S.S.). G.G. is supported by the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie Actions grant agreement no. 956477 (PIANO).

ABBREVIATIONS

AKT3, protein kinase B; ALK, anaplastic lymphoma kinase; ALS, autophagy-lysosome system; ATF6, activating transcription factor-6; Boc_2Lys, *tert*-butylcarbamate-protected lysine; Boc_3Arg, *tert*-butylcarbamate-protected arginine; BRD4, bromodomain-containing protein 4; CDK, cyclin-dependent kinase; eIF2α, eukaryotic initiation factor-2α; ER, endoplasmic reticulum; GST, glutathione S-transferase; HyTag, hydrophobic tag; IRE1, inositol-requiring transmembrane kinase/endoribonuclease 1; MCRs, multicomponent reactions; PARP1, poly (ADP-ribose) polymerase 1; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PKR, double-stranded RNA-dependent protein kinase; POI, protein of interest; PPI, protein−protein interaction; PROTACs, proteolysis targeting chimeras; SAR, structure−activity relationships; TG, thapsigargin; TPD, targeted protein degradation; UPR, unfolded protein response; UPS, ubiquitin-proteasome system

REFERENCES

(1) Xie, X.; Yu, T.; Li, X.; Zhang, N.; Foster, L. J.; Peng, C.; Huang, W.; He, G. Recent Advances in Targeting the "Undruggable" Proteins: From Drug Discovery to Clinical Trials. *Signal Transduct. Target. Ther.* **2023**, *8* (1), 335.

(2) Samarasinghe, K. T. G.; Crews, C. M. Targeted Protein Degradation: A Promise for Undruggable Proteins. *Cell Chem. Biol.* **2021**, *28* (7), 934−951.

(3) Kim, J.; Byun, I.; Kim, D. Y.; Joh, H.; Kim, H. J.; Lee, M. J. Targeted Protein Degradation Directly Engaging Lysosomes or Proteasomes. *Chem. Soc. Rev.* **2024**, *53* (7), 3253−3272.

(4) Békés, M.; Langley, D. R.; Crews, C. M. PROTAC Targeted Protein Degraders: The Past Is Prologue. *Nat. Rev. Drug Discovery* **2022**, *21* (3), 181−200.

(5) Song, J.; Hu, M.; Zhou, J.; Xie, S.; Li, T.; Li, Y. Targeted Protein Degradation in Drug Development: Recent Advances and Future Challenges. *Eur. J. Med. Chem.* **2023**, *261*, 115839.

(6) Neklesa, T. K.; Winkler, J. D.; Crews, C. M. Targeted Protein Degradation by PROTACs. *Pharmacol. Ther.* **2017**, *174*, 138−144.

(7) Zeng, S.; Huang, W.; Zheng, X.; Cheng, L.; Zhang, Z.; Wang, J.; Shen, Z. Proteolysis Targeting Chimera (PROTAC) in Drug Discovery Paradigm: Recent Progress and Future Challenges. *Eur. J. Med. Chem.* **2021**, *210*, 112981.

(8) Wang, X.; Qin, Z. L.; Li, N.; Jia, M. Q.; Liu, Q. G.; Bai, Y. R.; Song, J.; Yuan, S.; Zhang, S. Y. Annual Review of PROTAC Degraders as Anticancer Agents in 2022. *Eur. J. Med. Chem.* **2024**, *267*, 116166.

(9) He, Q.; Zhao, X.; Wu, D.; Jia, S.; Liu, C.; Cheng, Z.; Huang, F.; Chen, Y.; Lu, T.; Lu, S. Hydrophobic Tag-Based Protein Degradation: Development, Opportunity and Challenge. *Eur. J. Med. Chem.* **2023**, *260*, 115741.

(10) Xie, S.; Zhu, J.; Li, J.; Zhan, F.; Yao, H.; Xu, J.; Xu, S. Small-Molecule Hydrophobic Tagging: A Promising Strategy of Druglike Technology for Targeted Protein Degradation. *J. Med. Chem.* **2023**, *66* (16), 10917−10933.

(11) Neklesa, T. K.; Tae, H. S.; Schneekloth, A. R.; Stulberg, M. J.; Corson, T. W.; Sundberg, T. B.; Raina, K.; Holley, S. A.; Crews, C. M. Small-Molecule Hydrophobic Tagging-Induced Degradation of HaloTag Fusion Proteins. *Nat. Chem. Biol.* **2011**, *7* (8), 538−543.

(12) Long, M. J. C.; Gollapalli, D. R.; Hedstrom, L. Inhibitor Mediated Protein Degradation. *Chem. Biol.* **2012**, *19* (5), 629−637.

(13) Hachisu, M.; Seko, A.; Daikoku, S.; Takeda, Y.; Sakono, M.; Ito, Y. Hydrophobic Tagged Dihydrofolate Reductase for Creating Misfolded Glycoprotein Mimetics. *ChemBioChem* **2016**, *17* (4), 300−303.

(14) Go, A.; Jang, J. W.; Lee, W.; Ha, J. D.; Kim, H. J.; Nam, H. J. Augmentation of the Antitumor Effects of PARP Inhibitors in Triple-Negative Breast Cancer via Degradation by Hydrophobic Tagging Modulation. *Eur. J. Med. Chem.* **2020**, *204*, 112635.

(15) Asawa, Y.; Nishida, K.; Kawai, K.; Domae, K.; Ban, H. S.; Kitazaki, A.; Asami, H.; Kohno, J. Y.; Okada, S.; Tokuma, H.; Sakano, D.; Kume, S.; Tanaka, M.; Nakamura, H. Carborane as an Alternative Efficient Hydrophobic Tag for Protein Degradation. *Bioconjugate Chem.* **2021**, *32* (11), 2377−2385.

(16) Li, J.; Liu, T.; Song, Y.; Wang, M.; Liu, L.; Zhu, H.; Li, Q.; Lin, J.; Jiang, H.; Chen, K.; Zhao, K.; Wang, M.; Zhou, H.; Lin, H.; Luo, C. Discovery of Small-Molecule Degraders of the CDK9-Cyclin T1

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753

Complex for Targeting Transcriptional Addiction in Prostate Cancer. *J. Med. Chem.* **2022**, *65* (16), 11034−11057.
(17) Xie, S.; Zhan, F.; Zhu, J.; Sun, Y.; Zhu, H.; Liu, J.; Chen, J.; Zhu, Z.; Yang, D. H.; Chen, Z. S.; Yao, H.; Xu, J.; Xu, S. Discovery of Norbornene as a Novel Hydrophobic Tag Applied in Protein Degradation. *Angew. Chem., Int. Ed.* **2023**, *62* (13), No. e202217246.
(18) Ma, X.; Wang, X.; Chen, F.; Zou, W.; Ren, J.; Xin, L.; He, P.; Liang, J.; Xu, Z.; Dong, C.; Lan, K.; Wu, S.; Zhou, H. B. Novel Acyl Thiourea-Based Hydrophobic Tagging Degraders Exert Potent Anti-Influenza Activity through Two Distinct Endonuclease Polymerase Acidic-Targeted Degradation Pathways. *J. Med. Chem.* **2024**, *67* (11), 8791−8816.
(19) Xu, F.; Zhang, X.; Chen, Z.; He, S.; Guo, J.; Yu, L.; Wang, Y.; Hou, C.; Ai-Furas, H.; Zheng, Z.; Smaill, J. B.; Patterson, A. V.; Zhang, Z. M.; Chen, L.; Ren, X.; Ding, K. Discovery of Isoform-Selective Akt3 Degraders Overcoming Osimertinib-Induced Resistance in Non-Small Cell Lung Cancer Cells. *J. Med. Chem.* **2022**, *65* (20), 14032−14048.
(20) Shi, Y.; Long, M. J. C.; Rosenberg, M. M.; Li, S.; Kobjack, A.; Lessans, P.; Coffey, R. T.; Hedstrom, L. Boc3Arg-Linked Ligands Induce Degradation by Localizing Target Proteins to the 20S Proteasome. *ACS Chem. Biol.* **2016**, *11* (12), 3328−3337.
(21) Neklesa, T. K.; Noblin, D. J.; Kuzin, A.; Lew, S.; Seetharaman, J.; Acton, T. B.; Kornhaber, G.; Xiao, R.; Montelione, G. T.; Tong, L.; Crews, C. M. A Bidirectional System for the Dynamic Small Molecule Control of Intracellular Fusion Proteins. *ACS Chem. Biol.* **2013**, *8* (10), 2293−2300.
(22) Hirai, K.; Yamashita, H.; Tomoshige, S.; Mishima, Y.; Niwa, T.; Ohgane, K.; Ishii, M.; Kanamitsu, K.; Ikemi, Y.; Nakagawa, S.; Taguchi, H.; Sato, S.; Hashimoto, Y.; Ishikawa, M. Conversion of a PROTAC Mutant Huntingtin Degrader into Small-Molecule Hydrophobic Tags Focusing on Drug-like Properties. *ACS Med. Chem. Lett.* **2022**, *13* (3), 396−402.
(23) Gao, N.; Chu, T. T.; Li, Q. Q.; Lim, Y. J.; Qiu, T.; Ma, M. R.; Hu, Z. W.; Yang, X. F.; Chen, Y. X.; Zhao, Y. F.; Li, Y. M. Hydrophobic Tagging-Mediated Degradation of Alzheimer's Disease Related Tau. *RSC Adv.* **2017**, *7*, 40362−40366.
(24) Choi, S. R.; Wang, H. M.; Shin, M. H.; Lim, H. S. Hydrophobic Tagging-Mediated Degradation of Transcription Coactivator SRC-1. *Int. J. Mol. Sci.* **2021**, *22* (12), 6407.
(25) Wang, M.; Lin, R.; Li, J.; Suo, Y.; Gao, J.; Liu, L.; Zhou, L.; Ni, Y.; Yang, Z.; Zheng, J.; Lin, J.; Zhou, H.; Luo, C.; Lin, H. Discovery of LL-K8−22: A Selective, Durable, and Small-Molecule Degrader of the CDK8-Cyclin C Complex. *J. Med. Chem.* **2023**, *66* (7), 4932−4951.
(26) Xie, H.; Liang, J. J.; Wang, Y. L.; Hu, T. X.; Wang, J. Y.; Yang, R. H.; Yan, J. K.; Zhang, Q. R.; Xu, X.; Liu, H. M.; Ke, Y. The Design, Synthesis and Anti-Tumor Mechanism Study of New Androgen Receptor Degrader. *Eur. J. Med. Chem.* **2020**, *204*, 112512.
(27) Buskes, M. J.; Coffin, A.; Troast, D. M.; Stein, R.; Blanco, M. J. Accelerating Drug Discovery: Synthesis of Complex Chemotypes via Multicomponent Reactions. *ACS Med. Chem. Lett.* **2023**, *14* (4), 376−385.
(28) Bhela, I. P.; Ranza, A.; Balestrero, F. C.; Serafini, M.; Aprile, S.; Di Martino, R. M. C.; Condorelli, F.; Pirali, T. A Versatile and Sustainable Multicomponent Platform for the Synthesis of Protein Degraders: Proof-of-Concept Application to BRD4-Degrading PROTACs. *J. Med. Chem.* **2022**, *65* (22), 15282−15299.
(29) Duan, Y.; Guan, Y.; Qin, W.; Zhai, X.; Yu, B.; Liu, H. Targeting Brd4 for Cancer Therapy: Inhibitors and Degraders. *MedChemComm* **2018**, *9* (11), 1779−1802.
(30) Cao, C.; He, M.; Wang, L.; He, Y.; Rao, Y. Chemistries of Bifunctional PROTAC Degraders. *Chem. Soc. Rev.* **2022**, *51* (16), 7066−7114.
(31) Castiello, C.; Junghanns, P.; Mergel, A.; Jacob, C.; Ducho, C.; Valente, S.; Rotili, D.; Fioravanti, R.; Zwergel, C.; Mai, A. Green-MedChem: The Challenge in the next Decade toward Eco-Friendly Compounds and Processes in Drug Design. *Green Chem.* **2023**, *25* (6), 2109−2169.
(32) Bhela, I. P.; Serafini, M.; Del Grosso, E.; Tron, G. C.; Pirali, T. Tritylamine as an Ammonia Surrogate in the Ugi Reaction Provides Access to Unprecedented 5-Sulfamido Oxazoles Using Burgess-Type Reagents. *Org. Lett.* **2021**, *23* (9), 3610−3614.
(33) Giovenzana, G. B.; Tron, G. C.; Di Paola, S.; Menegotto, I. G.; Pirali, T. A Mimicry of Primary Amines by Bis-Secondary Diamines as Components in the Ugi Four-Component Reaction. *Angew. Chem. Int. Ed.* **2006**, *45* (7), 1099−1102.
(34) Klein, V. G.; Bond, A. G.; Craigon, C.; Lokey, R. S.; Ciulli, A. Amide-to-Ester Substitution as a Strategy for Optimizing PROTAC Permeability and Cellular Activity. *J. Med. Chem.* **2021**, *64* (24), 18082−18101.
(35) Kadowaki, H.; Nishitoh, H. Signaling Pathways from the Endoplasmic Reticulum and Their Roles in Disease. *Genes* **2013**, *4* (3), 306−333.
(36) Hu, J.; Pan, D.; Li, G.; Chen, K.; Hu, X. Regulation of Programmed Cell Death by Brd4. *Cell Death Dis.* **2022**, *13* (12), 1059.

https://doi.org/10.1021/acsomega.4c09726
ACS Omega 2025, 10, 4745−4753