doi:10.2533/chimia.2025.665 Chimia 79 (2025) 665–667

FH HES Universities of Applied Sciences
Fachhochschulen – Hautes Ecoles Spécialisées

Greener Synthesis of a Potent Proteolysis Targeting Chimera (PROTAC) Degrader of Indoleamine-2, 3-Dioxygenase 1 (hDOI1)

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Abstract: The synthesis of the potential indoleamine-2,3-dioxygenase 1 (IDO1) degrader proteolysis-targeting chimeras (PROTAC) using sustainable reagents and technologies such as ionic liquids and photochemical couplings was investigated. The ligand to the protein of interest, an indole derivative, was synthesized successfully for three substrates bearing different substituents (NO_2, $(CF_3)_2$ and H) over three steps with good to satisfactory results with overall yields ranging from 21 to 71%. However, the coupling with the ubiquitin ligase, pomalidomide, was unsuccessful due to the degradation of the reactive diazoketone functional group.

Keywords: Indoles · Photochemistry · Pomalidomide · PROTACS

1. Introduction

Proteolysis-targeting chimeras (PROTACs) are heterobifunctional molecules that can both bind to a target protein and a ubiquitin ligase, thus enabling the ubiquitination of the target and its subsequent degradation. This mechanism allows the selective degradation of proteins otherwise difficult to target by traditional small molecules.[1,2]

Herein, we report the synthesis of potential indoleamine 2,3-dioxygenase 1 degraders which have been proven to be interesting targets for cancer immunotherapy.[3,4] The family of PROTAC targeted in this study is composed of an indole derivative as a ligand to the protein of interest and of a pomalidomide moiety as the ubiquitin ligase. These two structures are linked by dioxane linker as shown in Fig. 1.

The choice of the indole derivative moiety was based on the biological inhibition tests against human indoleamine 2,3-dioxygenase (hIDO) and tryptophan 2,3-dioxygenase (TDO_2). Indeed, in the context of our collaboration with Prof. S. Sen, we have reported a library of C2-aroyl indole derivatives that has demonstrated substantial inhibition of hIDO1.[5] The most active compound *N*-(2-(2-(4-nitrobenzoyl)-1H-indol-3-yl)ethyl)acetamide **2a**, inhibits hIDO1 at 180 nM IC_{50} and also demonstrated the inhibition of the proliferation of numerous cancer cells such as HeLa, A549, MCF7, HCT116 and MDA-MB-231 in low to mid nanomolar range IC_{50}s. As a proof-of-concept study, compound **2a** was connected to pomalidomide **4**, an E3 ligase binding ligand, through dioxyalkyl linker **1** and it was observed that the resulting PROTAC molecule **6a** induced persistently moderate degradation of hIDO1 with maximum degradation of 59% in HeLa cells. Flow cytometric analysis indicated that IFN-γ induced an increase of IDO1, which was reduced by compound **6a** (Table 1).

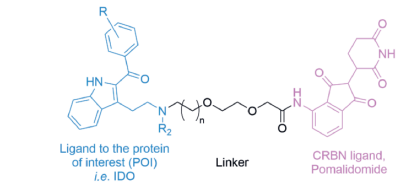
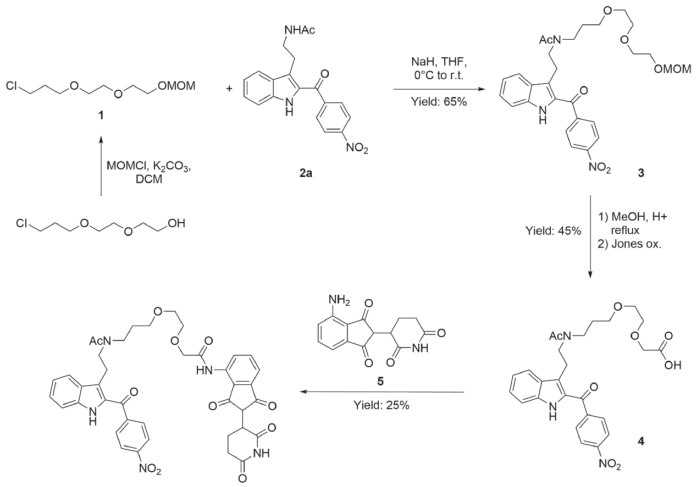


Fig. 1. General structure of a PROTAC.

Table 1. Synthesis of IDO-PROTAC degrader **6a** from **2a** and biological activity (IC_{50}) tests results of **2a**.



MCF7	A549	HeLa	HCT 116	MDA-MB-231
1.00 µM	0.76 µM	0.32 µM	> 5 µM	1.2 µM

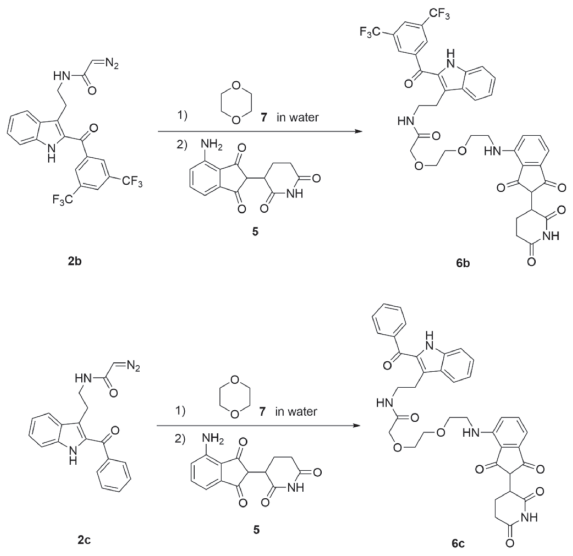
Based on these studies we herein propose to identify a PROTAC hIDO1 degrader that could inhibit the proliferation of lung/breast cancer cells. We aim to improve the lead **6a** to be a more potent PROTAC degrader of hIDO1 by connection of *N*-(2-(2-(3,5-bis(trifluoromethyl)benzoyl)-1H-indol-3-yl)ethyl) acetamide **2b** and methyl(2-(2-benzoyl-1H-indol-3-yl)ethyl) carbamate **2c** to obtain the PROTAC degrader **6b** and **6c** while performing the last synthetical step (coupling of the two biologically active moieties on the linker) in a photochemical manner using a blue LED as an eco-friendly alternative to an organic coupling agent (Scheme 1).

2. Results and Discussion

2.1 *Synthesis of Indole Moiety*

The first step consists of a Pictet-Spengler reaction between tryptamine and a benzaldehyde derivative to afford the tetrahydro-β-carboline ring. This reaction was carried out in an ionic liquid which offered both solvent and catalytic properties.

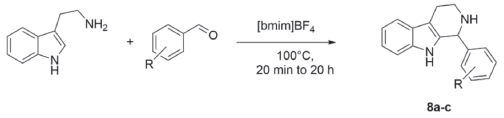
The reaction time was highly dependent on the substituent present on the benzaldehyde moiety, as electron withdrawing groups enhance the aldehyde electrophilic properties which in



Scheme 1. Eco-friendly coupling route.

return accelerates the formation of the imine intermediate. Thus, 4-nitrobenzaldehyde forms the tetrahydro-β-carboline product **8a** in only 20 minutes at room temperature (Table 2, Entry 1) and benzaldehyde requires consistent heat for 20 hours to yield the compound **8c** (Table 2, Entry 3).[6] By fine tuning the reaction time for each reaction, products could be isolated without presence of impurities or side products and could be readily used for the next step, an *N*-alkylation reaction.

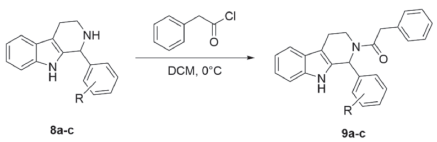
Table 2. Results of the Pictet-Spengler reaction.



Entry	Compound	R	Time [h]	Yield [%]
1	8a	4-NO_2	0.3	97
2	8b	3, 5-CF_3	8	76
3	8c	H	20	47

Alkylations were initially performed with acetyl chloride. This highly reactive partner yielded the desired product efficiently (up to 98% yield) with little to no side product. However, subsequent reactions could not be efficiently carried out. To solve this issue, new alkylating partners were investigated. Phenylacetyl chloride was selected for its increased reactivity over less activated partners such as methyl phenyl acetate. The bulkiness of the phenyl substituent helped stabilize the oxidation product. The *N*-alkylated products **9a-c** were obtained in good to excellent yield after 3 hours of reaction at room temperature (Table 3, Entry 1–3).[7] The isolated crude was used without further purification for the following step.

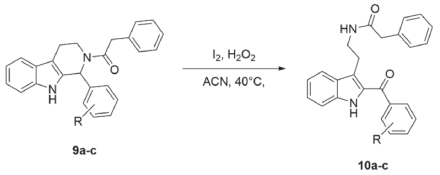
Table 3. Results *N*-alkylation.



Entry	Compound	R	Yield [%]
1	9a	4-NO_2	94
2	9b	3,5-CF_3	98
3	9c	H	50

The ring opening oxidation was smoothly carried out using iodine in the presence of hydrogen peroxide to form the strong oxidizing agent HOI. This step was successfully conducted and did not require any modification from the published route.[6] Again, the isolated products **10a-c** were pure enough to allow the subsequent step without purification (Table 4, Entry 1–3).

Table 4. Results ring opening oxidation.



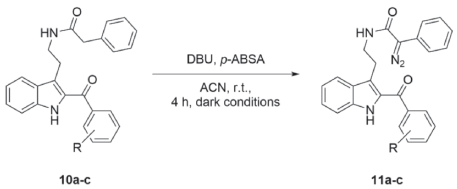
Entry	Compound	R	Time [h]	Yield [%]
1	10a	4-NO_2	4.5	60
2	10b	3,5-CF_3	4	96
3	10c	H	4.5	89

2.2 *Formation of the Photosensitive Diazo Ketone*

The photochemical coupling step requires a photolabile functional group, diazo, which can be installed by reaction with *p*-ABSA (4-acetamidobenzensulfonyl azide) as a diazo donor and under DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) catalysis.[8]

The characterization of these diazoketones is not trivial as they are sensitive to light and air. ^{1}H-NMR spectra indicated the formation of the diazoketone **11b** (Table 5, Entry 2). The other substrates **10a** and **10c**, however, did not show any conversion to **11a** and **11c** (Table 5, Entries 1 and 3).

Table 5. Results from diazoketone formation.



Entry	Compound	R	Yield
1	11a	4-NO_2	0
2	11b	3,5-CF_3	98
3	11c	H	0

2.3 *Photochemical Coupling Reaction*

The last step to obtain the desired PROTAC consists of a photochemical coupling reaction between the previously formed diazoketone and dioxane (which undergoes ring opening) followed by the addition of Pomalidomide, the ubiquitine ligase. To determine the absorption range and facilitate the LED selection, a UV absorption spectrum of the diazo compound **11b** was performed. A maximum absorption is achieved at 272 nm. Unfortunately, no lamp with such wavelength was available. Trials at 265 nm were performed, but with no conversion. It seems that the diazo compound undergoes degradation through Wolff rearrangement before being able to react with the linker and the ubiquitine ligase. This hypothesis was confirmed by elemental analysis that proved the presence of the ketone instead of the diazoketone. It seems that the diazo compound is not stable enough to be isolated. This

decomposition might be avoided by generating it *in situ* and performing the photochemical coupling as a one-pot reaction.

3. Conclusion

The synthesis of the potential indoleamine-2,3-dioxygenase 1 (hIDO1) degrader through sustainable steps was studied. The indole moiety was successfully obtained with global yields ranging from 21 to 72% depending on the benzaldehyde derivative selected. The diazoketone was only successfully formed for substrate **10b**, which quickly underwent Wolff rearrangement to form the corresponding ketene, which was further degraded into the ketone derivative according to elemental analysis. A solution to avoid degradation would be the *in situ* formation of the diazo followed by direct photochemical coupling with 1,4-dioxane and pomalidomide. This route was not explored due to a lack of material. Indeed, a lamp corresponding to the maximal absorbance wavelength of 272 nm and with sufficient power would be necessary, which was not available to us at the time of the experiment. In addition, a different route for the formation of the reactive diazoketone would be necessary for substrates **10a** and **10c** as they showed no reactivity under the conditions tested.

Acknowledgements

L. G. would like to thank the Chemistry Department, as well as the ChemTech Institute, of University of Applied Sciences Western Switzerland, Faculty of Engineering and Architecture, for the technical, operational, as well as the financial support. S. S. is thankful to Shiv Nadar Institution of Eminence, deemed to be University for funding and facility. We would like also to thank Prof. C. Bochet (UNIFR) for letting us use this equipment for photochemical reactions.

Received: June 17, 2025

[1] J. Liu, J. Ma, Y. Liu, J. Xia, Y. Li, Z. P. Wang, W. Wei, *Semin. Cancer Biol.* **2020**, *67*, 171, https://doi.org/10.1016/j.semcancer.2020.02.006.

[2] R. J. Deshaies, *Nat. Chem. Biol.* **2015**, *11*, 634, https://doi.org/10.1038/nchembio.1887.

[3] N. Pradhan, S. Paul, S. J. Deka, A. Roy, V. Trivedi, D. Manna, *ChemistrySelect* **2017**, *2*, 5511, https://doi.org/10.1002/slct.201700906.

[4] L. R. Bollu, P. V. Bommi, P. J. Monsen, L. Zhai, K. L. Lauing, A. Bell, M. Kim, E. Ladomersky, X. Yang, L. C. Platanias, D. E. Matei, M. G. Bonini, H. G. Munshi, R. Hashizume, J. D. Wu, B. Zhang, C. D. James, P. Chen, M. Kocherginsky, C. Horbinski, M. D. Cameron, A. A. Grigorescu, B. Yamini, R. V. Lukas, G. E. Schiltz, D. A. Wainwright, *J. Med. Chem.* **2022**, *65*, 15642, https://doi.org/10.1021/acs.jmedchem.2c00771.

[5] J. Chauhan, S. R. Maddi, K. D. Dubey, S. Sen, *Front. Chem.* **2021**, *9*, 691319, https://doi.org/10.3389/fchem.2021.691319.

[6] J. Chauhan, T. Luthra, S. Sen, *Eur. J. Org. Chem.* **2018**, *2018*, 4776, https://doi.org/10.1002/ejoc.201800879. "In a three neck flask, [bmim]BF_4 (10.0 ml) was loaded and dried under high vacuum for 30 minutes. The system was intertized with three vacuum/argon cycles. Then, tryptamine (2.01 g, 12.5 mmol, 1 eq.) was loaded and benzaldehyde derivative (10.0 mmol, 0.8 eq.) added dropwise. The mixture was heated up to 100°C and stirred for 30 min to 20 h depending on the benzaldehyde derivative used. The end of the reaction was checked by TLC (cyclohexane, EtOAc). The RM was diluted with EtOAc and extracted with 4 × 20 ml of toluene. The organic phase was dried under Na_2SO_4, filtered the solvent evaporated under reduced pressure to afford **8a-c**. In an argon purged three neck flask, **9a**, **b** or **c** (2.25 mmol, 1 eq.) was loaded. Acetonitrile (22 ml) and molecular iodine (0.19 g, 0.67 mmol, 0.30 eq.) were added. Then, hydrogen peroxide (35% aq. solution, 0.39 ml) was slowly added dropwise and heated up to 40°C. The RM was stirred for 4 – 4.5 h. The end of reaction was determined by TLC (cyclohexane/EtOAc). The reaction was quenched with saturated sodium thiosulfate and extracted with 3 × 20 ml of EtOAc. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford **10a** and **10c**. **10b** was isolated by filtration of the white precipitate and washing of the cake with iced water."

[7] P. V. Joshi, A. A. Sayed, A. RaviKumar, V. G. Puranik, S. S. Zinjarde, *Eur. J. Med. Chem.* **2017**, *136*, 246, https://doi.org/10.1016/j.ejmech.2017.05.002. "In an inertized three neck flask **8a**, **b** or **c** (8.8 mmol, 1 eq.) were loaded. Anhydrous DCM (50 ml) was added and the mixture was cooled down to 0°C. Phenylacetyl chloride (8.8 mmol, 1 eq.) was added dropwise and the reaction was stirred at 0°C for 3 h. The end of reaction was controlled by TLC (cyclohexane/EtOAc). The reaction was quenched with 30 ml of Na_2CO_3 aq. 10%. and transferred into a separating funnel and washed with 3 × 20 ml of water (until pH neutral). The organic phase was dried under Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to afford **9a** and **9c**. **9b** was isolated by filtration of the precipitate and washing of the cake with dichloromethane until disappearing of the yellow coloration."

[8] D. Maiti, R. Das, S. Sen, *Green Chem.* **2021**, *23*, 8533, https://doi.org/10.1039/D1GC02797B. "In an oven dried and inertized three neck flask wrapped in aluminium foil, **10b** (1.54 mmol, 1 eq.) was loaded and dissolved in acetonitrile (10 ml). DBU (1.85 mmol, 1.2 eq.) was added dropwise. the reaction mixture was stirred at r.t. for 10 min and *p*-ABSA (1.85 mmol, 1.2 eq.) was added. The reaction mixture was stirred for 4 h and reaction completion was check by TLC (toluene/EtOH 95:5). The solvent was evaporated under reduced pressure (without heating) and the crude was diluted in DCM and washed with 2 × 50 ml water and 2 × 5 ml brine. The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure (without heating)."