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Use of ionic liquids in amidation reactions for proteolysis targeting chimera synthesis†

Michela Eleuteri,* Jenny Desantis, Gabriele Cruciani, Raimondo Germani and Laura Goracci

Selective degradation of disease-causing proteins using proteolysis targeting chimeras (PROTACs) has gained great attention, thanks to its several advantages over traditional therapeutic modalities. Despite the advances made so far, the structural chemical complexity of PROTACs poses challenges in their synthetic approaches. PROTACs are typically prepared through a convergent approach, first synthesizing two fragments separately (target protein and E3 ligase ligands) and then coupling them to produce a fully assembled PROTAC. The amidation reaction represents the most common coupling exploited in PROTACs synthesis. Unfortunately, the overall isolated yields of such synthetic procedures are usually low due to one or more purification steps to obtain the final PROTAC with acceptable purity. In this work, we focused our attention on the optimization of the final amidation step for the synthesis of an anti-SARS-CoV-2 PROTAC by investigating different amidation coupling reagents and a range of alternative solvents, including ionic liquids (ILs). Among the ILs screened, [OMIM][ClO_4] emerged as a successful replacement for the commonly used DMF within the HATU-mediated amidation reaction, thus allowing the synthesis of the target PROTAC under mild and sustainable conditions in very high isolated yields. With the optimised conditions in hand, we explored the scalability of the synthetic approach and the substrate scope of the reaction by employing different E3 ligase ligand (VHL and CRBN)-based intermediates containing linkers of different lengths and compositions or by using different target protein ligands. Interestingly, in all cases, we obtained high isolated yields and complete conversion in short reaction times.

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Introduction

In the last two decades, proteolysis targeting chimeras (PROTACs) technology has emerged as a new therapeutic approach with the potential to revolutionize drug discovery.[1,2] Since the synthesis of the first PROTAC in 2001 by Crews and Deshaies,[3] PROTACs have received considerable attention from both academic and industrial laboratories worldwide due to their abilities to induce targeted degradation of pathogenic proteins.[4]

Structurally, PROTACs are heterobifunctional molecules comprising a ligand targeting a protein of interest (POI), a ligand binding an E3 ligase, and a connecting linker.[1] The formation of a ternary complex between the POI, PROTAC molecule, and the recruited E3 ligase brings the two proteins in spatial proximity to each other, thus facilitating ubiquitin transfer from the E3 ligase to the lysine residues of the POI. Once the POI is ubiquitinated, it is recognized and degraded by the 26S proteasome.[1] Among the three components, the linker moiety plays a key role in the biological activity of PROTACs. In fact, the length, composition, and flexibility of the linker are pivotal for the formation of a productive ternary complex and degradation activity. Additionally, linker–protein interactions can also significantly impact the stability of the POI–PROTAC–E3 ternary complex.[5,6] In general, the modular nature of PROTACs makes them well suited for parallel synthesis, enabling a rapid and efficient generation of PROTAC libraries in order to identify a promising degrader.[5]

Thanks to their event-driven mechanism of action, PROTACs possess several advantages over traditional occupancy-driven small molecule inhibitors, including overcoming potential resistance to therapeutic treatments.[7,8] To date, 26 PROTAC degraders have entered clinical studies.[9] Among them, PROTACs ARV-110 and ARV-471, both developed by Arvinas (for prostate cancer and breast cancer), are in phase II and III clinical trials, respectively.[10] So far, PROTAC technology has been successfully used to degrade several distinct target proteins associated with various diseases, such as cancer,

Department of Chemistry, Biology, and Biotechnology, University of Perugia, Italy. E-mail: michela.eleuteri1@studenti.unipg.it

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immune disorders, neurodegenerative conditions, cardiovascular diseases and viral infections.[7,11–13] Given the growing success of PROTAC technology, there is a lot of attention being paid to the optimization of their current synthetic strategies. In particular, the large size of these molecules (molecular weight of 600–1900 Da) coupled with their intricate structures demands increased synthetic efforts.

From a synthetic chemistry point of view, PROTAC derivatives are typically prepared through three main approaches: (1) coupling of the linker to the E3 ligand before attaching it to the POI (Approach A, Fig. 1), (2) functionalization of the POI with the linker followed by its coupling to the E3 ligand (Approach B, Fig. 1), and (3) binding of two linker fragments on both the POI and E3 ligands, followed by their final coupling (Approach C, Fig. 1).[5,14–16]

Notably, the amide bond is not only one of the most fundamental functional group linkages in biomolecules and pharmaceuticals but also the most exploited linkage in the design and synthesis of PROTACs. In fact, PROTAC components are often combined *via* late-stage amide couplings, due to the reliability and robustness of amide bond formation. According to the statistics made on the largest web-accessible database dedicated to PROTACs (PROTAC-DB 2.0, **https://cadd.zju.edu.cn/protacdb/**)[17] (Fig. 2), among the total of 3270 reported PROTACs (data accessed on 27th November 2023), 83% contain an amide bond as a connection between modules. More in detail, among them, the amide linkage is variously exploited to connect the linker moiety to the POI ligand (31%), the E3 ligase ligand (22%), both (16%), or even two E3 ligase ligands (1%). Additionally, to a lesser extent, it has also been used as a connection for two linker fragments following Approach C (13%).

Despite the wide use of amidation reactions, when the last coupling reaction for PROTACs synthesis is amidation, the isolated yields are commonly low, probably due to the formation of by-products and impurities. Thus, the purification step might be difficult and tricky, and sometimes two or three successive purifications are needed to obtain the final PROTAC compounds with acceptable purity for biological evaluation (>95%). Furthermore, only a few conditions for the amide coupling in the synthesis of PROTACs have been reported so far in the literature (Fig. 3).[5,14,18] The use of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) as an amidation coupling reagent in the presence of *N*,*N*-diisopropylethylamine (DIPEA) as the base remains the most dominant, followed, to a lesser extent, by other coupling agents and combinations of bases such as *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide

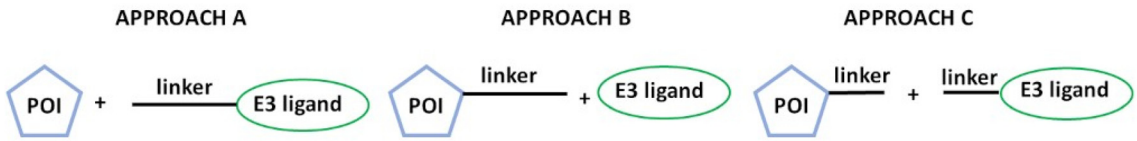


Fig. 1 A schematic representation of the three main synthetic approaches used for PROTAC synthesis.

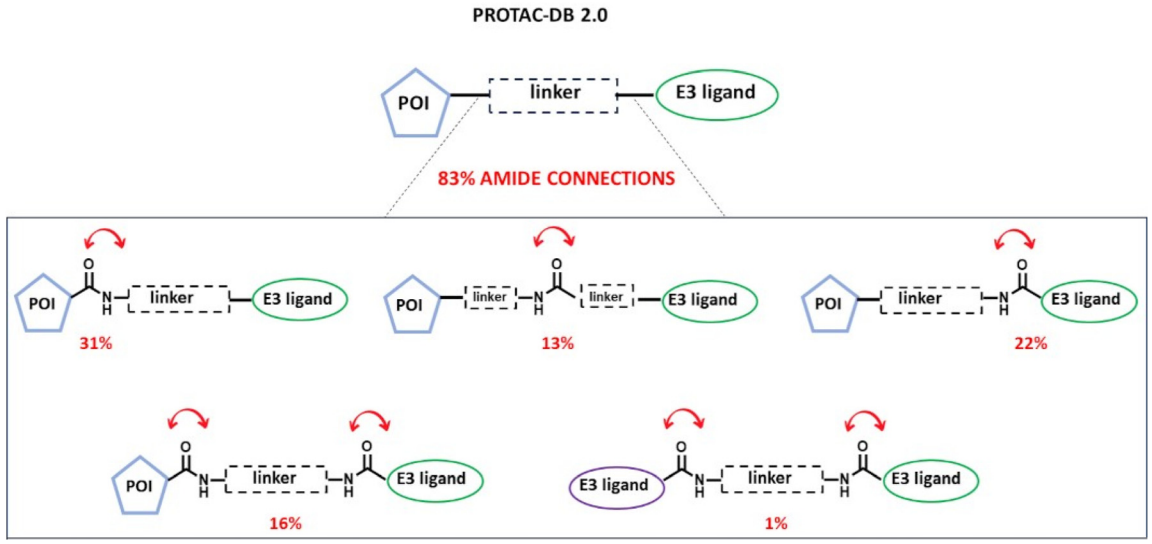


Fig. 2 Overview of the presence of the amide bond as a connection at different positions of a PROTAC molecule based on the statistics of the available structures deposited in PROTAC-DB 2.0 (data accessed on 27th November 2023).[17] Percentages indicate the relative abundance of the total of 3270 PROTACs endowed with one or two amide connections.

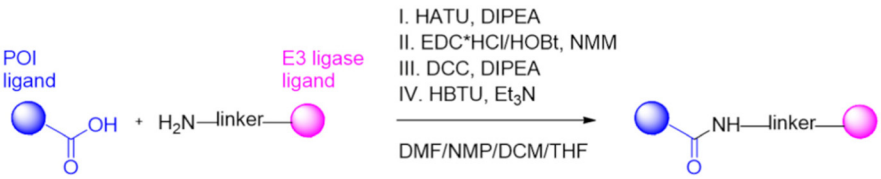


Fig. 3 Typical reported conditions in the literature for the last amide coupling for PROTACs synthesis.

hydrochloride (EDC*HCl)/1-hydroxybenzotriazole (HOBt) with *N*-methylmorpholine (NMM), dicyclohexylcarbodiimide (DCC) with DIPEA, and *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) with triethylamine (Et_3N).[5,15,18] Concerning the reaction solvent, these reactions are typically conducted in hazardous dipolar aprotic solvents such as anhydrous *N*,*N*-dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), dichloromethane (DCM) or tetrahydrofuran (THF) depending on the solubility of PROTAC building blocks. Among them, DMF is the most exploited one, thanks to its high solvency power.

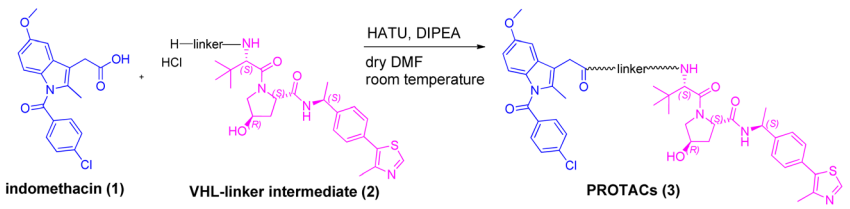
To date, our group's interest in targeted protein degradation applied to different research fields[19–22] led us to synthesize a wide structurally heterogeneous library of more than 100 PROTACs. During the preparation of this library, we obtained consistently low yields, particularly in the last coupling synthetic amidation step, thus recognizing the need for exploring and identifying better reaction parameters/conditions.

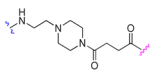
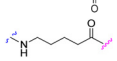
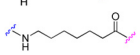
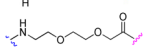
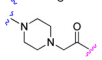
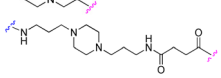
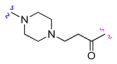
In this work, we report an in-depth study aimed at exploring and optimizing the final amidation step for the synthesis of PROTAC derivatives. Through the evaluation of alternative solvents, we identified an ionic liquid as a valid replacement for DMF within the HATU-mediated amidation reaction, thus allowing the synthesis of PROTACs under mild and sustainable conditions with very high yields.

Results and discussion

We began our investigation on PROTACs synthesis by focusing on the amidation reaction between indomethacin (INM) (**1**) and the suitable E3 ligase ligand-linker intermediate (Von Hippel–Lindau (VHL) as the E3 ligase) (**2**) to prepare anti-SARS-CoV-2 INM-based PROTACs (**3**), previously synthesized and reported by us (Table 1).[21,22] In particular, by following common Approach A (Fig. 1), PROTACs **3a–3g** were synthesized by an amidation reaction in the presence of HATU and DIPEA in anhydrous DMF at room temperature in poor isolated yields (17–32%) (Table 1).[21,22]

Table 1 Anti-SARS-CoV-2 INM-based PROTACs synthesised in our previous work[a]



INM-based PROTACs	Linker	Time (h)	Yield[b] (%)
3a		16	24
3b		16	28
3c		16	31
3d		5	17
3e		18	19
3f		3	28
3g		4	32

[a] Reactions were performed on a 0.059–0.139 mmol scale with **1** (1.0 equiv.), 1.0 equiv. of **2**, 1.25 equiv. of HATU, 3.0 equiv. of DIPEA, in dry DMF at 0.059–0.139 M concentration. [b] Isolated yields.

Indeed, in PROTACs synthesis, HATU is the coupling agent most used for amidation coupling (as reported in the literature, it reduces the risk of epimerization in the coupling of chiral compounds),[23,24] while DMF is the preferred solvent due to the high solubility that PROTAC building blocks have in it.[5,25] Initially, we selected the synthesis of INM-based PROTAC **3a**, emerged as one of the most active compounds in inhibiting SARS-CoV-2 *in vitro*, as a case study to investigate and optimize the reaction conditions.

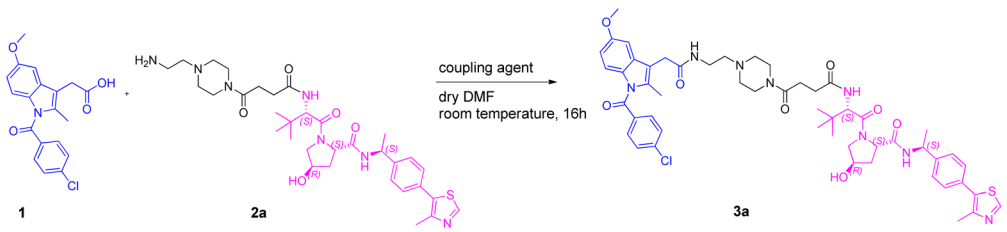
In our first attempt to explore the reaction conditions, the amidation reaction step for the synthesis of PROTAC **3a** was carried out using other coupling agents than HATU (Table 2).[26] In particular, five of the most common amide coupling agents or reagent combinations were selected, *i.e.*, (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU),[27–29] dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP),[30,31] EDC*HCl/HOBt,[31,32] (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP)[33] and HBTU[26] (Table 2, entries 1–5). All reactions were stirred in dry DMF at room temperature using DIPEA or Et_3N as the base and stopped, in the first evaluation, after 16 h. For comparison purposes, the reaction was re-run under the same conditions in the presence of HATU (Table 2, entry 6). All reactions proceeded until the complete conversion of both starting materials (as monitored by TLC), but the isolated yields (Table 2, entries 1–6) were anyway low (<34%) and in the same range as that of our case study (Table 2, entry 6).

Although COMU provided PROTAC **3a** in a slightly higher yield than HATU, we decided to use HATU for subsequent investigations as it is the 'gold standard' reagent[23] for amide formation and PROTACs synthesis in both liquid and solid phases showing good versatility and reliability. Additionally, it is known to reduce the risk of racemization in compounds that have multiple chiral centers.[34,35]

Thus, by maintaining HATU as the coupling agent and DIPEA as the base, different solvents than the hazardous DMF were evaluated, including emerging greener alternatives such as CPME and 2-Me–THF. All reactions were conducted at room temperature and stopped after 16 h. While DCM gave the same results as DMF (Table 3, entry 1), in the case of CPME and 2-Me–THF (Table 3, entries 2 and 3), the conversion was below 100% due to incomplete solubilization of the starting materials, thus affording lower final yields.

Aiming at exploring further sustainable and safe solvents able to replace DMF, we decided to explore the use of ionic liquids (ILs). Indeed, their unique features, such as low volatility, non-flammability, good thermal and chemical stability, and the ability to dissolve both organic and inorganic compounds,[36,37] represent intriguing advantages over conventional organic volatile, flammable, and toxic solvents.[38–40] The high efficiency of ILs as solvents and/or catalysts for the direct amidation of carboxylic acid and amine has already been reported in the literature, arousing considerable interest.[41–48] Additionally, ILs have been extensively used in pharmaceutical fields as reaction media for the synthesis of pharmaceutical compounds,[36,49,50] including non-steroidal anti-inflammatory drugs[51] and antiviral,[52,53] antimicrobial,[54,55] antimalarial,[56] and antitumor agents.[57,58] Here, a set of differently structured ILs were explored as reaction media for the synthesis of PROTAC **3a**, including room-temperature ionic liquids (RTILs), such as 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ([OMIM][NTf_2]), 1-methyl-3-octyl-imidazolium-hexafluorophosphate ([OMIM][PF_6]), 1-methyl-3-octyl-imidazolium-perchlorate ([OMIM][ClO_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][NTf_2]) (Table 3, entries 4–9), and solid-state ionic liquids (SSILs), such as tributylmethylammonium methanesulfonate ([TBMA][MsO]) (Table 3, entry 10). Among them, [OMIM][ClO_4] is the only IL that has never been reported before; conversely, the anion $[ClO_4]^-$ has already been widely reported in the lit-

Table 2 Coupling agent screening for the synthesis of PROTAC **3a**[a]

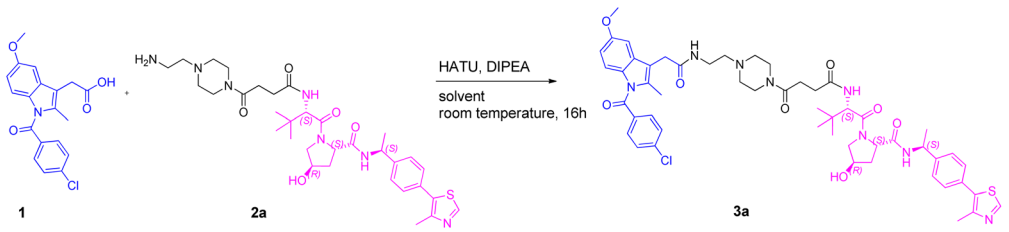


1 2a 3a

Entry	Coupling agent (1.25 eq.)	Base (3.0 eq.)	Solvent (0.056 M)	Time (h)	Conversion[b] (%)	Yield of **3a**[c] (%)
1	HBTU	Et_3N	DMF	16	100	25
2	DCC/DMAP	DIPEA	DMF	16	100	31
3	HOBt/EDC*HCl	DIPEA	DMF	16	100	23
4	PyBOP	DIPEA	DMF	16	100	26
5	COMU	DIPEA	DMF	16	100	34
6	HATU	DIPEA	DMF	16	100	28

[a] Reaction conditions: **1** (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), coupling agent (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), dry DMF (0.056 M), room temperature, 16 h. [b] Monitored by TLC with respect to consumption of both starting materials. [c] Isolated yield.

Table 3 Solvent screening for the HATU-mediated synthesis of PROTAC **3a**[a]



1 2a 3a

INM-based PROTACs	Coupling agent (1.25 eq.)	Base (3.0 eq.)	Solvent (0.056 M)	Time (h)	Conversion (%)	Yield of **3a**[d] (%)
1	HATU	DIPEA	DCM	16	100[b]	28
2	HATU	DIPEA	CPME	16	85.54[c]	14
3	HATU	DIPEA	2-Me–THF	16	82.13[c]	17
4	HATU	DIPEA	[OMIM][NTf_2]	16	100[b]	55
5	HATU	DIPEA	[OMIM][PF_6]	16	100[b]	68
6	HATU	DIPEA	[OMIM][ClO_4]	16	100[b]	75
7	HATU	DIPEA	[BMIM][BF_4]	16	100[b]	68
8	HATU	DIPEA	[BMIM][PF_6]	16	100[b]	73
9	HATU	DIPEA	[BMIM][NTf_2]	16	72.48[c]	30
10	HATU	DIPEA	[TBMA][MsO]	16	71.9[c]	32
11	—	—	[TBMA][PF_6]	—	—	—

[a] Reaction conditions: **1** (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), solvent (0.056 M), room temperature, 16 h. [b] As monitored by TLC with respect to consumption of both starting materials. [c] As monitored by HPLC with respect to consumption of **1**. [d] Isolated yield.

erature for the synthesis of ILs used for different purposes.[59–64]

Overall, as reported in Table 3, the use of RTILs proved beneficial for the synthesis of **3a** achieved under mild reaction conditions with an impressive improvement in the reaction yield, as compared to those obtained with DMF or other organic solvents. In fact, the replacement of DMF with [OMIM][PF_6] (entry 5), [OMIM][ClO_4] (entry 6), [BMIM][BF_4] (entry 7) and [BMIM][PF_6] (entry 8) afforded the highest yields, reaching up to 68–75%. Conversely, the reactions performed in [OMIM][NTf_2] (entry 4), [BMIM][NTf_2] (entry 9), and [TBMA][MsO] (entry 10, in this case, the reaction was performed at 75 °C) gave lower yields. Moreover, the use of [BMIM][NTf_2] or [TBMA][MsO] led to an incomplete conversion of **1**.

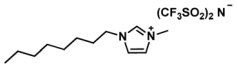
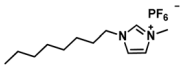
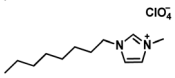
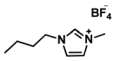
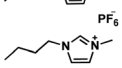
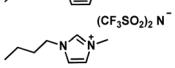
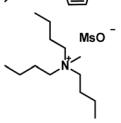
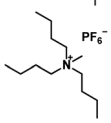
Since reactions performed in ILs with $[PF_6]^-$ anions provided high yields (Table 3, entries 5 and 8), we also synthesized [TBMA][PF_6] (entry 11). Unfortunately, [TBMA][PF_6] provided a solid at room temperature, with a high melting point (129.6–131.1 °C) (Table 4), thus not compatible with the mild reaction conditions applied to PROTAC synthesis.

By analyzing the structural properties of the ILs used for the synthesis of **3a**, it can be observed that RTILs with the large anion $[NTf_2]^-$ performed poorly (Table 3, entries 4 and 9) when compared to those with smaller anions, such as $[PF_6]^-$, $[ClO_4]^-$ and $[BF_4]^-$ (Table 3, entries 5–8). Although the overall properties of ILs result from the composite properties of the cation and anion, the latter plays a fundamental role in determining the physicochemical properties of the ILs including the melting point, viscosity and density.[65–69] According to the literature, the $[NTf_2]^-$ anion offers imidazolium-based ionic liquids with low viscosity, while the $[BF_4]^-$, $[PF_6]^-$ and $[ClO_4]^-$ anions contribute significantly to increasing the viscosity. By comparing the length of the alkyl chains on the imidazolium ring, explored in our study, with the anions, the viscosity can be broadly depicted as [BMIM][NTf_2] < [OMIM][NTf_2] < [BMIM][BF_4] < [BMIM][PF_6] < [OMIM][ClO_4] < [OMIM][PF_6] (Table 4). A different pattern can be observed for the density of the described RTILs, which is closely related to the mass of the anion. In particular, for the same cation, an increase in the mass of the anion corresponds to an increase in density as follows: [OMIM][ClO_4] < [OMIM][PF_6] < [OMIM][NTf_2] and [BMIM][BF_4] < [BMIM][PF_6] < [BMIM][NTf_2] (Table 4).

By analysing the structural and physicochemical properties of the ILs used for the synthesis of **3a**, it can be noted that among the RTILs explored, [OMIM][PF_6], [OMIM][ClO_4], [BMIM][BF_4], and [BMIM][PF_6] (Table 3 entries 5, 6, 7 and 8, respectively) allowed us to achieve the highest yields, and were all characterized by higher values of viscosity and lower values of density at 20–25 °C (Table 4).

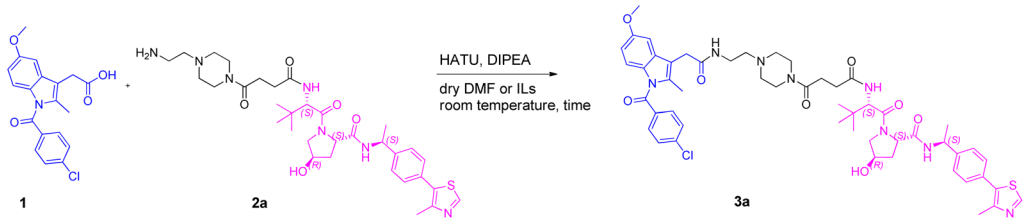
To further explore the reaction conditions with the four best performing ILs, the amidation reaction for the synthesis of **3a** in [OMIM][PF_6], [OMIM][ClO_4], [BMIM][BF_4], and [BMIM][PF_6] was repeated, and each reaction was stopped once the total conversion of the starting materials was achieved (Table 5, entries 1–4). Interestingly, as shown in Table 5, all four RTILs afforded PROTAC **3a** in high yields (68–77%) in a short reaction time from 1 h to 2 h. Among them, [OMIM][ClO_4] (Table 5, entry 1) furnished the target compound in the shortest reaction time (1 h), while a slightly longer reaction time (1.5–2 h) was required for the reactions in [OMIM][PF_6], [BMIM][BF_4] and [BMIM][PF_6] (Table 5, entries 2–4).

Table 4 Physical properties of ionic liquids used in this work

Ionic liquid	Structure	Molecular weight (g mol^{-1})	Viscosity[b] (cP)	Density[c] (g cm^{-3})	Melting point[d] (°C)	Physical state (25 °C)
[OMIM][NTf_2]		475.47	86 (25 °C)	1.32 (25 °C)	<RT	Liquid
[OMIM][PF_6]		340.29	608 (25 °C)	1.24 (24 °C)	−74	Liquid
[OMIM][ClO_4]		294.77	432.1 (20 °C)[a]	1.07 ± 0.02 (20 °C)[a]	−57/−58[a]	Liquid
[BMIM][BF_4]		226.02	233 (25 °C)	1.20 (25 °C)	−82	Liquid
[BMIM][PF_6]		284.19	400 (25 °C)	1.37 (25 °C)	−8	Liquid
[BMIM][NTf_2]		419.36	52 (25 °C)	1.42 (25 °C)	−4	Liquid
[TBMA][MsO]		295.48	—	1.02 (80 °C)	70–72	Solid
[TBMA][PF_6]		345.35	—	—	129.6–131.1[a]	Solid

[a] Values determined experimentally *in house*. [b] Data reported by the seller Iolitec; values reported for ILs that have a purity >99% and a water content <250 ppm for [BMIM][BF_4], [BMIM][PF_6], [OMIM][PF_6], and <100 ppm for [BMIM][NtF_2], [OMIM][NtF_2]. [c] Data reported by the seller Iolitec; values reported for ILs that have a purity >99% and a water content <250 ppm for [BMIM][BF_4], [BMIM][PF_6], [OMIM][PF_6], and <100 ppm for [BMIM][NtF_2], [OMIM][NtF_2]. [d] Data reported by the seller Iolitec; values reported for ILs that have a purity >99% and a water content <250 ppm for [BMIM][BF_4], [BMIM][PF_6], [OMIM][PF_6], and <100 ppm for [BMIM][NtF_2], [OMIM][NtF_2].

Table 5 Investigation of the reaction time for the HATU-mediated synthesis of **3a** in ILs[a]



1 **2a** **3a**

INM-based PROTACs	Coupling agent (1.25 eq.)	Base (3.0 eq.)	Solvent (0.056 M)	Time (h)	Conversion[b] (%)	Yield of **3a**[c] (%)
1	HATU	DIPEA	[OMIM][ClO_4]	1	100	75
2	HATU	DIPEA	[OMIM][PF_6]	2	100	69
3	HATU	DIPEA	[BMIM][BF_4]	1.5	100	68
4	HATU	DIPEA	[BMIM][PF_6]	2	100	77

[a] Reaction conditions: **1** (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), ILs (0.056 M), room temperature, 1–2 h. [b] As monitored by TLC with respect to consumption of both starting materials. [c] Isolated yield.

To deeply ascertain the reaction performance in RTILs [OMIM][PF_6], [OMIM][ClO_4], [BMIM][BF_4], and [BMIM][PF_6], each reaction was also analysed by HPLC at different time points (up to 120 minutes) to give a conversion curve, which could be directly compared across the solvent selection (Fig. 4). In particular, given the rapid solubilization of our PROTAC building blocks (**1** and **2a**) in [OMIM][ClO_4], the amidation reaction in this medium proceeded very quickly exhibiting the fastest conversion rate. Notably, in the case of [BMIM][BF_4] and [OMIM][PF_6], we observed a sudden jump in the conversion rate only after 30 minutes and 1 hour, respectively, which is due to the delay necessary to achieve a complete solubilization of the starting materials in the selected reaction medium.

Among the screened ILs, [OMIM][ClO_4] and [BMIM][PF_6] were selected for further studies considering their favourable

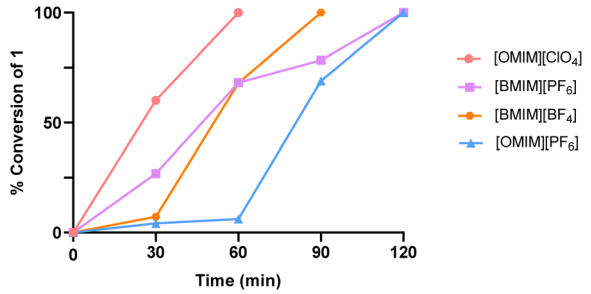


Fig. 4 Representation of conversion data as determined by HPLC: amidation reaction for the synthesis of **3a** using HATU with four different ILs.

balance in terms of conversion rate and yield. In particular, we evaluated the impact of the addition of a small amount of water (1–2% (v/v)) to [OMIM][ClO_4] and [BMIM][PF_6] on the final yield and reaction time. As illustrated in Table 6, the addition of 1% of water had no effect on the conversion rates as well as on the yields of the reactions (Table 6, entries 2 and 5), compared to the ones conducted in the absence of water (Table 6, entries 1 and 4), although in the case of [OMIM][ClO_4], an increased reaction time was required (Table 6, entry 2). Conversely, when 2% of water was added, both reactions (Table 6, entries 3 and 6) did not show complete conversion even after 24 h of reaction, thus affording lower yields.

Successively, we evaluated the impact of concentrations on the performance of the amidation reaction in both [OMIM][ClO_4] and [BMIM][PF_6]. As shown in Table 7, the doubling or halving of the final concentration of both ILs did not affect the reaction time or the conversion rate (Table 7, for [OMIM][ClO_4], compare entries 2 and 3 with entry 1, while for [BMIM][PF_6], compare entries 5 and 6 with entry 4). However, while halving the final IL concentration to 0.028 M showed a reduction in terms of yields compared to a concentration of 0.056 M (Table 7, entries 3 and 6 *vs.* entries 1 and 4, respectively), the higher concentration of ILs at 0.112 M slightly improved the final reaction yields (Table 7, entries 2 and 5 *vs.* entries 1 and 4, respectively).

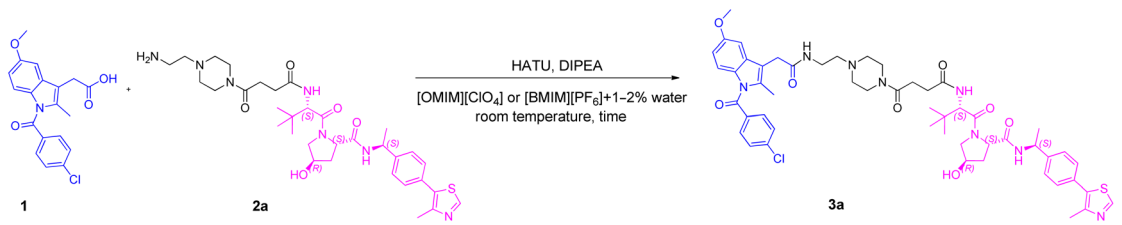
Although both [OMIM][ClO_4] and [BMIM][PF_6] emerged as good alternatives to DMF in the HATU-mediated amidation for the synthesis of **3a** with improved isolated final yields, we decided to select [OMIM][ClO_4] for further investigations due to its superior performance, lower density (1.07 ± 0.02 g cm^{-3}) than other conventional ILs (Table 4), excellent solvency power and also considering that the presence of fluorine atoms in the anion with respect to [BMIM][PF_6] can decompose and form toxic volatiles such as HF and phosphorus oxyfluoride.

Next, the optimized reaction conditions identified so far for the synthesis of PROTAC **3a** (1.0 equiv. of **1**, 1.0 equiv. of **2a**, 1.25 equiv. of HATU, 3.0 equiv. of DIPEA, 0.056 M [OMIM][ClO_4], and room temperature) were applied to explore the scalability of the synthetic approach. In particular, by employing the same reagent ratio, the reaction was performed on a 0.28 mmol scale affording PROTAC **3a** in high isolated yield (68%) with only a slight increase in the reaction time (2.5 h) compared to a smaller scale (1 h, Table 5, entry 1).

Thus, with the established optimal reaction conditions, the substrate scope of the reaction was successively explored. In particular, different intermediates bearing ligands for two E3 ligases (VHL and Cereblon) functionalized with linkers of different lengths and compositions were used as amine derivatives (Schemes 1 and 2) in the amidation coupling with INM. Moreover, different POI ligands (already exploited in the literature for PROTAC synthesis) were used as acid derivatives in the coupling with intermediate **2a** (Scheme 3).

Initially, **1** was coupled with a set of different VHL-linker intermediates (**2b–f**) to afford PROTACs **3b–f** (Scheme 1), pre-

Table 6 Effect of the addition of water to [OMIM][ClO_4] and [BMIM][PF_6] in terms of reaction time, conversion rate and yield[a]

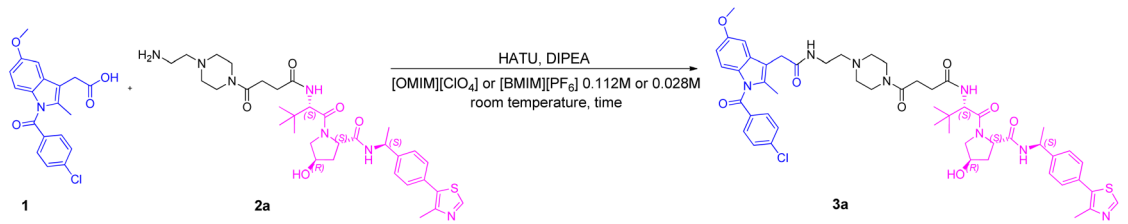


1 2a 3a

INM-based PROTACs	Coupling agent (1.25 eq.)	Base (3.0 eq.)	Solvent (0.056 M)	Time (h)	Conversion (%)	Yield of **3a**[d] (%)
1	HATU	DIPEA	[OMIM][ClO_4]	1	100[b]	75
2	HATU	DIPEA	[OMIM][ClO_4] + 1% H_2O	2	100[b]	73
3	HATU	DIPEA	[OMIM][ClO_4] + 2% H_2O	24	90.08[c]	55
4	HATU	DIPEA	[BMIM][PF_6]	2	100[b]	77
5	HATU	DIPEA	[BMIM][PF_6] + 1% H_2O	2	100[b]	66
6	HATU	DIPEA	[BMIM][PF_6] + 2% H_2O	24	81.68[c]	37

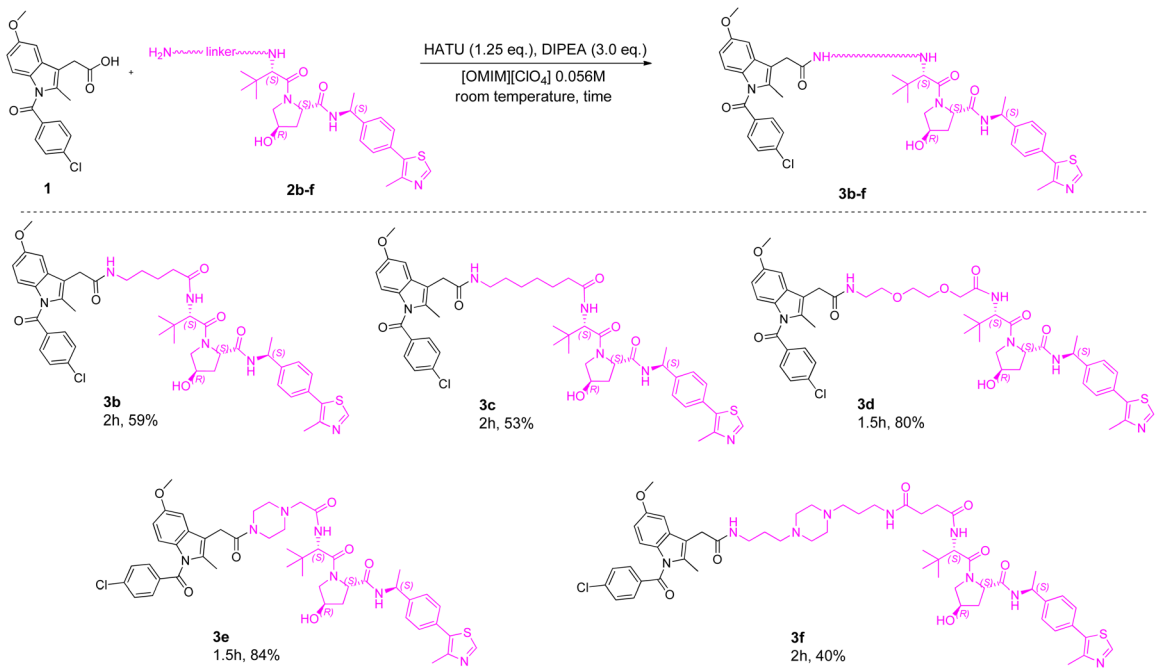
[a] Reaction conditions: **1** (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), IL (0.056 M) or IL with 1 or 2% H_2O (0.056 M), room temperature, 1–24 h. [b] As monitored by TLC with respect to consumption of both starting materials. [c] As monitored by HPLC with respect to consumption of **1**. [d] Isolated yield.

Table 7 Effect of the final IL concentration in terms of reaction time, conversion rate, and yield for the synthesis of **3a**[a]



INM-based PROTACs	Coupling agent (1.25 eq.)	Base (3.0 eq.)	Solvent	Time (h)	Conversion[b] (%)	Yield of **3a**[c] (%)
1	HATU	DIPEA	$[OMIM][ClO_4]$ 0.056 M	1	100	75
2	HATU	DIPEA	$[OMIM][ClO_4]$ 0.112 M	1	100	83
3	HATU	DIPEA	$[OMIM][ClO_4]$ 0.028 M	1	100	61
4	HATU	DIPEA	$[BMIM][PF_6]$ 0.056 M	2	100	77
5	HATU	DIPEA	$[BMIM][PF_6]$ 0.112 M	2	100	81
6	HATU	DIPEA	$[BMIM][PF_6]$ 0.028 M	2	100	73

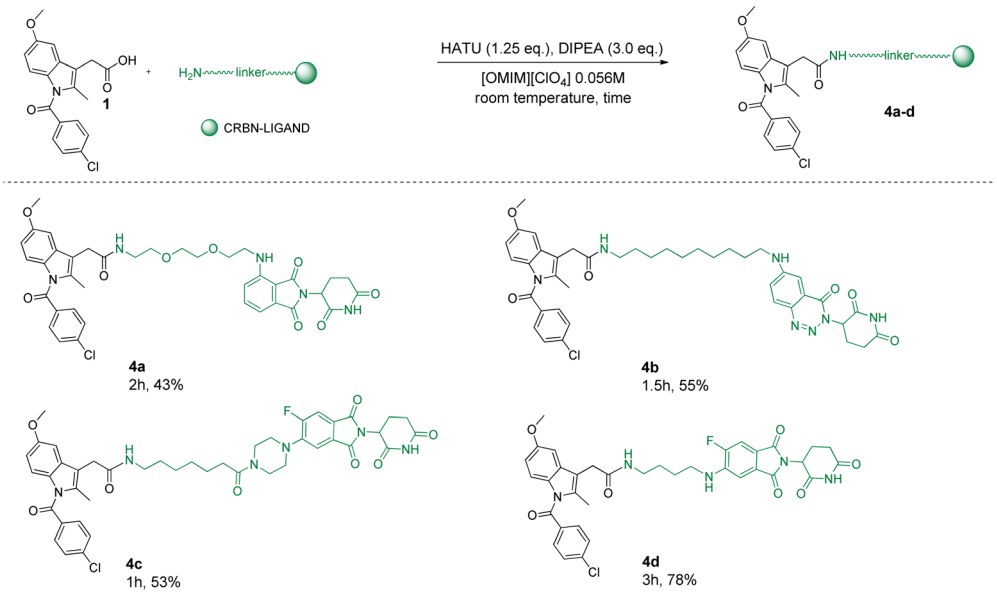
[a] Reaction conditions: **1** (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), IL (0.112 M or 0.028 M), room temperature, 1–2 h. [b] As monitored by TLC with respect to consumption of both starting materials. [c] Isolated yield.



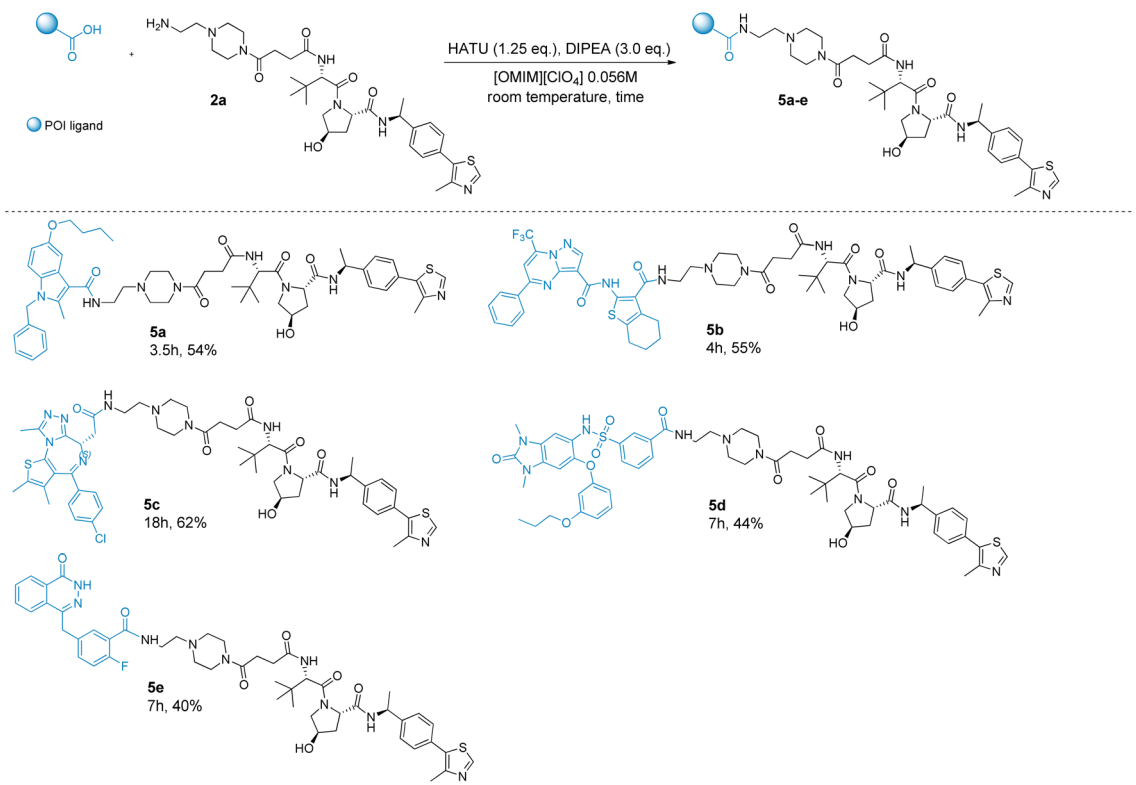
Scheme 1 The scope of VHL-based amine linker intermediates. Conditions: **1** (0.056 mmol, 1 equiv.), **2b–f** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), $[OMIM][ClO_4]$ (0.056 M), room temperature, 1.5–2 h. Reaction times and isolated yields are reported for each target compound.

viously synthesized with the DMF-based protocol[21,22] (Table 1). Overall, $[OMIM][ClO_4]$ was confirmed to be a good solvent in amidation coupling, affording target PROTACs **3b–f** in good to excellent yields (40–84%) and short reaction times (1.5–2 h). Additionally, compared with the yields obtained with DMF as the solvent, (Table 1), in all cases, the use of $[OMIM][ClO_4]$ gave higher isolated yields.

Then, to further expand the substrate chemical space, we decided to include Cereblon (CRBN) E3 ligase ligands, which together with VHL represent the most exploited E3 ligase in



Scheme 2 The scope of CRBN-based amine linker intermediates. Conditions: **1** (0.056 mmol, 1 equiv.), CRBN-linker intermediate (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), [OMIM][ClO_4] (0.056 M), room temperature, 1–3 h. Reaction times and isolated yields are reported for each target compound.



Scheme 3 The scope of the carboxylic acid derivative. Conditions: POI ligand (0.056 mmol, 1 equiv.), **2a** (0.056 mmol, 1 equiv.), HATU (0.070 mmol, 1.25 equiv.), DIPEA (0.168 mmol, 3 equiv.), [OMIM][ClO_4] (0.056 M), room temperature, 3.5–18 h. Reaction times and isolated yields are reported for each target compound.

the synthesis of PROTACs. In particular, **1** was coupled with various CRBN binders functionalized with linkers of different lengths and compositions leading to new PROTACs **4a–4d** (Scheme 2). Also in this case, all PROTACs were obtained in short reaction times (1–3 h) with high isolated yields, all above 50% (**4b–4d**) except for **4a** (43%). Notably, despite the observed

100% conversion rate, the isolated yields for these PROTACs were low. This discrepancy can be ascribed to the known hydrolytic degradation at the glutarimide and/or phthalimide rings of the thalidomide moiety that can occur not during the reaction itself but during the reaction work-up in aqueous media.[19,70–72] Nevertheless, it is important to note that, for example, compound **4d** was obtained in a significantly higher yield (78%) compared to the yield of the same reaction but with DMF as the reaction medium (38%).[73] Therefore, in this specific case, with a reference compound, we can confidently state that the use of [OMIM][ClO_4] as the reaction medium led to successful optimization.

Finally, as shown in Scheme 3, by maintaining the VHL-linker intermediate **2a**, different POI ligands than indomethacin **1** have been exploited leading to PROTACs **5a–e**. In particular, we decided to expand the POI ligand chemical space including common ligands used for PROTACs synthesis such as JQ1 (bromo-and-extra-terminal (BET) inhibitor),[74] olaparib (PARP1/2 inhibitor)[75] and IACs-7e (TRIM24 inhibitor).[76] Moreover, the 5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid derivative (anti-influenza compound)[77] and 1-benzyl-5-butoxy-2-methyl-1*H*-indole-3-carboxylic acid derivative (efflux pump inhibitor)[78] were also included for investigation purposes. For all the synthesized PROTACs **5a–5e**, we observed complete conversion, which occurred within a short reaction time for **5a** and **5b** (3.5–4 h). A longer reaction time was instead required in the case of **5c–5e** (7–18 h), due to the initial lower solubility of the selected POI ligands in the reaction medium (Scheme 3). Overall, while the yields for **5d** and **5e** were slightly lower (44% and 40%, respectively), compounds **5a–5c** were obtained in good to high yields (54–62%). Notably, although the reactions for the synthesis of PROTACs **5d** and **5e** proceeded with the complete conversion of the starting materials, the final isolated yield was low, due to the formation of traces of more than one side product/degradation product, which made the purification step more complicated and trickier, in turn affecting the rate of the isolated yield.

Overall, the results obtained herein confirmed that [OMIM][ClO_4], in addition to the general advantages related to the use of ILs, can be considered a suitable replacement for the classic DMF as the medium for amidation reactions during PROTACs synthesis.

Indeed, being characterized by very low density and high solvency power, [OMIM][ClO_4] permitted us to achieve generally high isolated yields through a complete conversion rate, a short reaction time, and mild reaction conditions.

Conclusions

In this work, we have evaluated several coupling agents and solvents in the amidation reaction for PROTACs synthesis. Among the coupling agents screened, HATU was selected for further evaluations in several solvents. In particular, we tried to replace the environmentally undesirable organic solvent commonly used in this reaction step, namely DMF, with potential greener alternatives. We found that ILs generally proved to be suitable replacements for DMF when compared to other organic solvents. Indeed, in most cases, the use of RTILs as solvents in the amidation coupling for the synthesis of our case study PROTAC **3a** resulted in an impressive improvement in isolated yields (up to 75–77%) with complete conversion (100%), mild reaction conditions (anhydrous conditions not required, room temperature), and short reaction time (up to 2 h). Among the ILs screened, [OMIM][ClO_4] was selected for further studies because of the favourable balance between results (times and yields) and its good physicochemical properties.

A scale-up experiment and the exploration of the reaction scope for PROTACs synthesis further confirmed that the employment of HATU/DIPEA in [OMIM][ClO_4] was a reliable protocol to obtain variously functionalized PROTACs in good to high isolated yields and short reaction times. Moreover, to the best of our knowledge, there have been no reports on the use of ILs for the synthesis of PROTACs. Given the promising results obtained in the present study, successive exploration will be focused on the possible application of ionic liquids as solvents in further types of reactions commonly used for PROTACs synthesis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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