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Catalytic Castagnoli–Cushman reaction-based synthesis of tetrahydroisoquinolone–glutarimide dyads and their evaluation as potential cereblon ligands

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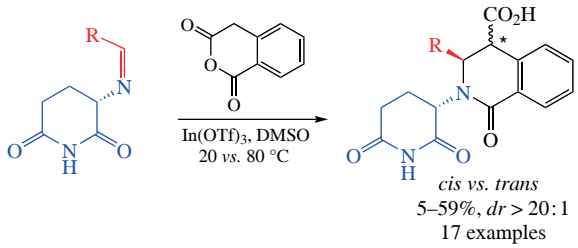
A series of imines derived from α-aminoglutarimide and alhehydes RCHO was introduced into the In(OTf)$_3$-catalyzed Castagnoli–Cushman reaction with homophthalic anhydrides thus yielding novel glutarimide–tetrahydroisoquinolone dyads with moderate yields. Carrying out the reaction at room temperature affords isomers with *cis*-orientation of the R substituent and carboxy group whereas heating to 80 °C promotes conversion into more stable *trans*-isomers. Some selected compounds were evaluated *in vitro*, revealing mild or no antiproliferative effects, and tested for cereblon-binding, with the best-performing compound showing an affinity in the range of canonical cereblon ligands.

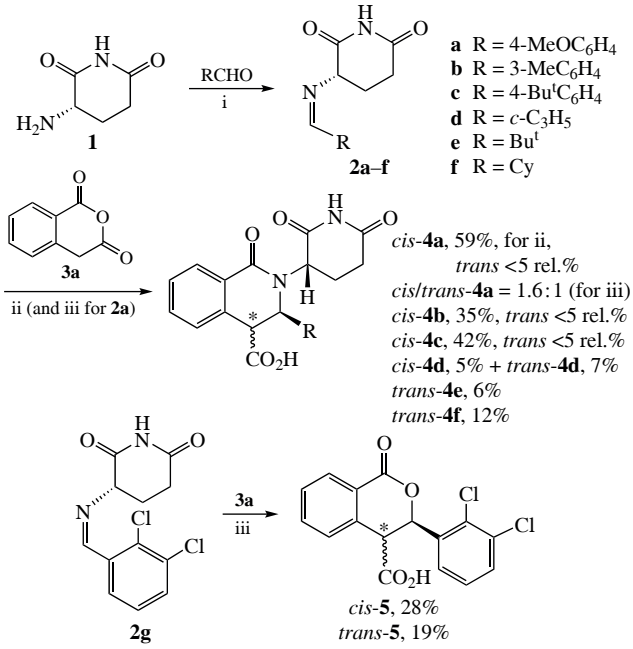


Keywords: PROTAC, glutarimide, imines, homophthalic anhydride, Castagnoli–Cushman reaction, tetrahydroisoquinolones.

Immunomodulatory imide drugs (IMiDs), including thalidomide, lenalidomide, and pomalidomide, are approved therapeutic agents for the treatment of multiple myeloma.[1,2] The mechanism of action of these agents is primarily mediated through their interaction with cereblon[3] (CRBN), an E3 ubiquitin ligase substrate receptor. Serving as molecular glues, upon binding to CRBN, IMiDs shift the substrate spectrum of CRBN away from its natural substrates towards the ubiquitination and subsequent proteasomal degradation of certain neo-substrates (*e.g.* transcription factors IKZF1 and IKZF3).[4–8] This targeted degradation leads to growth inhibition in multiple myeloma cells, underlying the therapeutic efficacy of these drugs. Recently, CRBN and its ligands have gained prominence as a key player for construction of drug agents called PROTACs (targeted protein degradation *via* proteolysis-targeting chimeras). PROTACs are bifunctional molecules that enable the ubiquitination and degradation of a target protein of interest by bringing it into proximity with an E3 ligase.[9,10] This strategy has rapidly advanced, leading to the development of numerous PROTACs efficient for degrading over 50 different proteins, many of which are clinically validated drug targets.[11–13] Most PROTACs employ IMiDs as CRBN-binding warheads.[14,15] However, the teratogenic risks associated with IMiDs have prompted the search for novel CRBN ligands with improved safety profiles.[16] Advances in understanding the structural requirements for effective CRBN binding, particularly the critical role of the glutarimide[17] moiety, have driven the design of new ligands aimed at engaging CRBN more safely and effectively.

Recently, our group began exploring the chemistry of α-aminoglutarimide **1** derivatives for the synthesis of new potential IMiD analogs and PROTACs. Compound **1** was converted *in situ* to imines **2** *via* a classic condensation with aldehydes and then subjected to the Ugi reaction with isocyanides and carboxylic acids to provide a series of glutarimide-based bisamides (see Online Supplementary Materials, Scheme S1).[18] In the present work we further expand the utilization of such imines to the Castagnoli–Cushman lactam synthesis[19,20] utilizing homophthalic anhydride (HPA) **3**. This approach allowed for the construction of a novel type of glutarimide derivatives **4** with a tetrahydroisoquinolone (THIQ) moiety attached to the α-position (Schemes 1 and 2).

Imines **2** were obtained from L-glutamic acid and aldehydes in MeOH in the presence of $MgSO_4$ at room temperature (see Scheme 1). In contrast to our previous studies,[18] where such imines were used without isolation, we had to add a purification step. The latter included the removal of methanol (incompatible with anhydride) and filtration of the residue dissolved in chloroform through Celite to absorb intensively colored polymeric by-products. We began investigating the target reaction between HPA **3** and imines with simple stirring of the reactants in DMSO at room temperature. In the case of electron-rich imine **2a**, ^{1}H NMR monitoring revealed the formation of the desired lactam **4a** although with low diastereoselectivity (both

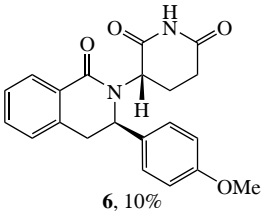


Scheme 1 *Reagents and conditions*: i, $MgSO_4$, MeOH, room temperature, 16 h; ii, $In(OTf)_3$ (0.05 equiv.), DMSO/DMSO-d_6, room temperature, 2 h; iii, the same, without $In(OTf)_3$.

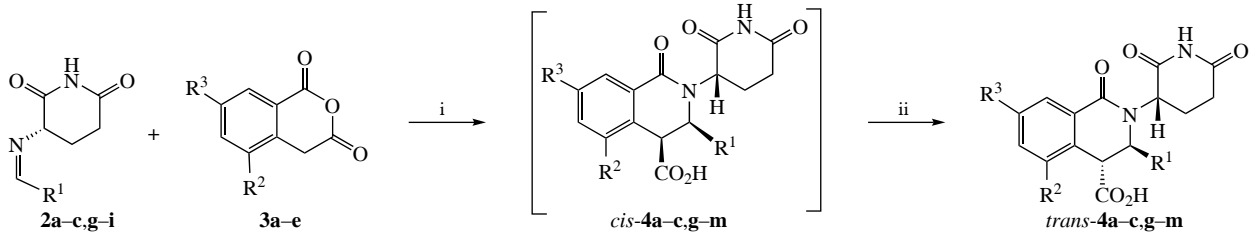
cis- and *trans*-orientations of the substituents at the newly formed lactam ring, see Scheme 2, conditions iii). Surprisingly, in the case of electron-poor imine **2g**, another type of product, namely, a mixture of diastereomeric lactones *cis*/*trans*-**5**, was formed. This could happen due to the low reactivity of imine **2g** towards HPA **3**, so the *in situ* hydrolysis of the imine could occur, which liberated much more reactive aldehyde 2,3-$Cl_2C_6H_3CHO$. Such lactone synthesis has been documented previously.[21] Since these results were unsatisfactory, we performed screening of the reaction conditions which included variations of solvent, temperature, reactant loading, and catalyst (see Online Supplementary Materials, Table S1). Performing the reaction **2a**+**3a** at room temperature in DMSO with indium triflate (5 mol%) for 2 h (see Scheme 1, conditions ii) gave the desired diastereomerically pure product *cis*-**4a**. In case of imine **2g**, under such conditions the similar *cis*-configured lactam was also formed, however we did not isolate and characterize it (see below for its further processing).

With the optimized conditions in hand, we proceeded with the preparation of a series of compounds **4**, varying the aldehyde component R (see Scheme 1). Compounds **4a–c** with aromatic substituent R were isolated as single *cis*-isomers in reasonable yields (35–59%). Compounds with aliphatic or alicyclic grouping R gave pure *trans*-isomers (*trans*-**4e**,**f**) or mixture (*cis*-**4d**+*trans*-**4d**; separated) with significantly lower yields (6–13%), which is quite common for the CCR of imines derived from enolizable aldehydes.

Reactions performed with other imines of type **2** under the same protocol led to the formation of *cis*-isomers of products **4** as well, but the latter were prone to rapid isomerization and could not be fully characterized. Therefore, we modified the reaction protocol to include additional heating at 80 °C after the CCR, which allowed complete isomerization and isolation of the thermally stable isomers *trans*-**4** to be performed (see Scheme 2). Along with abovementioned imines **2a–g**, imine **2h** (R^1 = 4-$Me_2NC_6H_4$) and imine **2i** (R^1 = 2-thienyl) were employed. Mostly, this thermal isomerization was carried out *in situ* without isolation of the *cis*-product, but for three cases *cis*-**4b**,**c**,**i** isomers were isolated and characterized. Substituted homophthalic anhydrides **3b–e** were also introduced into the CCR with glutarimide-based imines **2a**,**c** to afford products *trans*-**4j–m** in yields from 16 to 51%. Attempted acceleration the reaction by raising the temperature of the second step to 150 °C caused the decarboxylation of *trans*-**4a** into compound **6**.



The structure and configuration of compound *cis*-**4a** were confirmed by single crystal X-ray crystallography (Figure 1).†



3a $R^2 = R^3 = H$
3b $R^2 = F$, $R^3 = H$
3c $R^2 = H$, $R^3 = Br$
3d $R^2 = H$, $R^3 = Cl$
3e $R^2 = H$, $R^3 = F$

4a R^1 = 4-$MeOC_6H_4$, $R^2 = R^3 = H$, 20%
4b R^1 = 3-MeC_6H_4, $R^2 = R^3 = H$, 37%
4c R^1 = 4-$Bu^tC_6H_4$, $R^2 = R^3 = H$, 41%
4g R^1 = 2,3-$Cl_2C_6H_3$, $R^2 = R^3 = H$, 23%
4h R^1 = 4-$Me_2NC_6H_4$, $R^2 = R^3 = H$, 25%
4i R^1 = 2-thienyl, $R^2 = R^3 = H$, 12% (*dr* = 6.7:1)
4j R^1 = 4-$Bu^tC_6H_4$, $R^2 = F$, $R^3 = H$, 31%
4k R^1 = 4-$MeOC_6H_4$, $R^2 = H$, $R^3 = Br$, 23%
4l R^1 = 4-$MeOC_6H_4$, $R^2 = H$, $R^3 = Cl$, 16%
4m R^1 = 4-$MeOC_6H_4$, $R^2 = H$, $R^3 = F$, 51%

Scheme 2 *Reagents and conditions*: i, anhydride **3** (0.1–0.2 mmol), imine **2** (2 equiv.), $In(OTf)_3$ (0.05 equiv.), DMSO, room temperature, 2 h; ii, 80 °C, 1–6 days (in case of **4a**, K_2CO_3 was added). For **4a–c**,**i**: *cis*-isomers were isolated but not characterized due to partial isomerization and were taken into the final isomerization. For **4g**,**h**,**j–m**: *cis*-isomers were not isolated.

† *Crystal data for cis*-**4a**. Single crystals of $C_{25.14}H_{21.71}N_{2.29}O_{6.86}$ *cis*-**4a** were obtained from DMSO. A suitable crystal was selected and tested on an XtaLAB Synergy, Single source at home/near, HyPix diffractometer. The crystal was kept at 100.15 K during data collection. Using Olex2,[22] the structure was solved with the SHELXT[23] structure solution program using Intrinsic Phasing and refined with the SHELXL[24] refinement package using Least Squares minimization. Crystal data for $C_{25.142857}H_{21.714286}N_{2.285714}O_{6.857143}$ (M = 465.59 g mol^{-1}), orthorhombic, space group *Pbca* (no. 61), a = 12.6091(2), b = 15.9592(3) and c = 19.1587(4) Å, V = 3855.33(12) Å^3, Z = 7, T = 100.15 K, μ(CuKα) = 0.863 mm^{-1}, d_{calc} = 1.404 g cm^{-3}, 14651 reflections measured (9.232° ⩽ 2Θ ⩽ 160.314°), 3977 unique (R_{int} = 0.0534, R_{sigma} = 0.0468) which were used in all calculations. The final R_1 was 0.0531 [$I > 2\sigma(I)$] and wR_2 was 0.1504 (all data).

CCDC 2216208 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk.

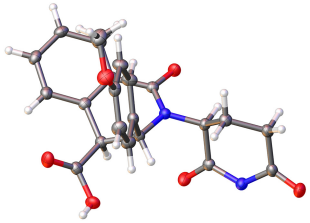


Figure 1 Crystal structure of compound *cis*-**4a** (ORTEP plot, 50% probability level).

The configuration of the lactam moiety (*cis*/*trans*) for other compounds was assigned from analysis of ^{1}H NMR data, specifically $^3J_{HH}$ coupling constants for vicinal CH protons (see Online Supplementary Materials, Table S2).

The synthesized compounds were evaluated for their anti-myeloma activity against the KMS-12-PE and MOLP-8 cell lines. After 72 h of exposure at a concentration of 30 μM, almost none of the compounds exhibited significant cytotoxic effects against these cancer cell lines. Furthermore, most of the compounds did not show any cytotoxicity towards normal human mononuclear cells (PMBC), indicating a selective safety profile (see Online Supplementary Materials, Figure S2). In fact, only *trans*-**4m** demonstrated significant cytotoxic properties against PBMC cell culture. These observations, along with the presence of the pharmacophoric glutarimide moiety, indicate that this series may include CRBN-binding compounds that lack molecular glue activity. Such compounds could be particularly valuable, as they offer potential for use in the design of PROTACs with a reduced risk of teratogenic or other toxic off-target effects associated with neo-substrate recruitment. Given these findings, we proceeded to assess the CRBN affinity of selected representatives using a microscale thermophoresis assay.[25]

To probe the CRBN binding of the obtained chemotype, we selected three non-cytotoxic derivatives *trans*-**4d**,**g**,**i** having *trans*-configuration at THIQ moiety. These compounds displayed CRBN binding affinities with K_i values ranging between 94.0 and 123.4 μM, which are at least one order of magnitude lower in potency as CRBN ligands compared to the reference drug Thalidomide that is commonly used in the design of PROTAC molecules (K_i of 8.7 μM) (Table S3).[25] Interestingly, *cis*-configured compounds demonstrated different K_i values. Compound *cis*-**4d** showed a K_i value of 106.81 μM (though with an unexpectedly large confidence interval), which was comparable to its *trans*-**4d**. In contrast, compound *cis*-**4a** exhibited a significant increase in CRBN binding affinity, with a K_i value of 23.32 μM, making it substantially more potent than the other evaluated compounds. Given this observation, we proceeded to profile the decarboxylated derivative **6**, which represents a decarboxylated analog of *cis*-**4a**. Notably, compound **6** exhibited even greater CRBN binding affinity, with a K_i value of 17.2 μM. This level of binding affinity was only two-fold lower than that of Thalidomide.

These results indicate a substantial improvement in CRBN binding affinity when transitioning from classical CCR products, such as *trans*-**4d**,**g**,**i**, to compounds *cis*-**4a** and **6**. Within the evaluated set of non-cytotoxic tetrahydroisoquinolone-glutarimide derivatives we observed that the *trans*-configuration of the carboxylic group, along with an aliphatic, aromatic, or heterocyclic periphery, appeared to be suboptimal for CRBN binding. In contrast, the *cis*-configuration of the carboxylic group, paired with an aromatic side chain as seen in compound *cis*-**4a**, resulted in potent CRBN binding affinity, which was further enhanced by the removal of the carboxylic group. Even though, this conclusion cannot be generalized due to the limited number of tested compounds, it showcases interesting structure–activity relationship within the explored chemotype. Furthermore, while a lipophilic molecular periphery in IMiD-like structures is generally considered favorable,[26] the presence of a charged carboxylic group (inherent to CCR products) has been shown in some cases to decrease CRBN binding affinity.[27] Despite this, the carboxylic group may serve as an important function for subsequent conjugation in the design of PROTAC molecules. In this context, our results provide insight into the structural features that can affect CRBN binding of the tetrahydro-isoquinolone-glutarimide derivatives, potentially guiding the design of more effective and safer CRBN-targeted molecules.

In conclusion, imines derived from α-aminoglutarimide were subjected to the Castagnoli–Cushman reaction with homophthalic anhydrides under indium triflate catalysis yielding novel glutarimide derivatives bearing a tetrahydroisoquinolone moiety. The primary *cis*-isomers of the products were converted into more stable *trans*-isomers under moderate heating or underwent decarboxylation at higher temperatures. Most of the compounds exhibited no significant effects on the viability of normal PBMC cells or myeloma KMS-12-PE and MOLP-8 cell lines, suggesting a lack of molecular glue activity typical of thalidomide and its analogs. While *trans*-configured derivatives *trans*-**4d**,**g**,**i** showed only weak CRBN binding with K_i values ranging from 94.0 to 123.4 μM, *cis*-substituted compound *cis*-**4a** exhibited a K_i value of 23.32 μM, which could be further improved on moving to the decarboxylated counterpart **6** (K_i of 17.2 μM), approaching the affinity of the reference drug Thalidomide.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7615.

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