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Synthesis of α-(azidomethyl)glutarimide and its application in construction of potential Cereblon ligands *via* the CuAAC reaction

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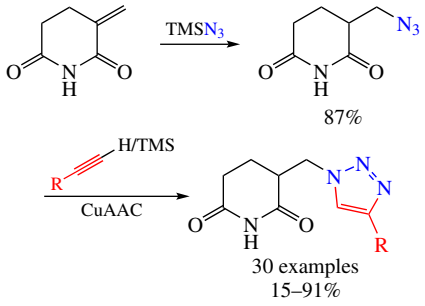
The Michael addition of tetramethylsilyl azide to 3-methylenepiperidine-2,6-dione afforded new glutarimide derivative, 3-(azidomethyl)piperidine-2,6-dione, which was introduced into the CuAAC click reaction with a variety of alkynes to afford thirty novel structurally diverse 1,2,3-triazoles. The cytotoxicity of the synthesized compounds was evaluated on multiple myeloma cell lines (MM1.S, KMS-12-PE), a leukemia cell line (NALM-6), and normal B-cells (WIL2-S) showing a noticeable effect on the MM1.S cell line. Selected compounds demonstrated significant Cereblon binding affinity in a microscale thermophoresis assay with one derivative outperforming the reference drug Pomalidomide.



Keywords: PROTAC, glutarimide, CuAAC, alkynes, azides, click reaction, 1,2,3-triazoles, Cereblon, Pomalidomide.

Cereblon (CRBN) is the substrate recognition component of the E3 ubiquitin ligase complex CRL4-CRBN, which also includes DNA damage-binding protein 1 (DDB1), cullin-4A (CUL4A), and RING-box protein 1 (RBX1).[1] The primary function of CRBN was largely unknown until 2010, when it was identified as the direct target of Thalidomide and its derivatives, known as immunomodulatory drugs (IMiDs).[2–4] Thalidomide,[2,5,6] Lenalidomide,[7] and Pomalidomide[7–9] (Figure 1), the most prominent IMiDs, bind to CRBN and alter its substrate specificity thus leading to the degradation of neo-substrates.[4,5,10] This

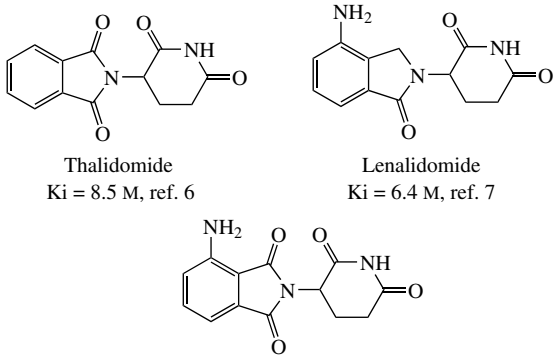


Figure 1 Structure and activity of the most prominent IMiDs.

mechanism mimics the recognition of natural substrates[10] and forms the basis of their therapeutic efficacy in treating multiple myeloma and certain other cancers.[5]

In recent years, CRBN has become a pivotal E3 ubiquitin ligase in the field of targeted protein degradation, particularly through the development of proteolysis-targeting chimeras (PROTACs).[11,12] Bifunctional molecules PROTACs bring a target protein (protein of interest, POI) into proximity with an E3 ligase thus facilitating the ubiquitination and degradation of the POIs. This approach has rapidly evolved leading to the creation of numerous PROTACs capable of degrading over 50 different proteins, many of which are clinically validated drug targets.[13–17] Most PROTACs utilize IMiDs as CRBN ligands due to their well-characterized binding properties.[18–20] However, the inherent teratogenic risks associated with IMiDs have driven the search for novel CRBN ligands with improved safety profiles. The structural requirements for effective CRBN binding have been increasingly understood, particularly the significance of the glutarimide moiety.[21,22] This understanding has prompted the development of new ligands designed to engage CRBN more safely and effectively.

In this work we aimed at preparation and evaluation of a series of novel glutarimide–triazole diads obtained from novel 3-(azidomethyl)piperidine-2,6-dione **1** *via* the CuAAC click reaction with acetylenes. Our studies began with preparation of azide **1**, a homologue of α-azidoglutarimide (Scheme 1). The glutarimide core was brought by 3-methylenepiperidine-2,6-

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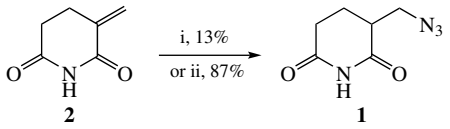
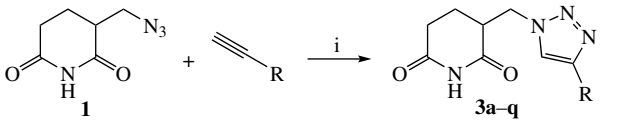


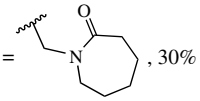
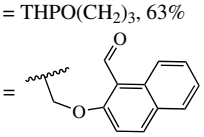
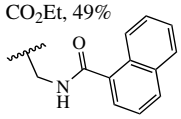
Scheme 1 *Reagents and conditions*: i, NaN_3, H_2O, AcOH, room temperature, 16 h; ii, $TMSN_3$, CH_2Cl_2, NEt_3, AcOH, room temperature.

dione **2** which was introduced into the Michael addition with an azide source. Our first attempt was to use sodium azide (in aq. AcOH at room temperature), which allowed isolation of the target compound with low yield of 13%. Alternative reaction conditions employing trimethylsilyl azide and the mixture of acetic acid and triethylamine improved the yield significantly to 87%. This procedure has proven to be scalable and was performed on gram quantities. The obtained azide **1** was found to be labile and should be stored at –20 °C in darkness (up to 2 months).

Having this key building block **1** in hand we proceeded with the preparation of a series of target 1,2,3-triazoles **3a**–**q** (Scheme 2) *via* azide–alkyne cycloaddition (CuAAC). To our delight, one of the most common protocols for CuAAC (terminal alkyne/copper acetate/sodium ascorbate/THF (aq.)/room temperature) worked well in this case providing one-step preparation of compounds **3a**–**q**, which were isolated after column chromatography or simple filtration with satisfying yields of 32–90%. The only exception was (trimethylsilyl)-acetylene $Me_3SiC{\equiv}CH$ which gave nothing of the required cycloaddition product.

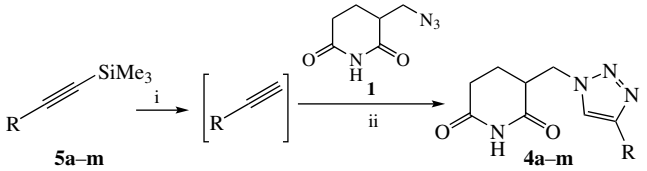
Compounds **4a**–**m** which required commercially unavailable alkynes were synthesized according to the modified two-step protocol including deprotection of TMS-alkynes **5a**–**m** (Scheme 3). The latter were prepared from $Me_3SiC{\equiv}CH$ *via* the classic Sonogashira coupling. The *in situ* deprotection was performed by quick pre-treatment of TMS-alkyne **5** with Bu_4NF for 20 min followed by addition of azide **1** and all other CuAAC components which afforded products **4a**–**m** (15–91%). This protocol allowed us to extend the novel triazole series to thirty compounds having large variety of side functions including aryls (*ortho*/*meta*/*para* substitution, with both EWG and EDG groups) and heteroaryls, as well as ester, carboxy, amide, urea, alcohol, acetal, amino and aldehyde functions. There was no obvious correlation between the nature of the substituent in acetylene and the reaction outcome. We suppose that the difference in isolated yields is mostly due to features in purification step rather than the different reactivity of alkynes. The structure of all compounds was supported by NMR spectroscopy and HRMS. ^{1}H NMR spectra of the obtained



a R = Ph, 68%
b R = CO_2Me, 62%
c R =  , 30%
d R = $THPO(CH_2)_3$, 63%
e R =  , 66%
f R = 4-$HC(O)C_6H_4OCH_2$, 77%
g R = 4-$Bu^tC_6H_4C(O)NHCH_2$, 96%
h R = $PhNHC(O)NHCH_2$, 32%
i R = 4-$MeOC_6H_4C(O)NHCH_2$, 75%
j R = 4-MeC_6H_4, 76%
k R = CO_2H, 48%
l R = 3-$ClC_6H_4C(O)NHCH_2$, 91%
m R = CO_2Et, 49%
n R =  , 82%
o R = $HO(CH_2)_2$, 82%
p R = $Me_2(HO)C$, 49%
q R = Me_2NCH_2, 30%

R = Me_3Si no reaction

Scheme 2 *Reagents and conditions*: i, $Cu(OAc)_2 \cdot H_2O$, Na ascorbate, THF, H_2O, room temperature, 16 h.



a R = 4-NCC_6H_4, 86%
b R = 3-$F_3CC_6H_4$, 49%
c R = 2-pyridyl, 29%
d R = 3-pyridyl, 33%
e R = 3-MeC_6H_4, 65%
f R = 2-$F_3CC_6H_4$, 31%
g R = 3-thienyl, 15%
h R = 4-$F_3CC_6H_4$, 43%
i R = 3-ClC_6H_4, 91%
j R = 2-MeC_6H_4, 67%
k R = 2-thienyl, 86%
l R = 3-FC_6H_4, 80%
m R = 2-FC_6H_4, 48%

Scheme 3 *Reagents and conditions*: i, Bu_4NF (1.2 equiv.), THF, room temperature, 20 min; ii, $Cu(OAc)_2 \cdot H_2O$, Na ascorbate, H_2O, room temperature, 16 h.

products revealed characteristic signals corresponding to 3-alkylpiperidine-2,6-dione moiety (five multiplets corresponding to the 3 diastereotopic CH_2-groups at ~1.7, 2.5, 4.6, 4.8 ppm, CH at ~3.2 ppm and NH at 10.9 ppm) and a novel 1,2,3-triazole moiety (one singlet around 7.8–8.7 ppm). Additionally, the structure of compound **4c** was supported by single-crystal X-ray crystallography (Figure 2).[†]

The cytotoxicity of the newly synthesized CRBN ligands was evaluated using a phenotypic screening approach.[25] Compounds **3a**–**q** and **4a**–**m** were subjected to an MTT-test at a single concentration of 50 μM, in triplicates, with an incubation time of 72 h. The screening was conducted on four different cell lines: multiple myeloma (MM1.S, KMS-12-PE), leukemia (NALM-6), and normal B-cells (WIL2-S). The results of the cytotoxicity assays are summarized in the heatmap shown in Figure 3. Interestingly, compounds **3b**,**e** and **4a**,**b**,**h** exhibited high levels of cytotoxicity against multiple myeloma MM1.S cells, which are sensitive to CRBN ligands. In contrast, the NALM-6 and KMS-12-PE cell lines showed varying degrees of sensitivity to the compounds, with generally lower cytotoxicity observed compared to MM1.S cells. Importantly, the WIL2-S normal B-cell line exhibited minimal cytotoxicity across the series, suggesting a favorable selectivity profile towards the malignant cells. In fact, most of the newly synthesized derivatives were less

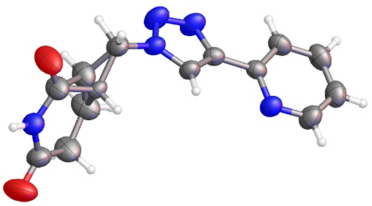


Figure 2 Crystal structure of compound **4c** (ORTEP plot, 50% probability level).

[†] *Crystal data for* **4c**. $C_{13}H_{11}N_5O_2$, M = 269.30 g mol^{-1}, monoclinic, space group $P2_1/c$ (no. 14), a = 5.5110(3), b = 10.4991(5) and c = 21.9469(12) Å, β = 94.480(5)°, V = 1265.98(12) Å^3, Z = 4, T = 290(11) K, μ(Cu Kα) = 0.837 mm^{-1}, d_{calc} = 1.413 g cm^{-3}, 5784 reflections measured (8.082° ⩽ 2θ ⩽ 138.22°), 2361 unique (R_{int} = 0.0323, R_{sigma} = 0.0481) which were used in all calculations. The final R_1 was 0.0675 [$I > 2\sigma(I)$] and wR_2 was 0.1909 (all data).

Single crystals were obtained from DMSO. A suitable crystal was selected and tested on a SuperNova, Single source at offset/far, HyPix3000 diffractometer. The crystal was kept at 290(11) K during data collection. Using Olex2,[23] the structure was solved with the SHELXS structure solution program using direct methods and refined with the SHELXL[24] refinement package using CGLS minimization.

CCDC 2351200 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.

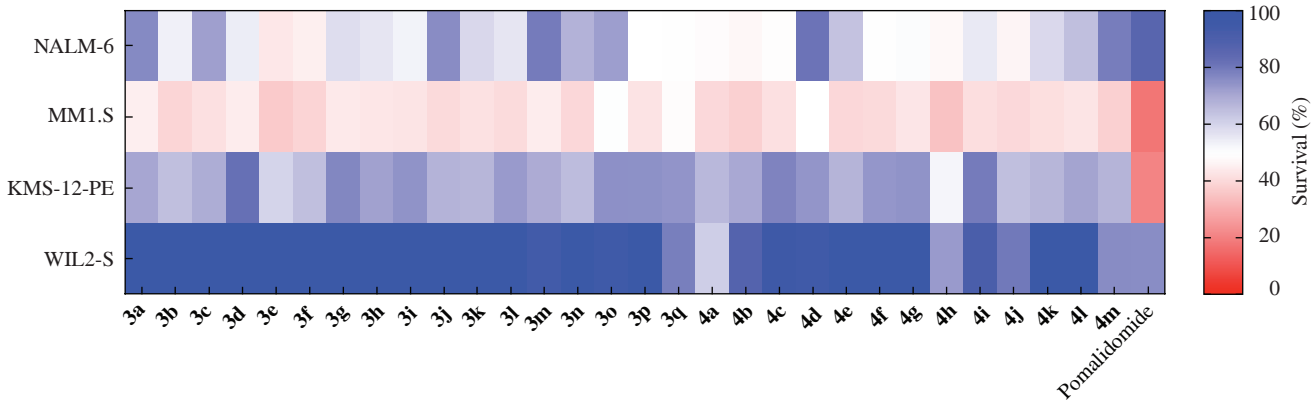


Figure 3 MTT assay results (50 μM, 72 h) against multiple myeloma (MM1.S, KMS-12-PE), leukemia (NALM-6), and normal B-cells (WIL2-S) for compounds **3** and **4**. Pomalidomide was used as a reference.

cytotoxic against WILS-2 than the reference IMiD Pomalidomide (see Figure 3).

Based on these results, we proceeded with the evaluation of selected representatives of the new chemotype for their CRBN binding affinities using a well-established microscale thermophoresis assay.[26] To our delight, some compounds demonstrated very strong binding to CRBN, with **3a** outperforming reference drug Pomalidomide in this assay[26] (see Online Supplementary Materials, Table S1). Interestingly, among the tested compounds, most of the derivatives incorporating lipophilic aromatic rings in the molecular periphery were more potent CRBN binders compared to their more polar counterparts.

In conclusion, we successfully implemented the CuAAC strategy for the preparation of a series of thirty novel structurally diverse glutarimide–triazole diades in which the key moieties were connected through a methylene linker (being homologs and close analogs of previously reported diades constructed without a linker). We have also developed a novel and efficient protocol for the preparation of the key starting material for this synthesis, 3-(azidomethyl)piperidine-2,6-dione, involving the Michael addition of trimethylsilyl azide to 3-methylenepiperidine-2,6-dione. Both terminal acetylenes and their TMS-derivatives can be successfully used in the examined type of CuAAC and a large number of functional groups was well-tolerated. The phenotypic screening outcomes highlight the promise of the 1,4-disubstituted 1,2,3-triazoles series as potential CRBN ligands with selective cytotoxicity towards multiple myeloma cells, warranting further investigation and optimization. Furthermore, selected compounds were profiled against CRBN using a microscale thermophoresis assay, revealing that several representatives exhibited low micromolar binding affinities to CRBN, with compound **3h** showing potency comparable to the reference drug Pomalidomide, and compound **3a** demonstrating even greater potency. These compounds can serve as valuable starting points for designing novel PROTAC molecules due to their enhanced CRBN binding affinity and non-cytotoxic effects in normal cells. Moreover, their selective cytotoxicity against multiple myeloma MM1.S cells makes this chemotype promising for the development of new antimyeloma IMiD analogs.

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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.7543.

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