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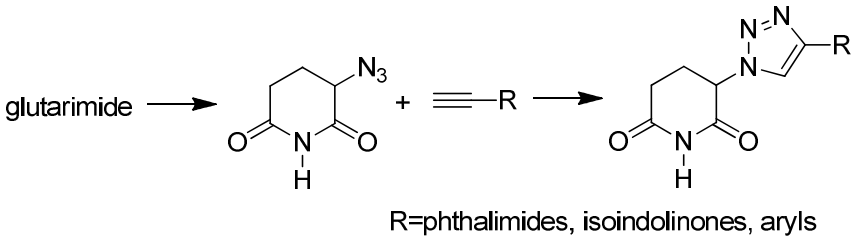
Luzzio, Ronnebaum Abstract Graphic



R=phthalimides, isoindolinones, aryls

Synthesis of 1,2,3-triazole 'click' analogues of thalidomide[†]

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Keywords: angiogenesis, cancer, EM-12, multiple myeloma, teratogenesis, thalidomide, TNF-α

Abstract

Click analogues of thalidomide were prepared from 3-azidoglutarimide and a diverse array of arylacetylenes and *N*-ethynyl/*N*-propargyl phthalimide derivatives. The sequence necessitated a new and scalable synthesis of the key click intermediate 3-azidoglutarimide. The dipolar cycloaddition reactions between the azidoglutarimide and the alkynyl coupling partners utilized a copper sulfate/sodium ascorbate reagent system in aqueous tetrahydrofuran and were first explored using substituted arylalkynes. Along with the click analogues of thalidomide, the click counterparts of the teratogenic and antiangiogenic thalidomide analogue EM-12 were prepared.

1. Introduction

In contrast to its notoriety as a potent embryotoxin, the diverse biological effects of thalidomide **1** (**Figure 1**) have proven beneficial.[1-2] The compound is an approved antilepromatic, and together with some of its analogues are active as immunosuppresants, immunomodulators, anti-inflammatories and anticancer agents.[3] As an experimental therapeutic, the antiangiogenic properties of thalidomide and closely-related analogues have gained much attention in the anticancer community.[4-8] The implication that **1** undergoes metabolic activation as an antiangiogenic agent was a consequence of in vivo metabolic studies in the rabbit and ex vivo

studies with human microsomal preparations.[9-13] At present, the structural elucidation and identity of the active antiangiogenic (or even teratogenic) species, if those indeed exist, has not been ascertained as well as its interaction with any purported molecular target.[14-15] Even though the suspected metabolites of thalidomide have been bioassay targets, most analogues have been prepared and evaluated for diverse activities without any considerations of metabolism in mind.[16-17] From the outset, however, a number of basic structural requirements became evident and were considered during the design of the most potent thalidomide analogues[18]: (1) the phthalimide portion of the molecule had to possess an intact five-member phthalimide or phthalimidine (lactam) ring; (2) the six-member glutarimide portion of the molecule had to

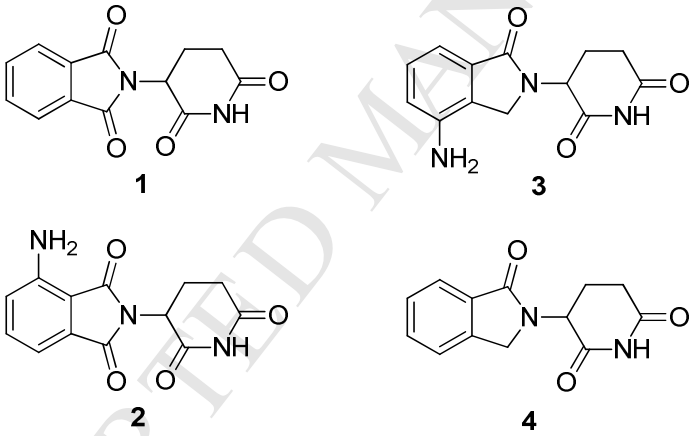


Figure 1. Thalidomide Analogues

possess an intact glutarimide ring with both carbonyls; (3) the imide (N-H) nitrogen had to possess the free hydrogen-not replaced with 'R' groups; (4) single or multiple substitutions on the aryl ring of the phthalimide while observing requirements 1-3 (above). While item 4 (above) has led to analogues of greater activity, this was specifically the case with the therapeutically-approved thalidomide analogues pomalidomide **2** and lenalidomide **3 (Figure 1)**, compounds which are used to treat multiple myeloma.[19-21] The structural basis for lenalidomide was derived

from the well-known thalidomide analogue EM-12 **4** (**Figure 1**), one of the early analogues investigated as a teratogen[22-23] and later found to have both anti-TNF-α and antiangiogenic activity.[24-25] Remarkably, very little change in the structure of **1** was responsible for delivering analogues of high therapeutic potency in **2** and **3**. While analogues involving substitution at just about every atom of the thalidomide core have been prepared, no thalidomide analogues designed with a "spacer" in between the phthalimide and glutarimide sections of the molecule have been examined. The concept is reminiscent of the 'fleximer' analogues in nucleoside chemistry whereby there is a heteroatom or carbon linker between portions of a nucleobase or a nucleobase and a sugar.[26] In the thalidomide series, such spacers could be in the form of an alkyl group, multivalent heteroatom or a carbocyclic or heterocyclic moiety (**Figure 2, 5a-c**). In terms of simplicity in synthesis, a carbon-based 'homoalkyl' analogue of thalidomide (**5a**) would be

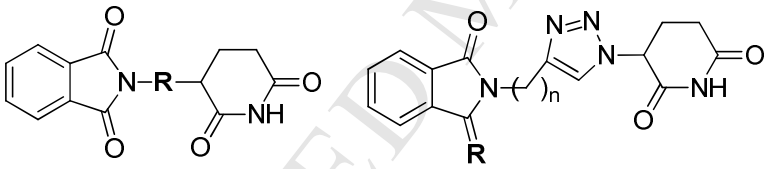


Figure 2. Thalidomide analogues having phthalimide-glutarimide 'spacers'

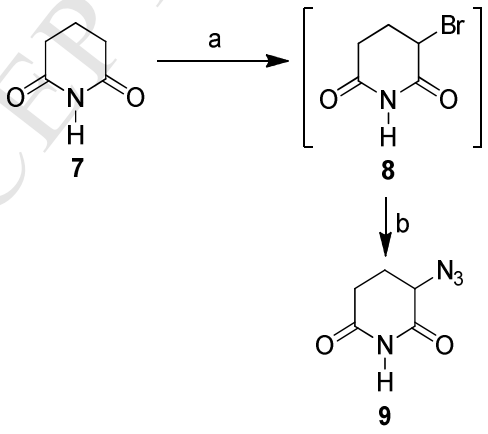
the most difficult to synthesize and would require carbon-carbon bond construction within the glutarimide framework. Heteroatoms such as nitrogen, oxygen, sulfur or phosphorus placed between the glutarimide and phthalimide rings (**5b**) may compromise stability while carbocycles between both rings (**5c**) will also require a significant synthetic commitment. The 1,2,3-triazole linker in **6**, formed by the click reaction of an *N*-substituted phthalimido azide, acetylene or propargyl group with a glutarimide-substituted acetylene or azide would be the most viable

route.[27-29] Moreover, the triazole-linked compounds may yield interesting bioassay results since these analogues exhibit both intact phthalimide and glutarimide rings and one or both moieties may associate with the molecular target.[30] Due to the ease of preparing many azides and acetylenes with simple substitution, the click route could be put to practice with relative ease and would only necessitate the exploration and adjustment of the conditions for the cycloaddition reaction. We describe herein the preparation of the first triazole spacer click analogues of thalidomide and its closely-related bioactive analogue EM-12 as well as their click homologues having a triazole-methylene spacer unit.

2. Results and Discussion

Our route to the 1,2,3-triazole series of thalidomide analogues **6** utilizes the 3-azido-substituted glutarimide **9** (**Scheme 1**). Commercially-available glutarimide **7** is reacted with bromine in chloroform at 100 °C which afforded the sensitive 3-bromoglutarimide **8** as a low-melting solid.[31] The α-bromolactam **8** was directly treated with excess sodium azide in acetone to provide

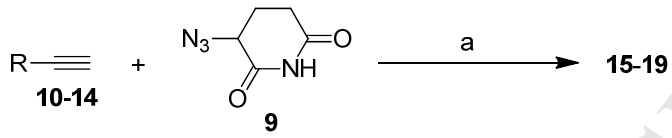
Scheme 1. Preparation of azidoglutarimide **9**.

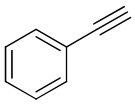
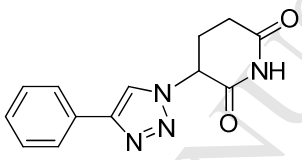
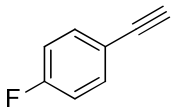
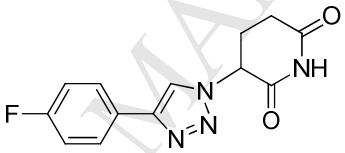
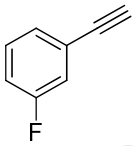
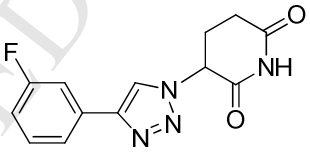
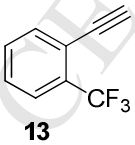
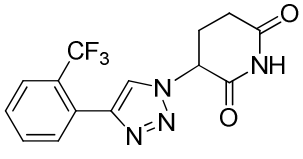
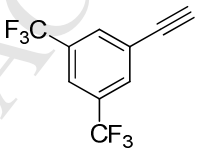
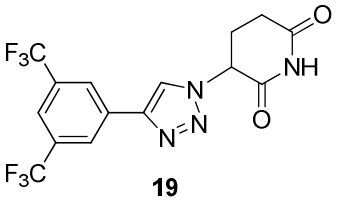


Reagents/Conditions: (a) $Br_2/CHCl_3$/100 °C/45 min; (b) NaN_3/Me_2CO/rt/16 h (53% from **7**).

the 3-azidoglutarimide **9** as an off-white solid in 53% yield (overall from glutarimide). The azidoglutarimide **9** was first evaluated for its reactivity with phenylacetylene **10** in a click reaction mediated by cupric sulfate/sodium ascorbate (**Table 1**). Using the reaction conditions

Table 1. Click products of arylacetylenes **10-14** with azidoglutarimide **9**.

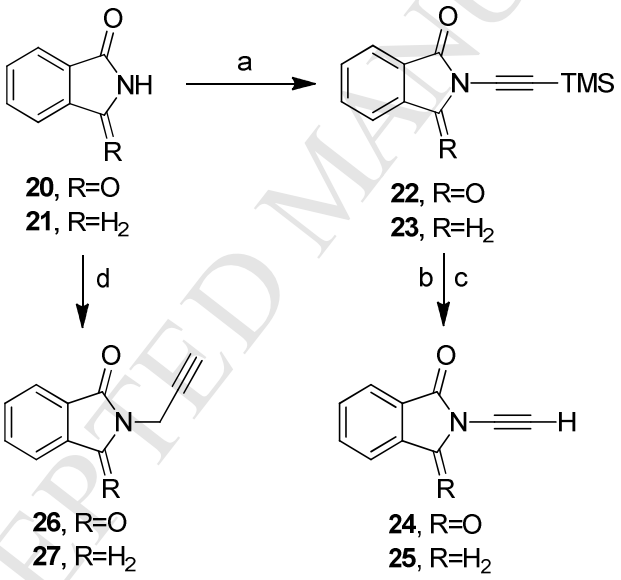


Acetylene	Product	Yield (%)
 **10**	 **15**	**78**
 **11**	 **16**	**94**
 **12**	 **17**	**98**
 **13**	 **18**	**84**
 **14**	 **19**	**76**

Reagents/Conditions: (a) $CuSO_4$/sodium ascorbate/THF/H_2O/rt/16 h

established with **9** and taking into consideration the success realized with earlier-investigated fluorinated analogues,[17] several commercially-available fluoro- and trifluoromethyl-substituted arylalkynes **11-14** were reacted with the glutarimide azide **9** and afforded the corresponding click products **15-19** (**Table 1**). The yield of the click products ranged from 76-98% and all the compounds were tractable solids and easily purified by column chromatography on silica gel. The synthesis of the phthalimide/isoindolinone and *N*-ethynyl/*N*-propargyl thalidomide reacting partners **24-27** are detailed in **Scheme 2**. The *N*-ethynylphthalimide click partner **24** was

Scheme 2. Synthesis of *N*-ethynyl and *N*-propargyl phthalimides and isoindolinones **24-27**.

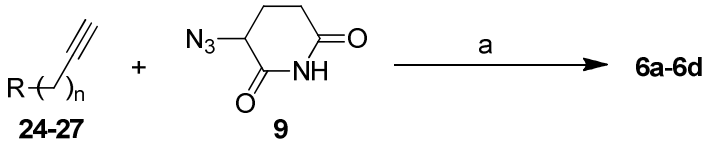


Reagents/Conditions: (a) ethynyltrimethylsilane/$Cu(OAc)_2$/O_2/pyridine/Na_2CO_3/70 °C/16 h (**22**, 59%; **23**, 22%); (b) R=O, TBAF/THF/AcOH/rt/16 h (**24**, 94%); (c) R=H_2 TBAF/THF/rt/16 h (**25**, 92%); (d) propargyl bromide/Cs_2CO_3/MeCN/80 °C/2 h (**26**, 69%; **27**, 65%).

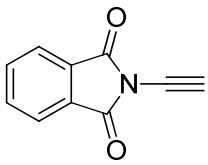
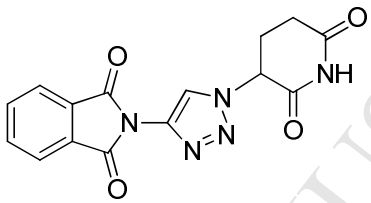
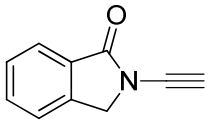
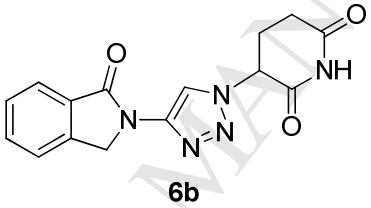
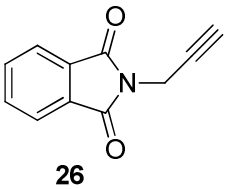
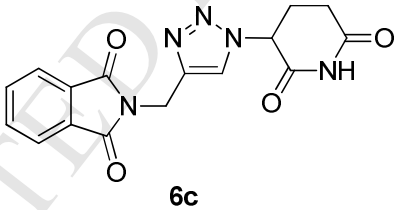
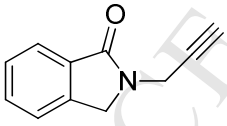
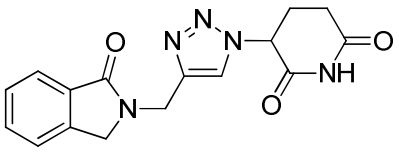
prepared from phthalimide **20** and TMS-acetylene by the method of Davies (**Scheme 2**).[32] Therefore, treatment of **20** with copper acetate, pyridine and ethynyltrimethylsilane in an atmosphere of oxygen provided the intermediate *N*-(trimethylsilylethynyl) phthalimide **22** as a

crystalline solid. Desilylation of the intermediate TMS-ethynylphthalimide **22** was accomplished with TBAF/acetic acid and provided the unstable ethynyl phthalimide **24** (**Scheme 2**). Not surprisingly, isoindolinone **21**, with its less-acidic or otherwise less-reactive proton, acted less favorably in the Davies ethynylation reaction and gave the trimethylsilyl isoindolinone **23** (22%) under similar conditions as **22**. In contrast to **22**, desilylation of **23** with TBAF only (THF/rt) gave the *N*-ethynyl isoindolinone **25** (92%) as a crystalline solid. The *N*-propargylation of phthalimide **20** and isoindolinone **21**, which gave the corresponding click partners **26** and **27**, was more straightforward than the *N*-ethynylation of **20** or **21**. Phthalimide and isoindolinone intermediates **26** and **27** were both prepared by the reaction of **20** or **21** with propargyl bromide in the presence of cesium carbonate in acetonitrile (**Scheme 2**).[15] Both propargylated intermediates (**26**, 69%, **27**, 65%) were obtained as crystalline compounds after column chromatography. The reaction of ethynyl phthalimide **24** and azidoglutarimide **9** gave click thalidomide derivative **6a** as a crystalline solid (83%) after purification by column chromatography (**Table 2**). Using the same reaction conditions as **6a**, the ethynyl isoindolinone **25** and azidoglutarimide **9** afforded the click EM-**12** analogue **6b** (78%) as a crystalline solid after column chromatography. Similarly, the *N*-propargyl phthalimide **26** and the *N*-propargylisoindolinone **26** were clicked with azidoglutarimide **9** giving the homomethylene thalidomide analogue **6c** and the homomethylene EM-12 analogue **6d** in 67% and 61% yield respectively. The regiochemistry of the dipolar cycloaddition click reaction involves products in which the substitution could be 1,4- or 1,5- on the triazole ring depending on the reaction conditions or reagents. All of the click products described herein (**Tables 1** and **2**) were formed as a result of copper (I) catalysis which gives exclusively the 1,4- or "anti"-substituted 1,2,3-triazole.[28]

Table 2. Click products of alkynyl phthalimides **24** and **26** and alkynyl isoindolinones **25** and **27** with azidoglutarimide **9**.



24-27 **9**

Alkyne	Product	Yield (%)
 **24**	 **6a**	**83**
 **25**	 **6b**	**78**
 **26**	 **6c**	**67**
 **27**	 **6d**	**61**

Reagents/Conditions: (a) $CuSO_4$/sodium ascorbate/THF/H_2O/rt/16 h

3. Conclusions

Synthetic routes to click analogues of thalidomide and its descarbonyl analogue EM-12 are detailed which utilize the key intermediate 3-azidoglutarimide along with *N*-ethynyl and *N*-

propargyl analogues of phthalimide as reacting components. The dipolar cycloaddition chemistry of the azidoglutarimide was explored using arylalkynes containing electron-withdrawing groups in place of the phthalimide group and yielded cycloaddition products in high yield using the $CuSO_4$/sodium ascorbate reaction conditions. The click thalidomide analogues will be evaluated in a full range of bioassay types and these results will be reported in due course.

4. Experimental

Solvents and reagents are ACS grade and were used as commercially supplied. Analytical thin-layer chromatography (TLC) utilized 0.25 mm pre-cut glass-backed plates (Merck, Silica Gel 60 F254). Thin-layer chromatograms were visualized during chromatographic and extraction runs by rapidly dipping the plates in anisaldehyde/ethanol/sulfuric acid stain or phosphomolybdic acid/ethanol stain and heating (hot plate). Gravity-column chromatography was carried out using silica gel 60 (E. Merck 7734, 70-230 mesh). Flash-column chromatography utilized silica gel 60 (E. Merck 9385, 230-400 mesh) with nitrogen gas pressurization. Melting points were taken on a Thomas Hoover apparatus. Extracts and chromatographic fractions were concentrated with a Büchi rotavapor under water aspirator vacuum. Nuclear magnetic resonance (^1H and ^{l3}C NMR) spectra were recorded with Varian VNMRS 400 or 700 MHz instruments using $CDCl_3$ as a solvent and TMS as internal standard. Infrared spectra (FTIR) were recorded with a Perkin-Elmer Spectrum 100 instrument and spectral values are reported as cm^{-1}. The measurement of high-resolution mass spectra (HRMS) were performed at the Texas A&M University Laboratory for Biological Mass Spectrometry. Elemental analysis was performed by Galbraith Laboratories, Knoxville, TN.

3-Azidopiperidine-2,6-dione 9: To a 48 mL glass pressure reaction vessel fitted with a Teflon screw cap was added piperidine-2,6-dione (2.00 g, 17.7 mmol) and Br_2 (2.82 g, 0.90 mL, 17.7 mmol) in chloroform (15 mL). The reaction mixture was then heated in a Kugelröhr oven at 110 °C (45 min). The solvent was removed and the crude bromoglutarimide **7** was then dissolved in acetone (5 mL) followed by the addition of sodium azide (3.44 g, 53.1 mmol) whereupon the reaction mixture turned blue-purple. The reaction mixture was stirred at room temperature (24 h) and then directly applied to a silica gel column. Elution with hexane/ethyl acetate (1:1) gave a mixture of unreacted 3-bromopiperidine-2,6-dione **7** and 3-azidopiperidine-2,6-dione **8** (1:1). The mixture of azide **8** and unreacted bromide **7** was again dissolved in acetone (5 mL) and sodium azide (1.15 g, 17.7 mmol) was added and stirring of the blue-purple reaction mixture was continued at room temperature (24 h). Column chromatography (hexane/ethyl acetate, 1:1) of the reaction mixture gave pure 3-azidopiperidine-2,6-dione **9** as an off-white amorphous solid (1.44 g, 53% from glutarimide): mp 144-145 °C; R_f 0.24 (TLC stains blue with heat); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (dd, *J*=9.6 Hz, 8.4 Hz, 1H), 2.78 (dt, *J*=18.4 Hz, 5.6 Hz, 1H) 2.63-2.54 (m, 1H), 2.23-2.16 (m, 1H), 2.04-1.95 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.7, 169.2, 58.2, 29.1, 24.0. FT-IR (neat) 3090, 2112, 1710, 1676 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_5H_6N_4O_2$: 155.0491, Found: 155.0569.

General Procedure for the preparation of click compounds 15-19: To a stirred solution of 3-azidopiperidine-2,6-dione **9** (20.0 mg, 0.13 mmol) and arylalkyne **10-14** (0.143 mmol) in tetrahydrofuran (750 μL) was added aqueous copper sulfate (52mM, 0.013 mmol, 250 μL). Sodium ascorbate (12.8 mg, 0.065 mmol) was then added ten minutes after the addition of the $CuSO_4$ solution. The reaction mixture was stirred (16 h) at room temperature followed by direct application to a gravity silica gel column and elution with chloroform/methanol (95:5).

Combination and concentration of the chromatographic fractions gave the pure triazoles **15-19** as white or off-white amorphous solids:

3-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 15: 3-(4-Phenyl-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione **15** was obtained as a white amorphous solid (26 mg, 78%): mp 208-210 °C; R_f 0.28 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 8.66 (s, 1H), 7.82-7.84 (d, *J*=7.2 Hz, 2H), 7.42-7.46 (m, 2H), 7.30-7.34 (m, 1H), 5.84 (dd, *J*=13.2, 5.2 Hz, 1H), 2.83-2.91 (m, 1H) 2.65-2.76 (m, 2H) 2.34-2.36 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 169.9, 146.7, 131.0, 129.4, 128.4, 125.5, 122.0, 59.6, 31.1, 24.8: IR (neat) 3090, 2930, 1732, 1699, 1676 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$; calcd for $C_{13}H_{13}N_4O_2$: 257.1039, Found: 257.1140.

3-(4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 16: 3-(4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione **16** was obtained as a white amorphous solid (64 mg, 94%): mp 221-222 °C; R_f 0.32 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.64 (s, 1H) 7.84-7.88 (m, 2H) 7.25-7.30 (m, 2H), 5.83 (dd, *J*=12.4, 4.8 Hz, 1H), 2.82-2.90 (m, 1H), 2.62-2.70 (m, 2H), 2.32-2.35 (m, 1H) ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 169.8, 163.5, 161.0, 145.8, 127.6 (d, *J*=7.6 Hz), 122.0, 116.4 (d, *J*=22.0 Hz), 59.6, 31.1, 24.8. IR (neat) 3194, 3105, 2916, 1731, 1708 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M+H]^+$; calcd for $C_{13}H_{12}FN_4O_2$: 275.0944, Found: 275.1014.

3-(4-(3-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 17: 3-(4-(3-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione **17** was obtained as a white solid (35 mg, 98%): mp 199-201 °C; R_f 0.375 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 8.74 (s, 1H), 7.65-7.69 (m, 1H), 7.46-7.51 (m, 2H), 7.13-7.18 (m, 1H), 5.85 (dd, *J*=12.4, 4.4

Hz, 1H), 2.83-2.89 (m, 1H), 2.62-2.71 (m, 2H), 2.34-2.37 (m, 1H) ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 169.8, 164.2, 161.8, 145.7, 132.4 (d, *J*=154.9 Hz), 122.9, 121.6, 115.2 (d, *J*=21.3 Hz), 112.1 (d, *J*=15.9 Hz), 59.7, 31.1, 24.8. IR (neat) 3098, 2917, 2853, 1732, 1705 cm-1. HRMS (ESI-TOF) *m/z* $[M+H]^+$; calcd for $C_{13}H_{12}FN_4O_2$: 275.0944, Found: 275.0837.

3-(4-(2-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 18: 3-(4-(2-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione was obtained as a white solid (36 mg, 84%): mp 155-157 °C; R_f 0.37 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 8.41 (s, 1H), 7.74-7.85 (m, 3H), 7.60-7.63 (m, 1H), 5.89 (dd, *J*=12.8, 5.2 Hz, 1H), 2.83-2.90 (m, 1H), 2.65-2.78 (m, 2H), 2.31-2.36 (m, 1H) ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9, 169.8, 143.7, 133.1, 132.2, 129.8 (q, *J*=2.2 Hz), 129.3, 126.8 (q, *J*=30.4 Hz), 126.7 (q, *J*=6.1 Hz), 124.8 (q, *J*=3.1 Hz), 124.4 (q, *J*=271.8 Hz), 59.7, 31.2, 24.8. IR (neat) 3220, 3094, 2957, 1732, 1701 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M-H]^-$; calcd for $C_{14}H_{10}F_3N_4O_2$ 323.0756, Found: 323.0714.

3-(4-(3,5-Bis(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 19: 3-(4-(3,5-bis(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione **19** was obtained as a white solid (38 mg, 76%): mp 115-117 °C; R_f 0.29 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.07 (s, 1H), 8.50 (s, 2H), 8.07 (s, 1H), 5.91 (dd, *J*=12.8, 5.2 Hz, 1H), 2.84-2.89 (m, 1H), 2.66-2.73 (m, 2H), 2.38-2.41 (m, 1H) ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 169.7, 144.1, 133.5, 131.5 (q, *J*=32.6 Hz), 125.7, 124.2, 123.2 (q, *J*=271.9 Hz), 121.7, 59.8, 31.1, 24.7. IR (neat) 3219, 3094, 2872, 1733, 1704 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M-H]^-$; calcd for $C_{15}H_9F_6N_4O_2$ 391.0630, Found: 391.0707

Isoindolin-1-one 21: In a 100 mL round bottom flask phthalimide **20** (5.00 g, 34.0 mmol) was dissolved in acetic acid (25 mL). A slurry of tin metal (9.68 g, 81.6 mmol) and concentrated hydrochloric acid (12.5 mL) was added to the phthalimide solution. The reaction mixture was heated under reflux at 120 °C (6 h) then diluted with dichloromethane (75 mL) and washed with brine (3 x 25 mL). Concentration of the extract followed by gravity- column chromatography (chloroform/methanol, 95:5) gave pure isoindolin-1-one as an off-white solid (1.75 g, 39% yield): mp 154-156 ºC (Lit.[35] 150-152 ºC). The spectroscopic data was consistent with the literature[34]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J*=8Hz, 1H), 7.75-7.59 (m, 1H), 7.47-7.49 (m, 2H), 4.47 (s, 2H).

2-(2-(Trimethylsilyl)ethynyl)isoindoline-1,3-dione 22: Prepared by the method of Davies (59%):[32] mp 92-94 ºC (Lit.[33] 94-96 ºC). The spectroscopic data was consistent with the literature.[32]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-7.93 (m, 2H), 7.78-7.81 (m, 2H), 0.273 (s, 9H).

2-(2-(Trimethylsilyl)ethynyl)isoindolin-1-one 23: To a 250 mL round bottom flask $Cu(OAc)_2$ (76.4 mg, 0.42 mmol), isoindolin-1-one (1.00 g, 7.51 mmol), Na_2CO_3 (216 mg, 4.2 mmol), and 4 Å molecular sieves (2.0 g) were combined. A solution of pyridine (324 mL, 4.2 mmol) in toluene (30 mL) was added to the reaction flask followed by sweeping with three volumes of O_2. The reaction flask was placed in an oil bath and stirred for 1 h (70ºC) until the reaction mixture was a bright green color. A solution of ethynyltrimethylsilane (298 µL, 2.1 mmol) in dry toluene (2 mL) was added to the mixture over 30 min. The reaction mixture was allowed to stir (4 h) during which time the reaction mixture changed to a black suspension. The reaction mixture was filtered through Celite®, concentrated under reduced pressure and purified by flash column chromatography (hexane/ethyl acetate, 5:1) to yield **23** as a fluffy white amorphous solid (106 mg, 22%): mp 134-136 °C; R_f 0.33 (hexane/ethyl acetate, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$)

δ 7.89 (d *J*=7.6 Hz, 1H), 7.62 (dd *J*=8.0, 7.6 Hz, 1H), 7.50 (dd *J*=8.0, 7.6 Hz, 1H), 7.44 (d *J*=8.0 Hz, 1H), 4.70 (s, 2H), 0.25 (s, 9H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 168.6, 140.8, 133.0, 129.6, 128.6, 124.6, 122.8, 92.8, 75.9, 52.5, 0.18. IR (neat) 2954, 2902, 2167, 1712 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Cl]^-$; calculated for $C_{13}H_{15}{}^{35}ClNOSi$ 264.0617, Found: 264.0688.

2-Ethynylisoindoline-1,3-dione 24: Prepared by the Method of Davies (94%):[32] mp 165-169 ºC (Lit.[33] 167-172 ºC). The spectroscopic data was consistent with the literature[32]: ^{1}H NMR (400 mHz, $CDCl_3$) δ 7.92-7.94 (m, 2H), 7.80-7.83 (m, 2H), 3.33 (s, 1H).

2-Ethynylisoindoline-1-one 25: 2-(2-(Trimethylsilyl)ethynyl)isoindolin-1-one **23** (72.0 mg, 0.313 mmol) was dissolved in THF (3.0 mL) while stirring followed by the addition of *tetra*-N-butylammonium fluoride (TBAF, 450 µL, 1 M solution). Stirring was continued (10 min) until the reaction was complete as indicated by TLC. The crude reaction mixture was directly submitted to gravity-column chromatography (hexanes/ethyl acetate) and afforded **25** as a white solid (45 mg, 92%): mp 92-94 °C; R_f 0.21 (hexane/ethyl acetate, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d *J*=7.2 Hz, 1H), 7.64 (dd *J*=7.2, 7.2 Hz, 1H), 7.51 (dd *J*=7.2, 7.2 Hz, 1H), 7.46 (d *J*=7.2 Hz, 1H), 4.71 (s, 2H), 3.08 (s, 1H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 169.1, 140.9, 133.2, 129.4, 128.7, 124.7, 122.9, 73.9, 61.7, 52.1. IR (neat) 3268, 2931, 2139, 1718 cm^{-1}. Anal. Calcd for $C_{10}H_7NO$ (157.17): C, 76.42; H, 4.49; N, 8.91; Found: C, 76.32; H, 4.68; N, 8.69.

2-(Prop-2-yn-1-yl)isoindoline-1,3-dione 26: Prepared by the method of Clayton (69%):[34] mp 146-148 ºC (Lit.[36] 149-150 ºC). The spectroscopic data was consistent with the literature[36]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80-7.91 (m, 2H), 7.74-7.76 (m, 2H), 4.47 (s, 2H), 2.24 (s, 1H).

2-(Prop-2-yn-1-yl)isoindolin-1-one 27: Isoindolin-1-one **21** (250 mg, 1.85 mmol) was dissolved in acetonitrile (9 mL) and cesium carbonate (2.40 g, 7.39 mmol) was added followed by

propargyl bromide (308 μL, 2.77 mmol). The reaction mixture was stirred at reflux (2 h). The reaction mixture was then dissolved in CH_2Cl_2 (10 mL) and washed with water (3 x 15 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Gravity-column chromatography of the residual oil (hexane/ethyl/acetate, 2:1) gave **27** as a yellow solid (205 mg, 65%): mp 79-81 °C; R_f 0.66 (ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d *J*=8.0 Hz, 1H), 7.52 (dd *J*=8.0, 7.2 Hz, 1H) 7.41-7.45 (m, 2H), 4.47 (s, 2H), 4.24 (s, 2H), 2.28 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 141.2, 132.1, 131.6, 128.1, 123.8, 122.8, 78.1, 72.5, 49.1, 31.7. IR (neat) 3290, 3243, 2118, 1701 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$; calculated for $C_{11}H_{10}NO$ 172.0762, Found: 172.0857.

General Procedure for the preparation of click products 6a-6d: 3-Azidopiperidine-2,6-dione **9** (0.25-0.5 mmol, 38-74 mg) and phthalimide or isoindolinone alkynes **24**, **26**, **25** or **27** (0.275-0.55 mmol, 1.1 equiv.) were dissolved in 25% aqueous THF (2.0 mL). The mixture was stirred (10 min) before addition of solid $CuSO_4$ (0.5 eq.) and sodium ascorbate (0.5 eq.) whereupon the reaction mixture became a teal-colored suspension and stirring was continued (16 h). The reaction mixture was concentrated under rotary evaporation while absorbing onto silica gel (70-230 mesh) and the resulting dry residue was applied to a silica gel chromatography column.

2-(1-(2,6-Dioxopiperidin-3-yl)-1*H*-1,2,3-triazol-4-yl)isoindoline-1,3-dione 6a: The crude reaction mixture was submitted to gravity-column chromatography (hexane/ethyl acetate, 1:9) to give triazole **6a** as a white amorphous powder (126 mg, 83%): mp 289-291 °C; R_f 0.38 (THF/hexane, 2:1); ^{1}H NMR δ (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 8.45 (s, 1H), 7.97-8.00 (m, 2H), 7.90-7.94 (m, 2H), 5.90 (dd *J*=12.8, 5.6 Hz, 1H), 2.82-2.86 (m, 1H), 2.66-2.75 (m, 2H), 2.34-2.37 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 169.5, 166.5, 136.1, 135.5, 131.8,

124.2, 122.9, 60.2, 31.2, 24.6. IR (neat) 3146, 3067, 2933, 1721, 1696 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M+H]^+$; calculated for $C_{15}H_{12}N_5O_4$ 326.0889, Found: 326.0961.

3-(4-(1-Oxoisoindolin-2-yl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 6b: The crude reaction mixture was submitted to gravity-column chromatography (chloroform/methanol, 95:5) and then recrystallized with methanol to give triazole **6b** as a white amorphous powder (70 mg, 78%): mp decomp>300 °C; R_f 0.41 (chloroform/methanol, 9:1); ^{1}H NMR (700 MHz, DMSO-d_6) δ 11.19 (s, 1H), 8.56 (s, 1H), 7.79 (d *J* = 7.7 Hz, 1H), 7.67-7.71 (m, 2H), 7.53 (dd *J*=7.7, 7.0 Hz, 1H), 5.85 (dd *J*=13.3, 5.6 Hz, 1H), 2.76-2.84 (m, 2H), 2.64-2.66 (m, 1H), 2.27-2.28 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 169.7, 165.6, 143.6, 142.4, 132.8, 131.6, 128.7, 124.3, 123.6, 113.8, 60.1, 49.4, 31.2, 24.5. IR (neat) 3180, 3087, 2923, 1735, 1697 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M+H]^+$; calculated for $C_{15}H_{14}N_5O_3$ 312.1097, Found: 312.1176.

2-((1-(2,6-Dioxopiperidin-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)isoindoline-1,3-dione 6c: The crude reaction mixture was submitted to gravity-column chromatography (chloroform/methanol, 97:3) to give triazole **6c** as a white amorphous powder (56 mg, 67%): mp 230-232 °C; R_f 0.44 (chloroform/methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1H), 8.16 (s, 1H), 7.83-7.89 (m, 4H), 5.74 (dd *J*=12.8, 8.0 Hz, 1H), 4.83 (s, 2H), 2.75-2.80 (m, 1H), 2.56-2.63 (m, 2H), 2.20-2.23 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 169.7, 167.7, 142.7, 135.0, 132.0, 123.7, 123.6, 59.5, 33.3, 31.1, 24.7. IR (neat) 3220, 2957, 2863, 1732, 1701. cm^{-1}; HRMS (ESI-TOF) m/z $[M+H]^+$; calculated for $C_{16}H_{14}N_5O_4$ 340.1046, Found: 340.0888.

3-(4-((1-Oxoisoindolin-2-yl)methyl)-1*H*-1,2,3-triazol-1-yl)piperidine-2,6-dione 6d: The crude reaction mixture was submitted to gravity-column chromatography (chloroform/methanol, 97:3) to give triazole **6d** as a white amorphous powder (53 mg, 61%): mp 212-215 °C; R_f 0.39

(chloroform /methanol, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.17 (s, 1H), 8.16 (s, 1H), 7.68 (d *J*=7.2 Hz, 1H), 7.56-7.57 (m, 2H), 7.44-7.49 (m, 1H), 5.76 (dd *J*=12.8 Hz, 5.2 Hz, 1H), 4.80 (s, 2H), 4.46 (s, 2H) 2.77-2.82 (m, 1H), 2.59-2.64 (m, 2H), 2.22-2.47 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 169.8, 167.5, 143.5, 142.2, 132.4, 131.9, 128.3, 123.9, 123.8, 123.3, 59.5, 49.9, 37.4, 31.1, 24.7. IR (neat) 3063, 2916, 2836, 1704, 1666 cm^{-1}. HRMS (ESI-TOF) *m/z* $[M+H]^+$; calculated for $C_{16}H_{16}N_5O_3$ 326.1253, Found: 326.1287.

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References and Notes

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Luzzio Ronnebaum Figurer

Luzzio_Ronnebaum Fig.2

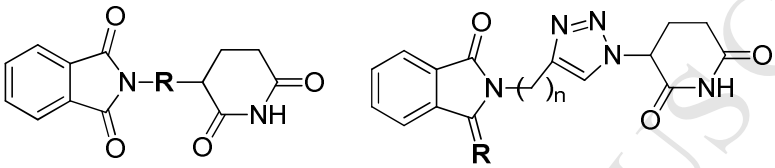

Figure 2. Thalidomide analogues having phthalimide-glutarimide 'spacers'