Electronic Supplementary Information

Structure-guided design of a truncated heterobivalent chemical probe degrader of IRE1α

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Hazards

All cereblon modulators should be treated as potential teratogens.

Figures

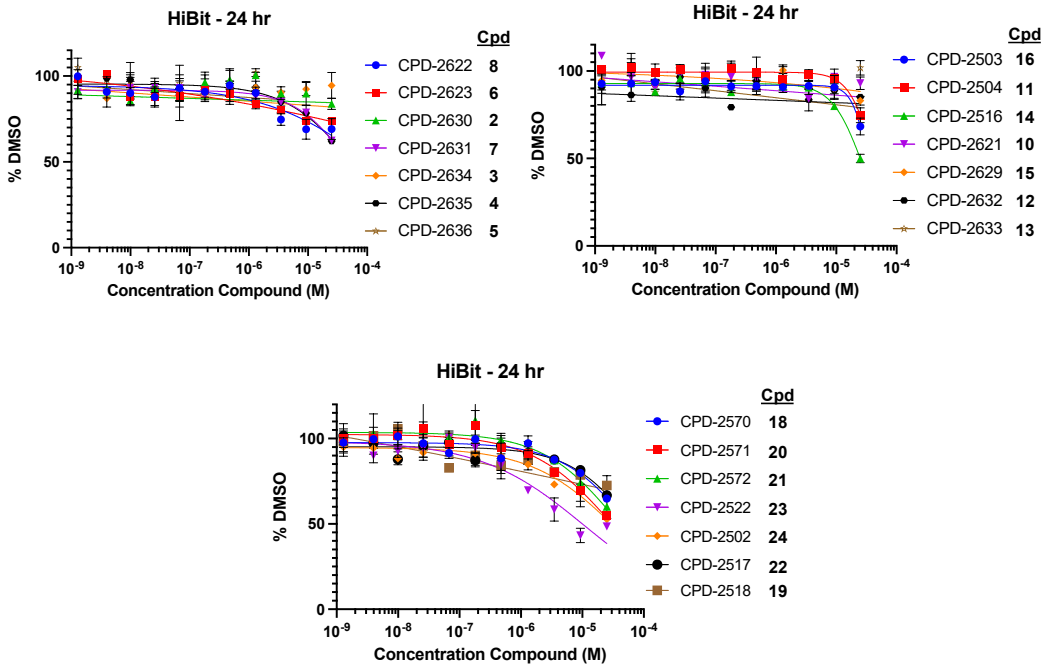
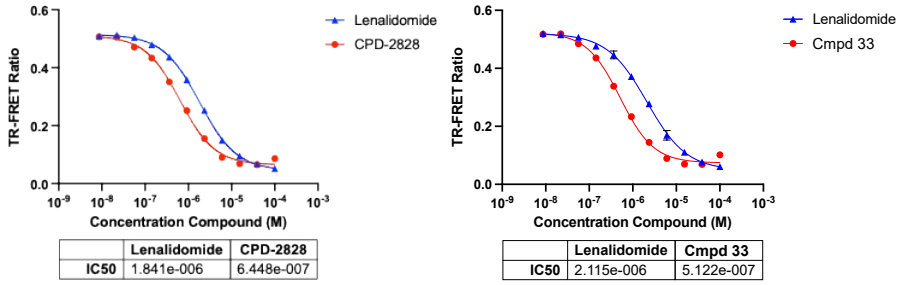


Fig. S1. Dose response curves for compounds in Fig. 3 in IRE1α HiBiT.



	Lenalidomide	CPD-2828
IC50	1.841e-006	6.448e-007

	Lenalidomide	Cmpd 33
IC50	2.115e-006	5.122e-007

Fig. S2. CRBN *in vitro* binding for **30** (CPD-2828) and cmpd **33** show similar potency. Lenalidomide is included as an assay positive control. Data are reported as the average of duplicates ± std dev.

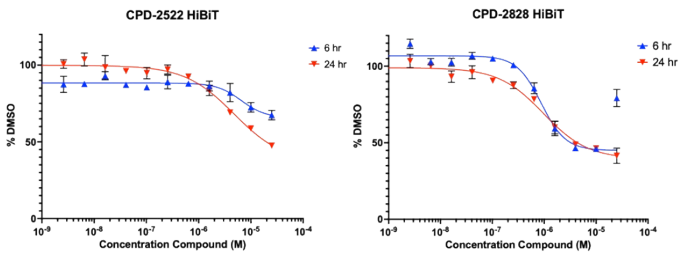


Fig. S3. IRE1α-SpyTag-HiBiT HEK293T cells were treated with dose titrations of **23** (CPD-2522) or **30** (CPD-2828). After the noted incubation time, the HiBiT signal was measured and normalized to DMSO in order to determine levels of degradation. Data are reported as the average of duplicates ± std dev.

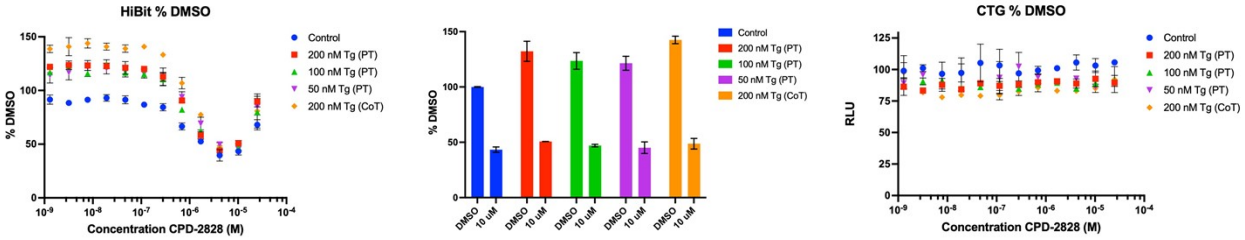
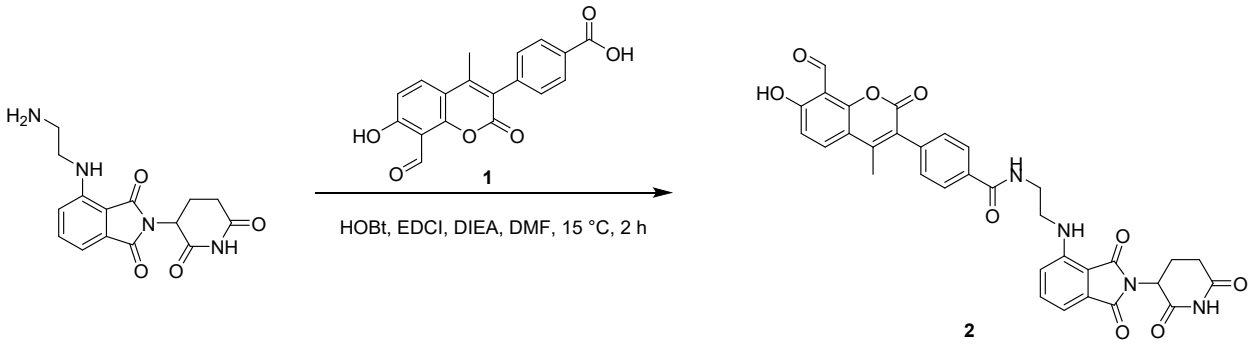


Fig. S4. HiBiT cells were pre-treated (PT) or co-treated (CoT) with thapsigargin (Tg) before dosing with CPD-2828 (**30**) for 6 hours. Tg clearly increased IRE1α levels, but the potency of CPD-2828 was not affected. A cell-titer glo (CTG) assay was performed to confirm that there was no effect on cell viability.

Chemical Syntheses

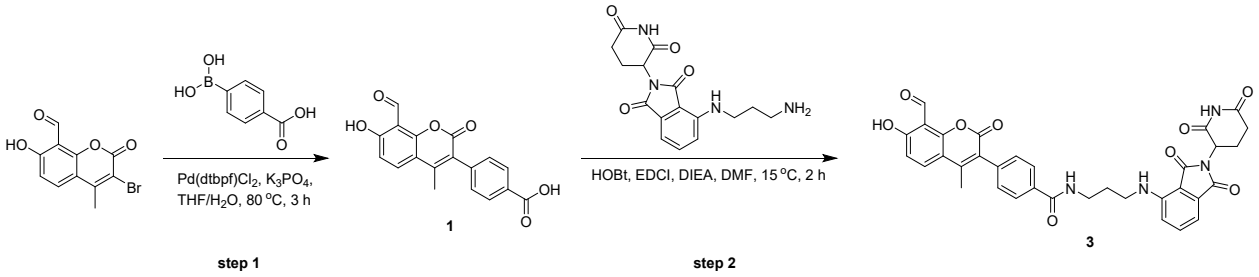
The purity of all tested compounds was >90% except for the following: **2** (82%); **5** (85%); **13** (79%); **15** (77%); **21** (86%); **26** (89%). Correcting for these purities would only slightly affect the potency data and does not significantly change interpretation of structure-activity relationships.

Synthesis of N-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (2)

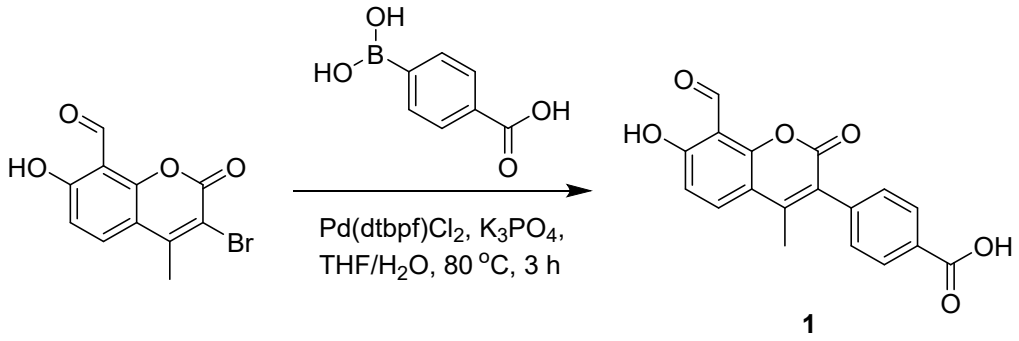


A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (30 mg, 92 μmol, 1 *eq*), 4-(2-aminoethylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (29 mg, 92 μmol, 1 *eq*), HOBt (15 mg, 111 μmol, 1.2 *eq*), DIEA (35 mg, 277 μmol, 48 μL, 3 *eq*) and EDCI (21 mg, 111 μmol, 1.2 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 15 °C for 2 hours under a N_2 atmosphere. The reaction mixture was quenched by the addition of H_2O (10 mL), and filtered to give a residue that was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 μm; mobile phase: [water (TFA)-ACN]; B%: 35%-65%, 8 minutes). Compound N-[2-[[2-(2,6-dioxo-3-piperidyl) -1,3-dioxo-isoindolin-4-yl]amino]ethyl]-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (1 mg, 1% yield, 82% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.91 (s, 1H), 11.09 (s, 1H), 10.50 (s, 1H), 8.76 (br s, 1H), 8.04 (d, J = 9.1 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.65 - 7.56 (m, 1H), 7.43 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.4 Hz, 1H), 7.07 - 6.99 (m, 2H), 6.86 (br s, 1H), 5.06 (dd, J = 4.8, 12.7 Hz, 1H), 3.54 - 3.47 (m, 4H), 2.93 - 2.82 (m, 1H), 2.61 - 2.53 (m, 2H), 2.26 (s, 3H), 2.05 - 1.97 (m, 1H).

Synthesis of N-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (3)

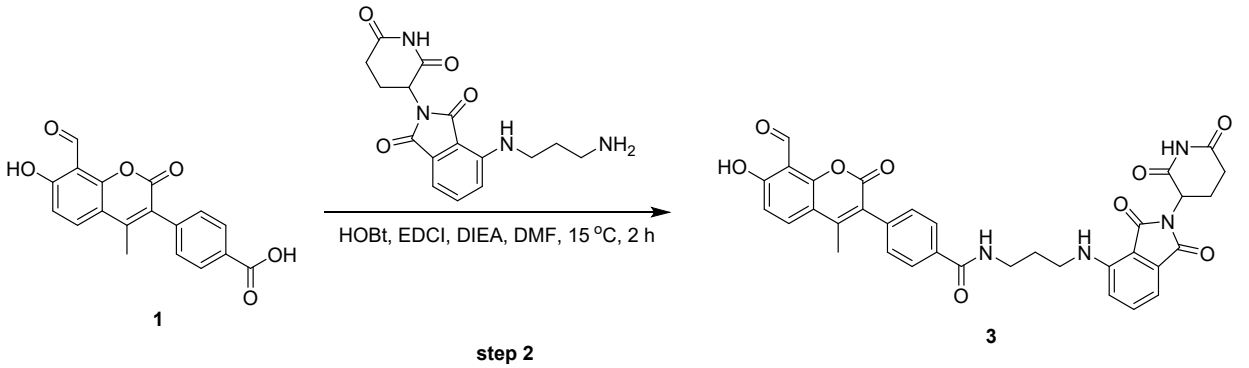


Step 1: Synthesis of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzoic acid



A mixture of 3-bromo-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (150 mg, 530 μmol, 1 *eq*), 4-boronobenzoic acid (105 mg, 635 μmol, 1.2 *eq*), ditert-butyl(cyclopentyl)phosphane dichloropalladium iron (35 mg, 53 μmol, 0.1 *eq*), K_3PO_4 (225 mg, 1.06 mmol, 2 *eq*) in THF (1 mL) and H_2O (0.2 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 80 °C for 3 hours under a N_2 atmosphere. The reaction mixture was quenched with H_2O (5 mL), and the pH adjusted to 9 with LiOH aq. The mixture was washed with EtOAc (5 mL) and the pH adjusted to 4 with 2M HCl, and the resulting solid precipitate was filtered to give the title compound (4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (110 mg, 64% yield) as a yellow solid which was used in the next step without further purification.

Step 2: Synthesis of N-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (3)



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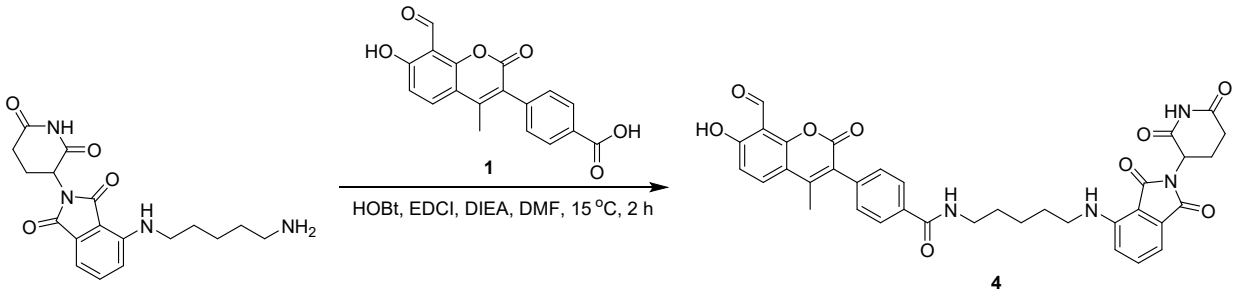
step 2

3

A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (35 mg, 108 μmol, 1 *eq*) , 4-(3- aminopropylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (35 mg, 107 μmol, 1 *eq*), HOBt (17 mg, 129 μmol, 1.2 *eq*), EDCI (24 mg, 129 μmol, 1.2 *eq*) and DIEA (42 mg, 324 μmol, 56 μL, 3 *eq*) in DMF (1 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of water (10 mL), and filtered to give a residue that was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 μm; mobile phase: [water (TFA)-ACN]; B%: 35%-65%, 8 minutes). The title compound N-[3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]propyl]-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (1 mg, 2% yield, 99% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 1.78 - 1.88 (m, 2 H), 2.00 - 2.07 (m, 1 H), 2.26 (s, 3 H), 2.53 - 2.61 (m, 2 H), 2.84 - 2.93 (m, 1 H), 3.39 - 3.46 (m, 4 H), 5.06 (dd, J=12.9, 5.3 Hz, 1 H), 6.78 (br t, J=5.8 Hz, 1 H), 6.97 - 7.06 (m, 2 H), 7.13 (d, J=8.6 Hz, 1 H), 7.42 (d, J=8.1 Hz, 2 H), 7.59 (t, J=7.8 Hz, 1 H), 7.92 (d, J=8.1 Hz, 2 H), 8.04 (d, J=9.0 Hz, 1 H), 8.63 (br t, J=5.4 Hz, 1 H), 10.50 (s, 1 H), 11.10 (s, 1 H), 11.91 (s, 1 H).

Synthesis of N-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (4)



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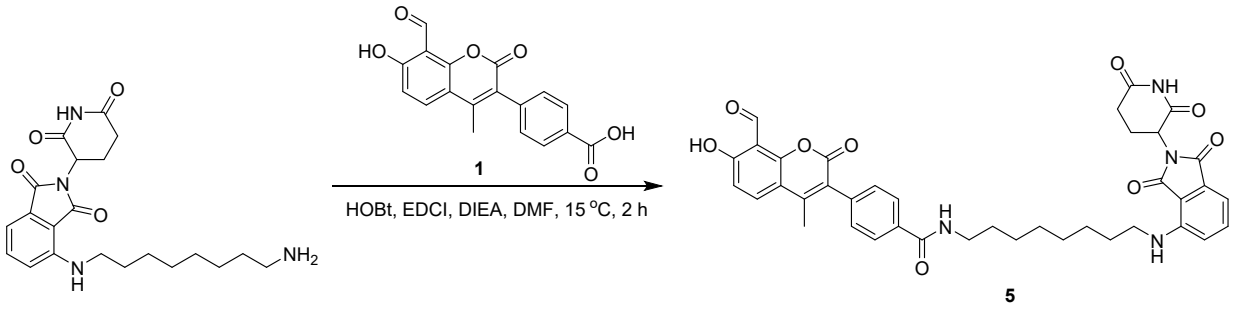
A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (30 mg, 92 µmol, 1 *eq*), 4-(5- aminopentylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (33 mg, 92 µmol, 1 *eq*), HOBt (15 mg, 111 µmol, 1.2 *eq*), EDCI (21 mg, 111 µmol, 1.2 *eq*) and DIEA (36 mg, 277 µmol, 48 µL, 3 *eq*) in DMF (1 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by addition H_2O (10 mL), filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 35%-65%, 8 minutes). The title compound N-[5-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]pentyl]-4-(8-formyl-7-hydroxy-4- methyl-2-oxo-chromen-3-yl)benzamide (3 mg, 4% yield, 93% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 1.38 - 1.45 (m, 2 H), 1.61 (dt, J=14.1, 7.1 Hz, 4 H), 1.99 - 2.04 (m, 1 H), 2.26 (s, 3 H), 2.58 (br d, J=17.0 Hz, 2 H), 2.85 - 2.90 (m, 1 H), 3.29 - 3.33 (m, 4 H), 5.05 (dd, J=13.0, 5.2 Hz, 1 H), 6.56 (br s, 1 H), 6.99 - 7.05 (m, 2 H), 7.11 (d, J=8.6 Hz, 1 H), 7.41 (d, J=8.1 Hz, 2 H), 7.58 (t, J=7.8 Hz, 1 H), 7.90 (d, J=8.1 Hz, 2 H), 8.04 (d, J=9.0 Hz, 1 H), 8.53 (br t, J=5.6 Hz, 1 H), 10.51 (s, 1 H), 11.09 (s, 1 H), 11.91 (s, 1 H).

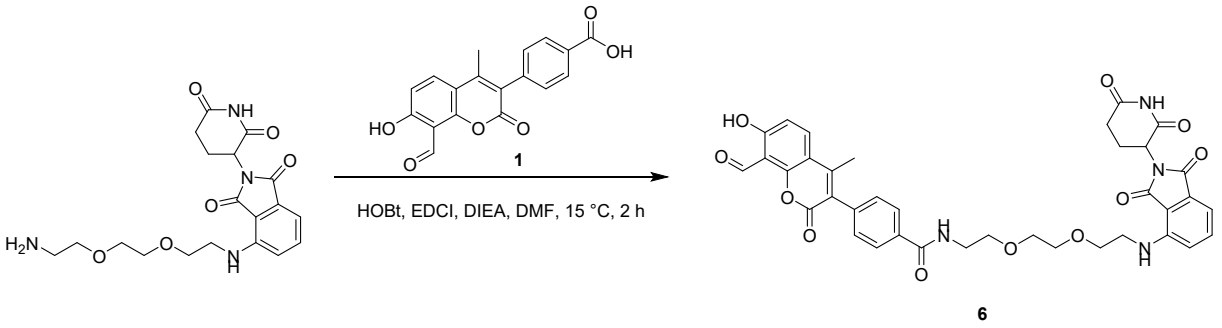
Synthesis of N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (5)



A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (30 mg, 92 µmol, 1 *eq*), 4-(8- aminooctylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (37 mg, 92 µmol, 1 *eq*), HOBt (15 mg, 111 µmol, 1.2 *eq*), EDCI (21 mg, 111 µmol, 1.2 *eq*) and DIEA (36 mg, 277 µmol, 48 µL, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 15 °C for 2 hr under N2 atmosphere. The reaction mixture was quenched by addition H_2O 10 mL, filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 40%-

70%, 8 minutes). The title compound N-[8-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]octyl]-4-(8-formyl-7-hydroxy-4- methyl-2-oxo-chromen-3-yl)benzamide (2 mg, 2% yield, 85% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 1.29 - 1.37 (m, 8 H), 1.51 - 1.60 (m, 4 H), 1.99 - 2.05 (m, 1 H), 2.25 (s, 3 H), 2.58 (br d, J=17.4 Hz, 2 H), 2.86 (br d, J=13.1 Hz, 1 H), 3.26 - 3.30 (m, 4 H), 5.05 (br dd, J=12.6, 5.1 Hz, 1 H), 6.53 (br s, 1 H), 6.99 - 7.12 (m, 3 H), 7.41 (br d, J=8.0 Hz, 2 H), 7.57 (br t, J=7.6 Hz, 1 H), 7.90 (br d, J=8.0 Hz, 2 H), 8.04 (d, J=9.0 Hz, 1 H), 8.50 (br t, J=5.4 Hz, 1 H), 10.50 (s, 1 H), 11.09 (s, 1 H), 11.91 (s, 1 H).

Synthesis of N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) amino) ethoxy) ethoxy)ethyl)-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl) benzamide (6)



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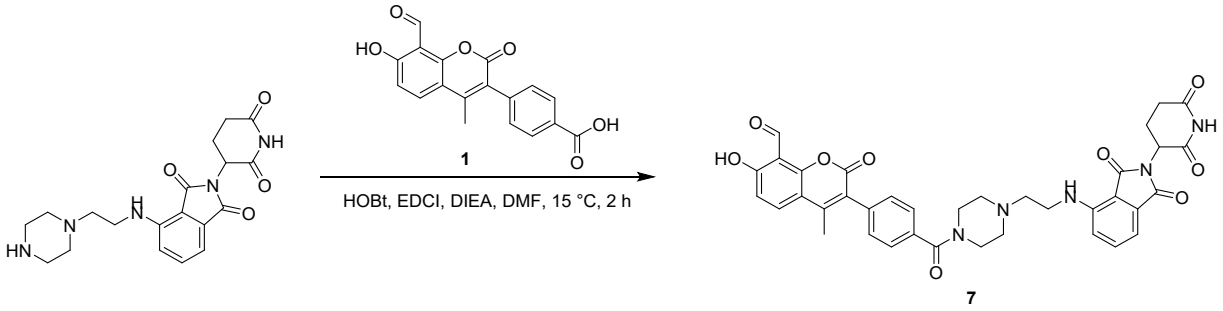
6

A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (35 mg, 107 µmol, 1 *eq*), 4-[2-[2-(2-aminoethoxy)ethoxy] ethylamino]-2-(2,6-dioxo-3-piperidyl) isoindoline-1,3-dione (43 mg, 107 µmol, 1 *eq*), HOBt (17 mg, 129 µmol, 1.2 *eq*), EDCI (24 mg, 129 µmol, 1.2 *eq*) and DIEA (41 mg, 323 µmol, 56 µL, 3 *eq*) in DMF (1 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of H_2O (10 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 35%-65%, 8 minutes). Compound N-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy]ethyl]-4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (3 mg, 4% yield, 95% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.91 (s, 1H), 11.09 (s, 1H), 10.50 (s, 1H), 8.56 (m, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.57 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.5 Hz, 1H), 7.02 (d, J = 8.0 Hz, 2H), 6.61 (br s, 1H), 5.05 (dd, J = 5.3, 12.9 Hz,

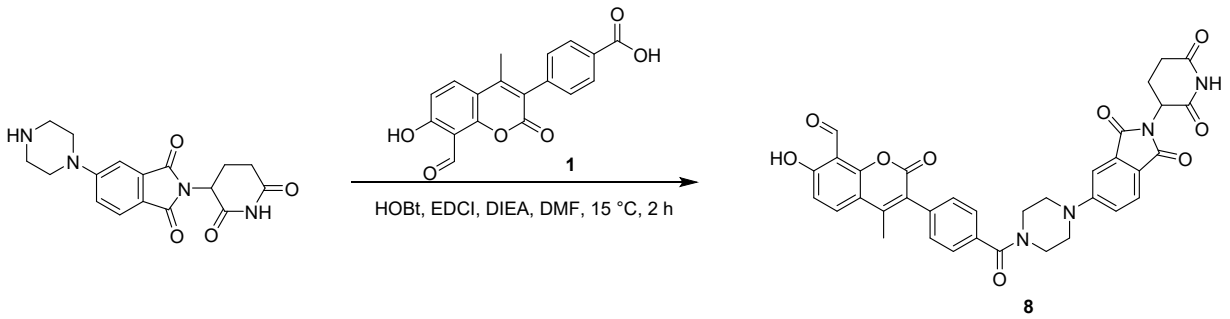
1H), 3.66 - 3.62 (m, 2H), 3.59 - 3.55 (m, 6H), 3.44 (m, 4H), 2.90 - 2.81 (m, 1H), 2.58 (m, 2H), 2.24 (s, 3H), 2.07 - 2.00 (m, 1H).

Synthesis of 3-(4-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) amino)ethyl) piperazine-1-carbonyl)phenyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (7)



A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (30 mg, 92 µmol, 1 *eq*), 2-(2,6-dioxo-3-piperidyl)-4-(2-piperazin-1-ylethylamino) isoindoline-1,3-dione (35 mg, 92 µmol, 1 *eq*), HOBt (15 mg, 111 µmol, 1.2 *eq*), EDCI (21 mg, 111 µmol, 1.2 *eq*) and DIEA (35 mg, 277 µmol, 48 µL, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 15 °C for 2 hr under a N_2 atmosphere. The reaction mixture was quenched by the addition of H_2O (10 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 25%-55%, 8 minutes). Compound 3-[4-[4-[2-[[2-(2,6-dioxo-3-piperidyl) -1,3-dioxo-isoindolin-4-yl]amino]ethyl]piperazine-1-carbonyl]phenyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (2 mg, 3% yield, 97% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.92 (br s, 1H), 11.11 (s, 1H), 10.54 - 10.45 (m, 1H), 8.05 (d, J = 9.1 Hz, 1H), 7.71 - 7.63 (m, 1H), 7.56 (br d, J = 7.5 Hz, 2H), 7.44 (br d, J = 7.8 Hz, 2H), 7.21 (br d, J = 8.6 Hz, 1H), 7.12 (br d, J = 7.1 Hz, 1H), 7.03 (br d, J = 8.9 Hz, 1H), 6.91 (br s, 1H), 5.07 (m, 1H), 3.72 (br s, 4H), 3.32 - 3.08 (m, 8H), 2.89 (br s, 1H), 2.57 (m, 2H), 2.28 (s, 3H), 2.06 - 2.00 (m, 1H).

Synthesis of 3-(4-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazine-1-carbonyl)phenyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (8)



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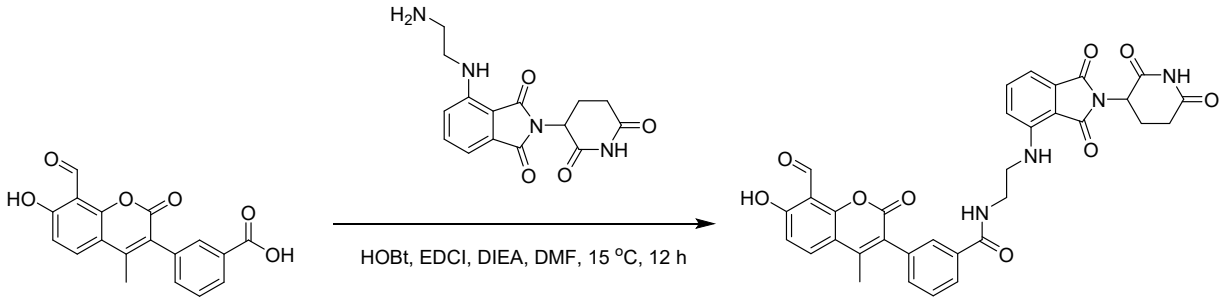
A mixture of 4-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (20 mg, 61 µmol, 1 *eq*), 2-(2,6-dioxo-3-piperidyl)-5-piperazin-1-yl-isoindoline-1,3-dione (28 mg, 61 µmol, 1 *eq*, TFA), HOBt (10 mg, 74 µmol, 1.2 *eq*), EDCI (14 mg, 74 µmol, 1.2 *eq*) and DIEA (23 mg, 185 µmol, 32 µL, 3 *eq*) in DMF (0.5 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched with H_2O (5 mL) and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 200 x 40 mm x 10 µm; mobile phase: [water (FA)-ACN]; B%: 35%-65%, 8 minutes). Compound 3-[4-[4-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]piperazine-1-carbonyl]phenyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (4 mg, 11% yield, 100% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.09 (s, 1H), 10.10 - 9.96 (m, 1H), 8.37 (br s, 2H), 7.76 - 7.64 (m, 2H), 7.52 (br d, J = 7.9 Hz, 2H), 7.39 (br d, J = 9.4 Hz, 3H), 7.27 (br d, J = 8.6 Hz, 1H), 5.08 (dd, J = 5.3, 12.9 Hz, 1H), 3.84 - 3.58 (m, 8H), 2.94 - 2.88 (m, 1H), 2.69 - 2.53 (m, 2H), 2.21 (br s, 3H), 2.05 - 2.00 (m, 1H).

Synthesis of N-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (10)



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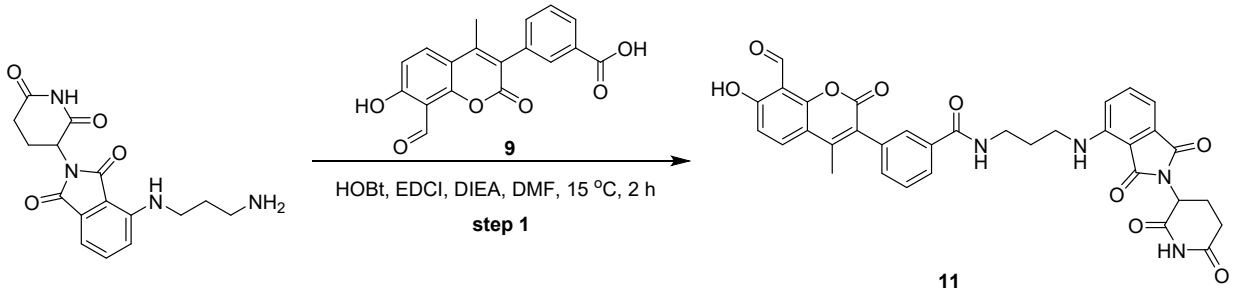
A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (10 mg, 30 μmol, 1 *eq*), 4-(2-aminoethylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (9 mg, 30 μmol, 1 *eq*), HOBt (5 mg, 37 μmol, 1.2 *eq*), EDCI (7 mg, 37 μmol, 1.2 *eq*) and DIEA (8 mg, 61 μmol, 10 μL, 2 *eq*) in DMF (0.2 mL) was stirred at 15 °C for 12 hr. The reaction mixture was quenched with H_2O (5 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 200 x 40 mm x 10 μm; mobile phase: [water (FA)-ACN]; B%: 35%-60%, 8 minutes). Compound N-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (3 mg, 15% yield, 97% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.10 (s, 1H), 10.02 (s, 1H), 8.77-8.74 (m, 1H), 7.84 (d, J=7.8 Hz, 1H), 7.75 (s, 1H), 7.67 (br d, J=9.0 Hz, 1H), 7.55 (m, 2H), 7.44 (br d, J=7.5 Hz, 1H), 7.26 (d, J=8.6 Hz, 1H), 7.02 (d, J=7.0 Hz, 1H), 6.85 (t, J=5.8 Hz, 1H), 6.47 (br d, J=9.3 Hz, 1H), 5.04 (dd, J=5.3, 12.8 Hz, 1H), 3.52 (m, 5H), 2.87 (m, 1H), 2.5-2.6 (m, 2H), 2.16 (s, 3H), 2.0-2.0 (m, 1H)

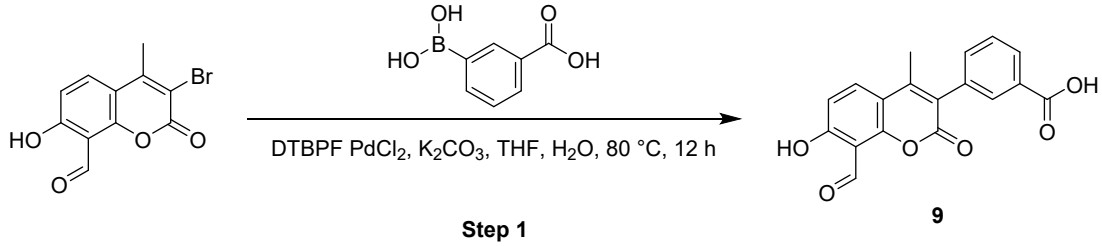
Synthesis of N-[3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]propyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (11)

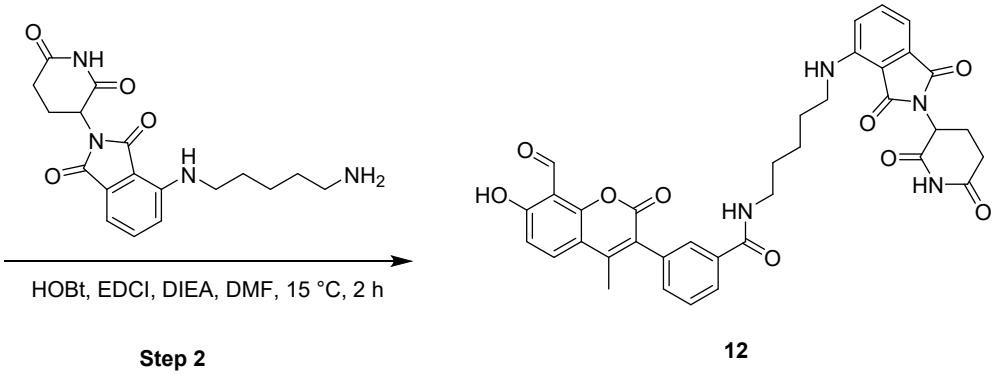


A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (40 mg, 123 μmol, 1 *eq*) , 4-(3-aminopropylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (54 mg, 123 μmol, 1 *eq*, TFA), HOBt (20 mg, 148 μmol, 1.2 *eq*), EDCI (28 mg, 148 μmol, 1.2 *eq*) and DIEA (48 mg, 370 μmol, 64 μL, 3 *eq*) in DMF (0.2 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of H_2O (3 mL) at 15 °C, yellow solid precipitated and the mixture was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 30%-70%, 8 minutes). Compound N-[3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]propyl]-

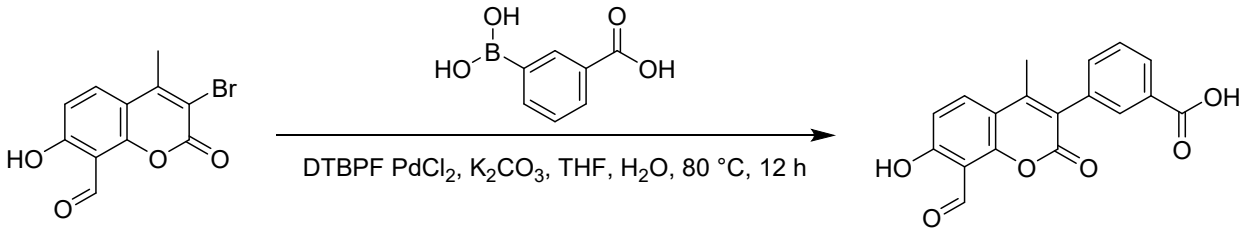
3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (2 mg, 2% yield, 94% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.90 (s, 1H), 11.09 (s, 1H), 10.51 (s, 1H), 8.61 (t, J = 5.7 Hz, 1H), 8.03 (br d, J = 9.4 Hz, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.79 (s, 1H), 7.62 - 7.54 (m, 2H), 7.48 (d, J = 7.8 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 7.06 - 6.97 (m, 2H), 6.76 (t, J = 5.8 Hz, 1H), 5.05 (dd, J = 5.4, 12.8 Hz, 1H), 3.39 (br dd, J = 6.3, 9.4 Hz, 4H), 2.93 - 2.84 (m, 1H), 2.63 - 2.57 (m, 2H), 2.26 (s, 3H), 2.07 - 1.98 (m, 1H), 1.87 - 1.78 (m, 2H).

Synthesis of N-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (12)





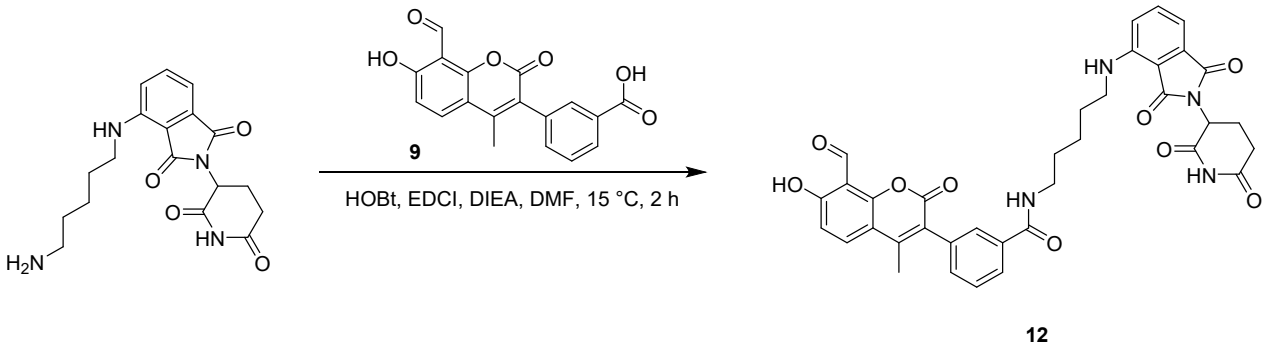
Step 1: Synthesis of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzoic acid



A mixture of 3-bromo-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (0.45 g, 1.59 mmol, 1 *eq*), 3-boronobenzoic acid (316 mg, 1.91 mmol, 1.2 *eq*), ditert-

butyl(cyclopentyl)phosphane;dichloropalladium;iron (103 mg, 158 μmol, 0.1 *eq*), K_3PO_4 (675 mg, 3.18 mmol, 2 *eq*) in THF (5 mL) and H_2O (1 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 80 °C for 12 hr under a N_2 atmosphere. The reaction mixture was quenched with H_2O (10 mL), and adjusted to pH 9 with LiOH aq. The mixture was washed with EtOAc (10 mL). The aqueous phase was combined and adjusted to pH 4 with 2 M HCl, and a grey solid precipitated that was filtered to give a residue, which was used in the next step without further purification. Compound 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (220 mg, 43% yield) was obtained as a grey solid.

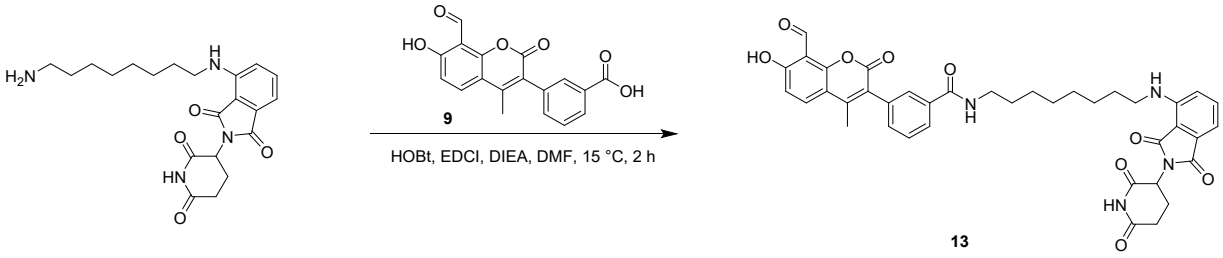
Step 2: Synthesis of N-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide



A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (50 mg, 154 μmol, 1 *eq*), 4-(5-aminopentylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (55 mg, 154 μmol, 1 *eq*), HOBt (25 mg, 185 μmol, 1.2 *eq*), EDCI (35 mg, 185 μmol, 1.2 *eq*) and DIEA (60 mg, 462 μmol, 80 μL, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 15 °C for 2 hr under a N_2 atmosphere. The reaction mixture was quenched by the addition of H_2O (10 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex C18 80 x 30 mm x 3 μm; mobile phase: [water (TFA)-ACN]; B%: 40%-70%, 8 minutes). Compound N-[5-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]pentyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (5 mg, 4% yield, 91% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.91 (s, 1H), 11.09 (s, 1H), 10.51 (s, 1H), 8.51 (br t, *J* = 5.4 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 7.91 - 7.84 (m, 1H), 7.78 (s, 1H), 7.62 - 7.52 (m, 2H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 7.03 - 7.00 (m, 1H), 6.54 (br s, 1H), 5.08 - 5.01 (m, 1H), 3.33 - 3.27 (m, 4H), 2.93 - 2.83 (m, 1H), 2.62

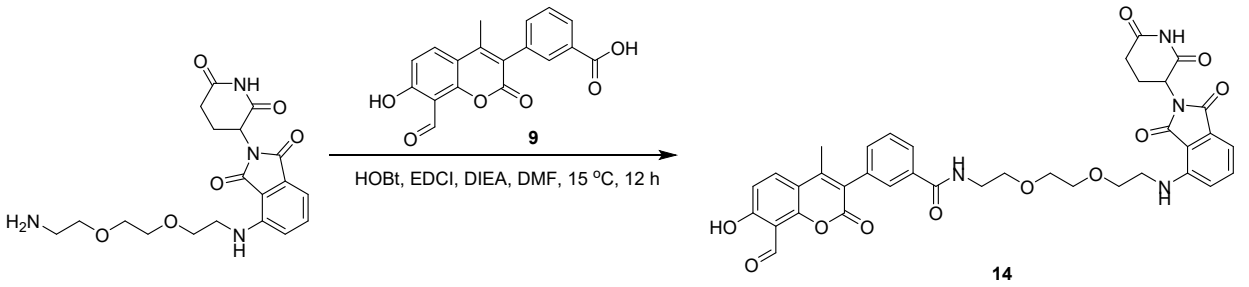
- 2.53 (m, 2H), 2.25 (s, 3H), 2.01 (br dd, *J* = 5.6, 10.6 Hz, 1H), 1.59 (qd, *J* = 7.0, 14.1 Hz, 4H), 1.45 - 1.36 (m, 2H).

Synthesis of N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)benzamide (13)



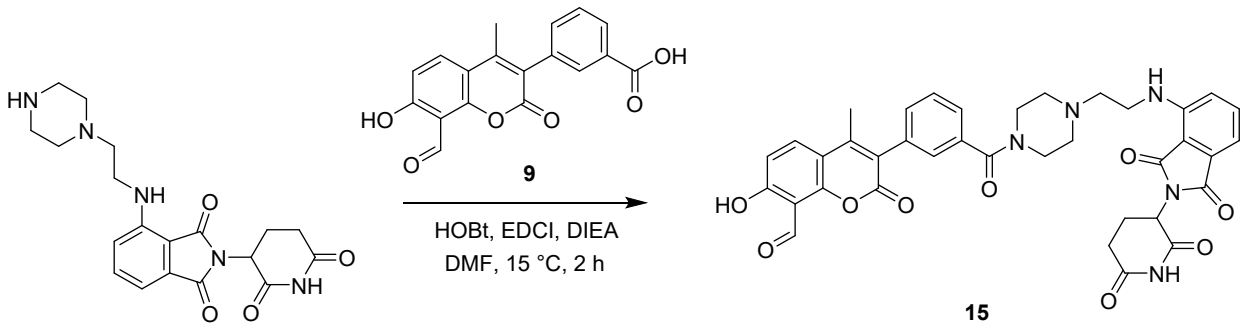
A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (50 mg, 154 µmol, 1 *eq*), 4-(8-aminooctylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (62 mg, 154 µmol, 1 *eq*), HOBt (25 mg, 185 µmol, 1.2 *eq*), EDCI (35 mg, 185 µmol, 1.2 *eq*) and DIEA (60 mg, 463 µmol, 80 µL, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 15 °C for 2 hr under a N_2 atmosphere. The reaction mixture was quenched by the addition of H_2O (10 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex C18 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 50%-80%, 8 minutes). Compound N-[8-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]octyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (2 mg, 1% yield, 79% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.90 (br s, 1H), 11.09 (br s, 1H), 10.50 (s, 1H), 8.48 (br s, 1H), 8.04 (br d, J = 9.0 Hz, 1H), 7.88 (br d, J = 7.6 Hz, 1H), 7.78 (br s, 1H), 7.59 - 7.52 (m, 2H), 7.49 - 7.43 (m, 1H), 7.10 - 6.99 (m, 3H), 6.52 (br s, 1H), 5.08 - 5.01 (m, 1H), 3.26 (br s, 4H), 2.93 - 2.84 (m, 1H), 2.63 - 2.54 (m, 2H), 2.25 (br s, 3H), 2.07 - 1.98 (m, 1H), 1.61 - 1.50 (m, 4H), 1.31 (br s, 8H).

Synthesis of N-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy]ethyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (14)



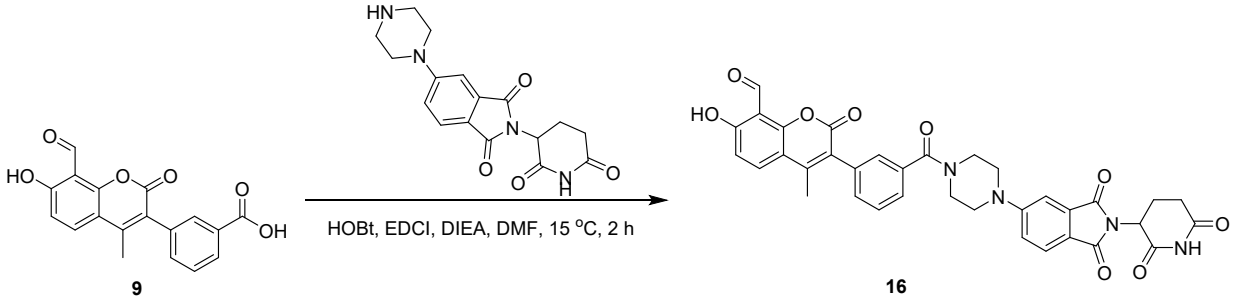
A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (50 mg, 154 µmol, 1 *eq*) , 4-[2-[2-(2-aminoethoxy)ethoxy]ethylamino]-2-(2,6-dioxo-3-piperidyl) isoindoline-1,3-dione (62 mg, 154 µmol, 1 *eq*), HOBt (25 mg, 185 µmol, 1.2 *eq*) , EDCI (35 mg, 185 µmol, 1.2 *eq*) and DIEA (59 mg, 462 µmol, 80 µL, 3 *eq*) in DMF (0.5 mL) was stirred at 15 °C for 12 hr. The reaction mixture was quenched by the addition of H_2O (3 mL) at 15 °C, and a yellow solid precipitated that was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 150 x 30 mm x 5 µm; mobile phase: [water (TFA)-ACN]; B%: 25%-55%, 8 minutes). Compound N-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy]ethyl]-3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzamide (1 mg, 1% yield, 97% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.91 (s, 1H), 11.09 (s, 1H), 10.50 (s, 1H), 8.54 (t, J = 5.4 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.79 (s, 1H), 7.59 - 7.51 (m, 2H), 7.50 - 7.44 (m, 1H), 7.10 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 6.59 (t, J = 5.4 Hz, 1H), 5.05 (dd, J = 5.4, 12.9 Hz, 1H), 3.61 - 3.54 (m, 8H), 3.44 (d, J = 2.8 Hz, 4H), 2.88 (m, 1H), 2.60 (s, 2H), 2.25 (s, 3H), 2.05 - 1.98 (m, 1H).

Synthesis of 3-(3-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl) piperazine-1-carbonyl)phenyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (15)



A mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (50 mg, 154 µmol, 1 *eq*), 2-(2,6-dioxo-3-piperidyl)-4-(2-piperazin-1-ylethylamino)isoindoline-1,3-dione (59 mg, 154 µmol, 1 *eq*), HOBT (25 mg, 185. µmol, 1.2 *eq*), EDCI (35 mg, 185 µmol, 1.2 *eq*) and DIEA (59 mg, 462 µmol, 80 µL, 3 *eq*) in DMF (0.5 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 15 °C for 2 hr under a N_2 atmosphere. The reaction mixture was quenched by the addition H_2O (10 mL), and filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex C18 80 x 30 mm x 3 µm; mobile phase: [water (TFA)-ACN]; B%: 20%-50%, 8 minutes). Compound 3-[3-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]piperazine-1-carbonyl]phenyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (5 mg, 3% yield, 77% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.98 - 11.87 (m, 1H), 11.11 (s, 1H), 10.50 (s, 1H), 8.05 (d, J = 9.1 Hz, 1H), 7.67 - 7.55 (m, 2H), 7.54 - 7.40 (m, 3H), 7.24 - 7.16 (m, 1H), 7.14 - 7.08 (m, 1H), 7.04 (br d, J = 9.0 Hz, 1H), 6.95 - 6.84 (m, 1H), 5.07 (br dd, J = 5.3, 12.5 Hz, 1H), 3.71 (br s, 4H), 3.34 - 3.06 (m, 8H), 2.94 - 2.87 (m, 1H), 2.69 - 2.57 (m, 2H), 2.30 (s, 3H), 2.05 - 2.00 (m, 1H).

Synthesis of 3-[3-[4-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]piperazine-1-carbonyl]phenyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (16)

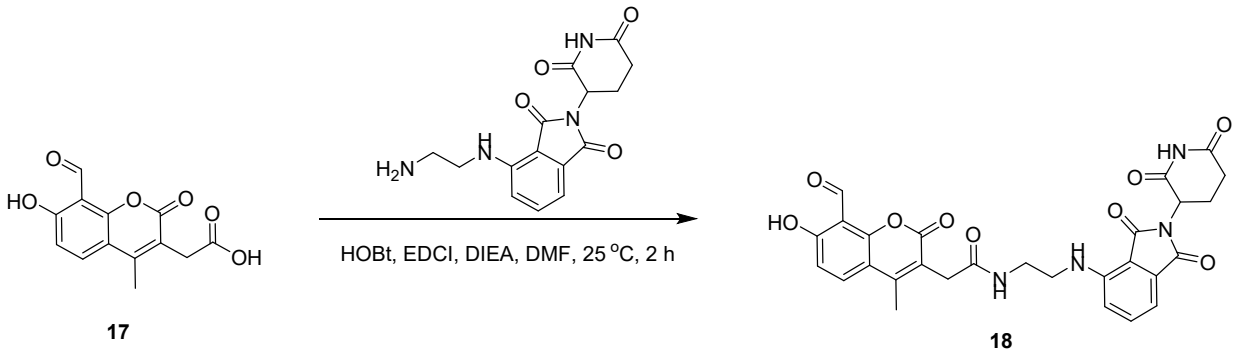


9 16

To a solution of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)benzoic acid (20 mg, 61 µmol, 1 *eq*) in DMF (1 mL) was added 2-(2,6-dioxo-3-piperidyl) -5-piperazin-1-yl-isoindoline-1,3-dione (28 mg, 61 µmol, 1 *eq*, TFA) DIEA (23 mg, 185 µmol, 32 µL, 3 *eq*) HOBt (10 mg, 74 µmol, 1.2 *eq*) and EDCI (14 mg, 74 µmol, 1.2 *eq*). The mixture was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of H_2O (3 mL) at 15 °C, and a yellow solid precipitated that was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 200 x 40 mm x 10 µm; mobile phase: [water (FA)-ACN]; B%: 25%-55%,

8 minutes). Compound 3-[3-[4-[2-(2,6-dioxo-3-piperidyl) -1,3-dioxo-isoindolin-5-yl]piperazine-1-carbonyl]phenyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (6 mg, 13 % yield, 95% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 12.02 - 11.76 (m, 1H), 11.09 (s, 1H), 10.46 (s, 1H), 8.00 (d, J = 9.2 Hz, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.58 - 7.51 (m, 1H), 7.50 - 7.45 (m, 1H), 7.42 - 7.38 (m, 2H), 7.34 (d, J = 2.0 Hz, 1H), 7.23 (dd, J = 2.0, 8.6 Hz, 1H), 6.98 (d, J = 9.2 Hz, 1H), 5.04 (dd, J = 5.2, 12.8 Hz, 1H), 3.89 - 3.72 (m, 2H), 3.68 - 3.42 (m, 6H), 2.92 - 2.77 (m, 1H), 2.65 - 2.47 (m, 2H), 2.26 (s, 3H), 2.03 - 1.93 (m, 1H).

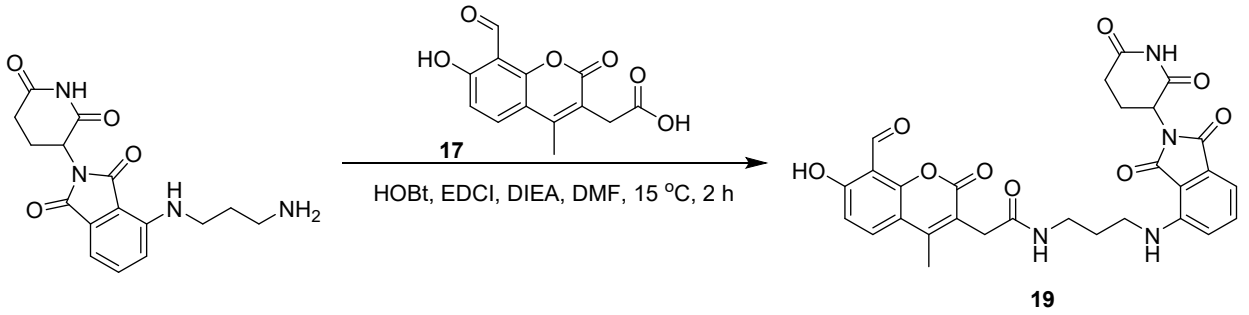
Synthesis of N-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (18)



A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (90 mg, 343 μmol, 1 *eq*), 4-(2-aminoethylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (108 mg, 343 μmol, 1 *eq*), HOBt (55 mg, 411 μmol, 1.2 *eq*), EDCI (78 mg, 411 μmol, 1.2 *eq*) and DIEA (133 mg, 1.03 mmol, 179 μL, 3 *eq*) in DMF (1 mL) was stirred at 25 °C for 2 hr. Brine (10 mL) was added to the reaction mixture, and a yellow solid formed, which was filtered. The cake was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Phenomenex Luna C18 150 x 30 mm x 5 μm; mobile phase: [water (TFA)-ACN]; B%: 20%-50%, 8 minutes). Compound N-[2-[[2-(2,6-dioxo-3-piperidyl) -1,3-dioxo-isoindolin-4-yl]amino]ethyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl) acetamide (9 mg, 4% yield, 95% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.89 (s, 1H), 11.17 (s, 1H), 10.53 (s, 1H), 8.21 (br t, J = 5.5 Hz, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.63 (dd, J = 7.3, 8.5 Hz, 1H), 7.23 (d, J = 8.6 Hz, 1H), 7.09 - 7.02 (m, 2H), 6.78 (br t, J = 5.8 Hz, 1H), 5.10 (dd, J = 5.4, 12.8 Hz, 1H),

3.54 (br s, 2H), 3.47 - 3.43 (m, 2H), 3.32 (m, 2H), 3.01 - 2.89 (m, 1H), 2.70 - 2.58 (m, 2H), 2.38 (s, 3H), 2.13 - 2.02 (m, 1H).

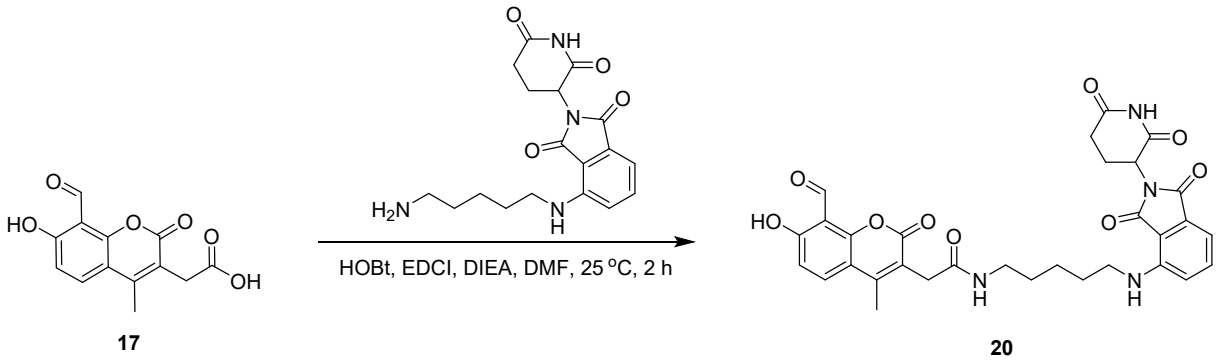
Synthesis of N-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-2H-chromen-3-yl)acetamide (19)



19

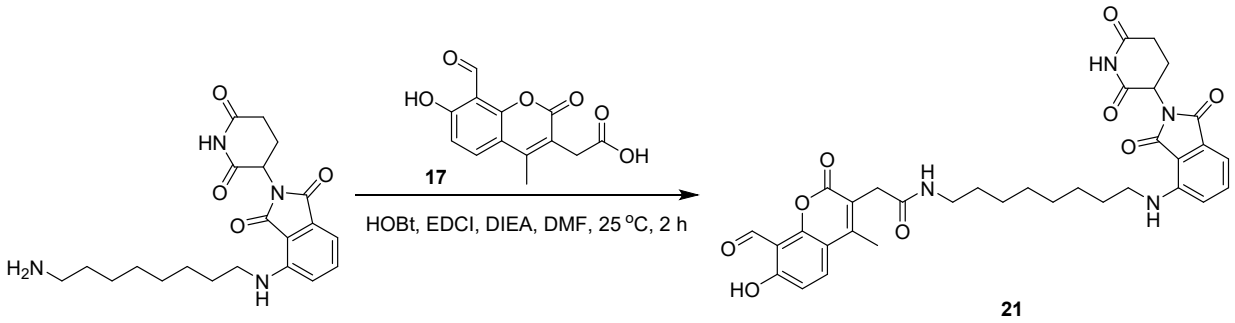
A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (30 mg, 114 µmol, 1 *eq*) , 4-(3-aminopropylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (38 mg, 114 µmol, 1 *eq*), HOBt (18 mg, 137 µmol, 1.2 *eq*), EDCI (26 mg, 137 µmol, 1.2 *eq*) and DIEA (44 mg, 343 µmol, 60 µL, 3 *eq*) in DMF (0.5 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of H_2O (3 mL) at 15 °C, and a yellow solid precipitated that was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 30%-60%, 8 minutes). Compound N-[3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]propyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (1 mg, 1% yield, 91% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.83 (s, 1H), 11.08 (s, 1H), 10.47 (s, 1H), 8.03 - 7.99 (m, 2H), 7.59 - 7.56 (m, 1H), 7.09 (m, 1H), 7.03 – 6.97 (m, 2H), 6.86 - 6.65 (m, 1H), 5.05 - 5.02 (m, 1H), 3.50 (br s, 2H), 3.13 (m, 2H), 2.98 - 2.86 (m, 1H), 2.68 - 2.61 (m, 2H), 2.46 - 2.33 (m, 5H), 2.18 - 2.01 (m, 1H), 1.87 - 1.59 (m, 2H).

Synthesis of N-[5-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]pentyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (20)



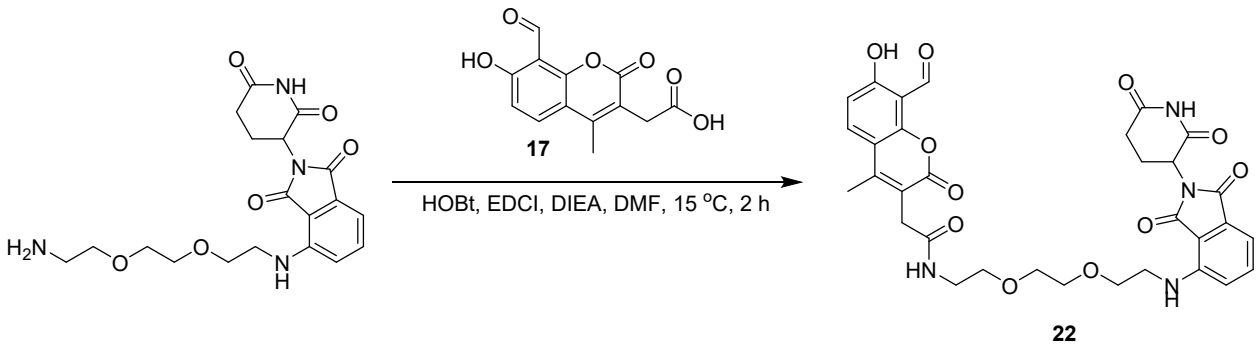
A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (90 mg, 343 µmol, 1 *eq*), 4-(5-aminopentylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (123 mg, 343 µmol, 1 *eq*), HOBt (55 mg, 411 µmol, 1.2 *eq*), EDCI (79 mg, 411 µmol, 1.2 *eq*) and DIEA (133 mg, 1.03 mmol, 179 µL, 3 *eq*) in DMF (1 mL) was stirred at 25 °C for 2 hr . Brine (10 mL), was added to the reaction mixture and a yellow solid precipitated which was filtered. The cake was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: C18-1 150 x 30 mm x 5 µm; mobile phase: [water (TFA)-ACN]; B%: 30%-75%, 8 minutes). Compound N-[5-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]pentyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (19 mg, 8% yield, 91% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.89 (br s, 1H), 11.15 (s, 1H), 10.52 (s, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.98 (br t, J = 5.5 Hz, 1H), 7.63 (dd, J = 7.2, 8.4 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.08 (d, J = 7.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.59 (br s, 1H), 5.11 (dd, J = 5.4, 12.9 Hz, 1H), 3.53 (s, 2H), 3.38 - 3.31 (m, 2H), 3.12 (q, J = 6.5 Hz, 2H), 2.99 - 2.89 (m, 1H), 2.67 - 2.59 (m, 2H), 2.40 (s, 3H), 2.14 - 2.04 (m, 1H), 1.64 (td, J = 7.3, 14.3 Hz, 2H), 1.51 (m, 2H), 1.44 - 1.37 (m, 2H).

Synthesis of N-[8-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]octyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (21)



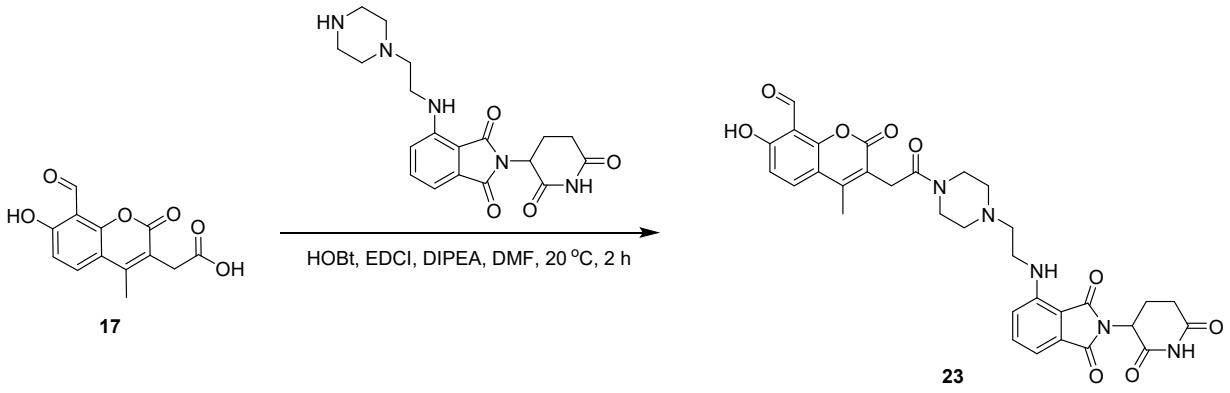
A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (90 mg, 343 µmol, 1 *eq*), 4-(8-aminooctylamino)-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (137 mg, 343 µmol, 1 *eq*), HOBt (55 mg, 411 µmol, 1.2 *eq*), EDCI (79 mg, 411 µmol, 1.2 *eq*) and DIEA (133 mg, 1.03 mmol, 179 µL, 3 *eq*) in DMF (1 mL) was stirred at 25 °C for 2 hr. Brine (10 mL) was added to the reaction mixture, and a yellow solid formed that was filtered. The cake was concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: C18-1 150 x 30 mm x 5 µm; mobile phase: [water (TFA)-ACN]; B%: 30%-75%, 8 minutes). Compound N-[8-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]octyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (9 mg, 3% yield, 86% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.88 (br s, 1H), 11.15 (s, 1H), 10.52 (s, 1H), 8.04 (d, J = 9.1 Hz, 1H), 7.95 (br t, J = 5.6 Hz, 1H), 7.66 - 7.61 (m, 1H), 7.17 - 7.12 (m, 1H), 7.07 (d, J = 7.1 Hz, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.58 (br s, 1H), 5.11 (dd, J = 5.3, 12.8 Hz, 1H), 3.53 (s, 2H), 3.34 (br s, 2H), 3.09 (q, J = 6.6 Hz, 2H), 3.01 - 2.88 (m, 1H), 2.65 - 2.58 (m, 2H), 2.41 (s, 3H), 2.13 - 2.05 (m, 1H), 1.64 - 1.55 (m, 2H), 1.48 - 1.43 (m, 2H), 1.37 – 1.33 (m, 8H).

Synthesis of N-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino] ethoxy]ethoxy]ethyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (22)



A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (30 mg, 114 µmol, 1 *eq*), 4-[2-[2-(2-aminoethoxy)ethoxy]ethylamino]-2-(2,6-dioxo-3-piperidyl) isoindoline-1,3-dione (46 mg, 114 µmol, 1 *eq*), HOBT (18 mg, 137 µmol, 1.2 *eq*), EDCI (26 mg, 137 µmol, 1.2 *eq*) and DIEA (44 mg, 343 µmol, 59 µL, 3 *eq*) in DMF (0.5 mL) was stirred at 15 °C for 2 hr. The reaction mixture was quenched by the addition of H_2O (3 mL) at 15 °C, and a yellow solid precipitated that was filtered to give a residue. The residue was purified by prep-HPLC (column: Phenomenex Luna C18 150 x 30 mm x 5 µm; mobile phase: [water (TFA)-ACN]; B%: 30%-50%, 8 minutes). Compound N-[2-[2-[2-[[2-(2,6-dioxo-3-piperidyl) -1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy]ethyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (1 mg, 2% yield, 91% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.89 - 11.77 (m, 1H), 11.09 (s, 1H), 10.46 (s, 1H), 8.11 - 7.90 (m, 2H), 7.58 (t, J = 8.2 Hz, 1H), 7.23 - 6.82 (m, 3H), 6.68 - 6.53 (m, 1H), 5.15 - 5.00 (m, 1H), 3.66 - 3.52 (m, 8H), 3.50 - 3.43 (m, 6H), 3.25 - 3.21 (m, 1H), 2.73 (m, 2H), 2.34 (d, J = 1.8 Hz, 3H), 2.08 - 2.00 (m, 1H).

Synthesis of 3-[2-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl] piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (23, CPD-2522)



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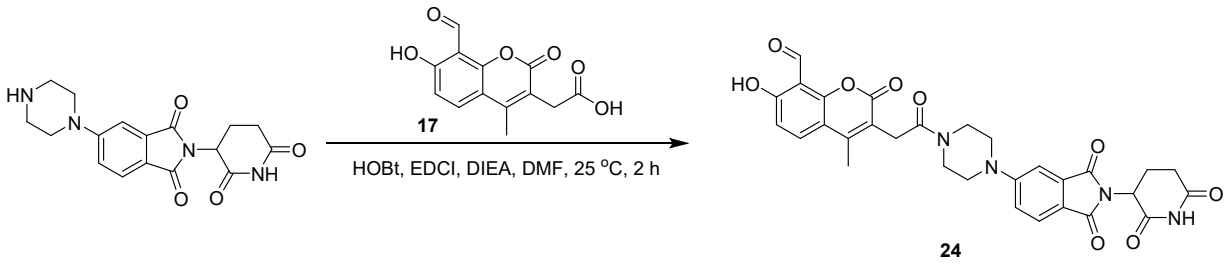
To the mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (50 mg, 190 μmol, 1 *eq*) and 2-(2,6-dioxo-3-piperidyl)-4-(2-piperazin-1-ylethylamino) isoindoline-1,3-dione (73 mg, 190 μmol, 1 *eq*) in DMF (0.5 mL) was added EDCI (43 mg, 228 μmol, 1.2 *eq*), HOBt (30 mg, 228 μmol, 1.2 *eq*) and DIPEA (49 mg, 381 μmol, 66 μL, 2 *eq*). The mixture was stirred for 2 h at 20 °C, and then concentrated under reduced pressure. The mixture was purified by prep-HPLC (column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 5%-35%, 8 minutes). Compound 3-[2-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (4 mg, 3% yield, 91% purity) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.10 (s, 1H), 10.47 (s, 1H), 8.00 (d, J = 9.1 Hz, 1H), 7.64 - 7.58 (m, 1H), 7.15 - 7.11 (m, 1H), 7.05 (d, J = 7.0 Hz, 1H), 6.99 (d, J = 9.0 Hz, 1H), 6.83 (br d, J = 3.8 Hz, 1H), 5.08 (dd, J = 5.4, 13.0 Hz, 1H), 3.73 - 3.69 (m, 2H), 3.66 - 3.59 (m, 3H), 3.48 (br s, 2H), 3.44 - 3.39 (m, 4H), 2.95 - 2.87 (m, 1H), 2.65 - 2.61 (m, 2H), 2.56 (m, 2H), 2.45 (br s, 2H), 2.33 (s, 3H), 2.08 - 1.99 (m, 1H).

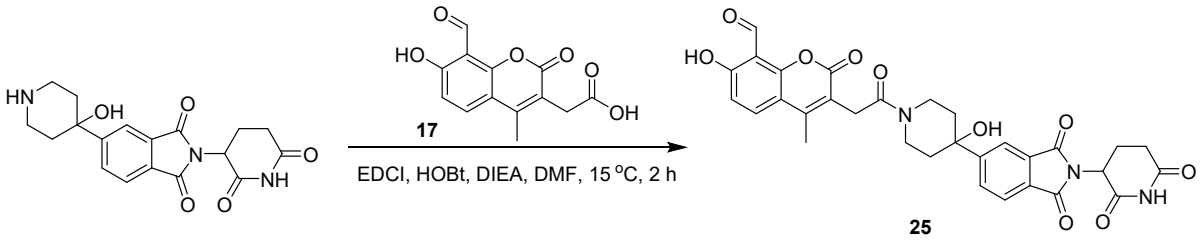
Synthesis of 3-(2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)piperazin-1-yl)-2-oxoethyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (24)



24

A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (30 mg, 114 µmol, 1), 2-(2,6-dioxo-3-piperidyl)-5-piperazin-1-yl-isoindoline-1,3-dione (52 mg, 114 µmol, 1 *eq*, TFA), HOBt (18 mg, 137 µmol, 1.2 *eq*), EDCI (26 mg, 137 µmol, 1.2 *eq*) and DIEA (44 mg, 343 µmol, 59 µL, 3 *eq*) in DMF (0.5 mL) was stirred at 25 °C for 2 hr. The reaction was diluted with DMSO (1 mL), and used for purification directly without further work-up. The residue was purified by prep-HPLC (FA condition: column: Phenomenex Luna C18 75 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 20%-60%, 8 minutes). Compound 3-[2-[4-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (4 mg, 6% yield, 93% purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 11.85 (s, 1H), 11.09 (s, 1H), 10.47 (s, 1H), 8.01 (d, J = 9.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 2.2, 8.6 Hz, 1H), 7.00 (d, J = 9.0 Hz, 1H), 5.09 (dd, J = 5.3, 12.8 Hz, 1H), 3.86 - 3.75 (m, 4H), 3.64 (br s, 4H), 3.52 (br d, J = 4.5 Hz, 2H), 2.99 - 2.83 (m, 1H), 2.69 - 2.56 (m, 2H), 2.36 (s, 3H), 2.06 - 1.98 (m, 1H).

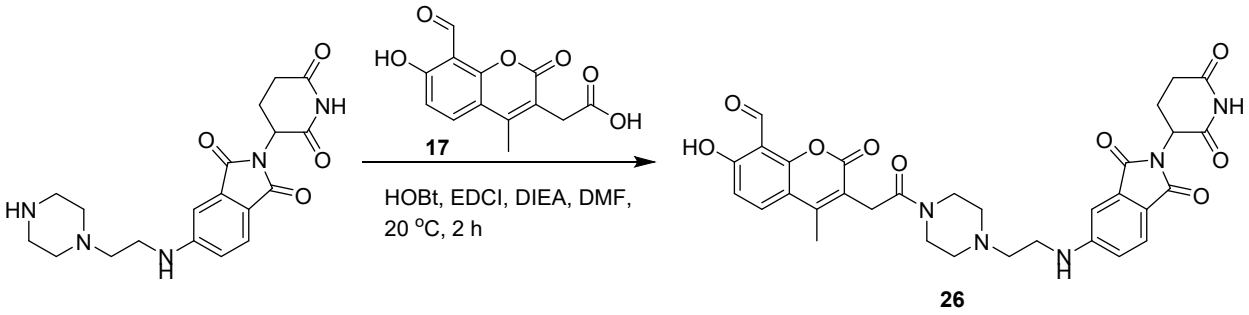
Synthesis of 3-(2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)-4-hydroxypiperidin-1-yl)-2-oxoethyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (25)



To a solution of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (33 mg, 125 µmol, 1 *eq*) and 2-(2,6-dioxo-3-piperidyl)-5-(4-hydroxy-4-piperidyl)isoindoline-1,3-dione (45 mg, 125 µmol, 1 *eq*) in DMF (1 mL) was added HOBt (20 mg, 151 µmol, 1.2 *eq*) and EDCI (29 mg, 151 µmol, 1.2 *eq*) and DIEA (32 mg, 251 µmol, 43 µL, 2 *eq*). The mixture was stirred at 15 °C for 2 hr. The residue was purified by prep-HPLC (FA condition column: Phenomenex Luna 80 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 25%-50%, 8 minutes). The title compound 3-[2-[4-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-4-hydroxy-1-piperidyl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (8 mg, 10% yield, 94% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.60 - 1.75 (m, 2 H),

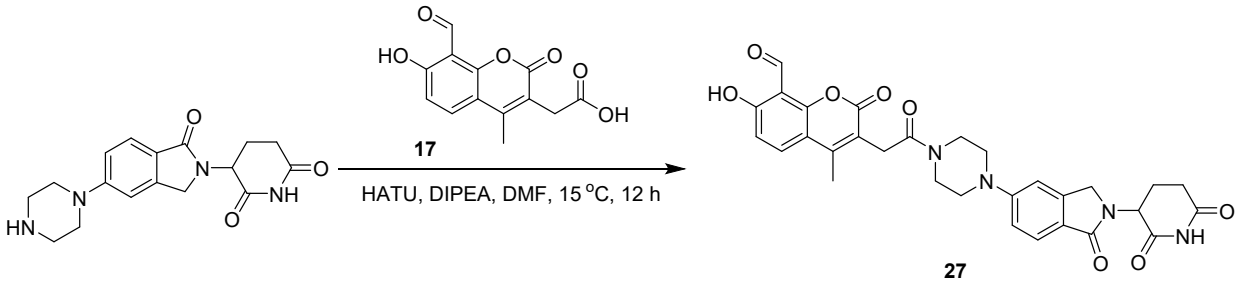
1.88 (m, 1 H), 2.01 - 2.10 (m, 1 H), 2.12 - 2.21 (m, 1 H), 2.37 (s, 3 H), 2.54 - 2.65 (m, 2 H), 2.85 - 2.95 (m, 1 H), 2.99 - 3.06 (m, 1 H), 3.55 (br t, *J*=12.1 Hz, 1 H), 3.70 - 3.82 (m, 2 H), 4.04 (br d, *J*=12.6 Hz, 1 H), 4.35 (br d, *J*=11.6 Hz, 1 H), 5.15 (dd, *J*=12.9, 5.3 Hz, 1 H), 5.63 (s, 1 H), 6.97 (d, *J*=9.0 Hz, 1 H), 7.91 (d, *J*=7.8 Hz, 1 H), 7.96 - 8.09 (m, 3 H), 10.47 (s, 1 H), 11.13 (s, 1 H), 11.40 - 12.24 (m, 1 H).

Synthesis of 3-[2-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]amino]ethyl] piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (26)



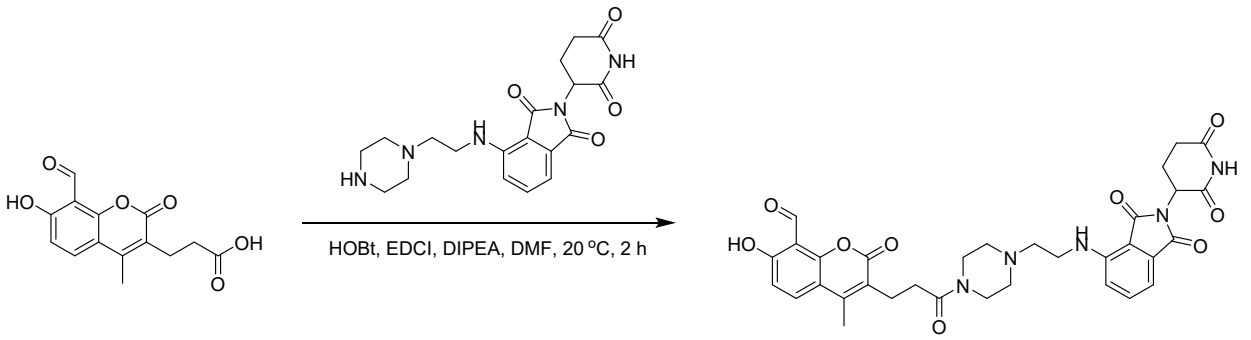
To a solution of 2-(2,6-dioxo-3-piperidyl)-5-(2-piperazin-1-ylethylamino)isoindoline-1,3-dione (50 mg, 118 μmol, 1 *eq*, HCl) and 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (31 mg, 118 μmol, 1 *eq*) in DMF (0.5 mL) was added HOBt (19 mg, 142 μmol, 1.2 *eq*) and DIEA (46 mg, 355 μmol, 62 μL, 3 *eq*) and EDCI (27 mg, 142 μmol, 1.2 *eq*). The mixture was stirred at 20 °C for 2 hr. The reaction was diluted with DMSO (1 mL), and used for purification directly without further work-up. The residue was purified by prep-HPLC (FA condition: column: Phenomenex Luna 80 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 10%-40%, 8 minutes) to yield the title compound (8 mg, 9% yield, 89% purity), obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.6 (m, 1H), 11.06 (s, 1H), 10.44 (s, 1H), 8.12 (s, 1H), 8.01 - 7.90 (m, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.05 - 6.98 (m, 2H), 6.95 (d, *J* = 9.2 Hz, 1H), 6.91 - 6.86 (m, 1H), 5.10 - 4.87 (m, 1H), 3.69 (s, 2H), 3.61 (br s, 2H), 3.46 (br s, 2H), 3.40 - 3.36 (m, 2H), 2.92 - 2.80 (m, 1H), 2.61 - 2.56 (m, 3H), 2.55 - 2.50 (m, 4H), 2.41 (br s, 1H), 2.30 (s, 3H), 2.03 - 1.94 (m, 1H).

Synthesis of 3-(2-(4-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)piperazin-1-yl)-2-oxoethyl)-7-hydroxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (27)



A mixture of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (50 mg, 190 µmol, 1 *eq*), HATU (145 mg, 381 µmol, 2 *eq*), DIPEA (49 mg, 381 µmol, 66 µL, 2 *eq*) in DMF (0.5 mL) was stirred for 0.5 hr at 15°C. Then 3-(1-oxo-5-piperazin-1-yl-isoindolin-2-yl)piperidine-2,6-dione (68 mg, 209 µmol, 1.1 *eq*) was added to the stirred solution at 15 °C. The mixture was stirred for 12 hr at 15°C and then concentrated under reduced pressure. The residue was purified by Prep-HPLC (column: Phenomenex C18 75 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 25%-55%, 8 minutes). The title compound 3-[2-[4-[2-(2,6-dioxo-3-piperidyl) -1-oxo-isoindolin-5-yl]piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (21 mg, 17% yield, 90% purity) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.92 - 2.02 (m, 1 H), 2.35 (s, 3 H), 2.37 - 2.44 (m, 1 H), 2.59 (m, 1 H), 2.85 - 2.96 (m, 1 H), 3.32 (br s, 2 H), 3.45 (br s, 2 H), 3.63 (br s, 2 H), 3.74 - 3.84 (m, 4 H), 4.20 - 4.38 (m, 2 H), 5.06 (dd, *J*=13.3, 5.1 Hz, 1 H), 6.99 (d, *J*=9.0 Hz, 1 H), 7.08 - 7.14 (m, 2 H), 7.56 (d, *J*=8.4 Hz, 1 H), 8.00 (d, *J*=9.0 Hz, 1 H), 10.46 (s, 1 H), 10.95 (s, 1 H), 11.84 (br s, 1 H).

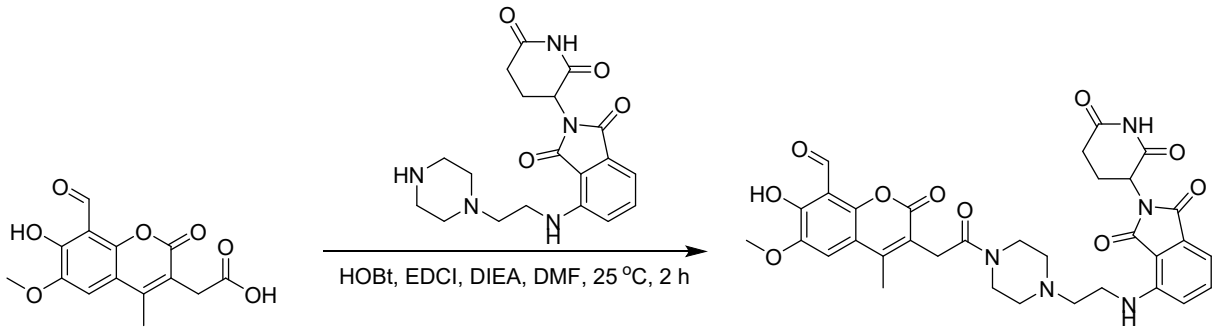
Synthesis of 3-[3-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl] amino] ethyl] piperazin-1-yl]-3-oxopropyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (28)



28

To the mixture of 3-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)propanoic acid (50 mg, 181 µmol, 1 *eq*) and 2-(2,6-dioxo-3-piperidyl)-4-(2-piperazin-1-ylethylamino)isoindoline-1,3-dione (70 mg, 181 µmol, 1 *eq*) in DMF (0.2 mL) was added EDCI (42 mg, 217 µmol, 1.2 *eq*), HOBt (29 mg, 217 µmol, 1.2 *eq*) and DIPEA (47 mg, 362 µmol, 63 µL, 2 *eq*). The mixture was stirred for 2 h at 20 °C. The mixture was concentrated under reduced pressure and purified by prep-HPLC (column: Phenomenex Luna C18 75 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 20%-50%, 8 minutes). Compound 3-[3-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]piperazin-1-yl]-3-oxopropyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (1 mg, 1% yield, 92% purity) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 11.10 (s, 1H), 10.40 (s, 1H), 7.90 - 7.83 (m, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 7.03 (d, J = 7.2 Hz, 1H), 6.86 - 6.76 (m, 2H), 5.09 - 5.03 (m, 1H), 3.51 - 3.42 (m, 8H), 2.93 - 2.85 (m, 1H), 2.78 - 2.73 (m, 2H), 2.62 - 2.58 (m, 2H), 2.57 (br dd, J = 1.8, 4.6 Hz, 2H), 2.44 - 2.40 (m, 4H), 2.38 (s, 3H), 2.04 - 1.99 (m, 1H).

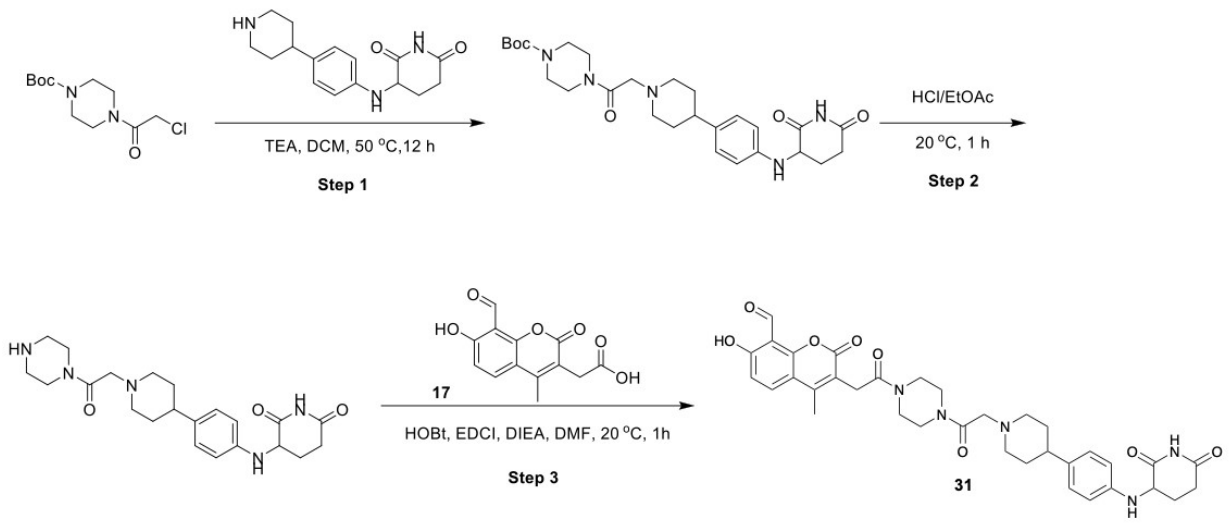
Synthesis of 3-(2-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl) piperazin-1-yl)-2-oxoethyl)-7-hydroxy-6-methoxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (29)



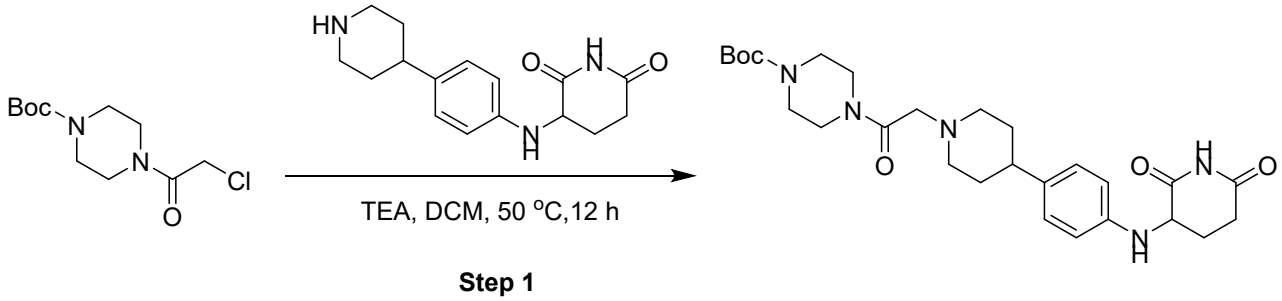
29

To a solution of 2-(8-formyl-7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (20 mg, 68 μmol, 1 *eq*) and 2-(2,6-dioxo-3-piperidyl)-4-(2-piperazin-1-ylethylamino)isoindoline-1,3-dione (26 mg, 68 μmol, 1 *eq*) in DMF (0.5 mL) was added HOBt (11 mg, 82 μmol, 1.2 *eq*) and EDCI (15 mg, 82 μmol, 1.2 *eq*) and DIEA (17 mg, 136 μmol, 23 μL, 2 *eq*). The mixture was stirred at 25 °C for 2 hr. The reaction was diluted with DMSO (1 mL) and used for purification directly without further work-up. The residue was purified by prep-HPLC (FA condition: column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 10%-50%, 8 minutes). The title compound 3-[2-[4-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethyl]piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-6-methoxy-4-methyl-2-oxo-chromene-8-carbaldehyde (3 mg, 7% yield, 95% purity) was obtained as yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.99 - 2.08 (m, 1 H), 2.36 (s, 3 H), 2.59 - 2.71 (m, 6 H), 2.84 - 2.94 (m, 1 H), 3.38 - 3.44 (m, 4 H), 3.48 (br s, 2 H), 3.63 (br s, 2 H), 3.71 (s, 2 H), 3.93 (s, 3 H), 5.08 (dd, *J*=12.9, 5.3 Hz, 1 H), 6.83 (br s, 1 H), 7.02 - 7.14 (m, 2 H), 7.43 - 7.64 (m, 2 H), 10.44 (s, 1 H), 11.09 (s, 1 H), 11.45 - 12.06 (m, 1 H).

Synthesis of 3-[2-[4-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino]phenyl]-1-piperidyl] acetyl] piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (31)

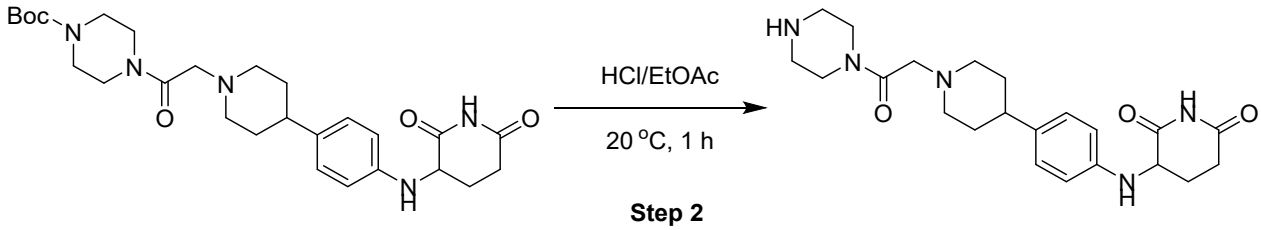


Step 1: Synthesis of tert-butyl 4-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino]phenyl]-1-piperidyl]acetyl]piperazine-1-carboxylate



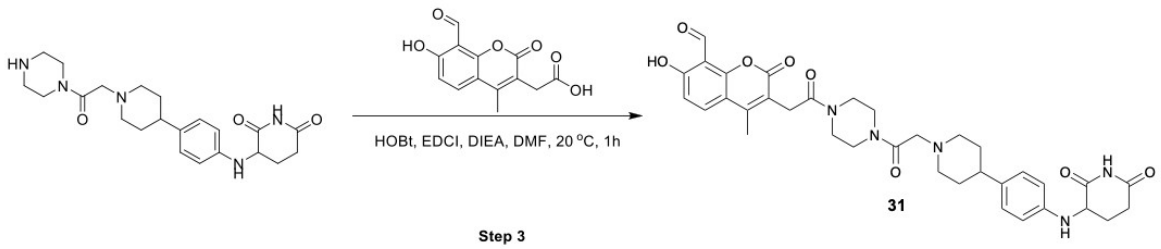
To a solution of tert-butyl 4-(2-chloroacetyl) piperazine-1-carboxylate (599 mg, 2.28 mmol, 2 *eq*), 3-[4-(4-piperidyl)aniline] piperidine-2,6-dione (328 mg, 1.14 mmol, 1 *eq*) in DCM (3 mL) was added TEA (692 mg, 6.84 mmol, 952 μL, 6 *eq*). The mixture was stirred at 50 °C for 12 hr. The reaction mixture was diluted with H_2O (20 mL) and extracted with DCM (10 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue that was purified by prep-HPLC (FA condition;). column: Phenomenex luna C18 80 x 40 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 1%-45%, 8 minutes. The title compound (65 mg, 11% yield) was obtained as a white solid.

Step 2: Synthesis of 3-[4-[1-(2-oxo-2-piperazin-1-yl-ethyl)-4-piperidyl] anilino] piperidine-2,6-dione



A solution of tert-butyl 4-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino] phenyl]-1-piperidyl] acetyl] piperazine-1-carboxylate (50 mg, 97 µmol, 1 *eq*) in HCl/EtOAc (10 mL) was stirred at 20 °C for 1 hr. Then EtOAc was added and the mixture filtered. The filter cake was collected to give the title compound (50 mg, crude) as a white solid, which was used in the next step directly without further purification.

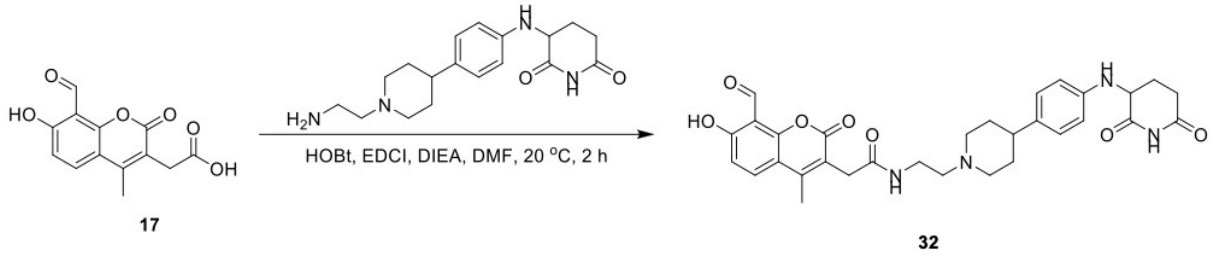
Step 3: Synthesis of 3-[2-[4-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino] phenyl]-1-piperidyl] acetyl]piperazin-1-yl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde



A mixture of 3-[4-[1-(2-oxo-2-piperazin-1-yl-ethyl)-4-piperidyl] anilino] piperidine-2,6-dione (50 mg, 120 µmol, 1*eq*), 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl) acetic acid (31 mg, 120 µmol, 1 *eq*), EDCI (27 mg, 145 µmol, 1.2 *eq*), HOBt (19 mg, 145 µmol, 1.2 *eq*) and DIEA

(78 mg, 604 μmol, 105 μL, 5 *eq*) in DMF (1 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 20 °C for 1 hr under a N_2 atmosphere. The mixture was quenched by adding 0.2 M HCl and filtered. The filter cake was collected and purified by prep-HPLC (FA condition;) column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 20%-50%, 8 minutes. The title compound (5 mg, 6% yield, 96 % purity) was obtained as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 10.76 (s, 1H), 10.44 (s, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.00 -6.89 (m, 3H), 6.61 (br d, *J* = 4.0 Hz, 2H), 5.64 (br d, *J* = 6.8 Hz, 1H), 4.25 (br s, 1H), 3.75 - 3.62 (m, 6H), 3.52 (m, 6H), 2.93 (br d, *J* = 9.2 Hz, 2H), 2.79 - 2.67 (m, 2H), 2.59 (br s, 1H), 2.32 (s, 3H), 2.18 - 2.05 (m, 4H), 1.90 - 1.81 (m, 1H), 1.73 - 1.66 (m, 2H), 1.64 - 1.55 (m, 2H).

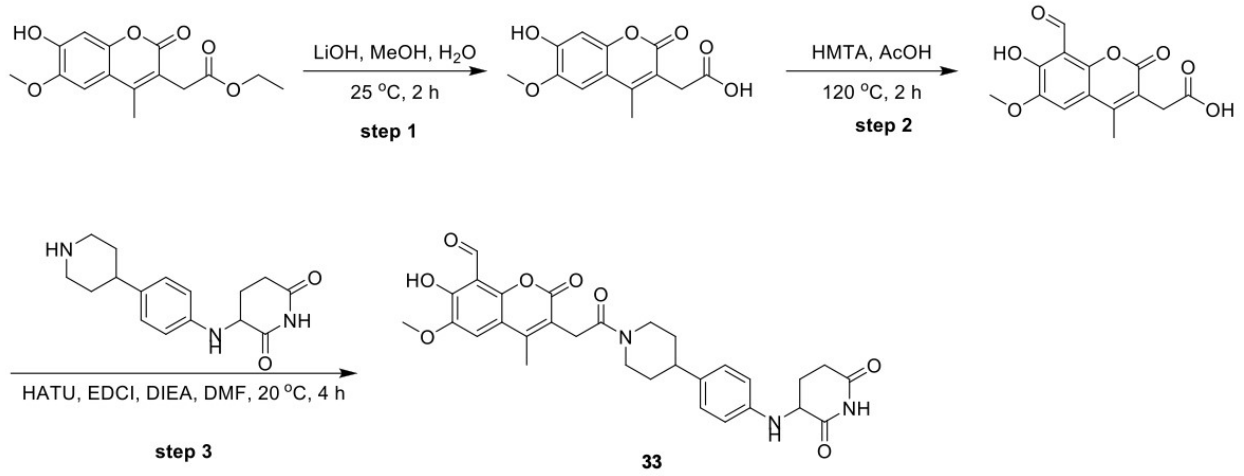
Synthesis of N-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino]phenyl]-1-piperidyl]ethyl]-2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetamide (32)



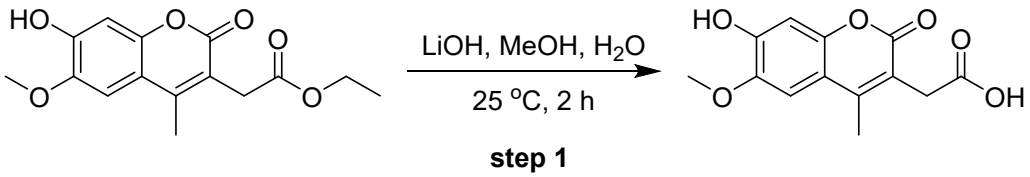
To a mixture of 3-[4-[1-(2-aminoethyl)-4-piperidyl] aniline]piperidine-2,6-dione (100 mg, 302 μmol, 1 *eq*) and 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (79 mg, 302 μmol, 1 *eq*) in DMF (1 mL) was added HOBt (49 mg, 363 μmol, 1.2 *eq*), EDCI (69 mg, 363 μmol, 1.2 *eq*) and DIEA (195 mg, 1.51 mmol, 263 μL, 5 *eq*) in one portion under N_2. The mixture was stirred at 20 °C for 2 hr. The mixture was concentrated to give a residue that was purified by prep-HPLC (column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 1%-40%, 8 minutes) to yield the title compound (7 mg, 4% yield, 95% purity) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 10.78 (s, 1H), 10.45 (s, 1H), 8.16 (s, 1H), 8.05 - 7.77 (m, 2H), 7.02 - 6.83 (m, 3H), 6.60 (br d, J = 8.0 Hz, 2H), 5.65 (br d, J = 7.2 Hz, 1H), 4.32 - 4.16 (m, 1H), 3.49 (s, 2H), 3.22 (br d, J = 6.0 Hz, 2H), 2.99 (br d, J = 10.4Hz, 2H), 2.83 - 2.66 (m, 1H),

2.64 - 2.52 (m, 2H), 2.45 (br t, J = 6.4 Hz, 2H), 2.40 - 2.26 (m, 4H), 2.17 - 2.06 (m, 3H), 1.93 - 1.80 (m, 1H), 1.73 - 1.63 (m, 2H), 1.62 - 1.46 (m, 2H).

Synthesis of 3-(2-(4-(4-((2,6-dioxopiperidin-3-yl)amino)phenyl)piperidin-1-yl)-2-oxoethyl)-7-hydroxy-6-methoxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde (33)

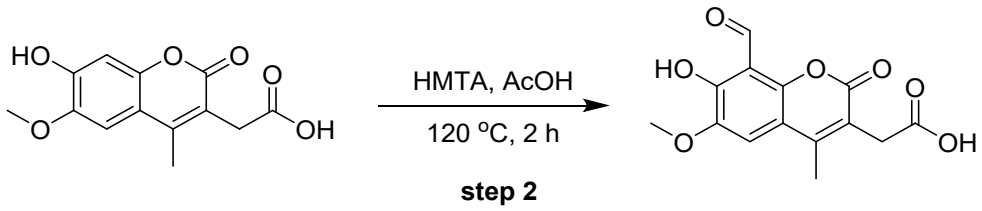


Step 1: Synthesis of 2-(7-hydroxy-6-methoxy-4-methyl-2-oxo-2H-chromen-3-yl)acetic acid



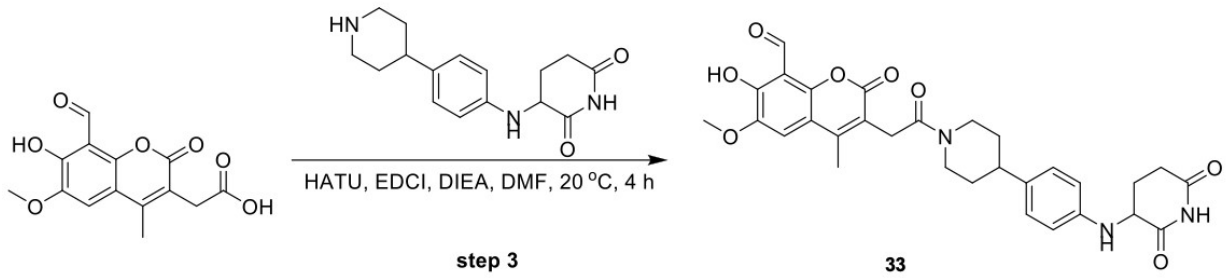
To a solution of 2-(7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (3.62 g, 13.69 mmol, 1 *eq*) and ethyl 2-(7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetate (4 g, 13.69 mmol, 1 *eq*) in MeOH (25 mL) and H_2O (25 mL) was added LiOH.H_2O (2.87 g, 68.43 mmol, 5 *eq*). The mixture was stirred at 25 °C for 2 hr. The pH was adjusted to 4 with HCl (2M). The reaction mixture was concentrated under reduced pressure. To the reaction mixture was added H_2O (200 mL), and a white solid formed. The solid was filtered. The cake was concentrated under reduced pressure. The title compound 2-(7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl) acetic acid (3 g, 83% yield) was obtained as a pink solid. It was used in the next step without further purification.

Step 2: Synthesis of 2-(8-formyl-7-hydroxy-6-methoxy-4-methyl-2-oxo-2H-chromen-3-yl) acetic acid



To a solution of 2-(7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (0.1 g, 378 µmol, 1 *eq*) in AcOH (20 mL) was added HMTA (212 mg, 1.51 mmol, 4 *eq*). The mixture was stirred at 120 °C for 2 hr. The reaction mixture was filtered and concentrated under reduced pressure. The concentrated solution was poured into H_2O (400 mL), and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (100 mL x 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue that was used directly in the next step without further purification. The title compound 2-(8-formyl-7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (63 mg, 57% yield) was obtained as a red solid.

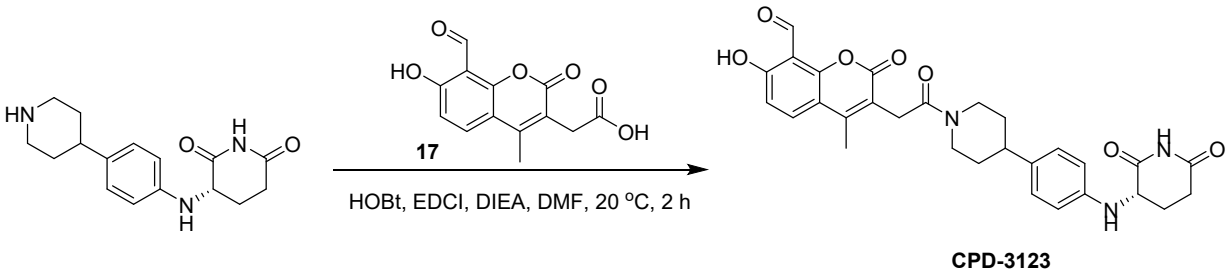
Step 3: Synthesis of 3-(2-(4-(4-((2,6-dioxopiperidin-3-yl)amino)phenyl)piperidin-1-yl)-2-oxoethyl)-7-hydroxy-6-methoxy-4-methyl-2-oxo-2H-chromene-8-carbaldehyde



A mixture of 3-[4-(4-piperidyl)anilino]piperidine-2,6-dione (50 mg, 174 µmol, 1.45 *eq*), 2-(8-formyl-7-hydroxy-6-methoxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (35 mg, 119 µmol, 1 *eq*), HATU (54 mg, 143 µmol, 1.2*eq*), EDCI (27 mg, 143 µmol, 1.2 *eq*) and DIEA (46 mg, 359 µmol, 62 µL, 3 *eq*) in DMF (1 mL) was degassed and purged with N_2 3 times, and then the mixture was stirred at 20 °C for 4 hr under a N_2 atmosphere. Water was then added and the mixture filtered.

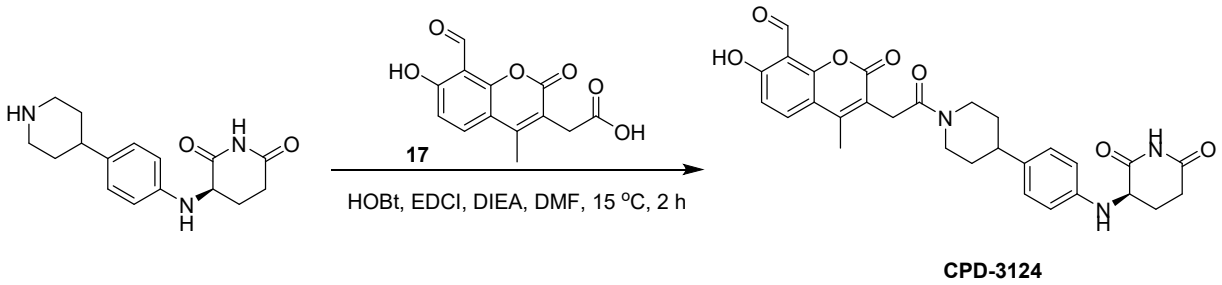
The filter cake was purified by prep-HPLC (FA condition). column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 30%-60%, 8 minutes. The title compound 3-[2-[4-[4-[(2,6-dioxo-3-piperidyl)amino]phenyl]-1-piperidyl]-2-oxo-ethyl]-7-hydroxy-6-methoxy-4-methyl-2-oxo-chromene-8-carbaldehyde (5 mg, 7% yield, 97% purity) was obtained as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.58 - 1.91 (m, 4 H) 2.11 (m, 1 H) 2.38 (s, 3 H) 2.52 - 2.69 (m, 4 H) 2.74 (m, 1 H) 3.18 (br t, *J*=12.0 Hz, 1 H) 3.66 - 3.81 (m, 2 H) 3.93 (s, 3 H) 4.13 - 4.51 (m, 3 H) 5.68 (d, *J*=7.5 Hz, 1 H) 6.63 (d, *J*=8.5 Hz, 2 H) 6.98 (d, *J*=8.4 Hz, 2 H) 7.47 (s, 1 H) 10.45 (s, 1 H) 10.77 (s, 1 H) 11.75 - 11.92 (m, 1 H).

Synthesis of 3-[2-[4-[4-[[(3S)-2,6-dioxo-3-piperidyl]amino]phenyl]-1-piperidyl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (35, CPD-3123)



To a solution of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl) acetic acid (91 mg, 348 μmol, 1 *eq*), (3S)-3-[4-(4-piperidyl)anilino]piperidine-2,6-dione (100 mg, 348 μmol, 1 *eq*) in DMF (1 mL) was added HOBt (235 mg, 1.74 mmol, 5 *eq*), DIEA (53 mg, 417 μmol, 73 μL, 1.2 *eq*) and EDCI (80 mg, 417 μmol, 1.2 *eq*). The mixture was stirred at 20 °C for 2 hr. Then 1M HCl (8 mL) was added and the mixture filtered. The filter cake was purified by prep-HPLC (FA condition). column: Phenomenex Luna C18 75 x 30 mm x 3 μm; mobile phase: [water (FA)-ACN]; B%: 20%-50%, 8 minutes, to yield the title compound (7 mg, 3% yield, 93% purity) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ = 10.77 (s, 1H), 10.46 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.02 -6.93 (m, 3H), 6.63 (d, *J* = 8.2 Hz, 2H), 5.68 (d, *J* = 7.5 Hz, 1H), 4.53 - 4.40 (m, 1H), 4.34 - 4.21 (m, 1H), 4.20 - 4.11 (m, 1H), 3.81 - 3.61 (m, 2H), 3.24 - 3.12 (m, 1H), 2.79 - 2.69 (m, 1H), 2.65 - 2.58 (m, 2H), 2.34 (s, 3H), 2.15 - 2.03 (m, 1H), 1.91 - 1.69 (m, 3H), 1.68 - 1.54 (m, 1H), 1.47 - 1.30 (m, 1H).

Synthesis of 3-[2-[4-[4-[[(3R)-2,6-dioxo-3-piperidyl]amino]phenyl]-1-piperidyl]-2-oxo-ethyl]-7-hydroxy-4-methyl-2-oxo-chromene-8-carbaldehyde (36, CPD-3124)



CPD-3124

To a solution of 2-(8-formyl-7-hydroxy-4-methyl-2-oxo-chromen-3-yl)acetic acid (91 mg, 348 µmol, 1 *eq*), (3R)-3-[4-(4-piperidyl) aniline] piperidine-2,6-dione (100 mg, 348 µmol, 1 *eq*) in DMF (1 mL) was added HOBt (56 mg, 417 µmol, 1.2 *eq*), DIEA (359 mg, 2.78 mmol, 484 µL, 8 *eq*) and EDCI (80 mg, 417 µmol, 1.2 *eq*). The mixture was stirred at 20 °C for 2 hr. Then 1M HCl (8 mL) was added and the mixture filtered. The filter cake was purified by prep-HPLC (FA condition, column: Phenomenex Luna C18 75 x 30 mm x 3 µm; mobile phase: [water (FA)-ACN]; B%: 20%-50%, 8 minutes) to yield the title compound (21 mg, 11% yield, 95% purity) as a yellow solid. ^{1}H NMR: (400 MHz, DMSO-d6) δ = 10.78 (s, 1H), 10.47 (s, 1H), 7.99 (s, 1H), 6.98 (br d, *J* = 8.0 Hz, 3H), 6.63 (d, *J* =8.0 Hz, 2H), 5.69 (br d, *J* = 7.6 Hz, 1H), 4.48 (br d, *J* = 12.8 Hz, 1H), 4.28 (ddd, *J* = 4.8, 7.2, 11.6 Hz, 1H), 4.17 (br d, *J* = 13.6 Hz, 1H), 3.84 - 3.64 (m, 2H), 3.23 - 3.15 (m, 1H), 2.81 - 2.70 (m, 1H), 2.66 - 2.58 (m, 2H), 2.34 (s, 3H), 2.16- 2.06 (m, 1H), 1.94 - 1.78 (m, 2H), 1.74 (br d, *J* = 13.2 Hz, 1H), 1.68 - 1.55 (m, 1H), 1.46 - 1.32 (m, 1H).

Experimental Procedures

Docking and Molecular Dynamics

Ternary complex modeling and compound docking were performed as previously described.[1]

RNase Activity Assay

This fluorescence-based protocol was modified from an RNA cleavage assay previously reported in the literature.[2] The assay contains 20 nM IRE1α protein (cat. no. E31-11G-10, SignalChem) and 100 nM of a single hairpin RNA substrate (5'-CAUGUCCGCAGCGCAUG-3') labelled with Alexa Fluor 647 fluorophore on the 5' terminus and a black hole quencher on the 3' terminus (custom design purchased from IDT). It is important to use RNase-free reagents and consumables for all parts of this assay. Necessary solutions and dilutions were made using Nuclease-free water (catalog #43-879-36, Fisher Scientific). When preparing the assay, all surfaces and pipettes were wiped down with RNase AWAY surface decontaminant (Catalog #21-402-178, Fisher Scientific). The reaction buffer was prepared as follows: 50 mM Tris, pH 7.0, 500 μM $MgCl_2$, 0.025% Tween-20, 2 mM DTT, and 63 μg/mL tRNA (from baker's yeast, catalog #10109495001, Sigma Aldrich). IRE1α protein was dissolved in the reaction buffer, and 45 μL of this reaction mixture was added to a black 384-well plate (781076, Greiner Bio-One) using multi-channel ClipTip pipette (ThermoFisher). Compounds dissolved in DMSO were dispensed using D300e Digital Dispenser (HP) in a 12-point dose response, in duplicates, and normalized to 0.5% final DMSO concentration. The plate was centrifuged at 500xg for 3 minutes. The plate was covered with foil and incubated with the compounds at room temperature for 4.5 hours. The RNA substrate was dissolved in the RNA substrate buffer (10 mM Tris pH 7.0 and 0.1 mM EDTA), and 5 μL of the RNA substrate mixture was added to each well of the plate using a multi-channel ClipTip pipette. The plate was centrifuged at 500xg for 3 minutes, and then the fluorescence (ex:625(30) nm, em: 680(30) nm)) was measured after 30 min incubation at RT on a Clariostar Plus plate reader (BMG). The data were analyzed using GraphPad Prism software and dose response curves were fitted using Variable Slope equation. Data are reported as the average ± standard deviation with n=2.

HiBiT Degradation Assay

This assay was performed as previously published using the IRE1α-SpyTag-HiBiT HEK293T cell line, without transfection of SpyCatcher-dTAG, using a duplicate plate to test cell viability with CTG (Promega).[1] For HiBiT assays with thapsigargin, cells were plated in the same manner at 5,000 viable cells/well final density. After allowing the cells to adhere overnight, thapsigargin was added at the indicated concentration 2 hours (pre-treatment, PT) or at the same time (co-treatment, CoT) as CPD-2828 (**30**). Total DMSO concentration was normalized to 1% across the plate. The plate was returned to the incubator at 37°C with 5% CO_2 and stopped after 6 hours by addition of the lystic HiBiT or CTG reagents. Data were analyzed using GraphPad Prism software and dose response curves were fitted using Variable Slope equation. Data are reported as the average ± standard deviation with n=2. D_{max} was determined to be the maximum level of degradation observed for each PROTAC.

Cellular Cereblon Engagement Assay

HEK293T cells stably expressing Nluc-CRBN were used as previously reported.[3]

Automated Capillary-Based Western Blot Analysis

HEK293T cells were plated at a density of 1.5 x 10^6 cells/well in DMEM supplemented with 10% FBS (ThermoScientific, catalog # A5256701). The cells were placed in an incubator at 37°C with 5% CO_2 overnight. The next day, the media was aspirated and replaced with 2 mL fresh media containing DMSO, 10 µM CPD-2522 or CPD-2828, normalized to a DMSO concentration of 1%. The plate was returned to the incubator for 24 hours. Cells were then washed three times with sterile PBS. The plate was placed on ice and 75 µL/well RIPA buffer containing cOmplete protease inhibitor cocktail (Roche, catalog # 11697498001) was added. Cells were allowed to lyse for 20 minutes on ice, then the lysate was collected in 1.5 mL microcentrifuge tubes. Samples were centrifuged at 14,000 rcf for 15 minutes, 4°C to remove insoluble cell components. The supernatant was collected and the protein concentration was determined using a BCA protein assay. Each sample was diluted to 1 mg/mL using 0.1X sample buffer (BioTechne Simple Western). Analysis of samples was performed following the Jess Simple Western protocol with EZ Standard Pack 3 (high MW, 66-440 kDa, catalog # PS-ST03EZ-8). The primary antibody solution was prepared by diluting IRE1α antibody (Cell Signaling, catalog # 3294T) 1:25 and diluting vinculin antibody (Novus, catalog # NB600-1293) 1:30. Both NIR and chemiluminescence imaging was performed with the high MW assay on the Simple Western Jess Automated Western Blot system. To quantify protein levels, corresponding peaks for IRE1α and vinculin were integrated and normalized in each sample by taking the ratio of the IRE1α:vinculin peak area. Samples are reported as the average % DMSO ± standard deviation of biological duplicates.

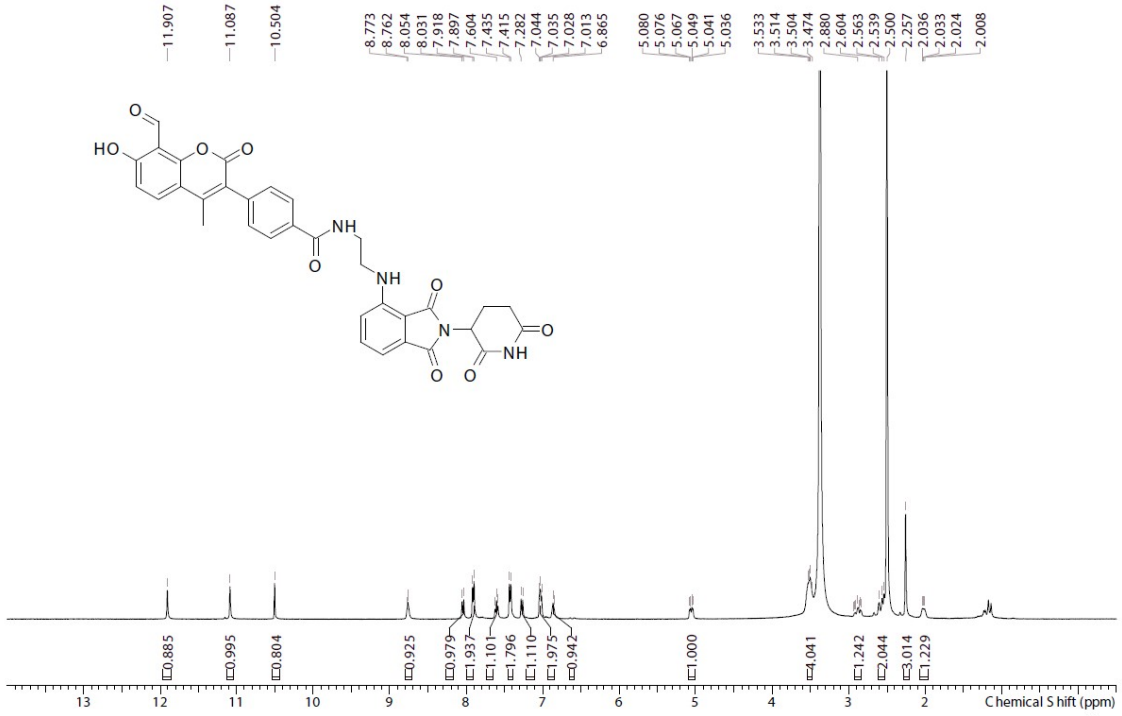
CRBN TR-FRET Assay for in vitro Binding

The assay was performed as previously published with biotinylated-CRBN-DDB1 and BODIPY-lenalidomide.[4] Briefly, 100 nM biotinylated-CRBN-DDB1, 2 nM terbium-streptavidin (ThermoFisher Scientific, catalog # PV3965), and 20 nM lenalidomide-BodipyFL in a buffer with 50 mM Tris at pH 7.5, 200 mM NaCl, 1 mM TCEP and 0.1% Poloxamer-188 (Sigma-Aldrich, catalog # P5556). The solution was protected from light and allowed to incubate for 15 minutes at room temperature. The mixture was then added to 384-well shallow well black plates (ThermoFisher Scientific, catalog # 264705) with a 15 µL assay volume. The compound titrations were performed using a d300e digital dispenser (HP), normalizing to a final DMSO concentration of 1%. After compounds were dispensed, fluorescence signal at 520 and 490 nm was recorded over five cycles with an excitation at 337 nm using a PHERAstar FSX microplate reader (BMG Labtech). The TR-FRET ratio was calculated as the emissions for 520/490 nm and plotted with compound concentration in GraphPad Prism. To calculate IC_{50}s, the data were fitted with a nonlinear fit variable slope model. Data are reported as the average ± standard deviation with n=2.

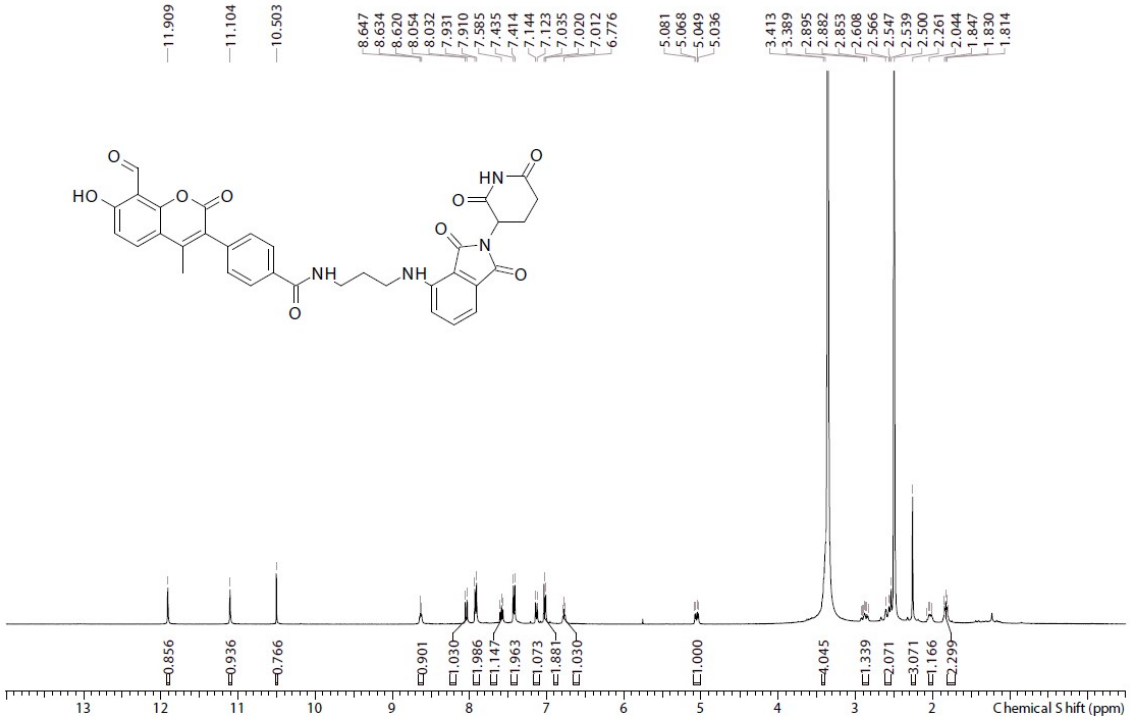
Quantitative Degradation Proteomics

HEK293T cells were treated with compounds at 10 μM in biological singlicate and DMSO vehicle control in biological triplicate for 5h. The sample preparation and analysis was performed as previously published.

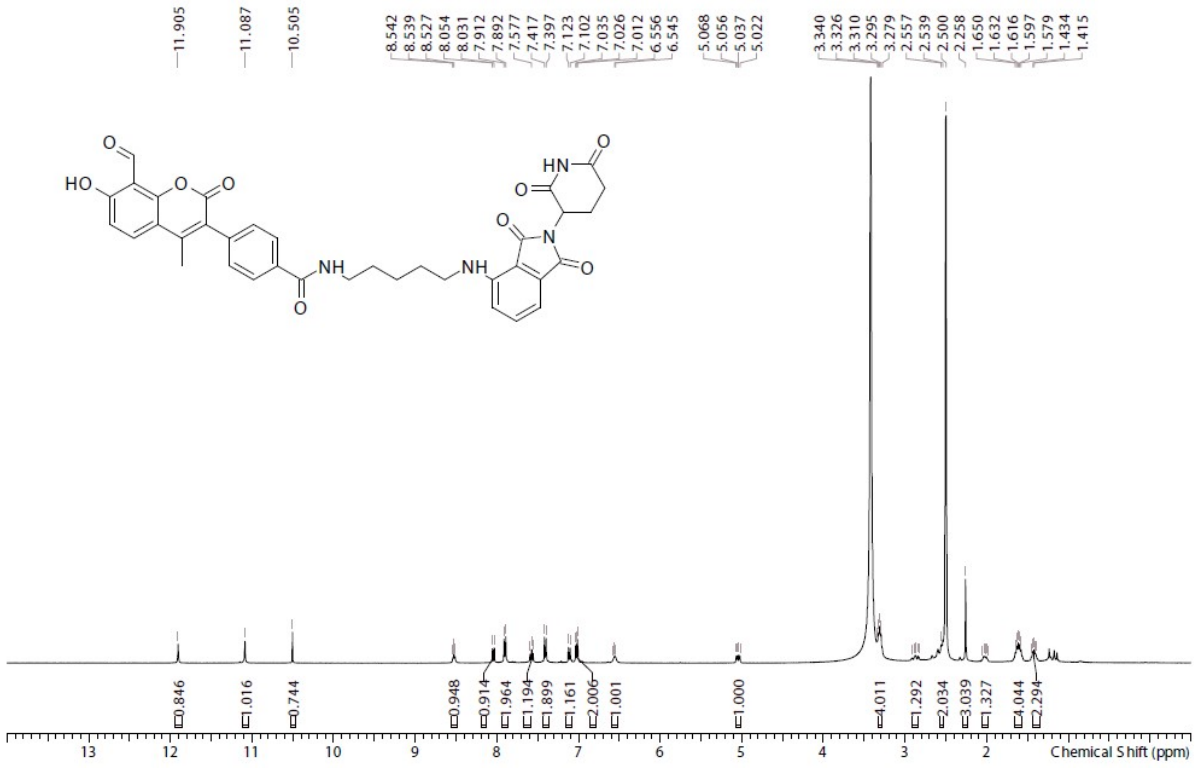
NMR spectra



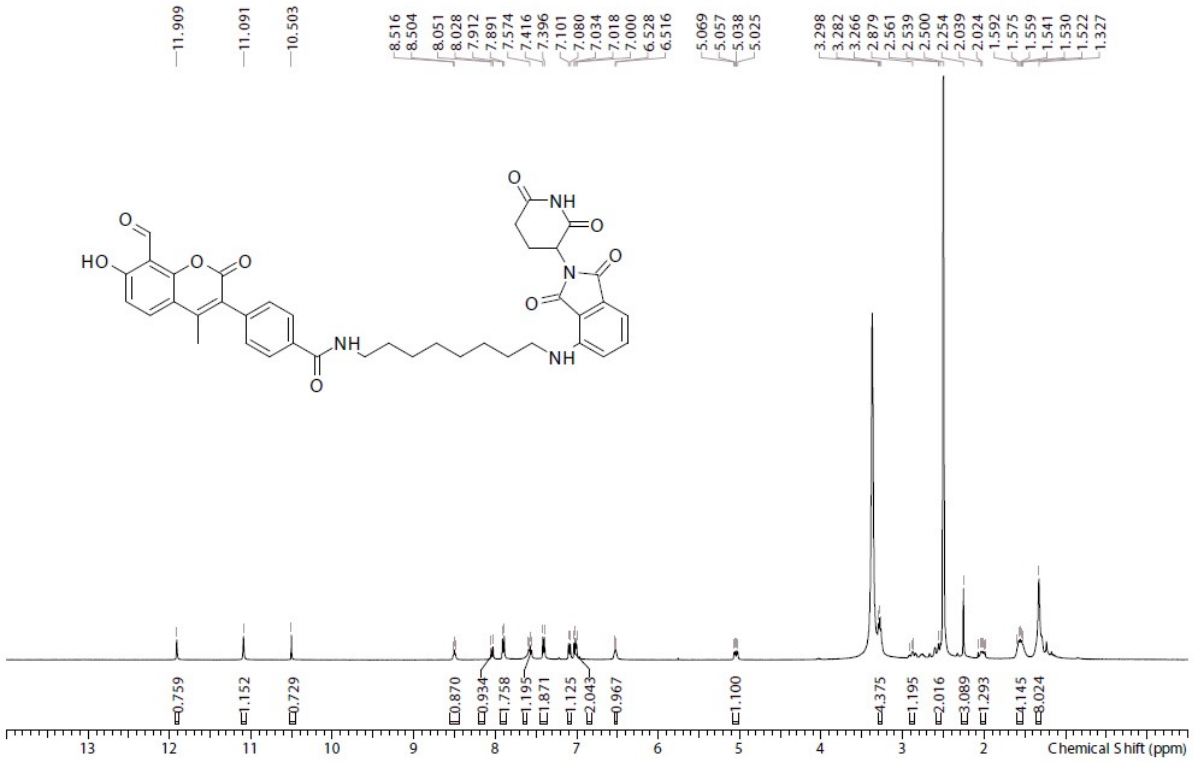
^{1}H NMR trace for compound 2.



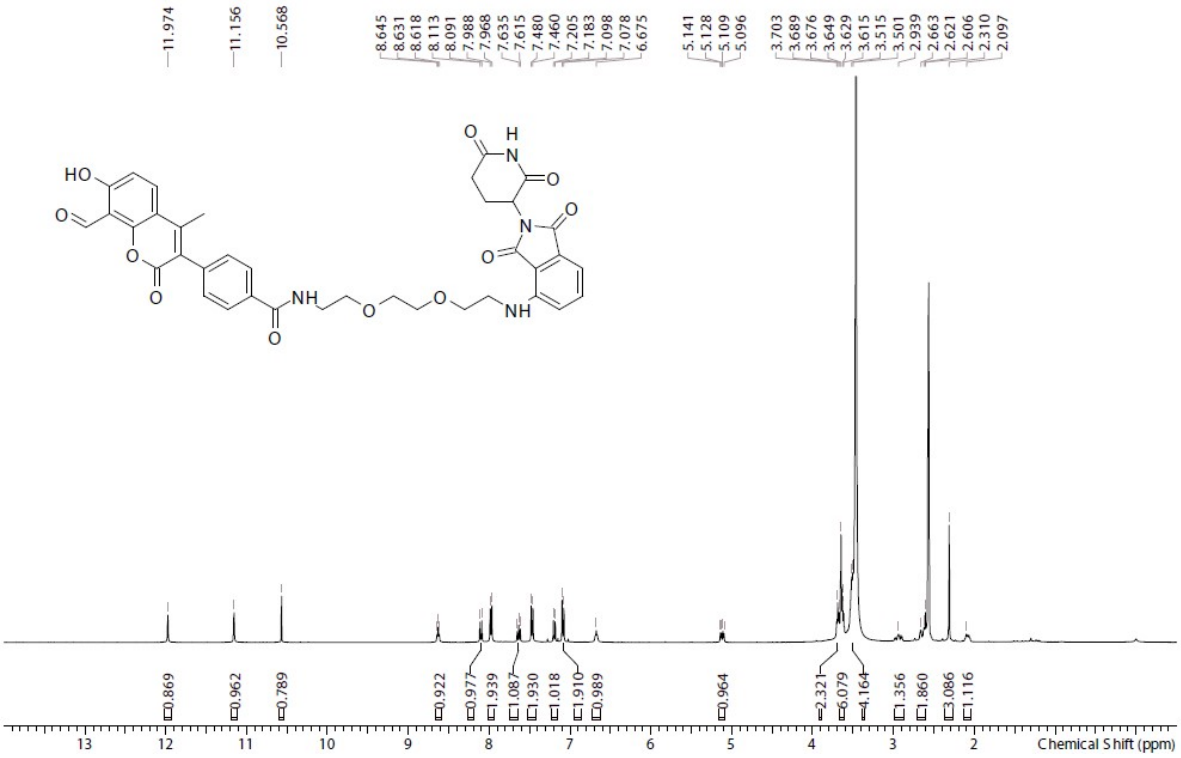
^{1}H NMR trace for compound 3.



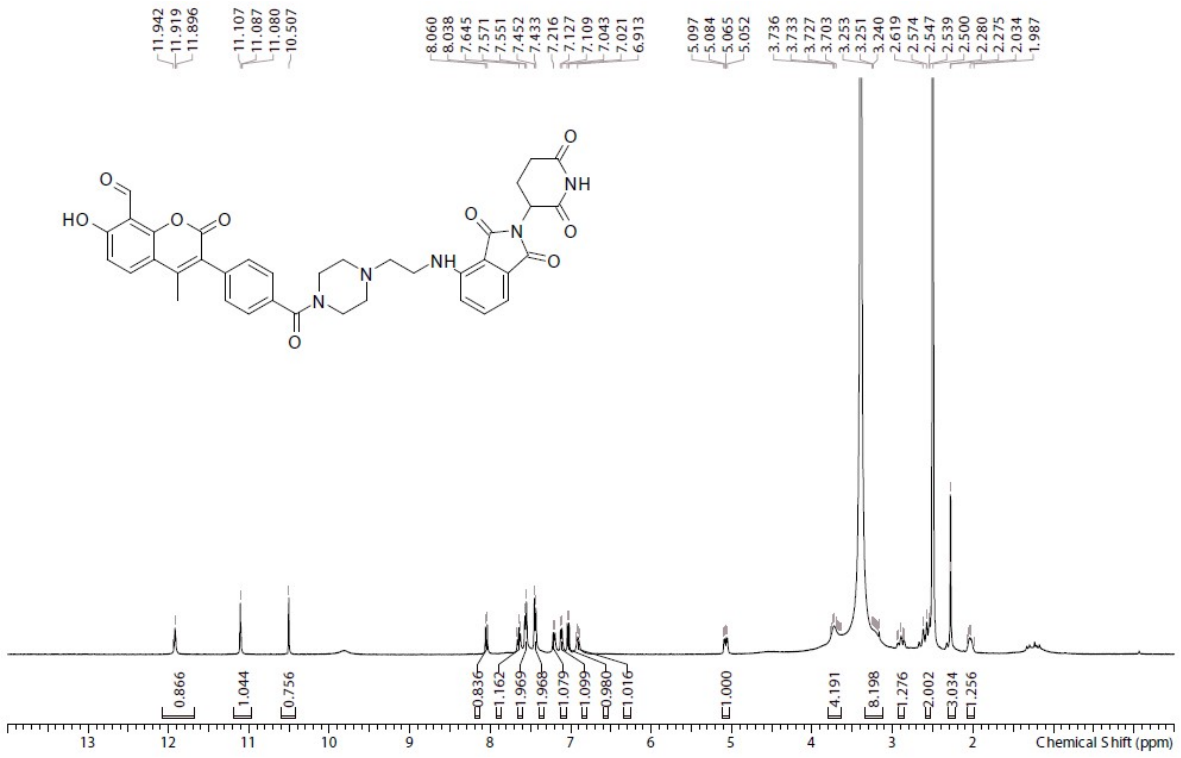
^{1}H NMR trace for compound 4.



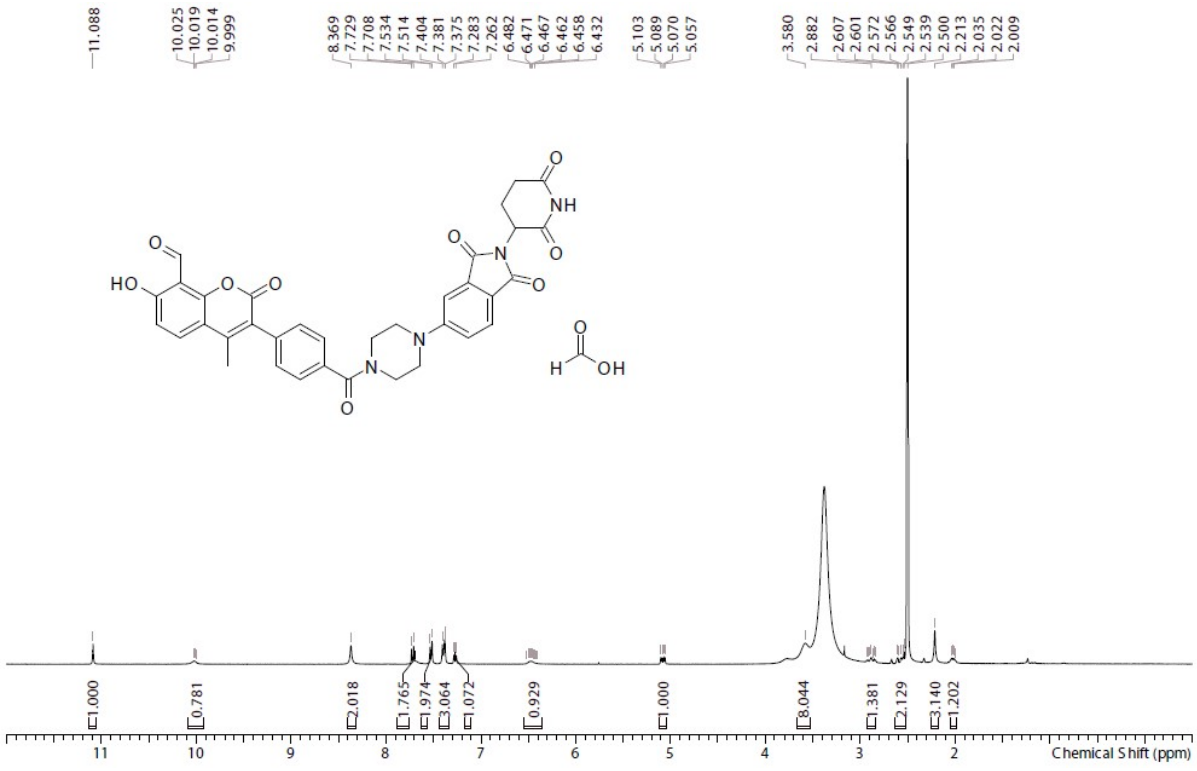
^{1}H NMR trace for compound 5.



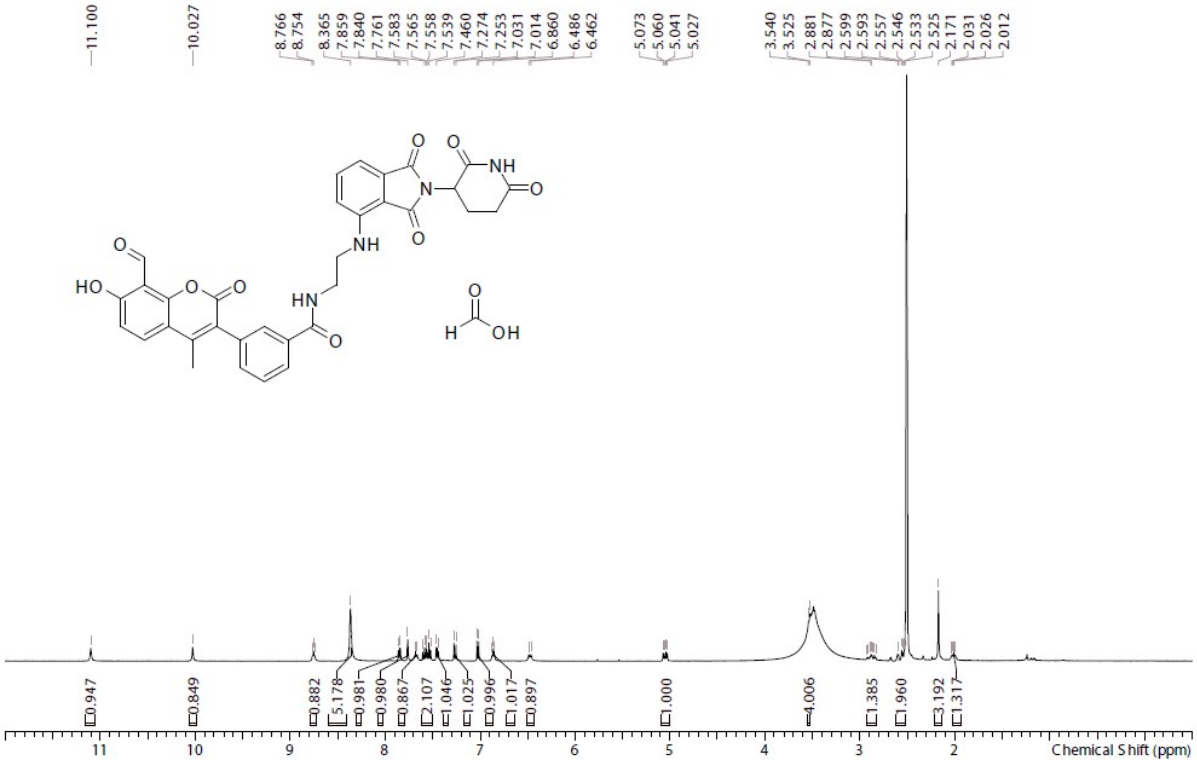
[1]H NMR trace for compound 6.



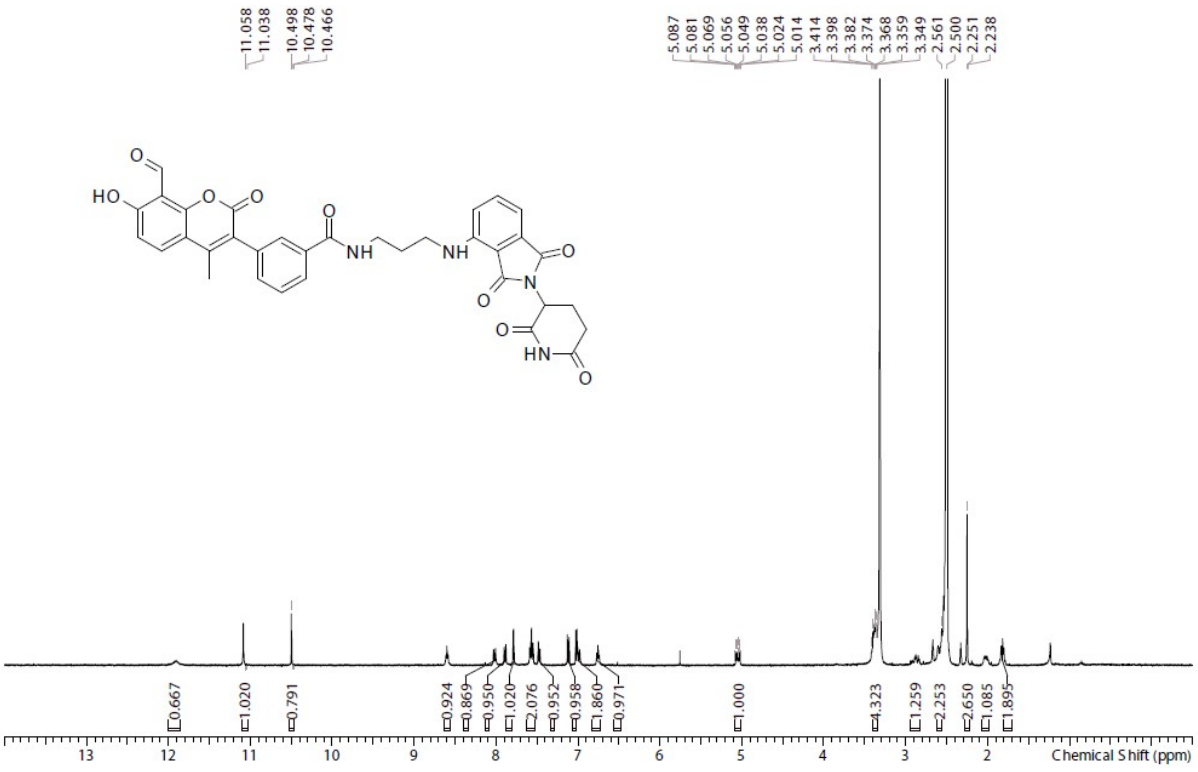
[1]H NMR trace for compound 7.



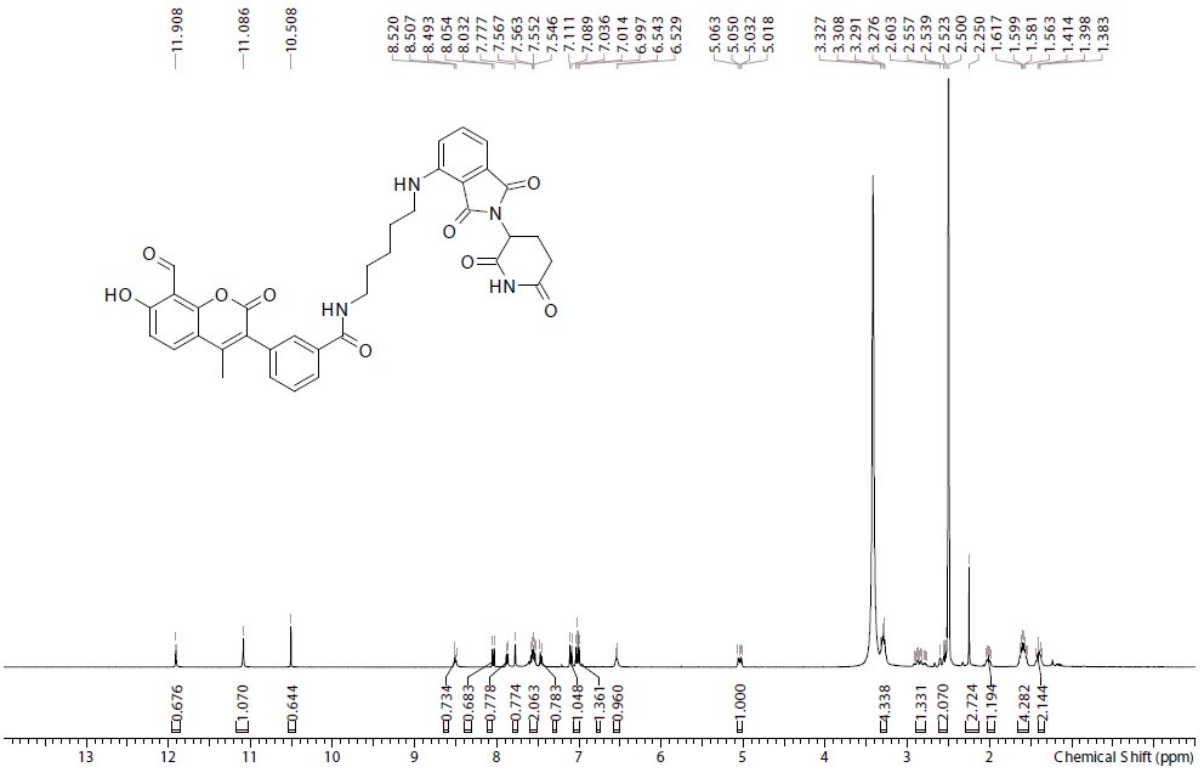
^{1}H NMR trace for compound 8.



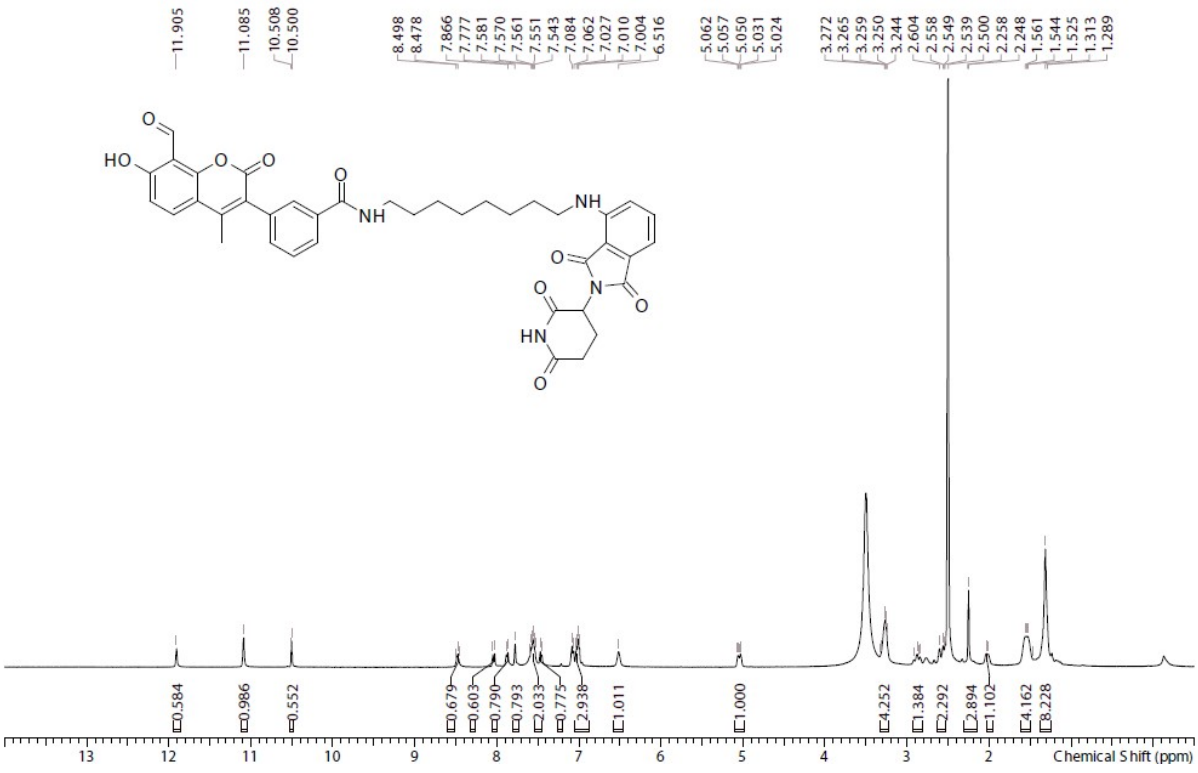
^{1}H NMR trace for compound 10.



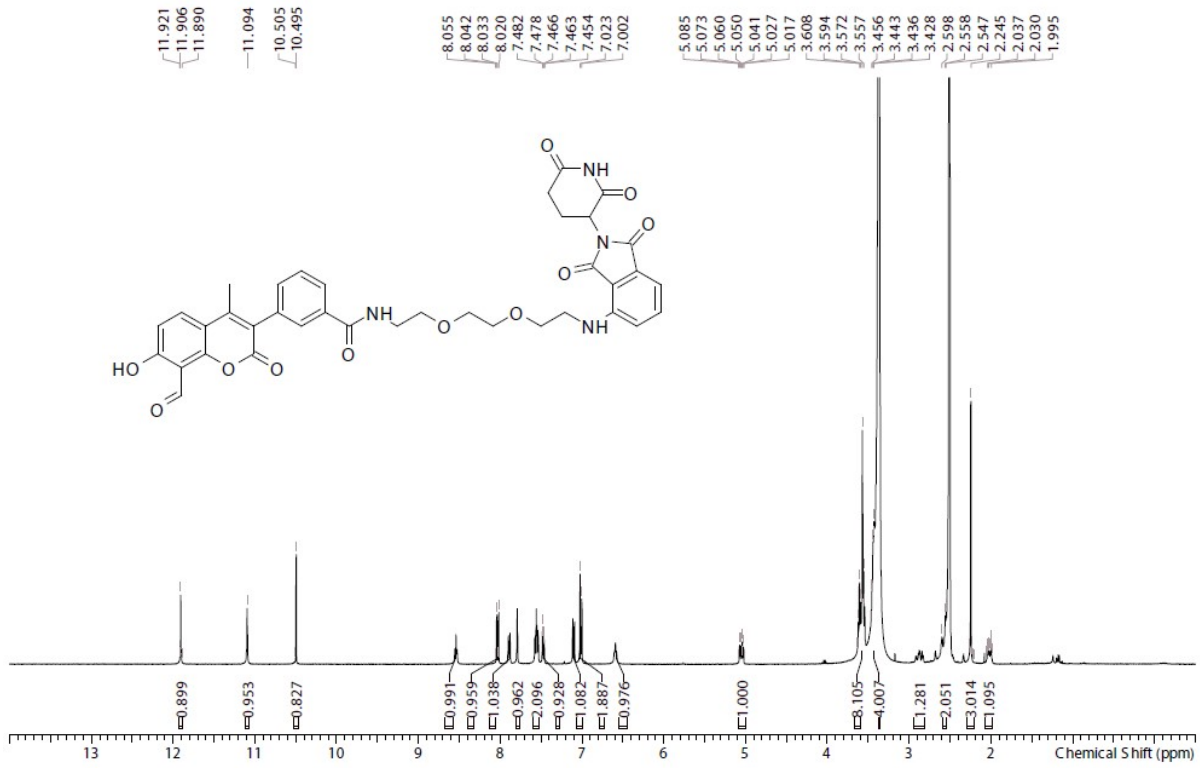
^{1}H NMR trace for compound 11.



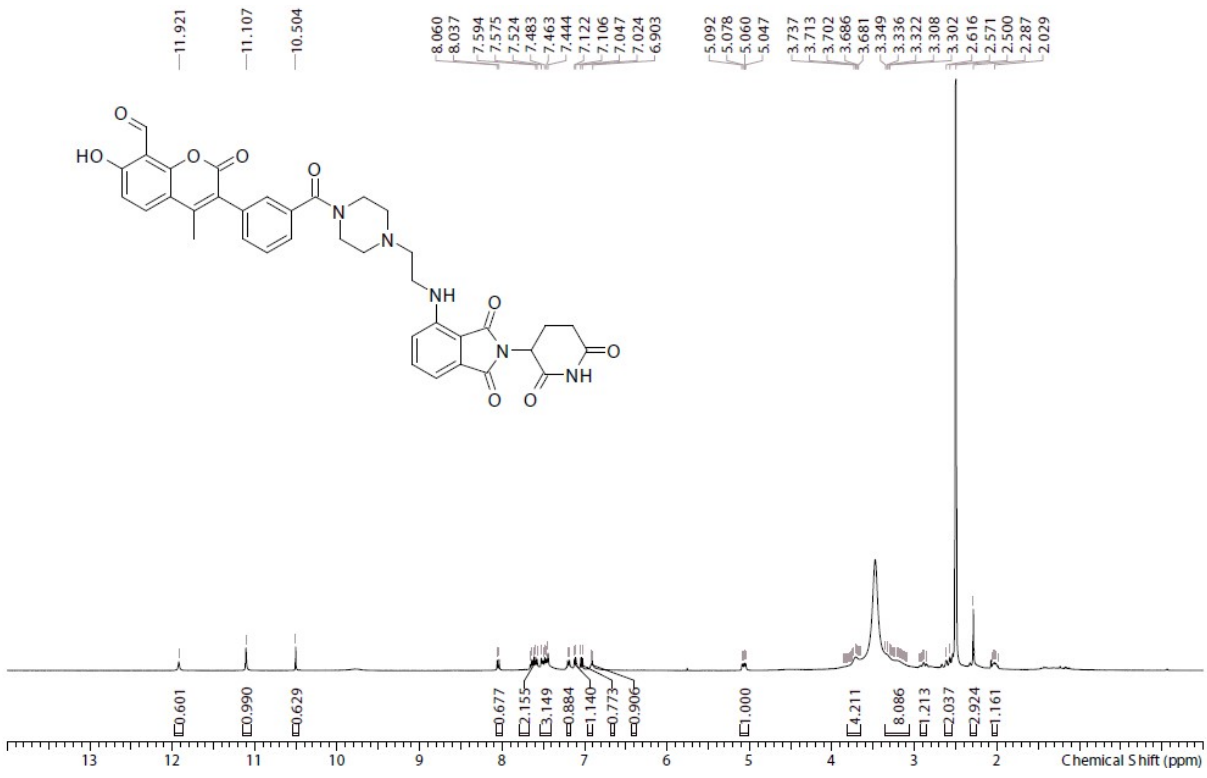
^{1}H NMR trace for compound 12.



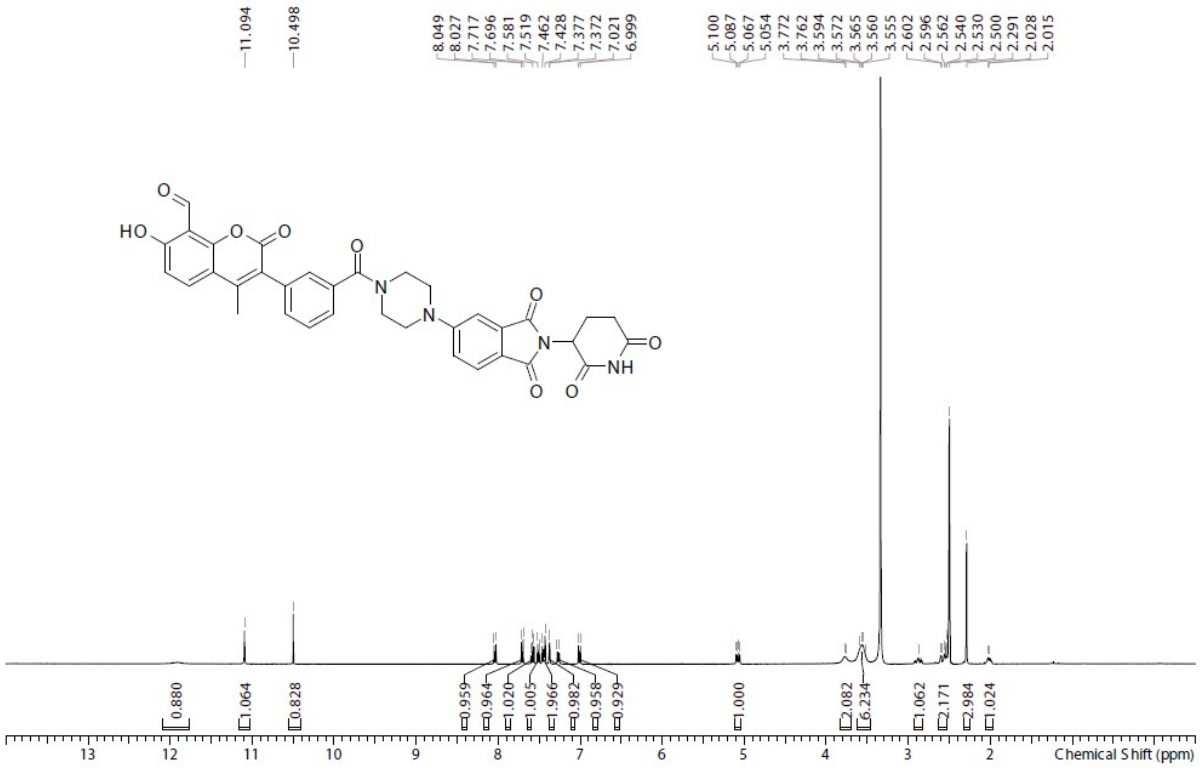
^{1}H NMR trace for compound 13.



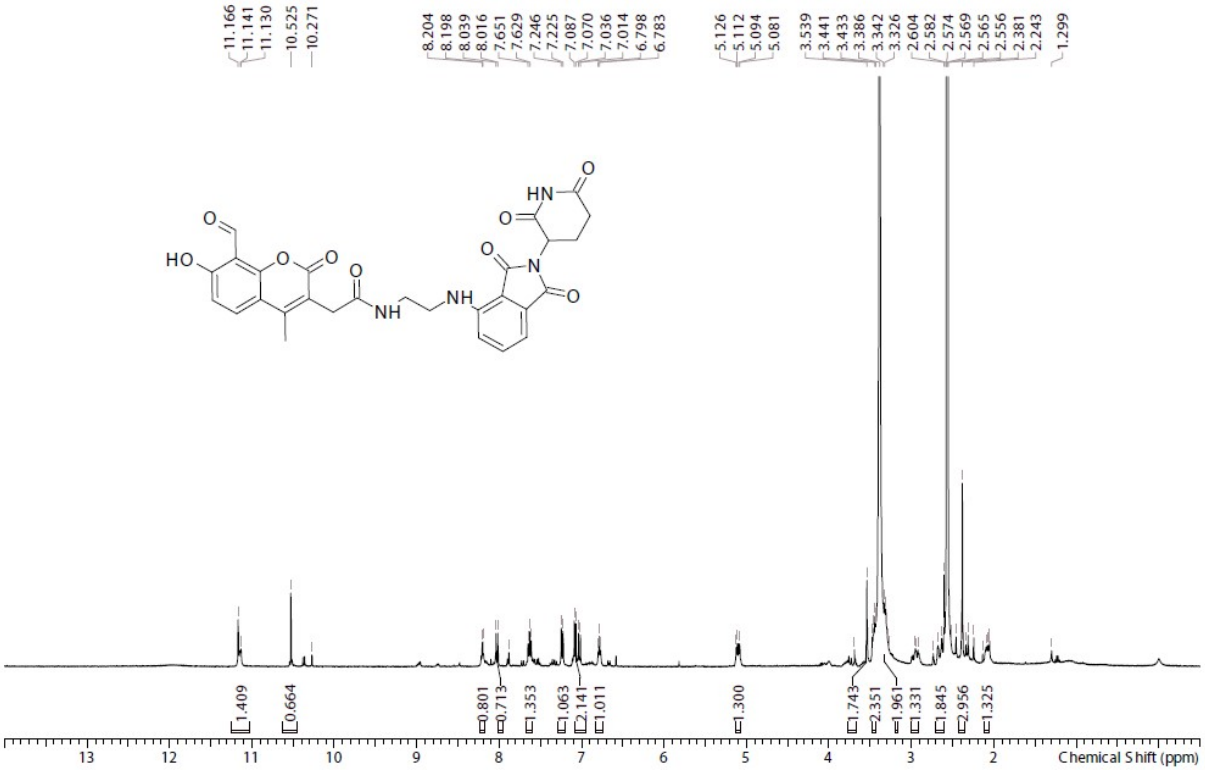
^{1}H NMR trace for compound 14.



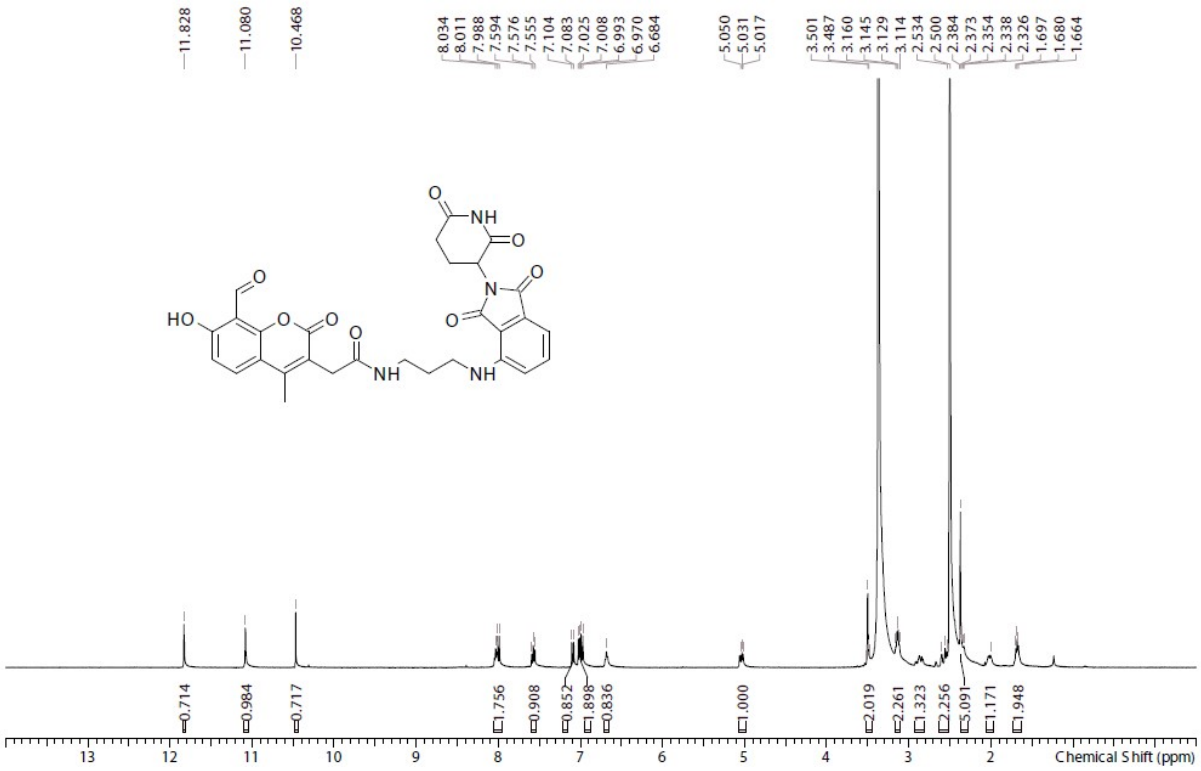
^{1}H NMR trace for compound 15.



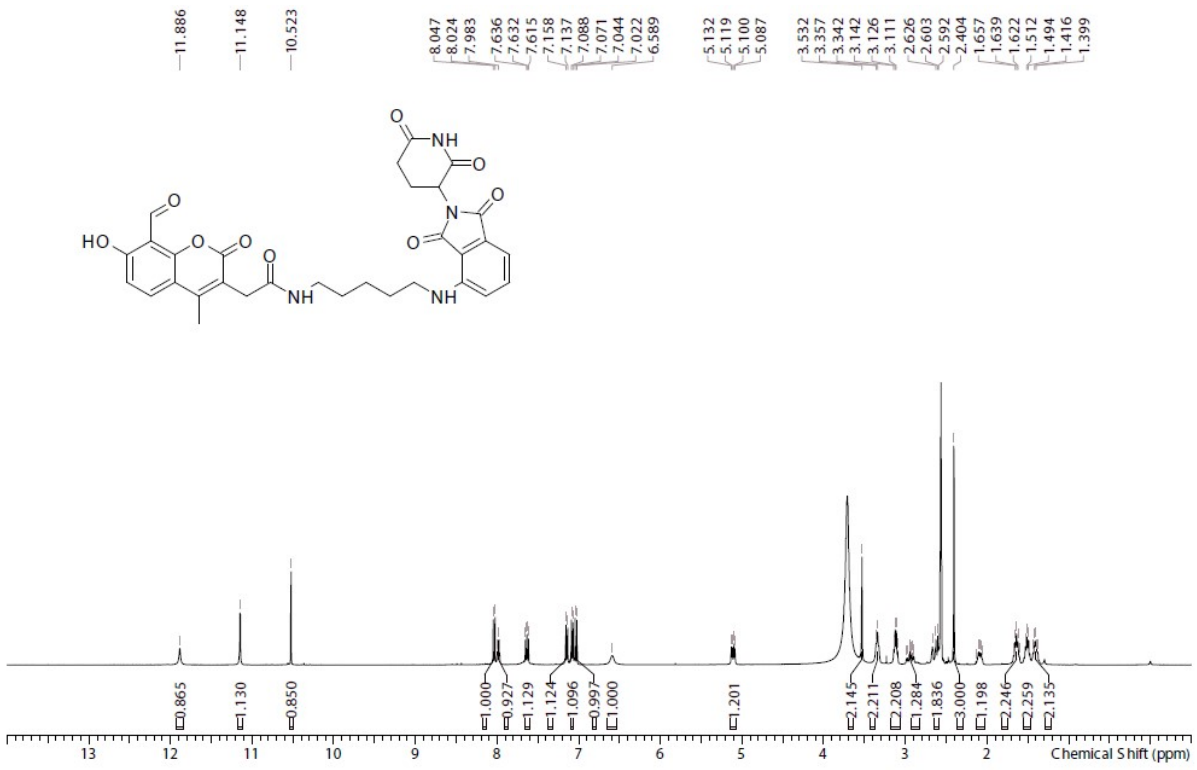
^{1}H NMR trace for compound 16.



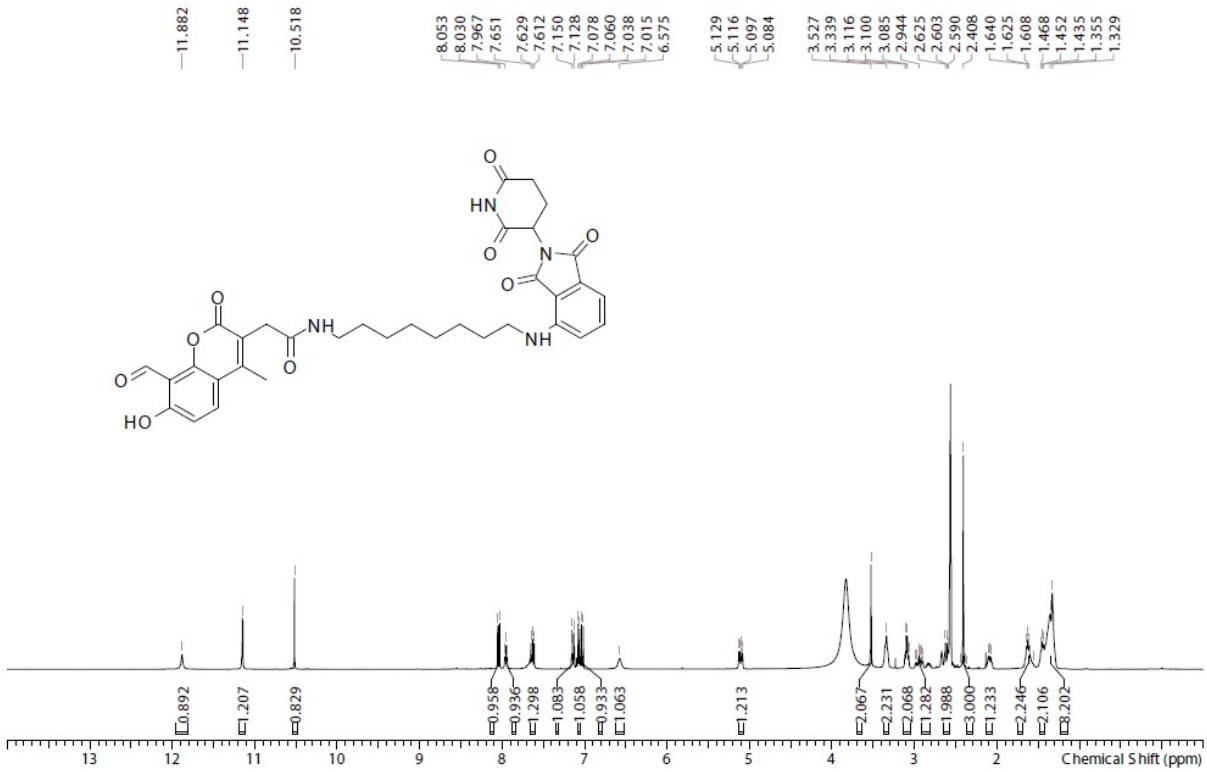
^{1}H NMR trace for compound 18.



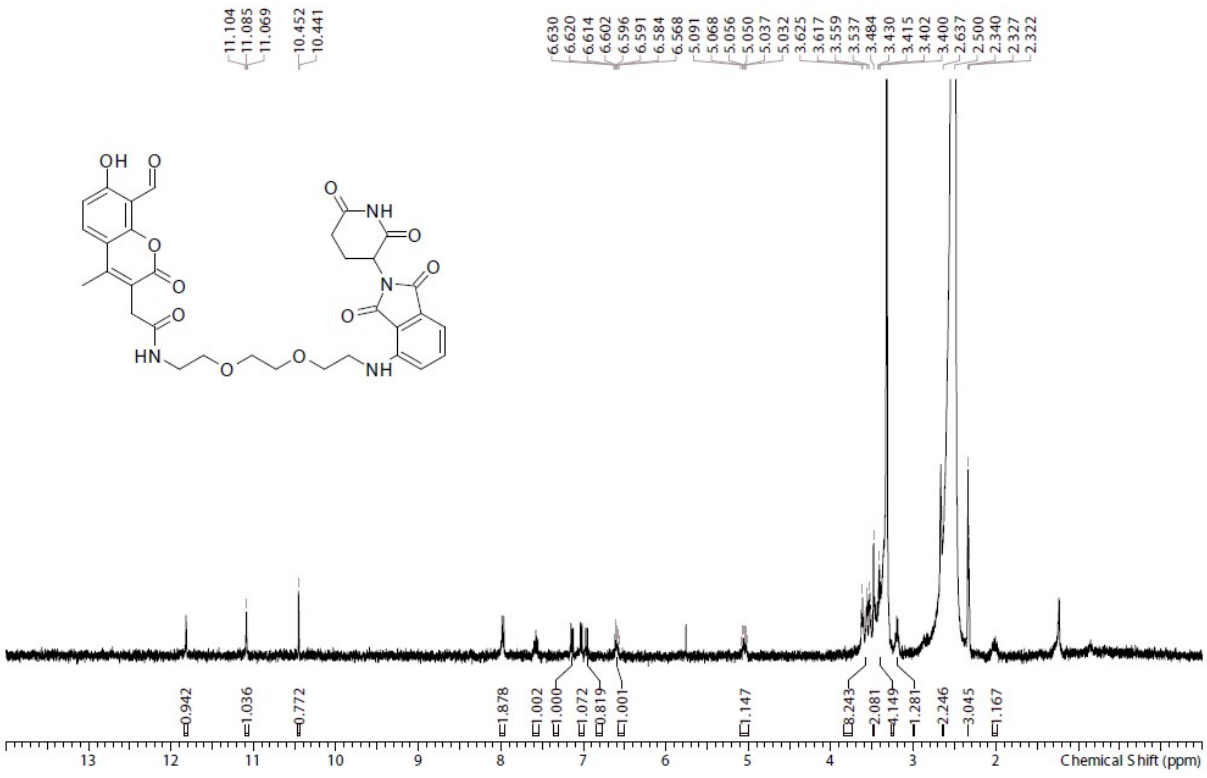
^{1}H NMR trace for compound 19.



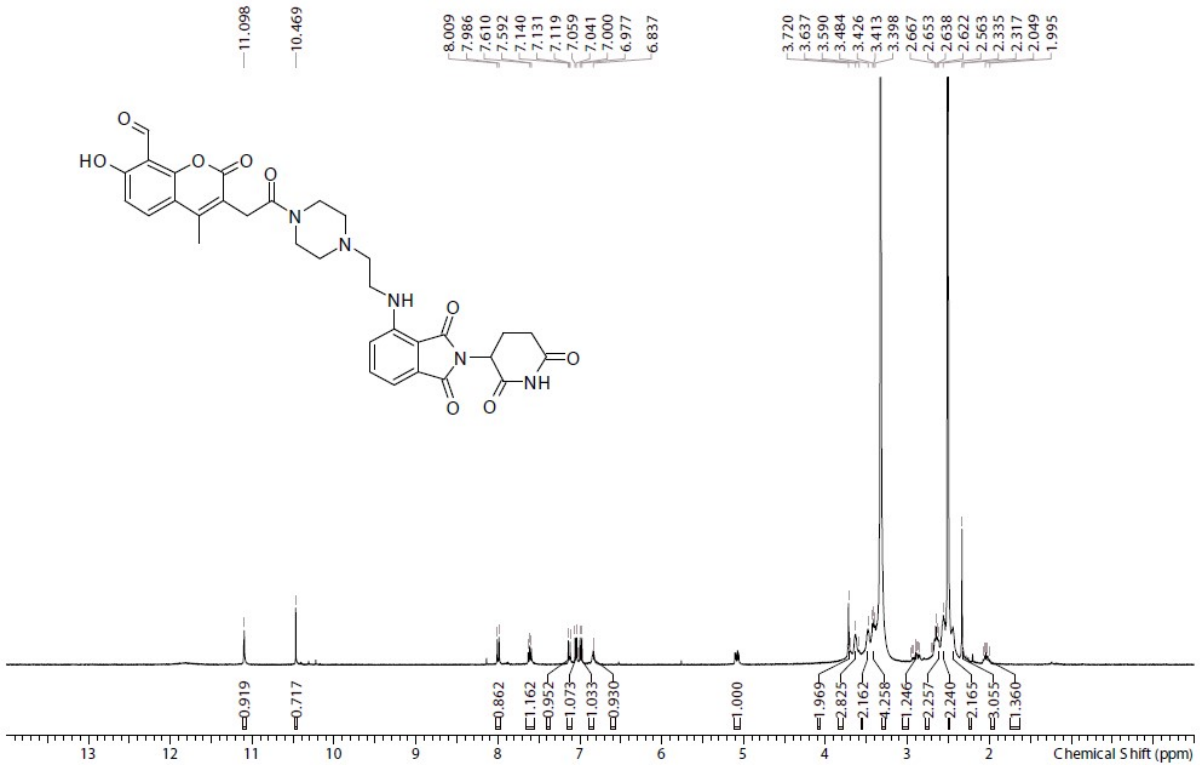
^{1}H NMR trace for compound 20.



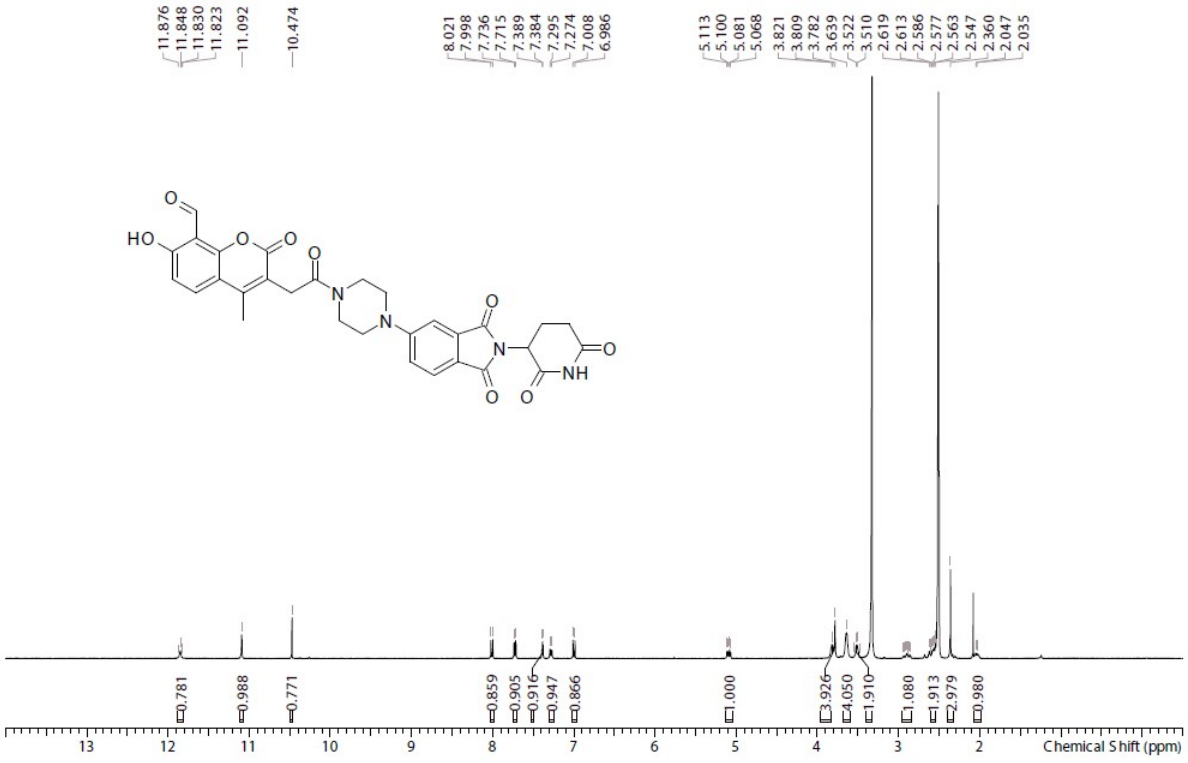
^{1}H NMR trace for compound 21.



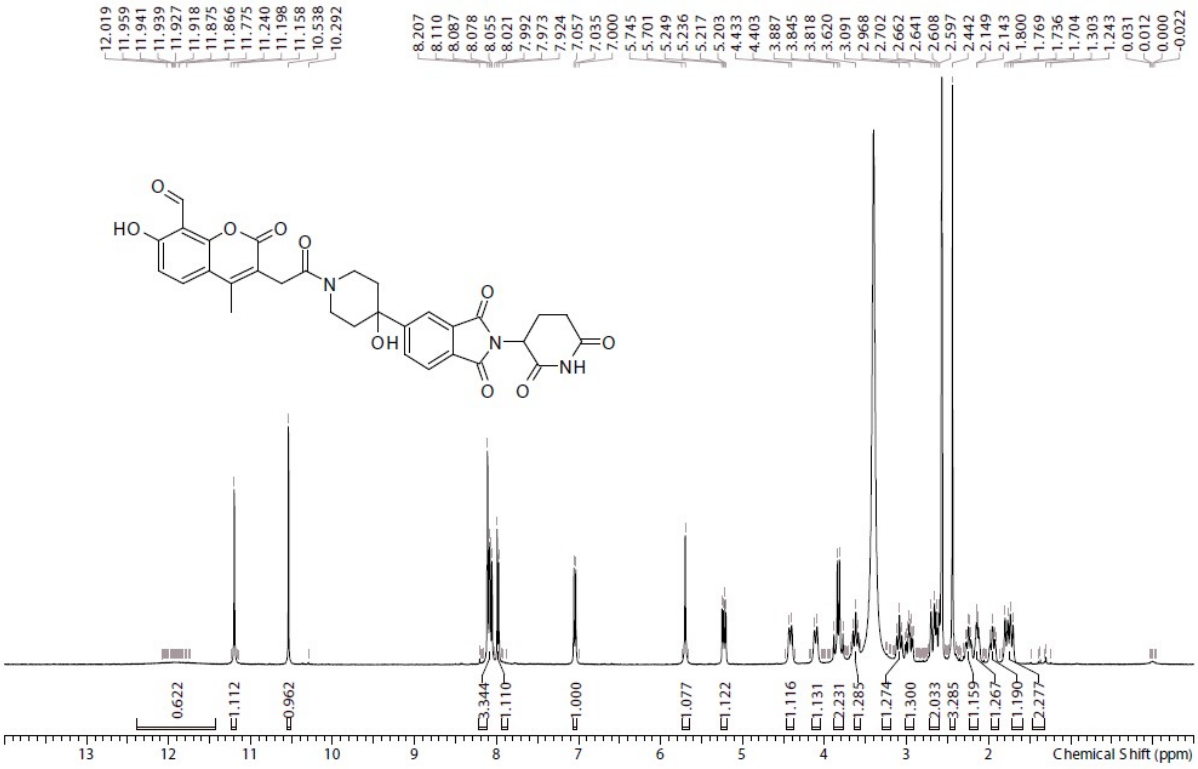
^{1}H NMR trace for compound 22.



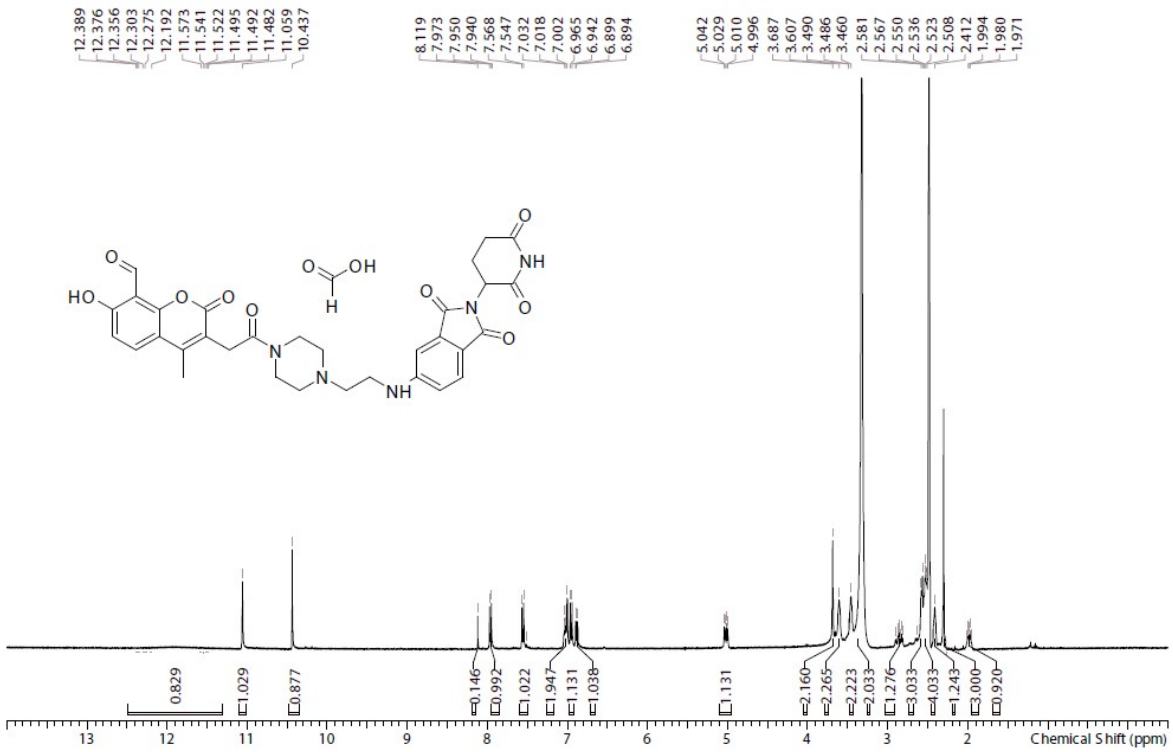
^{1}H NMR trace for compound 23 (CPD-2522).



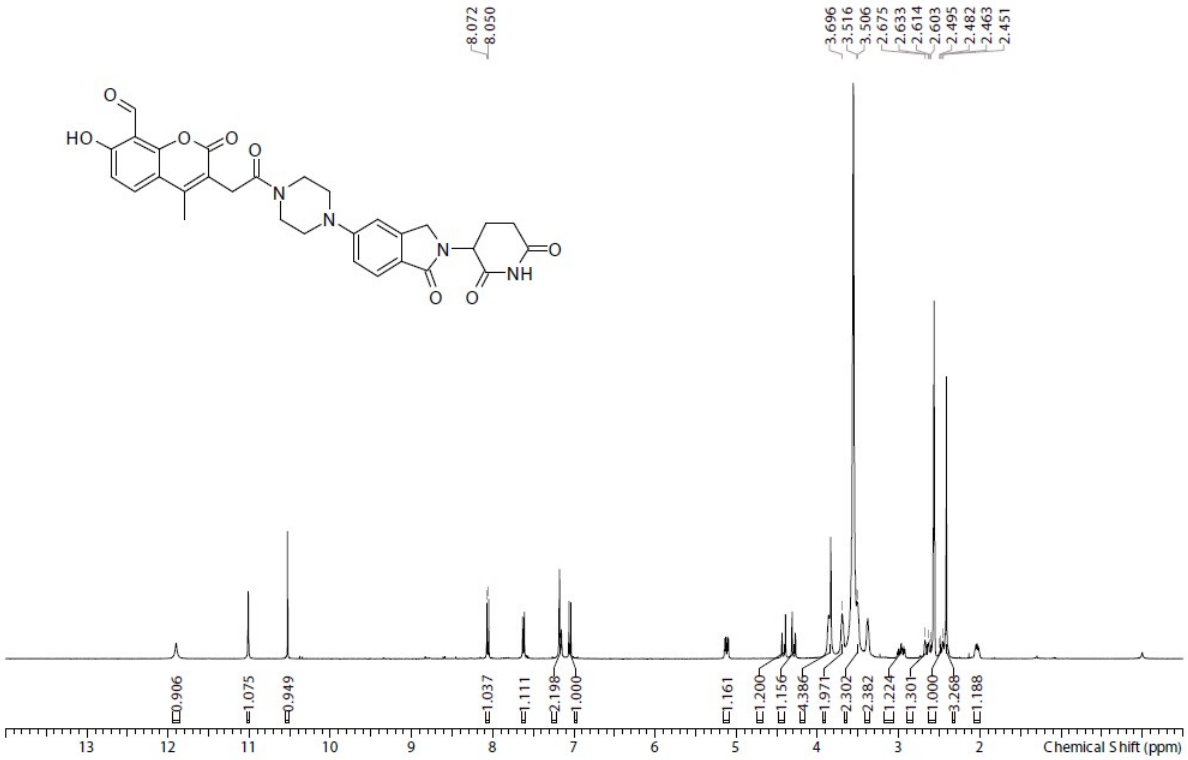
^{1}H NMR trace for compound 24.



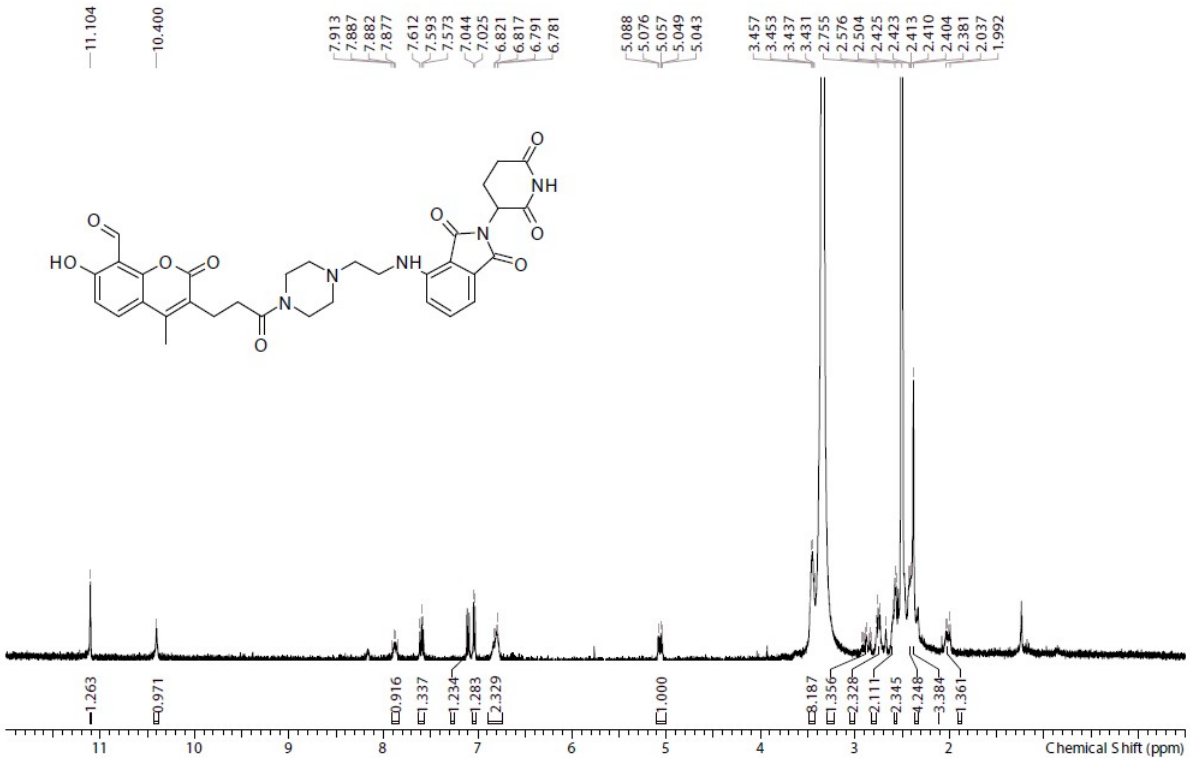
^{1}H NMR trace for compound 25.



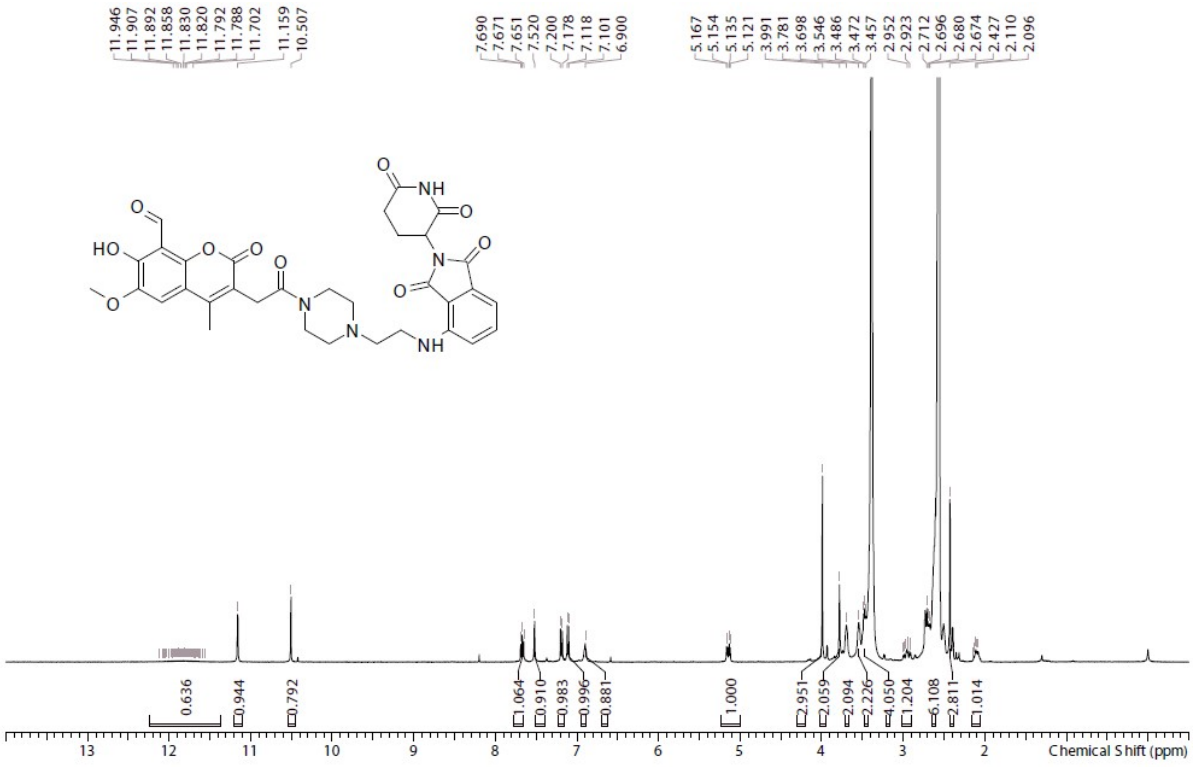
^{1}H NMR trace for compound 26.



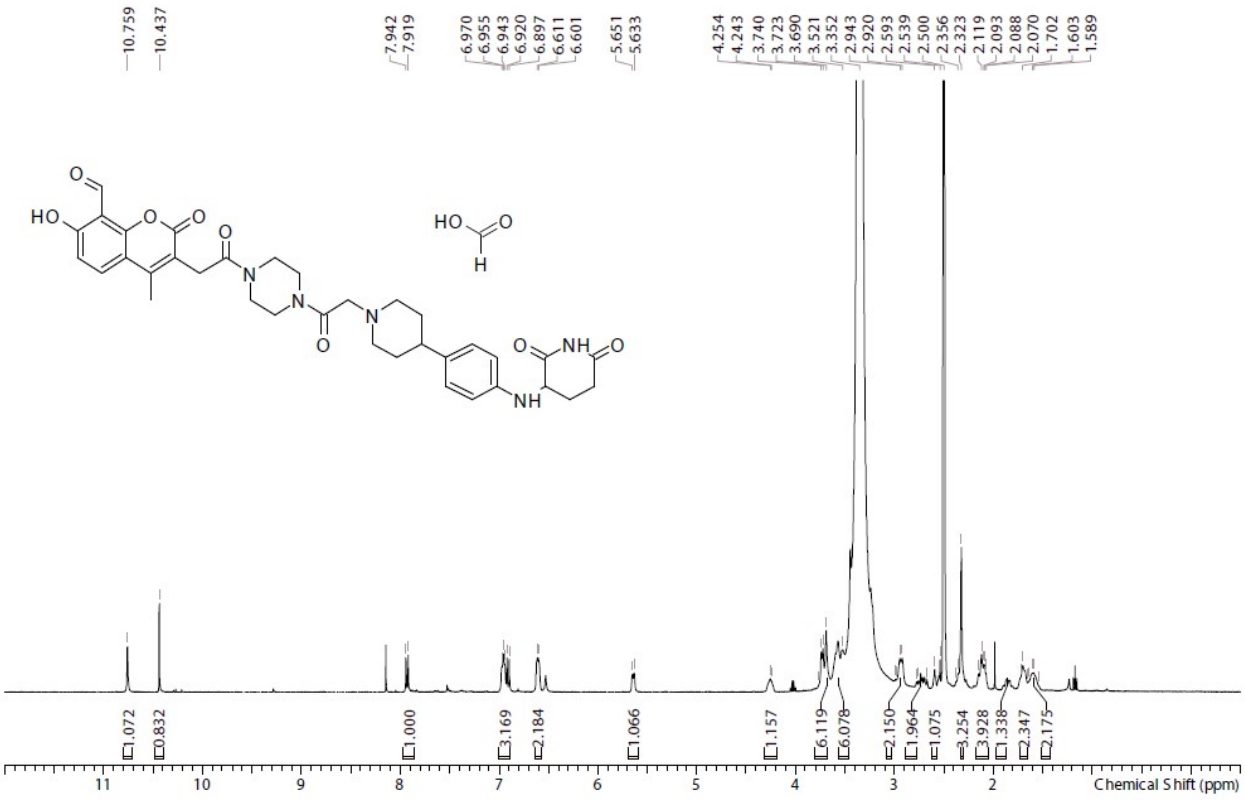
^{1}H NMR trace for compound 27.



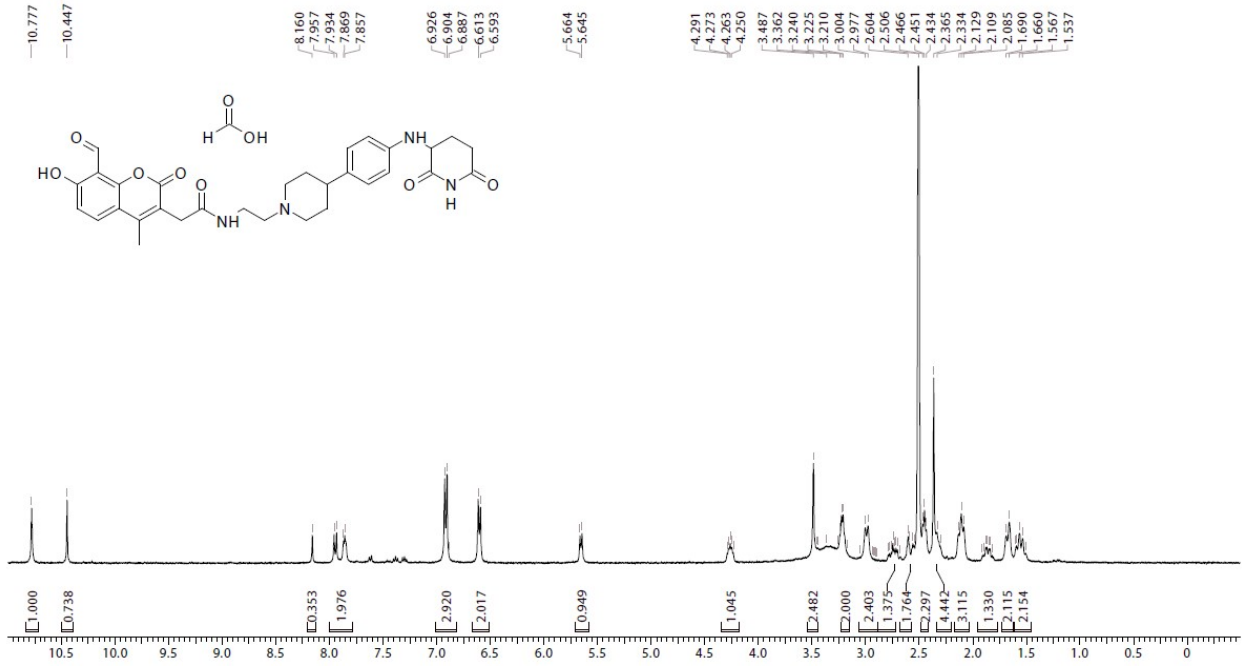
^{1}H NMR trace for compound 28.



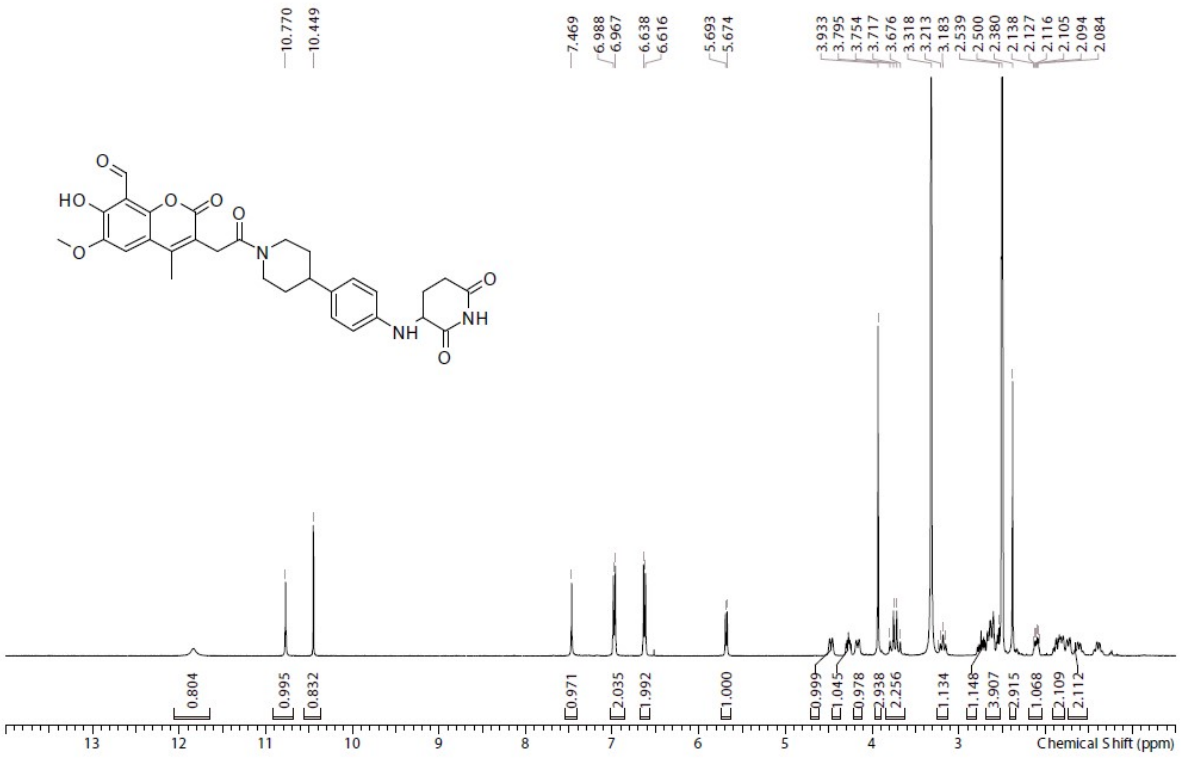
^{1}H NMR trace for compound 29.



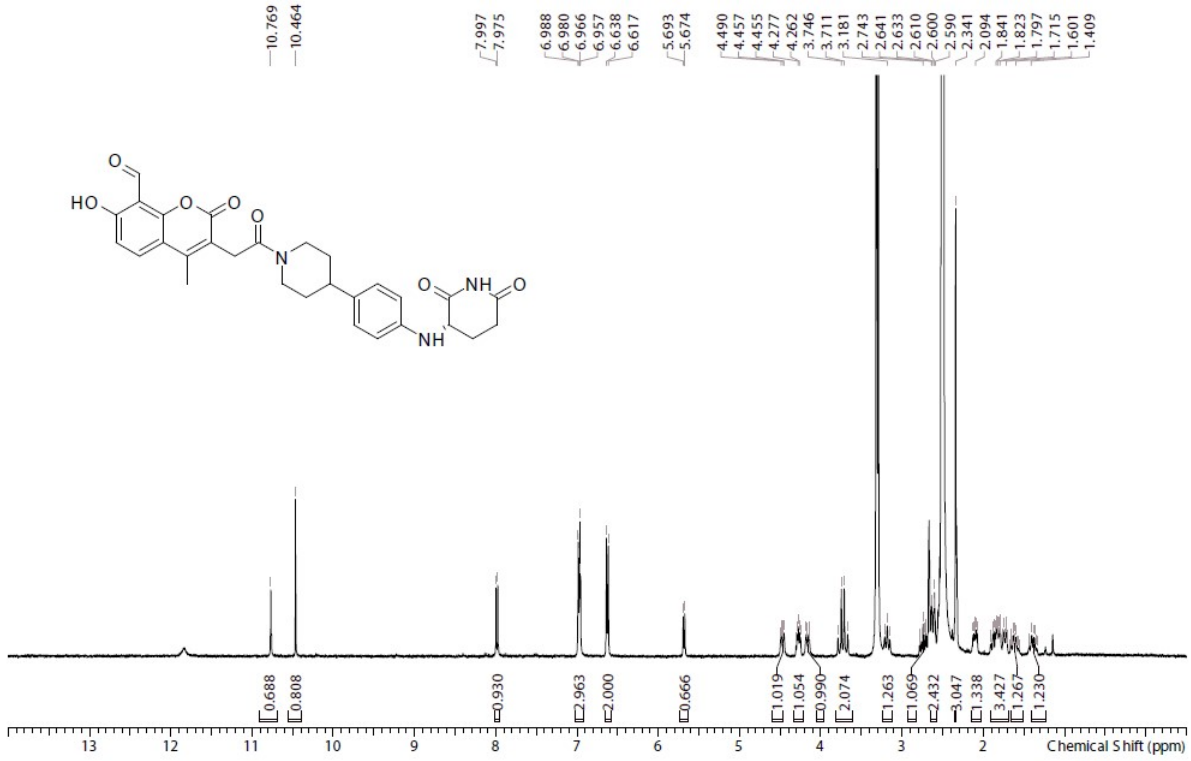
^{1}H NMR trace for compound 31.



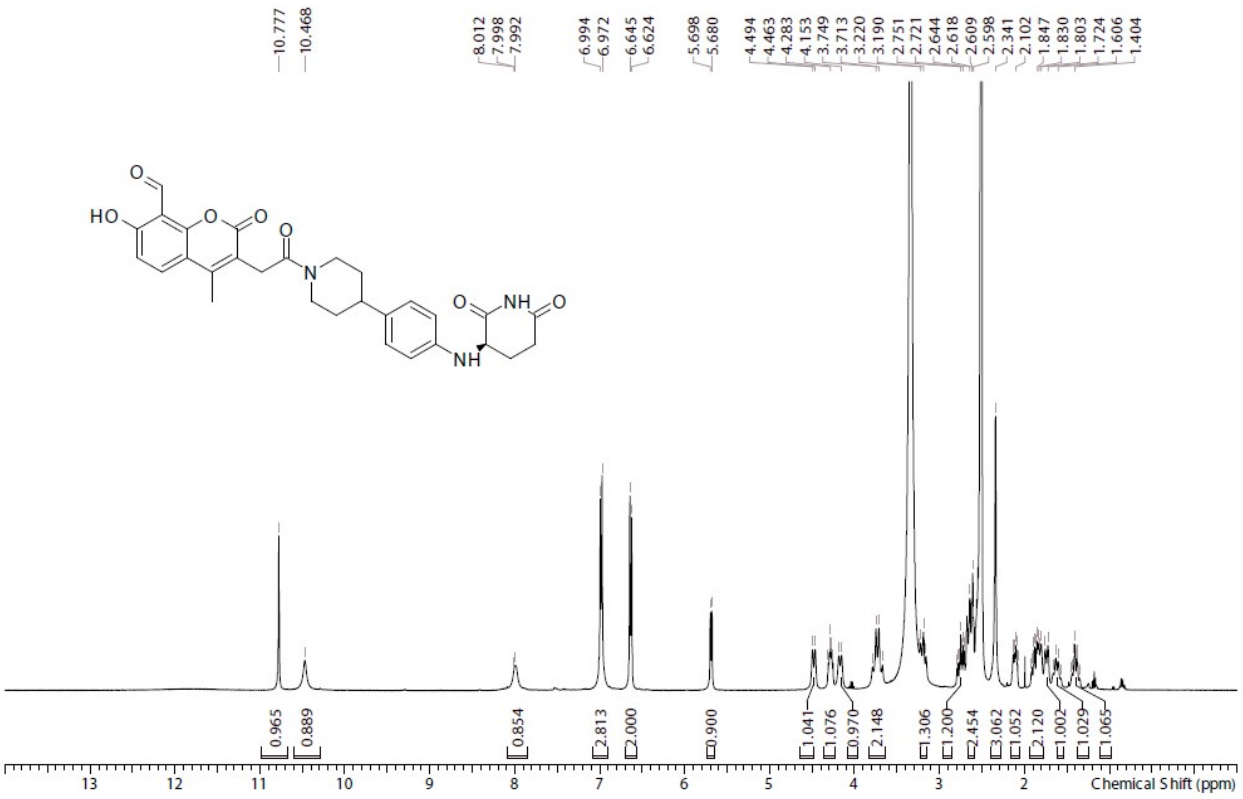
^{1}H NMR trace for compound 32.



^{1}H NMR trace for compound 33.



^{1}H NMR trace for compound 35 (CPD-3123).



[1]H NMR trace for compound 36 (CPD-3124).

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