Ru-PHOTACs Provide Photocontrol Over Protein Degradation with Optimized Properties for Biological Applications

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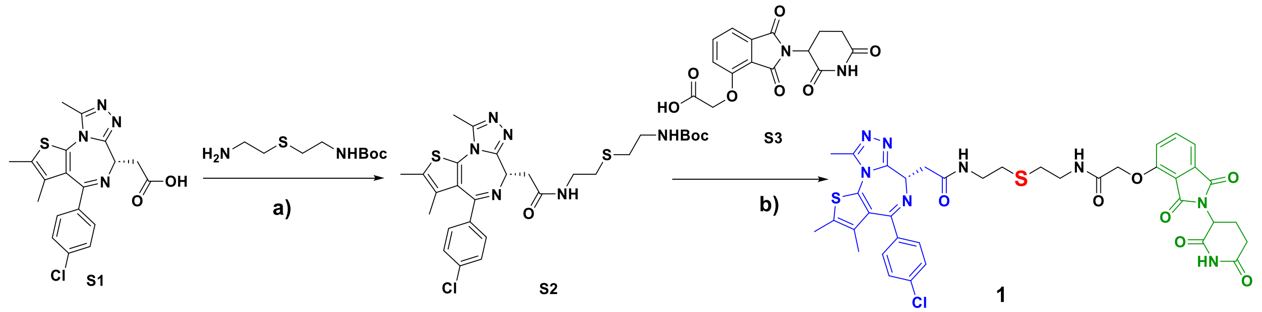
Supplementary Information

1. Synthesis.

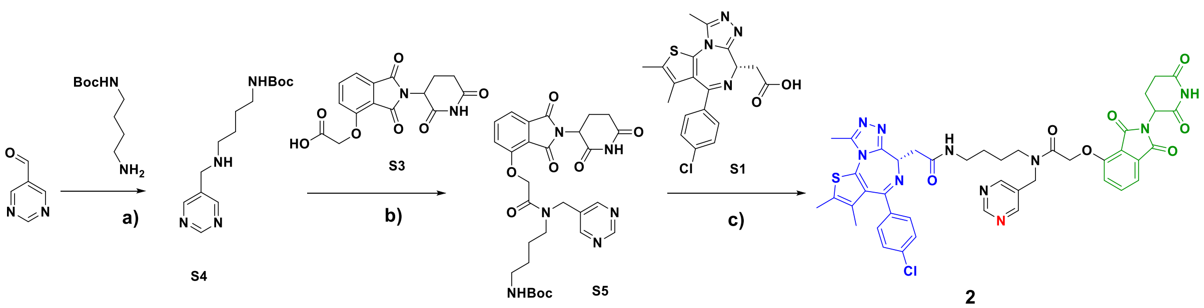


Scheme S1. Synthesis of PROTAC **1**: a) HBTU, DMAP, NEt_3, DMF, r.t.; b)(i) TFA, DCM, r.t.; (ii) 3, HBTU, DMAP, NEt_3, DMF, r.t.

The (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (S**1**) was purchased from Ambeed, 2-[[2-(2,6-dioxo-3-piperidinyl)-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]oxy]-acetic acid (S**3**) was synthesized according to the described methods.[1]

Synthesis of compound S2. (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (200 mg, 0.5 mmol), *N*-[2-[(2-aminoethyl)thio]ethyl]-1,1-dimethylethyl ester of carbamic acid (165 mg, 0.75 mmol), HBTU (284.4 mg, 0.75 mmol) and DMAP (6.1 mg, 0.05 mmol) were added to a flask followed by the addition of DMF (10 mL) and NEt_3 (152 mg, 1.5 mmol). The reaction mixture and stirred at room temperature overnight. After pouring the reaction mixture into water the precipitate was isolated under vacuum filtration to give 150 mg (50%) of compound S2. ^1H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.09 (t, *J* = 5.2 Hz, 1H), 5.24 (s, 1H), 4.64 (dd, *J* = 7.6, 6.0 Hz, 1H), 3.30–3.64 (m, 6H), 2.65–2.73 (m, 7H), 2.42 (s, 3H), 1.69 (s, 3H), 1.46 (s, 9 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.65, 163.96, 155.62, 149.89, 136.84, 136.53, 132.01, 130.93, 130.89, 130.47, 129.85, 128.73, 54.47, 39.31, 38.94, 32.11, 31.53, 28.42. ESI MS: m/z calculated for $C_{28}H_{35}ClN_6O_3S_2$ $[M + H]^+$ 603.19, found 603.2.

*Synthesis of PROTAC **1***. Compound **S2** (120 mg, 0.2 mmol) was added to a solution of TFA (2 mL) with DCM (5 mL). The reaction was stirred at room temperature for 2 h, followed by removal of the solvent under reduced pressure to give a solid (ESI MS: m/z calculated for $C_{23}H_{27}ClN_6OS_2$ $[M + H]^+$ 503.14, found 503.3). 2-[[2-(2,6-dioxo-3-piperidinyl)-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]oxy]-acetic acid (compound **S3**, 80 mg, 0.24 mmol), HBTU (114 mg, 0.3 mmol) and DMAP (2 mg, 0.02 mmol) were added to the residue. DMF (5 mL) and NEt_3 (61 mg, 0.6 mmol) were added to the reaction mixture and stirred at room temperature overnight. After pouring the reaction mixture into water the precipitate that formed was isolated under vacuum filtration, then purified by flash chromatography using a Biotage Isolera One system with a 40 g Sorbtech cartridge. Utilizing CH_2Cl_2 and CH_3OH as eluents gave 95 mg (59%) of **1**. ^1H NMR (400 MHz, $CDCl_3$): δ 9.56–9.75 (m, 1H), 7.86 (dt, *J* = 29.6, 6 Hz, 1H), 7.72 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.55 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.31–7.44 (m, 6H), 7.19 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.63–5.02 (m, 4H), 3.33–3.67 (m, 6H), 2.67–2.84 (m, 9H), 2.42 (s, 3H), 2.13–2.18 (m, 1H), 1.68 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.35, 171.21, 170.81, 170.76, 168.55, 168.42, 167.17, 167.16, 166.61, 166.29, 164.16, 164.05, 155.58, 154.58, 154.55, 149.94, 136.98, 136.78, 136.53, 133.57, 132.13, 130.94, 130.90, 130.47, 129.94, 129.88, 128.67, 119.92, 119.80, 118.29, 118.23, 117.49, 68.36, 68.24, 54.49, 53.44, 49.39, 39.28, 39.11, 39.05, 38.41, 38.29, 31.96, 31.86, 31.68, 31.56, 31.43, 31.57, 22.78, 22.71, 14.39, 13.11, 11.81. HRMS: m/z calculated for $C_{38}H_{37}ClN_8O_7S_2$ $[M + H]^+$ 817.1993, found 817.1973.

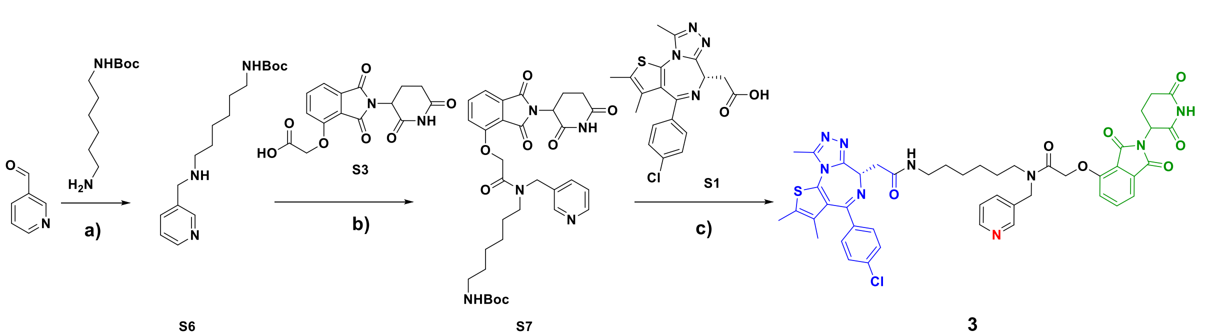


Scheme S2. Synthesis of PROTAC **2**: a) $Na(CH_3COO)_3BH$, AcOH, DCM; b) HBTU, DMAP, NEt_3, DMF, r.t.; c) (i) TFA, DCM, r.t.; (ii) S1, HBTU, DMAP, NEt_3, DMF, r.t.

Synthesis of compound S4. A mixture of 5-pyrimidinecarboxaldehyde (10 mmol), *N*-(tert-butoxycarbonyl)-1,4-butanediamine (10 mmol), sodium triacetoxyborohydride (10 mmol) and acetic acid (50 mmol) in dichloromethane (20 mL) was stirred at room temperature overnight. Dichloromethane (50 mL) was added, and the mixture was washed with saturated sodium bicarbonate (50 mL × 2). The organic solvent was removed under reduced pressure to give an oil. The product was purified by flash chromatography using a Biotage Isolera One system with 40 g Sorbtech cartridge with CH_2Cl_2 and CH_3OH as the eluents. ^1H NMR (400 MHz, $CDCl_3$): δ 9.13 (s, 1H), 8.73 (s, 2H), 4.76 (br s, 1H), 3.82 (s, 2H), 3.14 (br s 2H), 2.65–2.69 (m, 2H), 1.53–1.57 (m, 4H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.70, 156.71, 156.00, 133.40, 49.08, 48.95, 28.42, 27.84, 27.25. ESI MS: m/z calculated for $C_{14}H_{24}N_4O_2$ $[M + H]^+$ 281.2, found 281.2.

Synthesis of compound S5. 2-[[2-(2,6-dioxo-3-piperidinyl)-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]oxy]-acetic acid (compound **S3**, 400 mg, 1.2 mmol), HBTU (682 mg, 1.8 mmol) and DMAP (2 mg) were added to compound **S4** (500 mg, 1.8 mmol) in DMF (5 mL). Then NEt_3 (600 mg) was added to the reaction mixture and stirred at room temperature overnight. After pouring the reaction mixture into water a precipitate formed, isolated under vacuum filtration, and purified by flash chromatography using a Biotage Isolera One system with a 40 g Sorbtech cartridge. Utilizing CH_2Cl_2 and CH_3OH as the eluents gave 250 mg (35%) of compound **S5**. ^1H NMR (400 MHz, $CDCl_3$): δ 9.13 (s, 1H), 8.66 (s, 2H), 7.67 (t, J = 6.4 Hz, 1H), 7.53 (d, J = 5.6 Hz, 1H), 7.32 (d, J = 6.4 Hz, 1H), 5.14 (d, J = 11.6 Hz, 1H), 4.94–5.06 (m, 2H), 4.51–4.75 (m, 3H), 3.30–3.44 (m, 2H), 3.06–3.14 (m, 2H), 2.72–2.90 (m, 3H), 2.12–2.17 (m, 1H), 1.41–1.61 (m, 14H). ESI MS: m/z calculated for $C_{29}H_{34}N_6O_8$ $[M + H]^+$ 595.2, found 595.2.

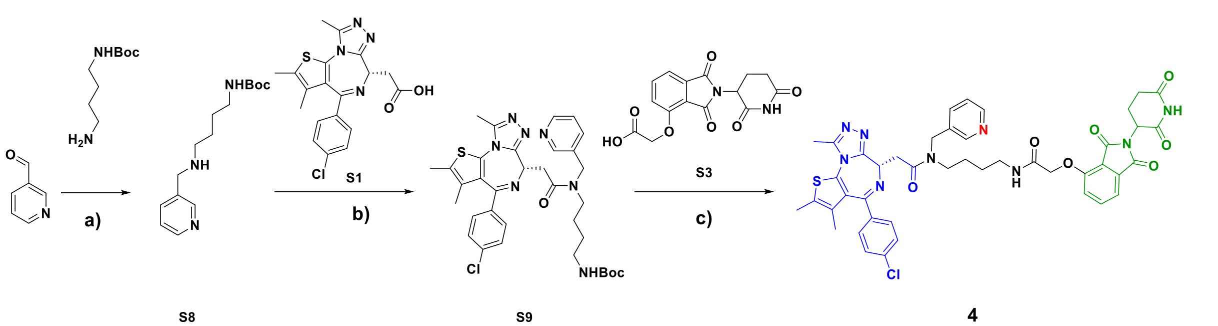
*Synthesis of PROTAC **2***. Compound **S5** (210 mg, 0.35 mmol) was added into a solution of TFA (2 mL) with DCM (5 mL). The reaction was stirred at room temperature for 2 h, followed by removal of the solvent under reduced pressure to give a solid residue. The mixture of compound **S1** (170 mg, 0.42 mmol), HBTU (200 mg, 0.53 mmol) and DMAP (2 mg) were added to the solid residue. DMF (5 mL) and NEt_3 (355 mg, 3.5 mmol) were added to the reaction mixture and stirred at room temperature overnight. After pouring the reaction mixture into water a precipitate was formed, then isolated under vacuum filtration and purified by flash chromatography using a Biotage Isolera One system with 12 g Sorbtech cartridge. Utilizing CH_2Cl_2 and CH_3OH as eluents gave 160 mg (52%) of **2**. ^1H NMR (400 MHz, $CDCl_3$): δ 9.09 (s, 1H), 8.65 (s, 2H), 7.64–7.68 (m, 1H), 7.50 (t, J = 6.0 Hz, 1H), 7.37–7.40 (m, 3H), 7.30 (d, J = 6.8 Hz, 2H), 7.10–7.18 (m, 1H), 4.42–5.15 (m, 5H), 3.15–3.63 (m, 6H), 2.66–2.83 (m, 2H), 2.63 (s, 3H), 2.38 (s, 3H), 2.09–2.15 (m, 1H), 1.40–1.82 (m, 9H). HRMS: m/z calculated for $C_{43}H_{41}ClN_{10}O_7S$ $[M + H]^+$ 877.2647, found 877.2627.



Scheme S3. Synthesis of PROTAC **3**: a) $Na(CH_3COO)_3BH$, AcOH, DCM; b) HBTU, DMAP, NEt_3, DMF, r.t.; c) (i) TFA, DCM, r.t.; (ii) S1, HBTU, DMAP, NEt_3, DMF, r.t.

Synthesis of PROTAC **3** has been conducted by the same synthetic route as for **2** utilizing 3-pyridinecarboxaldehyde and *N*-(tert-butoxycarbonyl)-1,6-hexanediamine at the first step.
Compound S7. Yield: 280 mg (55%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.48–8.55 (m, 2H), 7.32–7.73 (m, 4H), 7.24–7.27 (m, 1H), 4.55–5.13 (m, 6H), 2.76–3.43 (m, 7H), 2.13–2.16 (m, 1H), 1.28–1.73 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.28, 166.82, 165.53, 149.30, 149.05, 136.65, 135.90, 134.24, 133.93, 132.59, 123.66, 117.40, 117.17, 53.44, 49.29, 46.33, 31.53, 30.14, 29.88, 28.70, 28.43, 26.98, 26.52, 26.35, 22.57. ESI MS: m/z calculated for $C_{32}H_{39}N_5O_8$ [M] 621.3, found $[M + H]^+$ 622.4.

*Compound **3**.* Yield: 320 mg (78%). ^1H NMR (400 MHz, $CDCl_3$): 9.96–10.0 (m, 1H), 8.51–8.53 (m, 2H), 7.49–7.71 (m, 3H), 7.23–7.41 (m, 6H), 7.00–7.09 (m, 1H), 4.55–5.12 (m, 6H), 3.16–3.61 (m, 6H), 2.65–2.84 (m, 6H), 2.40 (s, 3H), 2.06–2.24 (m, 1H), 1.66 (s, 3H), 1.26–1.58 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.44, 171.36, 170.59, 168.95, 168.79, 167.42, 166.82, 165.55, 164.27, 164.02, 155.51, 149.28, 148.96, 136.77, 135.94, 133.93, 132.20, 132.12, 130.92, 130.87, 130.44, 129.87, 128.71, 123.66, 120.62, 117.39, 117.14, 69.16, 54.64, 49.29, 47.51, 46.32, 39.61, 39.41, 39.24, 31.53, 29.17, 28.60, 26.75, 26.66, 26.57, 26.48, 22.49, 14.38, 13.09, 11.80. HRMS: m/z calculated for $C_{46}H_{46}ClN_9O_7S$ $[M + H]^+$ 904.3008, found 904.2977.

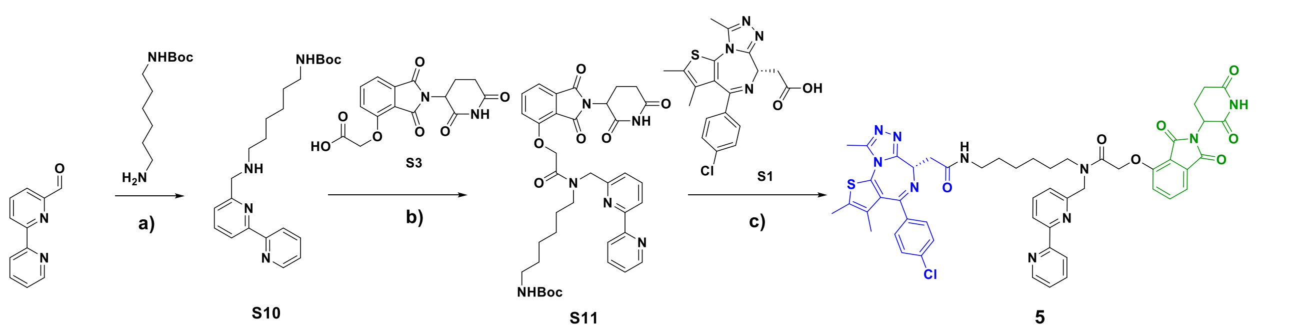


Scheme S4. Synthesis of compound **4**: a) $Na(CH_3COO)_3BH$, AcOH, DCM; b) HBTU, DMAP, NEt_3, DMF, r.t.; c) (i) TFA, DCM, r.t.; (ii) **S3**, HBTU, DMAP, NEt_3, DMF, r.t.

*Synthesis of compound **S8***. A mixture of 3-pyridinecarboxaldehyde (46 mmol), *N*-(tert-butoxycarbonyl)-1,4-butanediamine (46 mmol), sodium triacetoxyborohydride (46 mmol) and acetic acid (230 mmol) in dichloromethane (25 mL) was stirred at 40 °C in a pressure tube overnight. Dichloromethane (50 mL) was added, and the mixture was washed with saturated sodium bicarbonate (50 mL × 2). The organic solvent was removed under reduced pressure to give an oil. The product was purified by flash chromatography using a Biotage Isolera One system with 40 g Sorbtech cartridge with CH_2Cl_2 and CH_3OH as the eluents.

*Synthesis of compound **S9***. Compound **S1** (200 mg, 0.5 mmol), HBTU (284 mg, 0.75 mmol) and DMAP (2 mg) were added to compound **S8** (210 mg, 0.75 mmol) in DMF (10 mL). Then the reaction mixture was stirred at room temperature overnight. After pouring the reaction mixture into water a precipitate formed, isolated under vacuum filtration, and purified by flash chromatography using a Biotage Isolera One system with a 40 g Sorbtech cartridge and CH_2Cl_2 and CH_3OH as the eluents.

*Synthesis of PROTAC **4***. Compound **S9** (120 mg) was added into a solution of TFA (1 mL) with DCM (4 mL). The reaction was stirred at room temperature for 2 h, followed by removal of the solvent under reduced pressure to give a solid residue. The mixture of compound **S3** (72 mg, 0.22 mmol), HBTU (102 mg, 0.27 mmol) and DMAP (2 mg) were added to the solid residue. DMF (5 mL) and NEt_3 (182 mg, 1.8 mmol) were added to the reaction mixture and stirred at room temperature overnight. After pouring the reaction mixture into water a precipitate was formed, then isolated under vacuum filtration and purified by flash chromatography using a Biotage Isolera One system with 12 g Sorbtech cartridge and CH_2Cl_2 and CH_3OH as eluents. Yield 48 mg (30%) of **4**. ^{1}H NMR (400 MHz, $CDCl_3$): 9.52 (br s, 1H), 8.47–8.57 (m, 2H), 7.09–7.73 (m, 10H), 4.55–4.93 (m, 5H), 3.22–3.79 (m, 6H), 2.63–2.80 (m, 5H), 2.38 (s, 3H), 1.58–2.15 (m, 10H). HRMS: m/z calculated for $C_{44}H_{42}ClN_9O_7S$ $[M + H]^+$ 876.2695, found 876.2663.

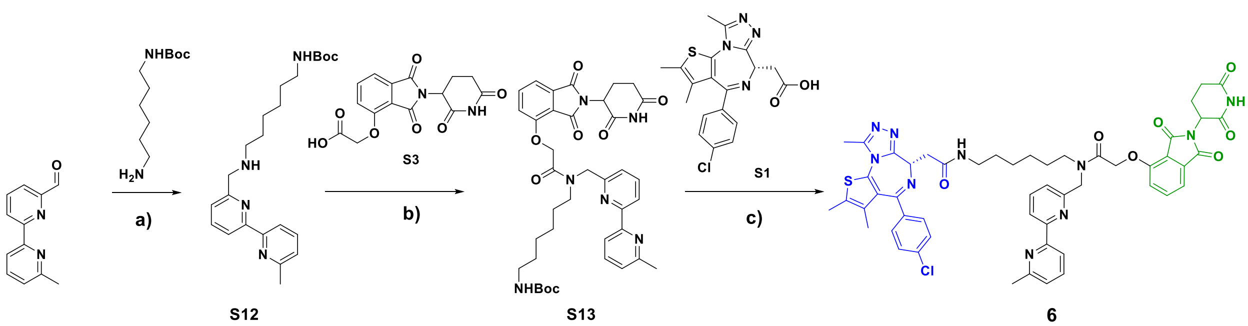


Scheme S5. Synthesis of PROTAC **5**: a) $Na(CH_3COO)_3BH$, AcOH, DCM; b) HBTU, DMAP, NEt_3, DMF, r.t.; c) (i) TFA, DCM, r.t.; (ii) S1, HBTU, DMAP, NEt_3, DMF, r.t.

Synthesis of PROTAC **5** has been conducted by the same synthetic route as for **2** utilizing [2,2'-bipyridine]-6-carbaldehyde and *N*-(tert-butoxycarbonyl)-1,6-hexanediamine at the first step.

Compound S11. Yield: 450 mg (50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H), 8.63–8.68 (m, 2H), 8.19–8.28 (m, 2H), 7.69–7.80 (m, 2H), 7.15–7.53 (m, 5H), 4.58–5.14 (m, 6H), 2.67–3.57 (m, 7H), 2.03–2.09 (m, 1H), 1.24–1.64 (m, 18H).

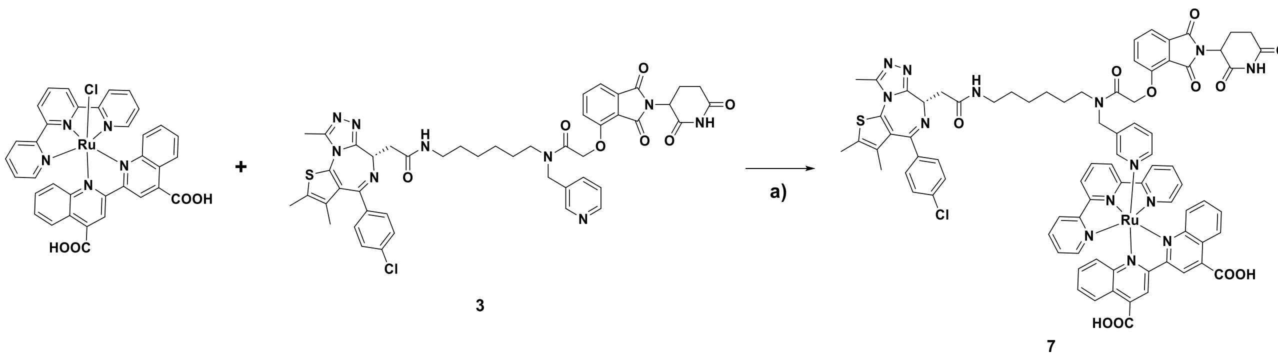
Compound ***5.*** Yield: 41 mg (53%). ^{1}H NMR (400 MHz, $CDCl_3$): 9.86 (s, 1H), 8.65–8.75 (m, 2H), 8.23–8.31 (m, 2H), 7.72–7.82 (m, 2H), 7.20–7.58 (m, 8H), 6.68–7.06 (m, 1H), 4.58–5.20 (m, 6H), 3.10–3.61 (m, 6H), 2.64–2.86 (m, 6H), 2.37 (s, 3H), 2.03–2.18 (m, 1H), 1.17–1.65 (m, 12H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.23, 171.06, 170.59, 170.55, 170.41, 169.07, 168.82, 167.88, 167.30, 166.86, 165.54, 165.40, 164.35, 156.30, 156.10, 155.82, 155.65, 155.63, 155.50, 155.36, 155.28, 149.98, 149.91, 149.14, 148.97, 137.92, 137.64, 137.21, 137.14, 136.80, 136.64, 136.37, 133.60, 132.11, 130.95, 130.93, 130.89, 130.51, 129.86, 128.72, 124.04, 123.87, 122.53, 121.60, 121.26, 121.12, 120.26, 120.09, 119.68, 117.28, 116.91, 116.51, 77.24, 54.69, 54.55, 53.44, 51.17, 49.32, 49.14, 49.06, 46.56, 39.77, 39.70, 39.42, 31.44, 29.21, 28.97, 28.88, 27.09, 26.94, 26.73, 26.60, 26.50, 26.27, 22.55, 14.38, 13.10, 11.82. HRMS: m/z calculated for $C_{51}H_{49}ClN_{10}O_7S$ $[M+H]^+$ 981.3273, found 981.3237 $[M + H]^+$.



Scheme S6 Synthesis of PROTAC **6**: a) $Na(CH_3COO)_3BH$, AcOH, DCM; b) HBTU, DMAP, NEt_3, DMF, r.t.; c) (i) TFA, DCM, r.t.; (ii) S1, HBTU, DMAP, NEt_3, DMF, r.t.

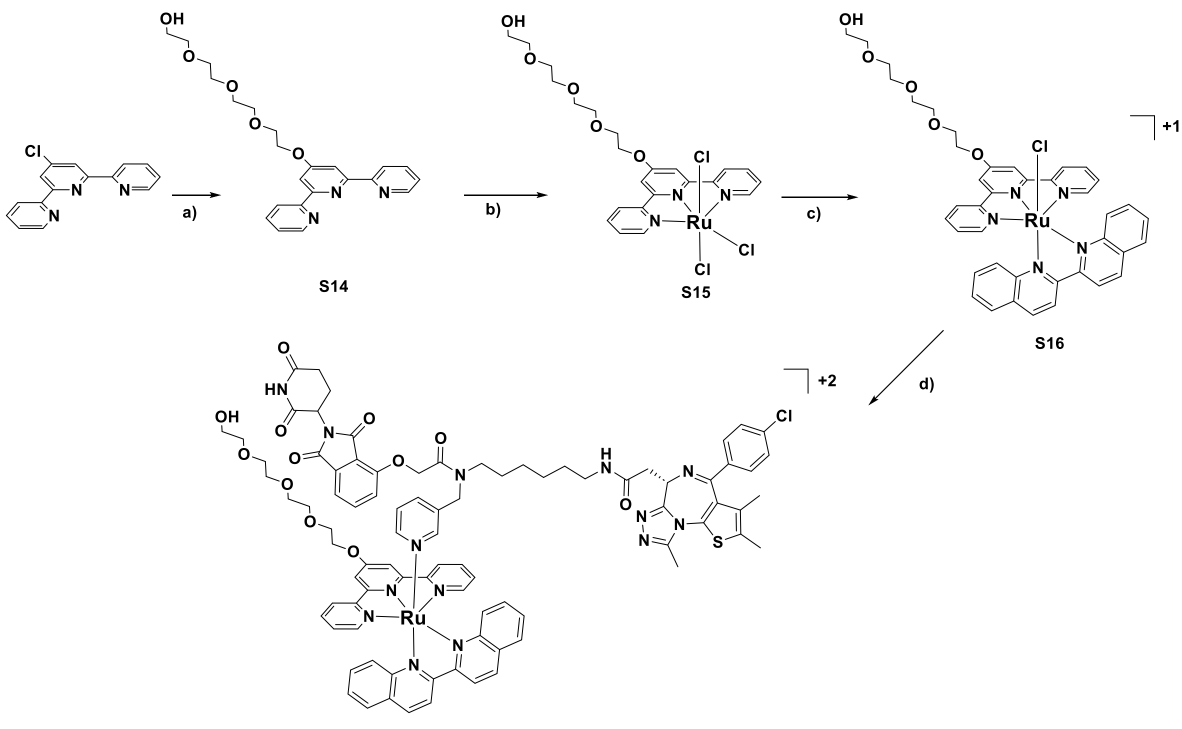
Synthesis of PROTAC **6** has been conducted by the same synthetic route as for **2** utilizing 6'-methyl-[2,2'-bipyridine]-6-carbaldehyde and *N*-(tert-butoxycarbonyl)-1,6-hexanediamine at the first step.
Compound S813. Yield: 70 mg (66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27–8.36 (m, 1H), 8.00–8.18 (m, 2H), 7.54–7.80 (m, 2H), 7.28–7.44 (m, 2H), 7.14–7.23 (m, 2H), 4.53–5.17 (m, 6H), 2.70–3.63 (m, 7H), 2.62 (s, 3H), 2.07–2.14 (m, 1H), 1.25–1.64 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.07, 167.79, 166.97, 165.45, 158.22, 158.06, 156.75, 155.94, 155.32, 155.07, 137.89, 137.65, 137.31, 137.13, 136.40, 133.70, 123.68, 123.44, 122.36, 121.40, 120.29, 119.82, 118.31, 118.08, 117.04, 116.53, 77.36, 53.57, 52.20, 49.39, 49.18, 31.54, 30.05, 28.57, 27.41, 26.61, 24.78, 22.69.

Compound ***6.*** Yield: 41 mg (53%). ^{1}H NMR (400 MHz, $CDCl_3$): 9.84 (s, 1H), 8.87 (s, 1H), 8.26–8.34 (m, 1H), 8.01–8.06 (m, 1H), 7.53–7.81 (m, 2H), 7.28–7.43 (m, 6H), 7.13–7.24 (m, 2H), 6.60–7.06 (m, 1H), 4.57–5.21 (m, 6H), 3.08–3.74 (m, 6H), 2.67–2.84 (m, 2H), 2.65 (s, 3H), 2.61 (s, 3H), 2.38 (s, 3H), 2.03–2.18 (m, 1H), 1.65 (s, 3H), 1.13–1.56 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.60, 171.51, 171.41, 171.33, 171.30, 170.68, 170.63, 170.50, 169.12, 168.90, 168.47, 168.44, 167.93, 167.37, 167.34, 166.99, 166.94, 165.65, 165.50, 164.40, 164.12, 164.07, 164.01, 158.12, 157.98, 156.66, 156.21, 155.97, 155.79, 155.74, 155.72, 155.64, 155.61, 155.50, 155.30, 155.06, 150.07, 149.99, 137.88, 137.61, 137.30, 137.11, 136.92, 136.86, 136.74, 136.72, 136.68, 136.43, 133.92, 133.66, 132.33, 132.24, 131.01, 130.95, 130.93, 130.58, 130.55, 129.98, 129.95, 128.80, 123.64, 123.59, 123.42, 122.33, 122.30, 121.47, 120.52, 120.42, 120.33, 120.27, 120.20, 119.77, 118.31, 118.08, 117.39, 117.36, 117.32, 116.98, 116.50, 77.36, 69.36, 68.45, 68.41, 58.45, 54.76, 54.71, 54.64, 54.11, 53.55, 52.06, 51.18, 50.95, 49.39, 49.35, 49.21, 48.94, 48.76, 46.68, 46.49, 39.82, 39.76, 39.52, 39.46, 39.33, 31.63, 31.53, 29.79, 29.41, 29.32, 29.29, 29.26, 28.96, 28.89, 28.65, 27.65, 27.22, 27.00, 26.87, 26.80, 26.68, 26.60, 26.57, 26.39, 24.75, 24.72, 22.64, 22.58, 22.10, 21.56, 18.52, 14.48, 13.18, 11.92. HRMS: m/z calculated for $C_{52}H_{51}ClN_{10}O_7S$ $[M+H]^+$ 995.3430, found 995.3392 $[M + H]^+$.



Scheme S5. Synthesis of Ru(II) complex **7**: a) MeOH : H_2O (4:1), 70 °C.

Synthesis of Ru(II) complex 7. [Ru(tpy)(bca)Cl]PF_6 (86 mg, 0.1 mmol) and 2-fold excess of PROTAC **3** (181 mg, 0.2 mmol) were added to 12 mL of degassed MeOH : H_2O (4:1) in a pressure tube. The mixture was stirred at 70 °C for 24 hours. The reaction mixture then cooled to room temperature and solvents removed under reduced pressure. The purification of the solid was carried out by flash chromatography (SiO_2, 2% water in MeOH, ramped to 30% H_2O) to give the pure complex. The product fractions were concentrated under reduced pressure producing a purple solid. Yield: 49 mg (31%). HRMS calcd for $C_{81}H_{69}ClN_{14}O_{11}RuS$ $[M]^{2+}$ 791.1862; found 791.1884 $[M]^{2+}$.



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Scheme S6. Synthesis of Ru(II) complex **8**: a) tetraethylene glycol, KOH, DMSO, 60 °C, b) $RuCl_3 \cdot 3H_2O$, EtOH, 80 °C, c) 2,2'-biquinoline, ethylene glycol, 180 °C, d) MeOH, 70 °C.

*Synthesis of Ru(II) complex **8**.*

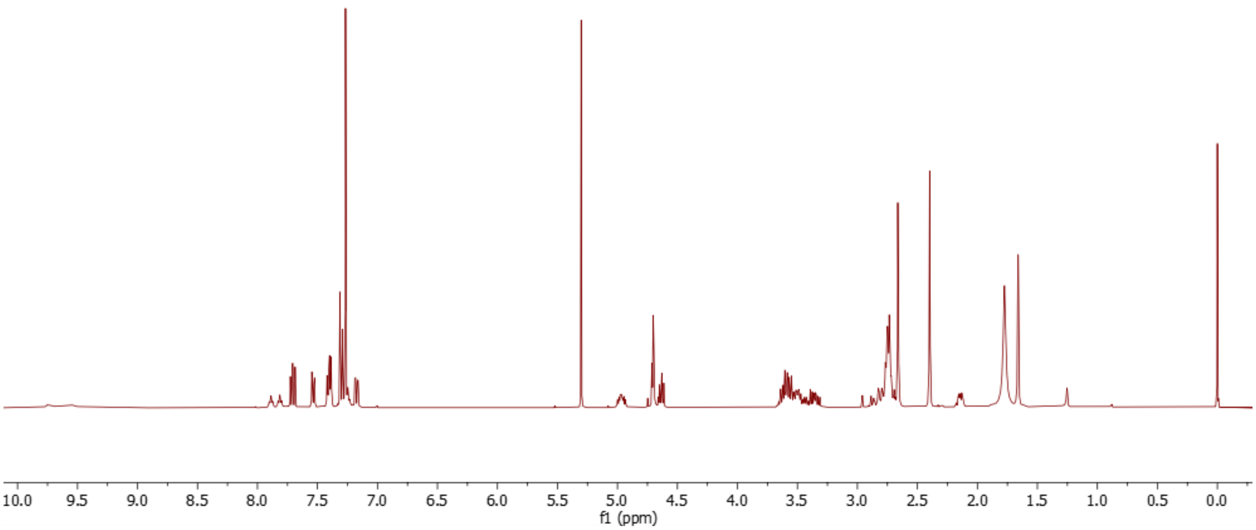
4'-4PEG-2,2' : 6',2''-terpyridine (**S14**) and [Ru(4'-4PEG-2,2' : 6',2''-terpyridine)]Cl_3 (**S15**) were synthesized by known procedures.[2-3]

[Ru(4'-4PEG-2,2' : 6',2''-terpyridine)]Cl_3 (140 mg, 0.22 mmol) and 2,2'-biquinoline (biq, 56 mg, 0.22 mmol) were added to 5 mL of degassed ethylene glycol in a pressure tube. The mixture was stirred at 180 °C for 1 hour. The reaction mixture then cooled to room temperature and transferred into 100 mL of H_2O. Saturated solution of KPF_6 was added and product was extracted into DCM. The solvent was removed under reduced pressure. The purification of the solid was carried out by flash chromatography (SiO_2, 2% MeOH in DCM, ramped to 10% MeOH) to give the product. The product fractions were concentrated under reduced pressure producing a purple solid.

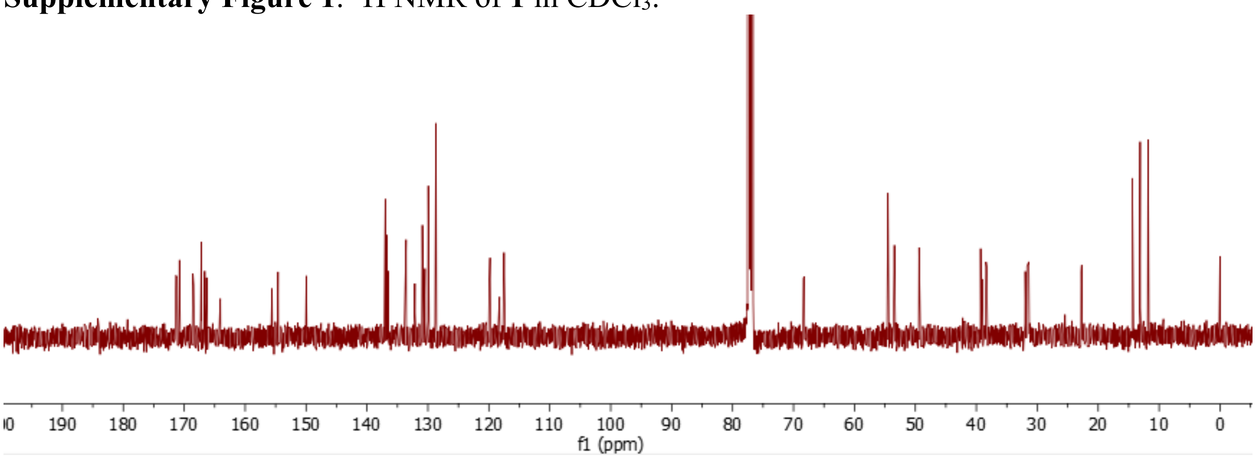
*Compound **S16**.* ^{1}H NMR (500 MHz, acetone-d6): δ 9.16 (d, *J* = 8.9 Hz, 1H), 9.01–9.10 (m, 2H), 8.92 (d, *J* = 8.9 Hz, 1H), 8.73 (dt, *J* = 8.1, 1.1 Hz, 2H), 8.59 (d, *J* = 5.8 Hz, 1H), 8.47 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.94–8.18 (m, 8H), 7.58 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.42–7.54 (m, 3H), 7.09 (d, *J* = 8.9 Hz, 1H), 4.71–4.79 (m, 2H), 4.01–4.11 (m, 2H), 3.76–3.79 (m, 2H), 3.69–3.71 (m, 2H), 3.60–3.65 (m, 8H), 3.52–3.54 (m, 2H).

*Compound **8**.* [Ru(4PEG-tpy)(biq)Cl](PF_6) (60 mg, 0.06 mmol) and PROTAC **3** (56 mg, 0.06 mmol) were added to 5 mL of degassed MeOH in a pressure tube. The mixture was stirred at 70 °C for 24 hours. The reaction mixture then cooled to room temperature, a saturated solution of KPF_6 was added and a purple solid was filtered off. The purification of the solid was carried out by flash chromatography (SiO_2, 0.2% KNO_3 and 5% water in MeCN, ramped to 15% H_2O) to give the pure complex. The product fractions were concentrated under reduced pressure producing a purple solid. The NO_3^- salt was dissolved in minimal water and converted to the PF_6^- salt upon the addition of a saturated solution of KPF_6. The precipitate was isolated by extraction into DCM and the solvent was removed under reduced pressure. Yield: 12 mg (10%). Purity by HPLC = 95 %. ^{1}H NMR (700 MHz, acetone-d6): δ 10.10–10.16 (m, 1H), 8.71–9.26 (m, 6H), 8.40–8.70 (m, 4H), 8.02–8.39 (m, 5H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.83–7.89 (m, 2H), 7.78–7.81 (m, 2H), 7.54–7.73 (m, 3H), 7.30–7.53 (m, 8H), 6.96–7.15 (m, 2H), 5.07–5.38 (m, 3H), 4.56–4.98 (m, 5H), 4.04–4.53 (m, 4H), 3.89–4.01(m, 2H), 3.50–3.82 (m, 9H), 3.02–3.42 (m, 7H), 2.34–2.71 (m, 6H), 0.79–1.72 (m, 13H). HRMS calcd for $C_{87}H_{85}ClN_{14}O_{12}RuS$ $[M]^{2+}$ 843.2462, $[M+H]^{3+}$ 562.5001; found 843.2444 $[M]^{2+}$, 562.5000 $[M+H]^{3+}$; UV/Vis (H_2O): λ_{max} ($\varepsilon \times 10^{-3}$) 540 nm (6.2).

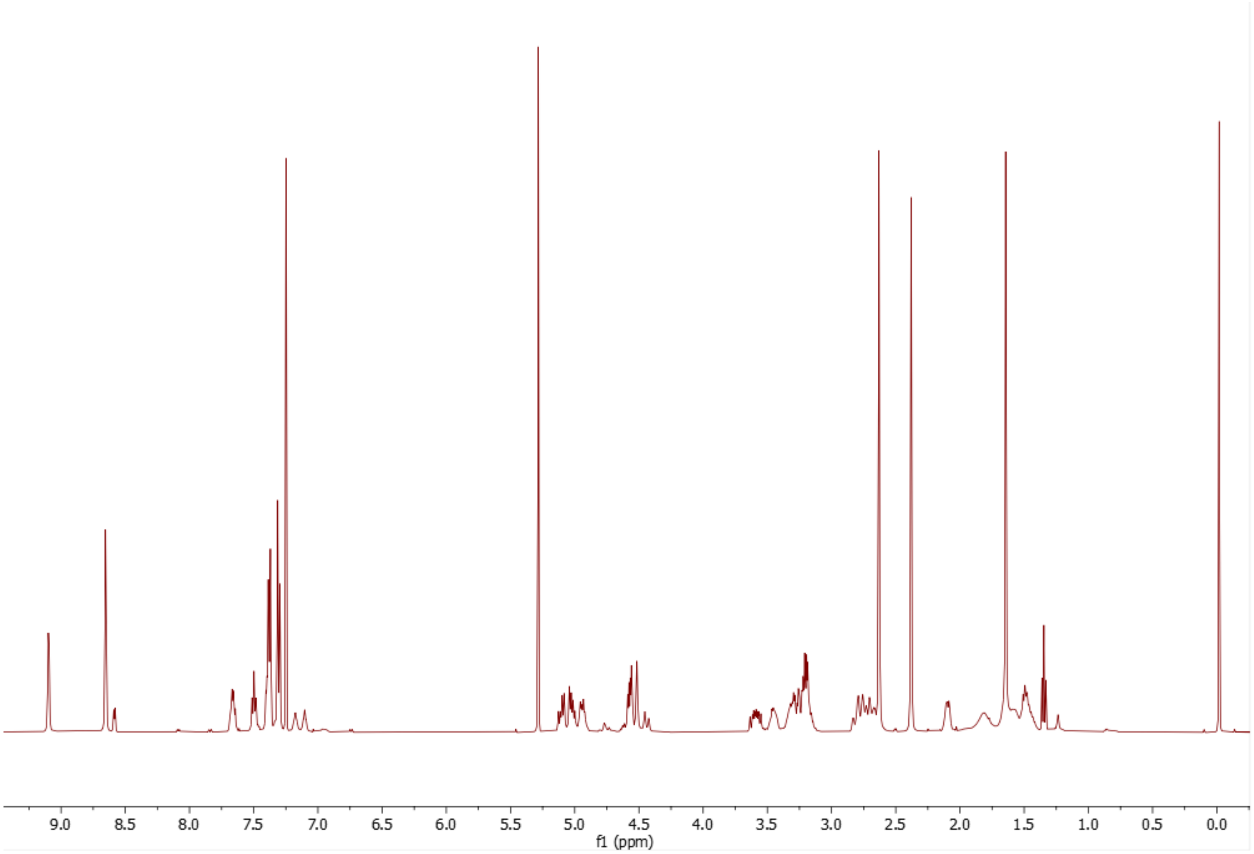
2. Additional Figures.



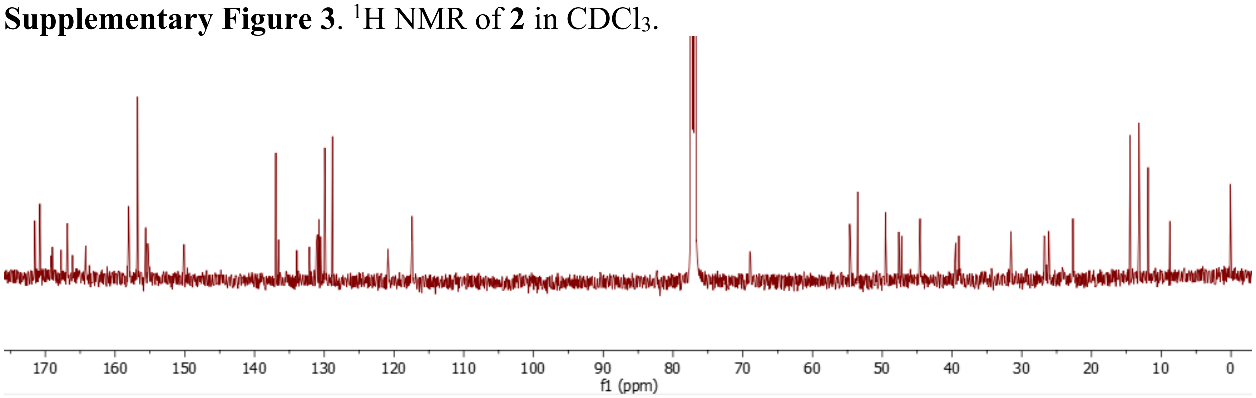
Supplementary Figure 1. ^{1}H NMR of **1** in $CDCl_3$.



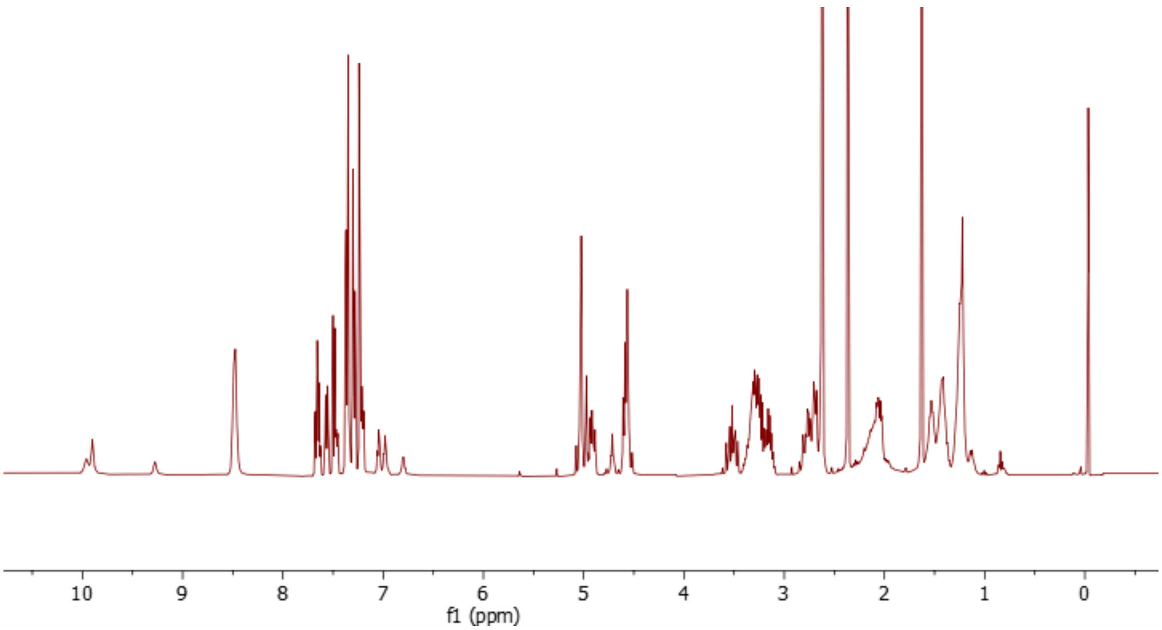
Supplementary Figure 2. ^{13}C NMR of **1** in $CDCl_3$.



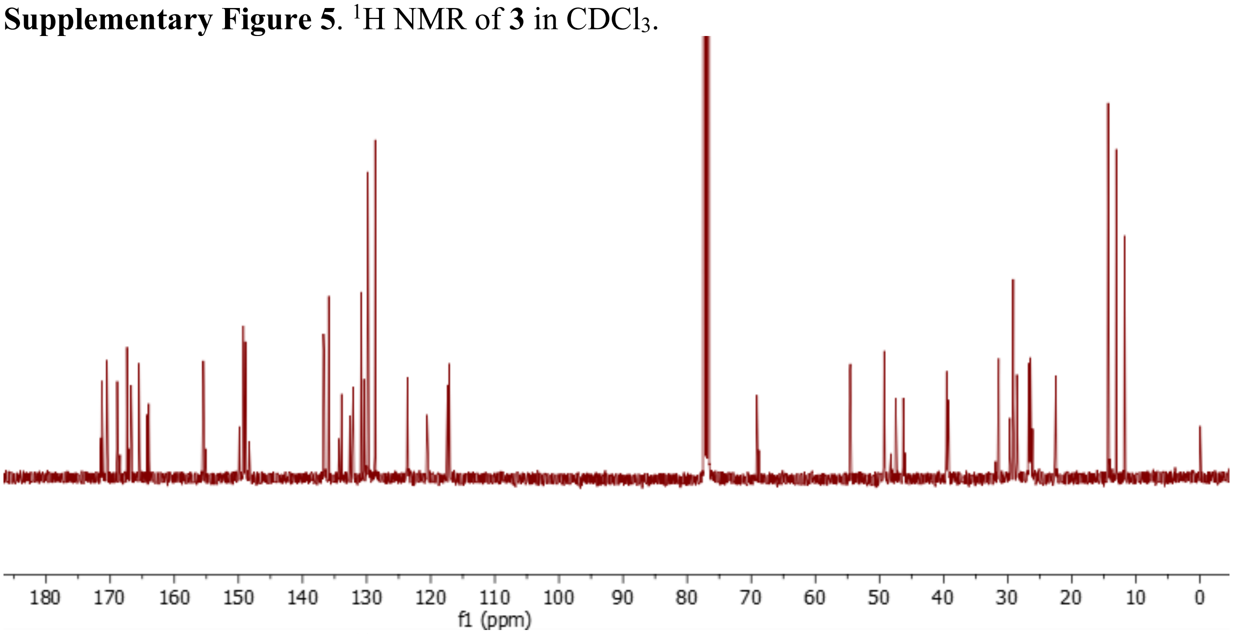
Supplementary Figure 3. ^{1}H NMR of **2** in $CDCl_3$.



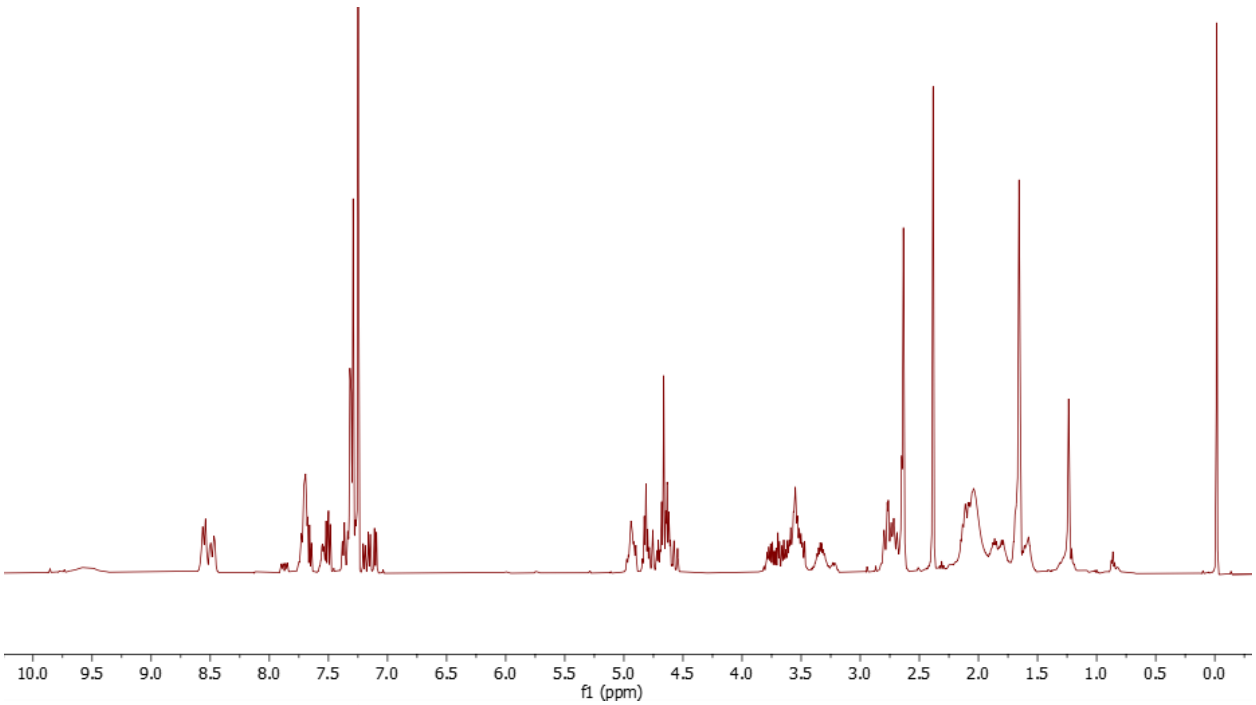
Supplementary Figure 4. ^{13}C NMR of **2** in $CDCl_3$.



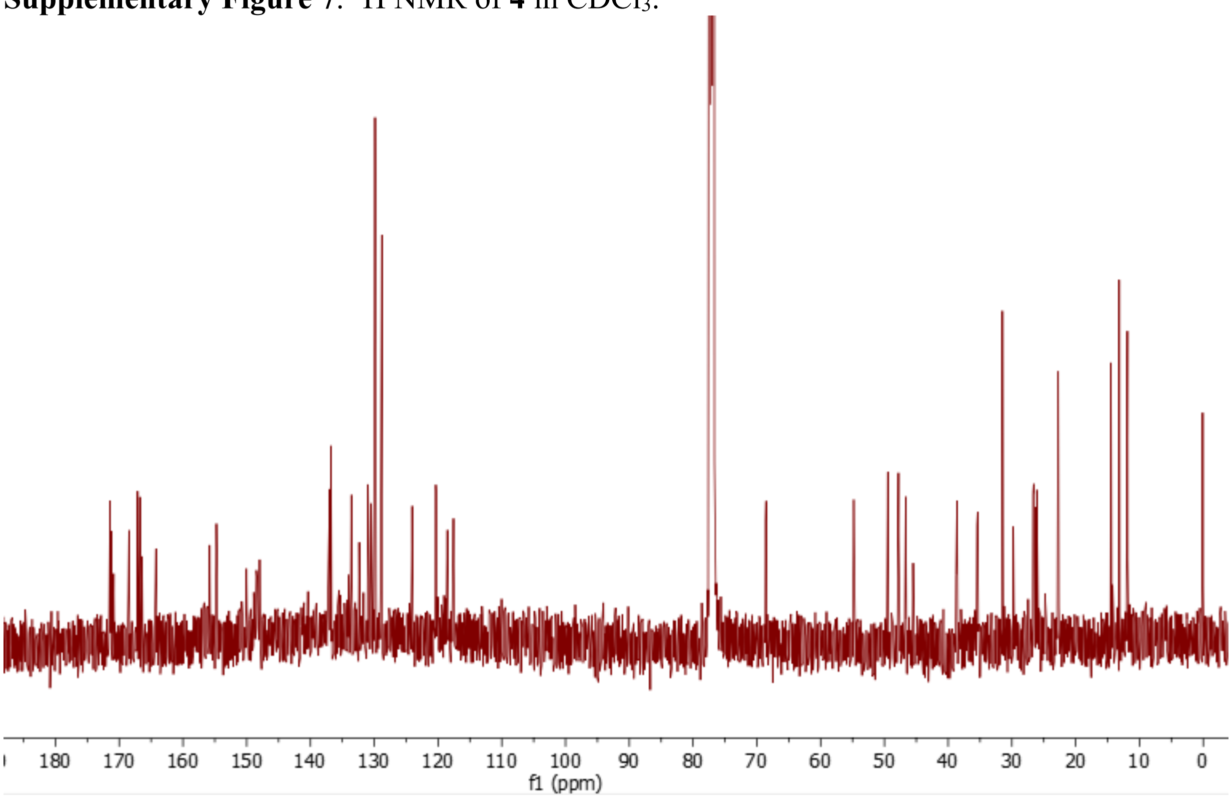
Supplementary Figure 5. ^{1}H NMR of **3** in $CDCl_3$.



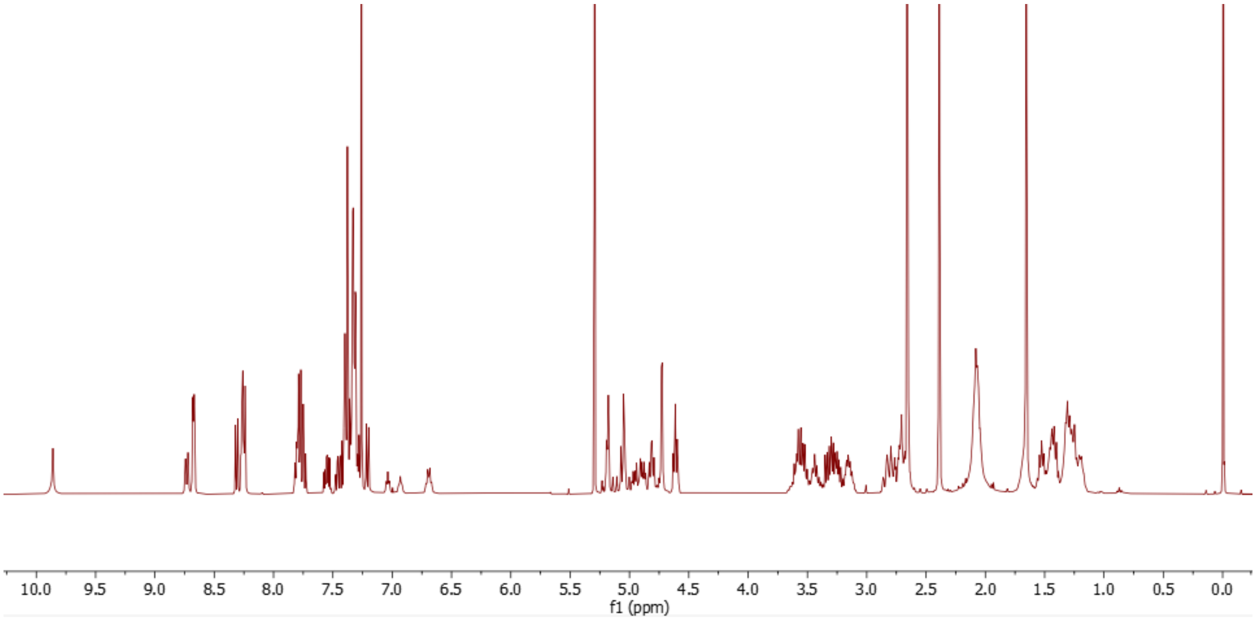
Supplementary Figure 6. ^{13}C NMR of **3** in $CDCl_3$.



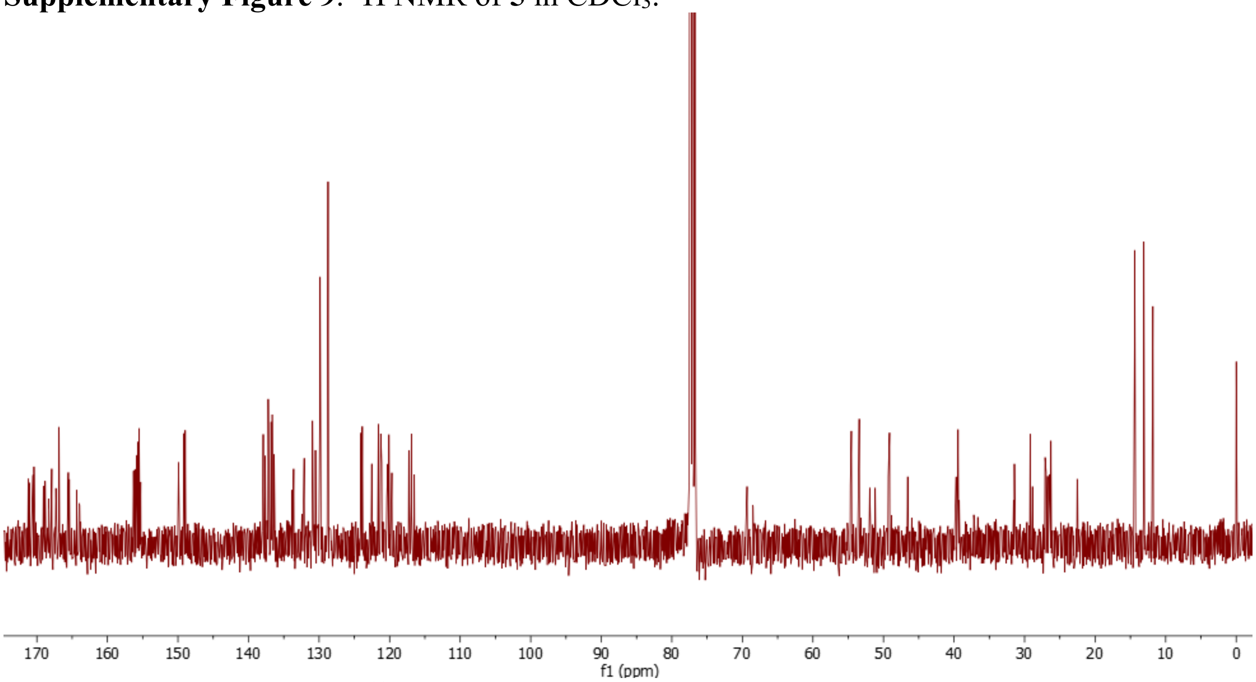
Supplementary Figure 7. ^{1}H NMR of **4** in $CDCl_3$.



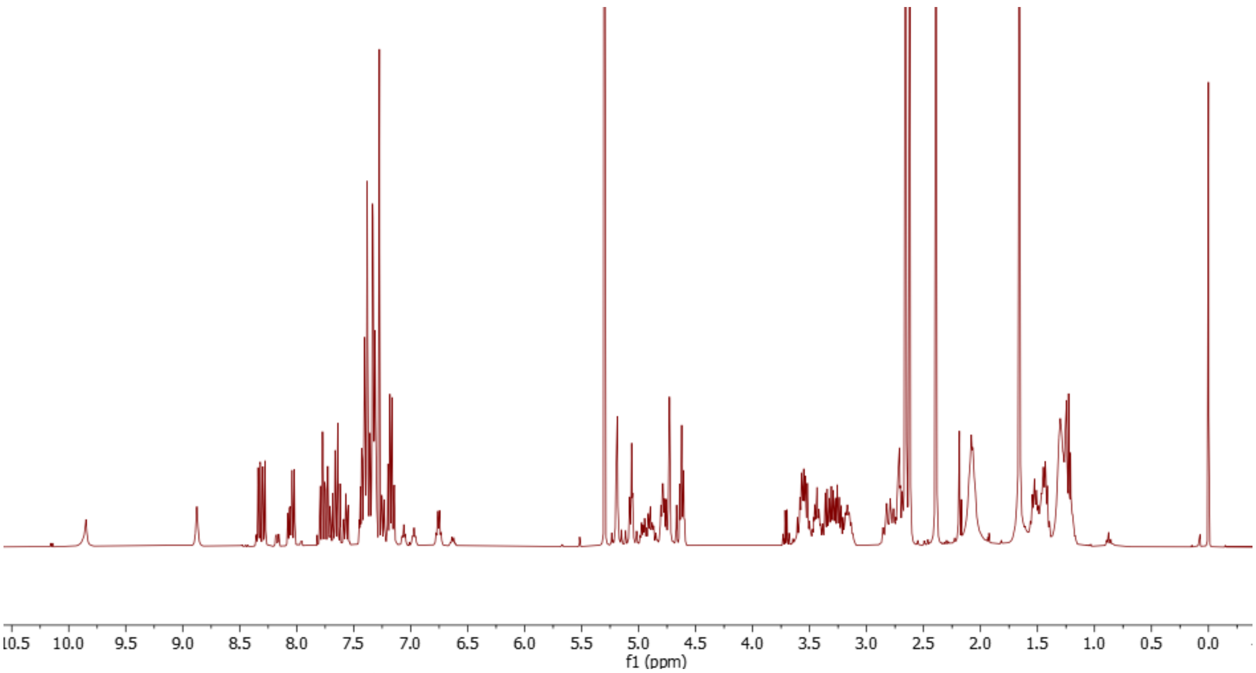
Supplementary Figure 8. ^{13}C NMR of **4** in $CDCl_3$.



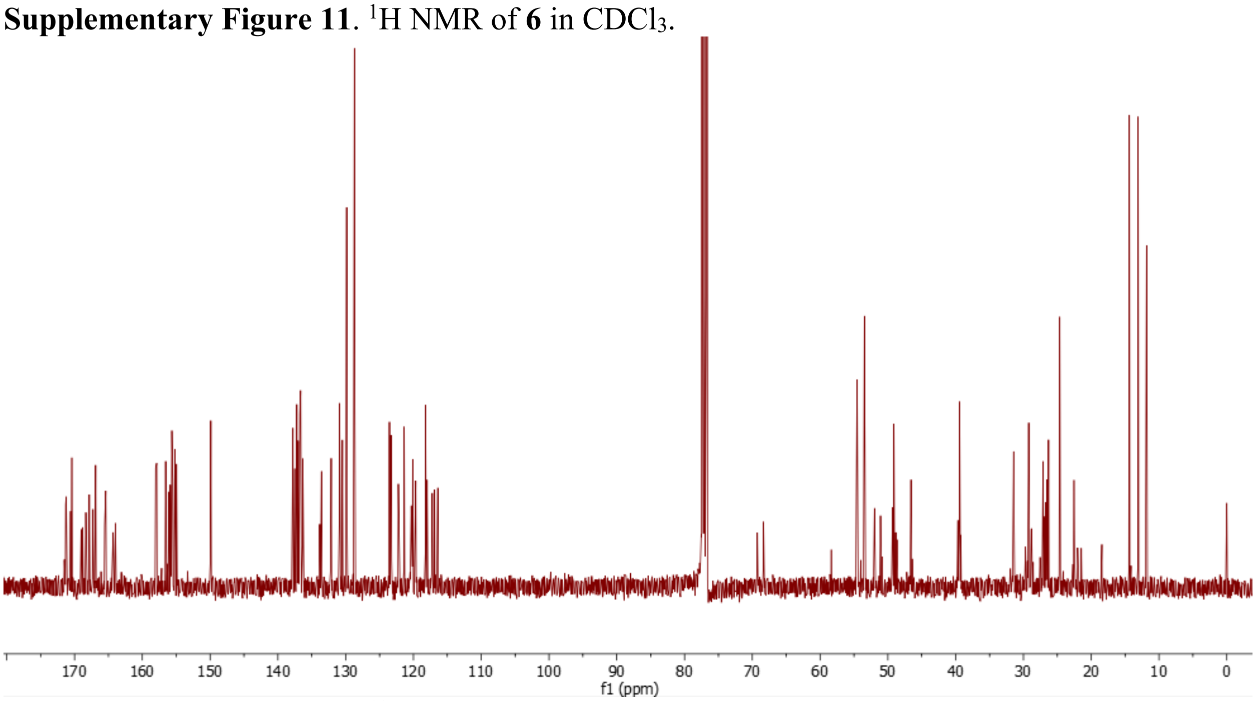
Supplementary Figure 9. ^{1}H NMR of **5** in $CDCl_3$.



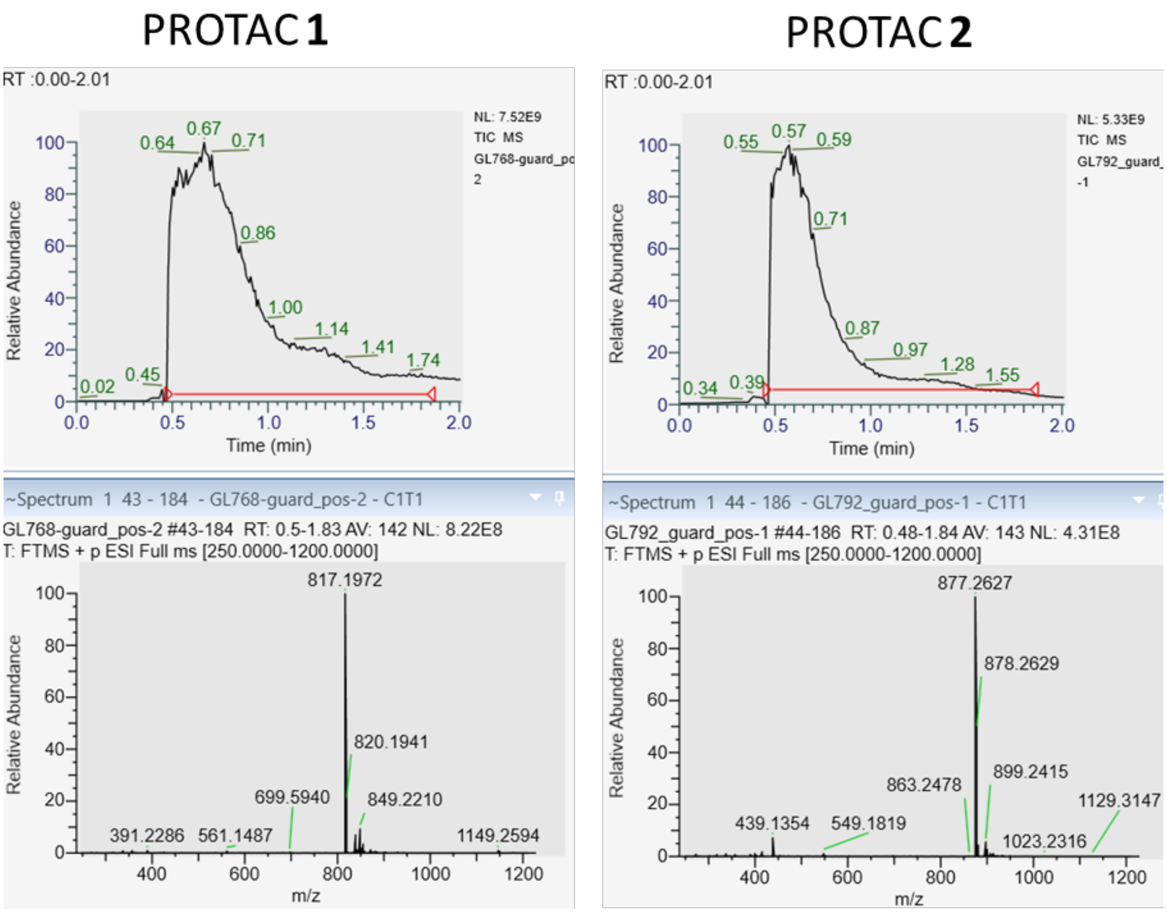
Supplementary Figure 10. ^{13}C NMR of **5** in $CDCl_3$.



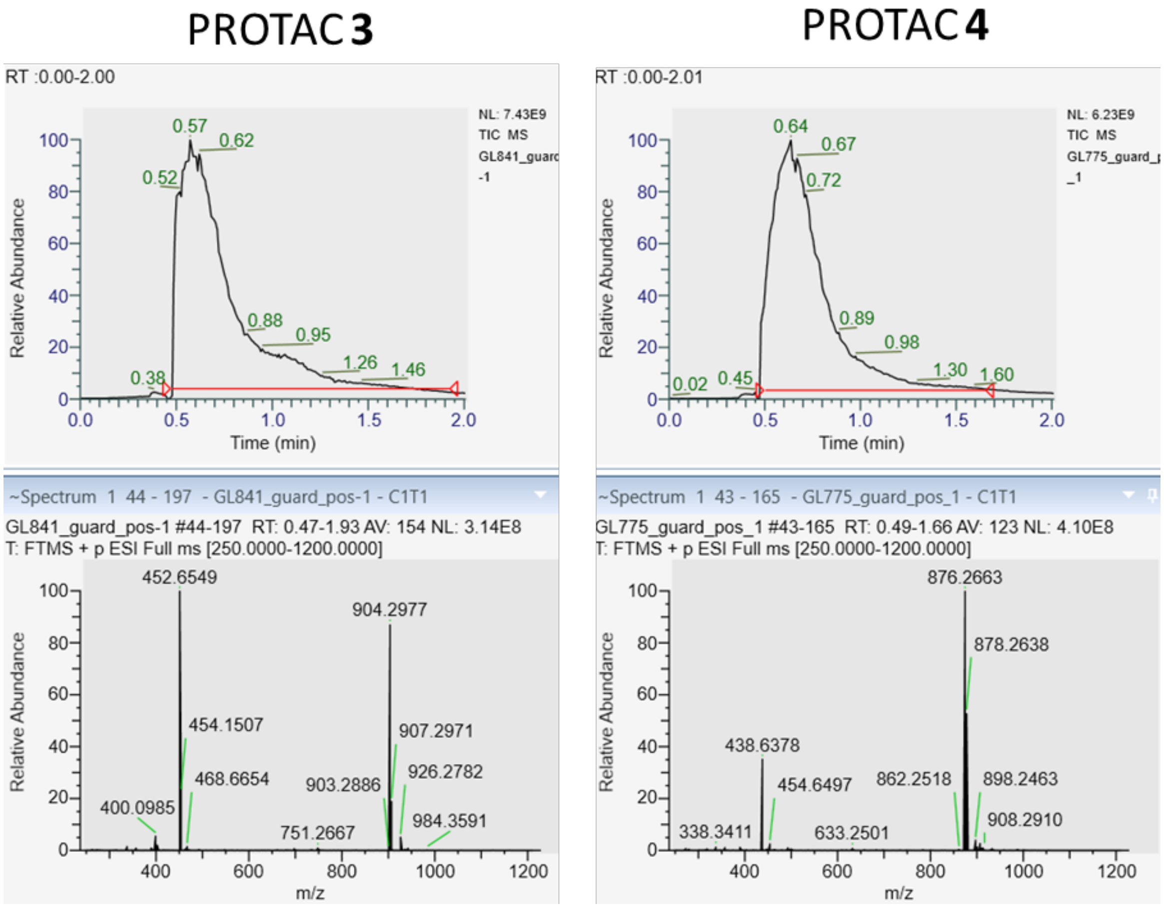
Supplementary Figure 11. ^{1}H NMR of **6** in $CDCl_3$.



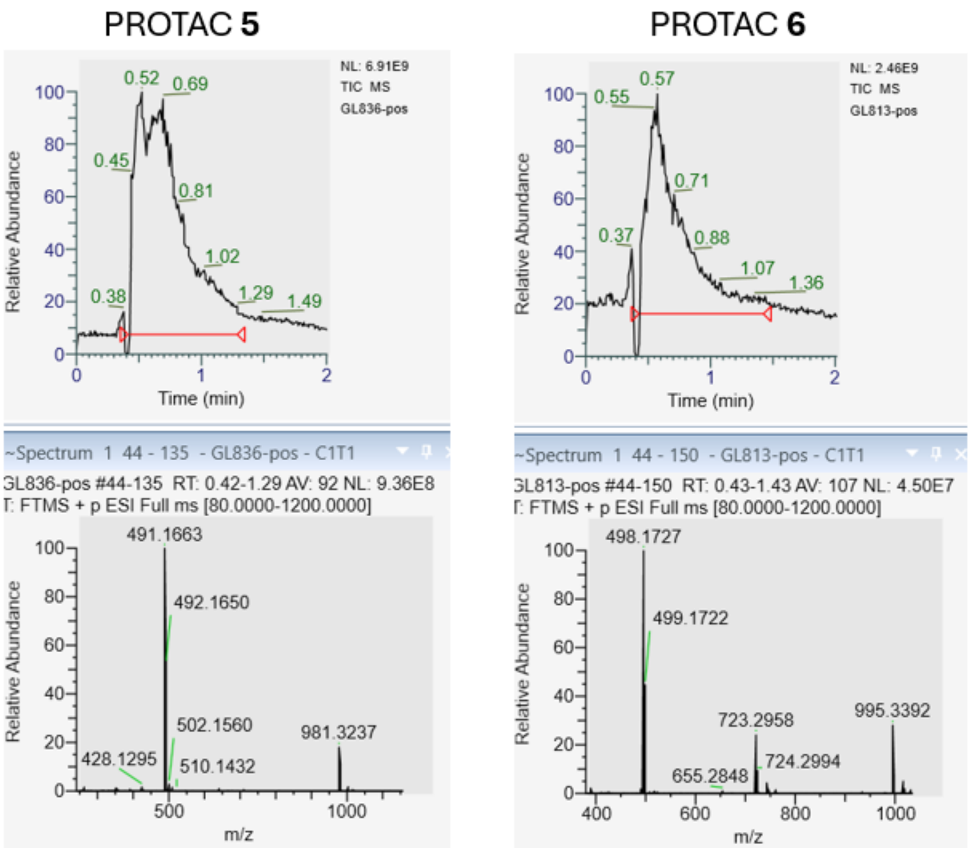
Supplementary Figure 12. ^{13}C NMR of **6** in $CDCl_3$.



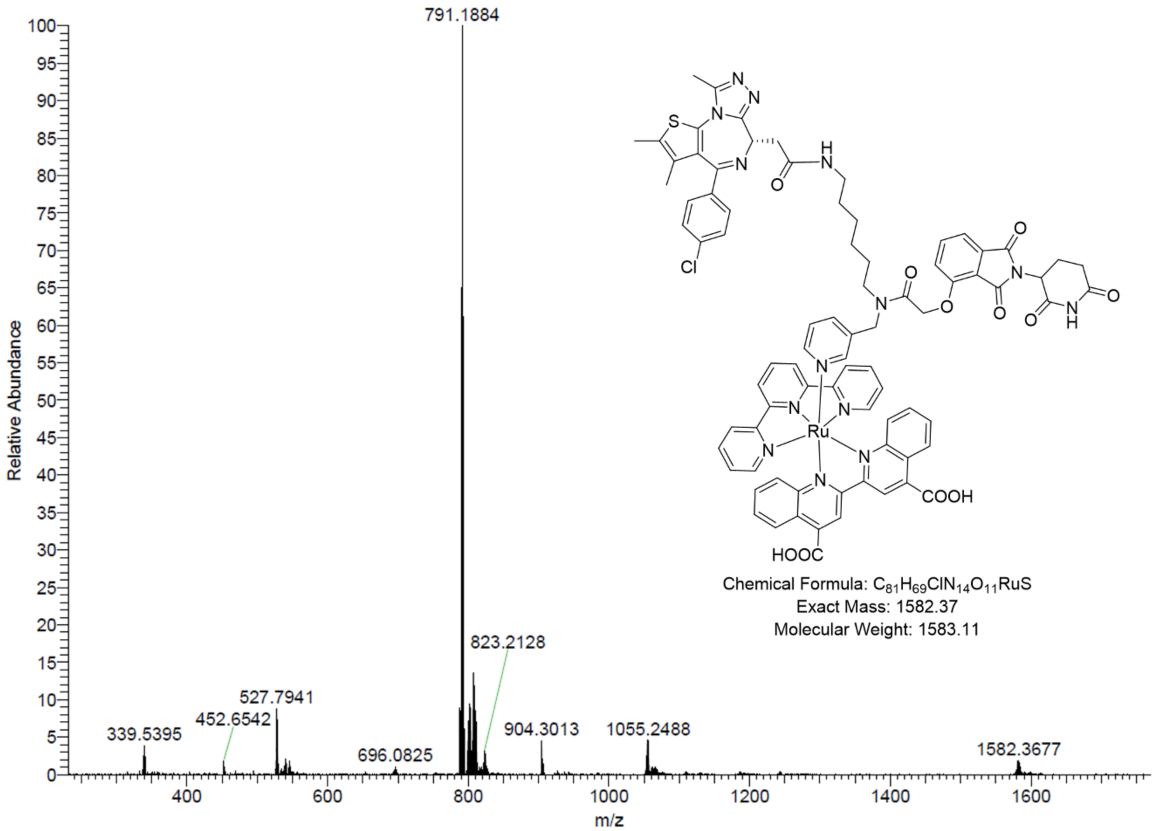
Supplementary Figure 13. HRMS for PROTACs **1** and **2**.

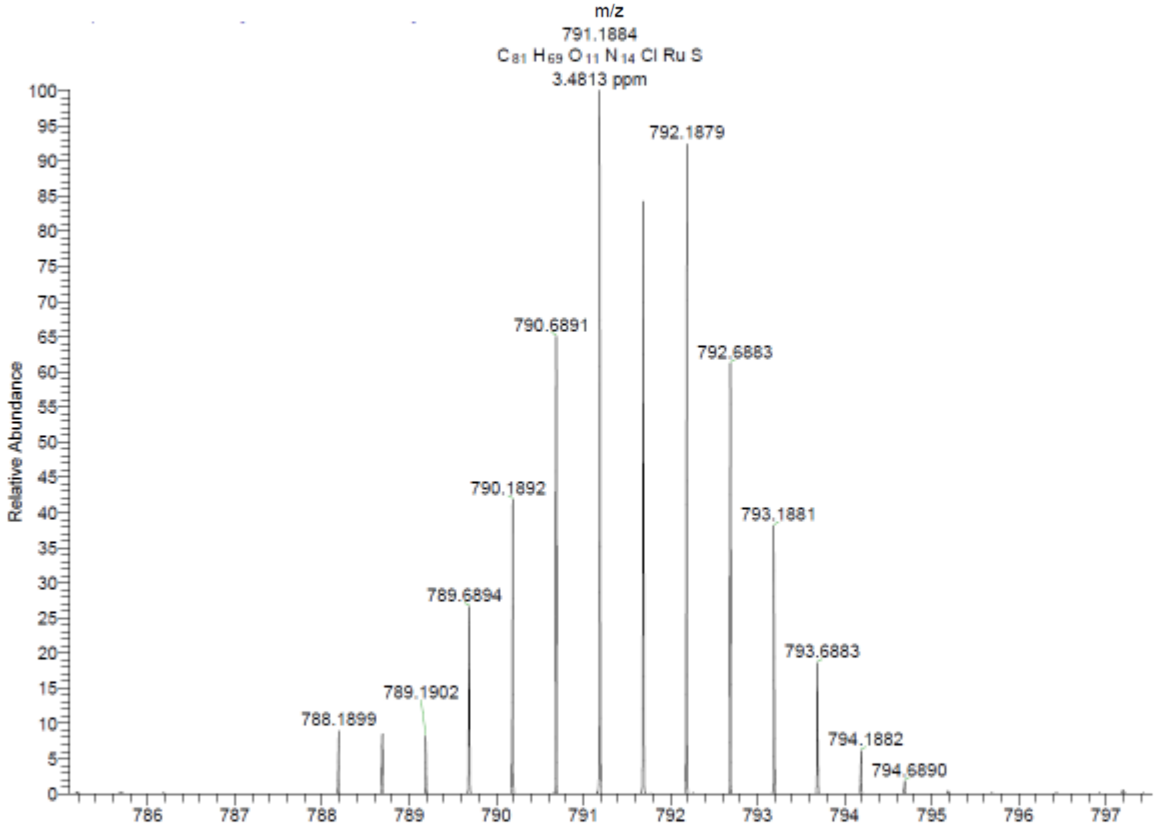


Supplementary Figure 14. HRMS for PROTACs **3** and **4**.

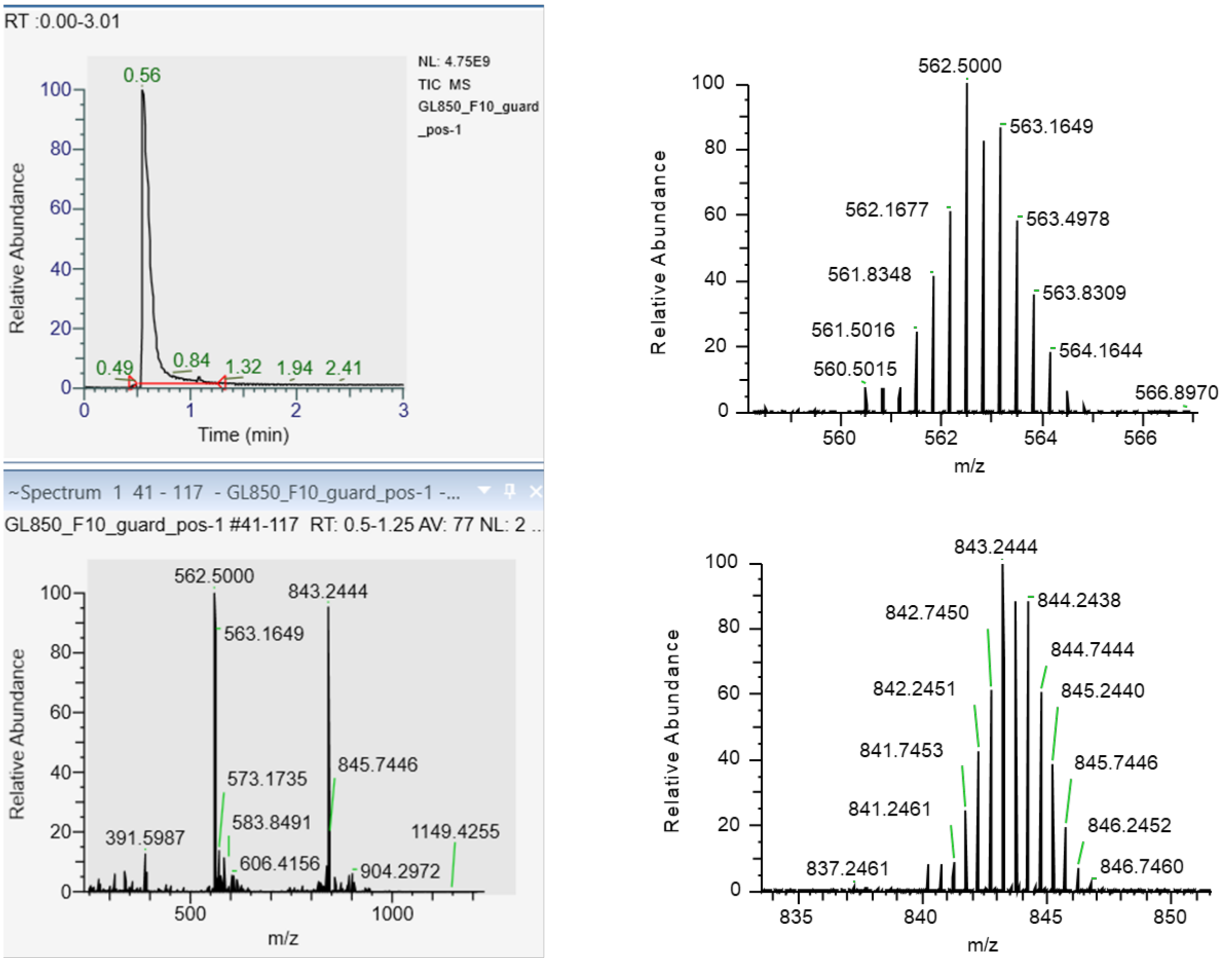


Supplementary Figure 15. HRMS for PROTACs **5** and **6**.

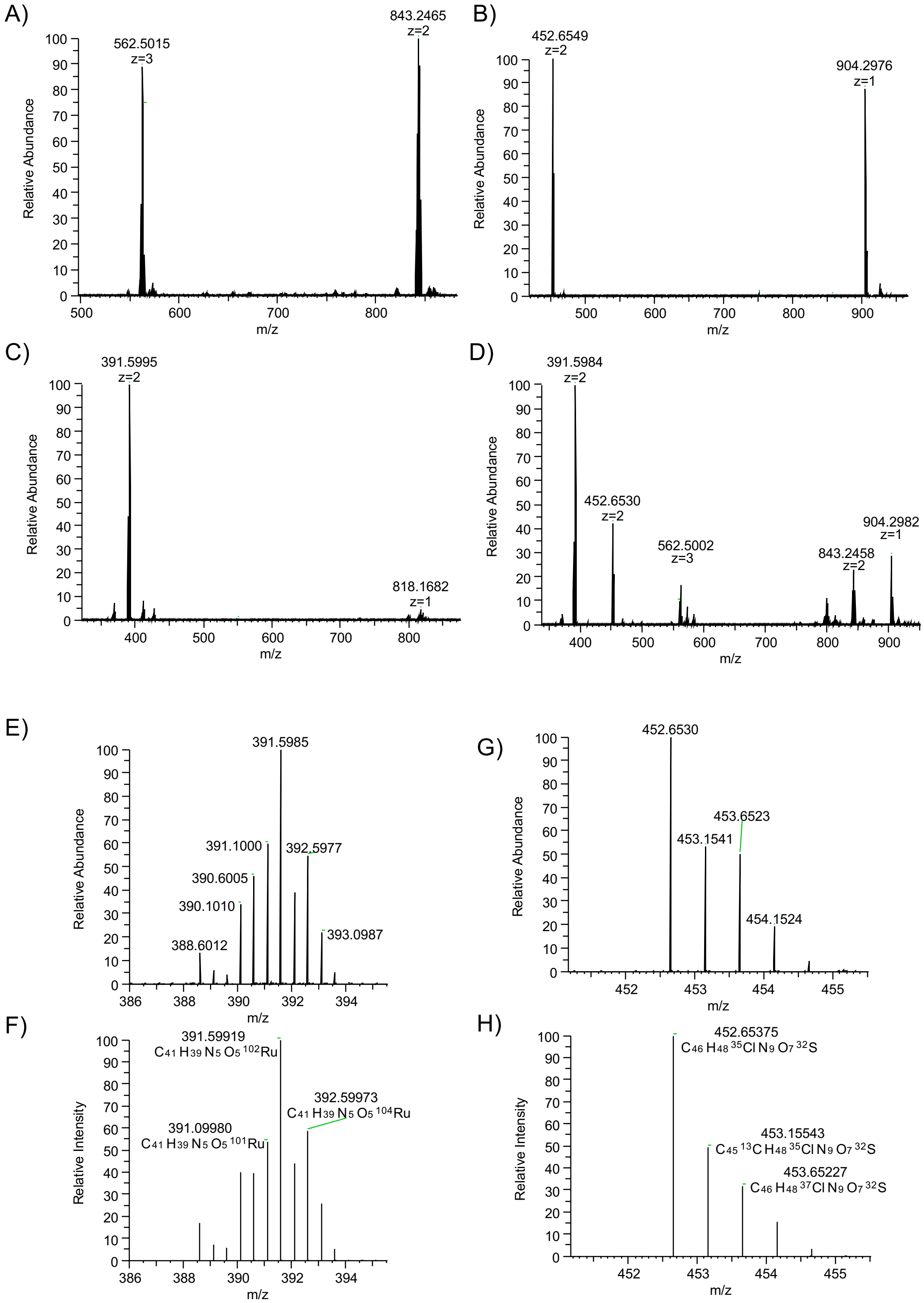




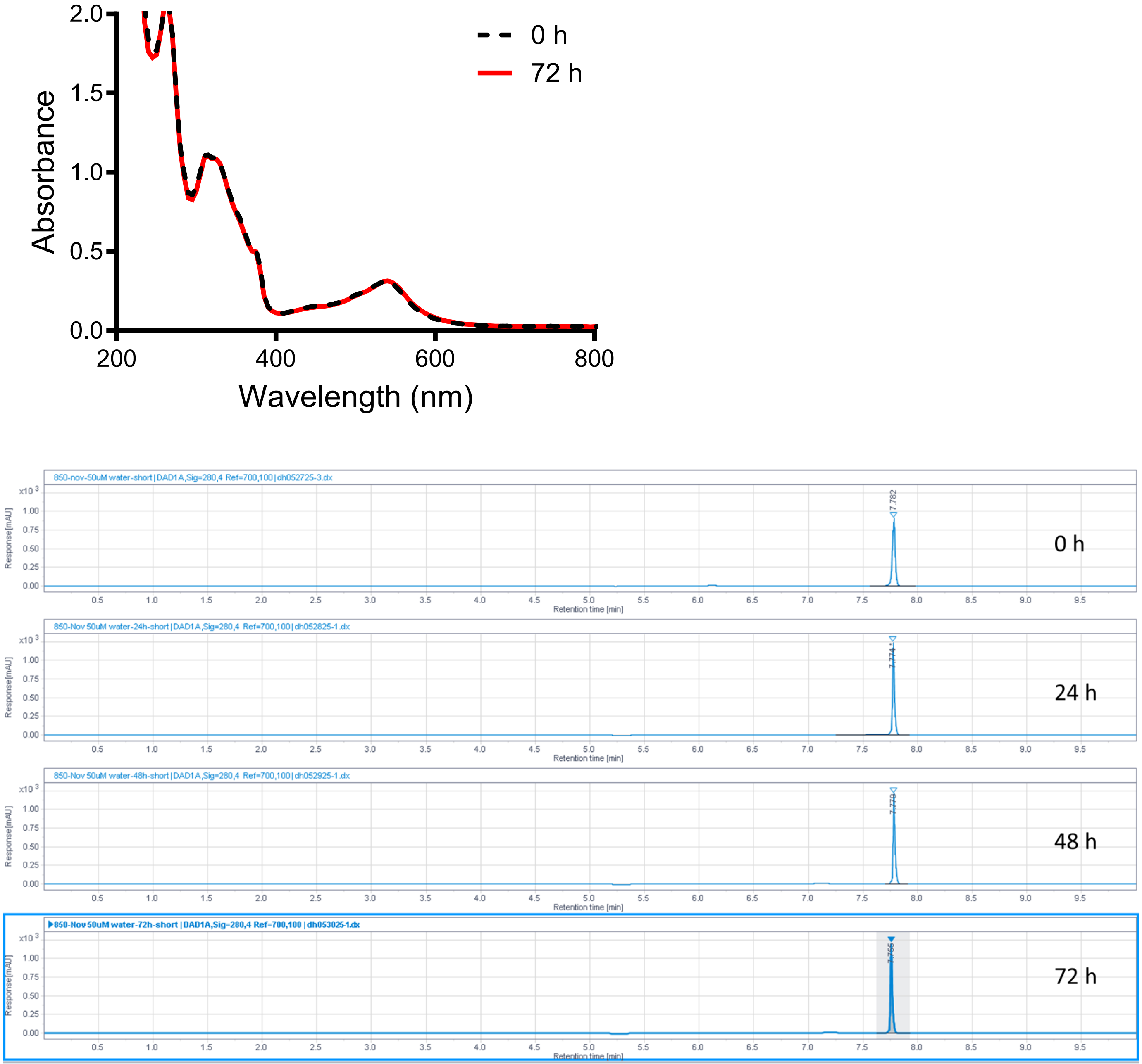
Supplementary Figure 16. HRMS of complex **7**, calcd for $C_{81}H_{69}ClN_{14}O_{11}RuS$ $[M]^{2+}$ 791.1862; found 791.1884 $[M]^{2+}$.



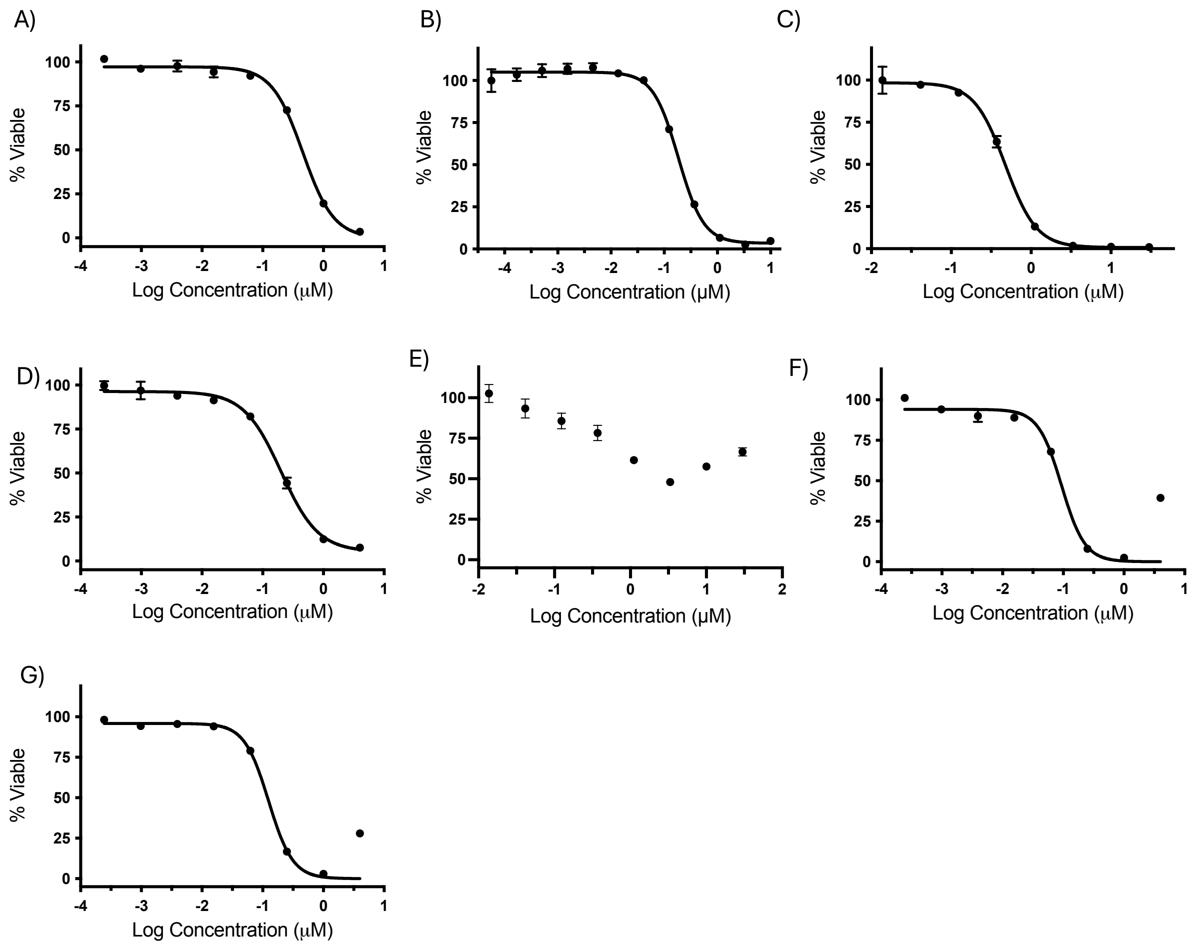
Supplementary Figure 17. HRMS of complex **8**, calcd for $C_{87}H_{85}ClN_{14}O_{12}RuS$ $[M]^{2+}$ 843.2462, $[M+H]^{3+}$ 562.5001; found 843.2444 $[M]^{2+}$, 562.5000$[M+H]^{3+}$.



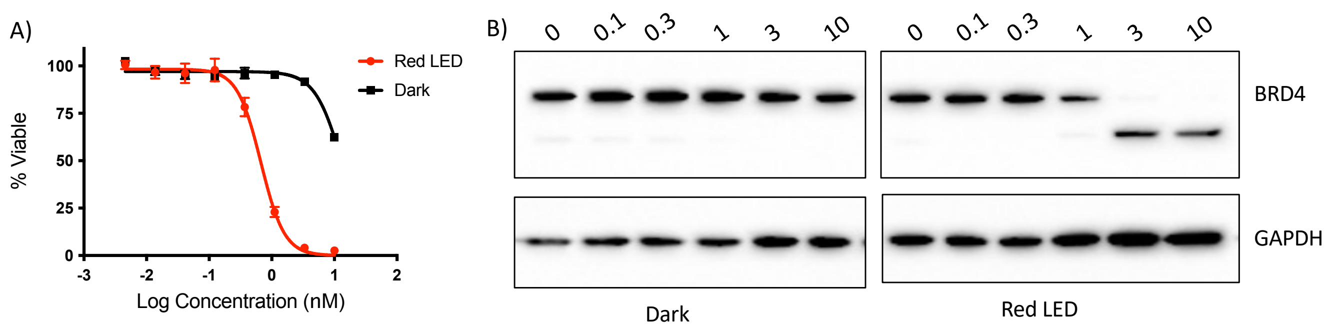
Supplementary Figure 18. HRMS data for A) complex **8**, B) PROTAC **3**, C) complex **9**, and D) complex **8** after 30 min irradiation with 660 nm light. E) and F) Expansion of D) and theoretical isotope pattern for **9**; G) and H) Expansion of D) and theoretical isotope pattern for **3**.



Supplementary Figure 19. Stability of Ru(II) complex **8** in water at 37 °C in the dark over the course of 0 (black dashed line) to 72 h (red line), followed by UV/vis absorption and studied by HPLC (short, 10 min method).



Supplementary Figure 20. Cytotoxicity dose responses for A) dBET1, B) **1**, C) **2**, D) **3**, E) **4**, F) **5**, and G) **6** in Ramos cells.

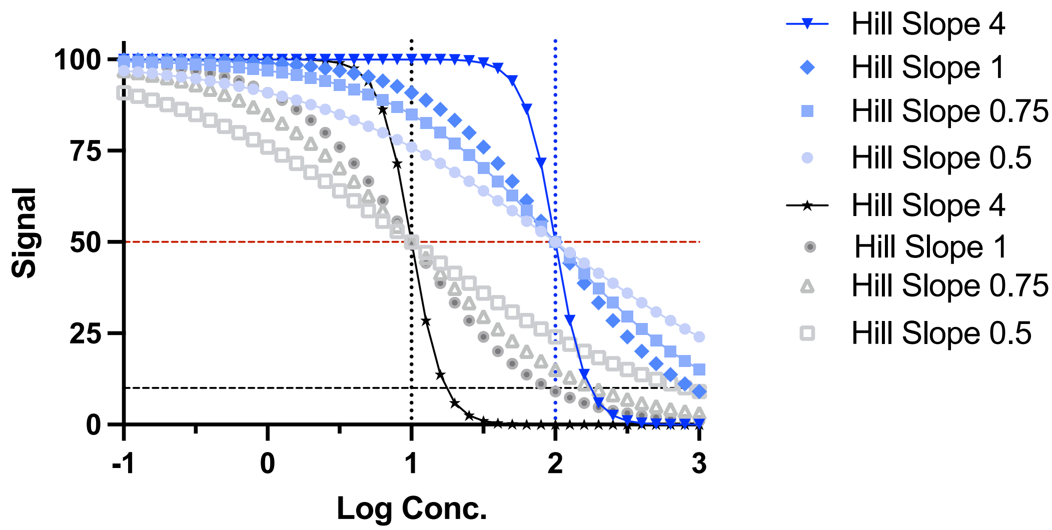


Supplementary Figure 21. Cytotoxicity dose responses (A) for **7** in Ramos cells and (B) immunoblot for BRD4 degradation without light and following 660 nm light activation.

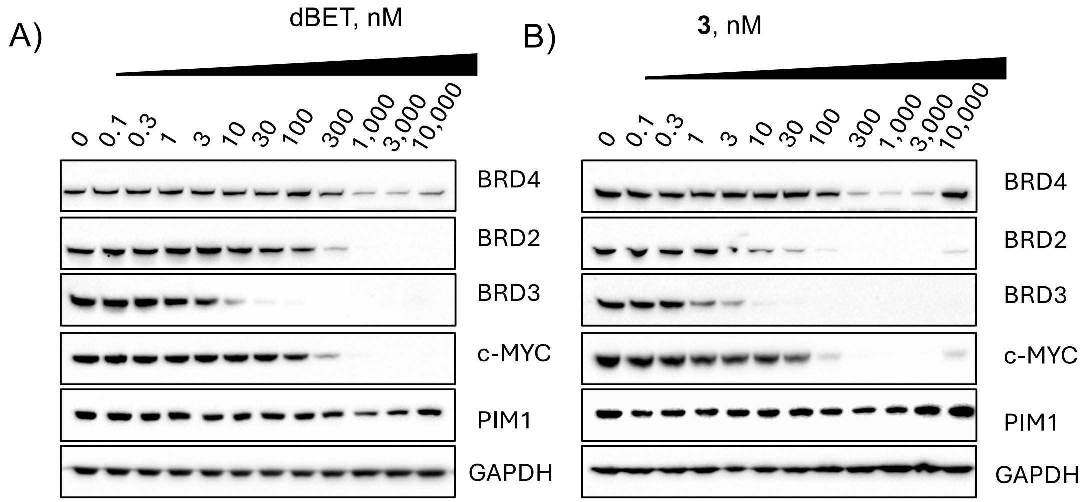
Supplementary Table 1. Cytotoxicity and BRD4 degradation potency of select PROTACs in SF8628 cells.

	Cytotoxicity	BRD4 Degradation
	EC$_{50}$, µM[a]	DC$_{50}$, µM[b]
Cmpd.	Dark	Dark
dBET1	3.43±0.57	0.3
3	1.59±0.09	0.3
5	0.26±0.0008	0.03
6	0.32±0.002	0.01

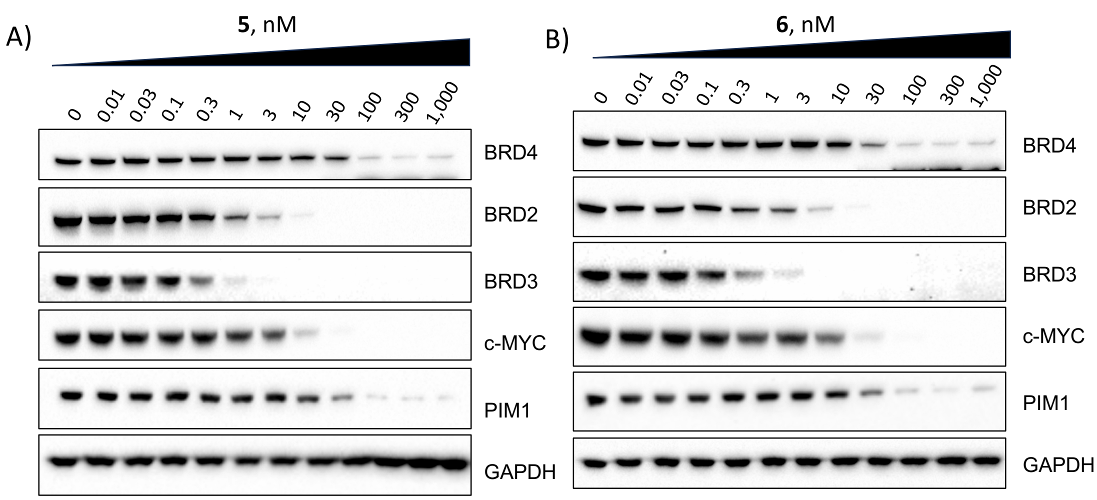
[a] EC$_{50}$ = effective concentration for 50% cytotoxicity. Evaluated in the SF8628 cell line (n=3). [b] DC$_{50}$ = effective concentration for 50% degradation.



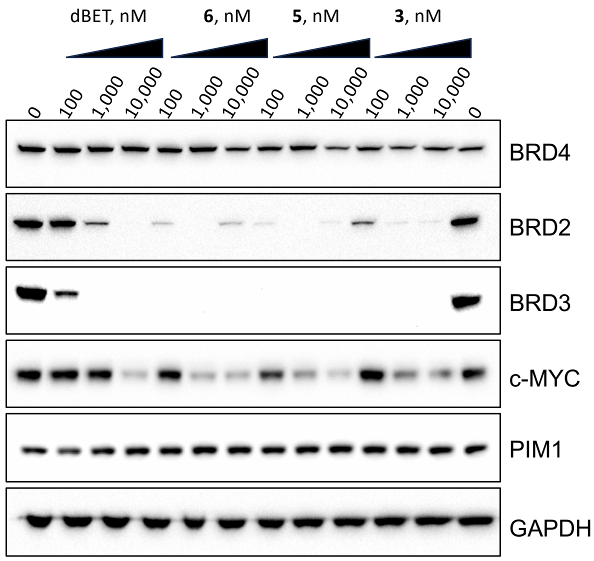
Supplementary Figure 22. Simulation of dose responses for different Hill coefficients with therapeutic windows of 10 (for example, for a PI of 10). The 50% response on the y-axis is marked with red dashes and the 90% response is marked with black dashes for ease of visualization. Note that a 90% response for an "active" agent with a Hill coefficient of 1 (placed at 1 on the x-axis) correlates to a 50% response for an "inactive" agent (at 2 on the x-axis; blue scale data). The window narrows for lower Hill coefficients (see grey scale data). For example, a Hill coefficient of 0.5 results in no viable therapeutic window.



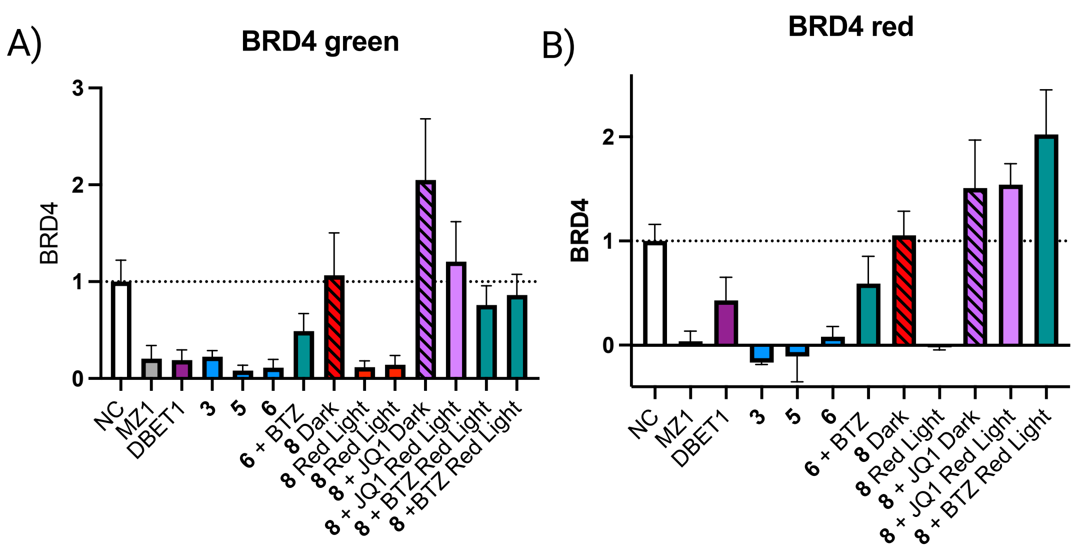
Supplementary Figure 23. Immunoblot dose responses for dBET1 (A) and **3** (B) in Ramos cells.



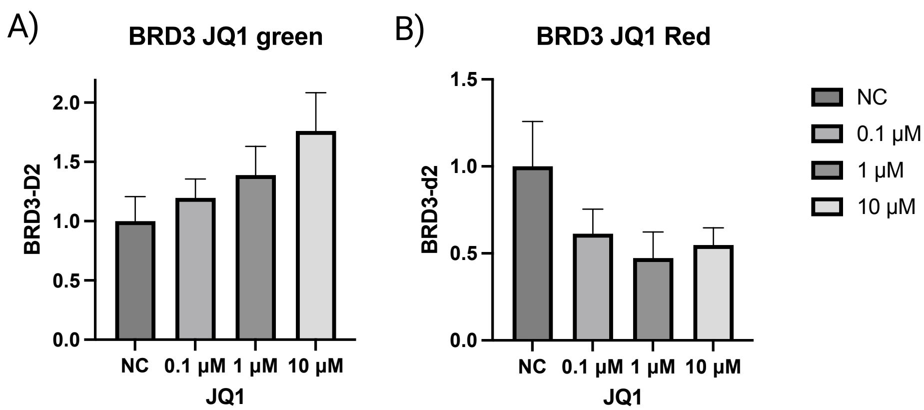
Supplementary Figure 24. Immunoblot dose responses for **5** (A) and **6** (B) in Ramos cells.



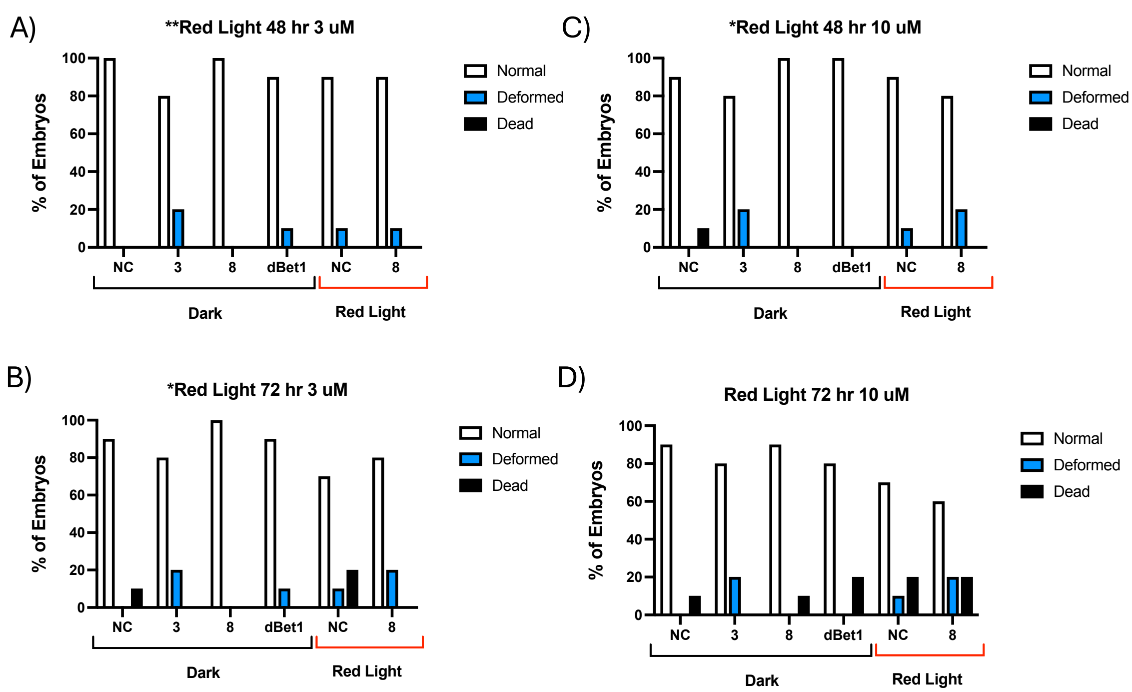
Supplementary Figure 25. Immunoblot dose responses for dBET**, 6**, **5**, and **3** in SF8628 cells.



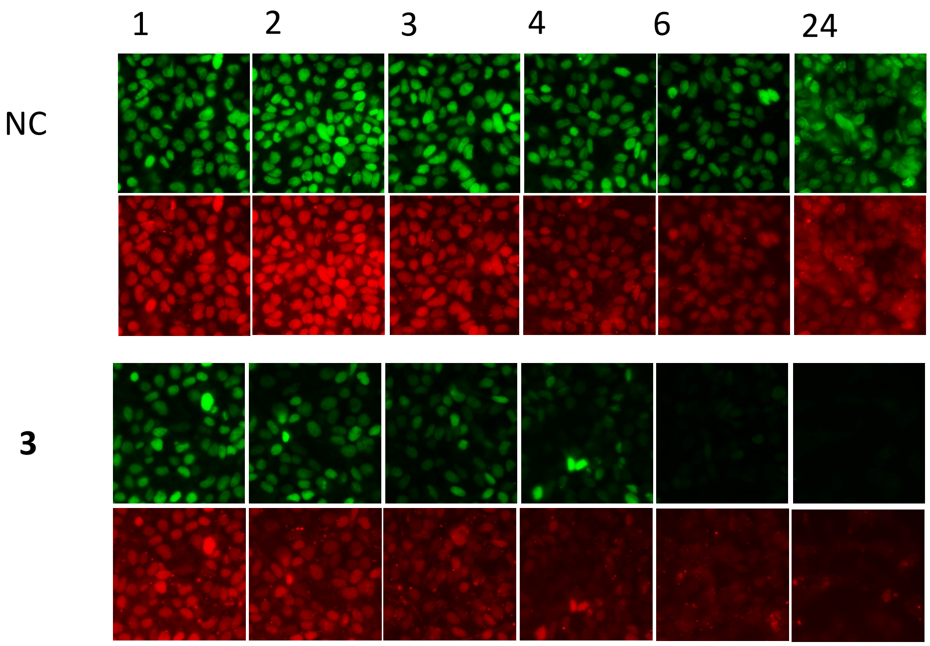
Supplementary Figure 26. Relative intensity of the emission of BRD4-Dendra2 fusion when observed using A) green emission vs. B) red. The emission of D2 was monitored 16 hours post compound treatment. In several cases experimental replicates are shown to demonstrate the robustness of the assay.



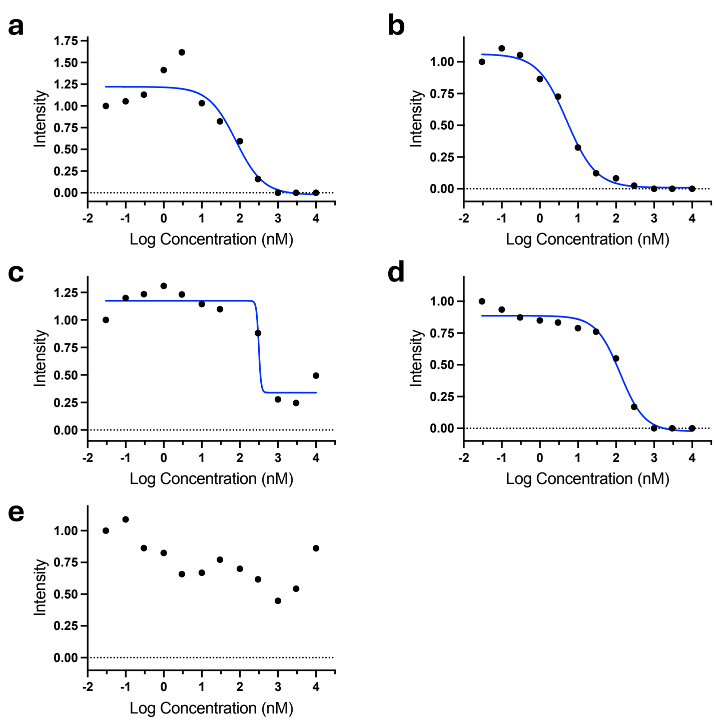
Supplementary Figure 27. Dose response for JQ1 in HEK Flp-In cell line. The emission of D2 was monitored in the green and red 16 hours post compound treatment.



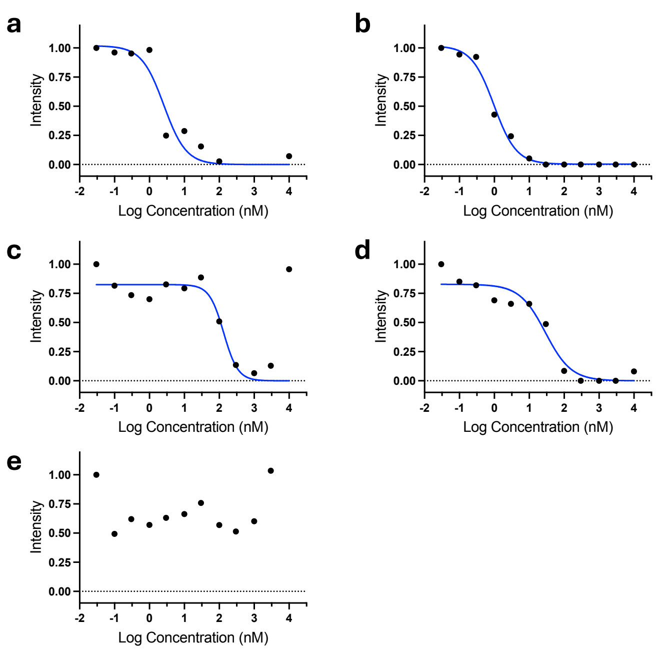
Supplementary Figure 28. Zebrafish embryos viability assessment exhibited both time and concentration dependent effects of compounds **3**, **8**, and dBET1. A) Compounds dosed at 3 µM concentrations with assessment at 48 hrs. B) Compounds dosed at 3 µM concentrations with assessment at 72 hrs. C) Compounds dosed at 10 µM concentrations with assessment at 48 hrs. D) Compounds dosed at 10 µM concentrations with assessment at 72 hrs. Organic PROTACs **3** and dBET1 exhibited an increase in deformed and dead embryos relative to the No Compound control. Light exposure also caused an increase in deformed and dead embryos, consistent with previous reports for the toxicity of red light treatment of zebrafish.[5] The effects of **8** in the dark matched the No Compound control, and **8** following irradiation was similar to irradiation alone.



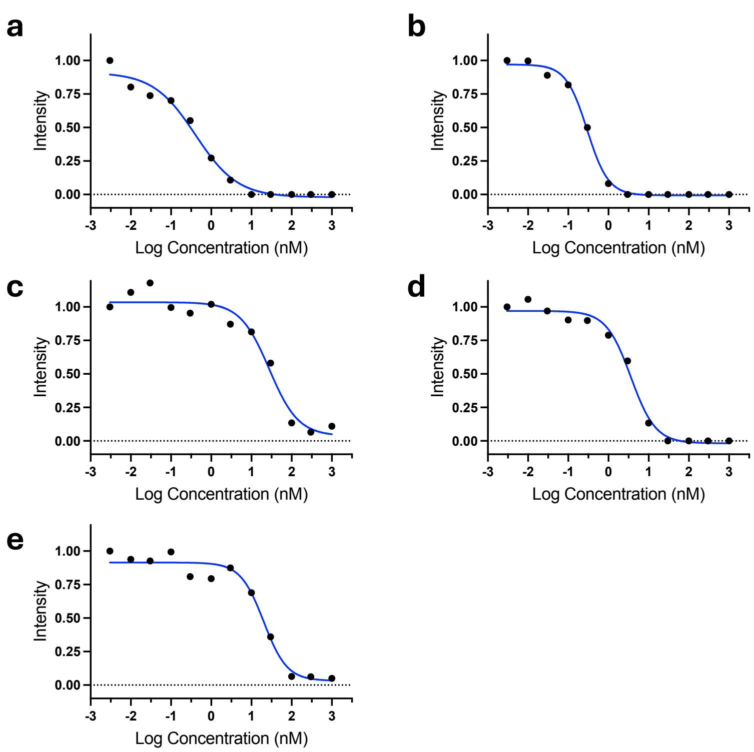
Supplementary Figure 29. Time course of emission for Dendra2 assay. The number at the top indicates hours.



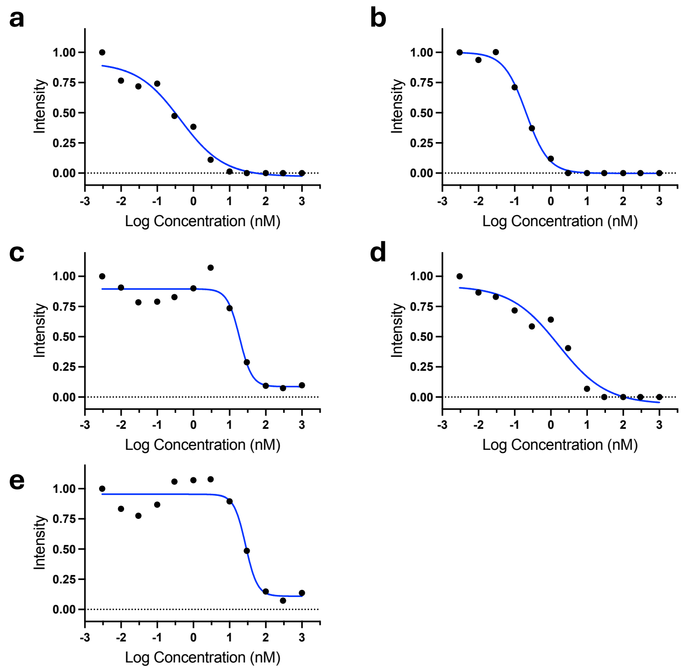
Supplementary Figure 30. Quantification of immunoblot dose responses for dBET1. **a** BRD2 (EC_{50} = 78 nM; Hill Slope = 1.2); **b** BRD3 (EC_{50} = 5 nM; Hill Slope = 1.1); **c** BRD4 (EC_{50} = 313 nM; Hill Slope = 14.1); **d** cMYC (EC_{50} = 129 nM; Hill Slope = 1.4); **e** PIM1 (N/A).



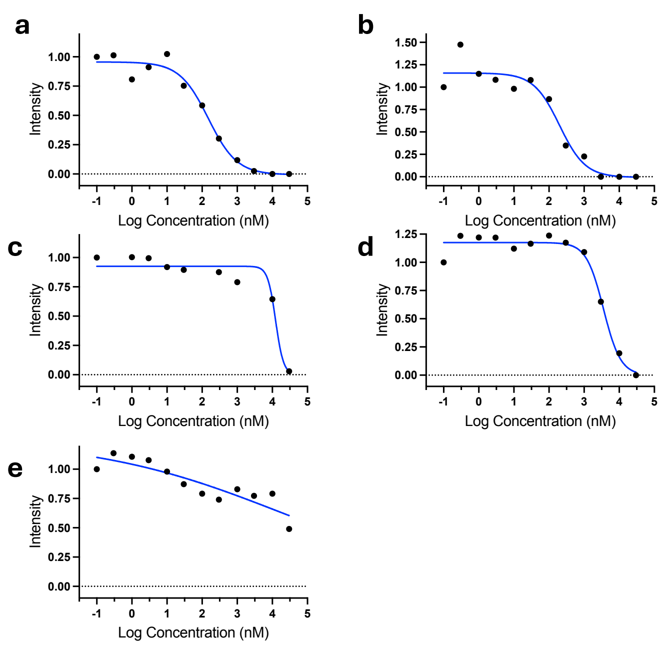
Supplementary Figure 31. Quantification of immunoblot dose responses for **3**. **a** BRD2 (EC_{50} = 2.6 nM; Hill Slope = 1.4); **b** BRD3 (EC_{50} = 1 nM; Hill Slope = 1.4); **c** BRD4 (EC_{50} = 133 nM; Hill Slope = 2.1); **d** cMYC (EC_{50} = 29 nM; Hill Slope = 1.2); **e** PIM1 (N/A).



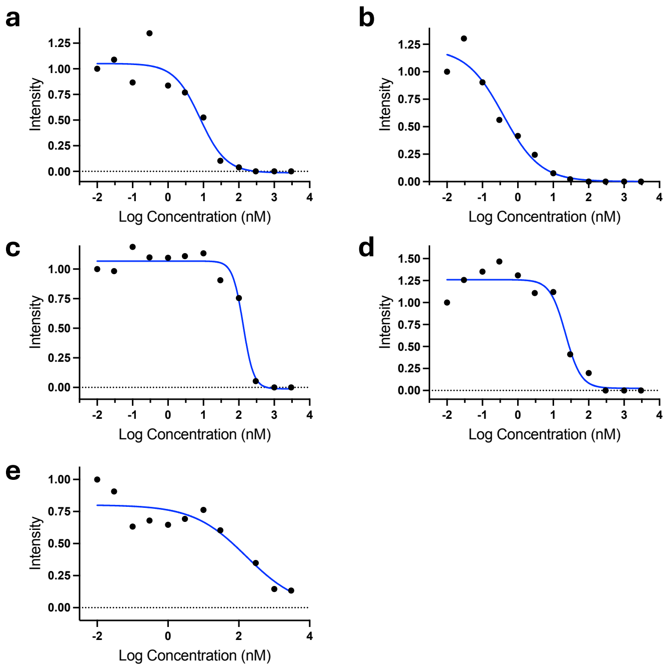
Supplementary Figure 32. Quantification of immunoblot dose responses for **5**. **a** BRD2 (EC_{50} = 0.4 nM; Hill Slope = 0.8); **b** BRD3 (EC_{50} = 0.3 nM; Hill Slope = 1.6); **c** BRD4 (EC_{50} = 27 nM; Hill Slope = 1.2); **d** cMYC (EC_{50} = 3.6 nM; Hill Slope = 1.4); **e** PIM1 (EC_{50} = 20 nM; Hill Slope = 1.5).



Supplementary Figure 33. Quantification of immunoblot dose responses for **6**. **a** BRD2 (EC_{50} = 0.4 nM; Hill Slope = 0.7); **b** BRD3 (EC_{50} = 0.2 nM; Hill Slope = 1.4); **c** BRD4 (EC_{50} = 19 nM; Hill Slope = 2.5); **d** cMYC (EC_{50} = 1.6 nM; Hill Slope = 0.7); **e** PIM1 (EC_{50} = 28 nM; Hill Slope = 2.7).



Supplementary Figure 34. Quantification of immunoblot dose responses for **8** (Dark). **a** BRD2 (EC_{50} = 150 nM; Hill Slope = 1.1); **b** BRD3 (EC_{50} = 200 nM; Hill Slope = 1.2); **c** BRD4 (EC_{50} = 12,395 nM; Hill Slope = 3.9); **d** cMYC (EC_{50} = 3,556 nM; Hill Slope = 1.8); **e** PIM1 (N/A).



Supplementary Figure 35. Quantification of immunoblot dose responses for **8** (Red Light). **a** BRD2 (EC_{50} = 8 nM; Hill Slope = 1.2); **b** BRD3 (EC_{50} = 0.4 nM; Hill Slope = 0.8); **c** BRD4 (EC_{50} = 132 nM; Hill Slope = 2.9); **d** cMYC (EC_{50} = 22 nM; Hill Slope = 2.0); **e** PIM1 (EC_{50} = 161 nM; Hill Slope = 0.6).

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5. Villamizar, N.; Vera, L. M.; Foulkes, N. S.; Sanchez-Vazquez, F. J., Effect of lighting conditions on zebrafish growth and development. *Zebrafish* **2014,** *11* (2), 173-81.