

BA-1/BA-3 preparation [1](<https://doi.org/10.1016/j.ejmech.2023.115975>)

Synthesis of **1**. 4-(diphenylamino) phenylboronic acid (433.7 mg, 1.50 mmol), pyridine-4-boronic acid (289.4 mg, 1.50 mmol), potassium carbonate (1382.1 mg, 10.00 mmol), and tetrakis (triphenylphosphine)- palladium (80.9 mg, 0.070 mmol) were dissolved into a mixture of THF (50 mL) and H₂O (5 mL), and the mixture was ultrasonically degassed and refluxed at 70 °C for 8 h under an N₂ atmosphere. TLC was used to determine the progress of the reaction (VPE: VEA =12:1). Then, **1** was purified by column chromatography on silica gel with a mixture of PE and EA as an eluent (10:1 by volume), obtaining a faint yellow powder (225.0 mg, 46.6 % yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.58 (d, J =5.6 Hz, 2H), 7.73 (d, J =8.3 Hz, 2H), 7.65 (d, J =5.6 Hz, 2H), 7.35 (t, J =7.7 Hz, 4H), 7.10 (t, J =7.5 Hz, 6H), 7.03 (d, J =8.3 Hz, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 150.62, 148.90, 147.14, 146.78, 130.46, 130.20, 128.27, 125.24, 124.32, 122.64, 120.89. Mp: 160–162 °C. MS (ESI-APCI): calculated for [C₂₃H₁₈N₂+H] +323.1; found 323.1. Synthesis of **2**. Potassium tert-butoxide (134.6 mg, 1.20 mmol) and 4-methylpyridine (93.0 mg, 1.00 mmol) were dissolved into DMF (20 mL), and the mixture was stirred at R.T. for 0.5 h under an N₂ atmosphere. Then, 4-(N, N-diphenylamino) benzaldehyde was added and the mixture was ultrasonically degassed and refluxed at 80 °C for 12 h under an N₂ atmosphere. TLC was used to determine the progress of the reaction (VPE: VEA =5:1). Then, **2** was purified by column chromatography on a silica gel with a mixture of PE and EA as an eluent (3:1 by volume), obtaining a yellow powder (298.0 mg, 85.6 % yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.51 (d, 2H), 7.55 (d, 2H), 7.51 (dd, J =6.8, 2.3 Hz, 3H), 7.46 (s, 1H), 7.33 (t, 4H), 7.14–7.01 (m, 7H), 6.96 (d, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 150.42, 148.16, 147.22, 145.02, 132.97, 130.42, 130.13, 128.76, 125.00, 124.44, 124.10, 122.73, 121.09. Mp: 130–131 °C. MS (ESI-APCI): calculated for [C₂₅H₂₀N₂+H] +349.1; found 349.1.

Synthesis of **4**. 4,7-dibromo-2,1,3-benzothiadiazole (437.7 mg, 1.50 mmol), 4-(diphenylamino)phenylboronic acid (433.7 mg, 1.50 mmol), potassium carbonate (1382.1 mg, 10.00 mmol), and tetrakis (triphenylphosphine)-palladium (80.9 mg, 0.070 mmol) were dissolved into a mixture of THF (50 mL) and H₂O (5 mL), and the

mixture was ultrasonically degassed and refluxed at 70 °C for 8 h under an N₂ atmosphere. TLC was used to determine the progress of the reaction (VPE: VEA =20:1). Then, **1** was purified by column chromatography on silica gel with a mixture of PE and EA as an eluent (10:1 by volume), obtaining a yellow powder (278.8 mg, 40.8 % yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.08 (d, *J* =7.7 Hz, 1H), 7.94–7.86 (m, 2H), 7.73 (d, *J* =7.6 Hz, 1H), 7.40–7.31 (m, 4H), 7.14–7.05 (m, 8H). ¹³C NMR (101 MHz, chloroform-*d*) δ 153.98, 153.17, 148.46, 147.35, 133.59, 132.38, 129.91, 129.86, 129.41, 127.33, 125.06, 123.53, 122.63, 112.20. Mp: 140–142 °C.

Synthesis of **3**. **4** (685.5 mg, 1.50 mmol), pyridine-4-boronic acid (184.6 mg, 1.50 mmol), potassium carbonate (1382.1 mg, 10.00 mmol), and tetrakis (triphenylphosphine)-palladium (80.9 mg, 0.070 mmol) were dissolved into the mixture of THF (50 mL) and H₂O (5 mL), and the mixture was ultrasonically degassed and refluxed at 70 °C for 8 h under an N₂ atmosphere. TLC was used to determine the progress of the reaction (VPE: VEA =2:1). Then, **1** was purified by column chromatography on silica gel with a mixture of PE and EA as an eluent (1:1 by volume), obtaining a deep yellow powder (236.9 mg, 34.6 % yield). ¹H NMR (400 MHz, chloroform-*d*) δ 8.80–8.74 (m, 2H), 7.97–7.91 (m, 2H), 7.88 (dd, *J* =8.0, 3.4 Hz, 3H), 7.78 (d, *J* =7.4 Hz, 1H), 7.30 (t, *J* =7.8 Hz, 4H), 7.24–7.17 (m, 7H), 7.08 (t, *J* =7.3 Hz, 2H). ¹³C NMR (101 MHz, chloroform-*d*) δ 154.08, 153.65, 150.19, 148.52, 147.35, 144.73, 134.72, 130.18, 130.08, 129.43, 129.29, 128.95, 126.87, 125.10, 123.56, 123.49, 122.57, 60.39, 14.21. Mp: 147–149 °C. MS (ESI-APCI): calculated for [C₂₃H₁₈N₂+H] +457.1; found 457.1.

General procedure for the preparation of **5**, **6**, and **7**. BA (456.7 mg, 1.00 mmol), 1,4-dibromobutane (855.6 mg, 4.00 mmol), and potassium carbonate (138.2 mg, 1.00 mmol) were dissolved into acetone (20 mL), and the mixture was refluxed at 65 °C for 4 h. TLC was used to determine the progress of the reaction (VPE: VEA =3:1). Then, **5**, **6**, or **7** were purified by column chromatography on a silica gel with a mixture of PE and EA as an eluent (2:1 by volume).

White powder **5** (500.0 mg, 84.7 % yield): ¹H NMR (400 MHz, chloroform-*d*) δ 4.73 (d, *J* =2.3 Hz, 1H), 4.60 (dd, *J* =2.4, 1.4 Hz, 1H), 4.19–4.04 (m, 2H), 3.44 (t, *J* =6.6

Hz, 2H), 3.18 (dd, $J=11.2, 5.0$ Hz, 1H), 3.05–2.94 (m, 1H), 2.27–2.14 (m, 2H), 2.02–1.75 (m, 6H), 1.68 (s, 4H), 1.67–1.45 (m, 3H), 1.45–1.39 (m, 2H), 1.43–1.35 (m, 5H), 1.38–1.32 (m, 1H), 1.35–1.22 (m, 2H), 1.24–0.99 (m, 2H), 0.96 (s, 6H), 0.92 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H), 0.72–0.64 (m, 1H). ^{13}C NMR (101 MHz, chloroform- d) δ 176.09, 150.52, 109.63, 78.99, 62.84, 56.59, 55.38, 50.59, 49.44, 47.03, 42.44, 40.74, 38.87, 38.75, 38.34, 37.22, 37.06, 34.36, 32.98, 32.20, 30.65, 29.68, 29.51, 28.00, 27.51, 27.43, 25.56, 20.93, 19.38, 18.31, 16.14, 16.05, 15.36, 14.71. Mp: 67–69 °C. $[\alpha]_{\text{D}}^{20} = +70.2^\circ$ (c 0.1, CH₃OH).

White powder **6** (512.5 mg, 86.8 % yield): ^1H NMR (400 MHz, chloroform- d) δ 5.29 (t, $J=3.7$ Hz, 1H), 4.05 (t, $J=6.2$ Hz, 2H), 3.43 (t, $J=6.7$ Hz, 2H), 3.21 (dd, $J=11.3, 4.8$ Hz, 1H), 2.86 (dd, $J=13.7, 4.5$ Hz, 1H), 2.03–1.82 (m, 5H), 1.85–1.72 (m, 2H), 1.75–1.64 (m, 1H), 1.67–1.57 (m, 4H), 1.61–1.43 (m, 4H), 1.47–1.40 (m, 1H), 1.40–1.24 (m, 3H), 1.13 (s, 3H), 1.24–1.02 (m, 3H), 0.99 (s, 3H), 0.97 (s, 1H), 0.95–0.88 (m, 9H), 0.76 (d, $J=19.7$ Hz, 7H). ^{13}C NMR (101 MHz, chloroform- d) δ 176.09, 150.52, 109.63, 78.99, 62.84, 56.59, 55.38, 50.59, 49.44, 47.03, 42.44, 40.74, 38.87, 38.75, 38.34, 37.22, 37.06, 34.36, 32.98, 32.20, 30.65, 29.68, 29.51, 28.00, 27.51, 27.43, 25.56, 20.93, 19.38, 18.31, 16.14, 16.05, 15.36, 14.71. Mp: 65–68 °C. $[\alpha]_{\text{D}}^{20} = +66.5^\circ$ (c 0.1, CH₃OH).

White powder **7** (522.6 mg, 88.5 % yield): ^1H NMR (400 MHz, chloroform- d) δ 5.24 (t, $J=3.7$ Hz, 1H), 4.18–3.93 (m, 2H), 3.43 (t, $J=6.7$ Hz, 2H), 3.21 (dd, $J=10.8, 5.0$ Hz, 1H), 2.22 (dd, $J=11.4, 1.8$ Hz, 1H), 2.09–1.93 (m, 2H), 1.95 (dd, $J=5.1, 2.0$ Hz, 1H), 1.97–1.86 (m, 3H), 1.83–1.21 (m, 14H), 1.08 (s, 3H), 0.99 (s, 3H), 0.97–0.90 (m, 6H), 0.86 (d, $J=6.4$ Hz, 3H), 0.77 (d, $J=12.7$ Hz, 6H), 0.72 (dd, $J=11.6, 1.8$ Hz, 1H). ^{13}C NMR (101 MHz, chloroform- d) δ 177.51, 138.24, 125.63, 79.03, 63.16, 55.24, 52.90, 48.14, 47.57, 42.09, 39.58, 39.10, 38.89, 38.76, 38.64, 37.00, 36.78, 33.06, 30.68, 29.53, 28.16, 27.98, 27.34, 27.25, 24.25, 23.56, 23.31, 21.17, 18.33, 17.17, 17.02, 15.63, 15.46. Mp: 51–53 °C. $[\alpha]_{\text{D}}^{20} = +88.1^\circ$ (c 0.1, CH₃OH).

General procedure for the preparation of BA-1 and BA-3. **1** (161.1 mg, 0.50 mmol), **2** (174.1 mg, 0.50 mmol), or **3** (228.1 mg, 0.50 mmol), and **5** (295.2 mg, 0.50 mmol), **6** (295.2 mg, 0.50 mmol), or **7** (295.2 mg, 0.50 mmol) were dissolved into acetonitrile

(10 mL), and the mixture was refluxed at 90 °C for 12 h. TLC was used to determine the progress of the reaction (VDCM:VMeOH =80:1). Then, BA-1 and BA-3 were purified by column chromatography on silica gel with a mixture of DCM and MeOH as an eluent (40:1 by volume).

Yellow powder **BA-1** (250.1 mg, 54.8 % yield): ¹H NMR (400 MHz, chloroform-d) δ 9.26 (d, *J* =6.7 Hz, 2H), 8.07 (d, *J* =6.6 Hz, 2H), 7.69–7.63 (m, 2H), 7.36 (t, *J* =7.8 Hz, 4H), 7.24–7.14 (m, 6H), 7.10–7.03 (m, 2H), 4.96 (t, *J* =7.5 Hz, 2H), 4.70 (d, *J* =2.3 Hz, 1H), 4.59 (t, *J* =1.9 Hz, 1H), 4.22–4.06 (m, 3H), 3.17 (dd, *J* =11.4, 4.6 Hz, 1H), 3.00–2.89 (m, 1H), 2.25–2.16 (m, 1H), 2.20–2.09 (m, 2H), 2.08 (d, *J* =8.6 Hz, 1H), 1.81 (q, *J* =6.6 Hz, 4H), 1.66 (s, 3H), 1.71–1.50 (m, 3H), 1.50–1.18 (m, 7H), 1.22–1.08 (m, 2H), 1.07–0.85 (m, 8H), 0.81 (s, 3H), 0.73 (d, *J* =10.0 Hz, 6H), 0.69–0.61 (m, 1H). ¹³C NMR (101 MHz, chloroform-d) δ 176.21, 155.08, 152.35, 150.41, 145.73, 144.21, 129.87, 129.02, 126.34, 125.50, 123.72, 122.63, 120.17, 109.70, 78.90, 62.52, 60.39, 59.70, 56.56, 55.31, 50.51, 49.38, 46.99, 42.40, 40.71, 38.84, 38.70, 38.25, 37.16, 37.03, 34.32, 32.07, 30.61, 29.69, 28.50, 27.99, 27.38, 25.50, 21.03, 20.91, 19.32, 18.27, 16.13, 16.09, 15.39, 14.67, 14.19. Mp: 169–178 °C. [α]_D²⁰ =+9.3° (c 0.1, CH₃OH). HPLC purity: 96.43 %. MS (ESI-APCI): calculated for [C₅₇H₇₃N₂O₃] +833.5; found 833.5.

Deep red powder **BA-3** (281.0 mg, 53.7 % yield): ¹H NMR (400 MHz, chloroform-d) δ 9.52–9.46 (m, 2H), 8.96–8.89 (m, 2H), 8.31 (d, *J* =7.7 Hz, 1H), 7.95–7.83 (m, 3H), 7.32 (dd, *J* =8.5, 7.3 Hz, 4H), 7.24–7.08 (m, 8H), 5.13 (t, *J* =7.5 Hz, 2H), 4.71 (d, *J* =2.3 Hz, 1H), 4.59 (t, *J* =1.9 Hz, 1H), 4.27–4.07 (m, 2H), 3.14 (dd, *J* =11.5, 4.5 Hz, 1H), 3.01–2.90 (m, 1H), 2.25–2.17 (m, 1H), 2.21–2.13 (m, 1H), 2.19–2.00 (m, 1H), 1.85 (h, *J* =6.3, 5.9 Hz, 3H), 1.66 (s, 3H), 1.62–1.51 (m, 1H), 1.48–1.18 (m, 6H), 1.17–1.09 (m, 1H), 1.06–0.85 (m, 7H), 0.87–0.80 (m, 1H), 0.78 (s, 3H), 0.65 (d, *J* =7.6 Hz, 6H). ¹³C NMR (101 MHz, chloroform-d) δ 176.26, 153.98, 153.02, 152.64, 150.38, 149.53, 146.92, 144.48, 138.47, 132.26, 130.49, 129.55, 128.46, 126.41, 126.34, 125.55, 124.13, 122.99, 121.64, 109.74, 78.88, 62.32, 60.42, 56.58, 55.25, 50.48, 49.39, 47.00, 42.40, 40.70, 38.79, 38.66, 38.25, 37.12, 34.30, 30.61, 29.70, 28.63, 27.93, 27.34, 25.60, 25.49, 20.89, 19.32, 18.20, 16.09, 16.04, 15.31, 14.66. Mp:

235–242 °C. $[\alpha]_{\text{He-Ne}}^{20} = +6.2^\circ$ (c 0.1, CH₃OH). HPLC purity: 97.53 %. MS (ESI-APCI): calculated for [C₆₃H₇₅N₄O₃S] +967.5; found 967.5.

Dimethyl sulfoxide (DMSO) was uniformly used as the solvent for preparing BA-1 and BA-3 stock solutions. The stock concentrations of BA-1 and BA-3 were both set to 20 mM. This concentration was determined based on the solubility of the compounds in DMSO and the experimental concentration range required for PDT on melanoma cells, which can ensure that the stock solution has good stability and can be diluted to the required working concentration without obvious precipitation. A two-step gradient dilution method was adopted to prepare the working concentration of BA-1 and BA-3 from the stock solution. First, the 20 mM stock solution was diluted with DMSO to a 2 mM intermediate dilution solution; then, the intermediate dilution solution was further diluted with complete Dulbecco's Modified Eagle Medium (DMEM) (containing 10% fetal bovine serum and 1% penicillin-streptomycin) to the working concentrations required for the experiment (the working concentration range of BA-1 was 0.5~10 μ M, and that of BA-3 was 0.5~20 μ M). During the dilution process, the solution was gently mixed by vortex oscillation for 30 seconds each time to ensure uniform concentration and avoid local concentration differences. After dilution to the working concentration, the final volume percentage of DMSO (solvent) in the cell culture medium was 0.1% for both BA-1 and BA-3 experimental groups. The vehicle control group was added with DMSO diluted with complete DMEM, and the final volume percentage of DMSO in the culture medium was also 0.1%, which was completely consistent with that of the BA-1 and BA-3 experimental groups. This ensures that the interference of DMSO itself on the experimental results can be effectively excluded, and the experimental effects can be accurately attributed to the compounds and PDT synergistic effect.

Table SI The antibodies involved in the article:

Antibody	Catalog number	Dilution	Supplier
C-MYC	67447-1-Ig	1:1000	Proteintech
PCNA	ab92552	1:5000	abcam

Antibody	Catalog number	Dilution	Supplier
MMP2	Ab86607	1:1000	abcam
GAPDH	60004-1-Ig	1:50000	Proteintech
Bax	50599-2	1:1000	Proteintech
Bcl-2	12789-1	1:1000	Proteintech
Cleaved Caspase-3	25128-1	1:1000	Proteintech
Cleaved Caspase-9	28794-1	1:5000	Proteintech

Table SII BA-AIEgens inhibits melanoma cell proliferation

Compoun	A375 cell IC ₅₀ (μM) ^a			B16F0 cell IC ₅₀ (μM) ^a		
d	Dark	Light	PI	Dark	Light	PI ^f
BA-1	2.69 ± 0.44 ^b	0.24 ± 0.03 ^b	11.2 1	4.76 ± 0.45 ^d	0.43 ± 0.04 ^d	11.0 7
BA-3	10.94 ± 1.57 ^c	0.26 ± 0.03 ^c	42.0 8	39.52 ± 4.1 ^e	0.49 ± 0.03 ^e	80.6 5

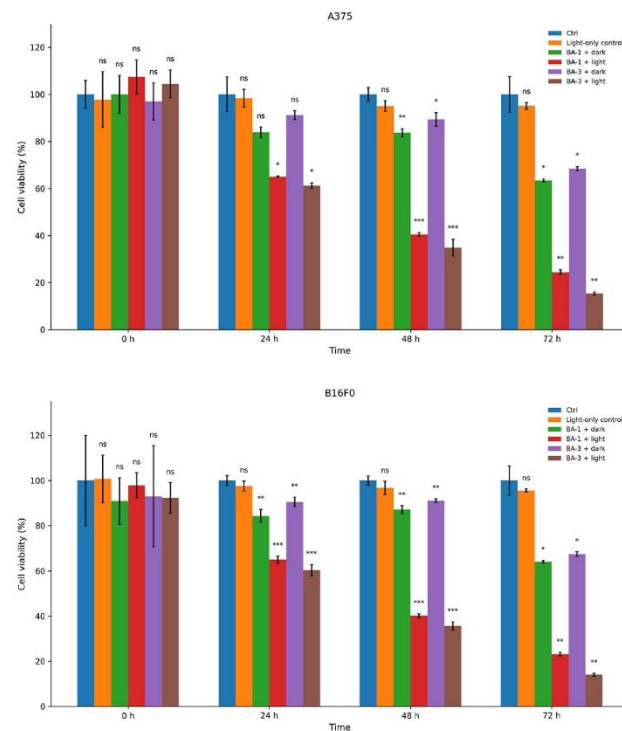


Figure SI CCK-8 analysis of A375 and B16F0 cell viability under different

treatments at 0, 24, 48, and 72 h. Data are shown as mean $\pm$ SD (n = 5). Statistical significance was determined versus the Ctrl group. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

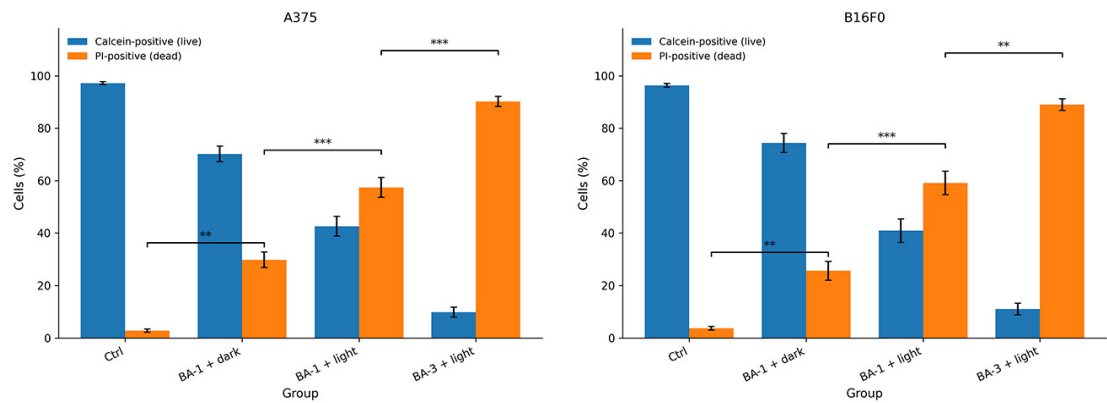


Figure SII Quantitative analysis of Calcein-positive (live) and PI-positive (dead) cells in each treatment group. Data are presented as mean $\pm$ SD (n = 3).

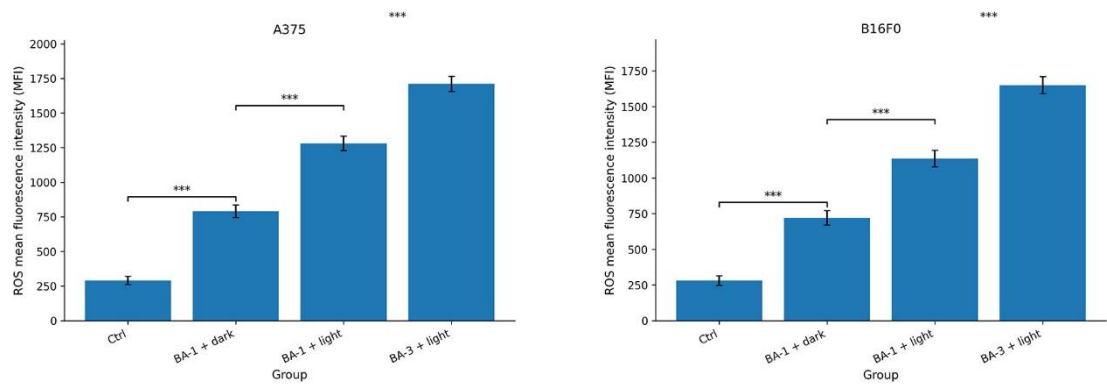


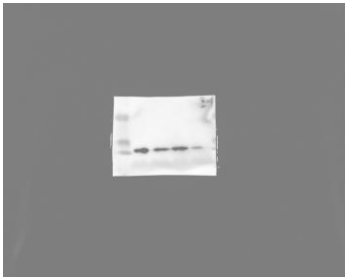
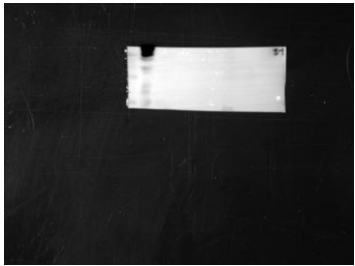
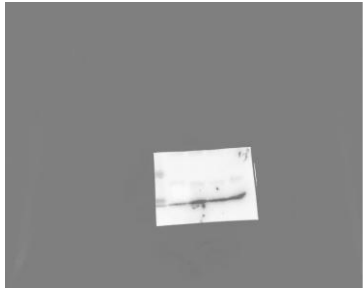
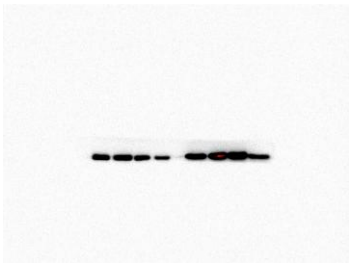
Figure SIII Quantitative analysis of intracellular ROS levels measured by flow cytometry and expressed as mean fluorescence intensity (MFI). Data are presented as mean $\pm$ SD (n = 3).

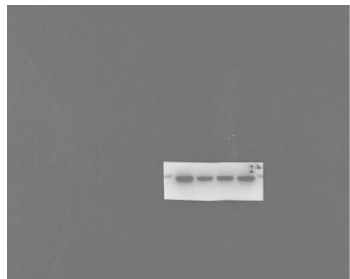
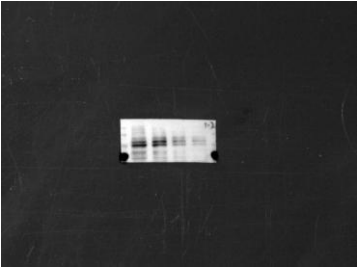
Reference

- Wang X, Zhang X, Zheng G, Dong M, Huang Z, Lin L, Yan K, Zheng J, Wang J: **Mitochondria-targeted pentacyclic triterpene NIR-AIE derivatives for enhanced chemotherapeutic and chemo-photodynamic combined therapy.** *Eur J Med Chem* 2024, **264**:115975.

Table SII The Western uncropped Gels and Blots involved in the article:

File label	Extracted band / strip
B16F0-Bax (Composite)	

B16F0-Bcl-2(Composite)	
B16F0-C-MYC	
B16F0-Cleaved caspase-3(Composite)	
B16F0-Cleaved caspase-9(Chemiluminescence)	
B16F0-Cleaved caspase-9(Composite)	
B16F0-GAPDH-A	

B16F0-GAPDH-B(Composite)	
B16F0-MMP2	
B16F0-PCNA(Composite)	

Western blotting images depicting the results of the second and third experimental replicates

 A375-MMP2 (Lane 3 to Lane 6)	 A375-GAPDH (The second result)	 A375-GAPDH(below) (The third result)	 A375-C-MYC (Above-Lane 6 to Lane 9)	 A375-C-MYC(Above)+Bcl-2(below)
 A375-Bcl-2 (The second result) .scn	 A375-Bax (The third result) .scn	 A375 (Lane 2 to Lane 5) +B16F0	 A375 (Lane 2 to Lane 5) +B16F0	 A375 (Lane 2 to Lane 5) +B16F0
 A375 (Lane 2 to Lane 5) +B16F0	 A375 (Lane 2 to Lane 5) +B16F0	 A375 (Lane 2 to Lane 5) +B16F0	 B16F0-β-actin (The third result) .scn	 B16F0-β-actin (The second result)
 B16F0-PCNA (Lane 6 to Lane 9)	 B16F0-MMP2 (The second result)	 B16F0-MMP2(The second line)+C-	 B16F0-C-MYC-(Lane 6 to Lane 9)	 B16F0-C-MYC-(below) (The second result)
 B16F0-Bcl-2 (The second result)	 B16F0-Bax(The second line)-	 A375-MMP2 (The second result)		