Supporting Information

Novel Anthraquinone Compounds Induce Cancer Cell Death Through Paraptosis

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Table of Contents

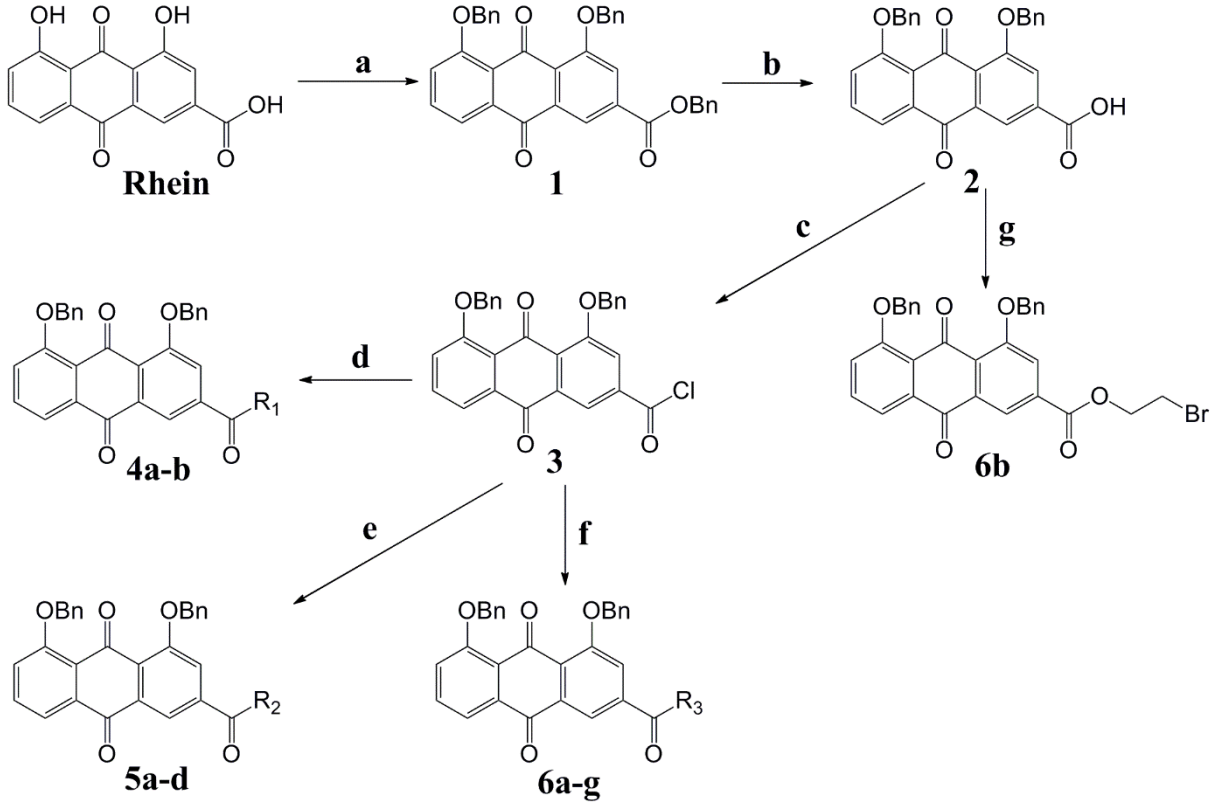
Materials and Methods

General Methods

All reactions were conducted under an inert gas atmosphere (nitrogen) using a Teflon-coated magnetic stir bar. All the reagents purchased from Aladdin were used without purification. Solvents were dried by standard methods. Reactions were monitored by thin layer chromatography(TLC) Merck, flash chromatography were performed on silica gel (100-200 mesh). Chemical shifts (δ) were reported in parts per million (ppm), and proton coupling patterns are described as s (singlet), dd (doublet of doublet), d (doublet), t (triplet), q (quartet) or m (multiplet). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-500 MHz spectrometer, or DMSO-d_6 on a Bruker-600 MHz spectrometer; tetramethylsilane (TMS) used as an internal standard. IR spectra were run on FI-IR Spectrometer (PerkineElmer). Melting points were measured in open capillaries and are uncorrected. All compounds were routinely checked by TLC with silica gel GF-254 aluminium plate and viewed under UV light at 254 nm.

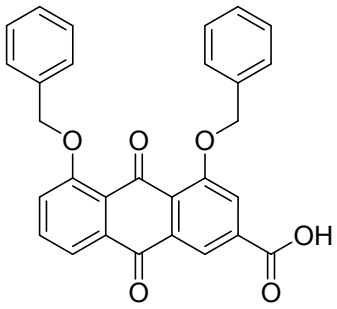
Synthesis and characterization data for anthraquinone compounds

To the best of our knowledge, the synthesis of compounds has never been reported before except for compound **6a**. All these target synthesized compounds were novel chemical entities characterized by IR, ^{1}H NMR, ^{13}C NMR, and high resolution mass (ESI) spectra. The results are in detail presented in the experimental part.



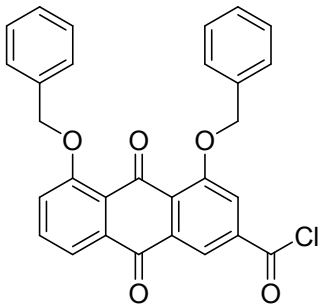
Scheme S1. Synthesis route of the target compound, reagents and conditions. a) DMF, K_2CO_3, Benzyl chloride, 60℃, 24~48 h; b) NaOH, EtOH/H_2O (3:1), 50℃, 4~6 h; c) DCM, Oxalyl chloride, DMF, rt~30℃, 0.5~1 h; d) DCM, K_2CO_3, rt, 0.5~1 h; e) DCM, K_2CO_3, rt, 1~2 h; f) DCM, K_2CO_3, rt, 1 h; g) DMF, K_2CO_3, 1,2-dibromoethane, 60℃, 4~6 h.

***Dibenzylrhein* (2)**



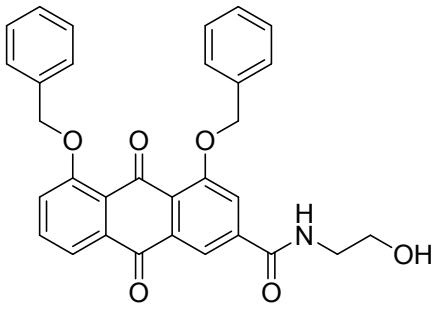
Rhein (248.2 mg, 1 mmol), potassium carbonate (2 g, 14.48 mmol) suspended in anhydrous *N,N*-dimethyl formamide (DMF, 30 mL) in a dry round bottom flask and warmed to 60℃. Then benzyl chloride (1 mL, 8.69 mmol) and sodium iodide(15 mg, 0.1 mmol) was added and stirred at 60 ℃ for 24~48 h under N_2. TLC (petroleum ether: ethyl acetate = 5:1) showed complete consumption of the starting material, the mixture's color turned from deep purple to yellow. The mixture was poured into dichloromethane (DCM, 100 mL), and washed with water (3×50 mL), dried with anhydrous sodium sulfate and evaporated. The residue was recrystallization from (DCM+MeOH) to give the desired product as a yellow solid compound **1**, yied 92%. Then compound **1** (480 mg, 0.86 mmol) was suspended in EtOH/H_2O (50 mL, 3: 1 v/v), NaOH (2 g, 50 mmol) was then added. The mixture was stirred at 50℃ for 4~6 h. The suspension was cooled down in an ice-bath and acidified with HCl (1 M). The precipitation was filtered to give yellow solid as the product of compound **2**, Yied 95%. Mp:251-252℃. IR (KBr, cm^{-1}) : 3401, 3064, 3033, 2920, 2866, 1671, 1599, 1585, 1554, 1498, 1467, 1444, 1431, 1375, 1313, 1288, 1233, 1115, 1082, 1062, 1030, 1002, 886, 794, 749, 733, 695.

1,8-Bis(benzyloxy)-9,10-anthraquinone-3-carbonyl chloride (3)



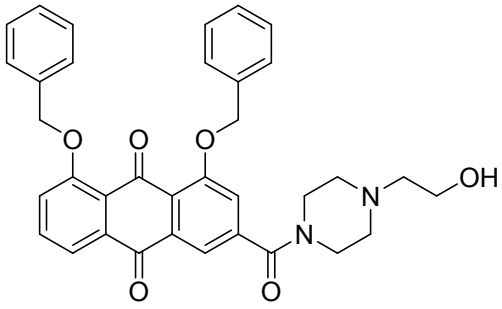
To anhydrous dichloromethane (5 mL) were added oxalyl chloride (100 uL, 9.66 mmol) and anhydrous dimethyl formamide (1 drop). The solution was stirred for 0.5 h at room temperature and was added dibenzylrhein (**2**) (100 mg, 0.215 mmol). The mixture was heated to 30℃ for 1 h. All solvents were removed under reduced pressure to get acyl chloride, which was dissolved in DCM (5 mL).

1,8-Bis(benzyloxy)-9,10-anthraquinone-*N*-(2-hydroxyethyl)-3-carboxamide (4a)



Potassium carbonate (1g, 7.24mmol) was added to a solution of 2-aminoethanol hydrochloride (105 mg, 1.075 mmol) in DCM (5 mL). Stirred at room temperature for 15 min, Then acid chloride (**3**) was slowly added and stirred at room temperature for 0.5~1 h. The mixture was poured into DCM (30 mL), and washed with water (3×30 mL), dried with anhydrous sodium sulfate and evaporated. The residue was purified by column chromatography on silica gel with DCM/MeOH (30:1, *V: V*) to give the pure title compounds **4a** as a yellow solid. Yield: 60%. Mp:187-189℃. IR (KBr, cm^{-1}) : 3510, 3313, 3065, 3034, 2934, 2877, 1672, 1601, 1585, 1543, 1498, 1451, 1418, 1383, 1330, 1293, 1237, 1127, 1082, 1060, 1030, 1001, 899, 834, 790, 745, 733, 695, 464. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.87 (s, 1H), 7.78 (d, *J* = 7.5 Hz, 1H), 7.65 – 7.55 (m, 5H), 7.43 – 7.37 (m, 4H), 7.36 – 7.30 (m, 3H), 7.05 (s, 1H), 5.29 (s, 2H), 5.27 (s, 2H), 3.84 (dd, *J* = 9.9, 5.1 Hz, 2H), 3.61 (dd, *J* = 10.0, 5.2 Hz, 2H), 2.79 (d, *J* = 3.9 Hz, 1H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.26, 181.70, 166.20, 158.69, 158.37, 138.82, 136.29, 136.02, 134.76, 134.50, 134.07, 128.65, 128.64, 127.92, 127.87, 126.95, 126.77, 124.44, 120.38, 119.56, 118.81, 116.07, 71.12, 71.11, 61.91, 43.01. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{31}H_{26}NO_6^+$:508.1754, found: 508.1761.

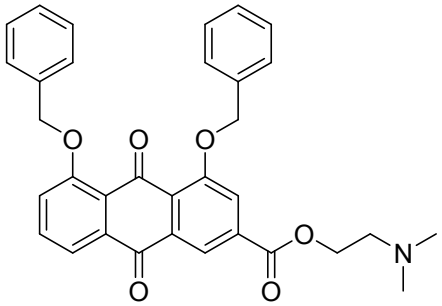
1,8-bis(benzyloxy)-9,10-anthraquinone-*N*-(4-(2-hydroxyethyl)piperazine)-3-carboxamide (4b)



Preparation of **4b** is followed the procedure for **4a** described above. Yellow solid, Yield: 75%. Mp:155-157℃. IR (KBr, cm^{-1}) : 3463, 3059, 3032, 2923, 2869, 2811, 1675, 1633, 1601, 1586, 1497, 1473, 1443, 1384, 1473, 1443, 1384, 1325, 1289, 1236, 1132, 1083, 1064, 1029, 1000, 977, 896, 792, 750, 736, 695, 467. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.83 (d, *J* = 1.4 Hz, 1H), 7.63 – 7.60 (m, 5H), 7.41 – 7.38 (m, 4H), 7.36 – 7.32 (m, 4H), 5.36 (s, 2H), 5.33 (s, 2H), 3.82 (s, 2H), 3.68 – 3.63 (m, 2H), 3.34 (s, 2H), 2.63 (s, 1H), 2.61 – 2.59 (m, 2H), 2.51 (s, 2H), 2.40 (s, 2H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.27, 181.61, 168.02, 158.50, 158.35, 140.61, 136.35, 136.08,

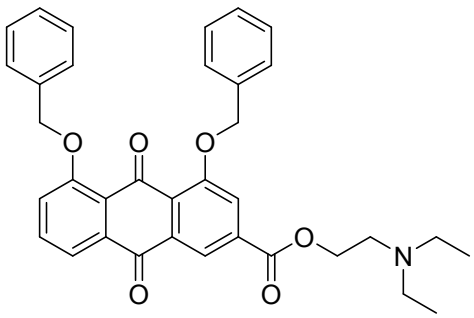
134.98, 134.65, 134.02, 128.69, 128.63, 127.90, 127.83, 126.73, 126.71, 125.39, 124.56, 120.37, 119.53, 118.43, 117.27, 71.19, 71.12, 59.28, 57.74, 53.08, 52.44, 47.48, 42.16. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{33}N_2O_6^+$: 577.2333, found : 577.2318.

1,8-bis(benzyloxy)-9,10-anthraquinone-O-(dimethylamino)ethyl-3-carboxylate (5a)



Potassium carbonate (1 g, 7.24 mmol) was added to a solution of *N*,*N*-dimethyl ethanolamine (0.1 mL) in DCM (5 mL), then acid chloride (**3**) was slowly added and stirred at room temperature for 2~4h. The mixture was poured into DCM (30mL), and washed with water (3×30mL), dried with anhydrous sodium sulfate and evaporated. The residue was purified by column chromatography on silica gel with DCM/MeOH (30:1, *V: V*) to give the pure title compounds **5a** as a yellow solid. Yield: 65%, Mp:133-134℃. IR (KBr, cm^{-1}) : 3064, 3032, 2924, 2858, 2825, 2774, 1723, 1672, 1600, 1586, 1498, 1454, 1422, 1383, 1333, 1290, 1230, 1155, 1115, 1082, 1063, 1031, 993, 889, 860, 792, 745, 695, 534, 465. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, *J* = 1.4 Hz, 1H), 8.00 (d, *J* = 1.4 Hz, 1H), 7.86 (dd, *J* = 7.6, 0.7 Hz, 1H), 7.66 – 7.59 (m, 5H), 7.42 – 7.38 (m, 4H), 7.36 – 7.32 (m, 3H), 5.37 (s, 2H), 5.31 (s, 2H), 4.49 (t, *J* = 5.8 Hz, 2H), 2.78 (t, *J* = 5.8 Hz, 2H), 2.37 (s, 6H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.15, 181.66, 164.97, 158.35, 158.32, 136.40, 136.13, 134.99, 134.73, 134.68, 134.05, 128.62, 128.60, 127.87, 127.79, 127.52, 126.87, 126.74, 124.67, 120.33, 120.27, 120.15, 119.54, 71.28, 71.12, 63.48, 57.54, 45.63. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{33}H_{30}NO_6^+$: 536.2067, found : 536.2066.

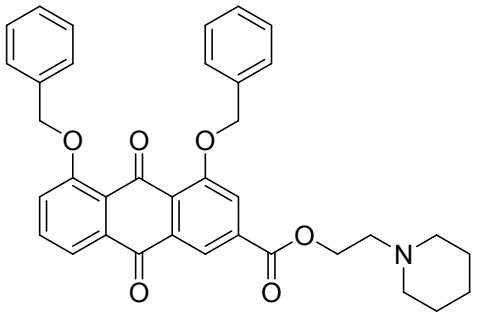
1,8-bis(benzyloxy)-9,10-anthraquinone-O-(diethylamino)ethyl-3-carboxylate (5b)



Preparation of **5b** is followed the procedure for **5a** described above. Yield: 64%. Mp:142-144℃. IR (KBr, cm^{-1}) : 3063, 3034, 2969, 2932, 2868, 2793, 1726, 1671, 1602, 1586, 1498, 1454, 1423, 1382, 1334, 1292, 1231, 1209, 1083, 1064, 1005, 888, 745, 731, 695, 466. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, *J* = 1.5 Hz, 1H), 8.00 (d, *J* = 1.5 Hz, 1H), 7.86 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.67 – 7.59 (m, 5H),

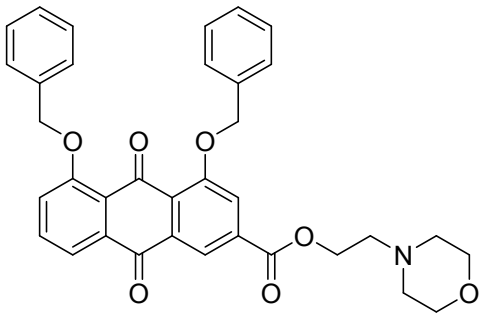
7.42 – 7.37 (m, 4H), 7.37 – 7.30 (m, 3H), 5.36 (s, 2H), 5.31 (s, 2H), 4.45 (t, J = 6.3 Hz, 2H), 2.90 (t, J = 6.3 Hz, 2H), 2.67 (q, J = 7.1 Hz, 4H), 1.10 (t, J = 7.1 Hz, 6H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.17, 181.72, 164.97, 158.32, 158.29, 136.37, 136.09, 134.99, 134.81, 134.73, 134.05, 127.86, 127.79, 127.47, 126.81, 126.70, 124.67, 120.28, 120.21, 120.02, 119.53, 71.21, 71.08, 64.16, 50.88, 47.82, 12.01. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{34}NO_6^+$: 564.2381, found : 564.2367.

1,8-bis(benzyloxy)-9,10-anthraquinone-O-(piperidin-1-yl)ethyl-3-carboxylate (5c)



Preparation of **5c** is followed the procedure for **5a** described above. Yield: 70%. Mp:153-155℃. IR (KBr, cm^{-1}) : 3089, 3064, 3031, 2934, 2854, 2783, 1716, 1671, 1601, 1586, 1498, 1453, 1442, 1423, 1383, 1335, 1290, 1229, 1114, 1083, 1063, 1030, 1002, 889, 745, 732, 694, 520, 460. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 1.5 Hz, 1H), 7.99 (d, J = 1.5 Hz, 1H), 7.88 – 7.81 (m, 1H), 7.66 – 7.58 (m, 5H), 7.42 – 7.38 (m, 4H), 7.36 – 7.32 (m, 3H), 5.37 (s, 2H), 5.30 (s, 2H), 4.53 (t, J = 6.1 Hz, 2H), 2.84 (t, J = 6.0 Hz, 2H), 2.58 (s, 4H), 1.66 – 1.62 (m, 4H), 1.47 (d, J = 4.9 Hz, 2H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.19, 181.69, 164.89, 158.32, 158.29, 136.38, 136.10, 134.98, 134.75, 134.70, 134.06, 128.63, 128.61, 127.87, 127.78, 127.45, 126.83, 126.70, 124.63, 120.25, 120.23, 120.05, 119.53, 71.21, 71.07, 63.18, 57.03, 57.03, 54.69, 25.69, 24.00. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{36}H_{34}NO_6^+$: 576.2381, found : 576.2390.

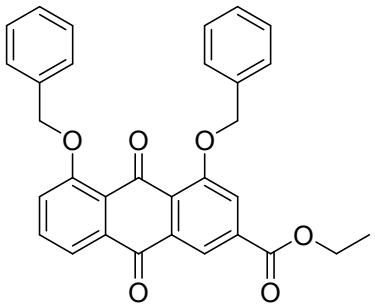
1,8-bis(benzyloxy)-9,10-anthraquinone-O-morpholinoethyl-3-carboxylate (5d)



Preparation of **5d** is followed the procedure for **5a** described above. Yield: 80%. Mp:159-161℃. IR (KBr, cm^{-1}) : 3089, 3064, 3030, 2961, 2890, 2853, 2819, 1717, 1670, 1601, 1587, 1498, 1453, 1423, 1384, 1335, 1292, 1228, 1117, 1082, 1063, 1031, 1002, 993, 887, 746, 734, 695, 510, 460. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (d, J = 1.5 Hz, 1H), 7.99 (d, J = 1.4 Hz, 1H), 7.86 (dd, J = 7.7, 0.9 Hz, 1H), 7.66 – 7.60 (m, 5H), 7.42 – 7.38 (m, 4H), 7.36 – 7.32 (m, 3H), 5.38 (s, 2H), 5.32 (s, 2H), 4.51 (t, J = 5.9 Hz, 2H), 3.73 (t, J = 6.0 Hz, 4H), 2.81 (t, J = 5.9 Hz, 2H), 2.59 (t, J = 5.4 Hz, 4H). ^{13}C NMR

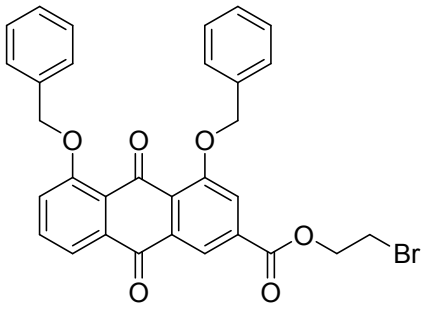
(125.8 MHz, $CDCl_3$) δ 183.17, 181.63, 164.90, 158.37, 158.34, 136.39, 136.09, 135.03, 134.71, 134.67, 134.07, 128.64, 128.61, 127.90, 127.80, 127.55, 126.85, 126.73, 126.66, 124.66, 120.30, 120.24, 120.11, 119.55, 71.28, 71.13, 66.83, 62.69, 56.98, 53.76. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{32}NO_7^+$: 578.2177, found : 578.2178.

Ethyl 1,8-bis(benzyloxy)-9,10-anthraquinone-3-carboxylate (6a)



Potassium carbonate (1 g, 7.24 mmol) was added to a solution of ethanol (2 mL) in DCM (5 mL), then acid chloride (**3**) was slowly added and stirred at room temperature for 1h. The mixture was poured into DCM (30 mL), and washed with water (3×30 mL), dried with anhydrous sodium sulfate and evaporated. The residue was purified by column chromatography on silica gel with DCM/PE (2:1, *V: V*) to give the desired product **6a** as a yellow solid. Yied 90%, Mp:176-178℃. IR (KBr, cm^{-1}) : 3090, 3064, 3033, 2987, 2904, 2868, 1717, 1670, 1601, 1587, 1498, 1470, 1454, 1444, 1423, 1367, 1336, 1292, 1229, 1083, 1064, 1031, 1004, 890, 745, 734, 694, 466. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (d, *J* = 1.5 Hz, 1H), 8.01 (d, *J* = 1.4 Hz, 1H), 7.87 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.67 – 7.60 (m, 5H), 7.42 – 7.38 (m, 4H), 7.36 – 7.32 (m, 3H), 5.38 (s, 2H), 5.33 (s, 2H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.27, 181.76, 164.98, 158.32, 158.29, 136.37, 136.11, 135.01, 134.99, 134.77, 134.04, 128.63, 128.61, 127.87, 127.80, 127.47, 126.86, 126.72, 124.73, 120.24, 120.22, 120.07, 119.56, 71.27, 71.12, 61.92, 14.30. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{31}H_{25}O_6^+$: 493.1646, found : 493.1659.

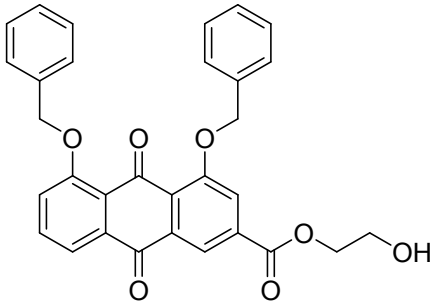
1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-bromoethyl)-3-carboxylate (6b)



Dibenzylrhein (100mg, 0.215 mmol), potassium carbonate (1g, 7.24 mmol) suspended in anhydrous dimethyl formamide (DMF, 5 mL) in a dry round bottom flask and warmed to 60℃. Then 1,2-dibromoethane (0.5 mL, 5.77 mmol) was added and stirred at 60℃ for 1 h under N_2. The mixture was poured into 30 mL dichloromethane, washed with brine, dried over Na_2SO_4, and

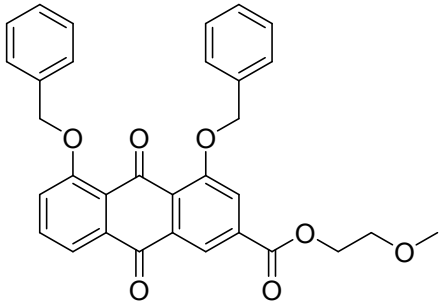
concentrated under reduced pressure to give a crude product. The residue was purified by column chromatography on silica gel (DCM/PE 2:1) to give the desired product **6b** as a yellow solid, yied 76%, Mp:209-210℃. IR (KBr, cm^{-1}) : 3089, 3065, 3029, 2923, 1717, 1672, 1603, 1588, 1498, 1449, 1423, 1377, 1337, 1293, 1231, 1219, 1099, 1082, 1065, 1030, 1013, 997, 891, 746, 730, 696, 574, 466. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, *J* = 1.5 Hz, 1H), 8.00 (d, *J* = 1.4 Hz, 1H), 7.87 (dd, *J* = 7.7, 0.8 Hz, 1H), 7.67 – 7.61 (m, 5H), 7.42 – 7.38 (m, 4H), 7.37 – 7.32 (m, 3H), 5.38 (s, 2H), 5.33 (s, 2H), 4.68 (t, *J* = 6.2 Hz, 2H), 3.67 (t, *J* = 6.2 Hz, 2H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.11, 181.67, 164.47, 158.33, 136.34, 136.00, 135.09, 134.71, 134.12, 134.04, 128.66, 128.63, 127.94, 127.83, 127.77, 126.90, 126.72, 124.65, 120.38, 120.25, 120.12, 119.57, 71.27, 71.10, 64.89, 28.30. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{31}H_{24}BrO_6^+$: 571.0751, found : 571.0756.

1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-hydroxyethyl)-3-carboxylate (6c)



Preparation of **6c** is followed the procedure for **6a** described above. Yield: 92%. Mp:194-196℃. IR (KBr, cm^{-1}) : 3416, 3277, 3065, 3031, 2926, 2870, 1724, 1673, 1600, 1586, 1498, 1442, 1423, 1384, 1335, 1290, 1229, 1155, 1115, 1081, 1060, 1030, 996, 904, 854, 792, 746, 696, 531, 465. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.26 (d, *J* = 1.4 Hz, 1H), 8.06 (d, *J* = 1.4 Hz, 1H), 7.83 – 7.77 (m, 1H), 7.76 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.69 – 7.62 (m, 5H), 7.45 – 7.40 (m, 4H), 7.39 – 7.34 (m, 2H), 5.42 (s, 2H), 5.34 (s, 2H), 5.04 (t, *J* = 5.7 Hz, 1H), 4.41 – 4.34 (m, 2H), 3.76 (dd, *J* = 9.9, 5.5 Hz, 2H). ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 182.64, 180.94, 164.55, 157.94, 157.84, 136.87, 136.65, 134.66, 134.64, 134.43, 134.23, 128.43, 127.77, 127.71, 127.01, 126.98, 126.91, 123.97, 120.70, 119.83, 119.01, 118.79, 70.55, 70.26, 67.55, 59.02. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{31}H_{25}O_7^+$: 509.1595, found : 509.1601.

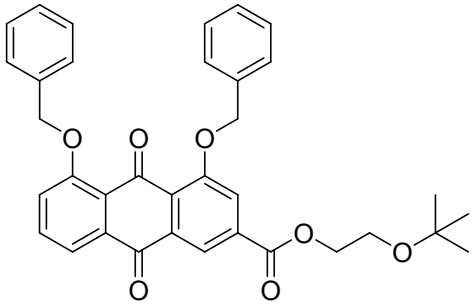
1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-methoxyethyl)-3-carboxylate (6d)



Preparation of **6d** is followed the procedure for **6a** described above. Yield: 88%. Mp:156-158℃. IR

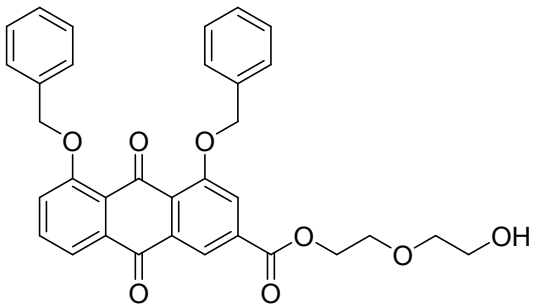
(KBr, cm^{-1}): 3090, 3066, 3031, 2986, 2932, 2892, 2823, 1718, 1674, 1600, 1586, 1498, 1455, 1443, 1423, 1384, 1369, 1337, 1291, 1269, 1230, 1130, 1111, 1083, 1058, 1031, 990, 909, 869, 795, 774, 745, 734, 695, 535, 464. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 1.5 Hz, 1H), 8.01 (d, *J* = 1.4 Hz, 1H), 7.87 (dd, *J* = 7.7, 0.8 Hz, 1H), 7.67 – 7.61 (m, 5H), 7.42 – 7.38 (m, 4H), 7.37 – 7.32 (m, 3H), 5.38 (s, 2H), 5.33 (s, 2H), 4.53 (t, *J* = 6.0 Hz, 2H), 3.76 (t, *J* = 6.0 Hz, 2H), 3.45 (s, 3H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.19, 181.74, 165.00, 158.31, 158.27, 136.35, 136.07, 135.00, 134.74, 134.58, 134.06, 128.62, 128.61, 127.88, 127.80, 127.59, 126.86, 126.71, 124.70, 120.40, 120.22, 120.17, 119.55, 71.27, 71.10, 70.29, 64.87, 59.12. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{32}H_{27}O_7^+$: 523.1751, found : 523.1755.

1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-(tert-butoxy)ethyl)-3-carboxylate (6e)



Preparation of **6e** is followed the procedure for **6a** described above. Yield: 85%. Mp:191-193℃. IR (KBr, cm^{-1}): 3065, 3034, 2969, 2909, 2870, 1716, 1669, 1602, 1587, 1498, 1453, 1424, 1338, 1291, 1230, 1201, 1084, 1065, 1031, 1009, 984, 889, 870, 747, 732, 696, 534, 466. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 1.5 Hz, 1H), 8.01 (d, *J* = 1.4 Hz, 1H), 7.87 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.68 – 7.59 (m, 5H), 7.43 – 7.38 (m, 4H), 7.37 – 7.31 (m, 3H), 5.36 (s, 2H), 5.32 (s, 2H), 4.47 (t, *J* = 6.0 Hz, 2H), 3.73 (t, *J* = 6.0 Hz, 2H), 1.25 (s, 9H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.16, 181.75, 164.99, 158.31, 158.27, 136.38, 136.09, 135.00, 134.81, 134.75, 134.04, 128.62, 128.61, 127.86, 127.79, 127.51, 126.81, 126.71, 124.71, 120.37, 120.20, 120.04, 119.54, 73.48, 71.21, 71.09, 65.64, 59.80, 27.51. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{33}O_7^+$: 565.2221, found : 565.2219.

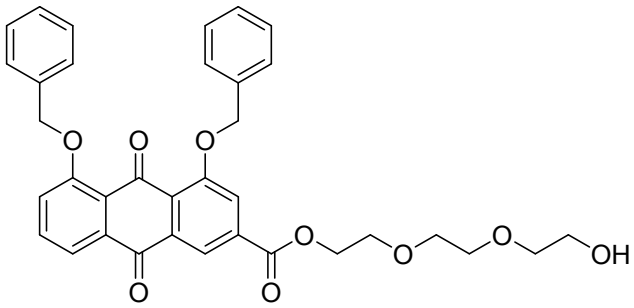
1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-hydroxyethoxy)ethyl)-3-carboxylate (6f)



Preparation of **6f** is followed the procedure for **6a** described above. Yield: 175-177℃. IR (KBr, cm^{-1}): 3512, 3088, 3063, 3028, 2948, 2918, 2866, 1712, 1669, 1603, 1587, 1498, 1471, 1454, 1423, 1378, 1337, 1293, 1223, 1132, 1118, 1099, 1063, 1031, 1009, 978, 892, 858, 746, 730, 697, 533, 467. ^{1}H

NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 1.4 Hz, 1H), 8.01 (d, *J* = 1.3 Hz, 1H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.67 – 7.61 (m, 5H), 7.42 – 7.38 (m, 4H), 7.37 – 7.32 (m, 3H), 5.38 (s, 2H), 5.33 (s, 2H), 4.57 – 4.52 (m, 2H), 3.90 – 3.86 (m, 2H), 3.79 (dd, *J* = 9.4, 5.5 Hz, 2H), 3.70 – 3.66 (m, 2H), 2.10 (t, *J* = 6.1 Hz, 1H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.23, 181.72, 165.01, 158.34, 136.35, 136.07, 135.03, 134.73, 134.52, 134.09, 128.64, 128.63, 127.90, 127.82, 127.63, 126.85, 126.72, 124.70, 120.36, 120.27, 120.11, 119.58, 72.48, 71.27, 71.12, 68.99, 64.82, 61.81. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{33}H_{29}O_8^+$: 553.1857, found 553.1859.

1,8-bis(benzyloxy)-9,10-anthraquinone-O-(2-(2-hydroxyethoxy)ethoxy)ethyl)-3-carboxylate(6g)



Preparation of **6g** is followed the procedure for **6a** described above. Yield: 80%. Mp:130-131℃. IR (KBr, cm^{-1}): 3536, 3091, 3065, 3034, 2889, 1723, 1673, 1600, 1587, 1498, 1442, 1424, 1381, 1355, 1334, 1288, 1269, 1233, 1211, 1105, 1080, 1062, 1030, 994, 941, 887, 873, 745, 731, 695, 534, 466. ^1H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, *J* = 1.5 Hz, 1H), 8.03 (d, *J* = 1.4 Hz, 1H), 7.85 (dd, *J* = 7.7, 0.8 Hz, 1H), 7.68 – 7.59 (m, 5H), 7.42 – 7.37 (m, 4H), 7.37 – 7.31 (m, 3H), 5.37 (s, 2H), 5.31 (s, 2H), 4.54 (dd, *J* = 5.4, 4.1 Hz, 2H), 3.88 (dd, *J* = 5.4, 4.1 Hz, 2H), 3.75 – 3.70 (m, 6H), 3.65 – 3.62 (m, 2H), 2.54 (s, 1H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 183.22, 181.67, 164.97, 158.31, 136.36, 136.13, 134.96, 134.69, 134.55, 134.06, 128.61, 128.60, 127.84, 127.78, 127.54, 126.84, 126.69, 124.65, 120.33, 120.22, 120.16, 119.53, 72.53, 71.23, 71.06, 70.77, 70.38, 69.01, 64.83, 61.80. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{35}H_{33}O_9^+$: 597.2119, found: 597.2106.

Cell culture:

CNE-1, CEN-2, HepG2 and SMMC-7721 cells lines were friendly provided by Guangxi Cancer Institute (Nanning, China). CNE-1, CEN-2 and SMMC-7721 cells were cultured in RPMI-1640 medium (Gibco), while HepG-2 cells were cultured in DMEM medium (Gibco). Both media were sup-plemented with 10% fetal calf serum (Gibco), penicillin (50 unit·mL^{-1}) and streptomycin (50 μg·mL^{-1}) and were kept in a cell incubator at 37℃ with humid atmosphere at 5% CO_2.

Determination of cell viability:

Cell viability was determined by MTT or CCK-8 assay.

MTT assay

For the cytotoxicity assays, cells were seeded into 96-well plate (4~5×10^3 in 100 μL per well). Test

compounds were dissolved or suspended in DMSO to make 10000 μM stock solutions and diluted with culture medium to various concentrations (final DMSO concentration < 0.5 %). Addition of test compound was performed immediately after adherent cells for 24 h. After incubation for 48 h at 37 ℃ in a humidified atmosphere with 5% CO_2, MTT (20 μL, 5 mg·mL^{-1} in phos-phate-buffered saline) was added to each well, and the plate was incubated for another 4h. The culture medium was then aspirated and DMSO (100 μL) was added to each well. The 96-well plate was read using a microarray reader for optical density at 490 nm. All tests were performed in triplicate and the optical absorption read-out was normalized to percentage of maximum cytotoxicity. The 50%-proliferation-inhibition concentration (IC_{50}) values were determined with the Log it method. For compounds with IC_{50} values <100 μM, the MTT assay was repeated.

CCK-8 assay

Cells were seeded into 96-well plate (4~5×10^3 in 100 μL per well). Test compounds were dissolved or suspended in DMSO to make 10000μM stock solutions and diluted with culture medium to various concentrations (final DMSO concentration < 0.5 %). Addition of test compound was performed immediately after adherent cells for 24 h. After incubation for 48 h at 37 ℃ in a humidified atmosphere with 5% CO_2, CCK-8 (10 μL, Bio-lifesci) was added to each well, and the plate was incubated for another 2~4 h. The 96-well plate was read using a microarray reader for optical density at 450 nm. All tests were performed in triplicate and the optical absorption read-out was normalized to percentage of maximum cytotoxicity. The viability of the cells was scored by the percentage of absorbance relative to control

Phase-contrast images of cancer cells treated with 4a

The logarithmic growth phase CNE-1, CNE-2, HepG2, SMMC-7721 cells were seeded into 96-well plate (4~5×10^3 in 100 μL per well). After the cells adhered for 24 h, the original medium was carefully discarded. In the control group, 100 μL of culture medium was added. In the experimental group were added with different concentrations of **4a** solution 100 μL, 37 ℃, 5% CO_2 incubator Continue training 48 h, under a light microscope.

SMMC-7721 cells morphological changes were observed by HE stain

Add 18×18 mm coverslips to a 6-well plate and logarithmic growth phase SMMC-7721 cells were seeded in 6-well plates at a density of 5×10^4 cells / mL with 2 ml per well. Cells adherent 24 h later, discard the original culture medium were added 0, 3, 5, 10 μM of **4a** solution 2 mL, set 37 ℃, 5% CO_2 incubator continued to train 48 h, remove the cell slide, washed with PBS three times. Join 95% ethanol fixed 20 min, PBS washed 2~3 times, each 1 min. Immersion hematoxylin dye (stained nucleus) staining 5 min, washed with distilled water. Observed in the microscope, if the nucleus too deep staining, with 1% hydrochloric acid solution color separation seconds. Dip in Eosin Y for 1min ， then washed with distilled water. Followed by 70%, 80%, 95% and 100% alcohol, one by one dehydration step by step, each 1 minute. Remove the slide or blow dry and finally drip glycerol

(or neutral gum), the cell side will be fixed down on the slide, save the cover, under a light microscope.

ER Tracker-red and Hoechst 33342 stain

The logarithmic growth phase SMMC-7721 cells were seeded in 30 mm laser confocal dish at a density of 1×10^4 cells / well. After the cells adhered for 24 h, the original culture medium was discarded, and added 0, 3, 5 μM **4a** 2 mL, 37℃, 5% CO_2 incubator for 48 h, remove the cell slide, with PBS washed 3 times. Add 1ml of ER Tracker stain, set 37℃, 5% CO_2 incubator for 15 min, then add 1 mL of Hoechst 33342 staining solution and continue staining for 15 min. The confocal dish was removed, washed 3 times with PBS, and the cells were observed by confocal laser scanning microscope.

Transmission electron microscopy (TEM)

SMMC-7721 cell aggregates were fixed with 2.5% glutaraldehyde-0.1 M NaH_2PO_4/Na_2HPO_4 phosphate buffer (pH 7.2) for 2 h at 4℃. After rinsing three times with phosphate buffer for 30 min, the samples were post-fixed with 1% OsO_4 for another 2 h at 4℃. Then, the samples were dehydrated in a graded series of ethanol- acetone with gradient ascent (50–70–90–100%), and embedded in epoxy resins. Subsequently, semi-thin sections (900 nm) were cut and stained with methylene blue for localization in light microscopy. Ultimately, ultrathin sections (110 nm) were stained with uranyl acetate and lead citrate, and examined under electron microscope (H-7650, Hitachi, Tokyo, Japan).

Apoptosis analysis

Apoptosis was discriminated with the Annexin V-FITC/Propidium Iodide (PI) test. Cells were seeded at 1×10^6/well in 10% FBS- RPMI-1640 into 6-well plates, and treated with compounds for 48 h. The cells were washed twice with cold Phosphate Buffered Saline (PBS) and then resuspend cells in 1×Binding Buffer [0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM $CaCl_2$)] at a concentration of 1×10^6 cells/ml. Transfer 100 μl of the solution (1×10^5 cells) to a 5 ml culture tube, and add 5 μl of Annexin V-FITC (BD, Pharmingen) and 5 μl PI to each tube. Gently vortex the cells and incubate for 15 minutes at RT (25℃) in the dark. Add 400 μl 1×Binding Buffer to each tube. Analysis was performed with the system software (CellQuest; BD Biosciences). Lower left quadrant, viable cells (annexin V-/PI-); lower right quadrant, early apoptotic cells (annexin V+/PI-); upper right quadrant, late apoptotic cells (annexin V+/PI+); upper left quadrant, necrotic cells (annexin V-/PI+). The percentage of cells positive for PI and/or Annexin V-PI was reported inside the quadrants.

Cell cycle analysis

The cells lines were treated with indicated concentrations of compounds **4a**. After incubated for 48 h, cells were washed twice with ice-cold PBS, fixed and permeabilized with ice-cold 70% ethanol at -20℃ overnight. The cells were treated with 100 μg/ml RNase A at 37℃ for 30 min after washed

with ice-cold PBS, and finally stained with 1 mg/ml propidium iodide (PI) in the dark at 4℃ for 30 min. Analysis was performed with the system software(CellQuest; BD Biosciences).

Western blotting analysis

The whole lysates of each group were collected to obtain the protein samples for Western blot analysis. The concentration of the total protein was quantified by BCA protein assay kit. 40 μg protein samples were separated on 10% SDS-PAGE gels, and electrophoresed in Tris-glycine buffer at a constant voltage of 100 V. The protein samples were subjected to transferring from the gel to nitrocellulose membrane (Millipore, MA, USA) at a constant voltage of 100 V for 1 h. The membranes were blocked with 5% skim milk for 2 h at room temperature, followed by incubation of indicated primary antibodies at 4℃ over night. The following primary antibodies were uesed, including GAPDH (Genesion, GP0003, China), Caspase-3 (Cell Signaling Technology, 9662T, USA) BiP/GRP78 (Cell Signaling Technology, 3177T, USA), p62 (MBL, PM045, Japan), LC3 (Novus, NB100-2331SS, USA). The membranes were washed with PBS for three times, and then incubated with secondary antibody (Anti-Rabbit IgG (H+L), DyLight 800 labeled or Anti-Mouse IgG (H+L) HSA, DyLight 680 labeled) for 1.5 h at room temperature in the darkness. Then the membranes were washed with PBST for three times in dark and the protein blots were visualized using the Odyssey Infrared Imaging System (odyssey, LI-COR biosciences, Bad Homburg, Germany). The intensity of the bands were quantitatively deternmined by Odyssey Infrared Imaging System.

Statistical Analysis

All the data of the experiments represented for three individual repeated experiments (n=3) which were shown as mean±standard deviation (SD). One-way ANONA was applied to assess the statistical differences of the means between multiple groups, and the difference between two groups was analyzed by two-tailed Students t-test. P-value<0.05 was considered to be statistically significant. Figures were obtained by the Statistical Analysis System (GraphPad Prism 5, GraphPad Software, Inc., San Diego, CA).

Table S1. Structure of compounds **4a-b**, **5a-d** and **6a-g**.

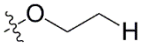
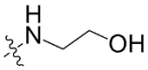
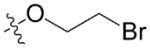
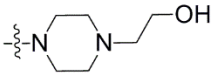
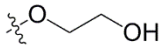
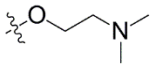
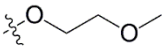
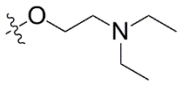
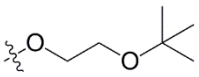
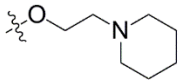
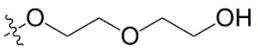
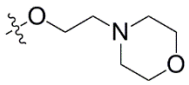
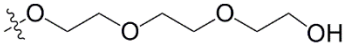
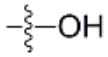
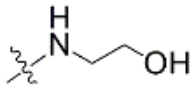
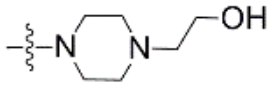
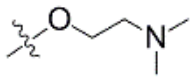
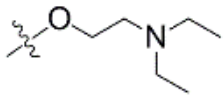
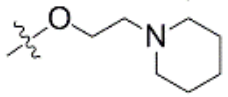
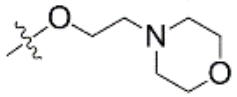
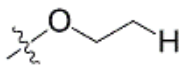
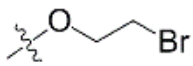
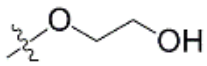
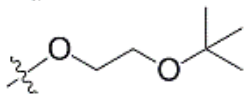
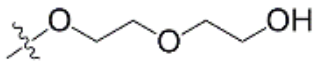
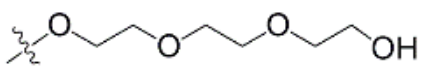
Compond	R	Yied/%	mp/℃	Compond	R	Yied/%	mp/℃
4		86	251-252	**6a**		90	176-178
4a		60	187-189	**6b**		76	209-210
4b		75	155-157	**6c**		40	194-196
5a		65	133-134	**6d**		88	156-158
5b		64	142-144	**6e**		85	191-193
5c		70	153-155	**6f**		78	175-177
5d		80	159-161	**6g**		80	130-131

Table 1. Anthraquinone compounds on cells inhibition in vitro.

Compond	R	IC_{50} (uM)[a]			
		CNE-1	CNE-2	HepG2	SMMC-7721
Rhein		>100	>100	>100	>100
2		19.6±2.2	21.3±1.4	41.2±2.5	72.2±5.3
4a		4.7±1.2[b]	2.1±0.2[b]	3.2±1.4[b]	1.5±0.3[b]
4b		6.7±0.3	7.0±0.3	12.1±1.5	15.2±2.7
5a		6.5±0.5	6.7±0.2	15.5±1.4	17.6±1.2
5b		6.3±0.5	6.6±0.9	14.4±0.8	15.3±1.3
5c		5.4±0.4	4.8±0.3	12.6±2.6	14.6±1.7
5d		27.7±4.5	38.4±3.1	>100	>100
6a		>100	>100	>100	>100
6b		>100	>100	>100	>100
6c		>100	>100	>100	>100
6d		>100	>100	>100	>100
6e		>100	>100	>100	>100
6f		>100	>100	>100	>100
6g		50.3±3.0	56.8±3.6	>100	>100
Cisplatin		8.2±0.5	7.7±0.2	16.5±0.9	18.2±2.2

[a] MTT assay after 48 h incubation (Mean±SD of three independent experiments). [b] CCK-8 assay after 48 h incubation (Mean±SD of three independent experiments).

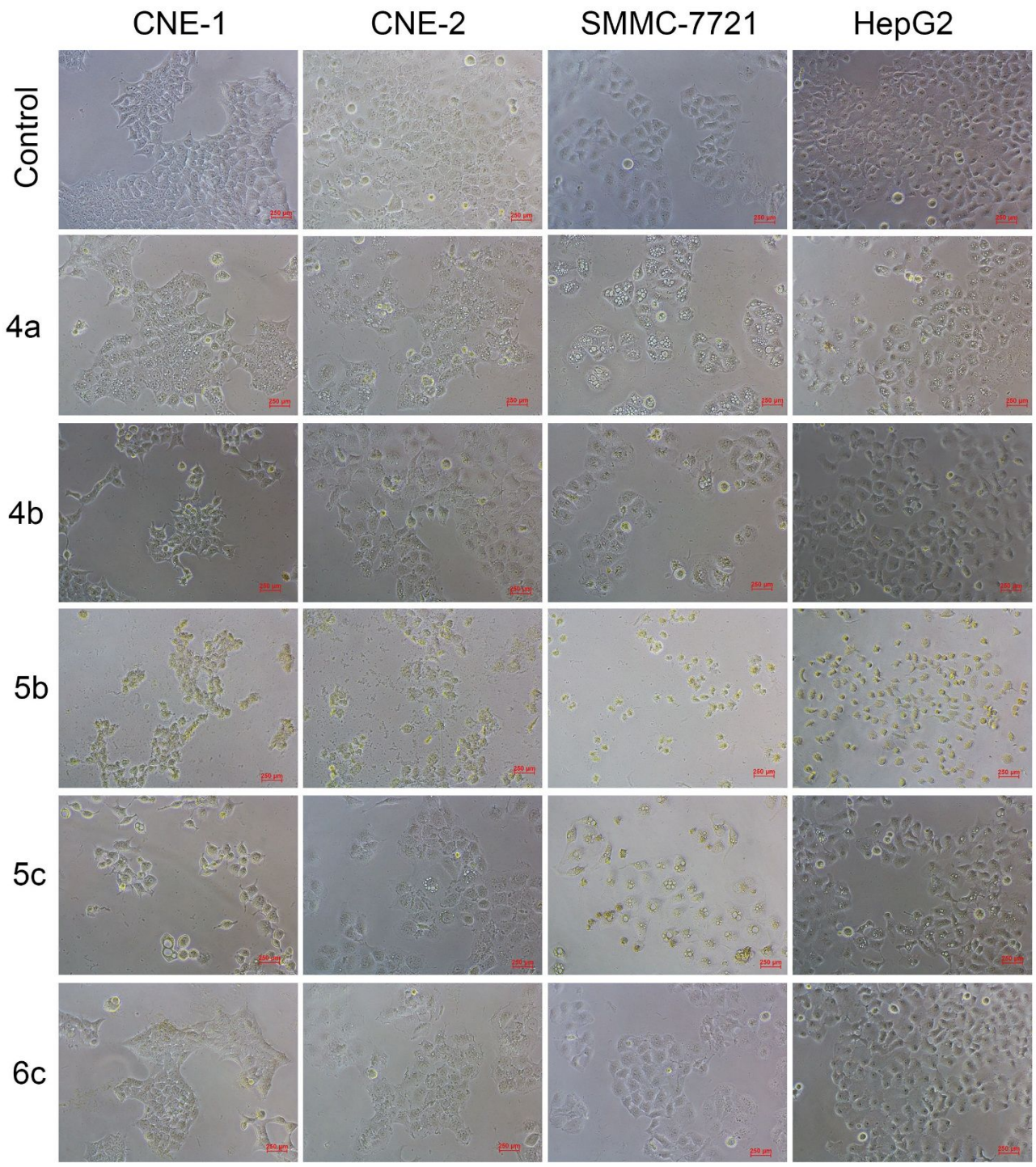


Figure S1. Phase-contrast images of nasopharyngeal carcinoma CNE-1, CNE-2, liver cancer SMMC-7721, HepG2 cells treated with different compounds after 5 μM treatment for 24 h. All images were acquired at the same magnification. (200×)

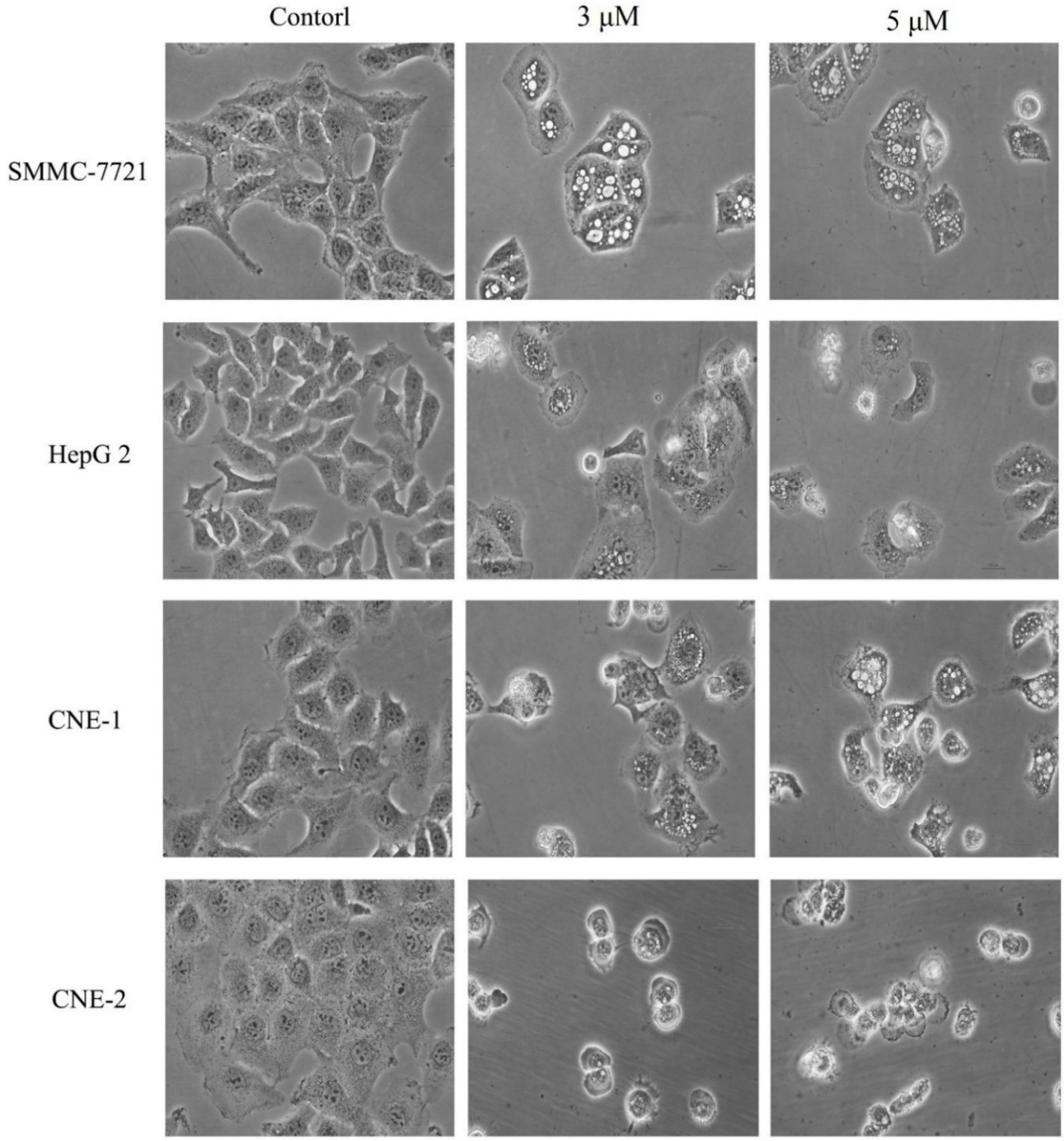


Figure S2. Phase-contrast images of liver cancer SMMC7721, HepG2, nasopharyngeal carcinoma CNE-1, CNE-2 cells treated with **4a** for 48 h. All images were acquired at the same magnification. (200×)

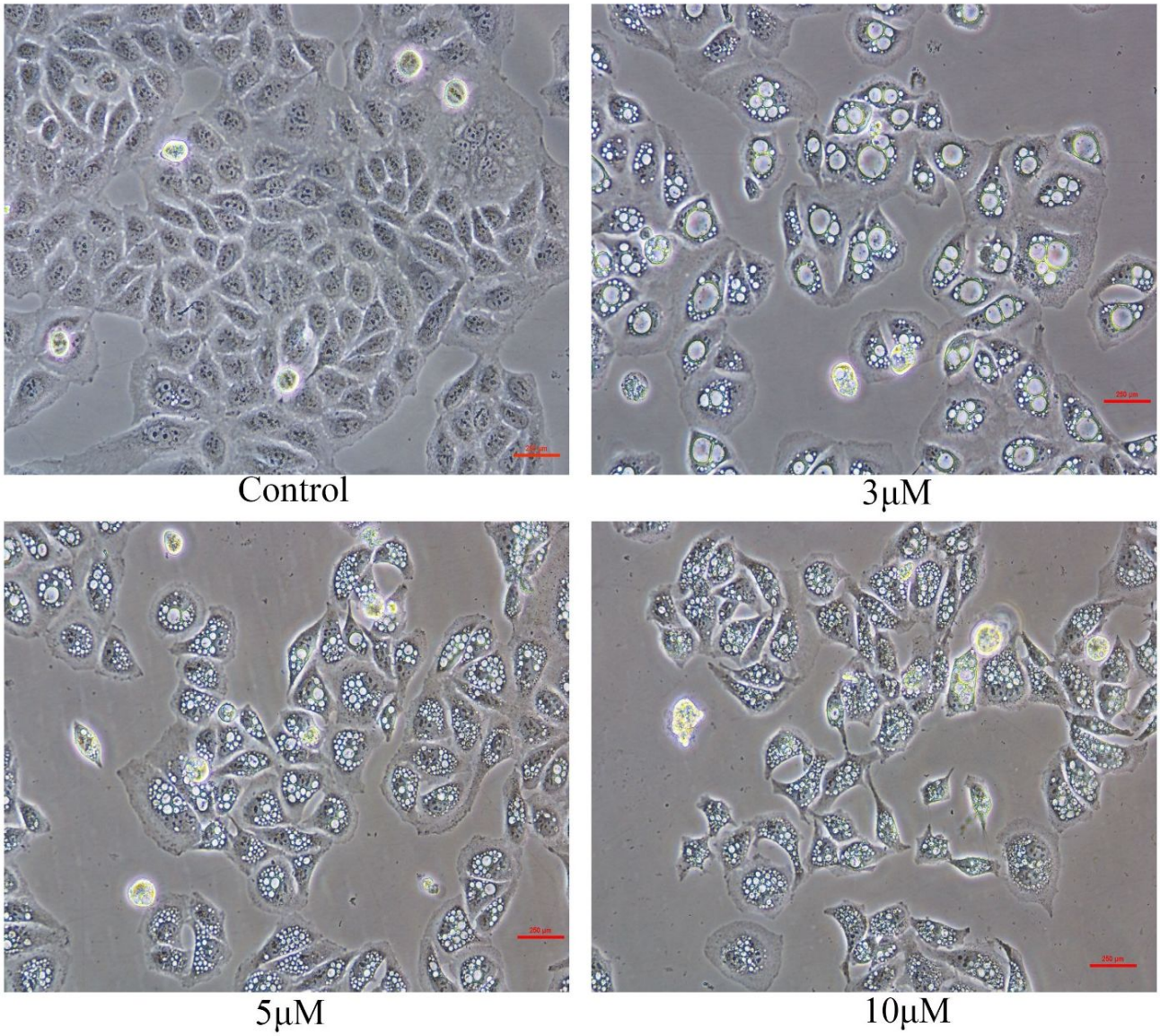


Figure S3. Phase-contrast images of SMMC-7721 cells treated with **4a** at indicated concentration after treatment for 48 h. All images were acquired at the same magnification. (200×)

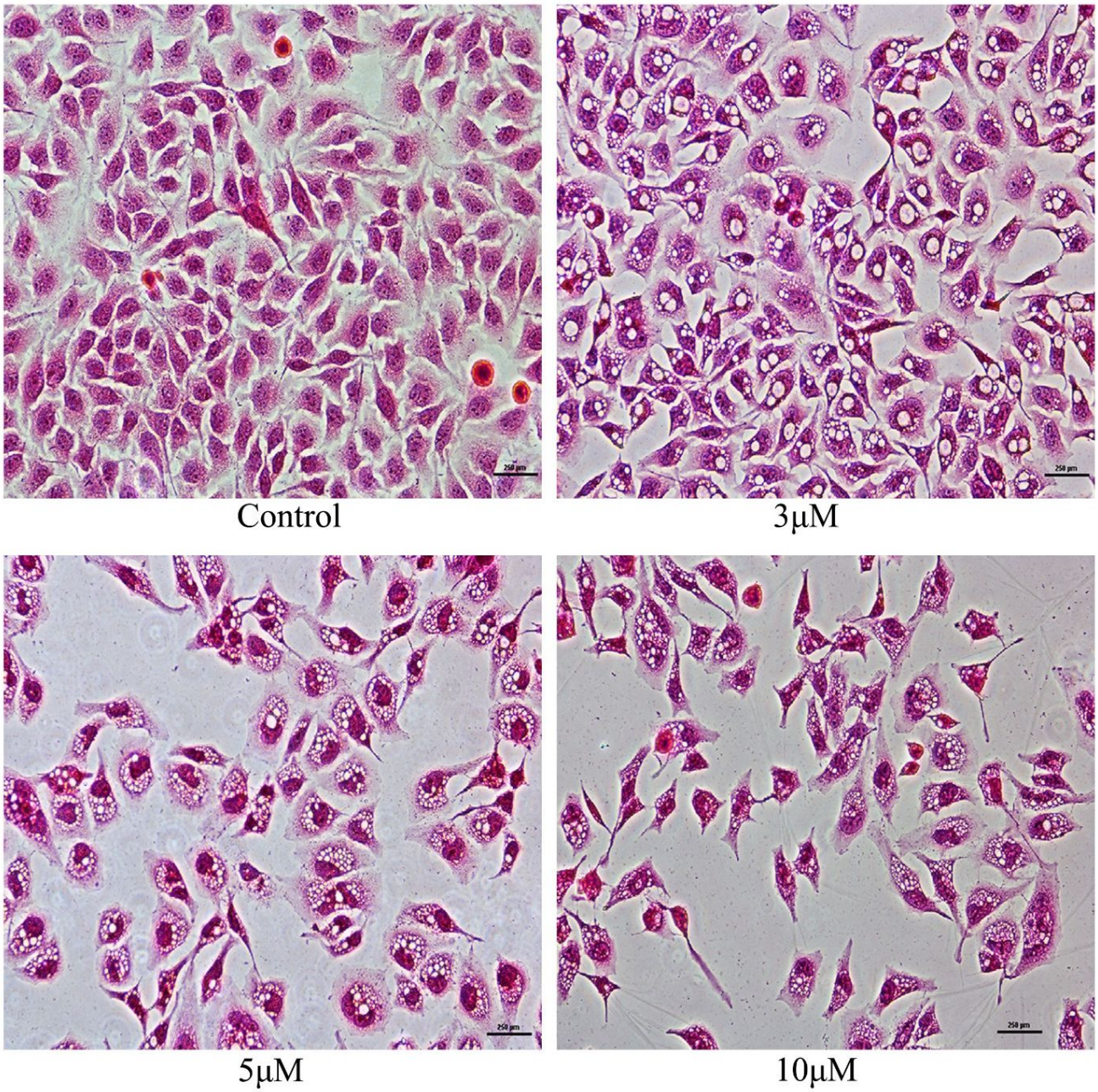


Figure S4. HE staining after SMMC-7721 cells treated with **4a** at indicated concentration after treatment for 48 h. All images were acquired at the same magnification. (200×)

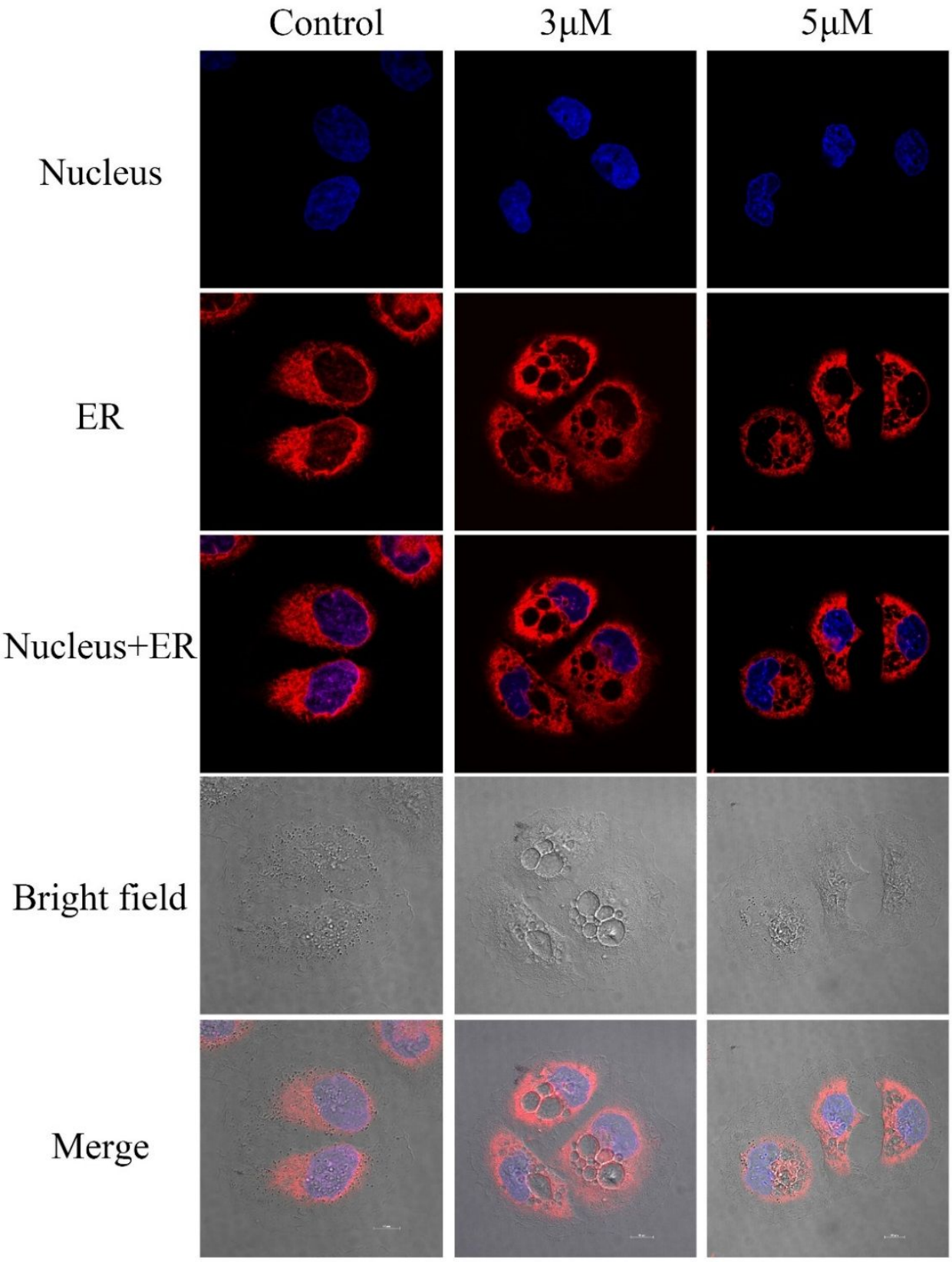


Figure S5. SMMC-7721 Cells were incubated with **4a** for 48 h and analysed using the ER-Tracker Red and Hoechst 33342 assay. Images were obtained by laser scanning confocal microscope at the same magnification. (600×)

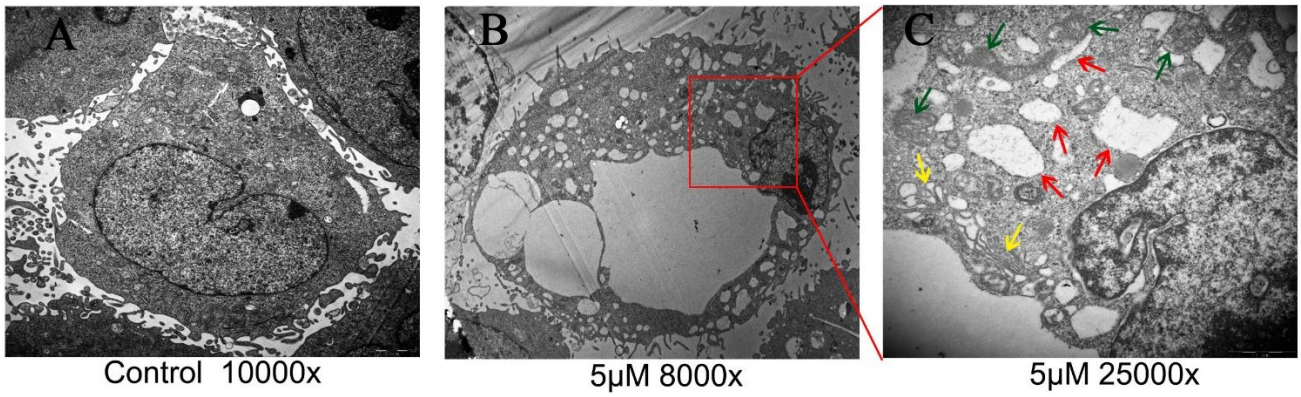


Figure S6. TEM imaging of control and treated (**4a**, 5 μM) SMMC-7721 cells. The ER, mitochondria and golgi apparatus are indicated by red arrowheads, green arrowheads and yellow arrowheads, respectively.

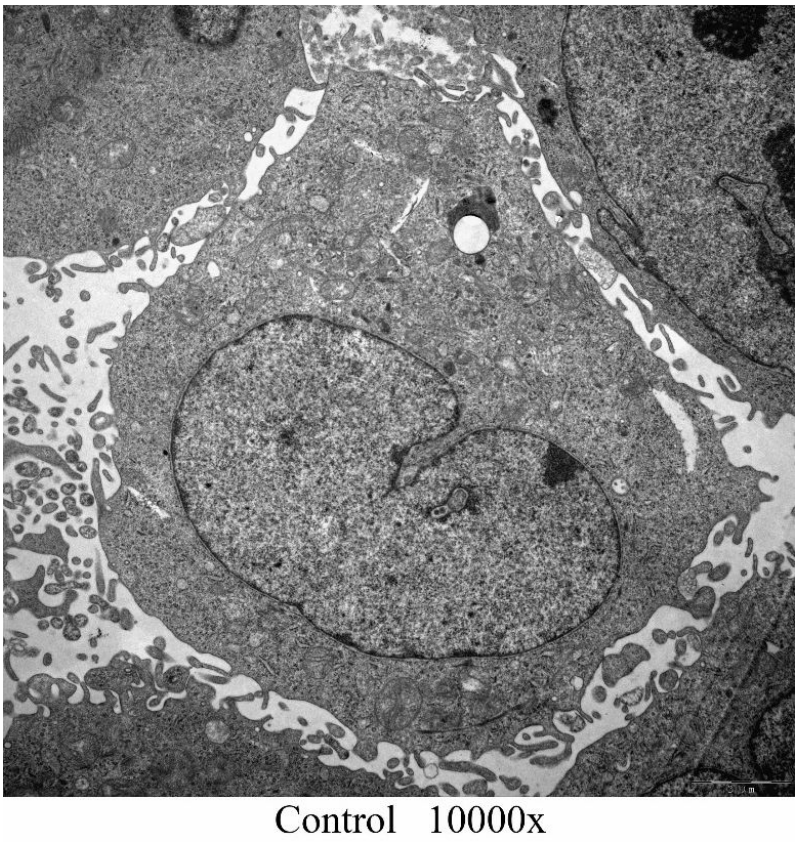
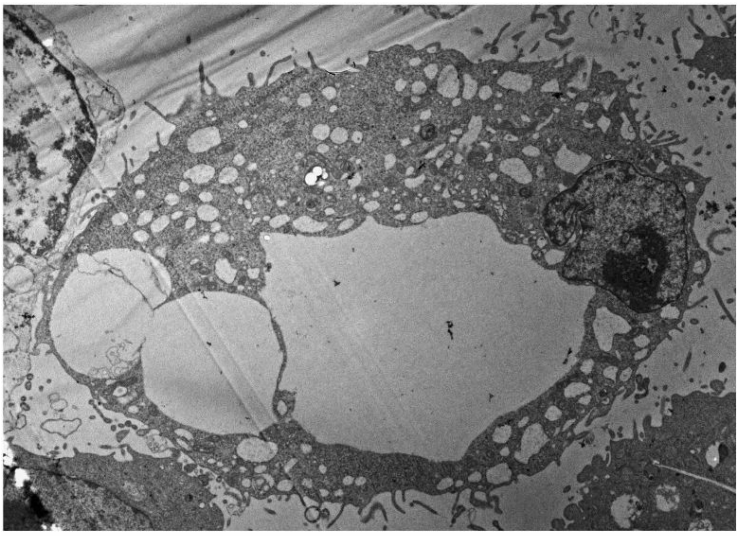
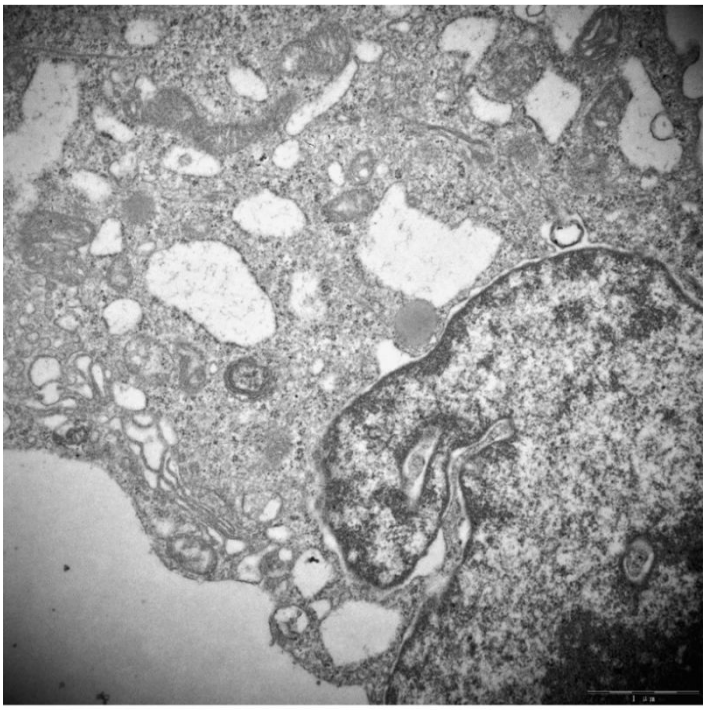


Figure S7. TEM imaging of control SMMC-7721 cells. (10000×)



5μM 8000x

Figure S8. TEM imaging of treated (**4a**, 5 μM) SMMC-7721 cells (8000×). Several large vacuoles and many small vacuoles are present in the cytoplasm, cell membrane structure remains intact, the nucleus is concentrated and smaller, autophagosome and apoptotic bodies did not find.



5μM 25000x

Figure S9. TEM imaging of treated (**4a**, 5 μM) SMMC-7721 cells (25000×). Partially enlarged view of Figure S8, Further observation showed that vacuoles originated from the expansion and swelling of the rough ER, and nothing in the vacuole, large amounts of ribosomes were observed on the outside of the swollen ER, and Golgi apparatus.

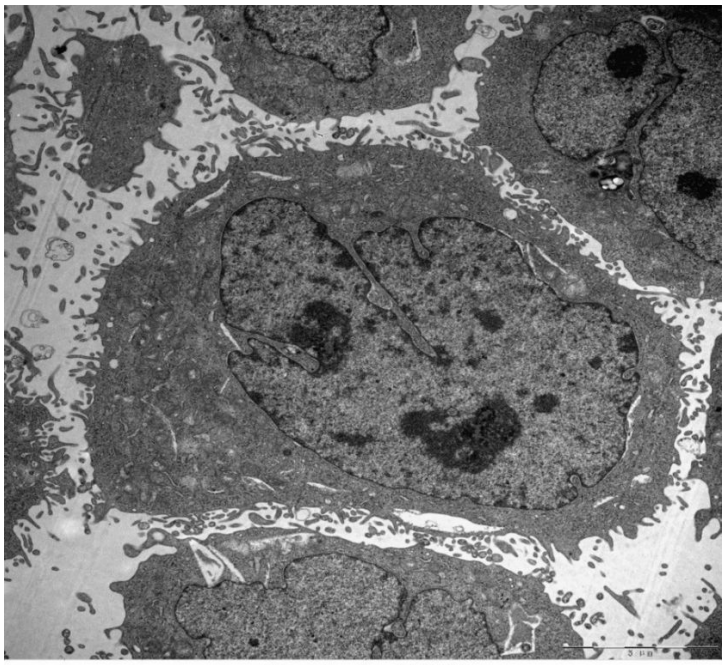


Figure S10. TEM imaging of control SMMC-7721 cells. (7000×)

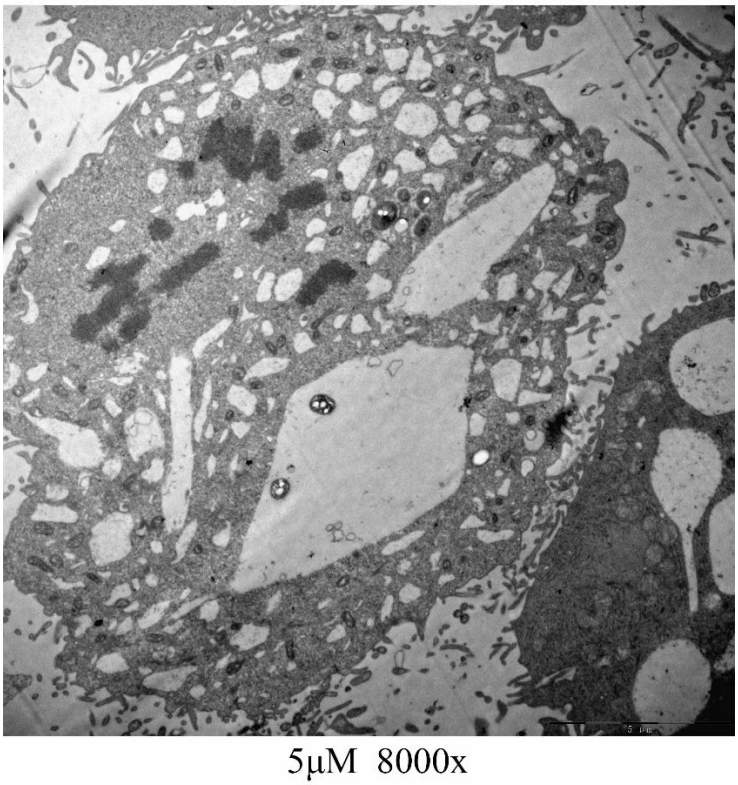


Figure S11. TEM imaging of treated (**4a**, 5 μM) SMMC-7721 cells (8000×). As shown in the figure, cell contour and membrane remain intact, a large number of vacuoles appear in the cytoplasm and the endoplasmic reticulum expands.

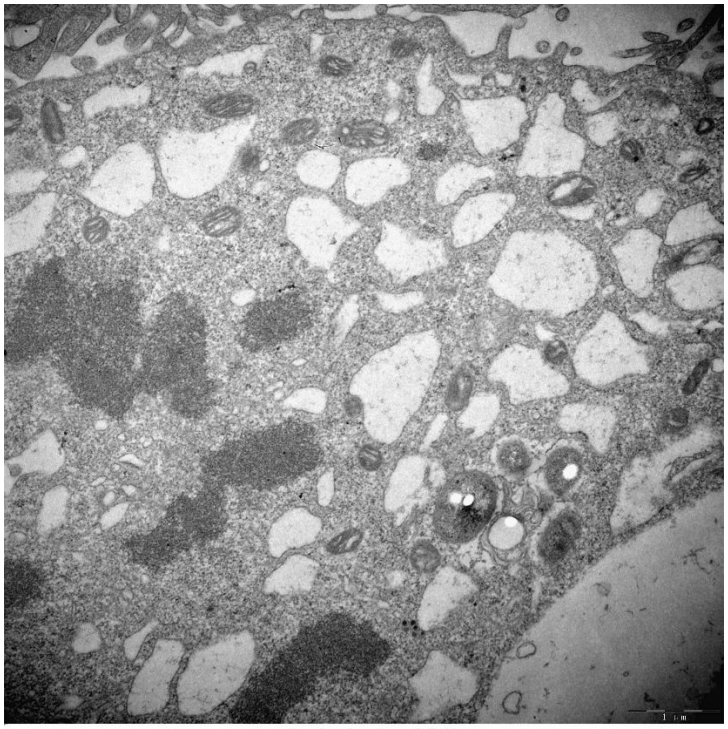


Figure S12. TEM imaging of treated (**4a**, 5 μM) SMMC-7721 cells (20000×). Partially enlarged view of Figure S11, vacuoles originated from the expansion and swelling of the rough ER, nothing in the vacuole; mitochondrial swelling, vacuoles, and ridges disappearing.

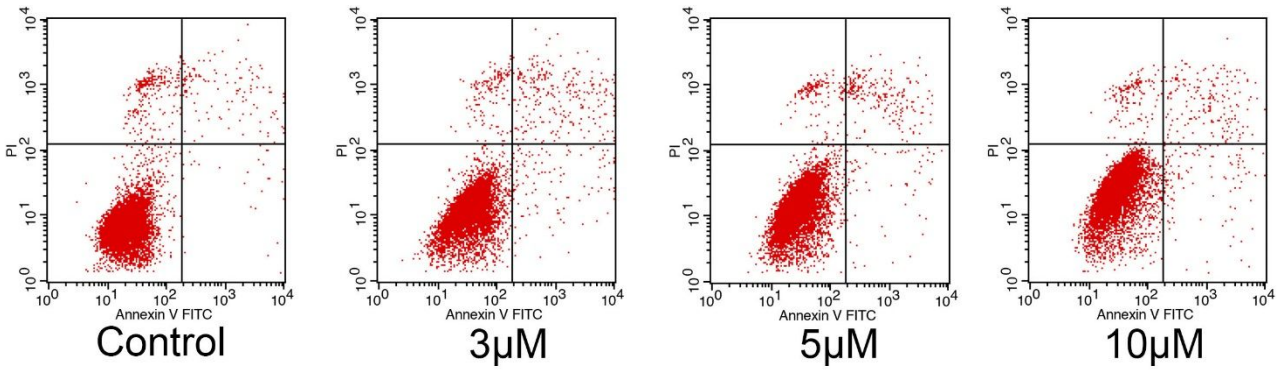
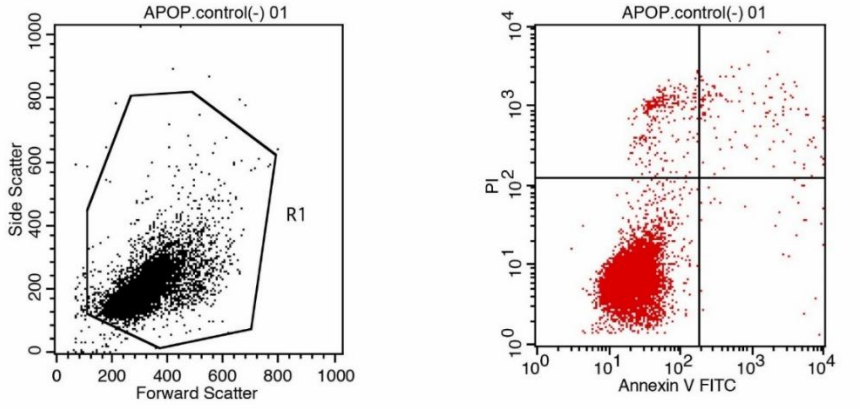


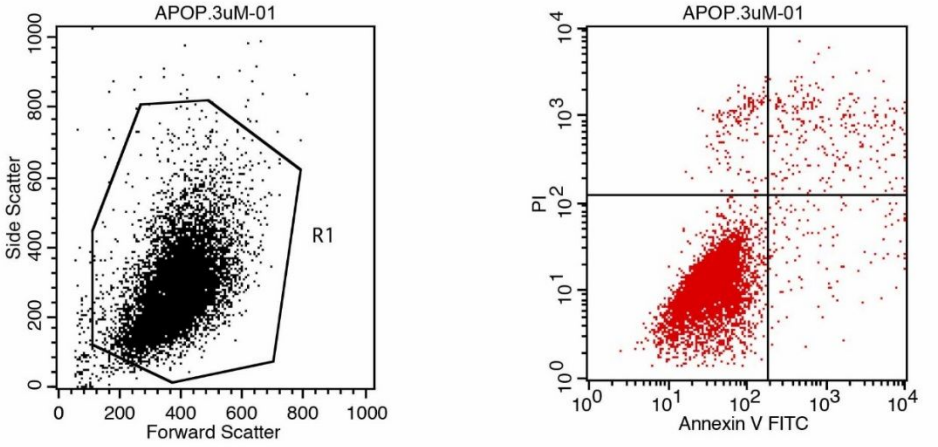
Figure S13. Flow cytometry analysis of apoptosis. Cells were exposed to 0~10 μM **4a** for 48 h. Cells were collected and stained with Annexin V-fluorescein isothiocyanate (FITC) and PI.



File: APOP.control(-) 01 Gated Events: 9905
Total Events: 10000

Quad	Events	% Gated	% Total
UL	255	2.57	2.55
UR	124	1.25	1.24
LL	9497	95.88	94.97
LR	29	0.29	0.29

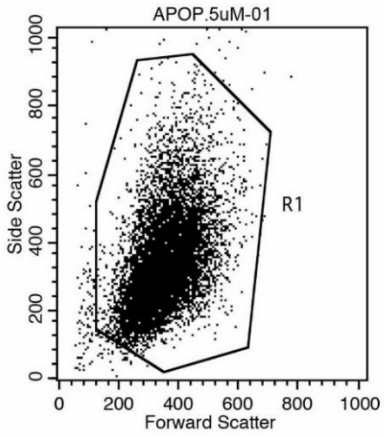
Figure S14. Flow cytometry analysis of apoptosis. SMMC-7721cells were exposed to 0 μM **4a** for 48 h. Cells were collected and stained with Annexin V-fluorescein isothiocyanate (FITC) and PI.

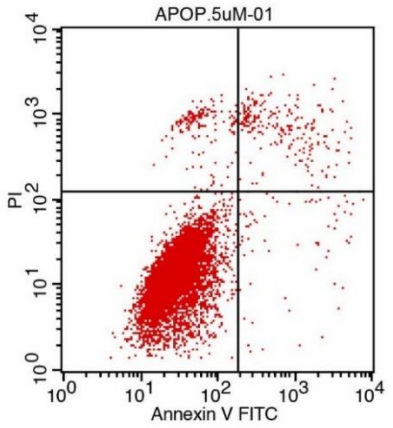


File: APOP.3uM-01 Total Gated Events: 9824
Events: 10000

Quad	Events	% Gated	% Total
UL	150	1.53	1.50
UR	262	2.67	2.62
LL	9301	94.68	93.01
LR	111	1.13	1.11

Figure S15. Flow cytometry analysis of apoptosis. SMMC-7721cells were exposed to 3 μM **4a** for 48 h. Cells were collected and stained with Annexin V-fluorescein isothiocyanate (FITC) and PI.

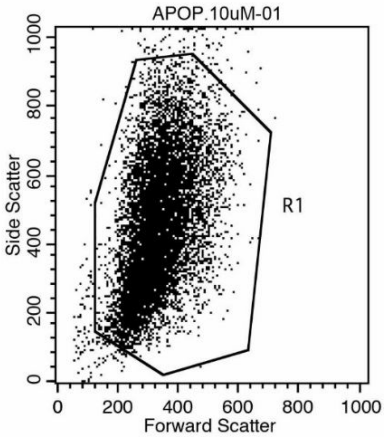


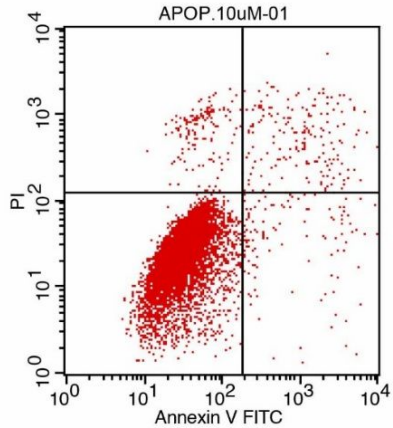


File: APOP.5uM-01 Gated Events: 9833
Total Events: 10000

Quad	Events	% Gated	% Total
UL	164	1.67	1.64
UR	230	2.34	2.30
LL	9368	95.27	93.68
LR	71	0.72	0.71

Figure S16. Flow cytometry analysis of apoptosis. SMMC-7721cells were exposed to 5 μM **4a** for 48 h. Cells were collected and stained with Annexin V-fluorescein isothiocyanate (FITC) and PI.





File: APOP.10uM-01 Gated Events: 9771
Total Events: 10000

Quad	Events	% Gated	% Total
UL	147	1.50	1.47
UR	187	1.91	1.87
LL	9311	95.29	93.11
LR	126	1.29	1.26

Figure S17. Flow cytometry analysis of apoptosis. SMMC-7721cells were exposed to 10 μM **4a** for 48 h. Cells were collected and stained with Annexin V-fluorescein isothiocyanate (FITC) and PI.

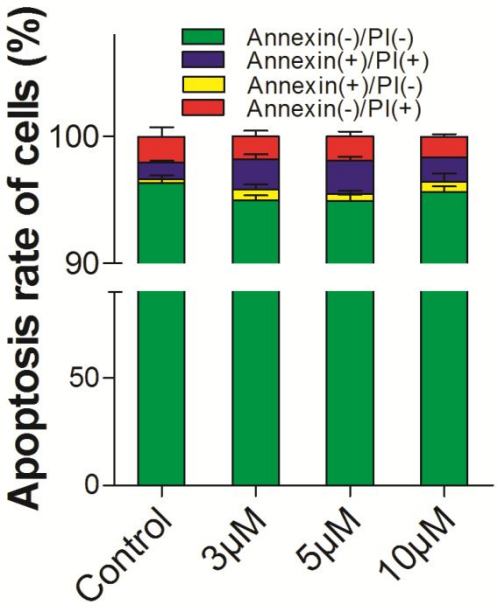


Figure S18. Frequency distribution of apoptosis in MMC-7721 cells were exposed to 0~10 μM **4a** for 48 h.

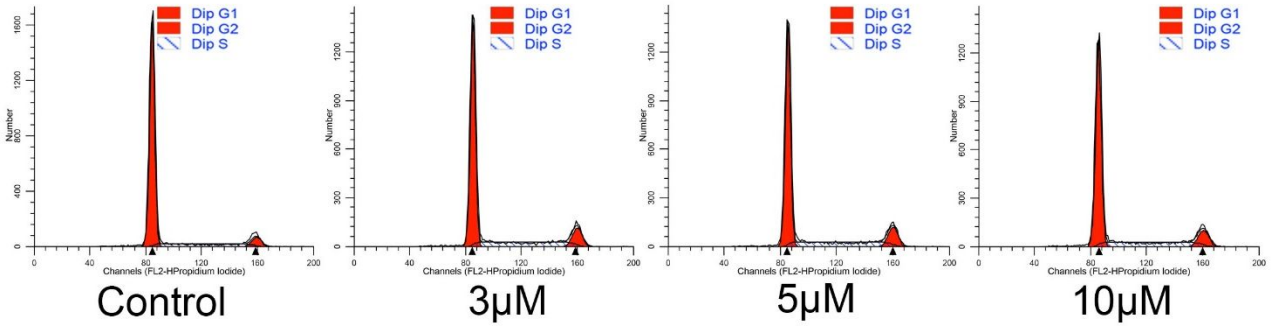


Figure S19. Flow cytometry analysis of cell cycle populations after treatment with 0~10 μM **4a** for 48 h.

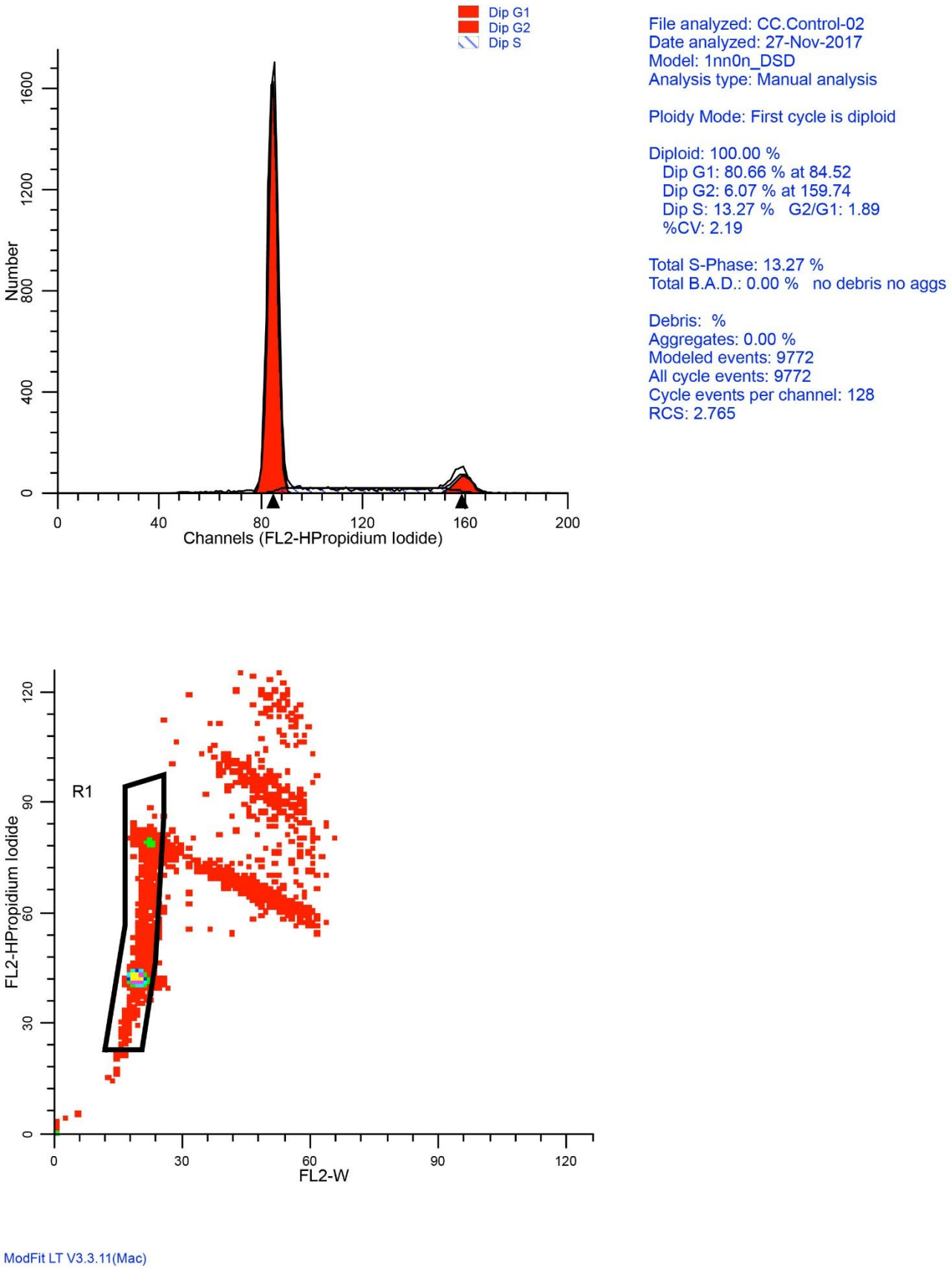


Figure S20. Flow cytometry analysis of cell cycle. SMMC-7721cells were exposed to 0 μM **4a** for 48 h.

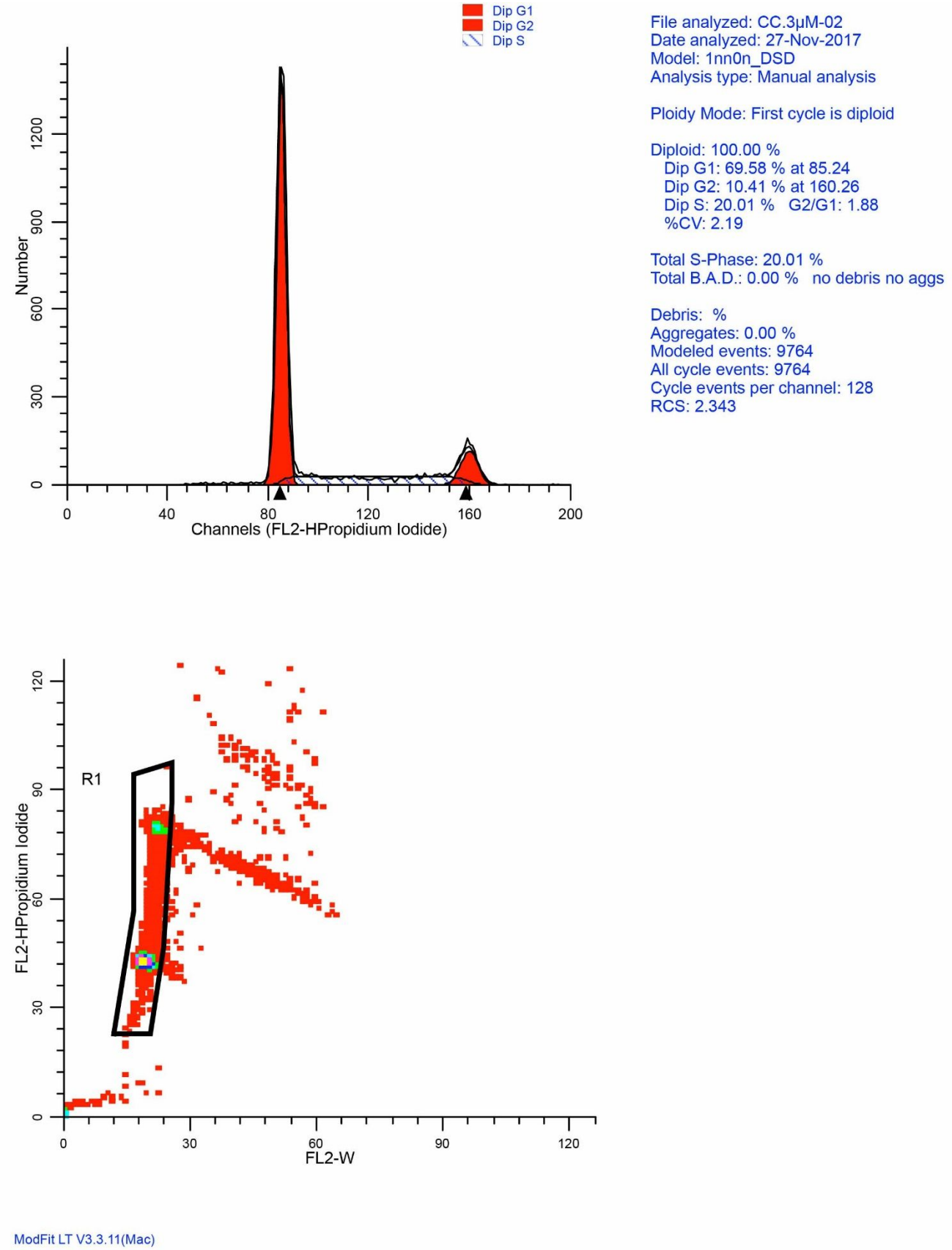


Figure S21. Flow cytometry analysis of cell cycle. SMMC-7721cells were exposed to 3 μM **4a** for 48 h.

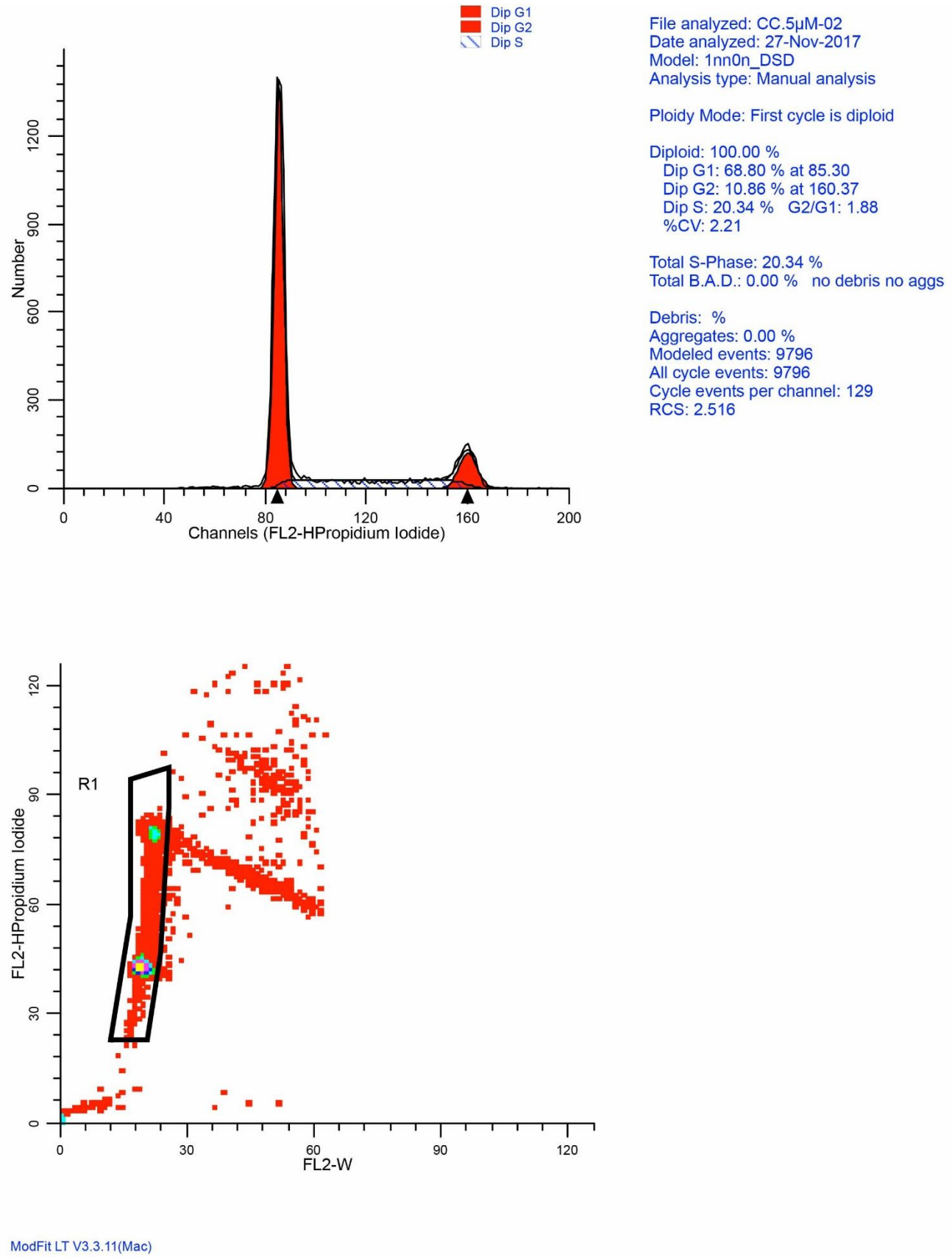


Figure S22. Flow cytometry analysis of cell cycle. SMMC-7721cells were exposed to 5 μM **4a** for 48 h.

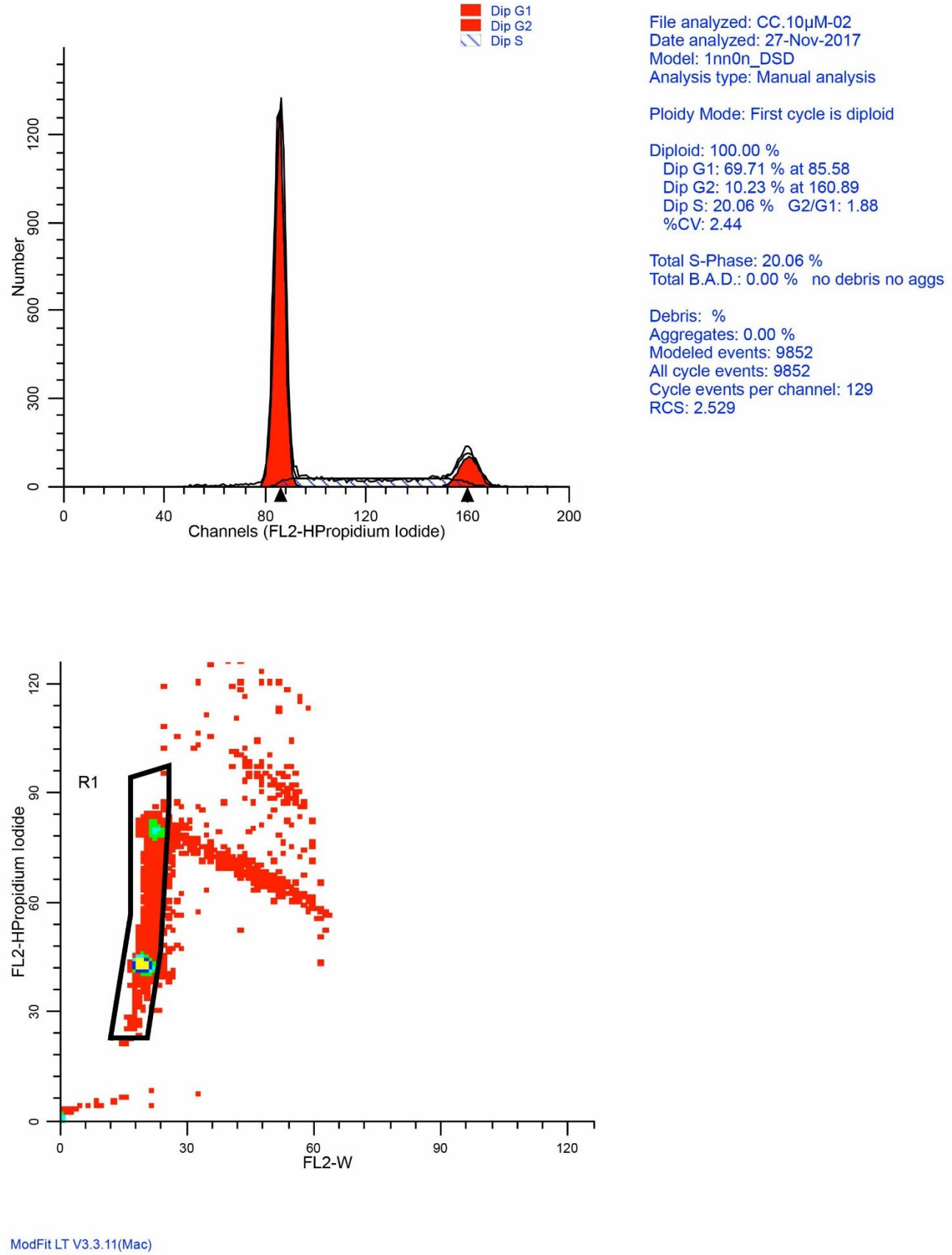


Figure S23. Flow cytometry analysis of cell cycle. SMMC-7721cells were exposed to 10 μM **4a** for 48 h.

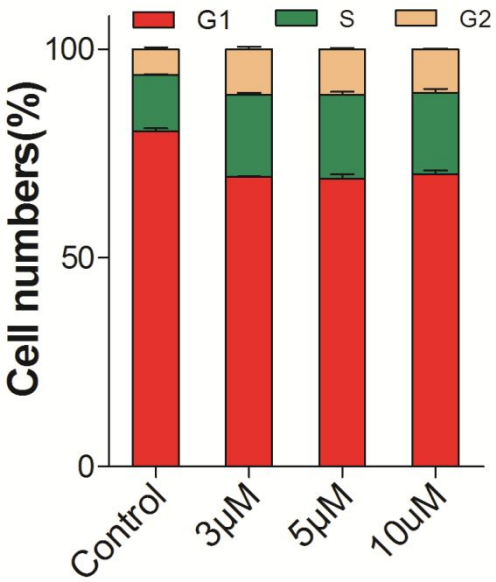


Figure S24. Frequency distribution of cell cycle in MMC-7721 cells were exposed to 0~10 μM **4a** for 48 h.

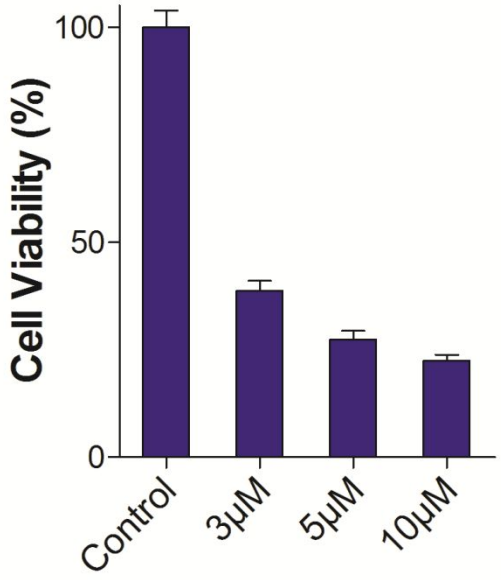


Figure S25. CCK-8 assays were performed after treatment SMMC-7721 cells with 0, 3, 5, 10 μM **4a** for 48 h

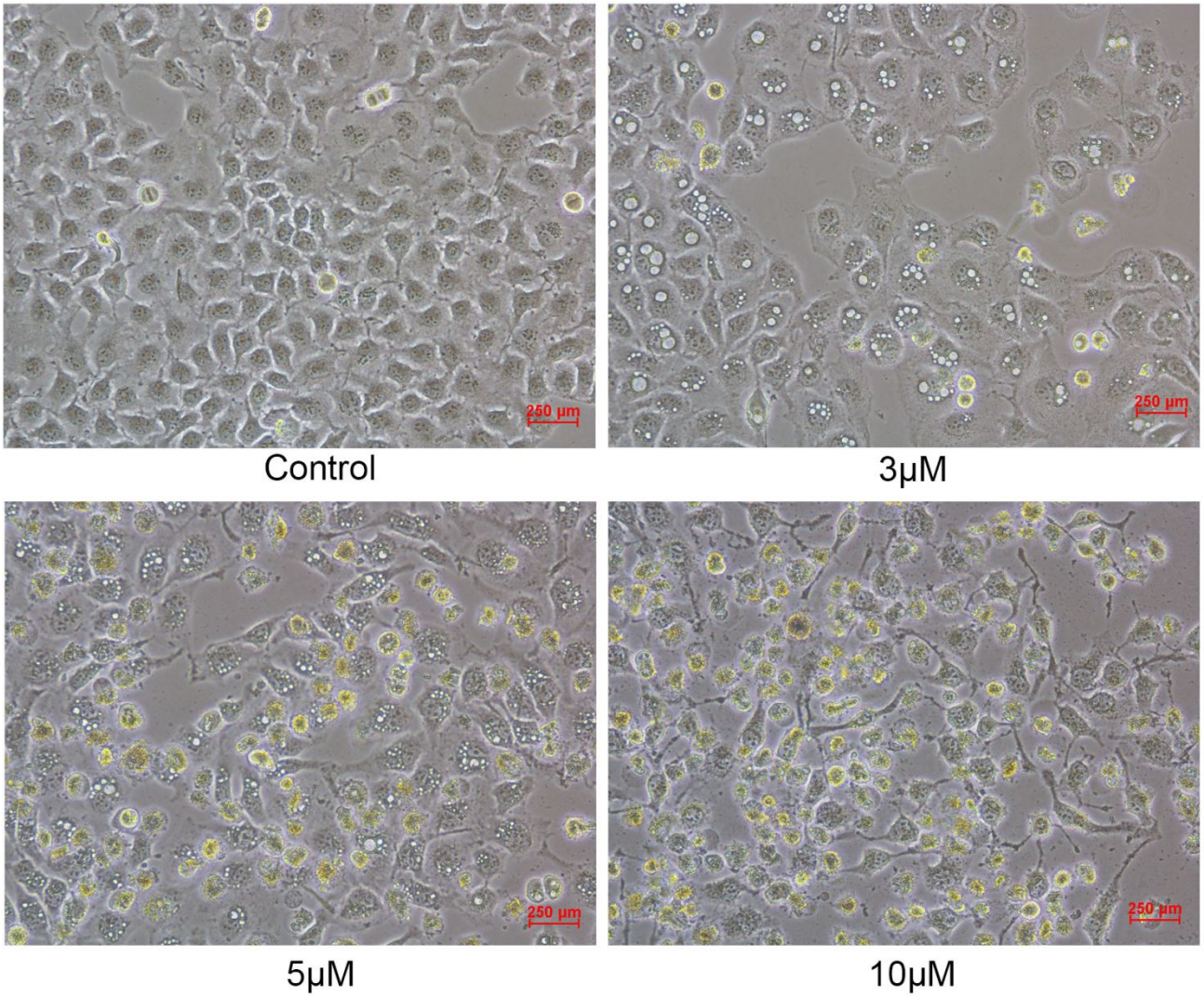


Figure S26. Phase-contrast images of SMMC-7721 cells treated with **5c** at indicated concentration after treatment for 48 h. All images were acquired at the same magnification. (200×)

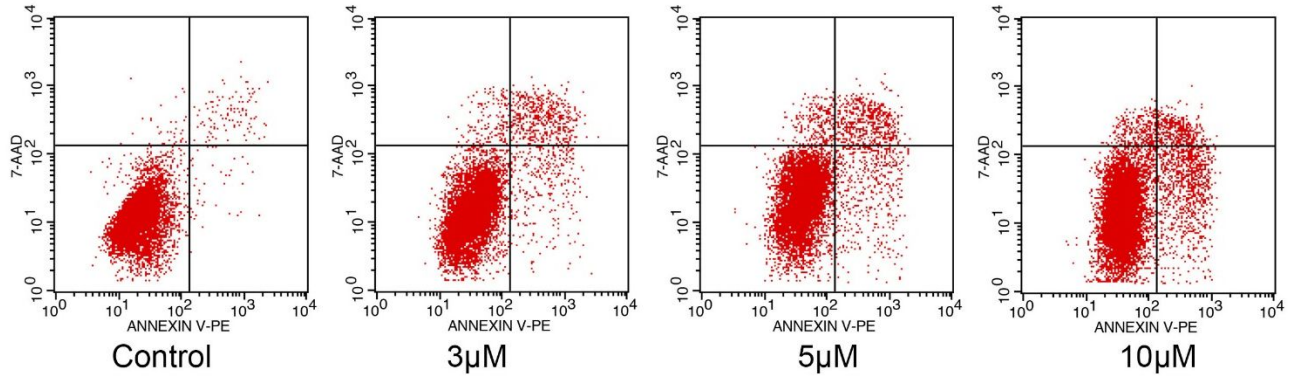
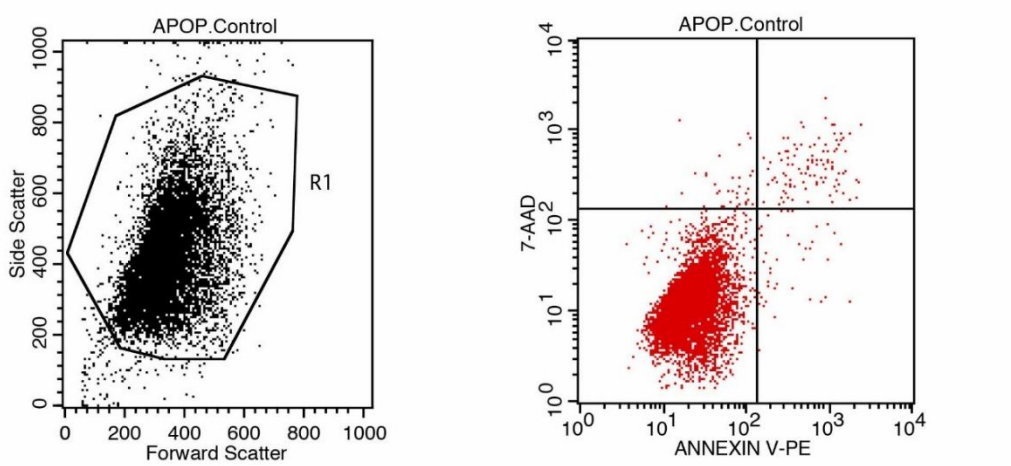


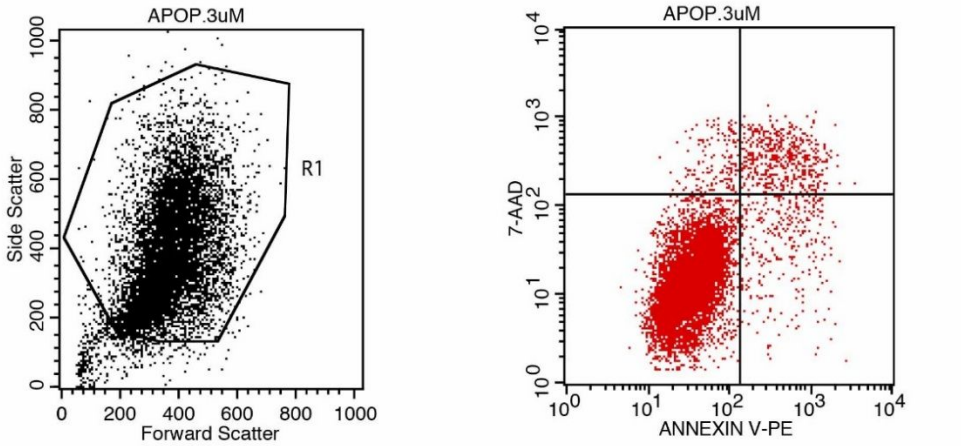
Figure S27. Flow cytometry analysis of apoptosis. SMMC-7721 cells were exposed to 0~10 μM **5c** for 48 h. Cells were collected and stained with Annexin V-PE and 7-AAD.



File: APOP.Control Gate: G1
Gated Events: 9792 Total Events: 10000

Quad	Events	% Gated	% Total
UL	36	0.37	0.36
UR	107	1.09	1.07
LL	9605	98.09	96.05
LR	44	0.45	0.44

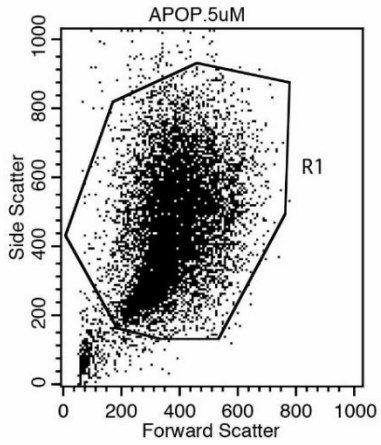
Figure S28. Flow cytometry analysis of apoptosis. Cells were exposed to 0 μM **5c** for 48 h. Cells were collected and stained with Annexin V-PE and 7-AAD.

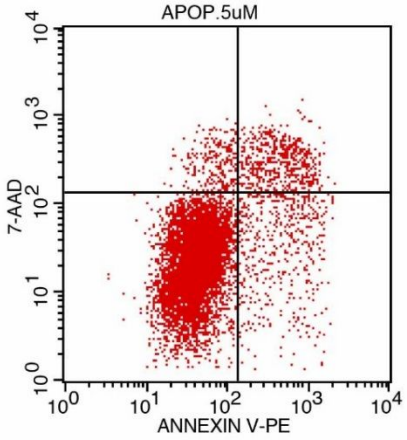


File: APOP.3uM Gate: G1
Gated Events: 9483 Total Events: 10000

Quad	Events	% Gated	% Total
UL	158	1.67	1.58
UR	525	5.54	5.25
LL	8488	89.51	84.88
LR	312	3.29	3.12

Figure S29. Flow cytometry analysis of apoptosis. Cells were exposed to 3 μM **5c** for 48 h. Cells were collected and stained with Annexin V-PE and 7-AAD.

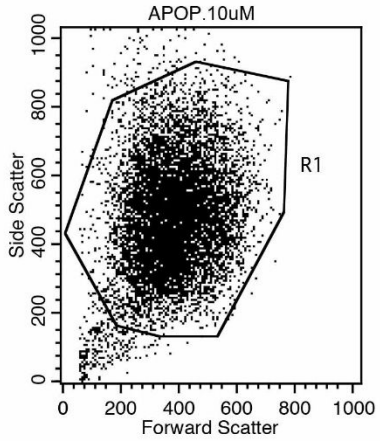


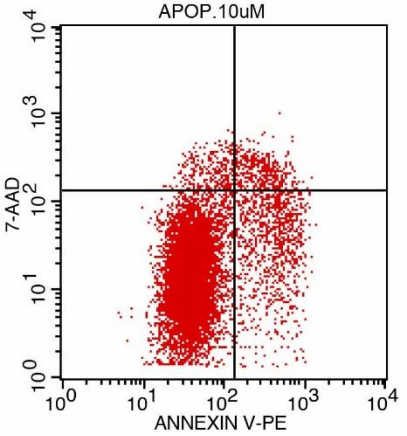


File: APOP.5uM Gate: G1
Gated Events: 9526 Total Events: 10000

Quad	Events	% Gated	% Total
UL	233	2.45	2.33
UR	557	5.85	5.57
LL	8229	86.38	82.29
LR	507	5.32	5.07

Figure S30. Flow cytometry analysis of apoptosis. Cells were exposed to 5 μM **5c** for 48 h. Cells were collected and stained with Annexin V-PE and 7-AAD.





File: APOP.10uM Gate: G1
Gated Events: 9634 Total Events: 10000

Quad	Events	% Gated	% Total
UL	280	2.91	2.80
UR	412	4.28	4.12
LL	7992	82.96	79.92
LR	950	9.86	9.50

Figure S31. Flow cytometry analysis of apoptosis. Cells were exposed to 10 μM **5c** for 48 h. Cells were collected and stained with Annexin V-PE and 7-AAD.

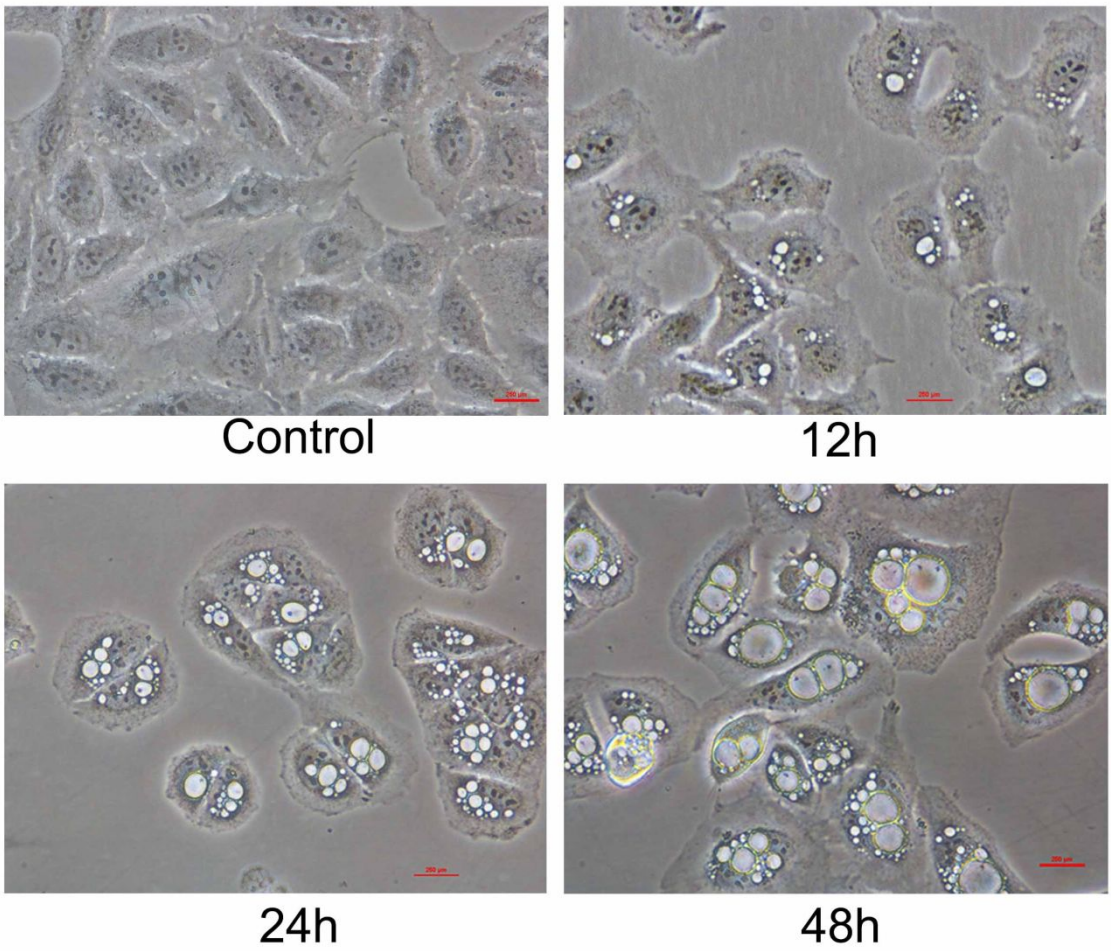


Figure S32. Phase-contrast images of SMMC-7721 cells treated with **4a** at indicated time points after 3 μM treatment, all images were acquired at the same magnification. (400×)

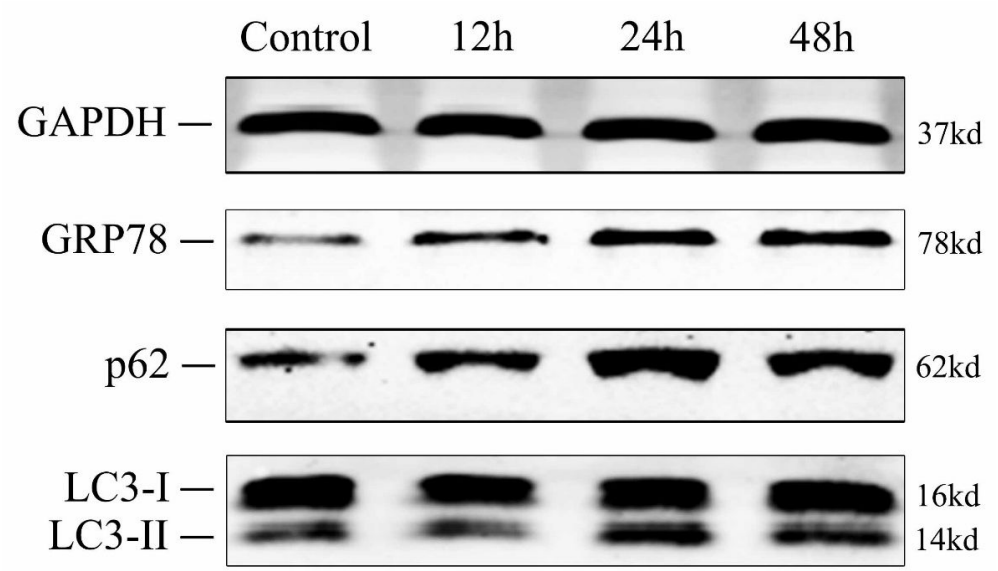


Figure S33. Western blotting analysis the expression of GRP78, p62, LC3 after **4a** (3μM) treatment at different time points in SMMC-7721 cells, GAPDH is shown as a loading control.

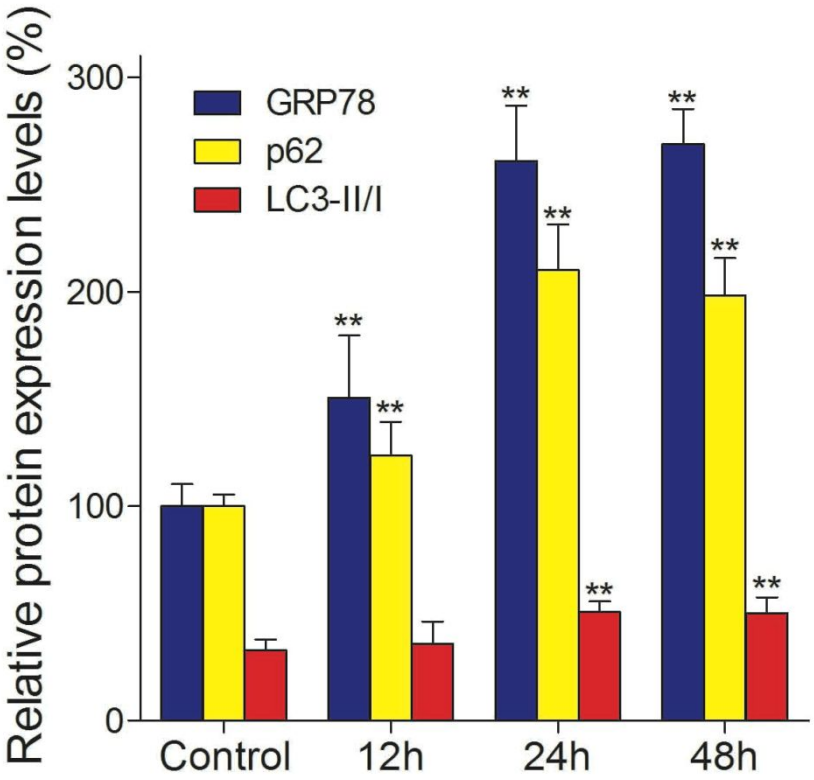


Figure S34. Statistical analysis histogram of related protein expression, **=*P*<0.01.

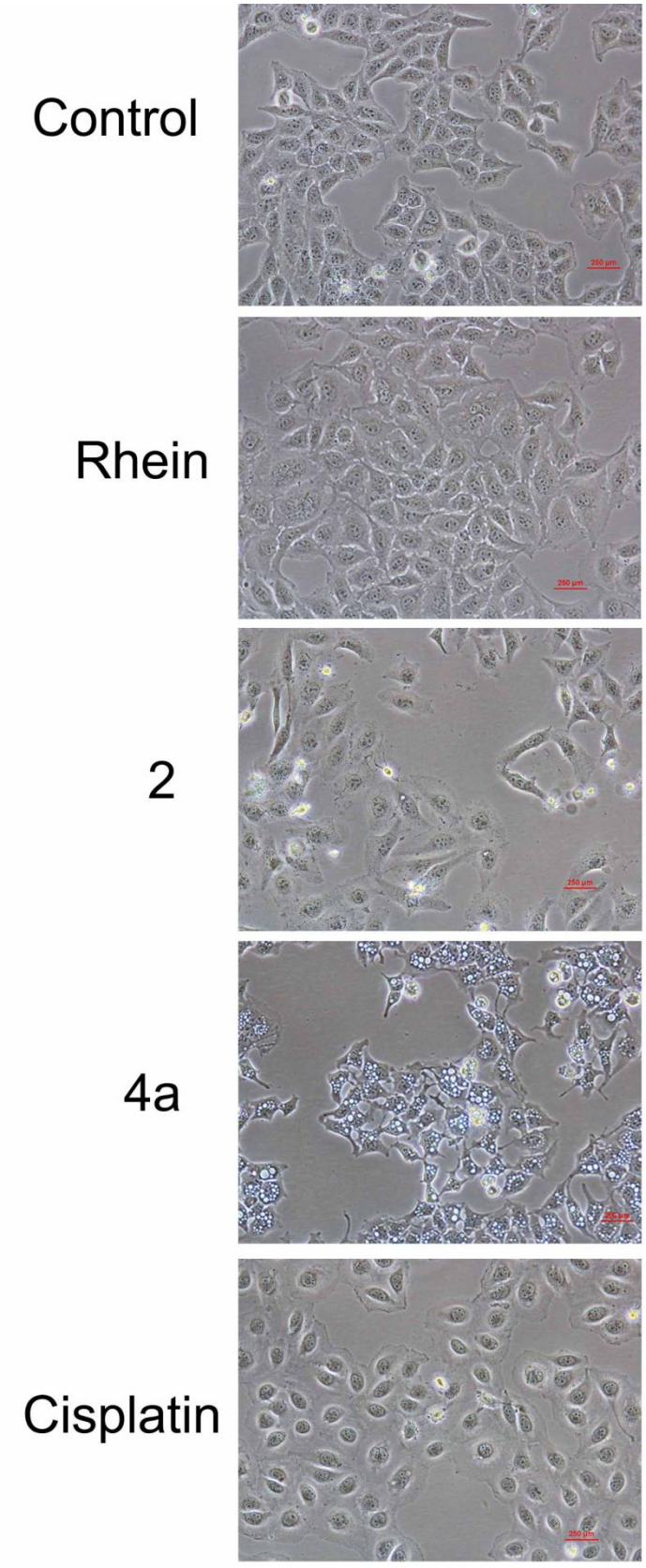


Figure S35. Phase-contrast images of SMMC-7721 cells treated with Rhein, **2**, **4a** and Cisplatin after 10 μM treatment for 48 h, all images were acquired at the same magnification. (200×)

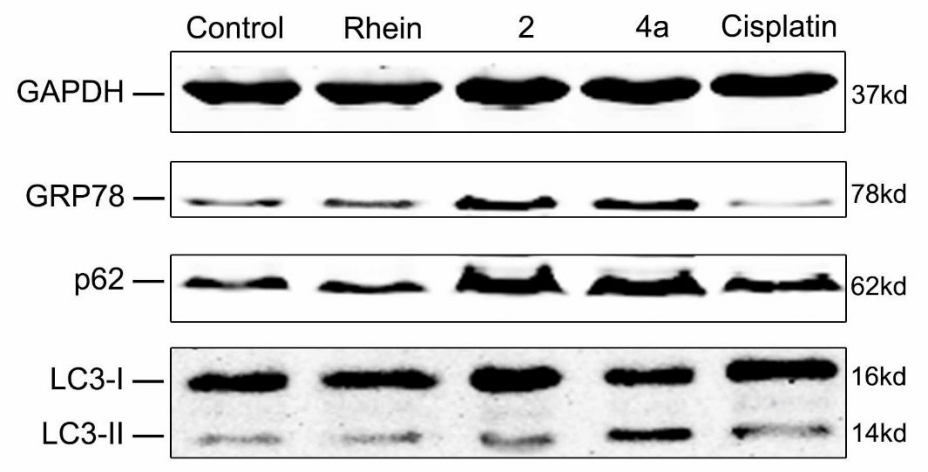


Figure S36. Western blotting analysis the expression of GRP78, p62, LC3 after Rhein, **2**, **4a**, Cisplatin treatment at the same concentration in SMMC-7721 cells, GAPDH is shown as a loading control.

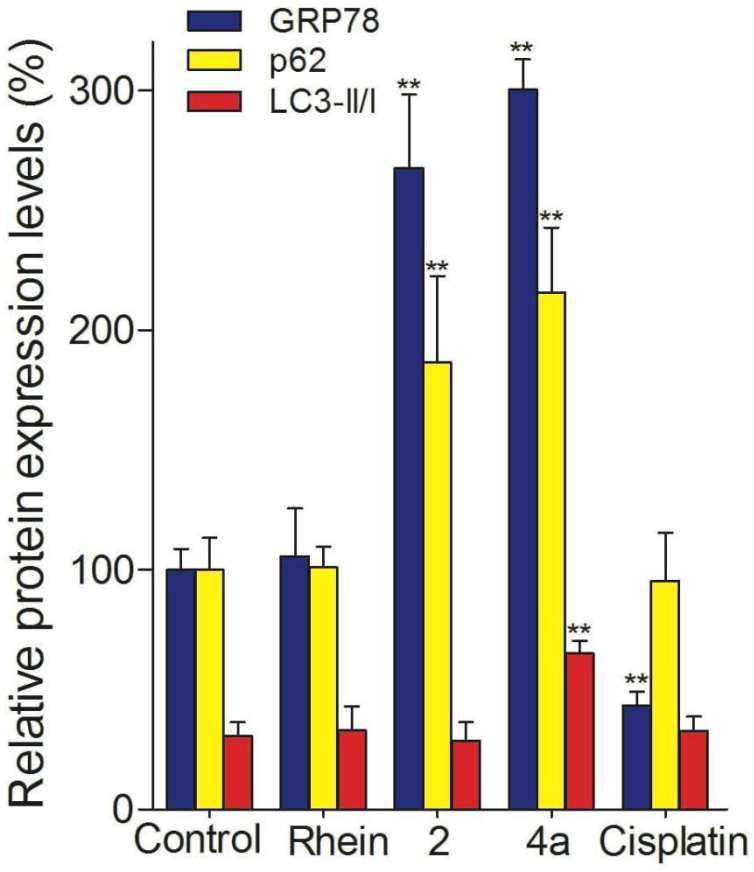


Figure S37. Statistical analysis histogram of related protein expression, **=P<0.01.

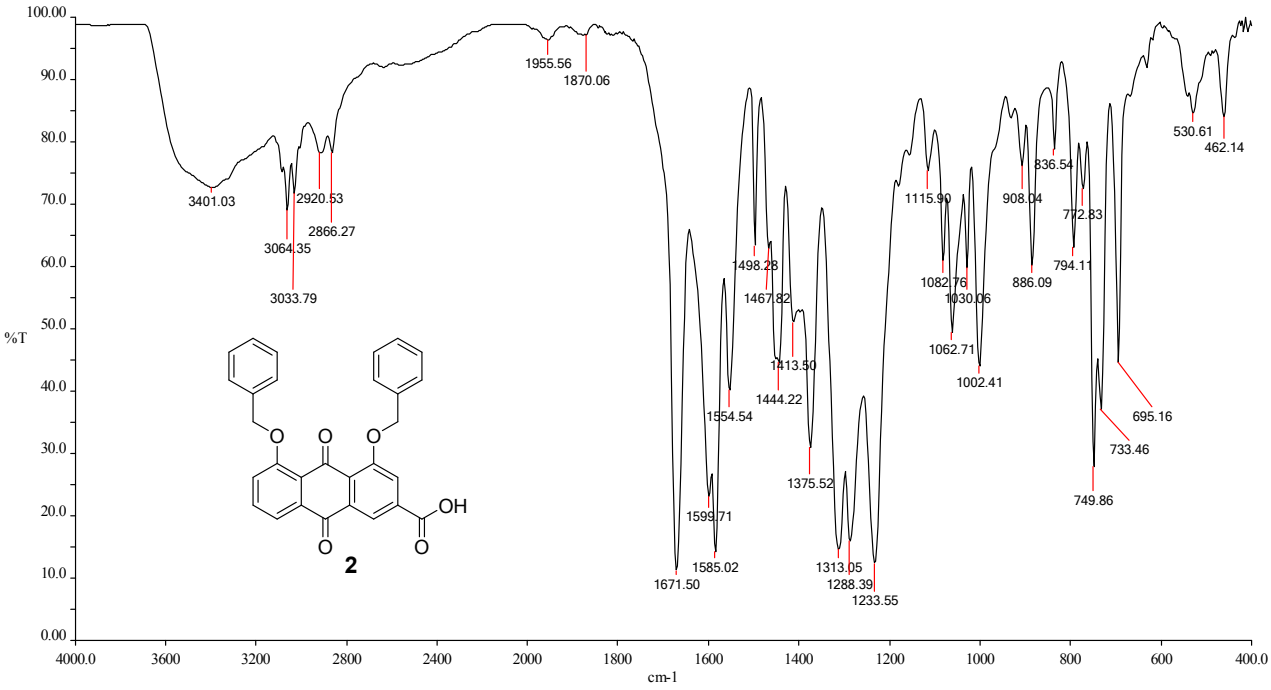


Figure S38. IR spectrum of compound **2**.

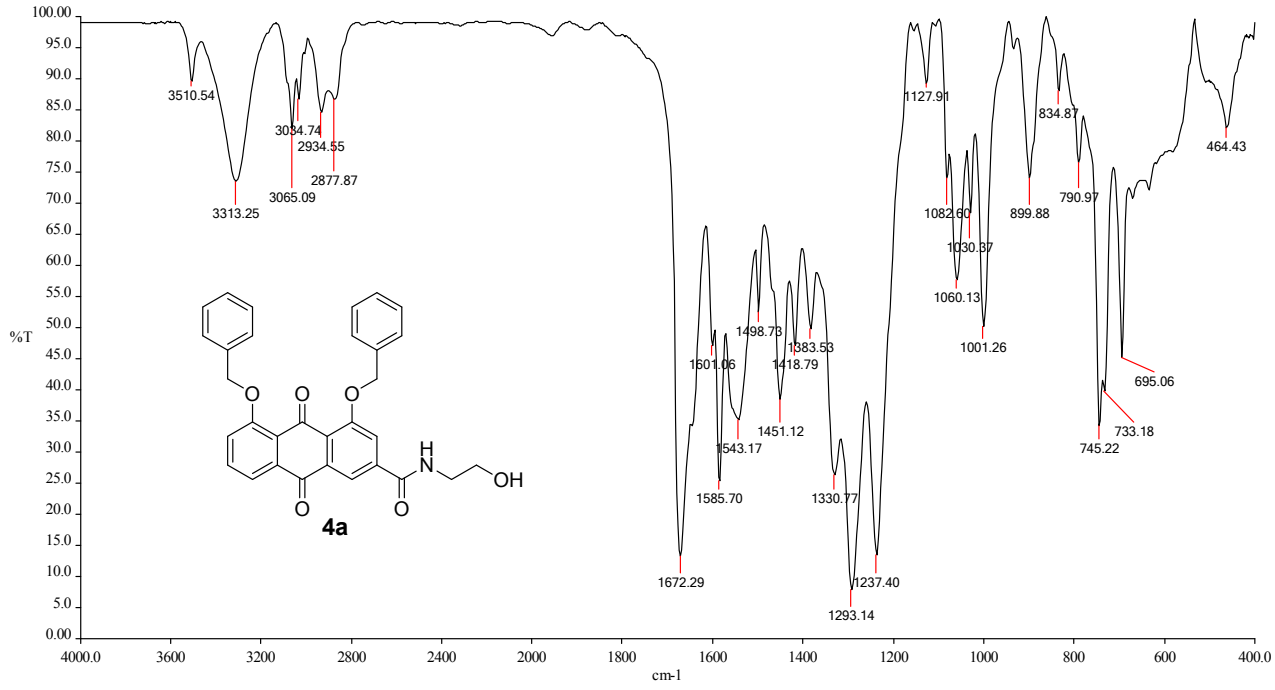


Figure S39. IR spectrum of compound **4a**.

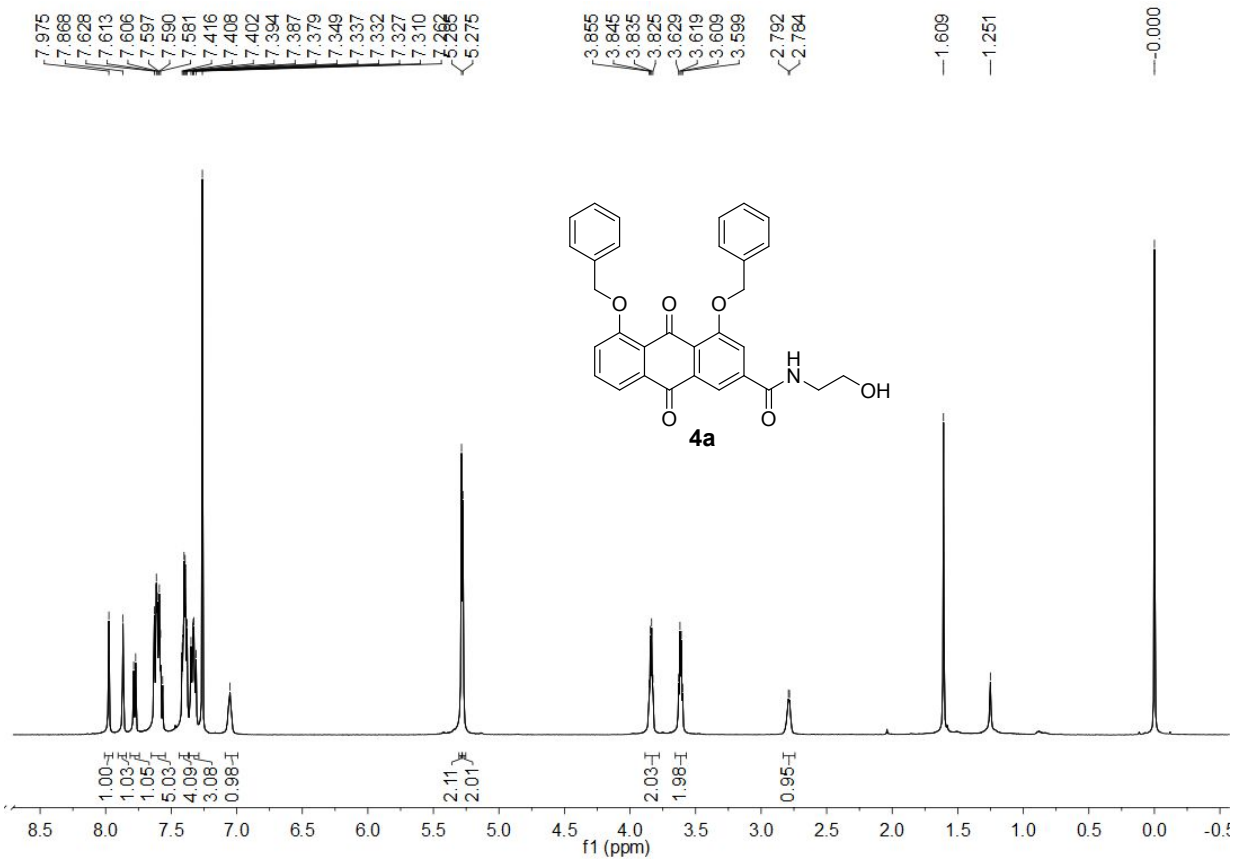


Figure S40. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **4a**.

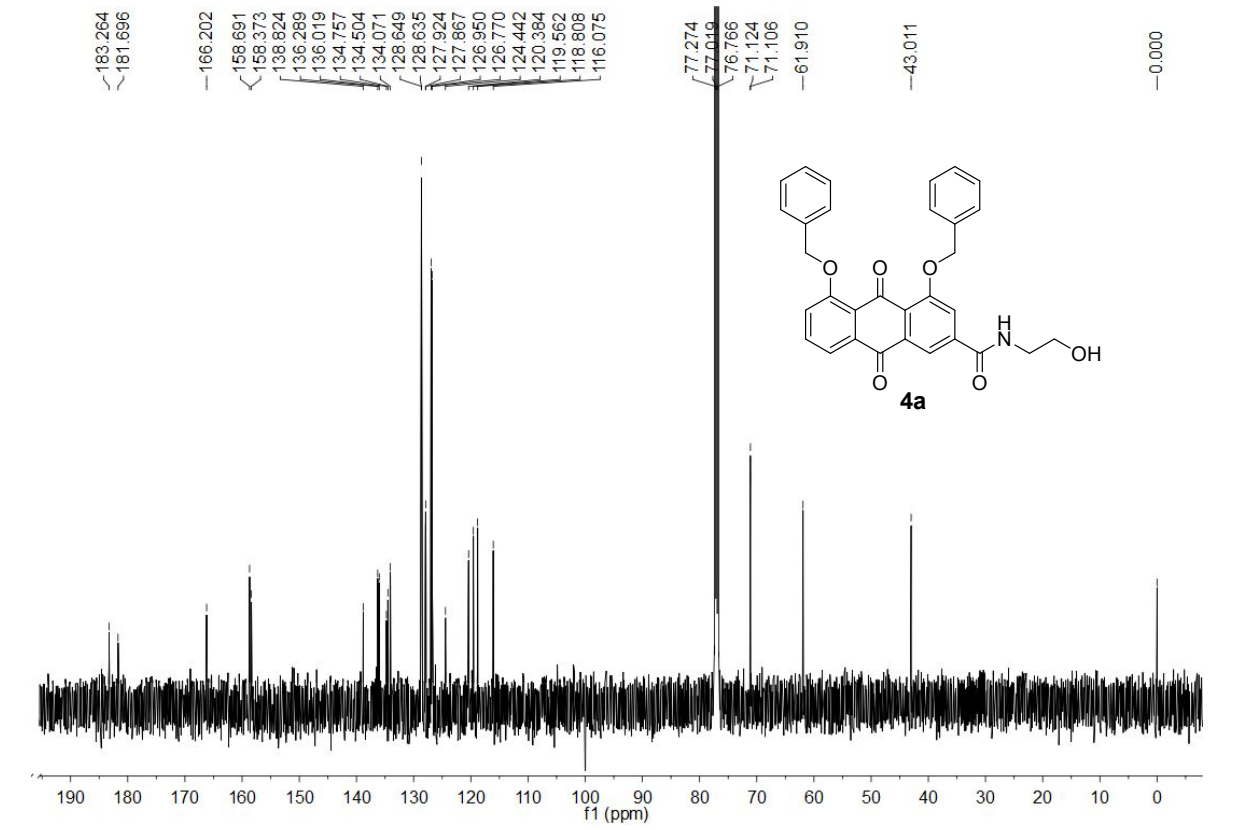


Figure S41. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **4a**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000067.d	Acquisition Date	10/18/2017 9:22:32 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 Ip
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	1048576
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

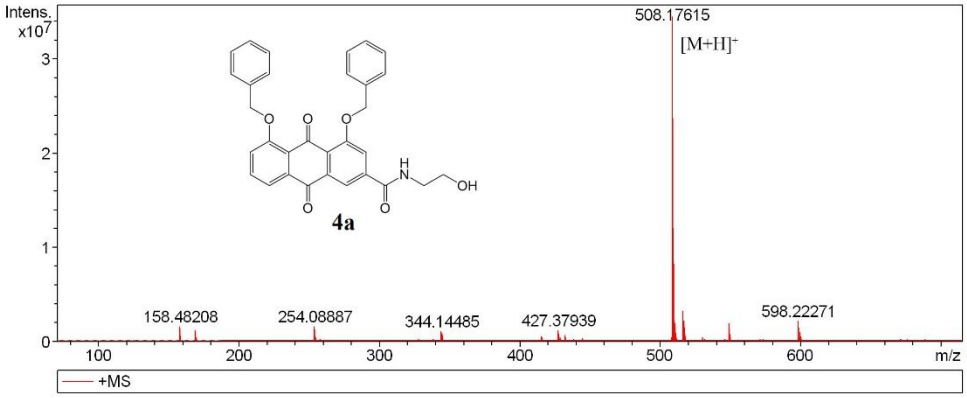


Figure S42. HRMS (ESI) spectrum of compound **4a**.

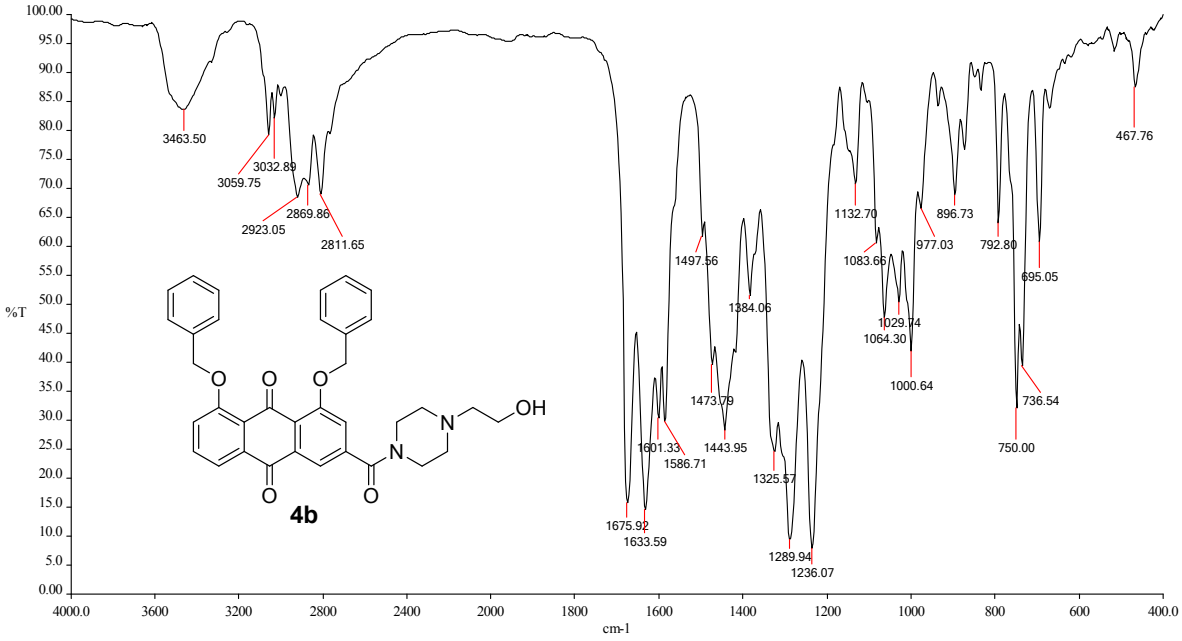


Figure S43. IR spectrum of compound **4b**.

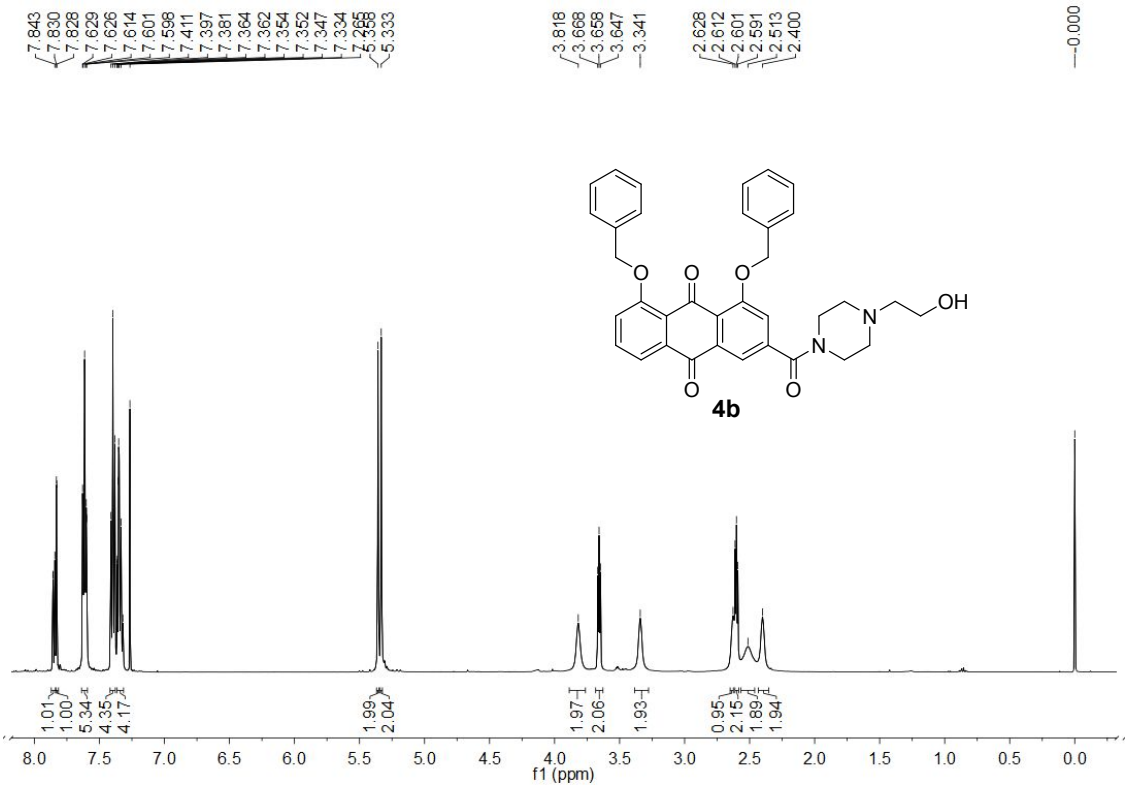


Figure S44. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **4b**.

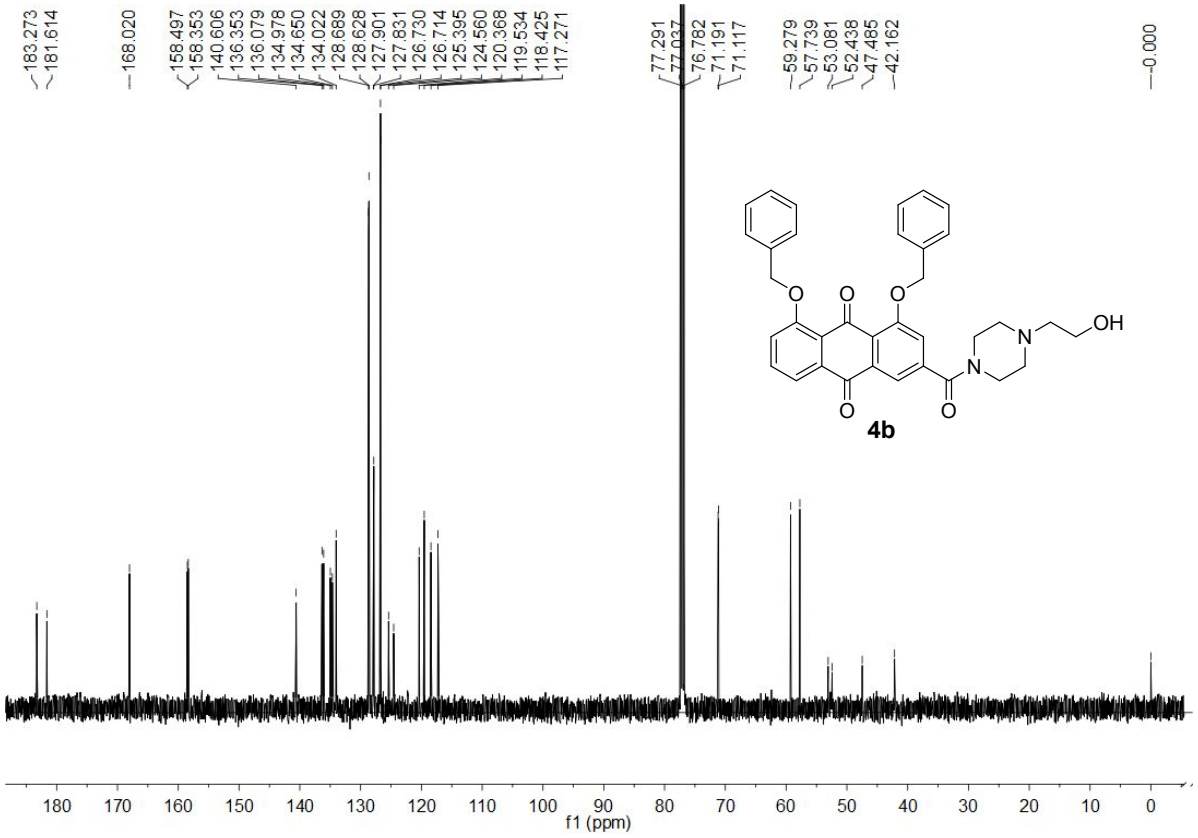


Figure S45. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **4b**.

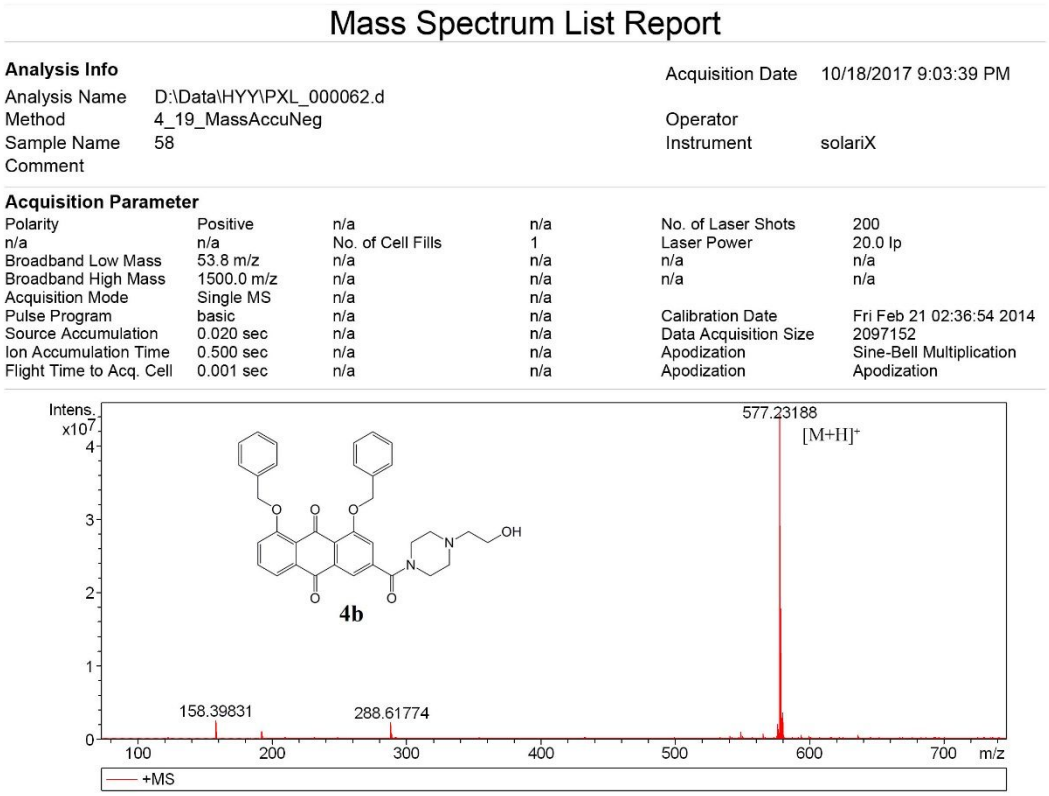


Figure S46. HRMS (ESI) spectrum of compound **4b**.

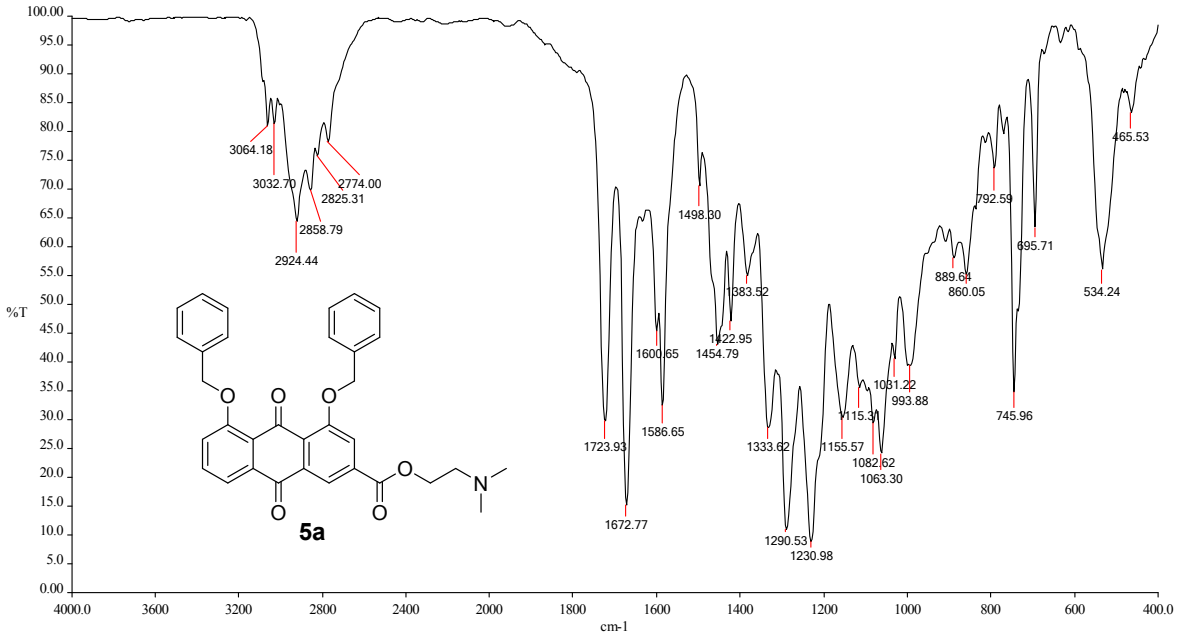


Figure S47. IR spectrum of compound **5a**.

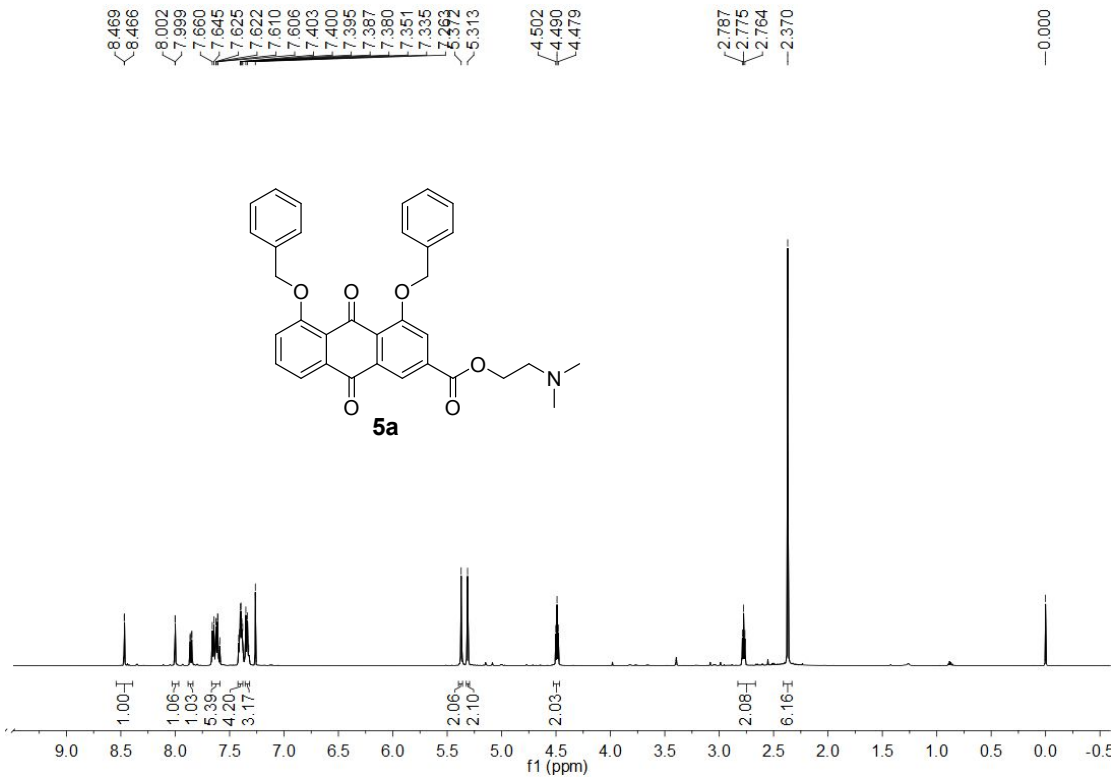


Figure S48. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **5a**.

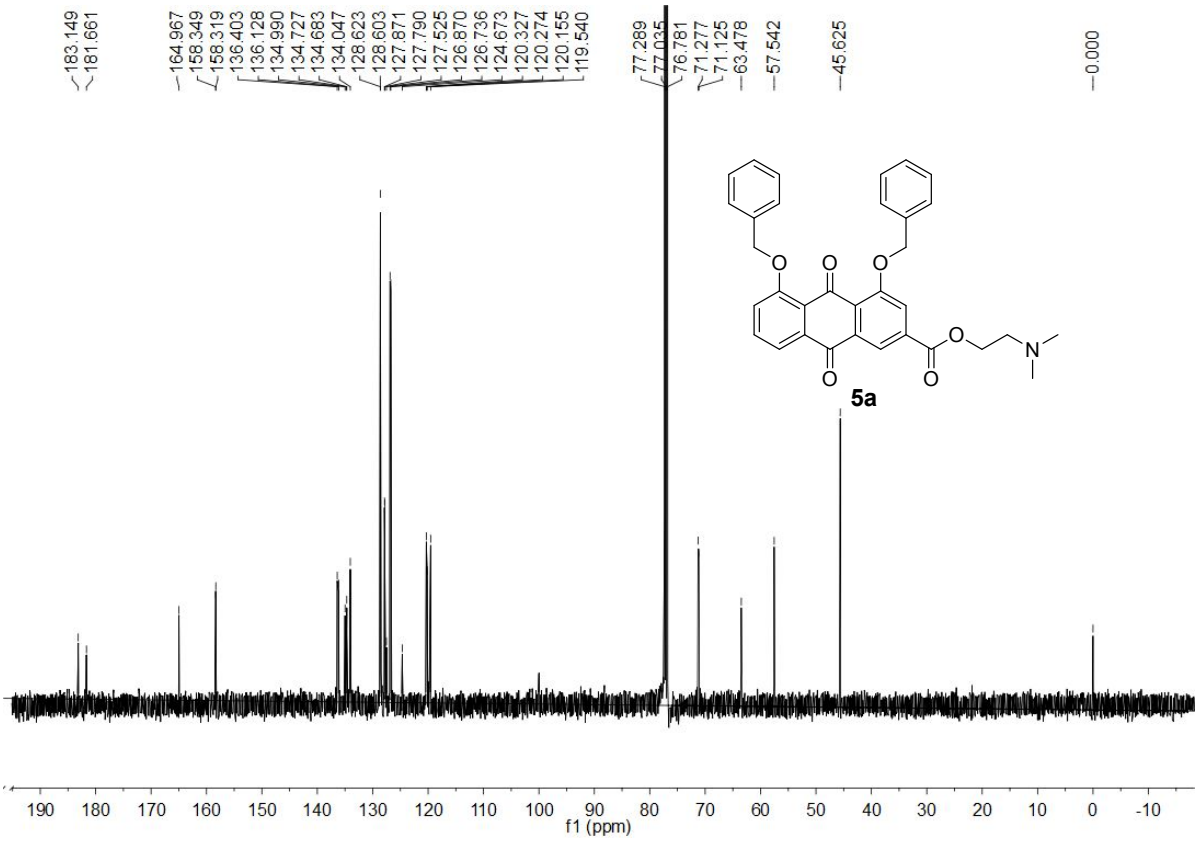


Figure S49. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **5a**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\YY_000003.d	Acquisition Date	10/9/2017 10:05:28 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1000.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	2097152
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

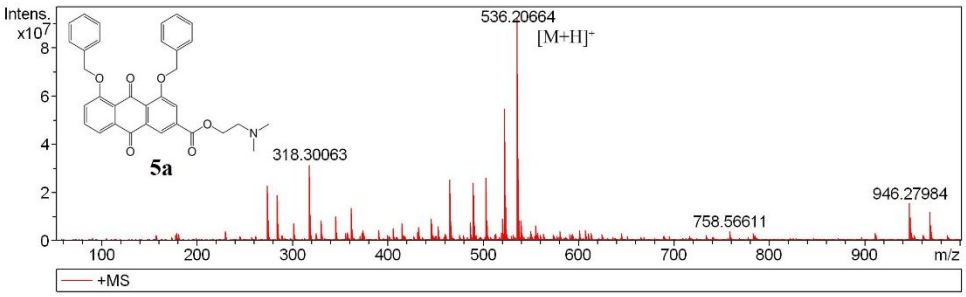


Figure S50. IR spectrum of compound **5a**.

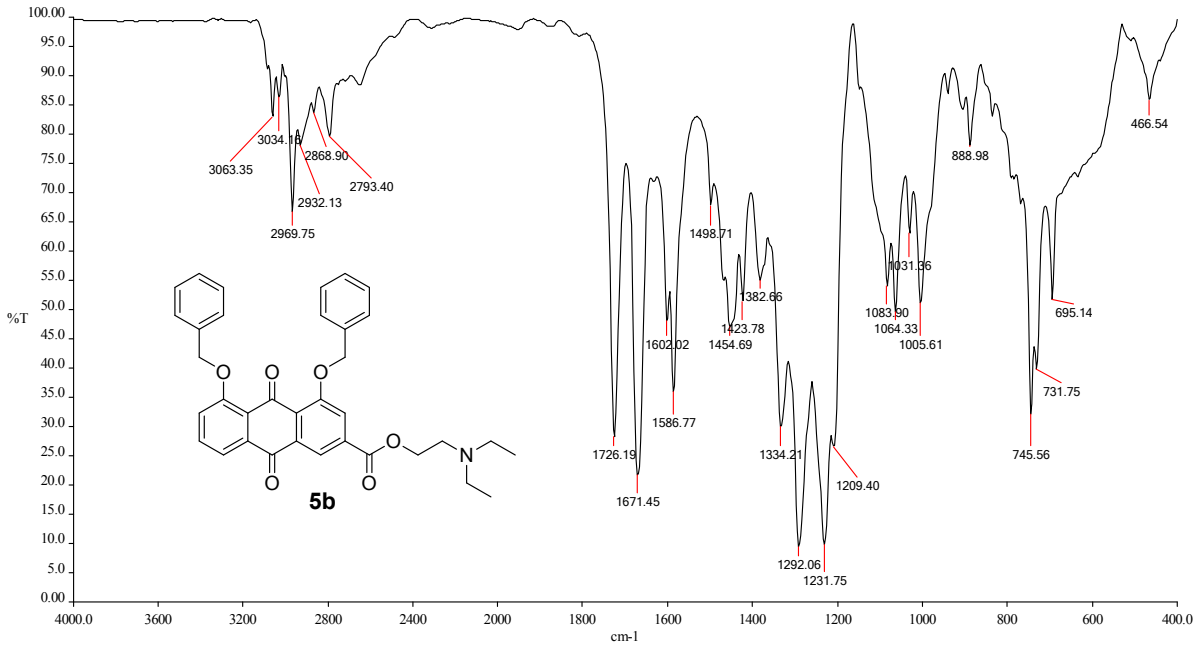


Figure S51. IR spectrum of compound **5b**.

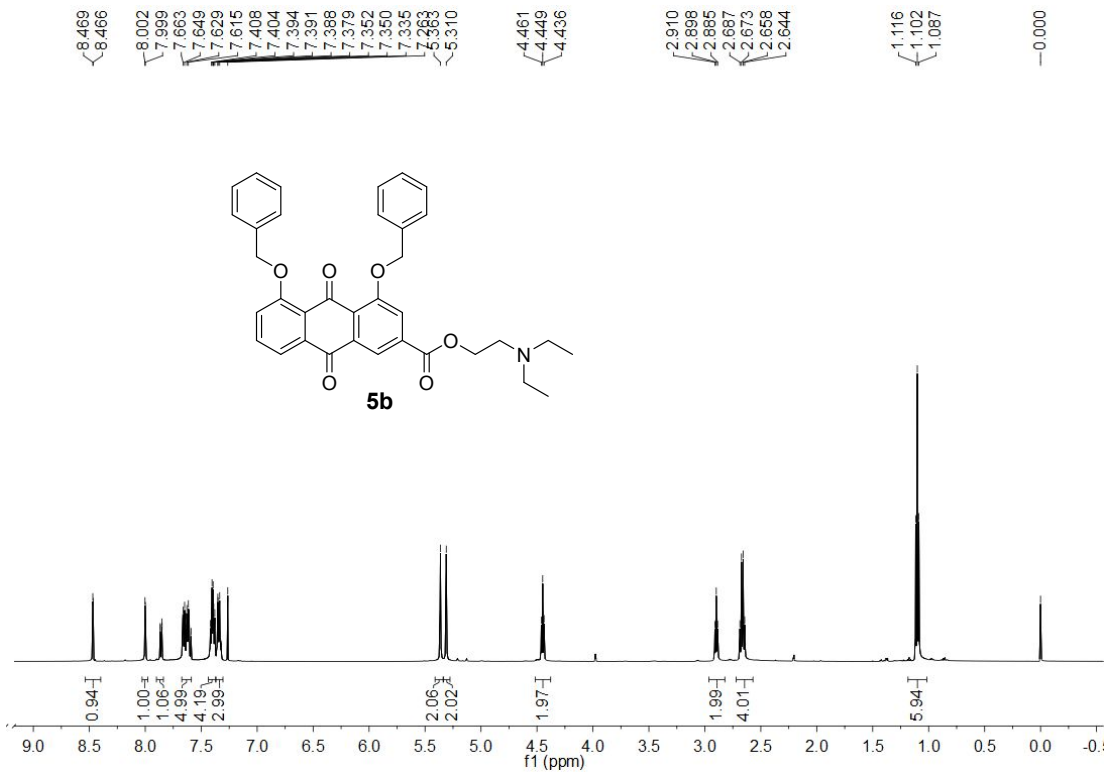


Figure S52. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **5b**.

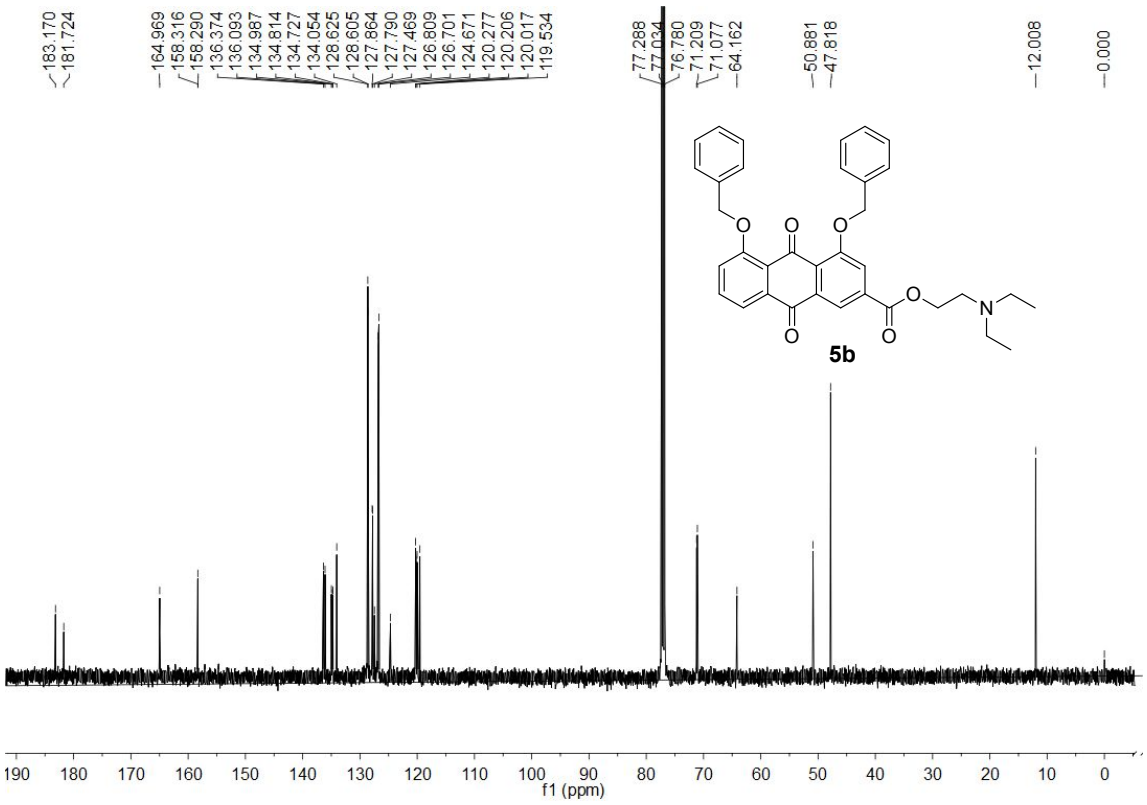


Figure S53. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **5b**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000054.d	Acquisition Date	10/18/2017 8:16:12 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	2097152
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

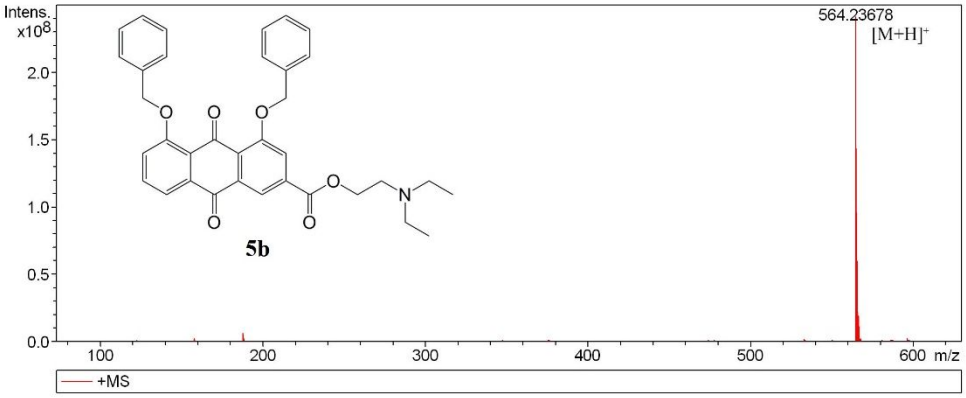


Figure S54. HRMS (ESI) spectrum of compound **5b**.

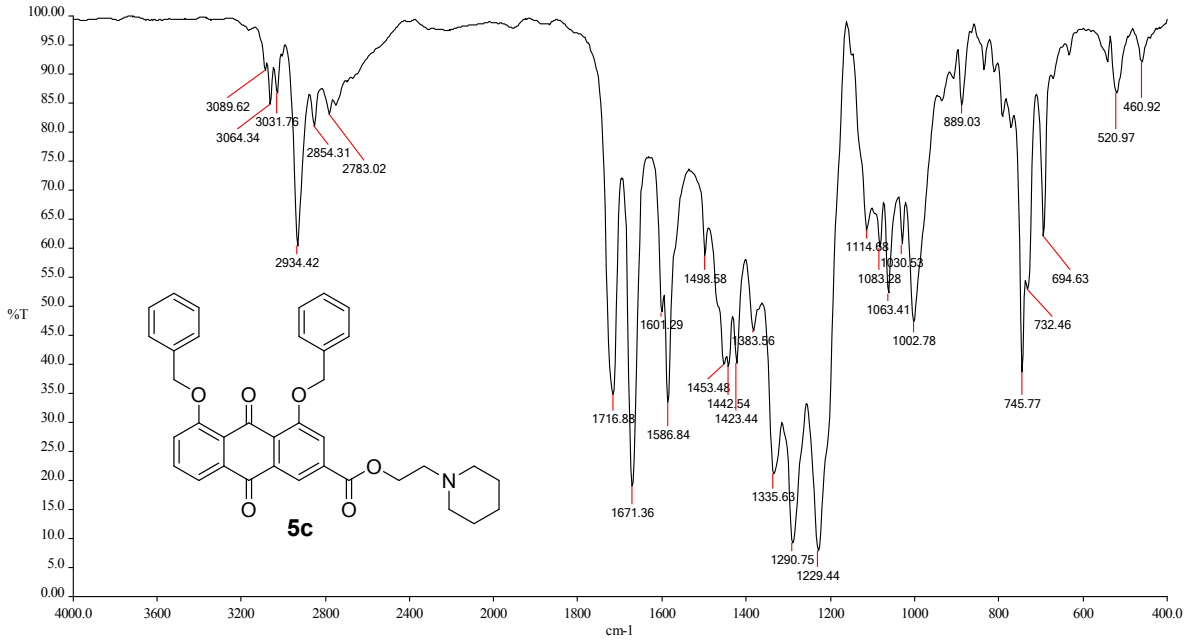


Figure S55. IR spectrum of compound **5c**.

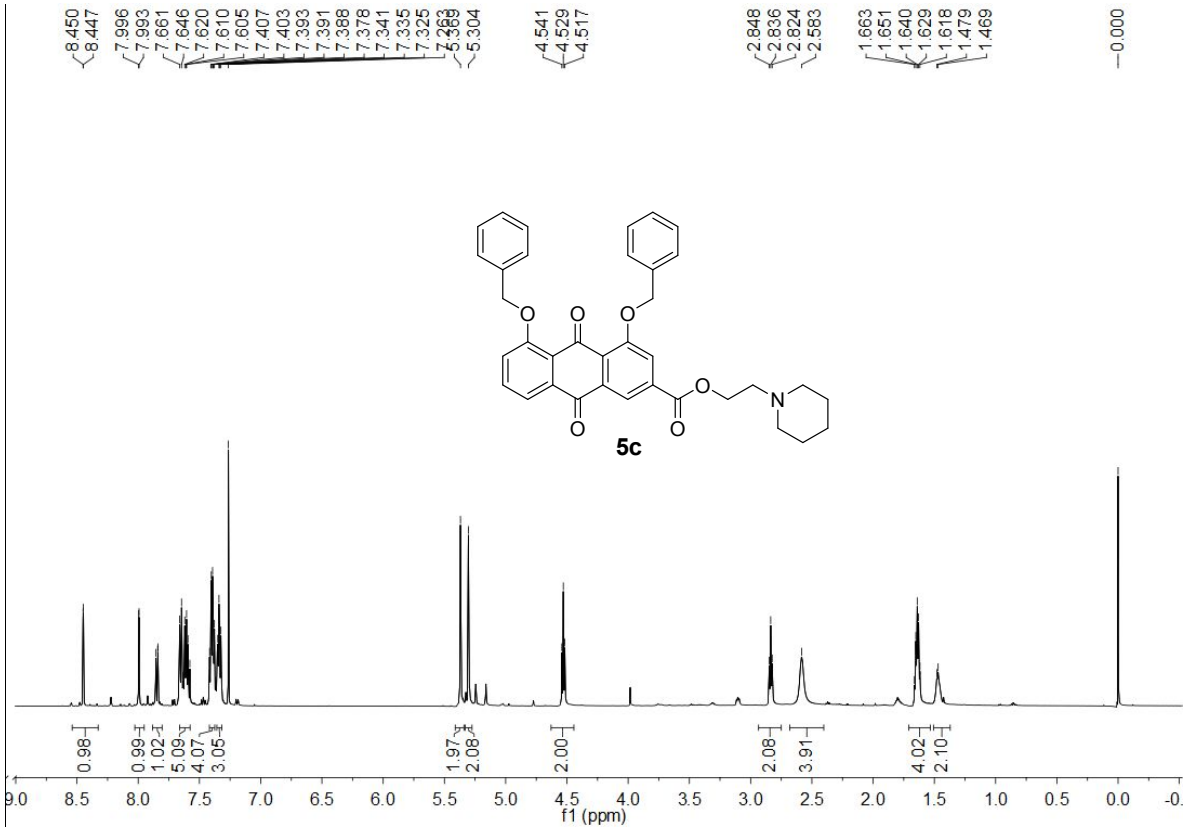


Figure S56. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **5c**.

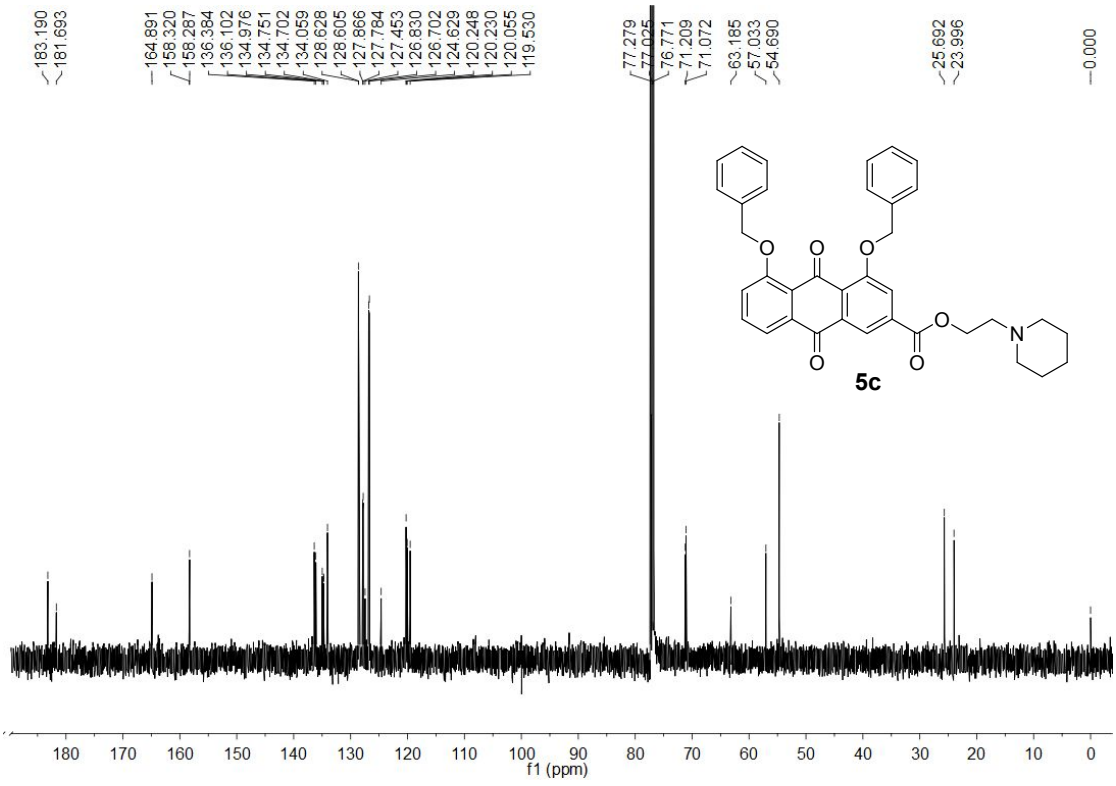


Figure S57. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **5c**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000063.d	Acquisition Date	10/18/2017 9:06:22 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 Ip
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	1048576
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

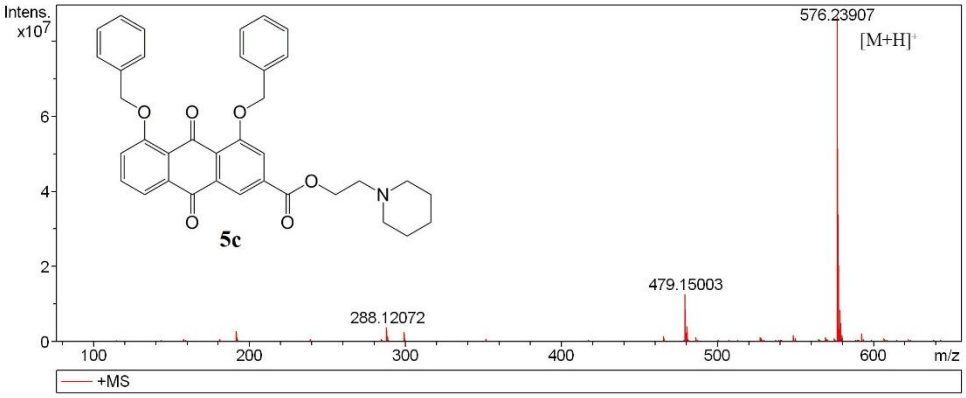


Figure S58. HRMS (ESI) spectrum of compound **5c**.

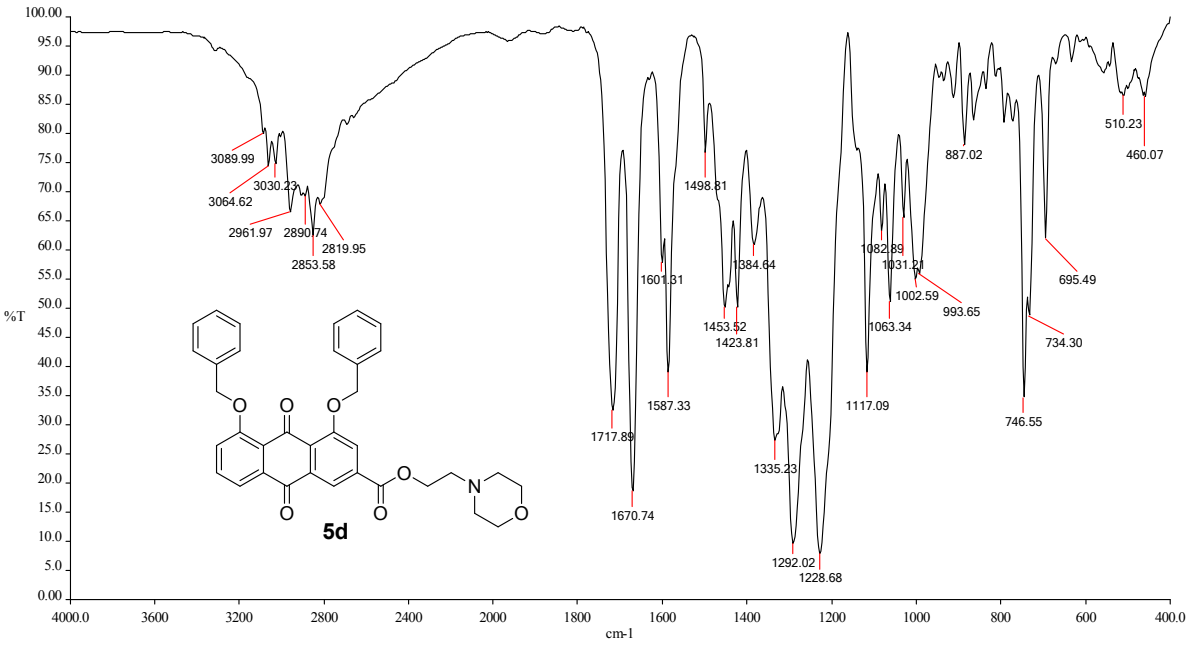


Figure S59. IR spectrum of compound **5d**.

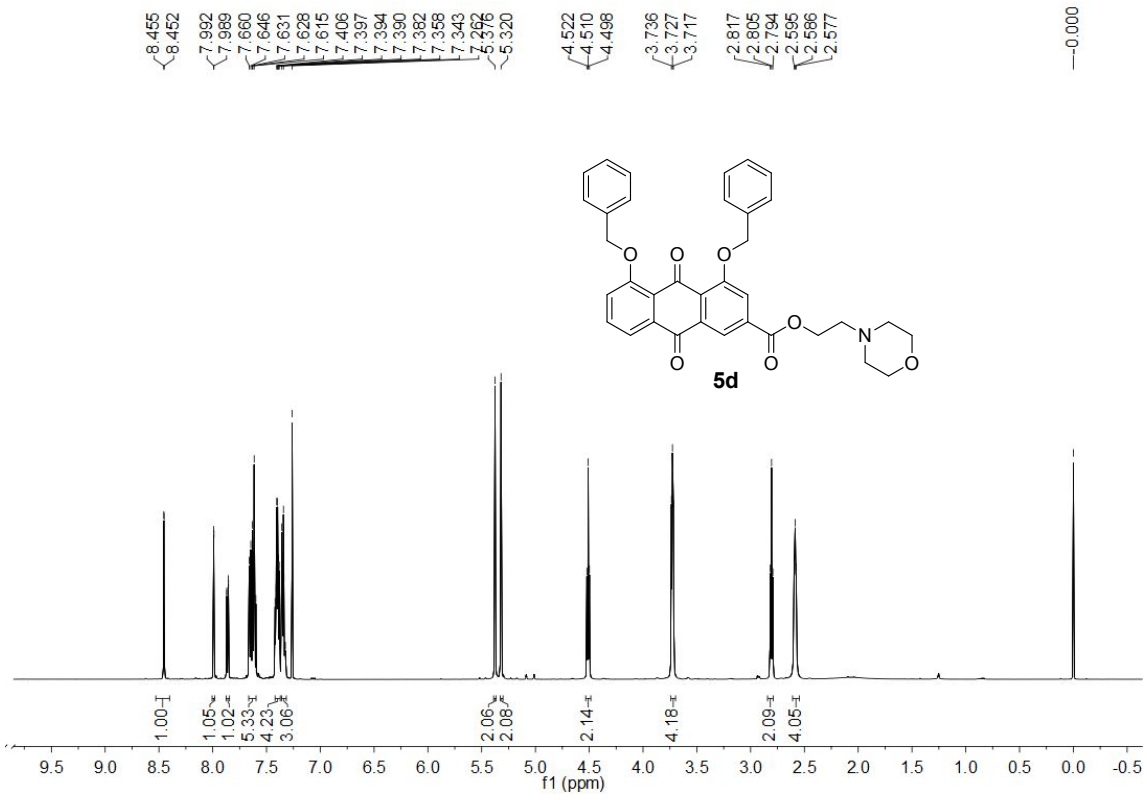


Figure S60. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **5d**.

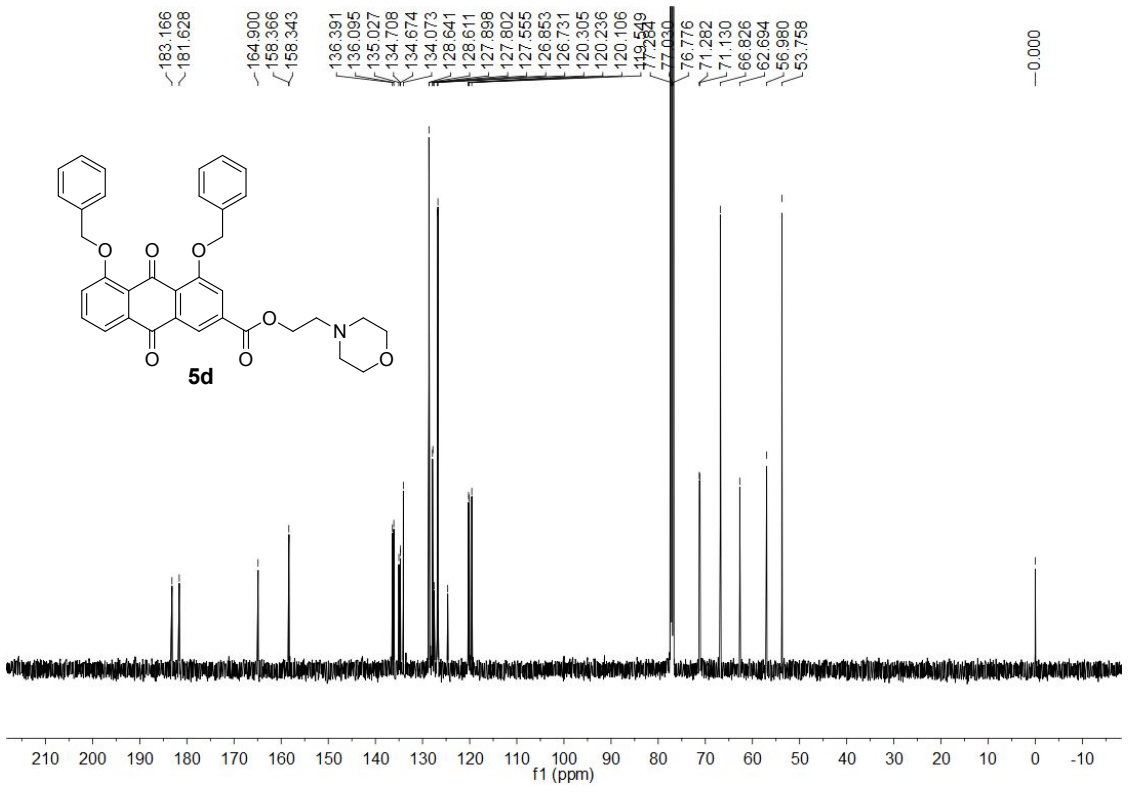


Figure S61. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **5d**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000068.d	Acquisition Date	10/18/2017 9:26:51 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	1048576
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

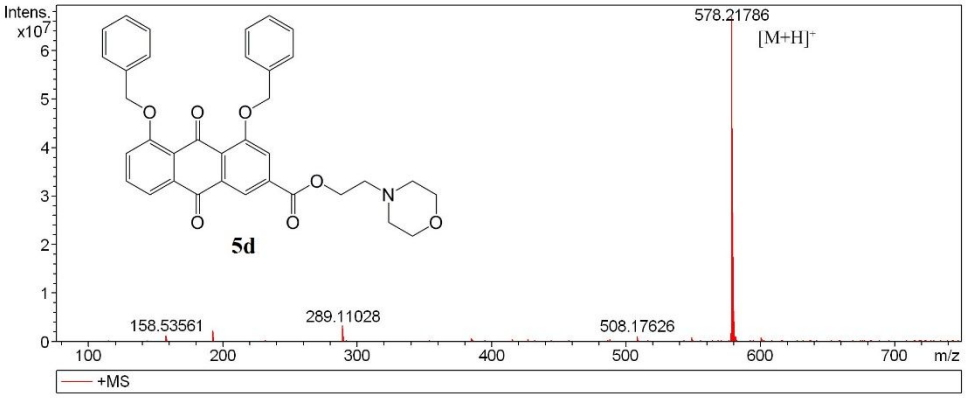


Figure S62. HRMS (ESI) spectrum of compound **5d**.

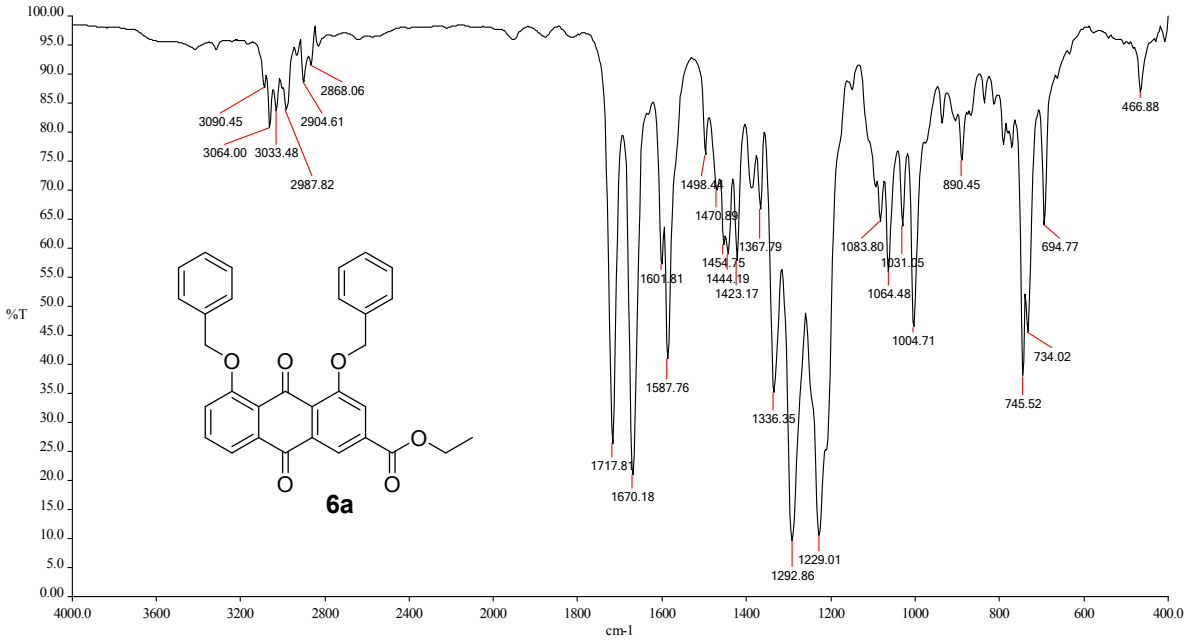


Figure S63. IR spectrum of compound **6a**.

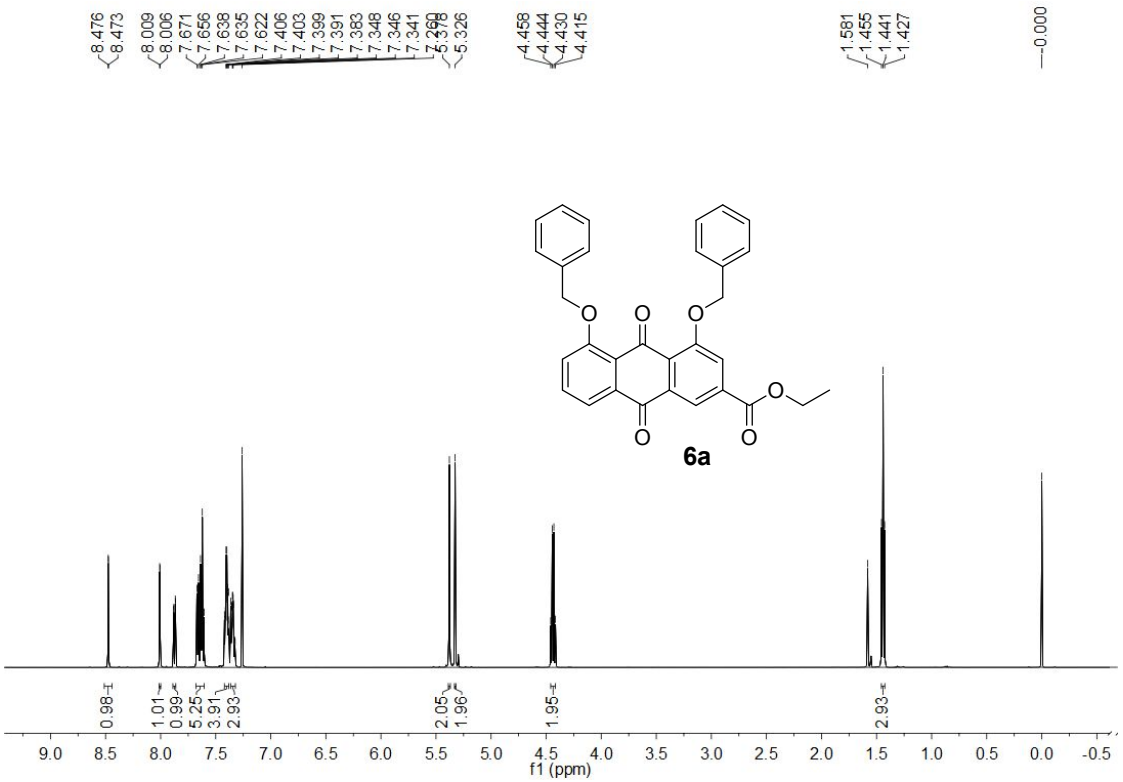


Figure S64. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6a**.

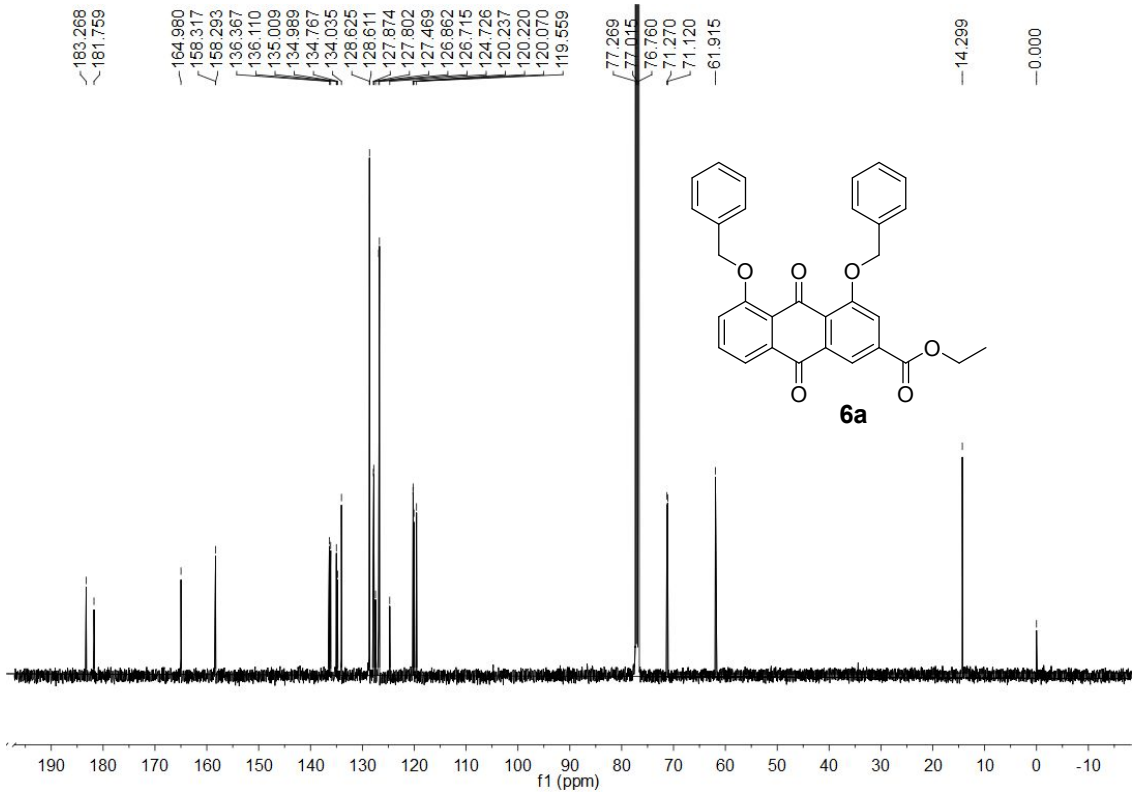


Figure S65. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6a**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000056.d	Acquisition Date	10/18/2017 8:46:49 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	2097152
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

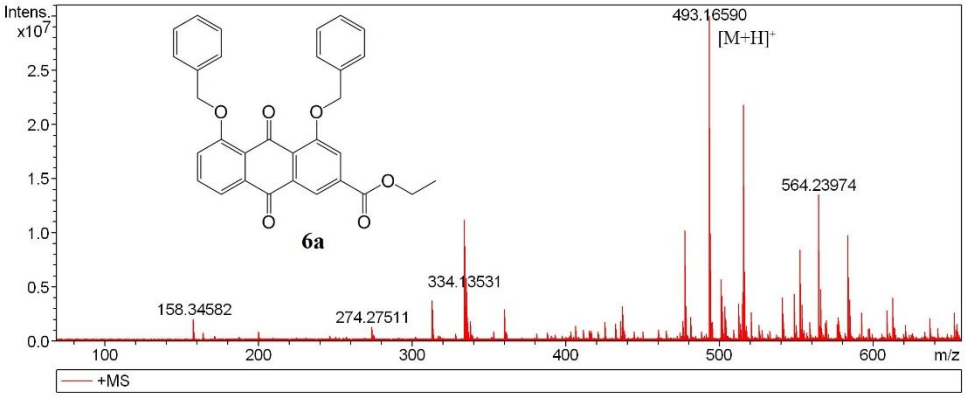


Figure S66. HRMS (ESI) spectrum of compound **6a**.

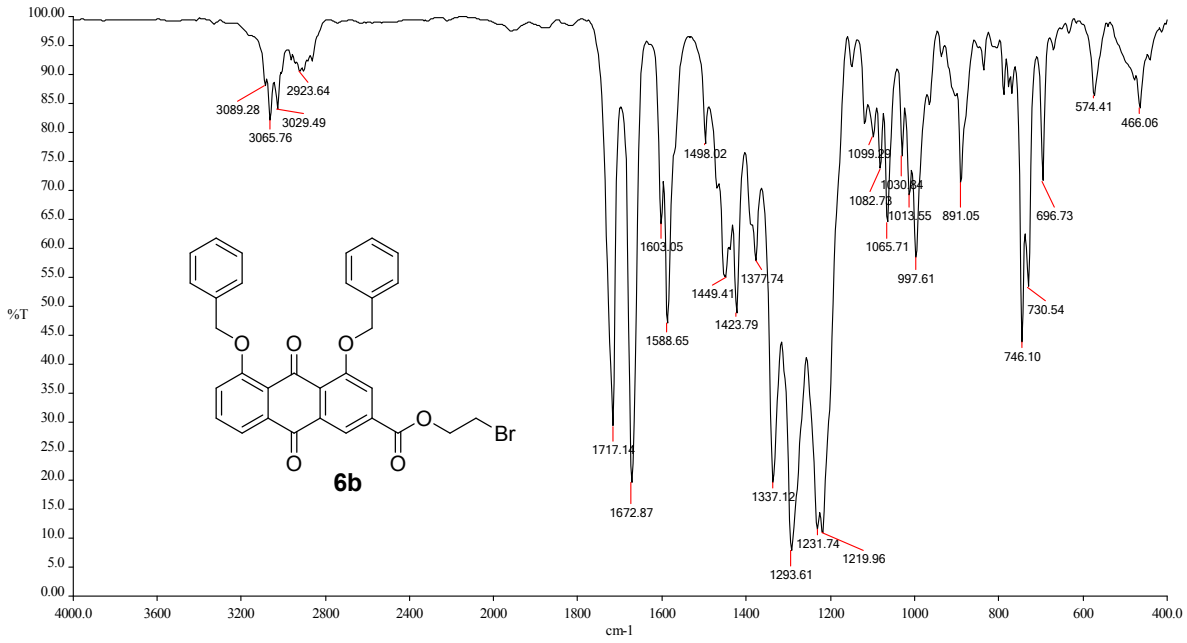


Figure S67. IR spectrum of compound **6b**.

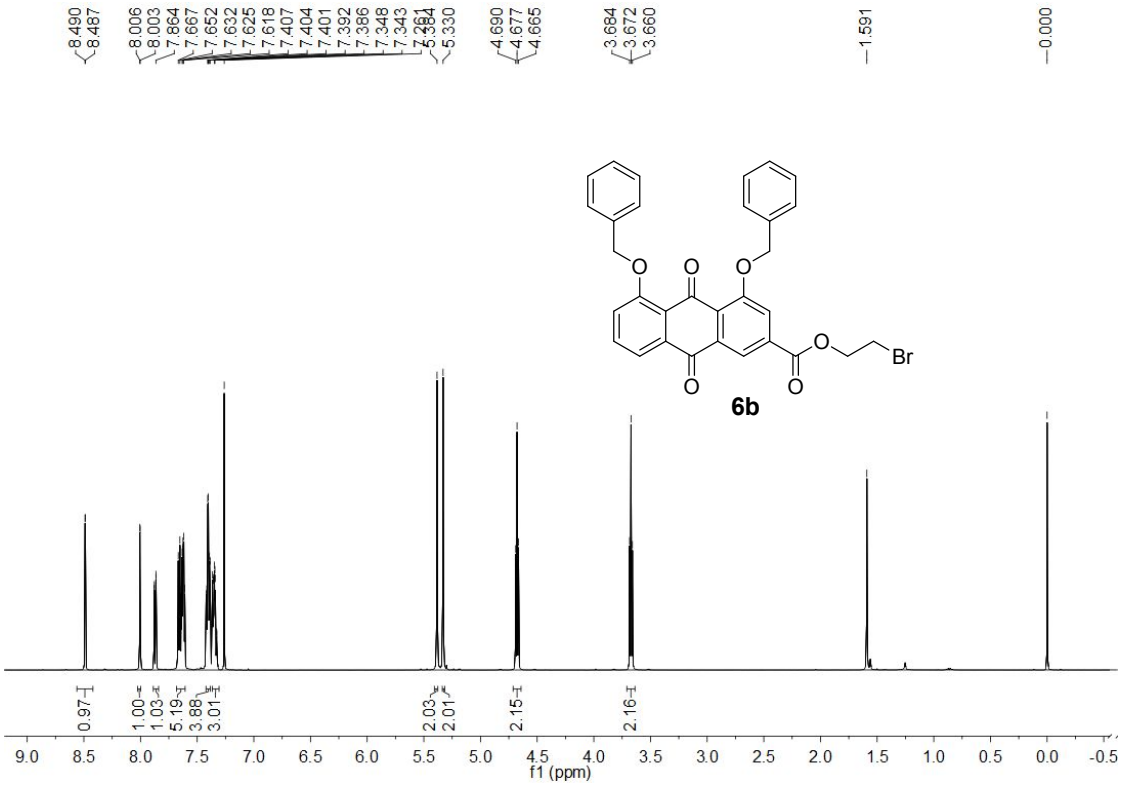


Figure S68. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6b**.

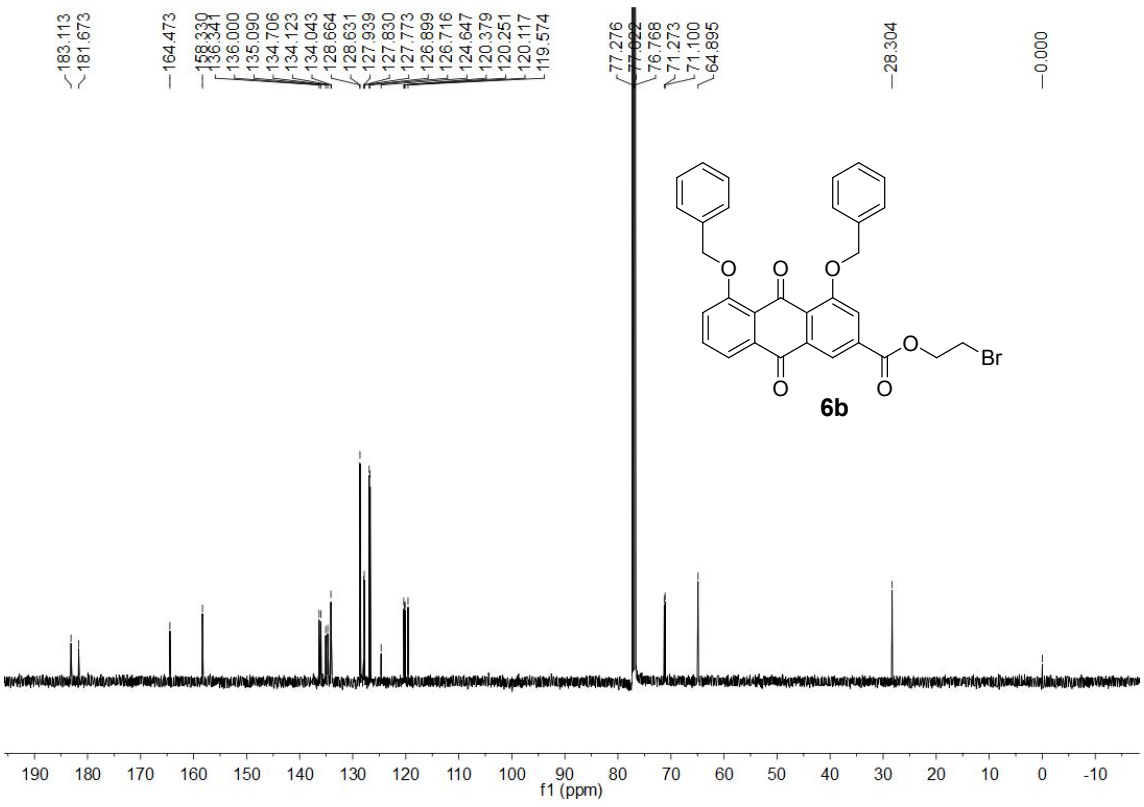


Figure S69. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6b**.

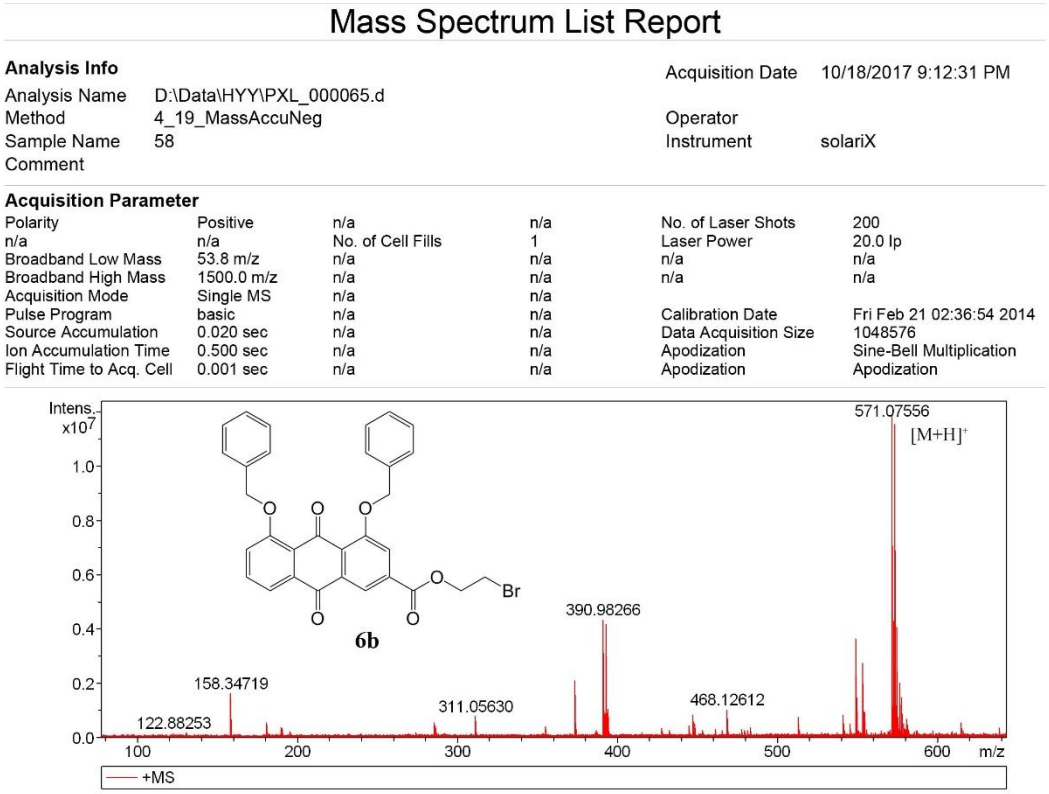


Figure S70. HRMS (ESI) spectrum of compound **6b**.

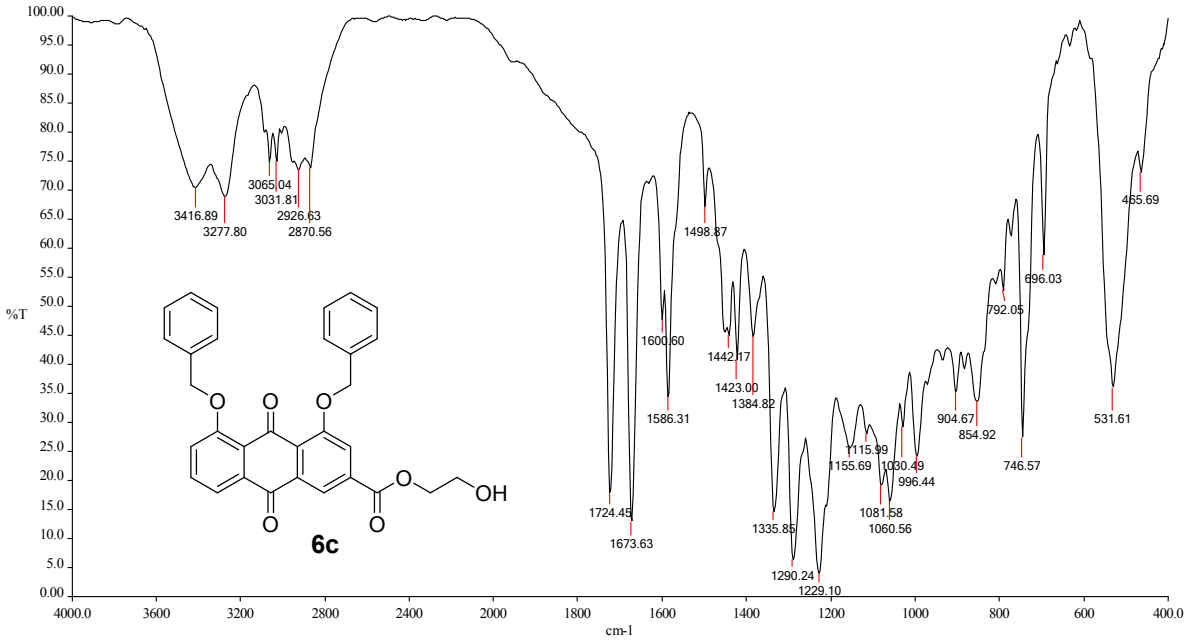


Figure S71. IR spectrum of compound **6c**.

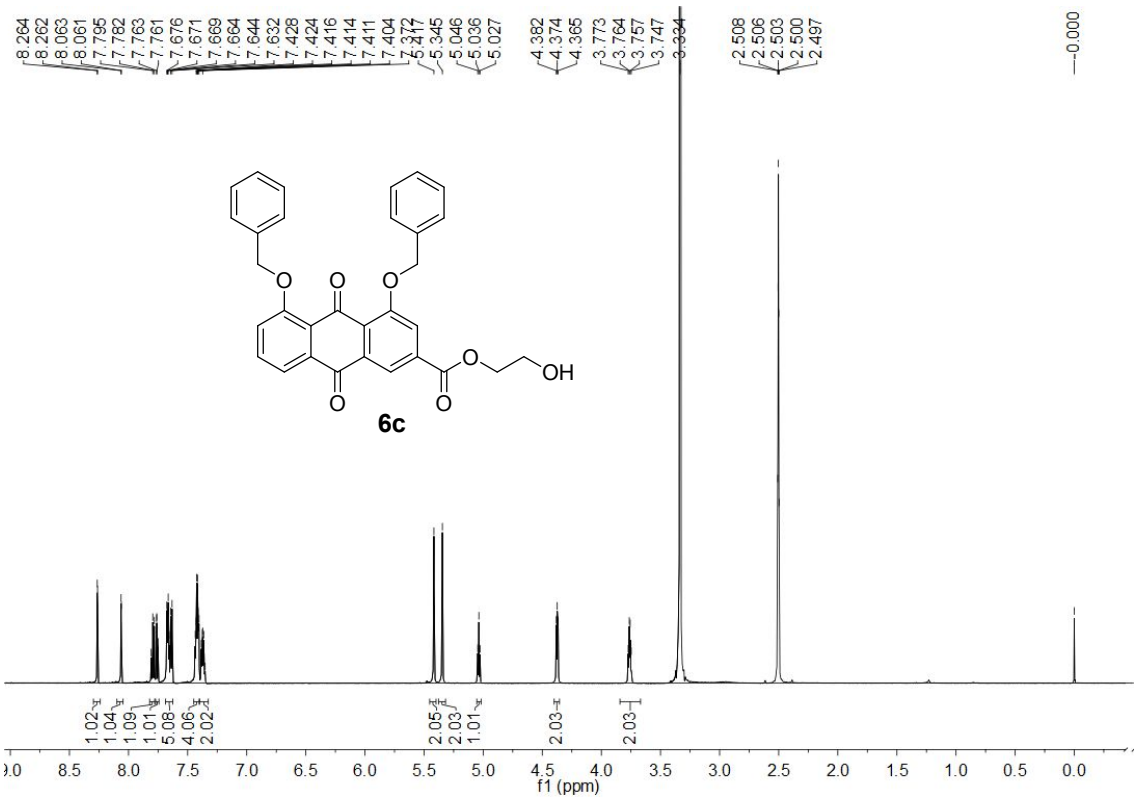


Figure S72. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **6c**.

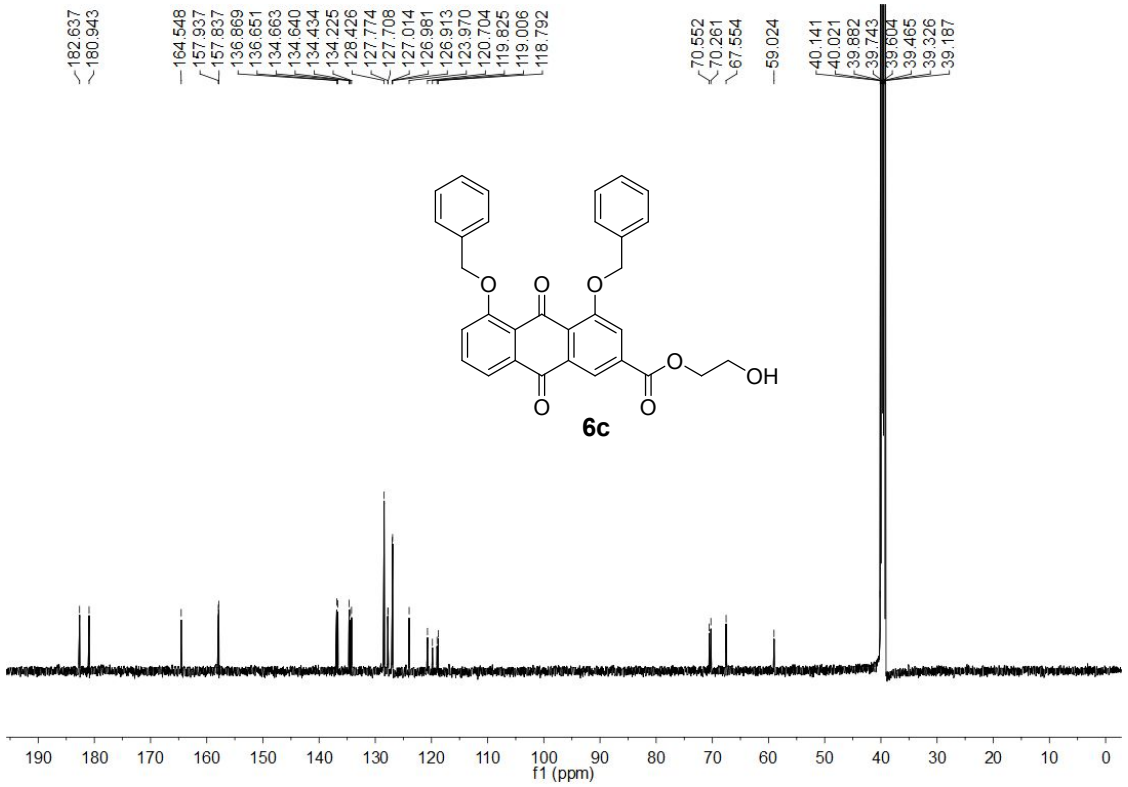


Figure S73. ^{13}C NMR (150.9 MHz, DMSO-d_6) spectrum of compound **6c**.

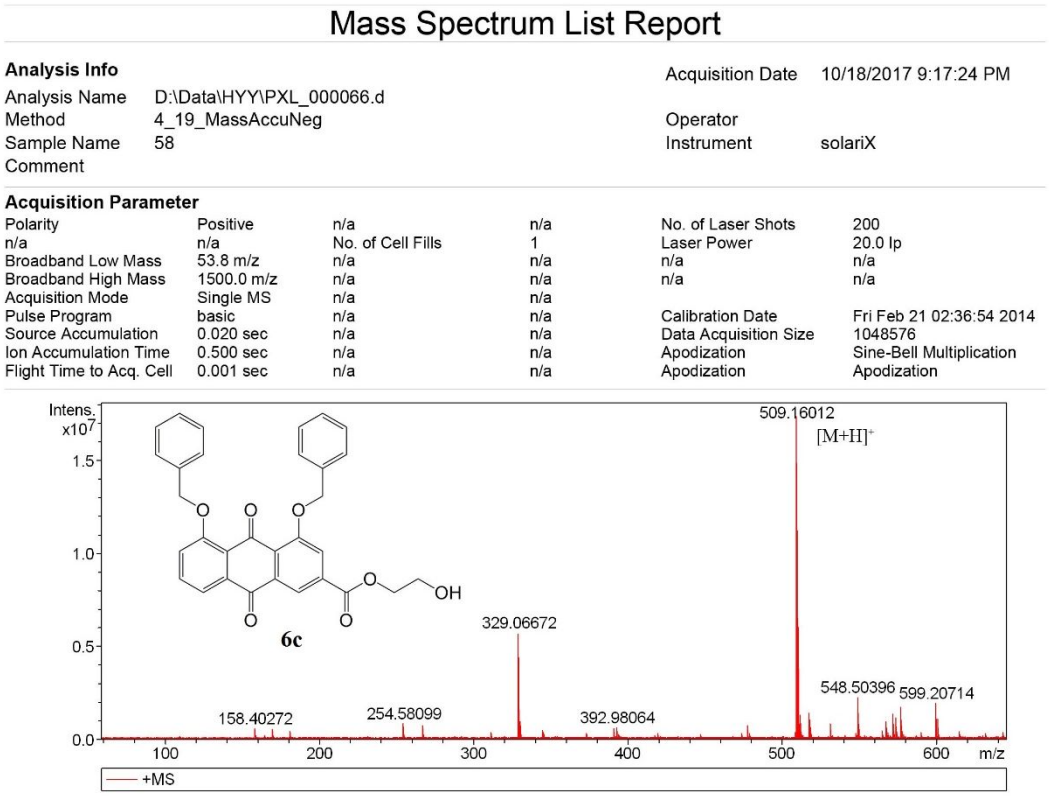


Figure S74. HRMS (ESI) spectrum of compound **6c**.

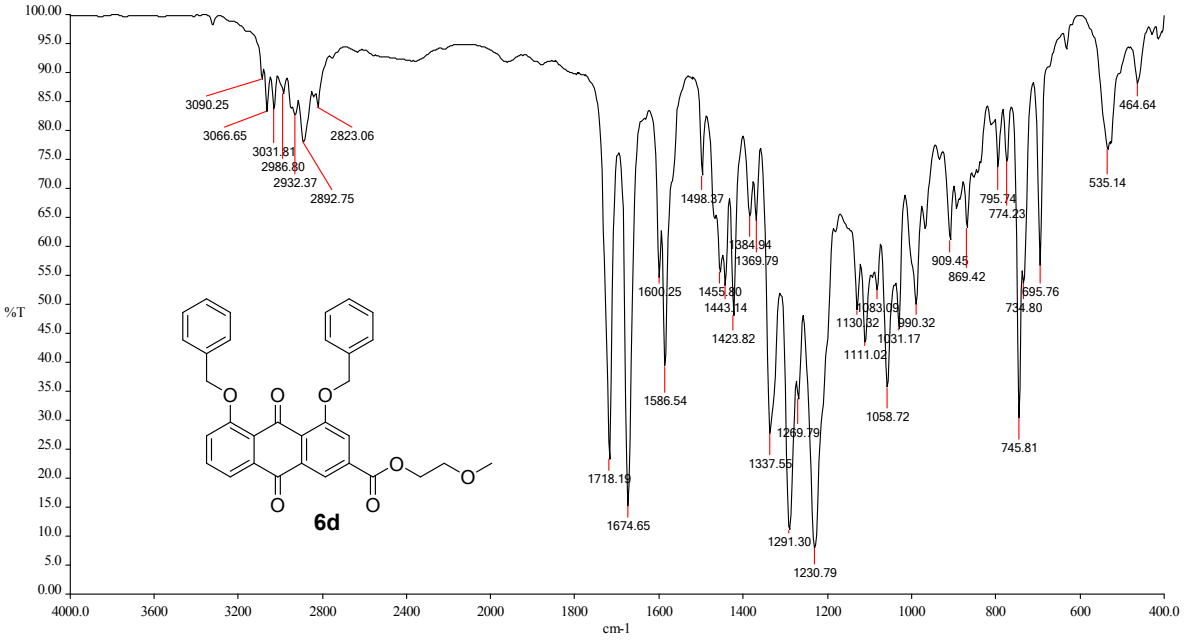


Figure S75. IR spectrum of compound **6d**.

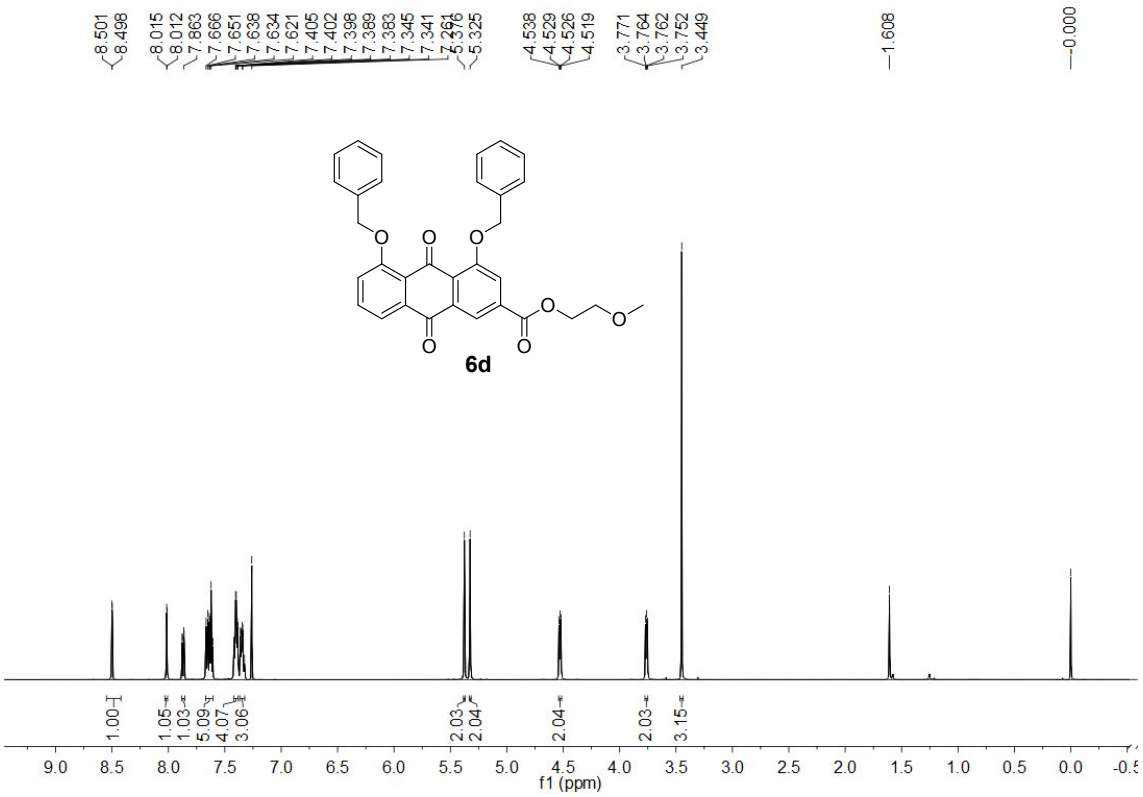


Figure S76. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6d**.

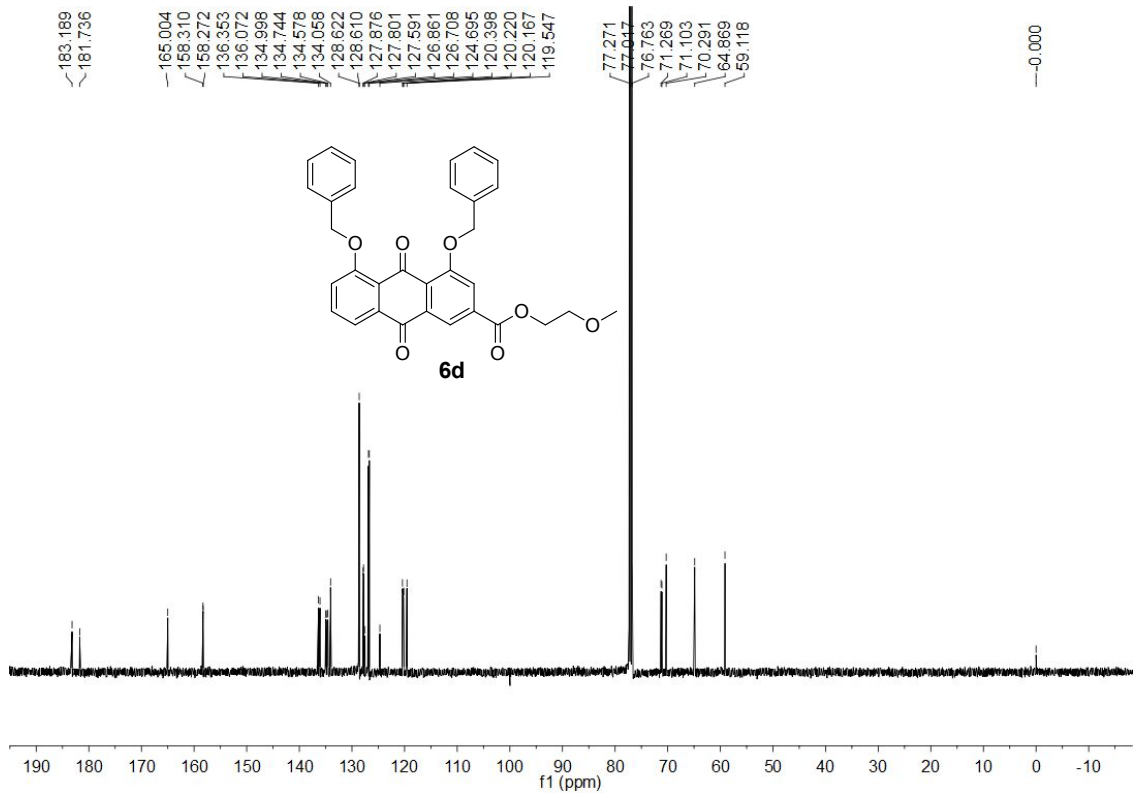


Figure S77. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6d**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000069.d	Acquisition Date	10/18/2017 9:30:22 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 Ip
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	1048576
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

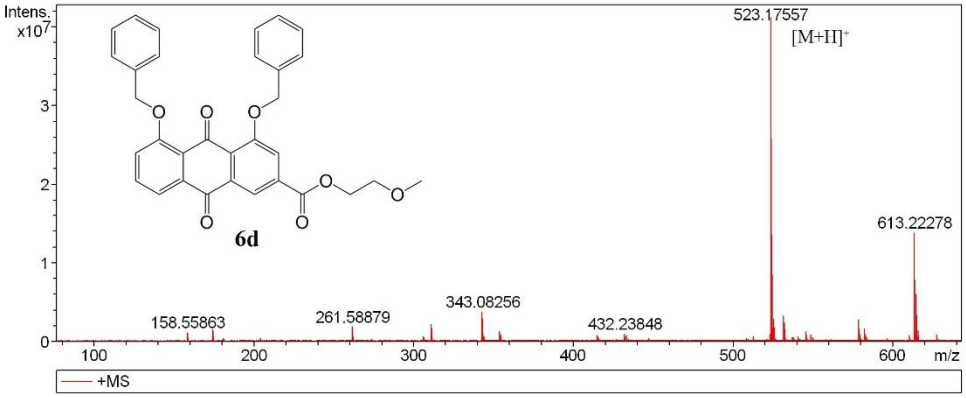


Figure S78. HRMS (ESI) spectrum of compound **6d**.

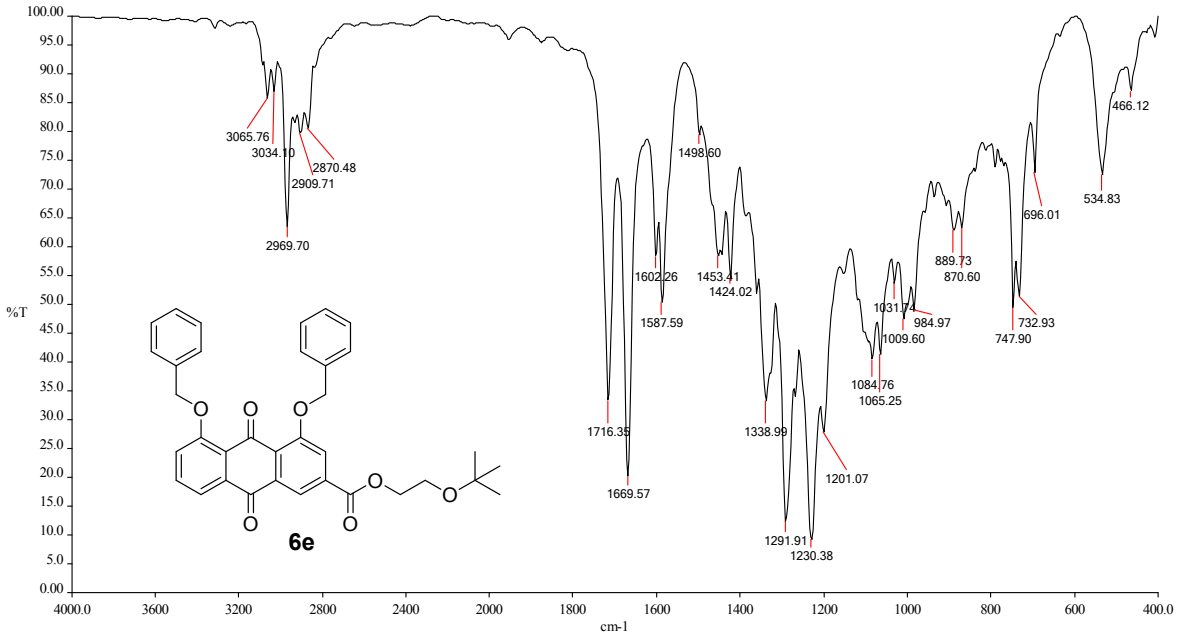


Figure S79. IR spectrum of compound **6e**.

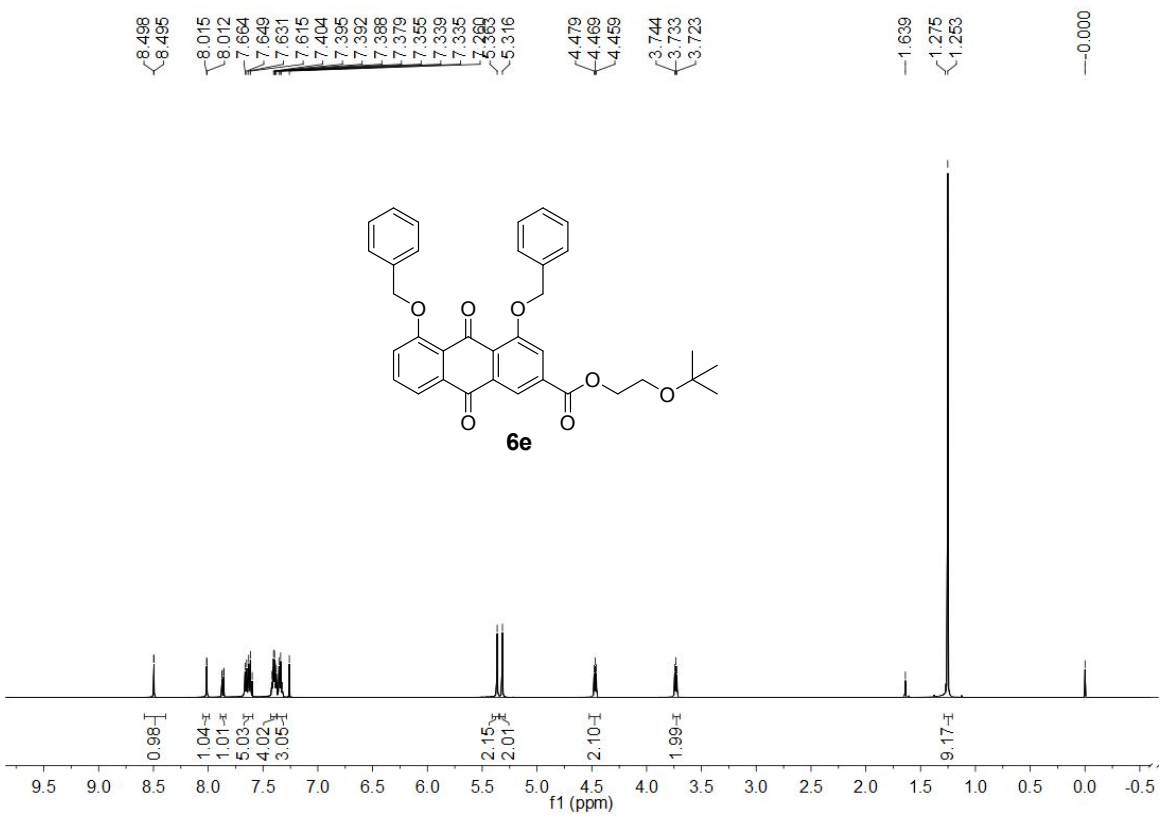


Figure S80. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6e**.

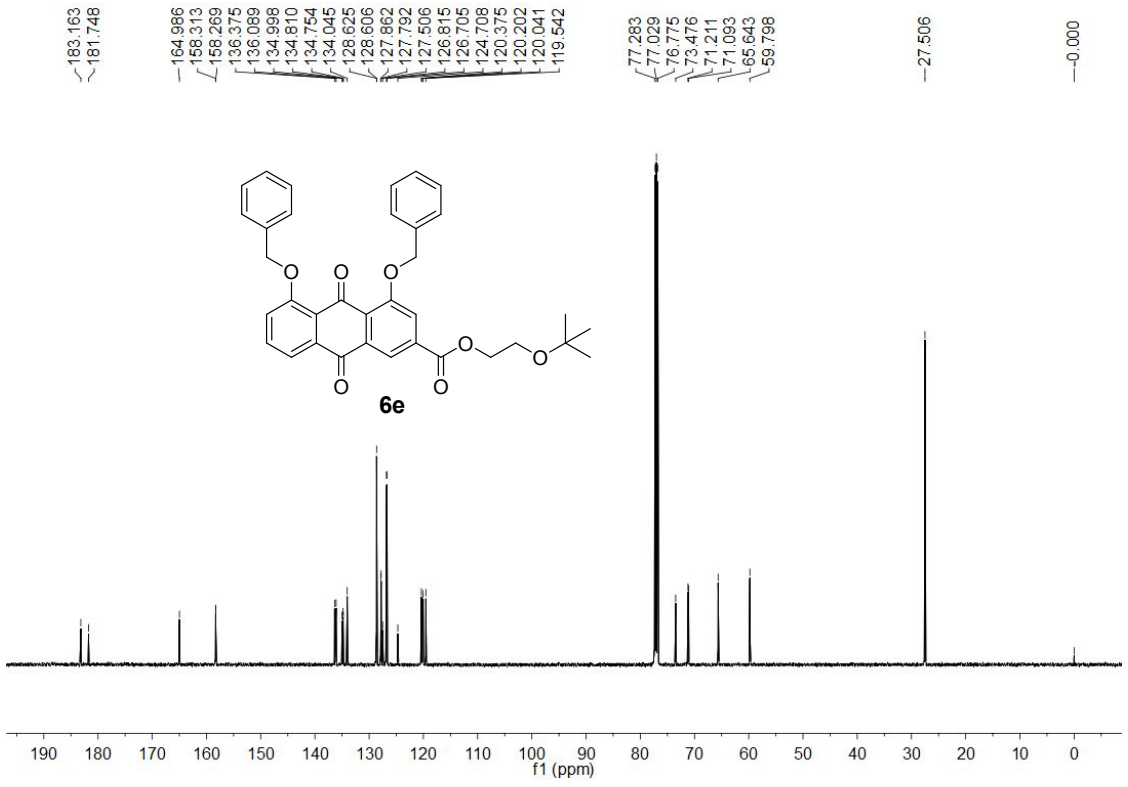


Figure S81. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6e**.

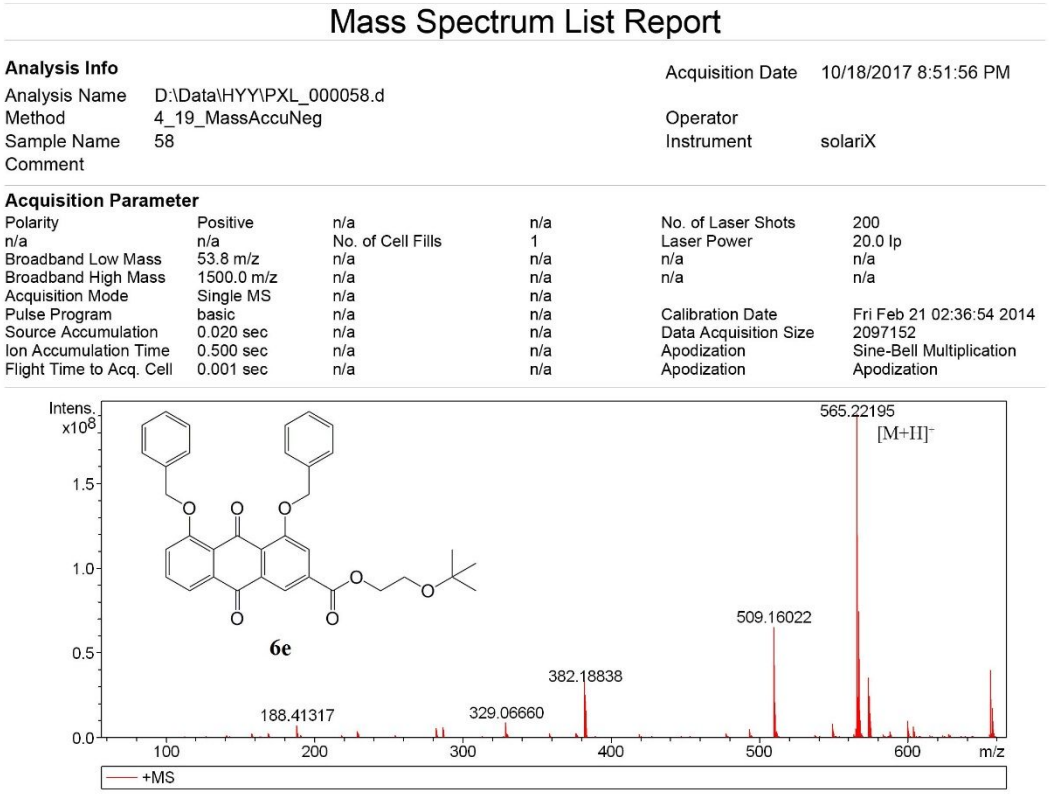


Figure S82. HRMS (ESI) spectrum of compound **6e**.

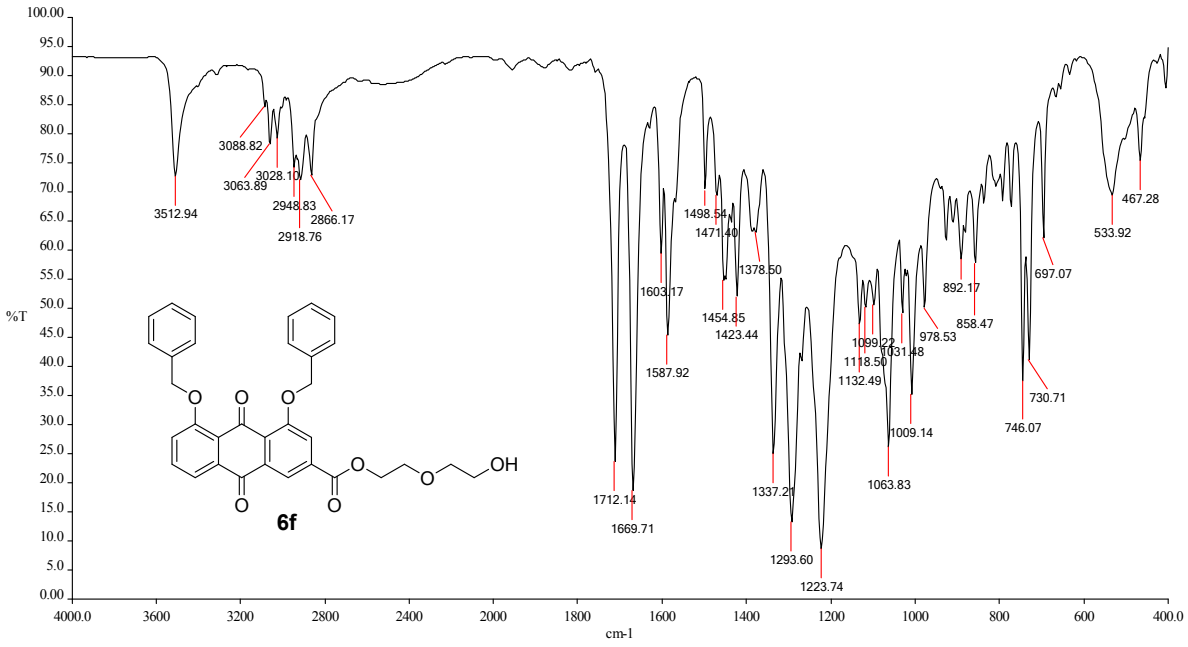


Figure S83. IR spectrum of compound **6f**.

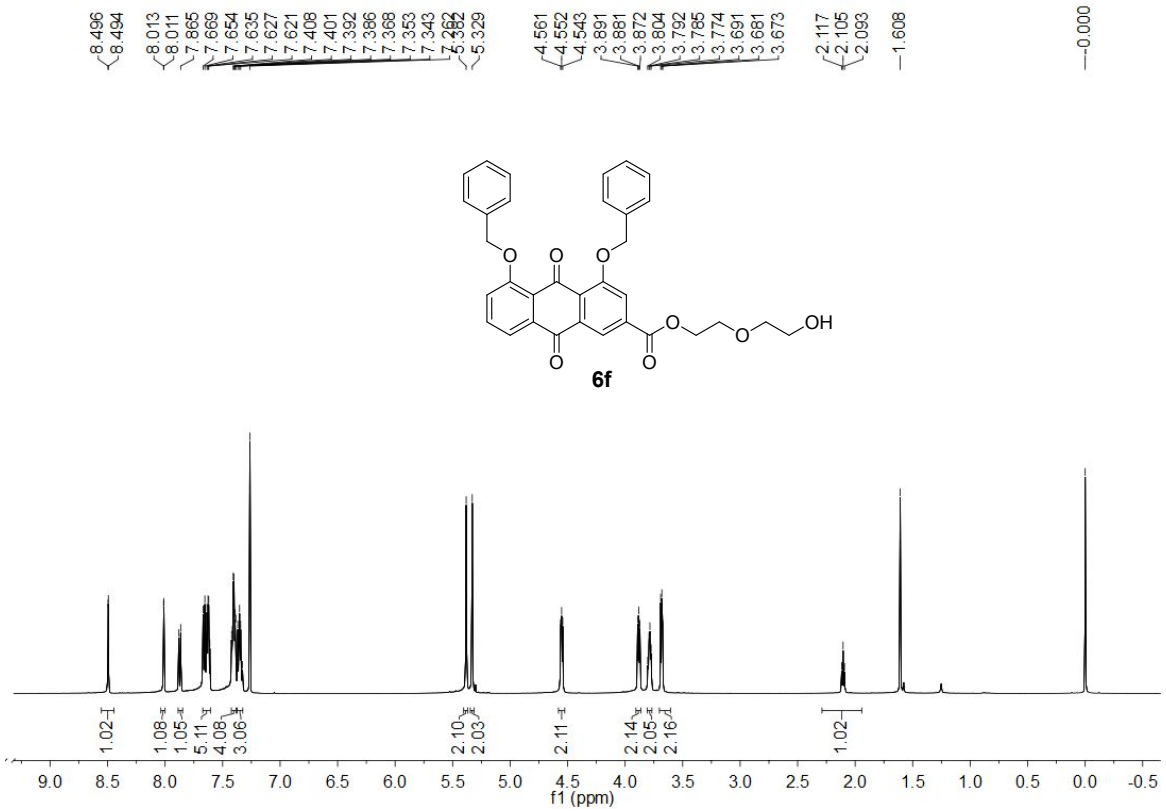


Figure S84. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6f**.

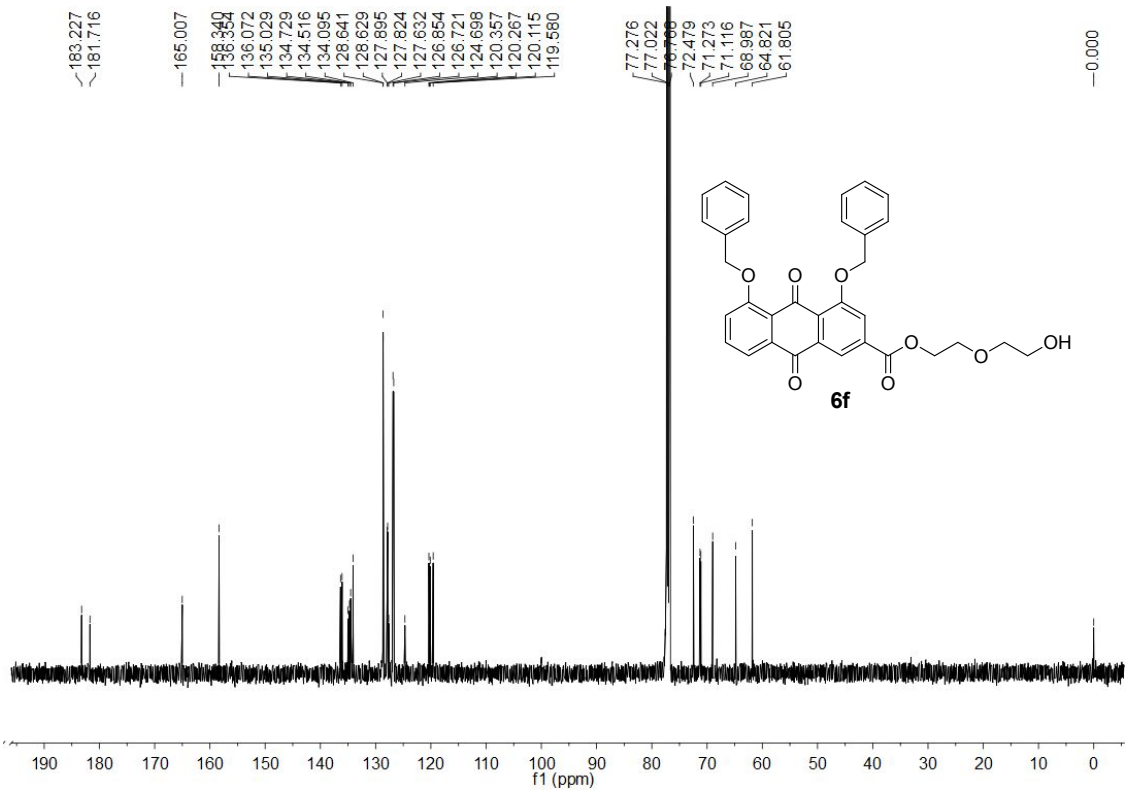


Figure S85. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6f**.

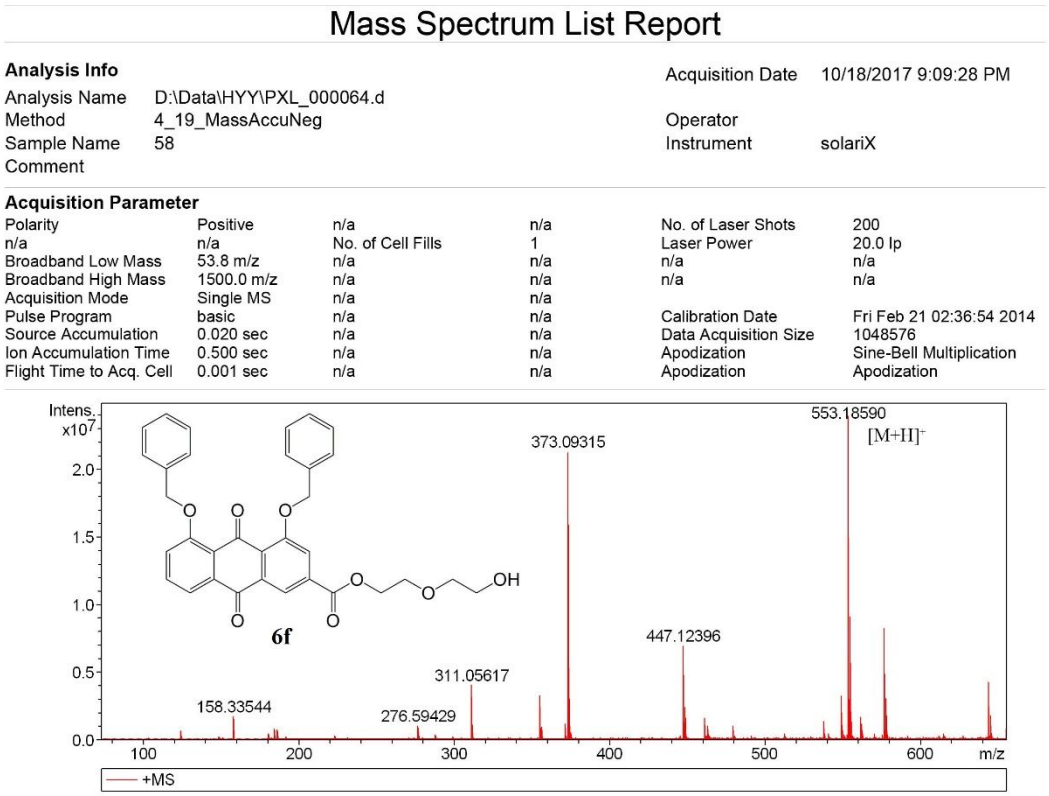


Figure S86. HRMS (ESI) spectrum of compound **6f**.

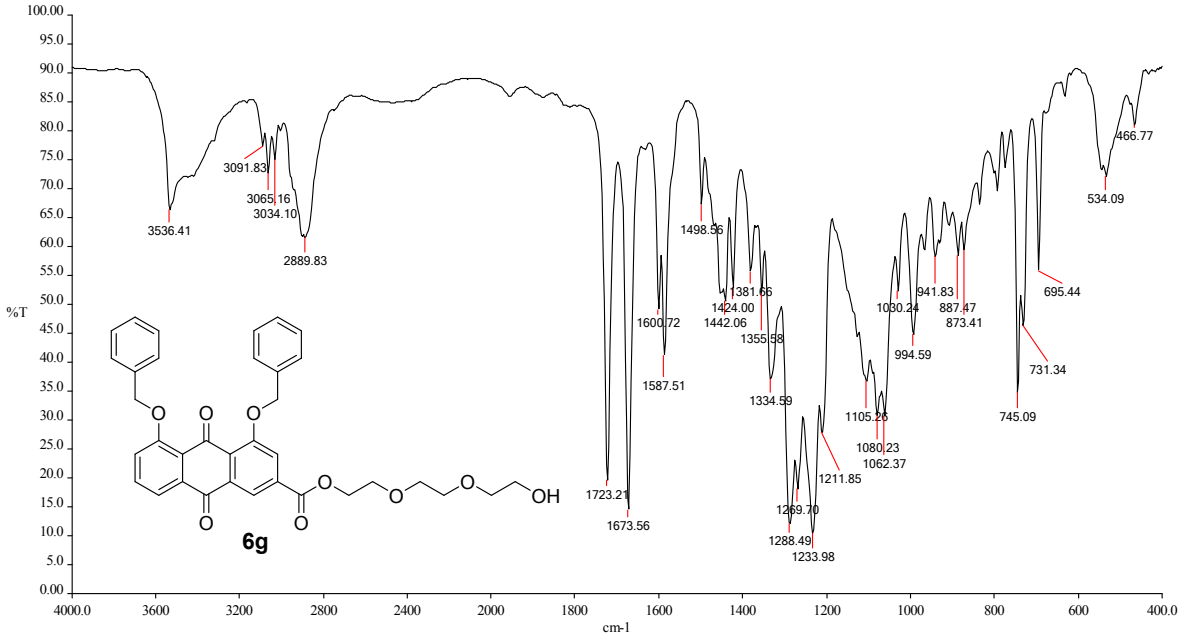


Figure S87. IR spectrum of compound **6g**.

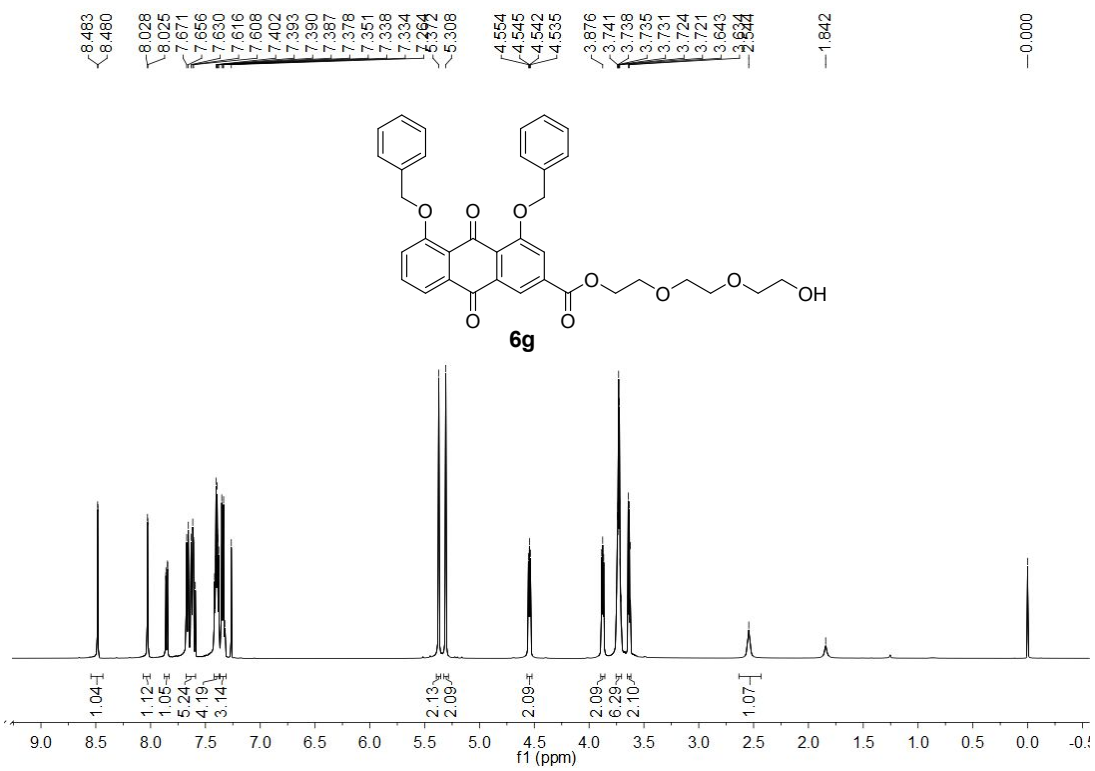


Figure S88. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of compound **6g**.

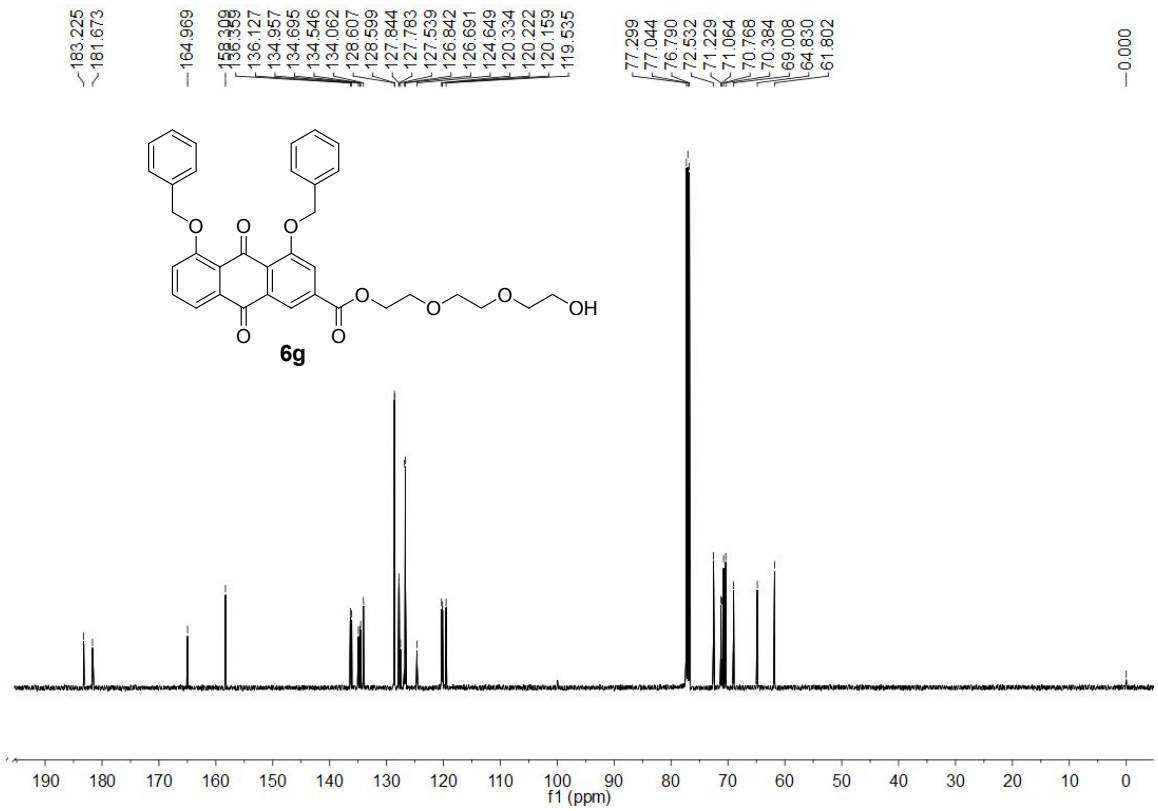


Figure S89. ^{13}C NMR (125.8 MHz, $CDCl_3$) spectrum of compound **6g**.

Mass Spectrum List Report

Analysis Info

Analysis Name	D:\Data\HYY\PXL_000053.d	Acquisition Date	10/18/2017 8:11:09 PM
Method	4_19_MassAccuNeg	Operator	
Sample Name	58	Instrument	solariX
Comment			

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1500.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Source Accumulation	0.020 sec	n/a	n/a	Data Acquisition Size	2097152
Ion Accumulation Time	0.500 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization

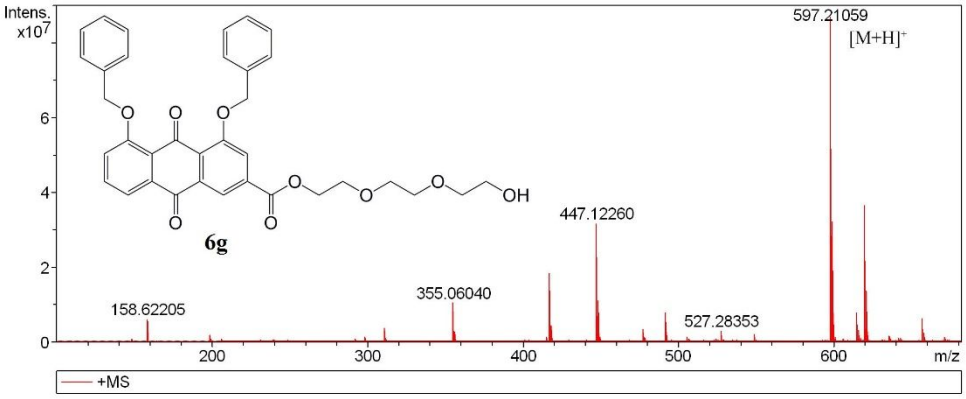


Figure S90. HRMS (ESI) spectrum of compound **6g**.