The application of nitrogen heterocycles on mitochondrial-targeting fluorescent markers with neutral skeleton

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1. Experimental section

1.1 Apparatus

^{1}H NMR spectrum was obtained by Varian INOVA-400 MHz spectrometer. ^{13}C NMR spectrum was recorded by Bruker DD2-600 MHZ spectrometer. And tetramethylsilane (TMS) was used as internal standard in spectrum above. High resolution mass spectrum was acquired on Finnigan MAT95 mass spectrometer. The Bruker VERTEX70 IR spectrometer was used to record the infrared spectra (IR). Melting points were measured on X-4 microscope electron thermal apparatus (Taike, China). UV–vis spectrum was obtained with Shimadzu UV-1800 spectrophotometer. Fluorescence emission spectra were performed on Shimadzu RF-5301PC spectrophotometer. The Leica TCS SP5 II confocal laser scanning microscope was used to implement confocal fluorescence experiments.

1.2 Synthesis

7-(Diethylamino)-4-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2H-chromen-2-one ***(6)***. Compound **5** (2.0 mmol, 618.1 mg), bis(pinacolato)diboron (B_2Pin_2, 2.5 mmol, 634.8 mg), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$, 0.2 mmol, 146.3 mg) and Potassium phosphate, (K_3PO_4, 4.0 mmol，849.1mg) were dissolved in 25.0 mL 1,4-dioxane and reacted at 100 °C for 12h under N_2 protection. After cooling to the room temperature, the mixture was filtered and the generating filtrate was evaporated in vacuum. Pure compound **6** was obtained through silica gel flash chromatography (PE: EA = 5:1), faint yellow solid, 244.9 mg, yield: 35%, mp: 170.2-172.5 °C. IR *v* (KBr, cm^{-1}): 1698, 1625, 1596, 1436, 1360, 1345, 1382, 1250, 1189, 1095, 962, 890, 698. ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 7.52 (d, 1H, *J* = 9.0, Ar-*H*), 6.67 (d, 1H, *J* = 8.9, Ar-*H*), 6.48 (s, 1H, Ar-*H*), 3.43 (q, *J* = 6.9 Hz, 4H, 2 × C*H*$_2$), 2.37 (s, 3H, C*H*$_3$), 1.30 (s, 12H, 4 × C*H*$_3$), 1.12 (t, *J* = 6.1 Hz, 6H, 2 × C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 163.4, 159.1, 156.4, 150.8, 125.9, 109.5, 108.1, 97.4, 84.0, 44.7, 24.8, 18.0, 12.5. HRMS (ESI$^+$): m/z calcd $C_{20}H_{29}BNO_4^+$ for $[M+H^+]^+$ = 358.2184, found: 358.2186.

9-(Diethylamino)-5-oxo-5H-benzo[a]phenoxazin-3-yl trifluoromethanesulfonate ***(8).*** Compound **7** (1.0 mmol, 334.1 mg) and N-Phenyl-bis(trifluoromethanesulfonimide) (Tf_2NPh, 2.0 mmol, 714.5 mg) were dissolved in 20.0 mL dry acetonitrile. Then triethylamine (2.0 mmol, 0.3 mL) was added to the mixture. The reaction was stirred at room temperature for 24h. And the crude product was purify by silica gel flash chromatography (DCM: MeOH = 100: 1) to give the compound **8** (330.9 mg) as green solid in 71%, mp: 202.4-202.9 °C. IR *v* (KBr, cm^{-1}): 3078, 2988, 1650, 1630, 1570, 1394, 804, 775, 763, 612. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.70 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 8.15 (s, 1H, Ar-*H*), 7.58 (d, *J* = 5.6 Hz, 1H, Ar-*H*), 7.55 (d, *J* = 5.8 Hz, 1H, Ar-*H*), 6.66 (d, *J* = 9.1 Hz, 1H, Ar-*H*), 6.43 (s, 1H, Ar-*H*), 6.38 (s, 1H, Ar-*H*), 3.48 (q, *J* = 7.1 Hz, 4H, 2 × C*H*$_2$), 1.26 (t, *J* = 7.1 Hz, 6H, 2 × C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 181.3, 152.3, 151.4, 150.5, 147.0, 137.6, 133.4, 131.7, 131.5, 126.5, 125.2, 124.0, 118.1, 110.3, 105.5, 96.1, 45.2, 12.6. HRMS (ESI$^+$): m/z calcd $C_{21}H_{18}F_3N_2O_5S^+$ for $[M+H^+]^+$ = 467.0883, found: 467.0885.

9-(Diethylamino)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5H-benzo[a]phenoxazin-5-one ***(9)***. Compound **9** was prepared by the similar procedure of compound **8**. Green solid, 790.7 mg, yield: 89%. mp: 199.8-202.3 °C. IR *v* (KBr, cm^{-1}): 1772, 1716, 1698, 1684, 1541, 1521, 1410, 1357, 1243, 920, 884, 611. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.78 (s, 1H, Ar-*H*), 8.61 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 8.10 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 7.58 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 6.63 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 6.43 (s, 1H, Ar-*H*), 6.37 (s, 1H, Ar-*H*), 3.45 (q, *J* = 6.7 Hz, 4H, 2 × C*H*$_2$), 1.38 (s, 12H, 4 × CH3), 1.25 (t, *J* = 7.1 Hz, 6H, 2 × C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 183.8, 152.2, 150.8, 146.7, 139.9, 136.9, 134.1, 132.7, 131.2, 130.8, 125.0, 122.8, 109.7, 105.8, 96.2, 84.1, 83.1, 45.1, 24.9, 24.5, 12.6. HRMS (ESI$^+$): m/z calcd $C_{26}H_{30}BN_2O_4^+$ for $[M+H^+]^+$ = 445.2293, found: 445.2327.

tert-Butyl 5-(7-(diethylamino)-4-methyl-2-oxo-2*H*-chromen-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-1-carboxylate (**12a**) was prepared by general procedure (I) for Suzuki couple reaction from compounds **6** and **10a**. Faint yellow solid, 147.9 mg, yield 33%. Mp: 168.8-169.9 °C. IR *v* (KBr, cm^{-1}):1764, 1592, 1526, 1435, 1146, 1079, 1042, 1001, 825, 763, 738, 698. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.65 (s, 1H, Ar-*H*), 8.20 (s, 1H, Ar-*H*), 8.15 (s, 1H, Ar-*H*), 7.49 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 6.67 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 6.57 (s, 1H, Ar-*H*), 3.45 (q, *J* = 7.0 Hz, 4H, 2 × C*H*$_2$), 2.29 (s, 3H, C*H*$_3$), 1.75 (s, 9H, 3 × C*H*$_3$), 1.24 (t, *J* = 7.0 Hz, 6H, 2 × C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 162.2, 156.1, 152.9, 151.6, 150.5, 150.3, 147.5, 136.3, 132.2, 125.5, 119.3, 115.4, 109.1, 108.8, 108.4, 97.7, 85.9, 44.7, 28.1, 18.4, 12.5. HRMS (ESI$^+$): m/z calcd $C_{25}H_{29}N_4O_4^+$ for $[M+H^+]^+$ = 449.2153, found: 449.2133.

tert-Butyl 5-(2-butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[de]isoquinolin-6-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-1-carboxylate (**13a**) was prepared by general procedure (I) for Suzuki couple reaction from compounds **11** and **10a**. White solid, 150.5 mg, yield 32%. Mp: 210.6-212.2 °C. IR *v* (KBr, cm^{-1}): 1749, 1699, 1659, 1387, 1361, 1246, 1233, 1148, 1071, 1066, 1050, 878, 845, 781, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.91 (s, 1H, Ar-*H*), 8.70 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 8.68 (d, *J* = 7.4 Hz, 1H, Ar-*H*), 8.31 (s, 1H, Ar-*H*), 8.26 (s, 1H, Ar-*H*), 8.13 (d, *J* = 8.4Hz, 1H, Ar-*H*), 7.77-7.73 (m, 2H, Ar-*H*), 4.23 (t, *J* = 7.5 Hz, 2H, C*H*$_2$) , 1.78 (s, 9H, 3 × C*H*$_3$), 1.74-1.71 (m, 2H, C*H*$_2$), 1.52-1.45 (m, 2H, C*H*$_2$), 1.00 (t, *J* = 7.3 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 164.0, 163.8, 151.6, 151.5, 147.7, 142.1, 137.4, 131.4, 131.4, 131.0, 130.6, 130.6, 130.2, 128.7, 127.5, 123.2, 122.9, 117.6, 85.9, 40.4, 30.2, 28.1, 20.4, 13.8. HRMS (ESI$^+$): m/z calcd $C_{27}H_{26}N_4NaO_4^+$ for $[M+Na^+]^+$ = 493.1846, found: 493.1804.

tert-Butyl 5-(9-(diethylamino)-5-oxo-5*H*-benzo[a]phenoxazin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-1-carboxylate (**14a**) was prepared by general procedure (I) for Suzuki couple reaction from compounds **9** and **10a**. Brown solid, 183.8 mg, yield 35%. Mp: 200.1-203.0 °C. IR *v* (KBr, cm^{-1}): 1757, 1639, 1626, 1582, 1487, 1428, 1408, 1385, 1370, 1320, 1178, 1147, 1077, 899, 854. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 9.11 (s, 1H, Ar-*H*), 8.75 (d, *J* = 7.0 Hz, 1H, Ar-*H*), 8.56 (s, 1H, Ar-*H*), 8.41 (s, 1H, Ar-*H*), 8.26

(s, 1H, Ar-*H*), 7.96 (d, *J* = 7.9 Hz, 1H, Ar-*H*), 7.63 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 6.69 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 6.48 (s, 1H, Ar-*H*), 6.43 (s, 1H, Ar-*H*), 3.48 (q, *J* = 5.8 Hz, 4H, 2 × CH_2), 1.77 (s, 9H, 3 ×CH_3). 1.27 (t, *J* = 5.4 Hz, 6H, 2 × CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 183.2, 152.4, 151.5, 151.0, 150.1, 147.8, 146.9, 139.2, 138.7, 137.6, 132.3, 132.1, 131.6, 131.3, 129.8, 128.4, 125.2, 125.0, 124.4, 118.0, 110.0, 105.7, 96.3, 85.5, 45.1, 28.1, 12.6. HRMS (ESI$^+$): m/z calcd $C_{31}H_{30}N_5O_4^+$ for $[M+H^+]^+$ = 536.2292, found: 536.2271.

tert-Butyl 5-(7-(diethylamino)-4-methyl-2-oxo-2*H*-chromen-3-yl)-1*H*-indazole-1-carboxylate (**12b**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **6** and **10b**. Faint yellow solid, 192.3 mg, yield 43%. Mp: 154.5-155.9 °C. IR *v* (KBr, cm^{-1}): 1681, 1616, 1581, 1469, 1442, 1380, 1197, 870, 841, 785, 674, 668. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.23 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 8.19 (s, 1H, Ar-*H*), 7.68 (s, 1H, Ar-*H*), 7.48 (d, J = 8.8 Hz, 2H, Ar-*H*), 6.64 (d, *J* = 9.0 Hz, 2H, Ar-*H*), 6.57 (s, 1H, Ar-*H*), 3.44 (q, *J* = 7.0 Hz, 4H, 2 × CH_2), 1.74 (s, 9H, CH_3), 2.24 (s, 3H, CH_3), 1.22 (t, *J* = 6.0 Hz, 6H, 2 × CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 162.2, 155.1, 150.4, 149.1, 148.8, 139.6, 139.0, 131.7, 131.0, 126.1, 126.0, 123.0, 120.2, 114.4, 109.4, 108.7, 97.5, 84.9, 44.8, 28.2, 24.8, 16.4, 12.4. HRMS (ESI$^+$): m/z calcd $C_{26}H_{30}N_3O_4^+$ for $[M+H^+]^+$ = 448.2231, found: 448.2222.

tert-Butyl 5-(2-butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)-1*H*-indazole-1-carboxylate (**13b**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **11** and **10b**. White solid, 300.3 mg, yield 64%. Mp: 203.8-204.9 °C. IR *v* (KBr, cm^{-1}):1733, 1701, 1652, 1591, 1388, 1371, 1168, 1073, 869, 837, 785. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.66 (t, *J* = 8.0 Hz, 2H, Ar-*H*), , 8.37 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 8.29 (s, 1H, Ar-*H*), 8.21 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 7.88 (s, 1H, Ar-*H*), 7.75 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 7.72 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 7.68 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 4.22 (t, *J* = 7.4 Hz, 2H, CH_2), 1.78 (s, 9H, 3 × CH_3), 1.76-1.71 (m, 2H, CH_2), 1.50-1.44 (m, 2H, CH_2), 1.00 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 164.2, 164.0, 149.1, 146.0, 139.5, 139.5, 134.4, 132.2, 131.2, 130.8, 130.7, 130.2, 128.7, 128.2, 127.0, 126.1, 123.0, 122.2, 122.1, 114.8, 85.4, 40.3, 30.2, 28.2, 20.4, 13.8. HRMS (ESI$^+$): m/z calcd $C_{28}H_{27}N_3NaO_4^+$ for $[M+Na^+]^+$ = 492.1894, found: 492.1881.

tert-Butyl 5-(9-(diethylamino)-5-oxo-5*H*-benzo[*a*]phenoxazin-3-yl)-1*H*-indazole-1-carboxylate (**14b**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **10** and **13**. Brown solid, 192.3 mg, yield 43%. Mp: 205.7-206.2 °C. IR *v* (KBr, cm^{-1}): 3070, 1772, 1684, 1489, 1386, 1280, 1154, 1042, 1030, 844, 830, 774. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.72 (d, *J* = 8.3 Hz, 1H, Ar-*H*), 8.59 (s, 1H, Ar-*H*), 8.28 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 8.25 (s, 1H, Ar-*H*), 8.10 (s, 1H, Ar-*H*), 8.00 (d, *J* = 8.3 Hz, 1H, Ar-*H*), 7.95 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 7.63 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 6.69 (d, *J* = 9.1 Hz, 1H, Ar-*H*), 6.49 (s, 1H, Ar-*H*), 6.43 (s, 1H, Ar-*H*), 3.47 (q, *J* = 6.9 Hz, 4H, 2 × CH_2), 1.76 (s, 9H, CH_3). 1.27 (t, *J* = 7.0 Hz, 6H, 2 × CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 183.5, 152.2, 150.7, 149.1, 146.7, 141.7, 139.8, 139.4, 139.3, 135.9, 132.1, 131.1, 130.9, 129.8, 128.6, 126.68, 125.1, 124.6, 124.1, 119.5, 114.9, 109.8, 105.7, 96.2, 85.1, 45.1, 28.2, 12.6. HRMS (ESI$^+$): m/z calcd $C_{32}H_{31}N_4O_4^+$ for $[M+H^+]^+$ = 535.2340, found: 535.2329.

tert-Butyl 5-(7-(diethylamino)-4-methyl-2-oxo-2*H*-chromen-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate (**12c**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **9** and **10c**. Faint yellow solid, 176.8 mg, yield 40%. Mp: 183.6-185.0 °C. IR *v* (KBr, cm^{-1}): 1748, 1700, 1441, 1357, 1264, 1186, 1089, 1046, 968, 844, 781. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.38 (s, 1H, Ar-*H*), 7.93 (s, 1H, Ar-*H*), 7.66 (d, *J* = 3.3 Hz, 1H, Ar-*H*), 7.47 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 6.64 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 6.56 (s, 1H, Ar-*H*), 6.54 (d, *J* = 3.3 Hz, 1H, Ar-*H*), 3.44 (q, *J* = 7.0 Hz, 4H, 2 × CH_2), 2.27 (s, 3H, CH_3), 1.69 (s, 9H, 3 × CH_3), 1.23 (t, *J* = 6.7 Hz, 6H, 2 × CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 162.1, 155.2, 150.4, 149.3, 147.9, 147.5, 146.7, 131.4, 126.9, 126.2, 126.1, 122.7, 117.9, 109.4, 108.7, 104.7, 97.5, 84.1, 44.8, 28.1, 16.5, 12.4. HRMS (ESI$^+$): m/z calcd $C_{26}H_{30}N_3O_4^+$ for $[M+H^+]^+$ = 448.2231, found: 448.2227.

tert-Butyl 5-(2-butyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)-1*H*-pyrrolo[2,3-*b*] pyridine-1-carboxylate (**13c**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **11** and **10c**. White solid, 243.9 mg, yield 52%. Mp: 206.6-208.2 °C. IR *v* (KBr, cm^{-1}): 1766, 1698, 1655, 1590, 1369, 1288, 1201, 1149, 1097, 784, 759, 737. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.68 (d, *J* = 7.6 Hz, 1H, Ar-*H*), 8.66-8.64 (m, 2H, Ar-*H*), 8.22 (d, *J* = 8.4 Hz, 1H, Ar-*H*), 8.04 (s, 1H, Ar-*H*), 7.78 (d, *J* = 3.8 Hz, 1H, Ar-*H*), 7.75 (d, *J* = 7.6Hz, 1H, Ar-*H*), 7.71 (d, *J* = 8.1 Hz, 1H, Ar-*H*), 4.23 (t, *J* = 7.5 Hz, 2H, CH_2), 1.79-1.75 (m, 2H, CH_2), 1.72 (s, 9H, 3 × CH_3), 1.50-1.45 (m, 2H, CH_2), 1.00 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 164.2, 164.0, 148.1, 147.8, 145.7, 143.7, 132.1, 131.3, 130.7, 130.4, 130.1, 129.55, 128.6, 128.5, 127.9, 127.1, 123.0, 122.8, 122.3, 104.5, 84.6, 40.3, 30.2, 28.1, 20.4, 13.8. HRMS (ESI$^+$): m/z calcd $C_{28}H_{27}N_3NaO_4^+$ for $[M+Na^+]^+$ = 492.1894, found: 492.1891.

tert-Butyl 5-(9-(diethylamino)-5-oxo-5*H*-benzo[*a*]phenoxazin-3-yl)-1*H*-pyrrolo[2,3-*b*]pyridine-1-carboxylate (**14c**) was synthesized by general procedure (I) for Suzuki couple reaction from compounds **9** and **10c**. Brown solid, 183.8 mg, yield 35%. Mp: 206.9-207.9 °C. IR *v* (KBr, cm^{-1}): 1750, 1627, 1584, 1352, 1278, 1180, 1096, 898, 830, 696. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 9.11 (s, 1H, Ar-*H*), 8.75 (d, *J* = 7.0 Hz, 1H, Ar-*H*), 8.56 (s, 1H, Ar-*H*), 8.41 (s, 1H, Ar-*H*), 8.26 (s, 1H, Ar-*H*), 7.96 (d, *J* = 7.9 Hz, 1H, Ar-*H*), 7.63 (d, *J* = 7.8 Hz, 1H, Ar-*H*), 6.69 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 6.48 (s, 1H, Ar-*H*), 6.43 (s, 1H, Ar-*H*), 3.48 (q, *J* = 5.8 Hz, 4H, 2 × CH_2), 1.77 (s, 9H, 3 ×CH_3). 1.27 (t, *J* = 5.4 Hz, 6H, 2 × CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ(ppm): 183.2, 152.4, 151.5, 151.0, 150.1, 147.8, 146.9, 139.2, 138.7, 137.6, 132.3, 132.1, 131.6, 131.3, 129.8, 128.4, 125.2, 125.0, 124.4, 118.0, 110.0, 105.7, 96.3, 85.5, 45.1, 28.1, 12.6. HRMS (ESI$^+$): m/z calcd $C_{32}H_{32}N_4NaO_4^+$ for $[M+Na^+]^+$ = 557.2129, found: 557.2097.

1.3 Preparation of test solvents

Dyes **1a-b**, **2a-c**, **3a-c** and **4a-c** were dissolved in several kinds of organic solvent respectively as stock solvents at the concentration of 100 μM. More specifically, toluene (TOL), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), ethanol (EtOH), dimethyl sulfoxide (DMSO) were used to observe the solvation effect of them. The test solution of each dye in each solvent was diluted to 10 μM in 10 mL volumetric flask from 1 mL relevant stock solution.

Besides, H_2O was also chose as one of the test projects in solvation effect. For coumarin derivatives, such as **1a** and **2a-c**, the test solution of them contained 10% DMSO. For 1,8-Naphthalimide derivatives, such as **3a-c**, they contained 20% DMSO. For Nile Red derivatives, such as **1b** and **4a-c**, they contained 30% DMSO. All of aqueous solution tested in solvent effect were obtained from 1 ml stock solution of DMSO (100 μM) and diluted by appropriate DMSO and distilled water.

1.4 The test of photo stability

Test solvents of dyes **1a-b**, **2a-c**, **3a-c** and **4a-c** that used for photofading experiment were dissolved in acetonitrile at the concentration of 10 μM. Preparing methods of them were the same as the preparation of test solution for solvation effect in the last section. Samples were irradiated by a Philips iodine-tungsten lamp (500 W) with a distance of 25 cm. Sodium nitrite aqueous solution with the layer thickness of 8 cm and the concentration of 60 $g \cdot L^{-1}$ in a transparent container was placed between samples and light source to reduce the interference of the heat and short wavelength light. The remaining absorption of samples was used to assess the photo stability of them. Furthermore, coumarin, 1,8-Naphthalimide and Nile Red was selected as control group respectively. And the specific data of that were acquired by calculating the change of the peak value of absorption every 30 minutes in continuous irradiation for 6h.

1.5 Determination of the relative fluorescence quantum yields

The fluorescence quantum yield of dyes **1a-b**, **2a-c**, **3a-c** and **4a-c** was tested and calculated by following equation:

$\Phi_x/\Phi_{st} = [A_{st}/A_x][n_x^2/n_{st}^2][D_x/D_{st}]$

where st: standard, x: sample, Φ: quantum yield, A: absorbance at the excitation wavelength, D: area under the fluorescence spectra on an energy scale, n: the refractive index of the solution. Harmin (Φ = 0.45 in 0.1 M H_2SO_4) was used as standard of coumarin (**1a** and **2a-c**) and Naphthalimide derivatives (**3a-c**). Cresyl violet (Φ = 0.578 in ethanol) was utilized as standard of Nile Red derivatives (**1b** and **4a-c**).

1.6 The test of cytotoxicity

The CCK-8 method was used to verify the cytotoxicity of dyes **1a-b**, **2a-c**, **3a-c** and **4a-c.** The survival rate of HeLa and L929 cells was calculated with the following equation:

survival rate (%) = $(A_{sample} - A_b)/(A_c - A_b)$

where A_c: negative control (including media and cells, no test substance), A_b: blank (including test substance and media, no cells).

2. Table

Table. S1 Optical properties of dyes 1a-b, 2a-c, 3a-c and 4a-c

Dyes	Solvents	$\lambda_{Abs,max}$[a]	$\lambda_{Em,max}$[a]	Stokes shift[a]	Φ[b]	ε[c]
1a	TOL	390	506	116	0.50	3.09
1a	THF	388	522	134	0.35	3.21
1a	CH_2Cl_2	393	544	151	0.33	3.47
1a	EtOH	394	ND[d]	ND[d]	ND[d]	2.88
1a	DMSO	395	ND[d]	ND[d]	ND[d]	2.48
1a	H_2O	403	ND[d]	ND[d]	ND[d]	3.15
1b	TOL	543	604	61	0.71	4.67
1b	THF	546	610	64	0.89	4.96
1b	CH_2Cl_2	554	614	60	0.79	4.81
1b	EtOH	561	639	78	0.59	4.27
1b	DMSO	566	641	75	0.56	4.52
1b	H_2O	536	ND[d]	ND[d]	ND[d]	1.90
2a	TOL	375	448	73	0.39	5.11
2a	THF	377	450	73	0.40	5.36
2a	CH_2Cl_2	382	453	71	0.51	4.55
2a	EtOH	386	468	82	0.54	5.23
2a	DMSO	385	469	84	0.49	5.19
2a	H_2O	383	468	85	0.56	5.26
3a	TOL	357	436	79	0.39	0.49
3a	THF	356	441	85	0.46	0.61
3a	CH_2Cl_2	355	448	93	0.49	0.64
3a	EtOH	357	470	113	0.33	0.62
3a	DMSO	361	466	105	0.25	0.59
3a	H_2O	359	476	117	0.03	0.58
4a	TOL	537	588	51	0.83	4.48
4a	THF	539	602	63	0.94	4.80
4a	CH_2Cl_2	550	610	60	0.95	4.95
4a	EtOH	561	640	79	0.55	4.82
4a	DMSO	561	640	79	0.68	4.76
4a	H_2O	605	ND[d]	ND[d]	NDd	3.81
2b	TOL	359	448	89	0.34	3.20
2b	THF	365	445	80	0.33	3.27
2b	CH_2Cl_2	375	451	76	0.70	3.42
2b	EtOH	385	460	75	0.88	3.14
2b	DMSO	382	466	84	0.78	3.08
2b	H_2O	390	479	89	0.45	2.71
3b	TOL	364	457	93	0.66	1.82
3b	THF	363	468	105	0.68	1.96
3b	CH_2Cl_2	363	473	110	0.59	2.08
3b	EtOH	363	504	141	0.33	1.94
3b	DMSO	366	505	139	0.47	1.99
3b	H_2O	364	501	137	0.07	1.08
4b	TOL	529	574	45	0.84	4.99
4b	THF	536	597	61	0.70	5.18
4b	CH_2Cl_2	544	610	66	0.80	5.49
4b	EtOH	553	640	87	0.57	5.24
4b	DMSO	555	642	87	0.64	5.02
4b	H_2O	530	ND[d]	ND[d]	ND[d]	2.63
2c	TOL	374	447	73	0.51	2.92
2c	THF	372	452	80	0.48	2.98
2c	EtOH	382	468	86	0.69	2.97
2c	DMSO	384	465	81	0.56	3.04
2c	H_2O	393	479	86	0.63	2.86
3c	TOL	360	450	90	0.35	2.15

Table. S1 (continued)

Dyes	Solvents	$\lambda_{Abs,max}$[a]	$\lambda_{Em,max}$[a]	Stokes shift[a]	Φ[b]	ε[c]
3c	THF	359	454	95	0.19	2.24
3c	CH_2Cl_2	355	450	95	0.22	2.20
3c	EtOH	360	ND[d]	ND[d]	ND[d]	2.25
3c	DMSO	364	ND[d]	ND[d]	ND[d]	2.12
3c	H_2O	359	ND[d]	ND[d]	ND[d]	2.19
4c	TOL	535	583	48	0.79	3.74
4c	THF	535	599	64	0.90	4.19
4c	CH_2Cl_2	548	607	59	0.85	4.07
4c	EtOH	558	641	83	0.60	4.15
4c	DMSO	560	642	82	0.62	4.06
4c	H_2O	503	ND[d]	ND[d]	ND[d]	2.73

[a] Reported in nm. [b] Cresyl violet (Φ = 0.578 in ethanol) and Harmine (Φ =0.45 in 0.1 M H_2SO_4) were used as the reference compounds. [c] reported in 104 M^{-1} cm^{-1}. [d] Not detectable.

Table. S2 Chromaticity coordinate of dyes 1a-b, 2a-c, 3a-c and 4a-c

Dyes	Solvents	CIE X	CIE Y	Dyes	Solvents	CIE X	CIE Y
1a	TOL	0.26	0.45	**3a**	$CHCl_3$	0.15	0.08
1a	THF	0.32	0.52	**3a**	EtOH	0.15	0.19
1a	CH_2Cl_2	0.38	0.51	**3a**	DMSO	0.15	0.19
1a	$CHCl_3$	0.35	0.50	**3a**	H_2O	0.17	0.26
1b	TOL	0.63	0.37	**3b**	TOL	0.14	0.12
1b	THF	0.64	0.36	**3b**	THF	0.15	0.19
1b	CH_2Cl_2	0.65	0.35	**3b**	CH_2Cl_2	0.15	0.23
1b	$CHCl_3$	0.65	0.35	**3b**	$CHCl_3$	0.15	0.20
1b	EtOH	0.69	0.31	**3b**	EtOH	0.24	0.48
1b	DMSO	0.69	0.31	**3b**	DMSO	0.24	0.48
2a	TOL	0.15	0.10	**3b**	H_2O	0.18	0.29
2a	THF	0.15	0.12	**3c**	TOL	0.15	0.10
2a	CH_2Cl_2	0.14	0.11	**3c**	THF	0.13	0.12
2a	$CHCl_3$	0.14	0.10	**3c**	CH_2Cl_2	0.14	0.11
2a	EtOH	0.14	0.16	**3c**	$CHCl_3$	0.15	0.15
2a	DMSO	0.14	0.17	**4a**	TOL	0.57	0.43
2a	H_2O	0.14	0.16	**4a**	THF	0.62	0.38
2b	TOL	0.15	0.11	**4a**	CH_2Cl_2	0.64	0.36
2b	THF	0.15	0.12	**4a**	$CHCl_3$	0.64	0.36
2b	CH_2Cl_2	0.15	0.12	**4a**	EtOH	0.68	0.32
2b	$CHCl_3$	0.15	0.11	**4a**	DMSO	0.68	0.32
2b	EtOH	0.14	0.14	**4b**	TOL	0.57	0.43
2b	DMSO	0.14	0.17	**4b**	THF	0.61	0.39
2b	H_2O	0.14	0.26	**4b**	CH_2Cl_2	0.64	0.36
2c	TOL	0.15	0.11	**4b**	$CHCl_3$	0.63	0.37
2c	THF	0.15	0.11	**4b**	EtOH	0.69	0.31
2c	CH_2Cl_2	0.16	0.12	**4b**	DMSO	0.69	0.31
2c	$CHCl_3$	0.15	0.10	**4c**	TOL	0.57	0.43
2c	EtOH	0.14	0.15	**4c**	THF	0.61	0.39
2c	DMSO	0.14	0.16	**4c**	CH_2Cl_2	0.63	0.37
2c	H_2O	0.14	0.27	**4c**	$CHCl_3$	0.63	0.37
3a	TOL	0.15	0.07	**4c**	EtOH	0.67	0.33
3a	THF	0.15	0.08	**4c**	DMSO	0.68	0.32
3a	CH_2Cl_2	0.15	0.09				

3.Figures

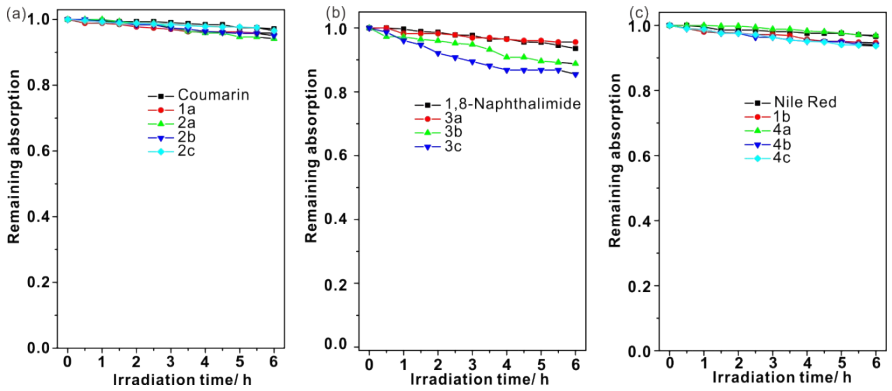

Fig. S1 Photofading behaviors of dyes **1a-b**, **2a-c**, **3a-c** and **4a-c** in acetonitrile.

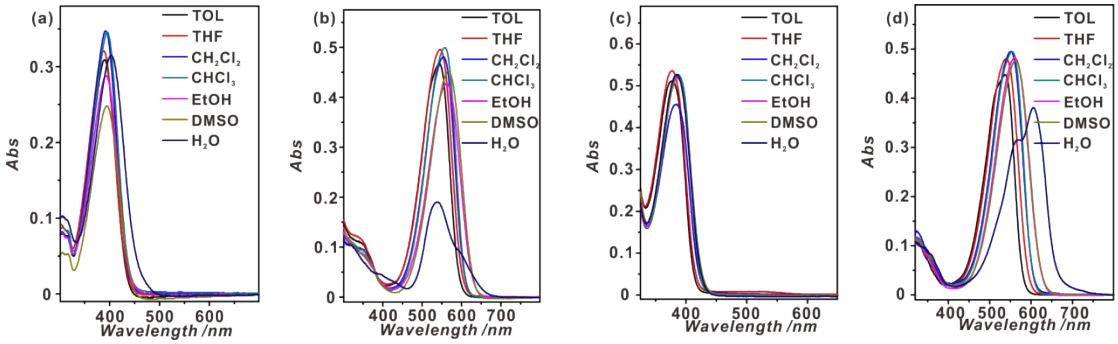

Fig. S2 Absorption spectra of **1a** (a), **1b** (b), **2a** (c) and **4a** (d) with the concentration of 10 μM in different solvents.

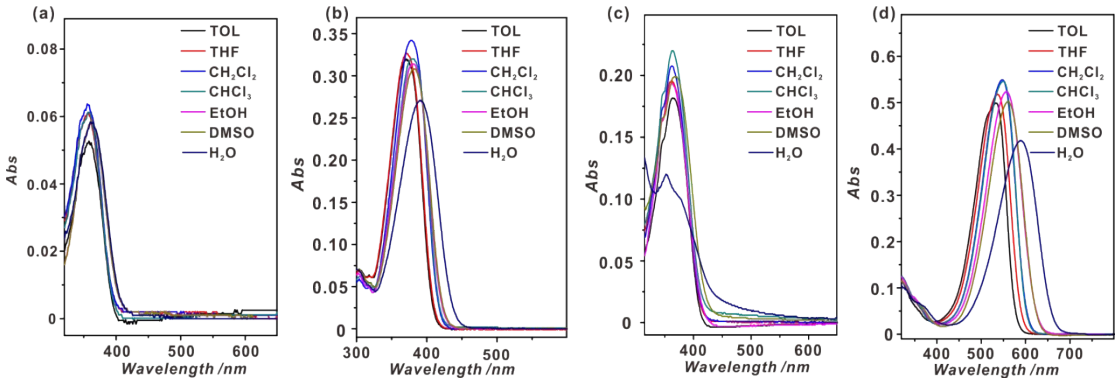

Fig. S3 Absorption spectra of **3a** (a), **2b** (b), **3b** (c) and **4b** (d) with the concentration of 10 μM in different solvents.

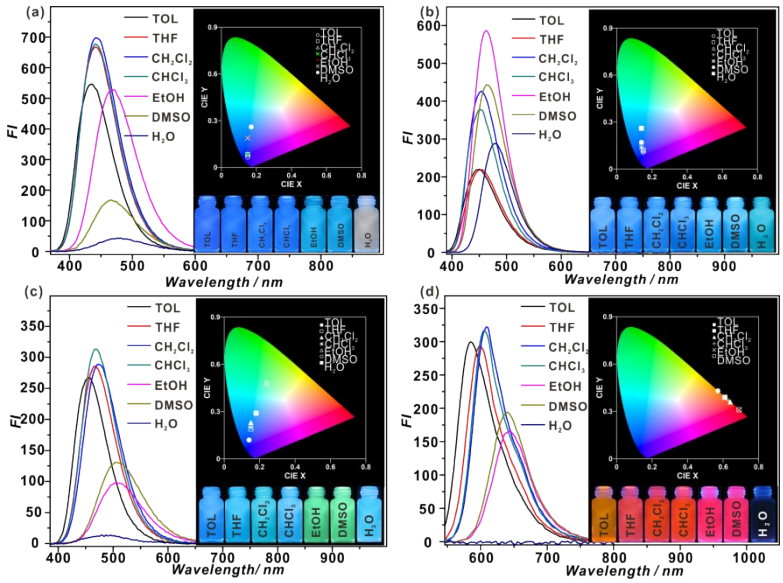

Fig. S4 Emission spectra of **3a** (a), **2b** (b), **3b** (c) and **4b** (d) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **3a** was excited at 360 nm, slit widths: 3 nm/3 nm; **2b** was excited at 380 nm, slit widths: 3 nm/1.5 nm; **3b** was excited at 374 nm, slit widths: 3 nm/1.5 nm; **4b** was excited at 560 nm, slit widths: 1.5 nm/1.5 nm.

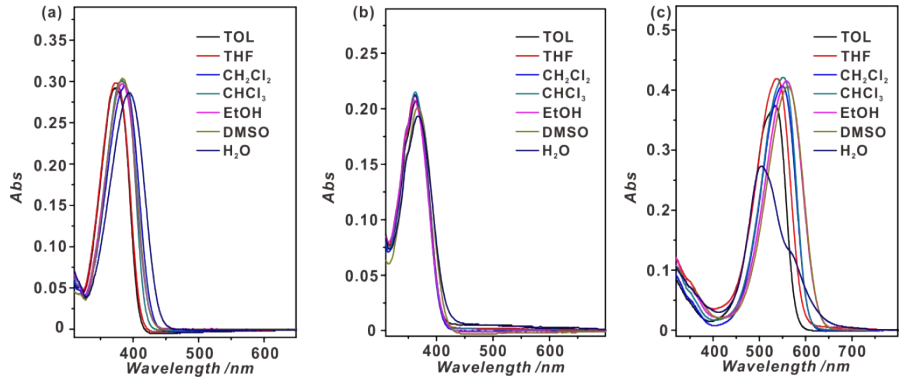

Fig. S5 Absorption spectra of **2c** (a), **3c** (b) and **4c** (c) and with the concentration of 10 μM in different solvents.

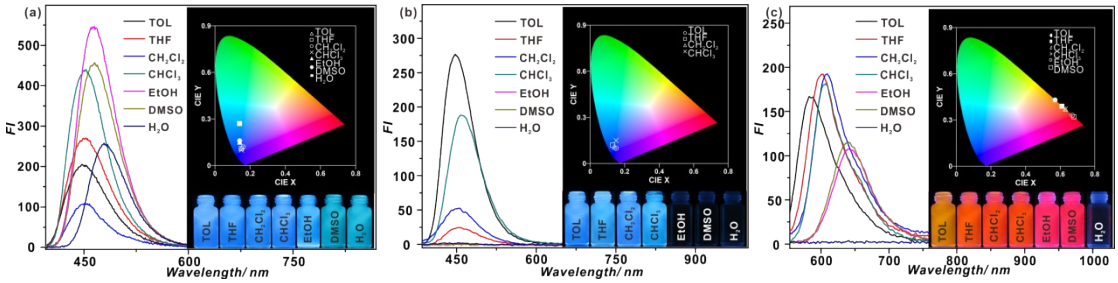

Fig. S6 Emission spectra of **2c** (a), **3c** (b) and **4c** (c) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **2c** was excited at 385 nm, slit widths: 3 nm/1.5 nm; **3c** was excited at 360 nm, slit widths: 3 nm/1.5 nm; **4c** was excited at 540 nm, slit widths: 1.5 nm/1.5 nm.

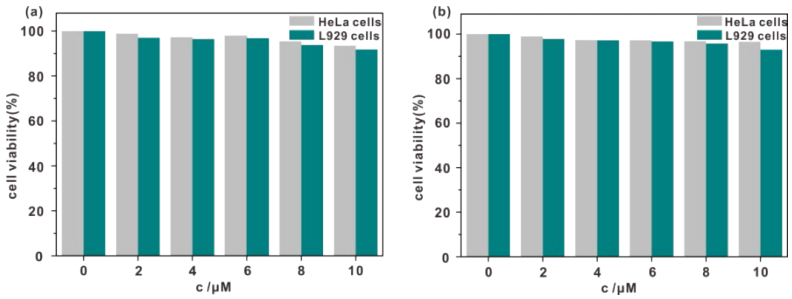

Fig. S7 Percentages of cell viabilities of HeLa and L929 cells after treatment with dyes **1a** (a) and **1b** (b) for 6 hours.

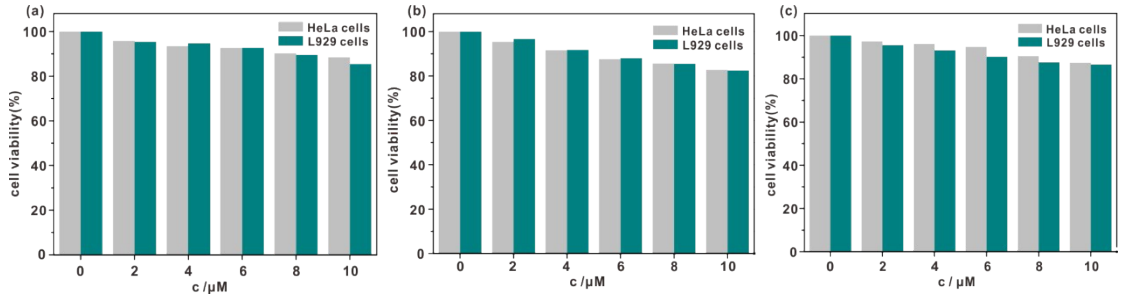

Fig. S8 Percentages of cell viabilities of HeLa and L929 cells after treatment with dyes **2a** (a), **3a** (b) and **4a** (c) for 6 hours.

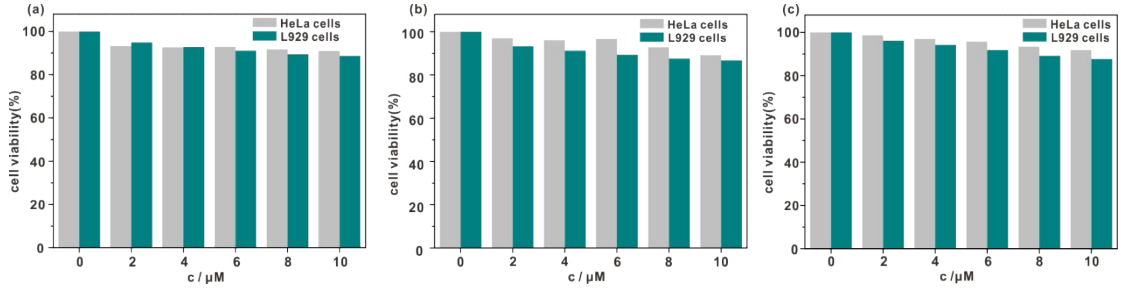

Fig. S9 Percentages of cell viabilities of HeLa and L929 cells after treatment with dyes **2b** (a), **3b** (b) and **4b** (c) for 6 hours.

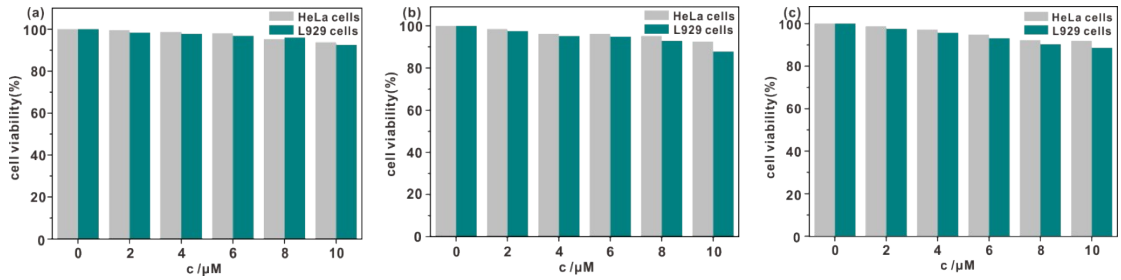

Fig. S10 Percentages of cell viabilities of HeLa and L929 cells after treatment with dyes **2c** (a), **3c** (b) and **4c** (c) for 6 hours.

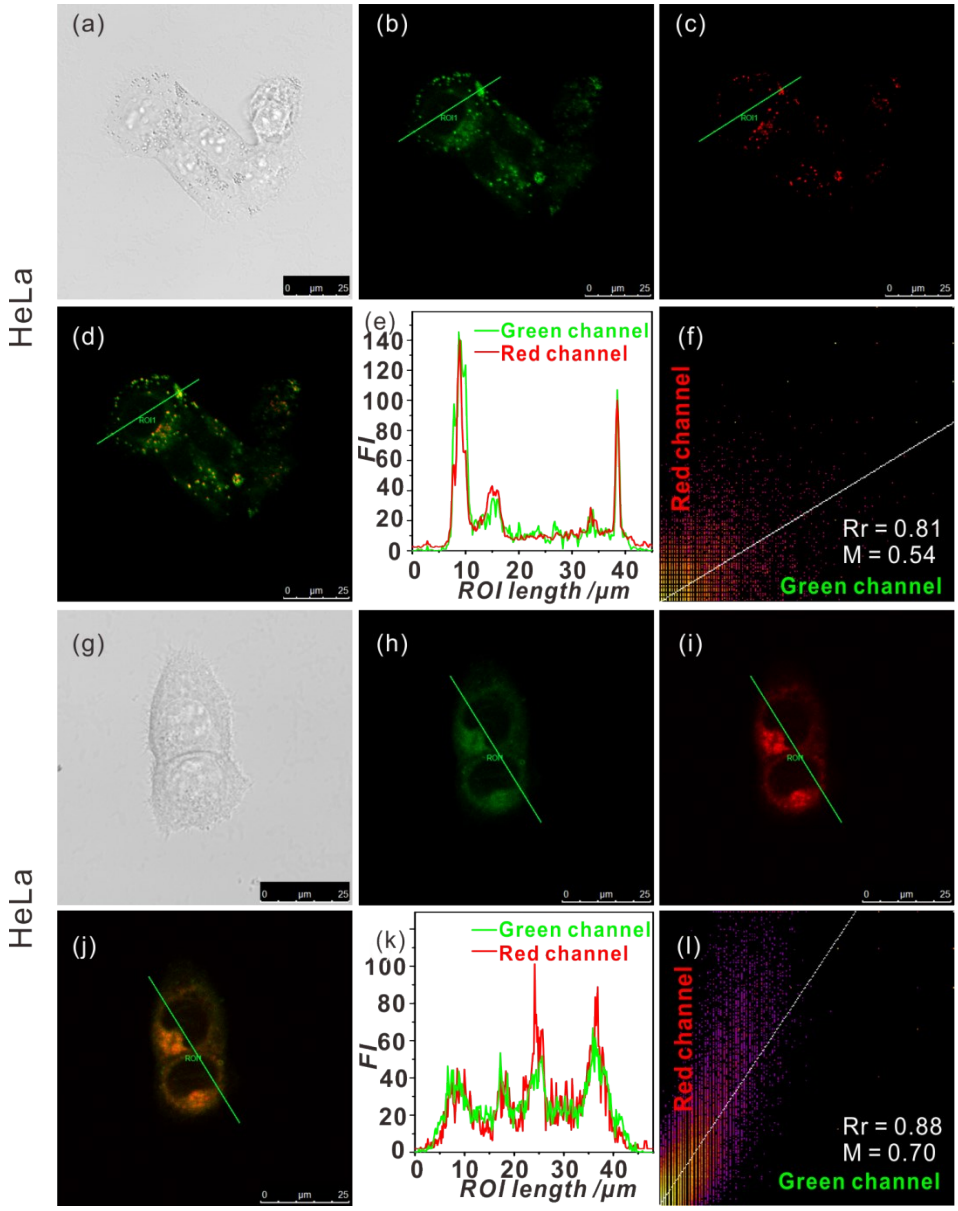

Fig. S11 Confocal fluorescence image of HeLa cells with dye **1a**. (a, g) bright field images; (b, h) confocal image (green channel) of cells with **1a** (1 μM); (c) confocal image (red channel) of cells with Nile Red (1 μM); (i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of green and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells; (f, l) fluorescence intensity correlation plot of green channel and red channel.

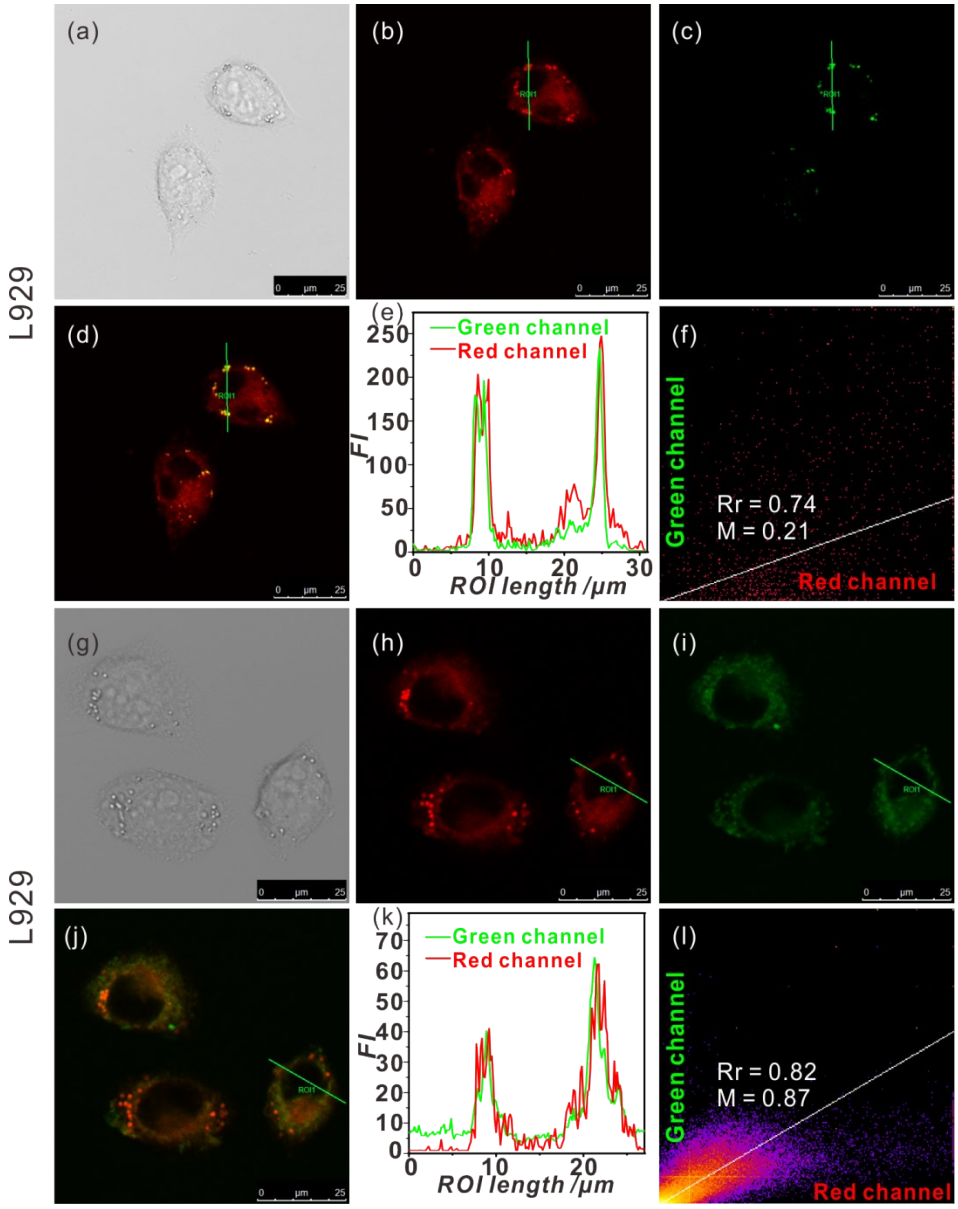

Fig. S12 Confocal fluorescence image of L929 cells with dye **1b**. (a, g) bright field images; (b, h) confocal image (red channel) of cells with **1b** (1 μM); (c) confocal image (green channel) of cells with lipid droplets probe reported by previous literature (1 μM); (i) confocal image (green channel) of cells with Mito-Tracker® Green FM (100 nM); (d, j) merged images of green and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells; (f, l) fluorescence intensity correlation plot of green channel and red channel.

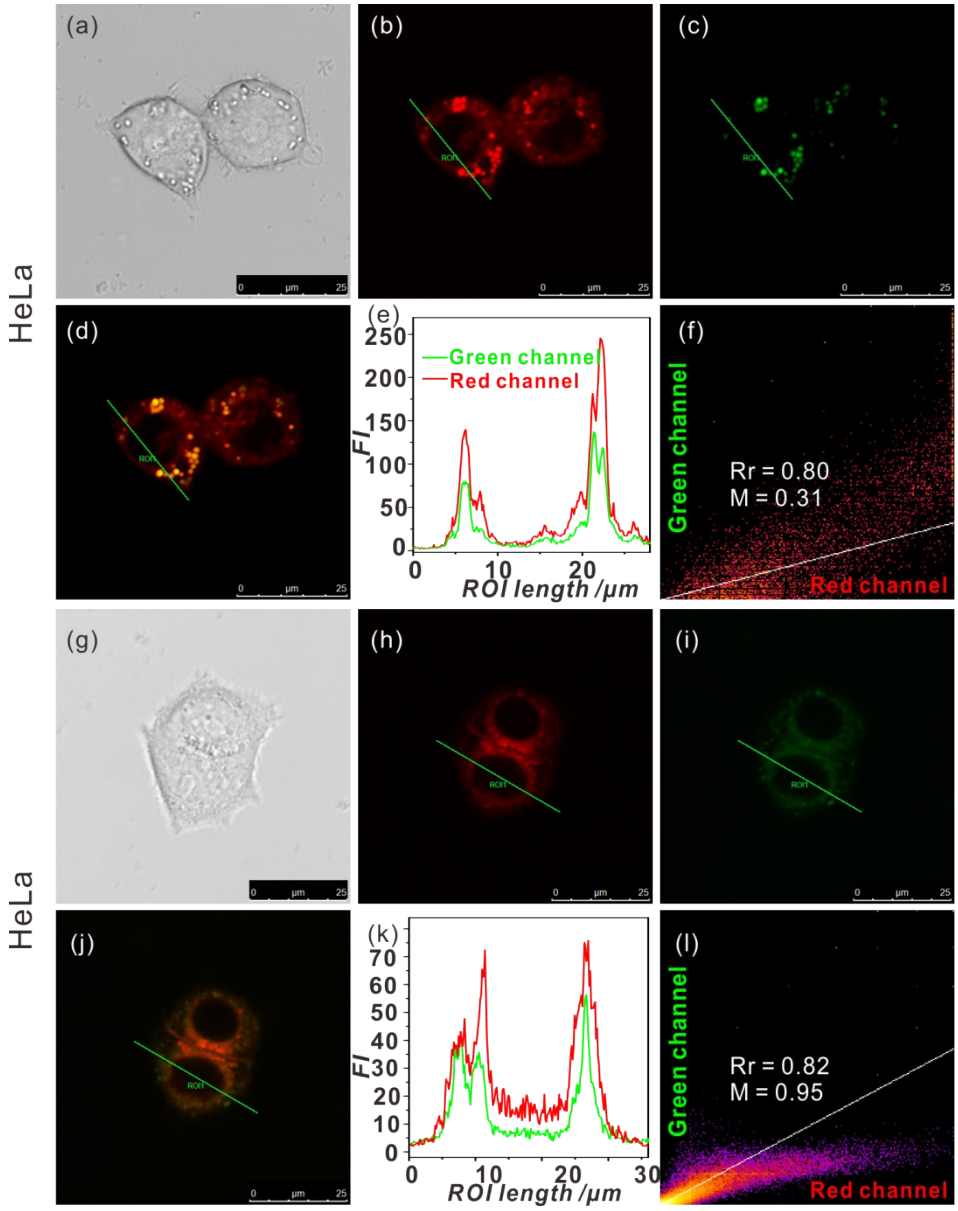

Fig. S13 Confocal fluorescence image of HeLa cells with dye **1b**. (a, g) bright field images; (b, h) confocal image (red channel) of cells with **1b** (1 μM); (c) confocal image (green channel) of cells with lipid droplets probe reported by previous literature (1 μM); (i) confocal image (green channel) of cells with Mito-Tracker® Green FM (100 nM); (d, i) merged images of green and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells; (f, l) fluorescence intensity correlation plot of green channel and red channel.

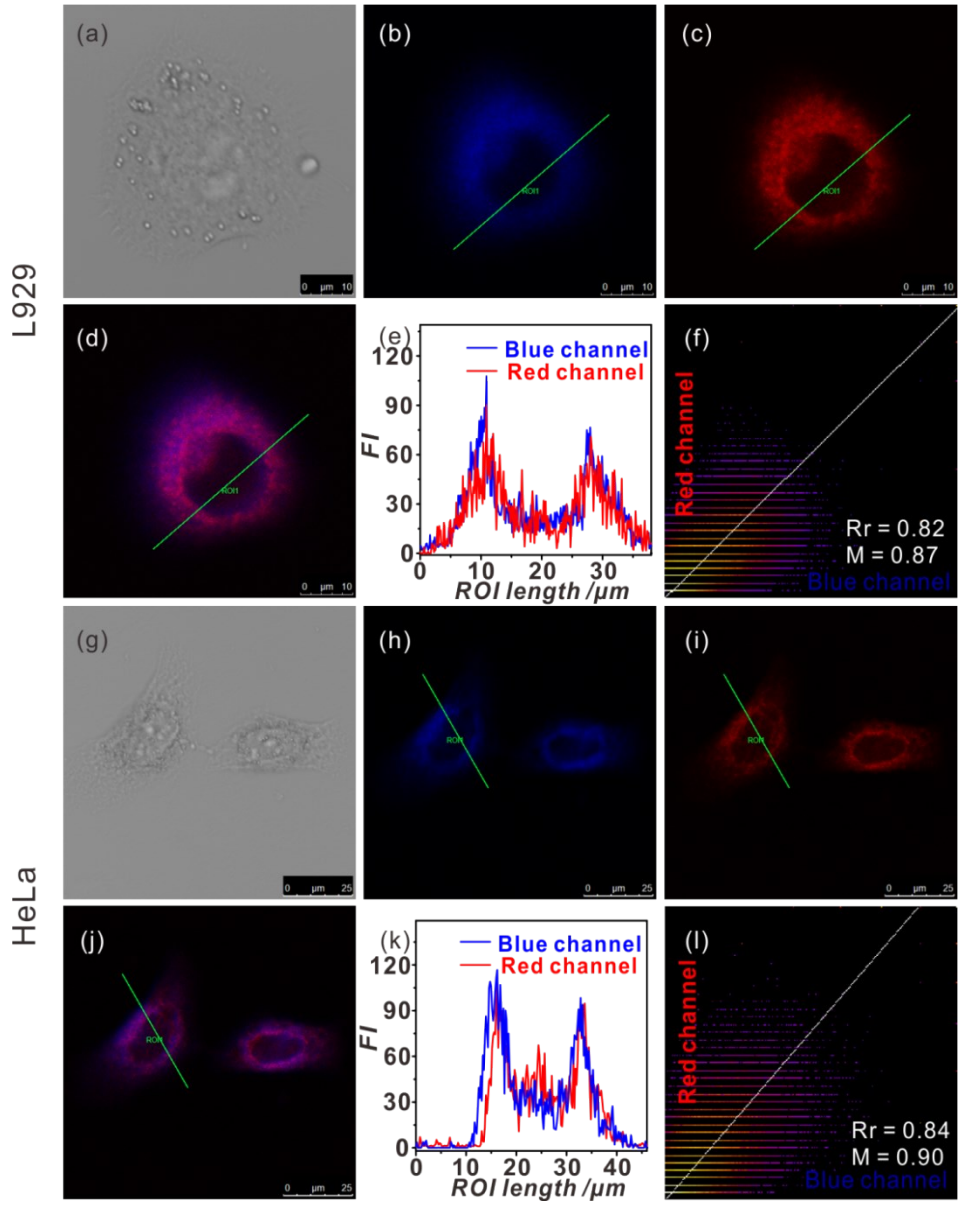

Fig. S14 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **3a**. (a, g) bright field images; (b, h) confocal image (blue channel) of cells with dye **3a** (1 μM); (c, i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of blue and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of blue channel and red channel.

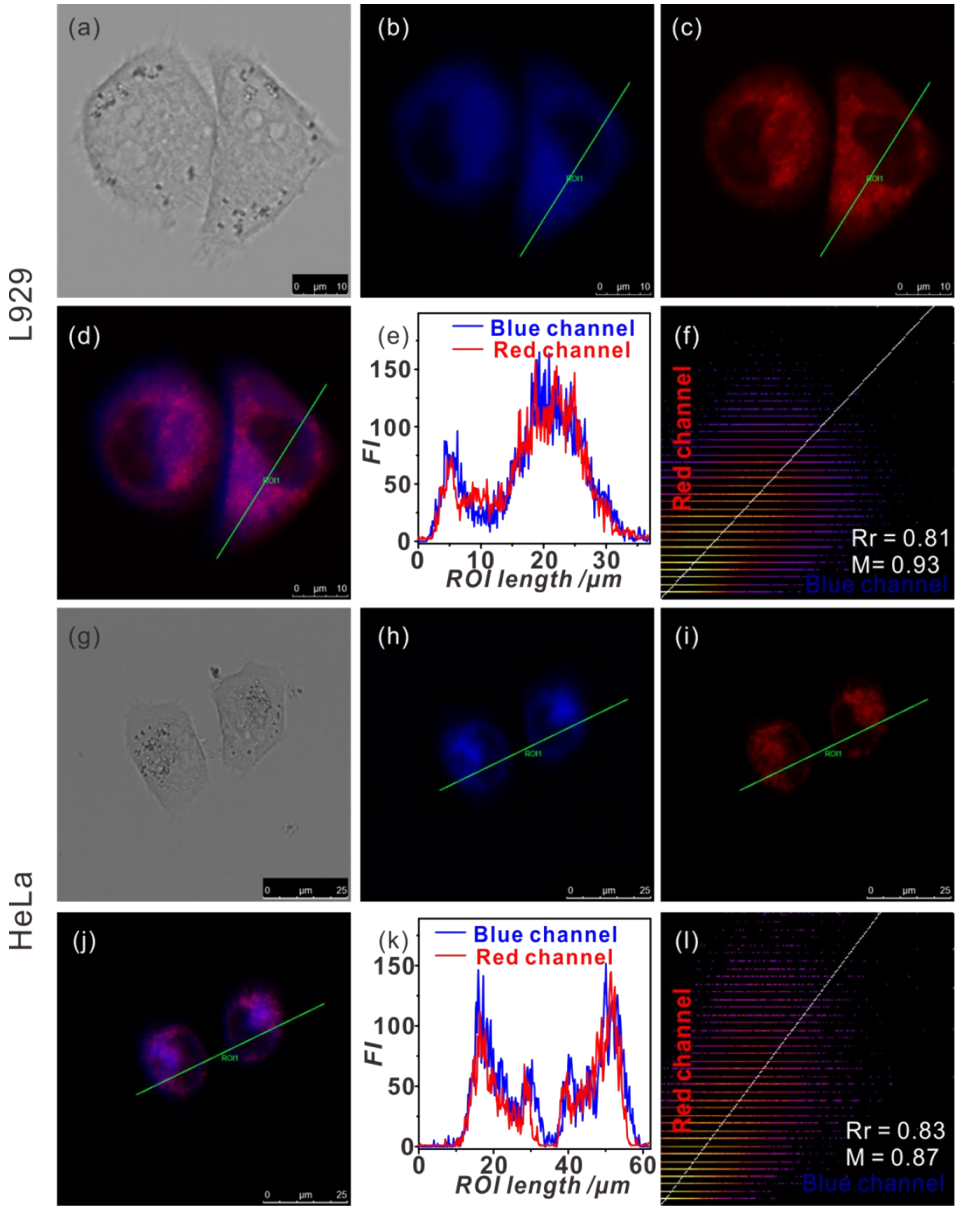

Fig. S15 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **2b**. (a, g) bright field images; (b, h) confocal image (blue channel) of cells with dye **2b** (1 μM); (c, i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of blue and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of blue channel and red channel.

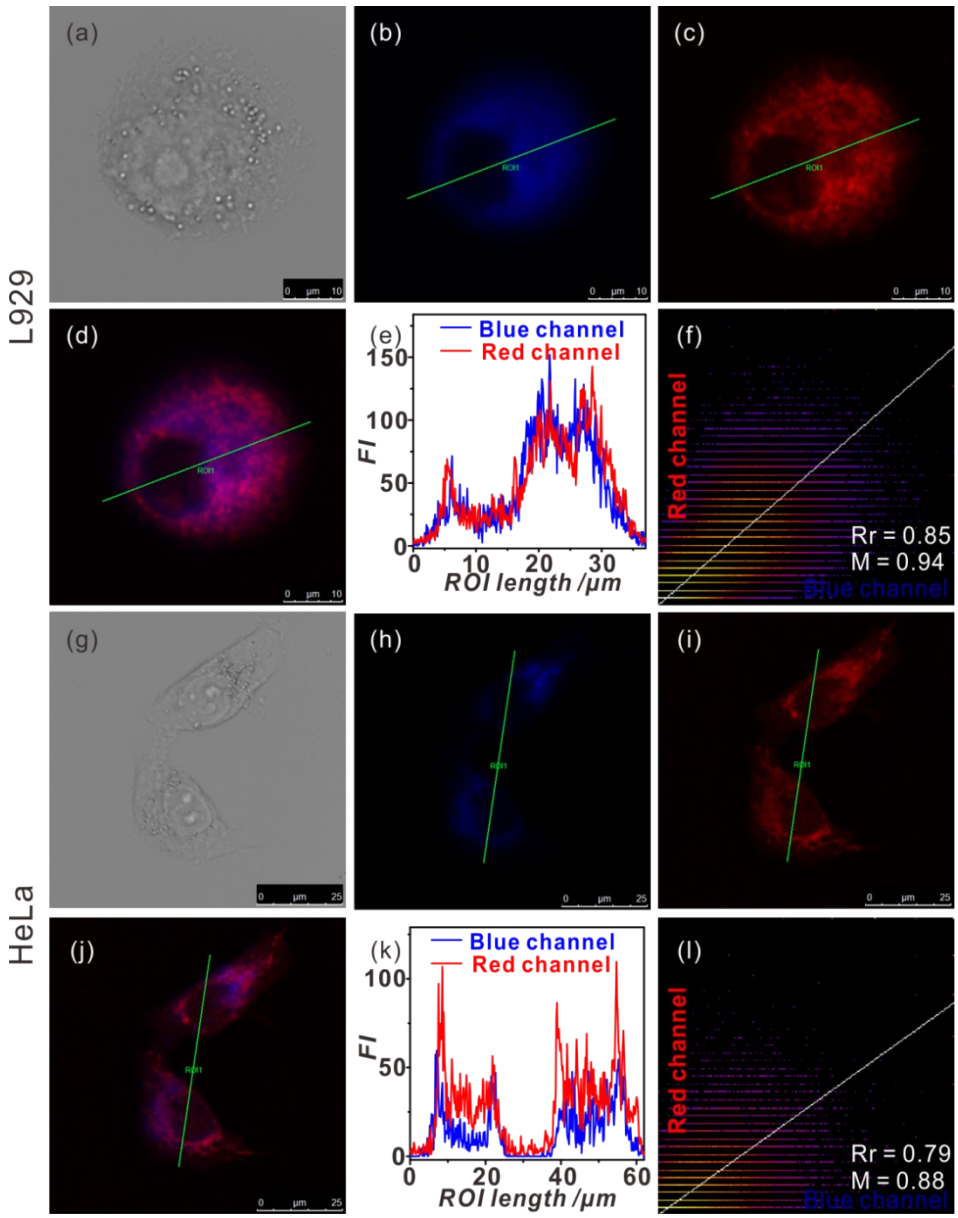

Fig. S16 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **3b**. (a, g) bright field images; (b, h) confocal image (blue channel) of cells with dye **3b** (1 μM); (c, i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of blue and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of blue channel and red channel.

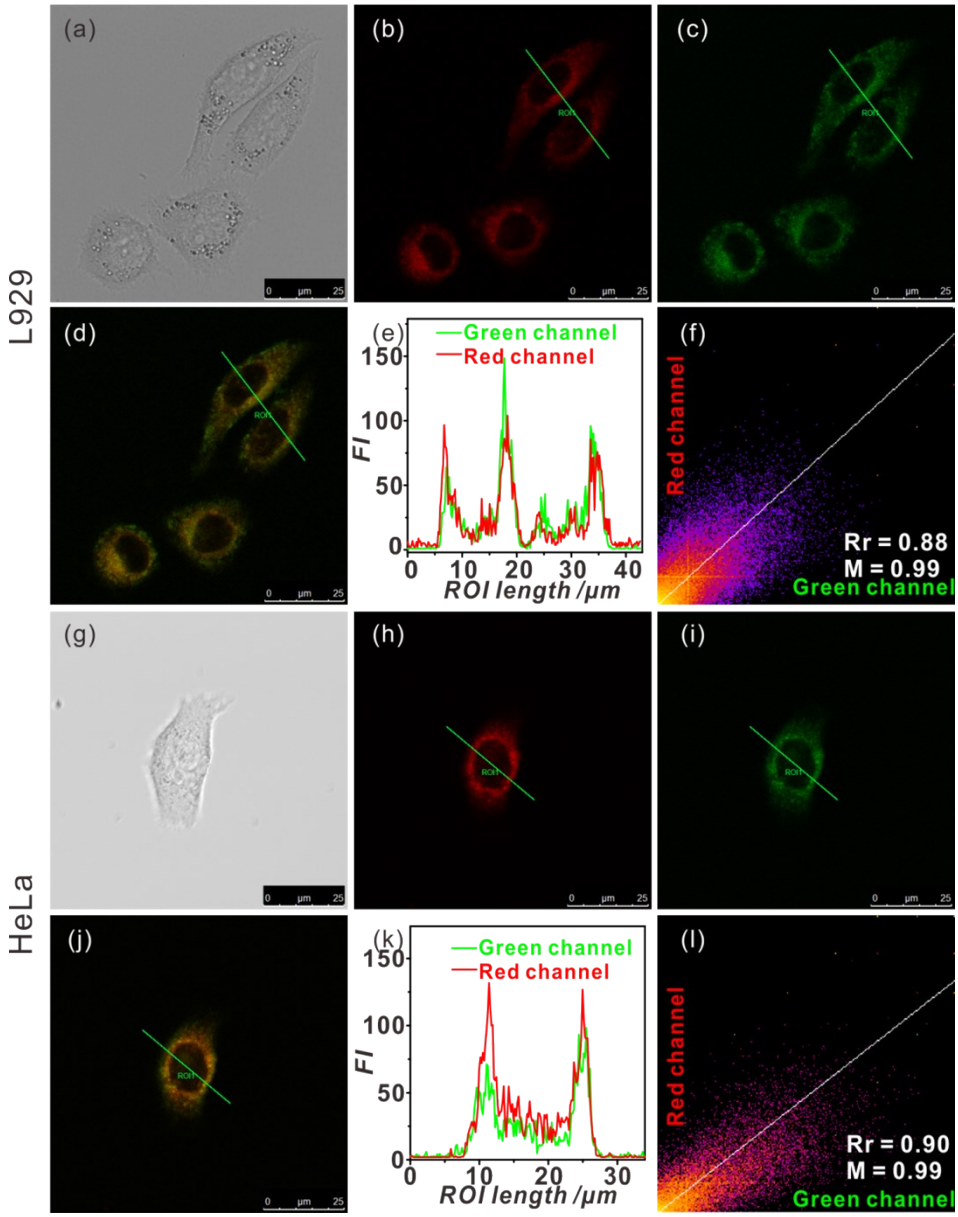

Fig. S17 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **4b**. (a, g) bright field images; (b, h) confocal image (red channel) of cells with dye **4b** (1 μM); (c, i) confocal image (green channel) of cells with Mito-Tracker® Green FM (100 nM); (d, j) merged images of green and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of green channel and red channel.

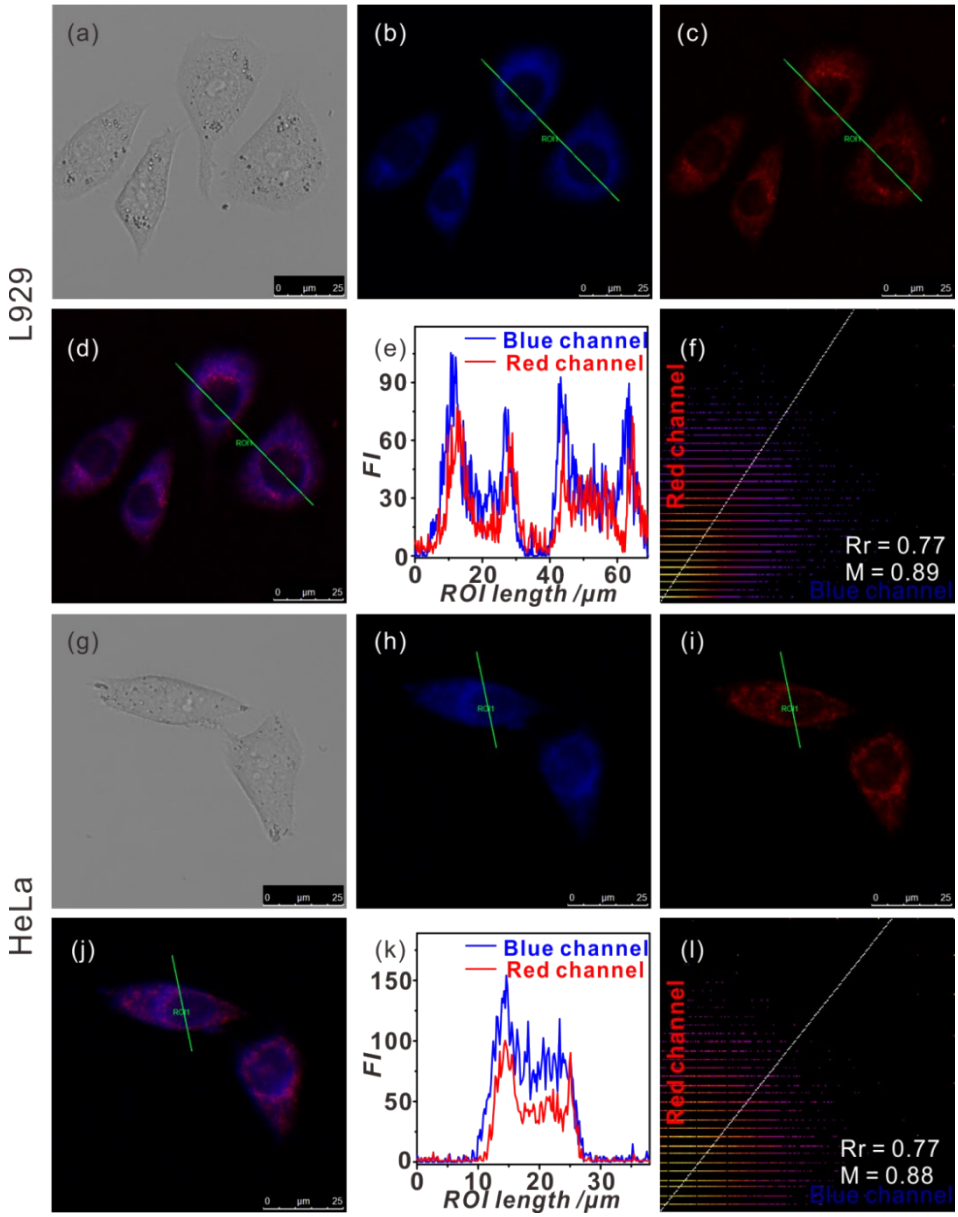

Fig. S18 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **2c**. (a, g) bright field images; (b, h) confocal image (blue channel) of cells with dye **2c** (1 μM); (c, i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of blue and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of blue channel and red channel.

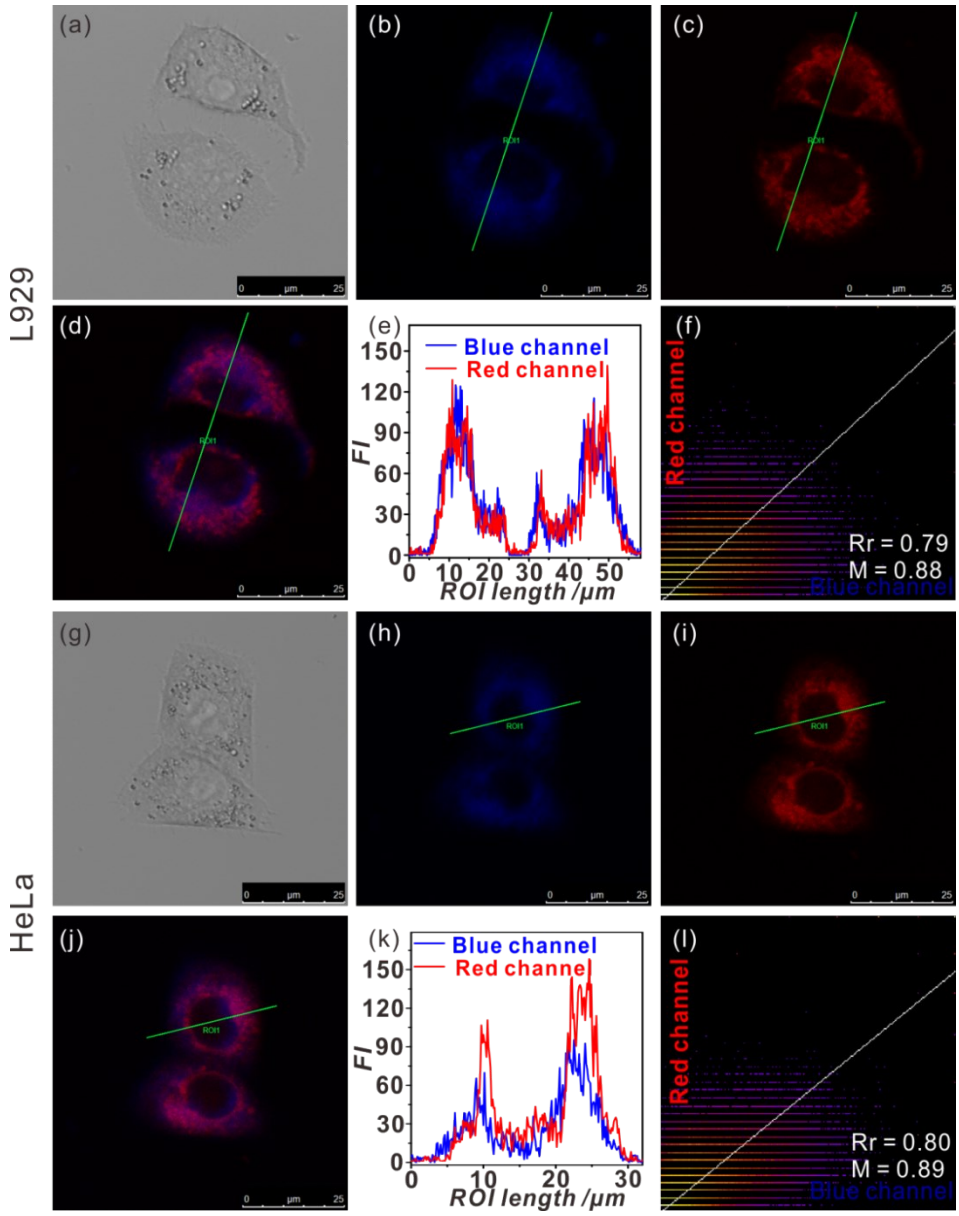

Fig. S19 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **3c**. (a, g) bright field images; (b, h) confocal image (blue channel) of cells with dye **3c** (1 μM); (c, i) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d, j) merged images of blue and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of blue channel and red channel.

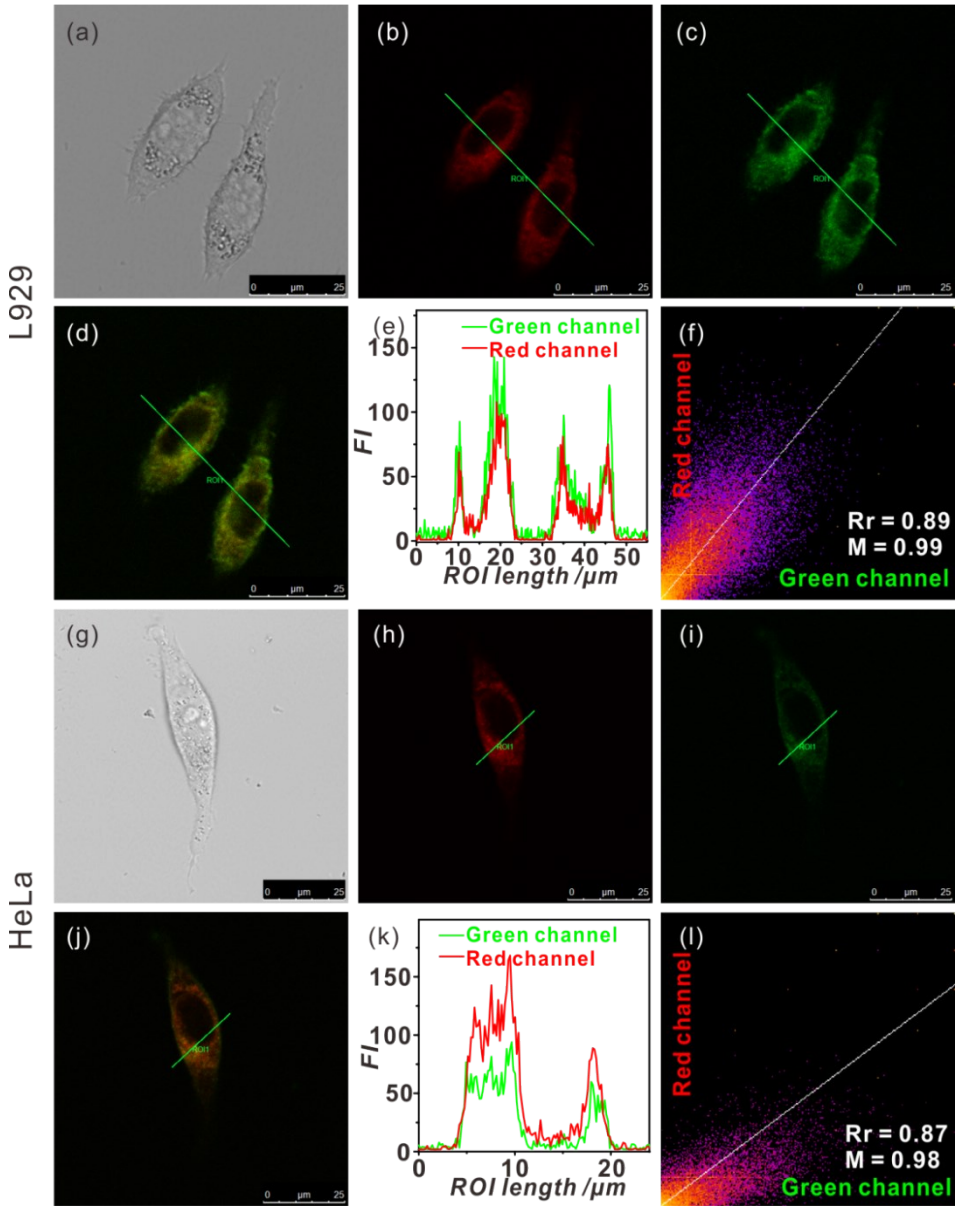

Fig. S20 Confocal fluorescence image of L929 (a-f) and HeLa (g-l) cells with dye **4c**. (a, g) bright field images; (b, h) confocal image (red channel) of cells with dye **4c** (1 μM); (c, i) confocal image (green channel) of cells with Mito-Tracker® Green FM (100 nM); (d, j) merged images of green and red channels; (e, k) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l) fluorescence intensity correlation plot of green channel and red channel.

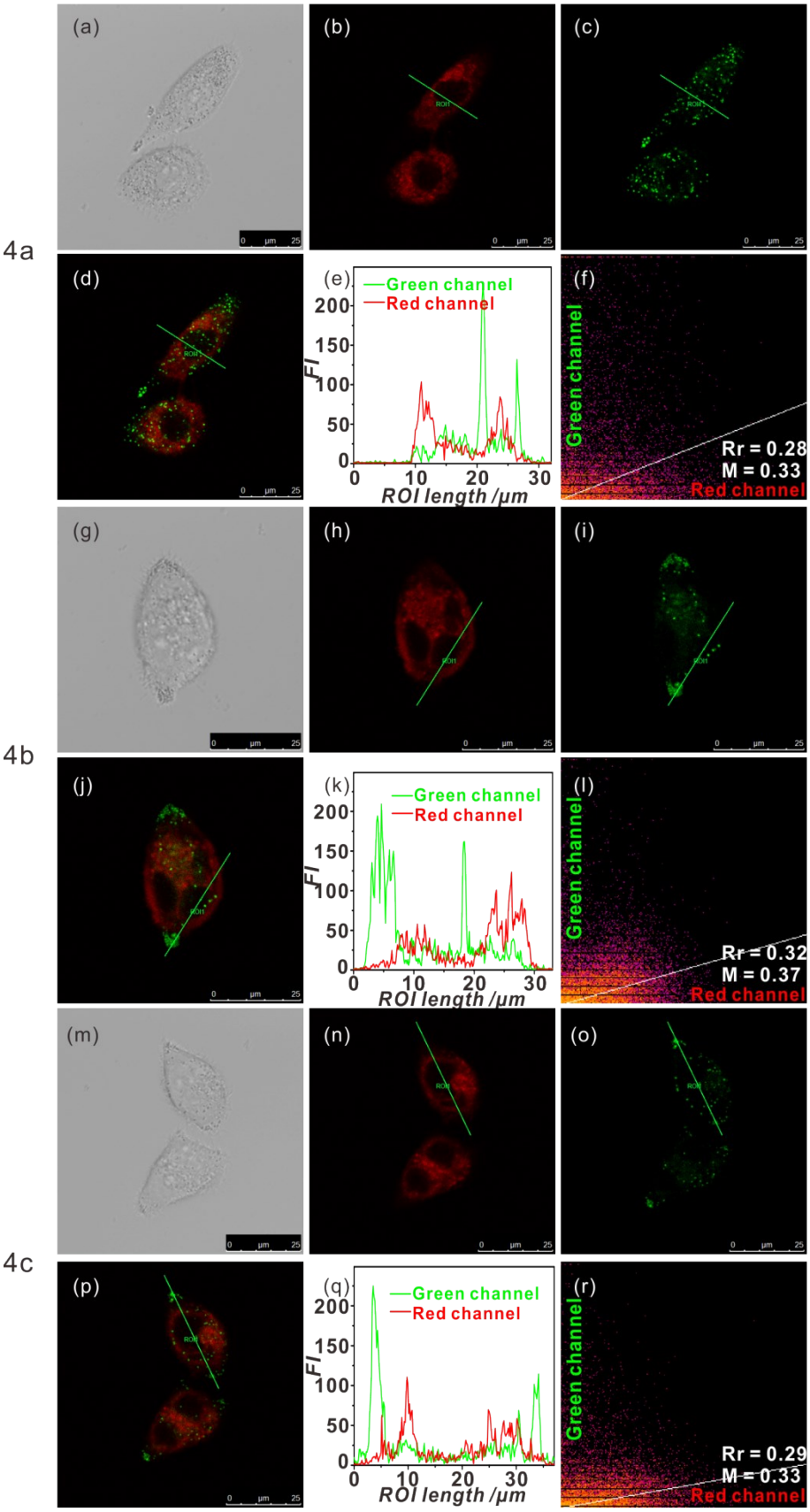

Fig. S21 Confocal fluorescence image of dyes **4a** (a-f), **4b** (g-l) and **4c** (m-r) in HeLa cells. (a, g, m) bright field images; (b, h, n) confocal image (red channel) of cells with dyes **4a**-**c** (1 μM); (c, i, o) confocal image (green channel) of cells with lipid droplets probe reported by previous literature (1 μM); (d, j, p) merged images of green and red channels; (e, k, q) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l, r) fluorescence intensity correlation plot of green channel and red channel.

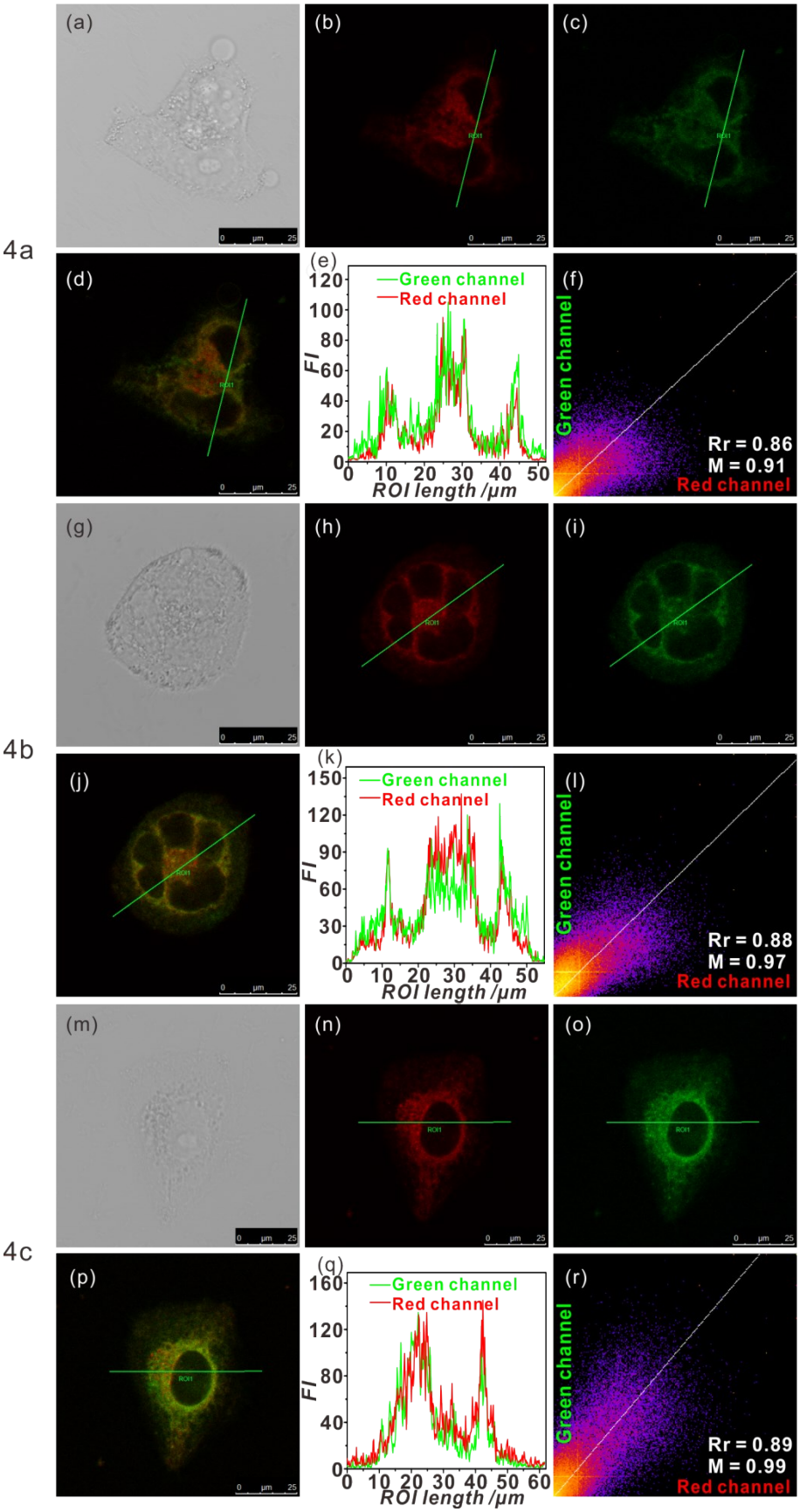

Fig. S22 Confocal fluorescence image of dyes **4a** (a-f), **4b** (g-l) and **4c** (m-r) in fixed HeLa cells. (a, g, m) bright field images; (b, h, n) confocal image (red channel) of cells with dyes **4a**-**c** (1 μM); (c, i, o) confocal image (green channel) of cells with Mito-Tracker® Green FM (100 nM); (d, j, p) merged images of green and red channels; (e, k, q) fluorescence intensities of the regions of interest (ROIs) across the cells, (f, l, r) fluorescence intensity correlation plot of green channel and red channel.

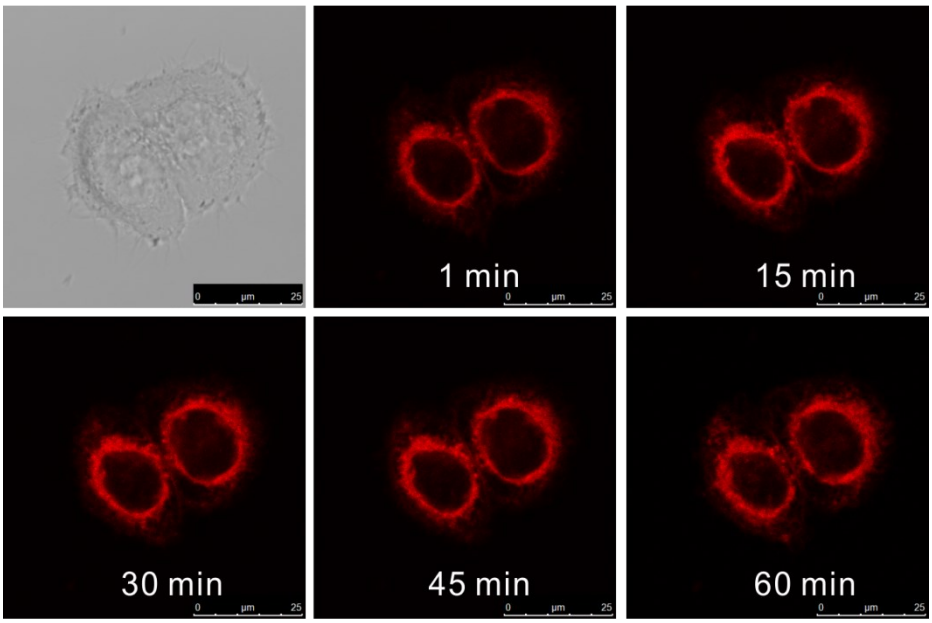

Fig. S23 Photobleaching study of dye **4c** in HeLa cells.

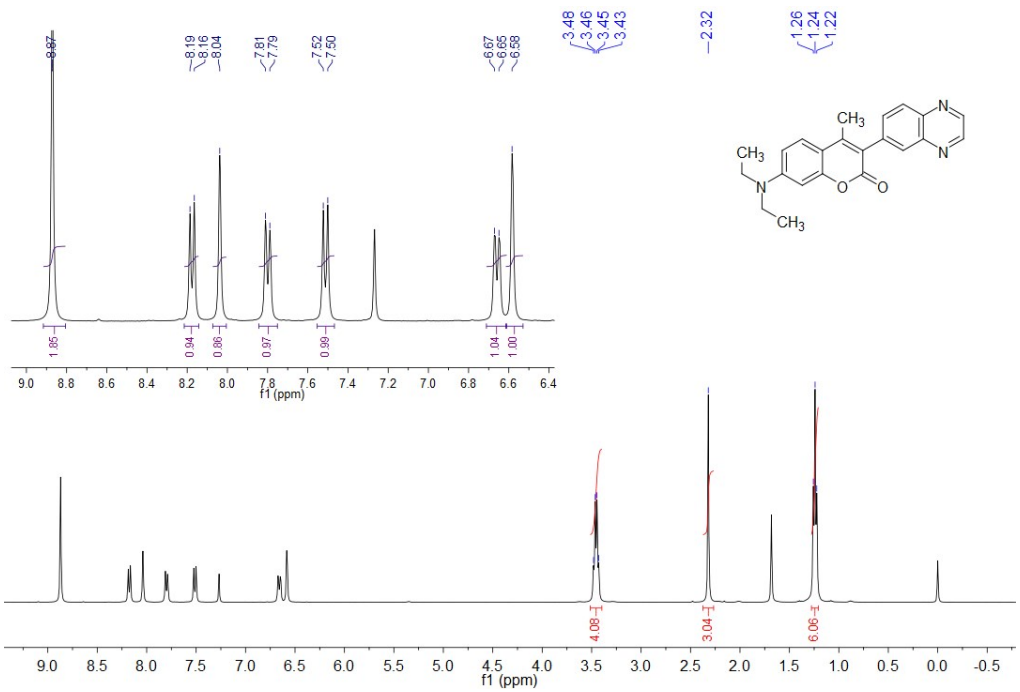

Fig. S24 ^{1}H NMR spectrum of dye **1a**.

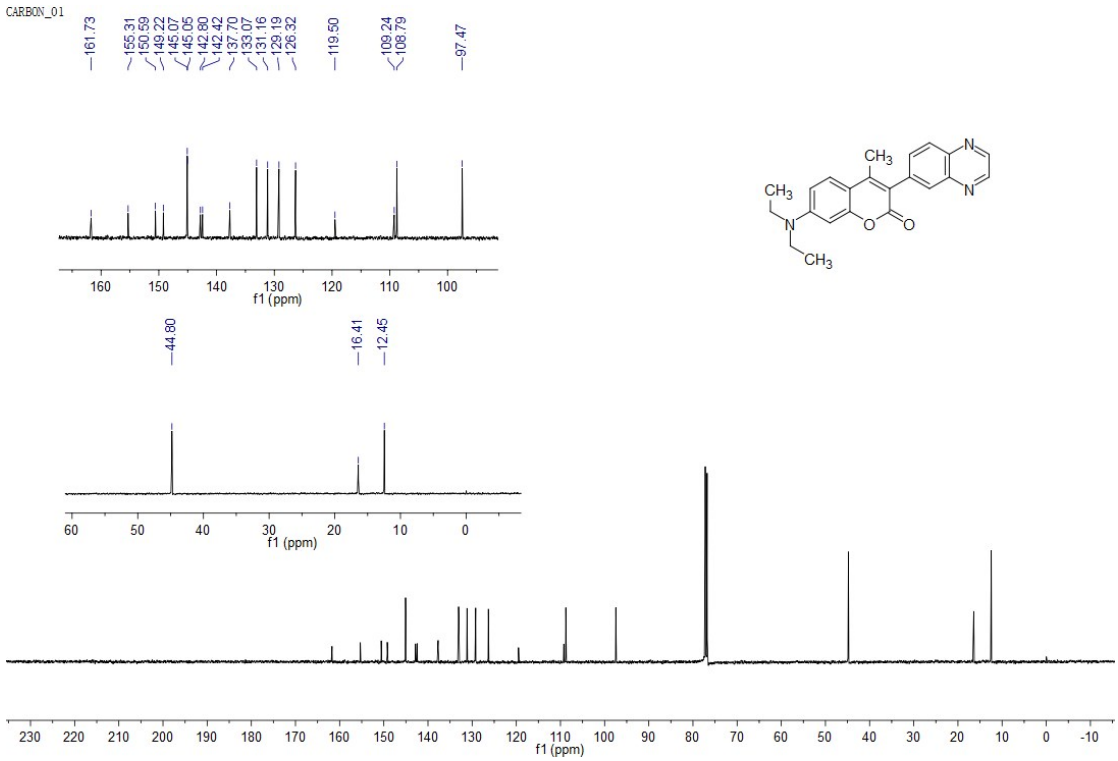

Fig. S25 ^{13}C NMR spectrum of dye **1a**.

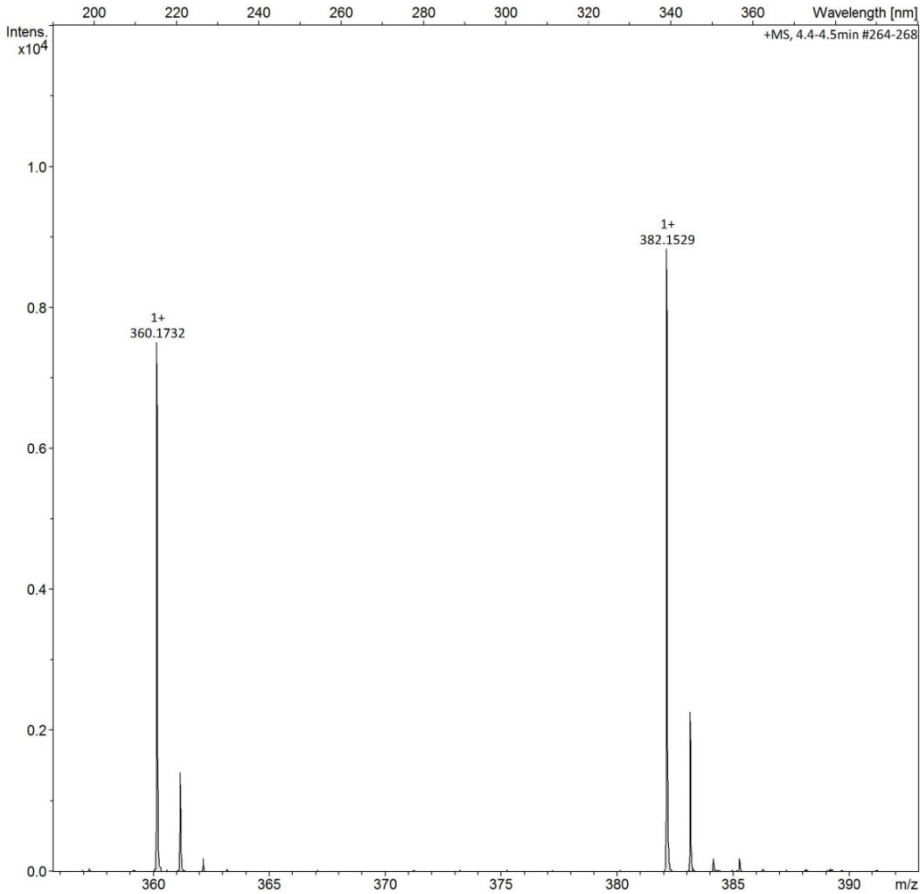

Fig. S26 HRMS (ESI^+) spectrum of dye **1a**.

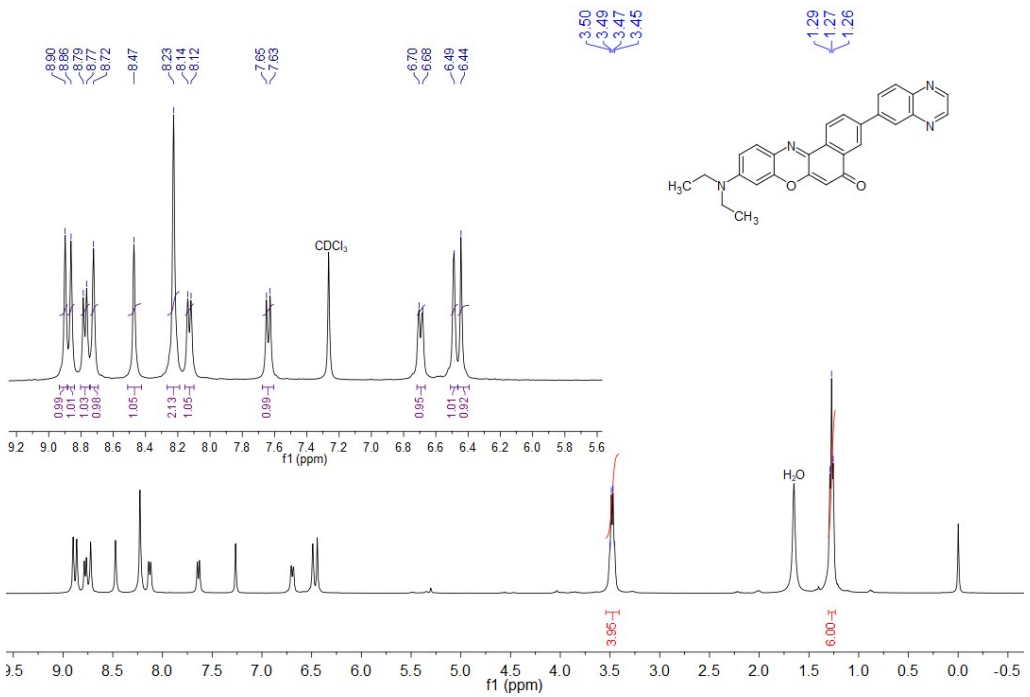

Fig. S27 ^1H NMR spectrum of dye **1b**.

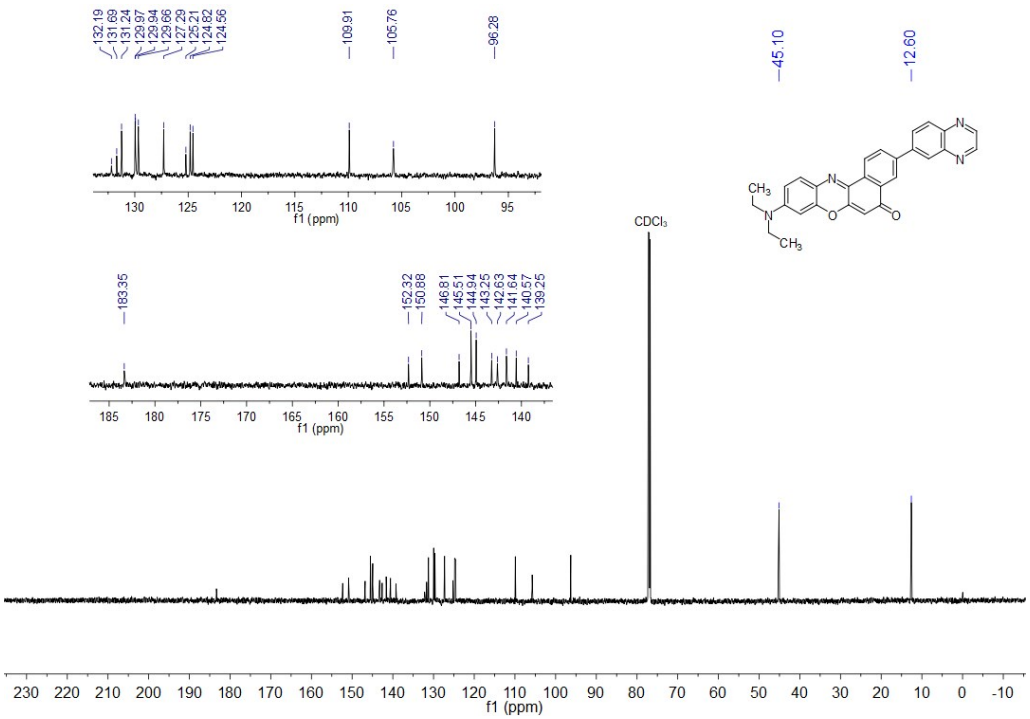

Fig. S28 ^{13}C NMR spectrum of dye **1b**.

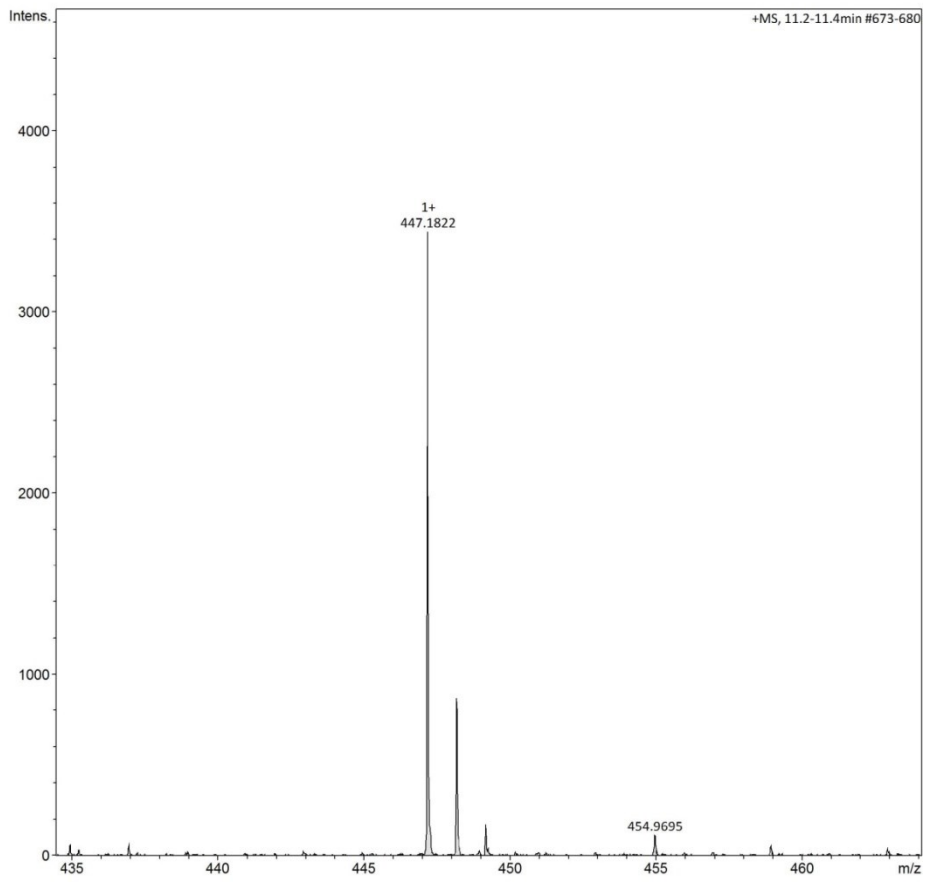

Fig. S29 HRMS (ESI$^+$) spectrum of dye **1b**.

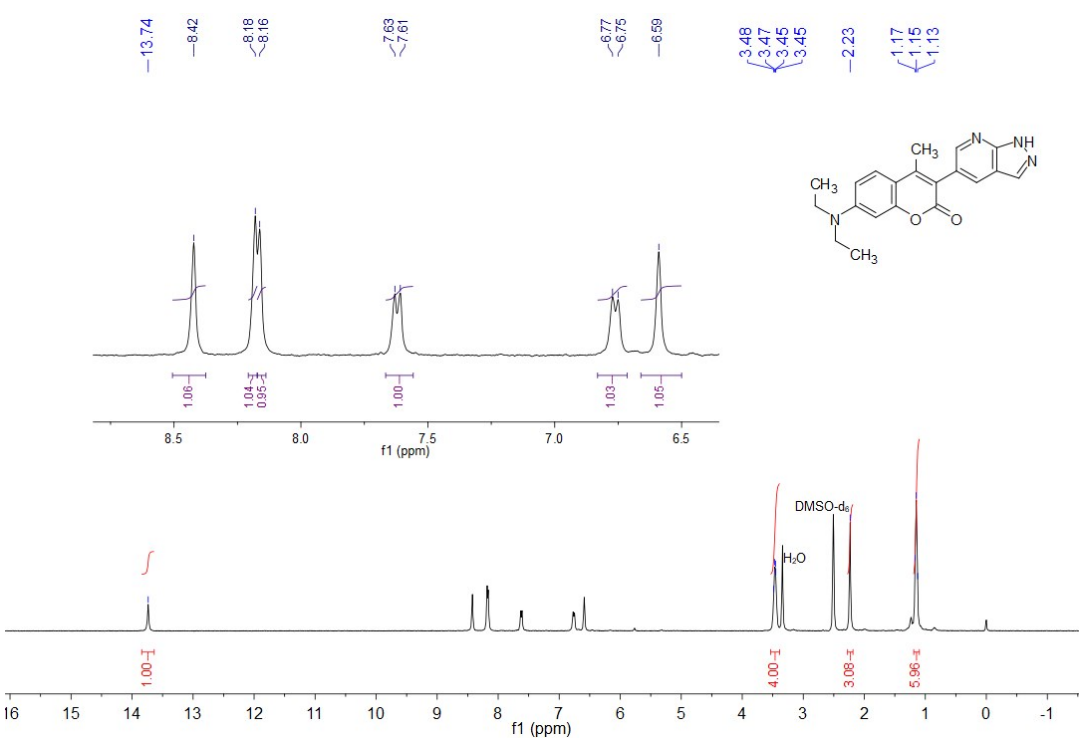

Fig.S30 ^{1}H NMR spectrum of dye **2a**.

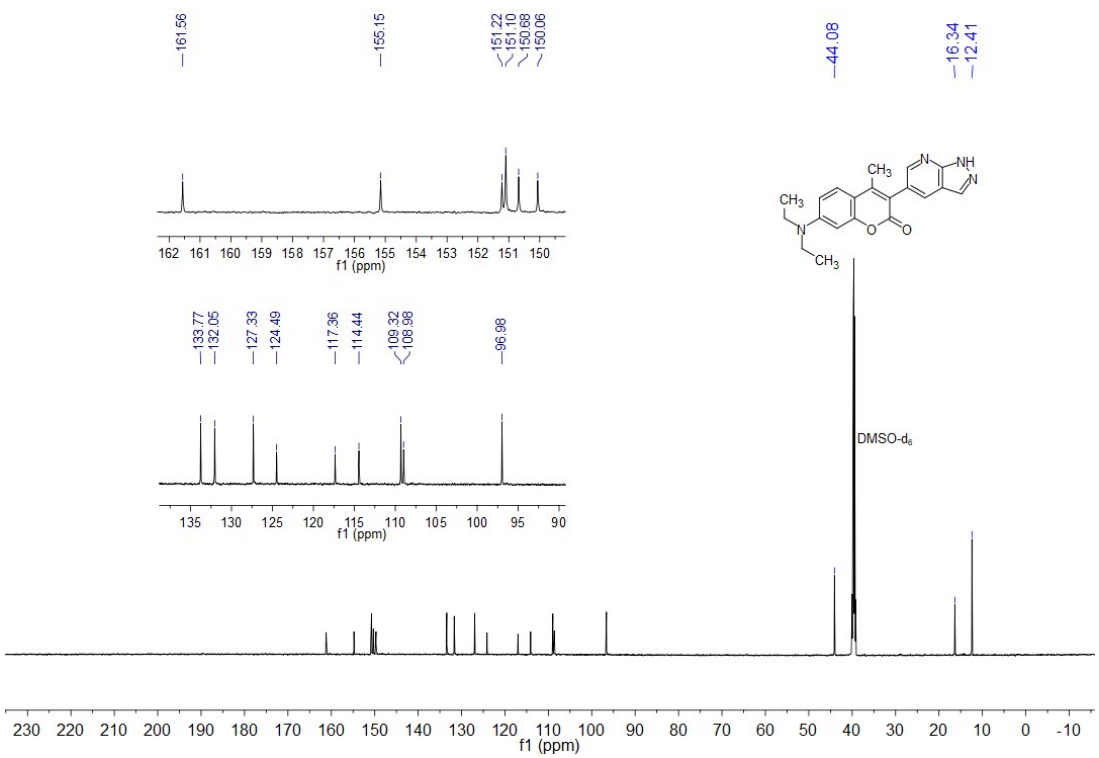

Fig. S31 ^{13}C NMR spectrum of dye **2a**.

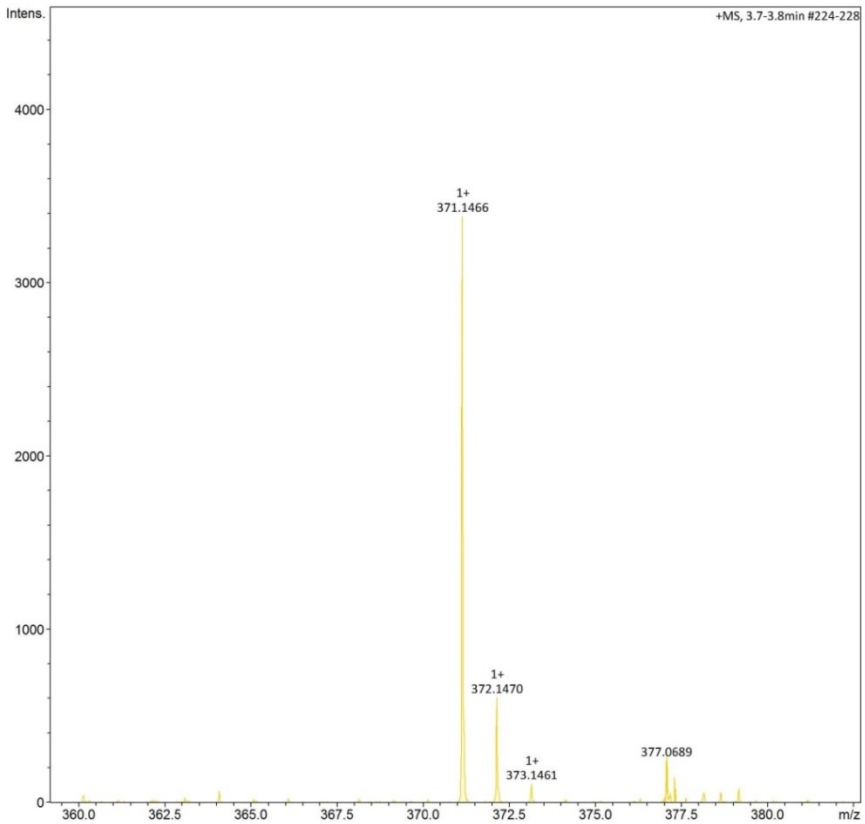

Fig. S32 HRMS (ESI^+) spectrum of dye **2a**.

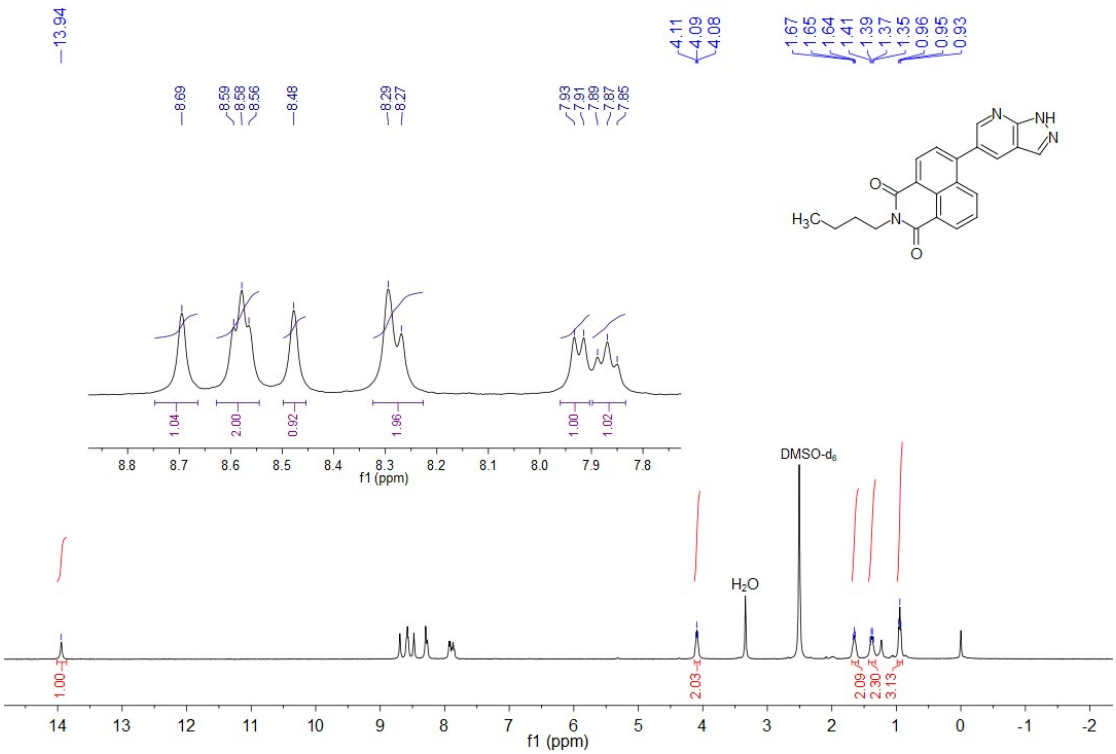

Fig. S33 ^1H NMR spectrum of dye **3a**.

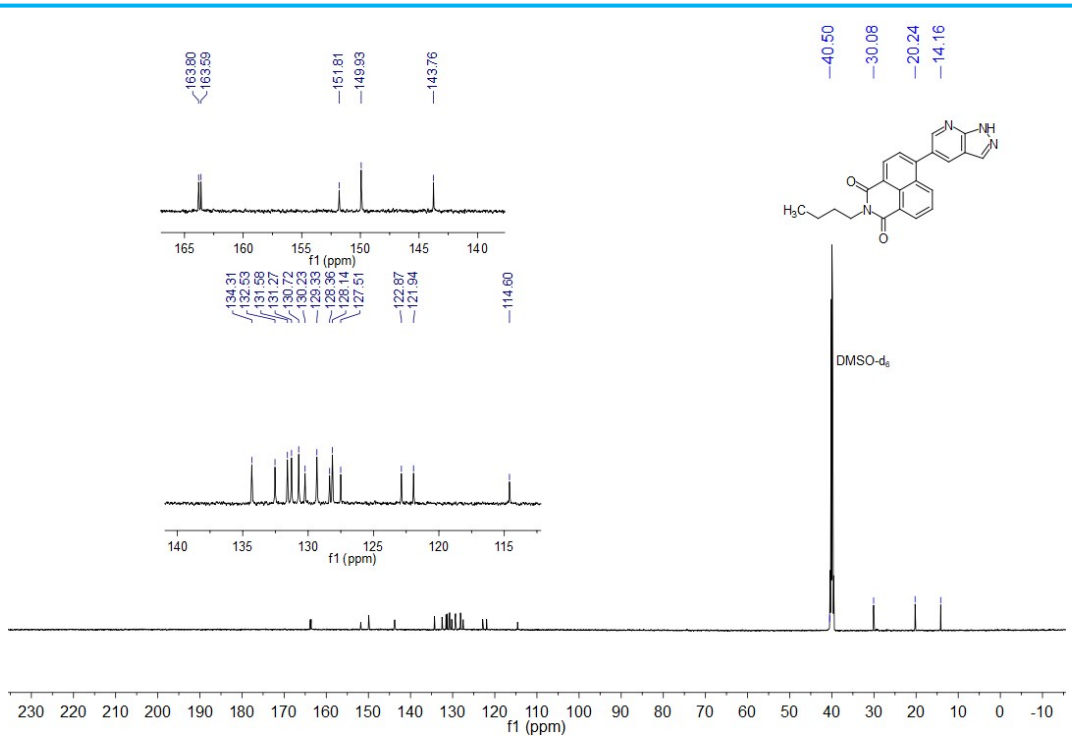

Fig. S34 ^{13}C NMR spectrum of dye **3a**.

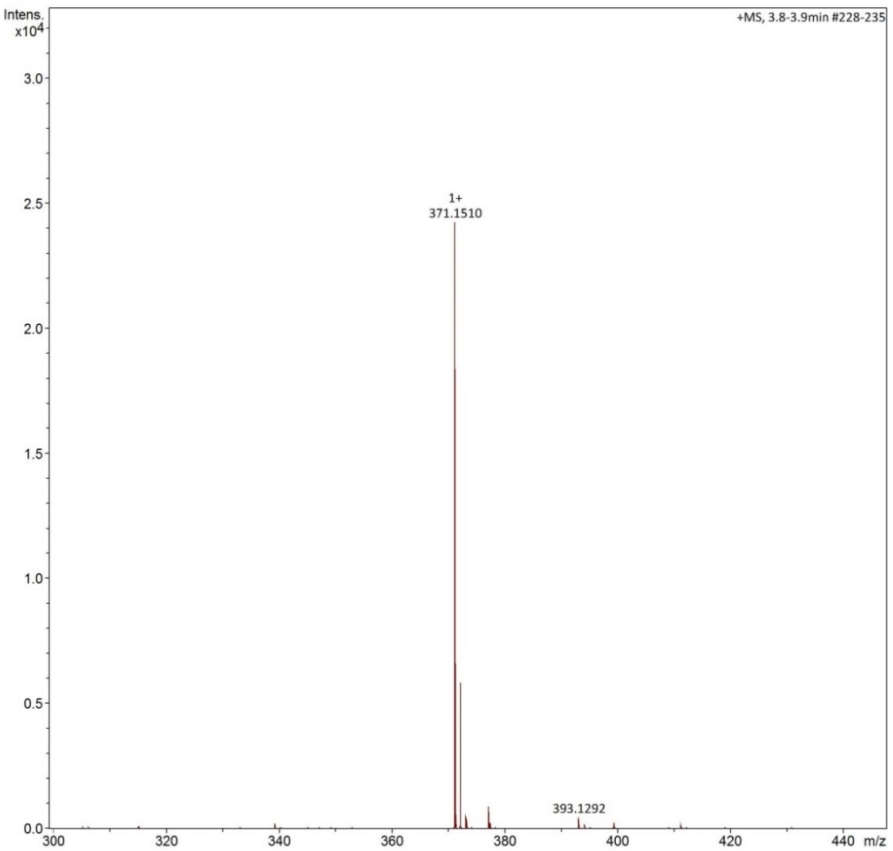

Fig. S35 HRMS (ESI$^+$) spectrum of dye **3a**.

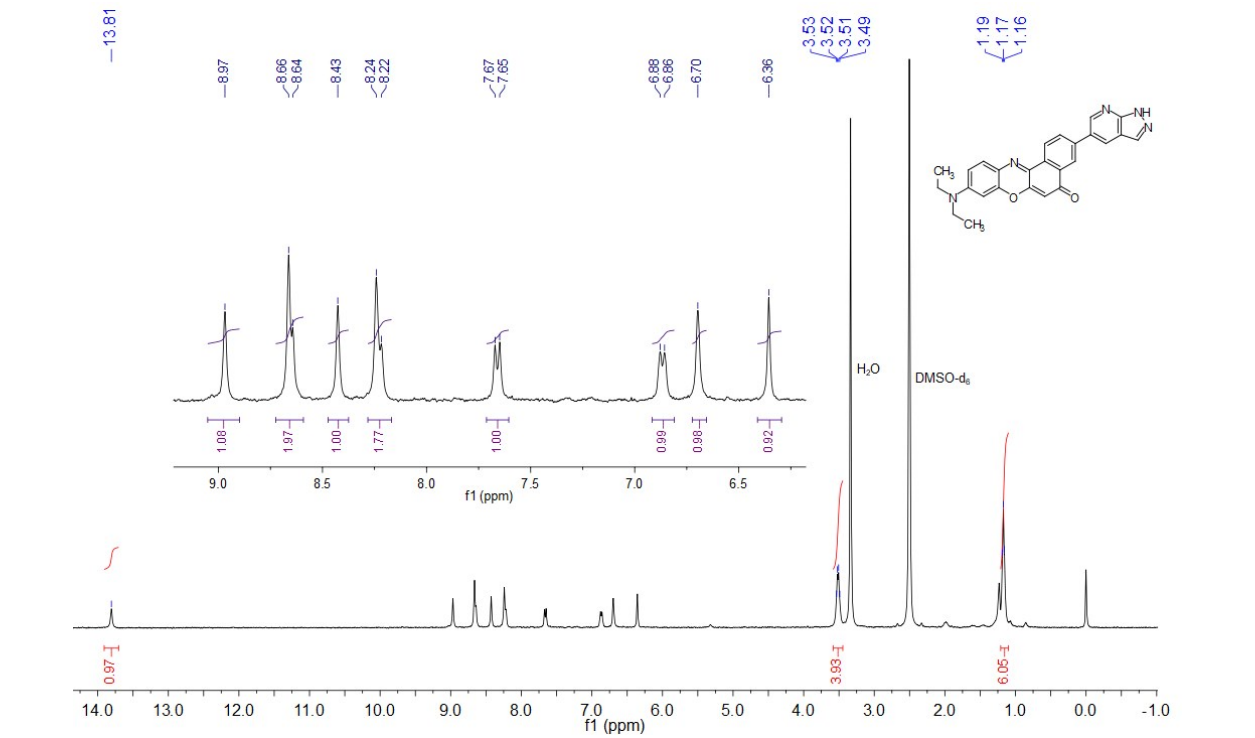

Fig. S36 ^{1}H NMR spectrum of dye **4a**.

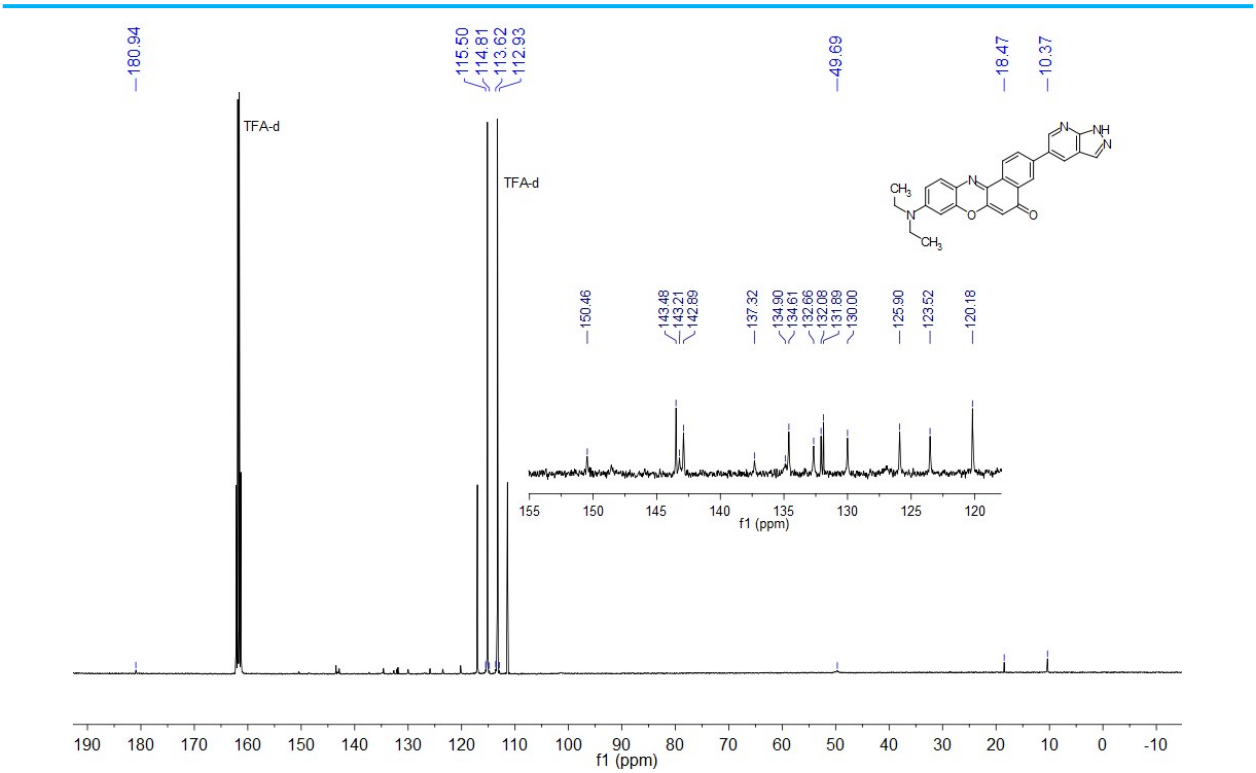

Fig. S37 ^{13}C NMR spectrum of dye **4a**.

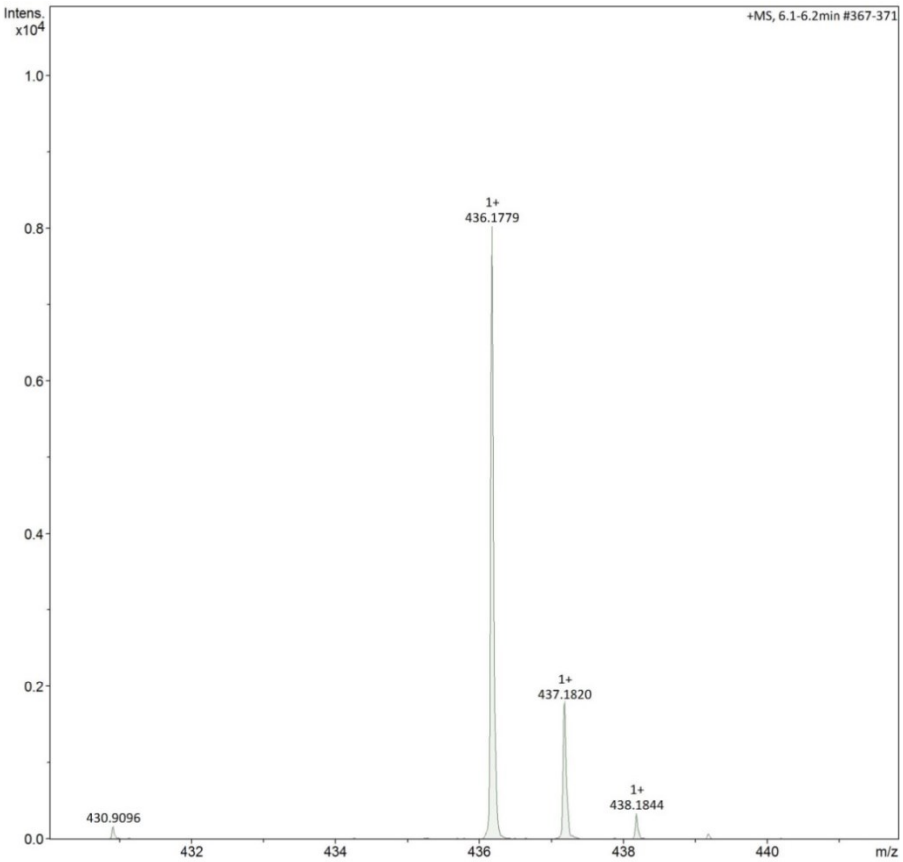

Fig. S38 HRMS (ESI$^+$) spectrum of dye **4a**.

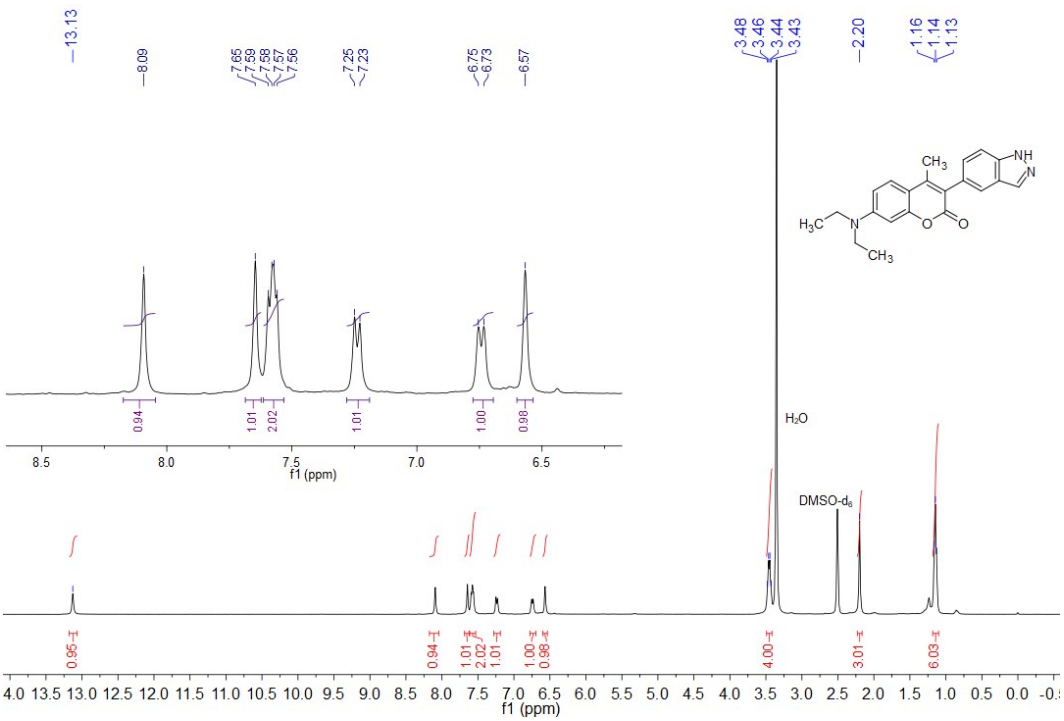

Fig. S39 ^{1}H NMR spectrum of dye **2b**.

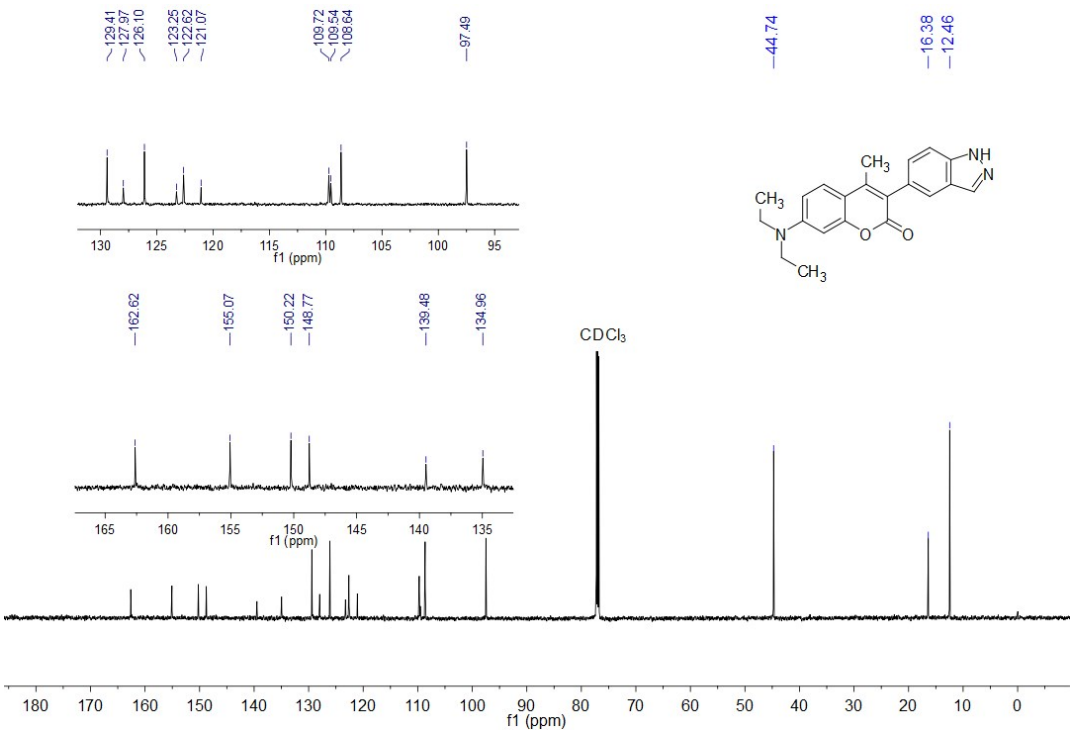

Fig. S40 ^{13}C NMR spectrum of dye **2b**.

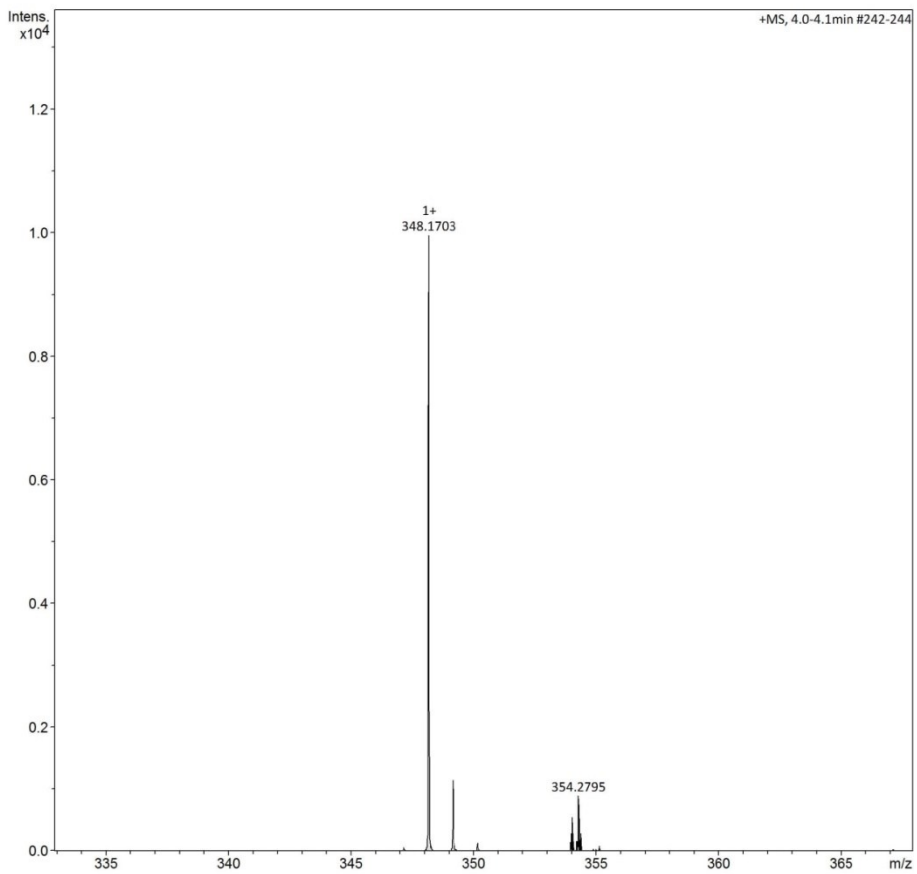

Fig. S41 HRMS (ESI$^+$) spectrum of dye **2b**.

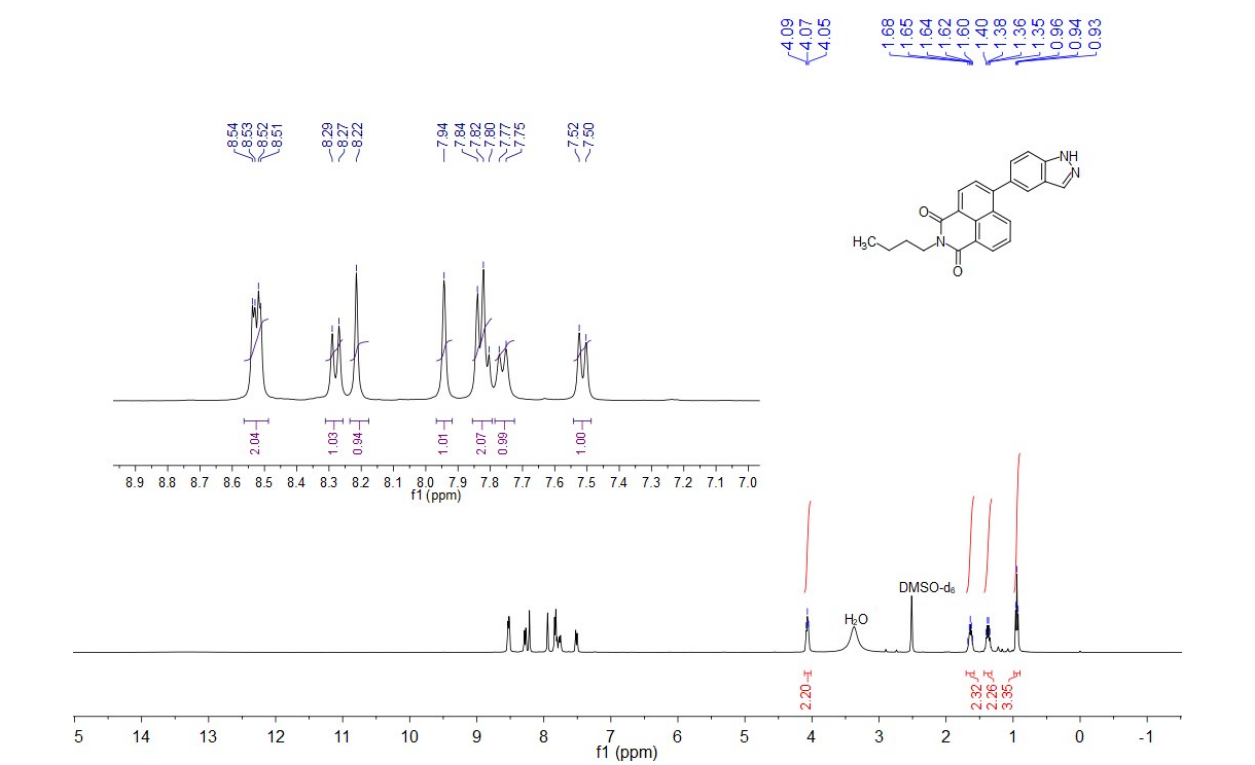

Fig. S42 ^{1}H NMR spectrum of dye **3b**.

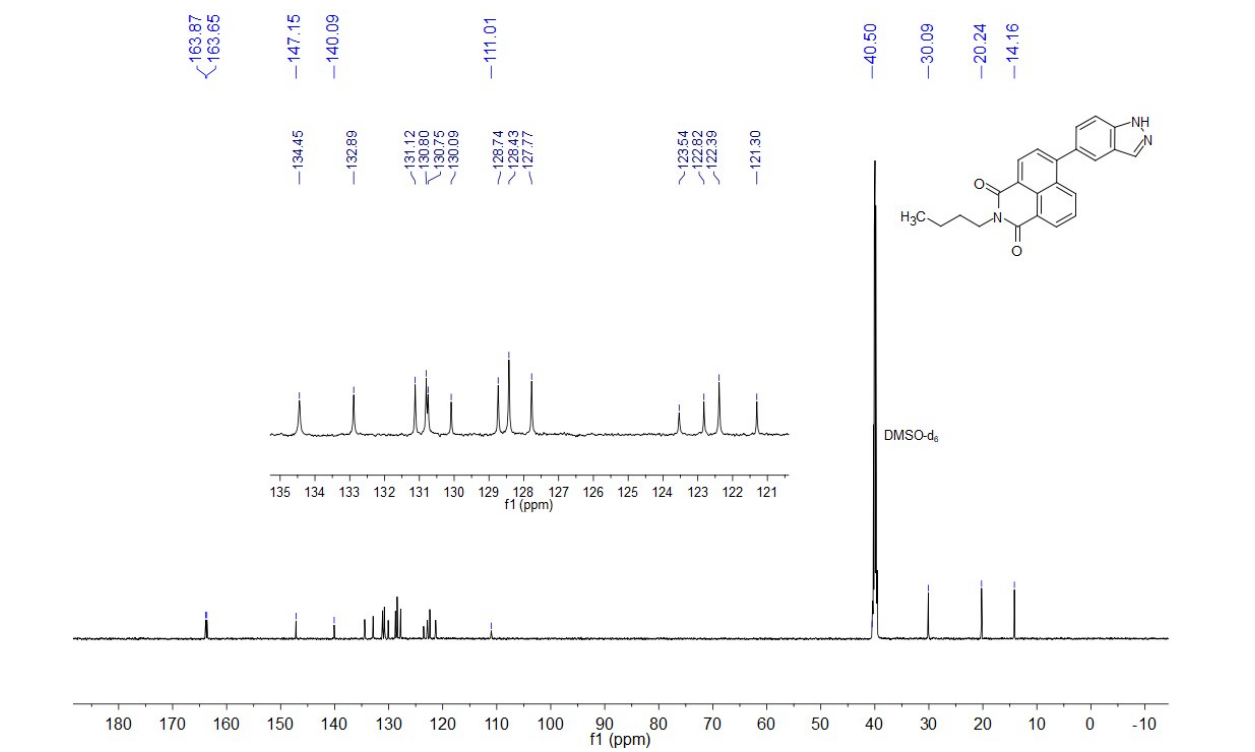

Fig. S43 ^{13}C NMR spectrum of dye **3b.**

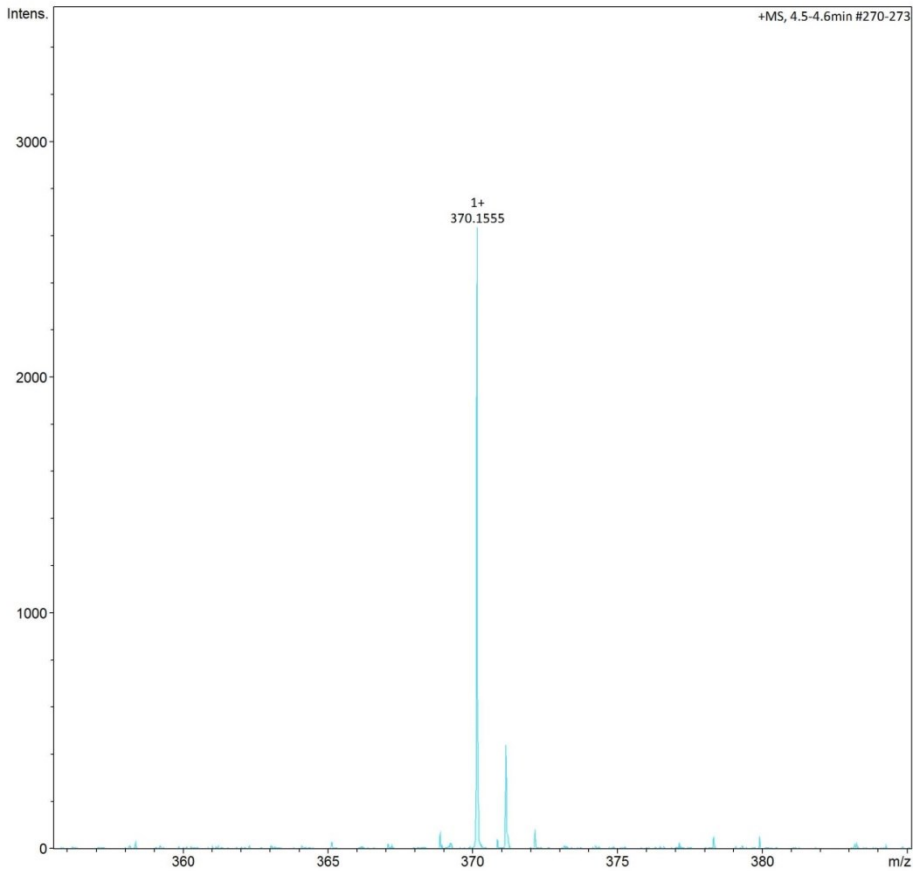

Fig. S44 HRMS (ESI^+) spectrum of dye **3b**.

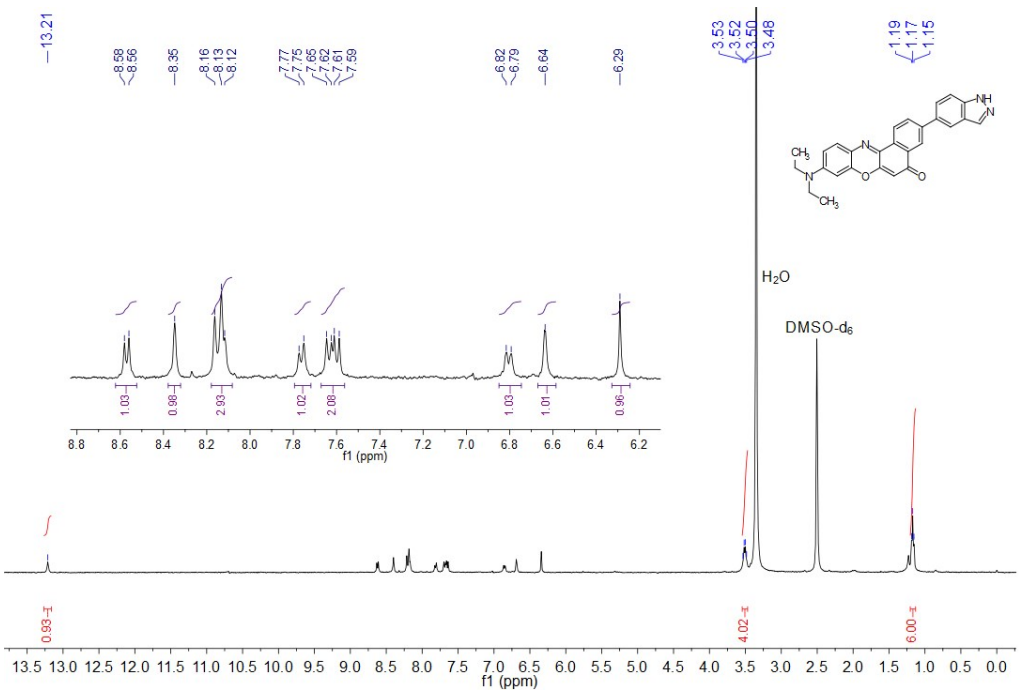

Fig. S45 ^1H NMR spectrum of dye **4b**.

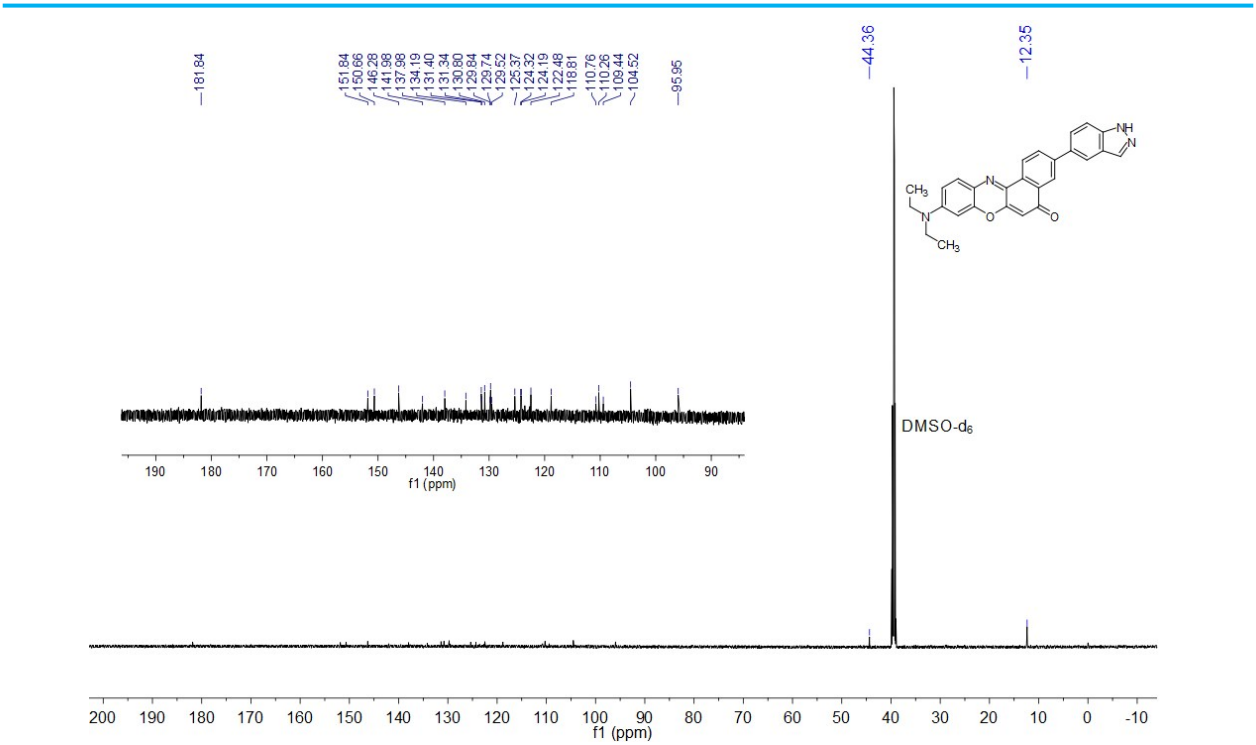

Fig. S46 ^{13}C NMR spectrum of dye **4b**.

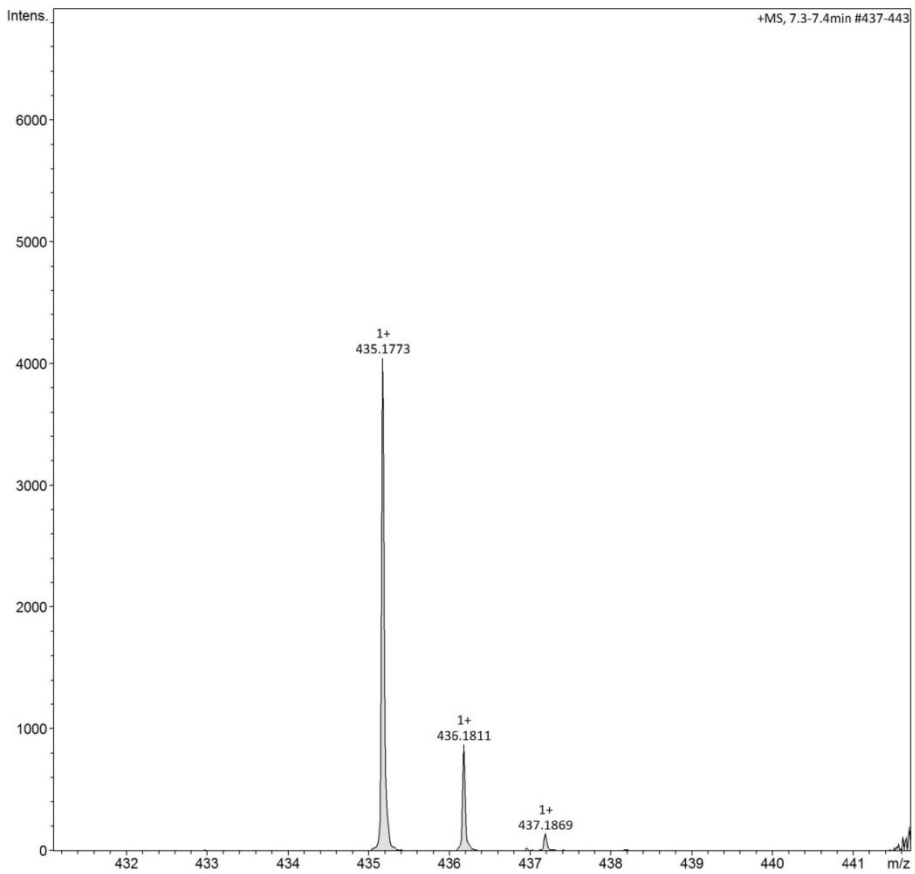

Fig. S47 HRMS (ESI$^+$) spectrum of dye **4b**.

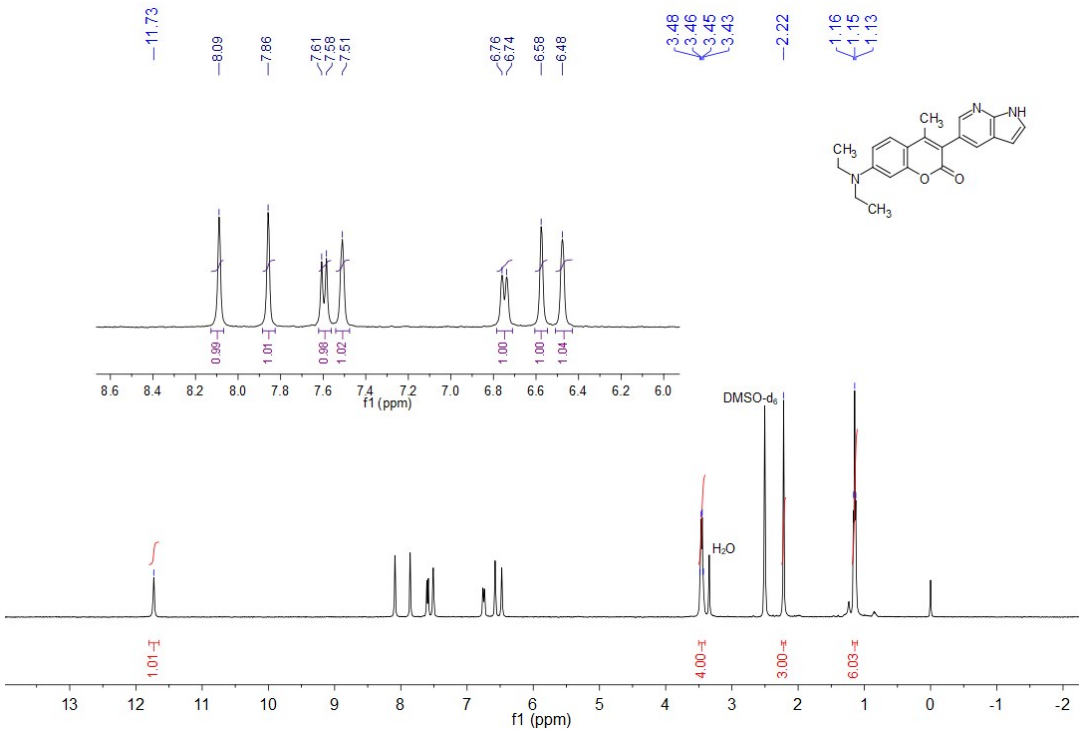

Fig. S48 ^{1}H NMR spectrum of dye **2c**.

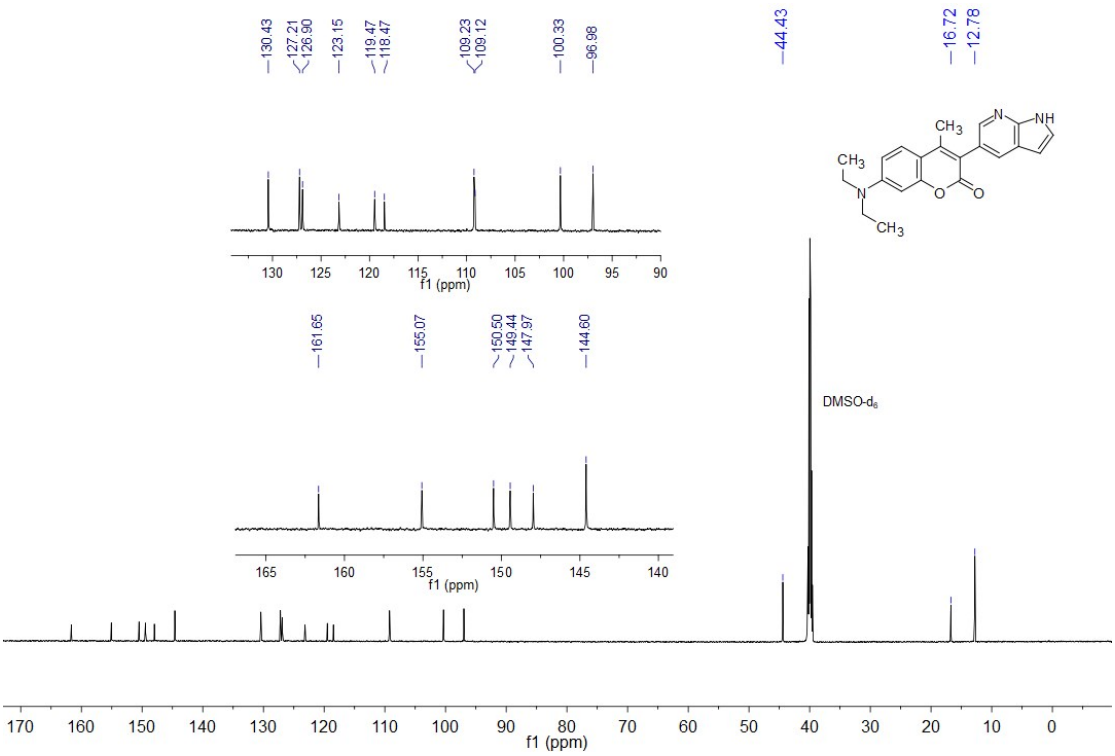

Fig. S49 ^{13}C NMR spectrum of dye **2c**.

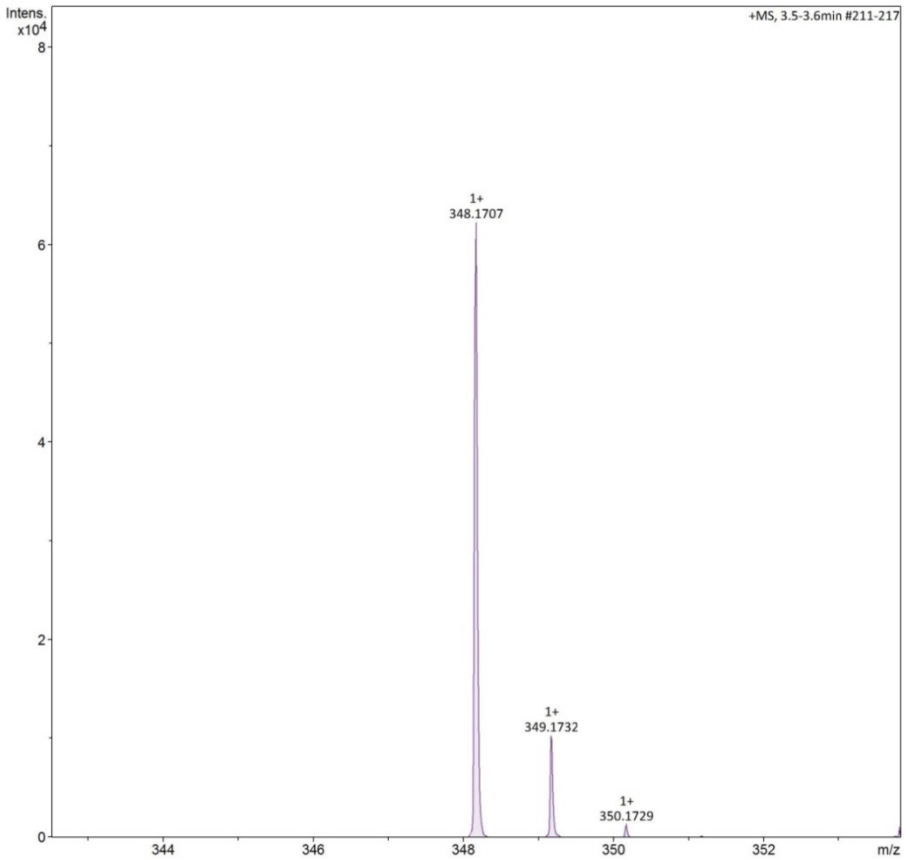

Fig. S50 HRMS (ESI$^+$) spectrum of dye **2c**.

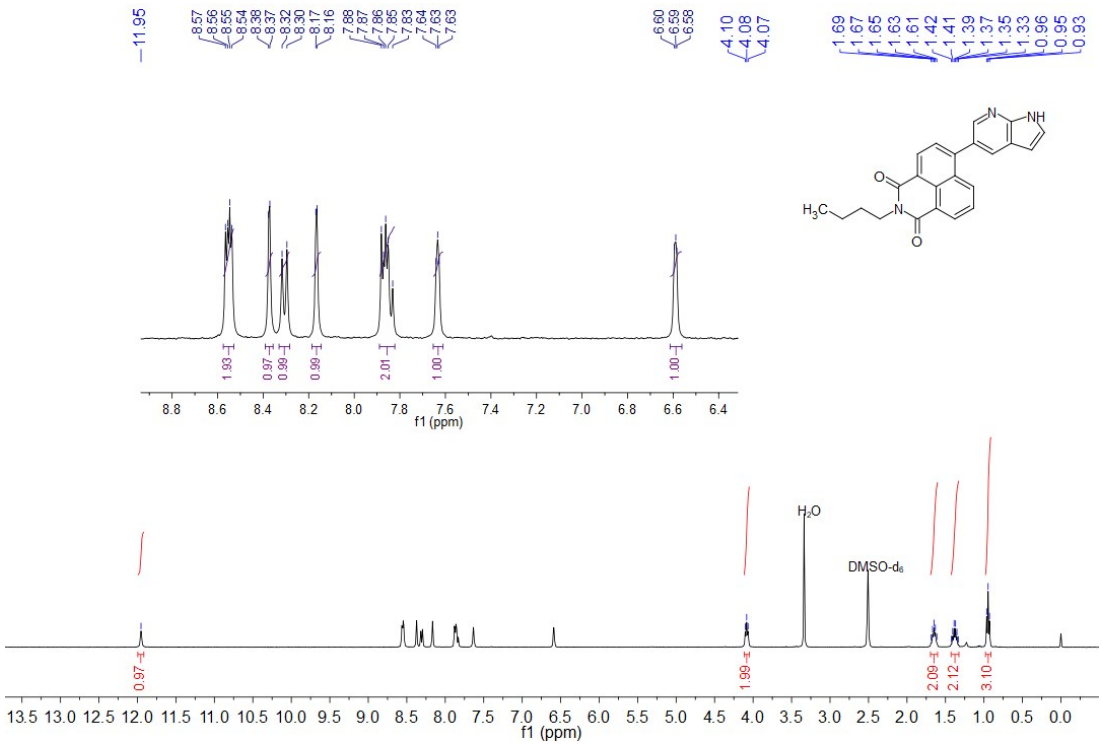

Fig. S51 ^{1}H NMR spectrum of dye **3c**.

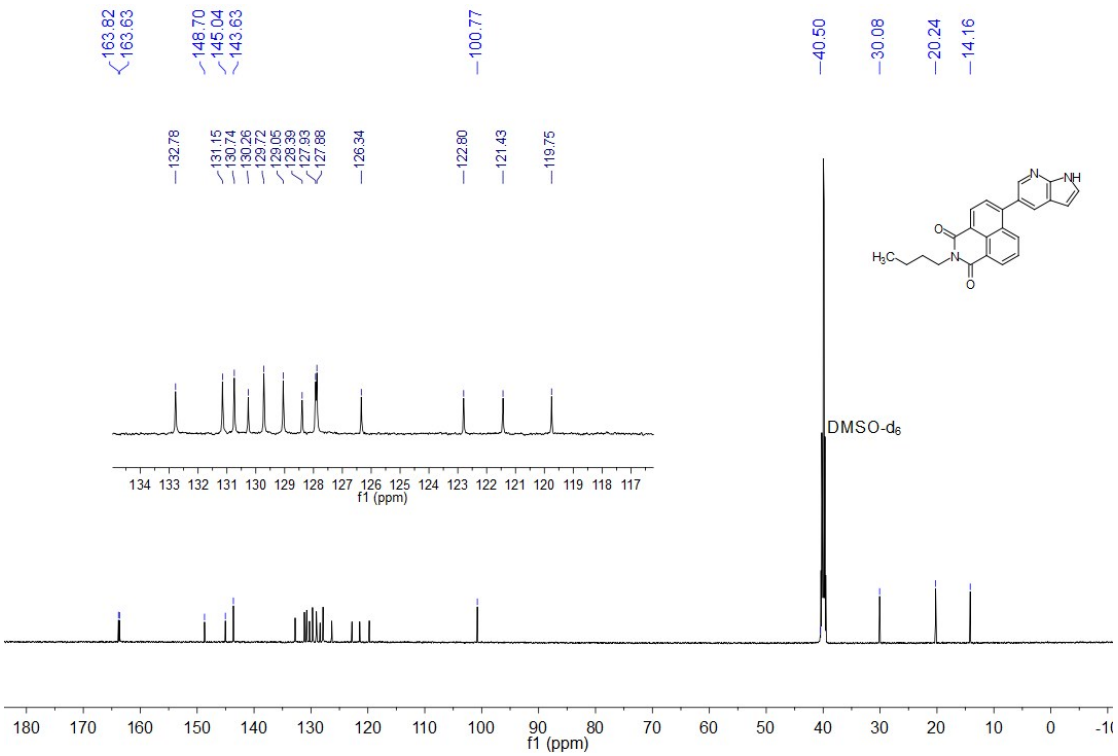

Fig. S52 ^{13}C NMR spectrum of dye **3c**.

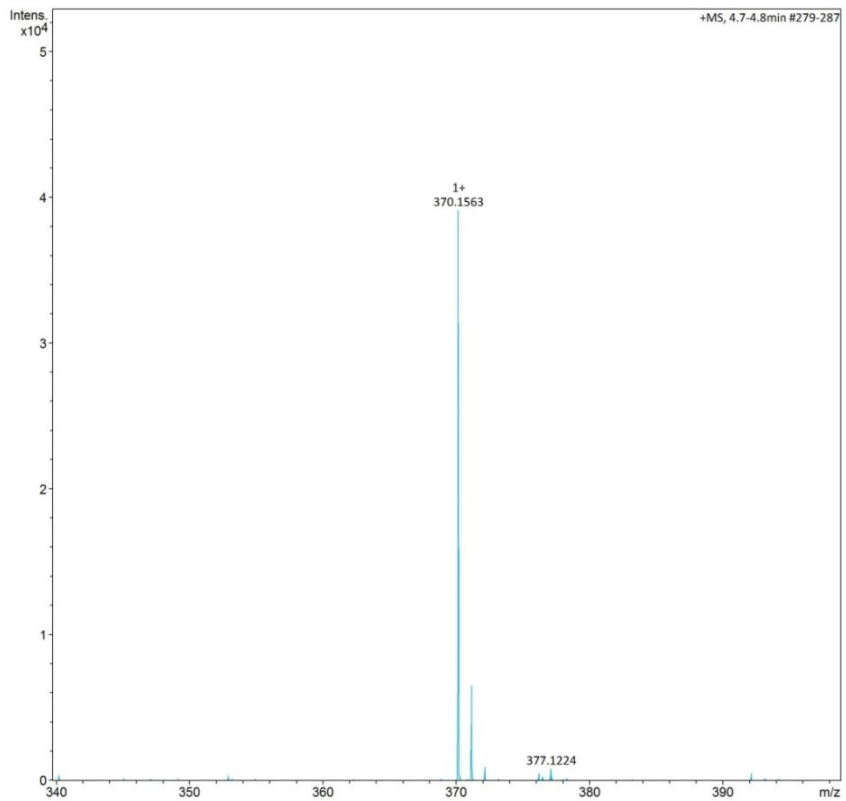

Fig. S53 HRMS (ESI$^+$) spectrum of dye **3c**.

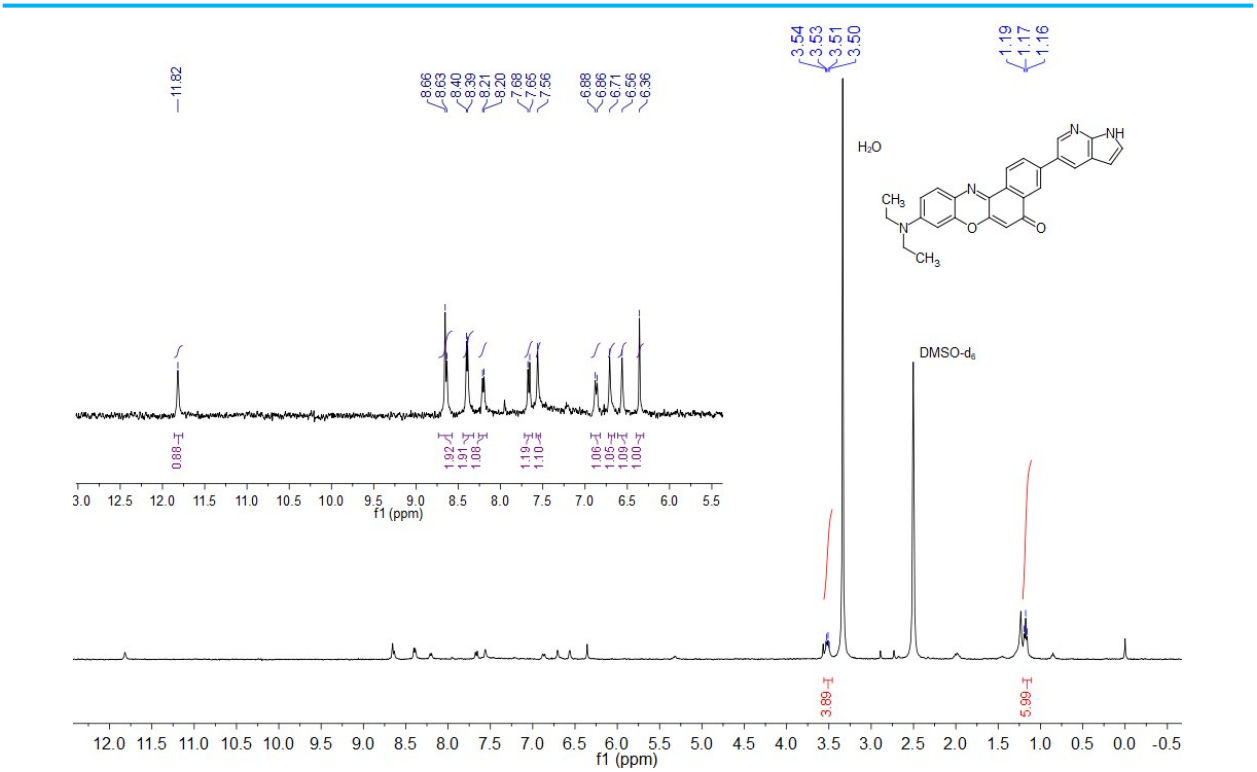

Fig. S54 ^{1}H NMR spectrum of dye **4c**.

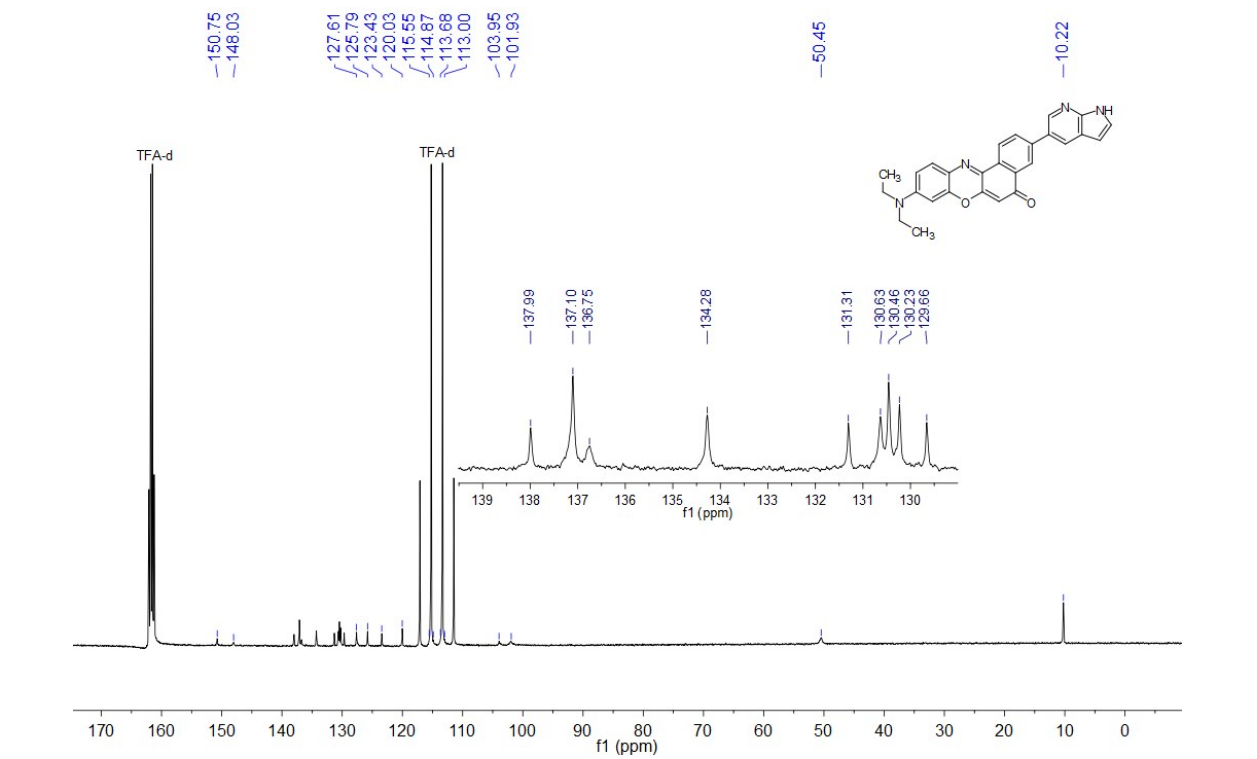

Fig. S55 ^{13}C NMR spectrum of dye **4c**.

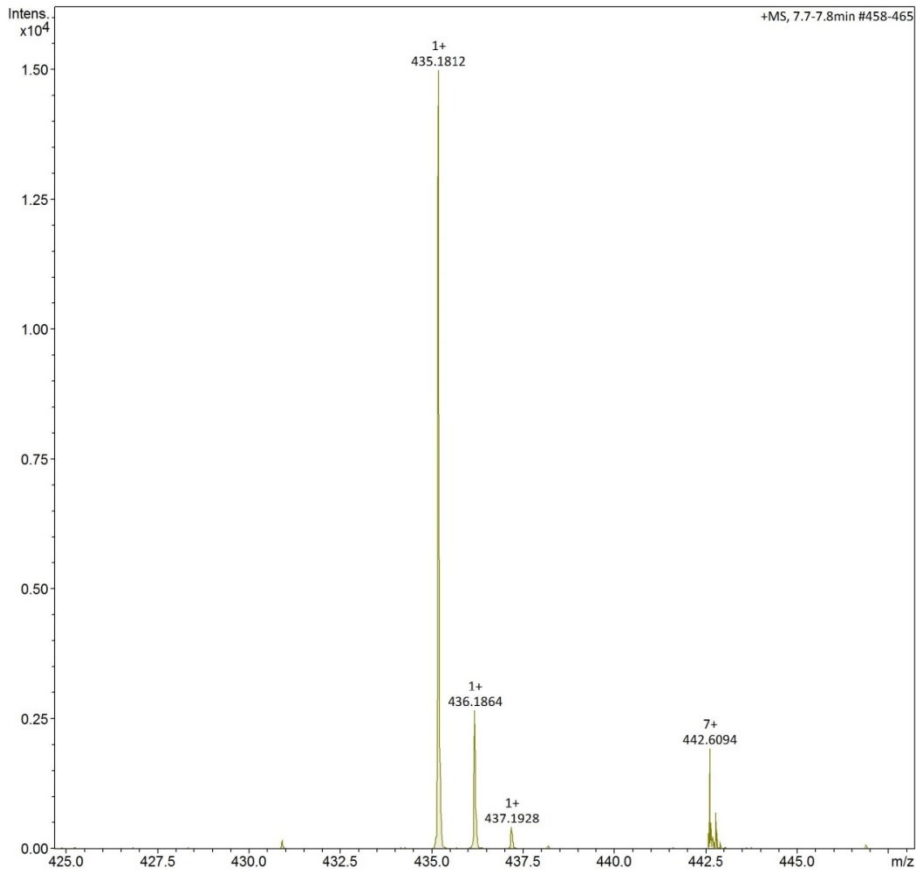

Fig. S56 HRMS (ESI$^+$) spectrum of dye **4c**.