Squaraine-based AIEgens for Reversible Mechanochromism, Sensitive and Selective Hypochlorite Detection and Photostable Far-red Fluorescence Cell Imaging

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Materials and method

Materials: All reagents and solvents were chemical pure (CP) grade or analytical reagent (AR) grade and were used as received unless indicated. Compounds **1**, **3**, **7** and **10** were synthesized following the methods reported by previous literature[1-4].

Measurements: ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV 400 or 600 spectrometer at 298 K in $CDCl_3$. UV-vis-NIR absorption spectra were recorded on a PerkinElmer LAMBDA 750 spectrophotometer. Fluorescent emission spectra were collected on an OmniFluo-960 fluorophotometer at 298 K. Dynamic light scattering (DLS) measurements were conducted using a Zetasizer Nano equipment. High-resolution mass spectrum were measured on an Ion Spec 4.7 Tesla FTMS instrument. The single crystal data of **TPE-SQ1** was collected on Rigaku Saturn diffractometer with CCD area detector. All calculations were performed using the SHELXL97 and crystal structure crystallographic software packages. Absolute fluorescence quantum yield were measured by FLS1000 Photoluminescence Spectrometer. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed using a Netzsch STA449F3 instrument under a nitrogen atmosphere with a heating rate of 10 $^{o}C\ min^{-1}$. Bioimages in 4T1 cells were obtained using an Olympus Fluoview FV 1200 laser scanning confocal microscopy.

Compound 4: A mixture of compound **1** (0.41 g, 1.00 mmol), $Pd_2(dba)_3$ (0.20 g, 0.22 mmol), and sodium tert-butoxide (0.13 g, 1.40 mmol) was added into a Schlenk flask, which was then

evacuated and deoxygenated with nitrogen gas three times. Afterwards, 80 mL of dry toluene, aniline (**2**, 0.11 g, 1.20 mmol) and tri-tert-butylphosphine (0.13 g, 0.66 mmol) were added, and then the resulting mixture was heated to 115 °C for 17 h. After cooling down to room temperature, the mixture was extracted with dichloromethane (DCM) three times. The organic layer was collected, washed with water and dried with anhydrous Na_2SO_4. After concentration using a rotary evaporator, the crude product was purified by column chromatography on the silica gel (DCM/petroleum ether = 1/3, V/V) to obtain a yellow-green solid compound (0.22 g, 50.9%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 7.28 – 7.24 (m, 2H), 7.19 – 7.07 (m, 13H, ArH), 7.07 – 7.01 (m, 4H, ArH), 6.92 (d, *J* = 8.5 Hz, 3H, ArH), 6.82 (d, *J* = 8.4 Hz, 2H, ArH), 5.67 (s, 1H,-NH).

TPE-SQ1: A mixture of compound **4** (0.10 g, 0.24 mmol) and squaric acid (0.01 g, 0.10 mmol) was added into a Schlenk flask, which was then evacuated and deoxygenated with nitrogen gas three times. Afterwards, dry n-butanol (2 mL) and dry toluene (2 mL) were injected into the Schlenk flask, respectively, and the resulting mixture was then heated to 110 °C under reflux for 24 h. After cooling down to room temperature, the product was precipitated from the reaction solution, filtered under reduced pressure and washed with methanol. Finally, the crude product was purified by column chromatography on the silica gel (DCM) to obtain a yellow solid compound (0.08 g, 85.0%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.38 (m, 6H, ArH), 7.20 – 6.98 (m, 38H, ArH), 6.92 (d, *J* = 8.2 Hz, 4H, ArH). Due to the poor solubility of **TPE-SQ1**, its ^{13}C NMR was not tested. HR-ESI-MS: m/z calcd. for $C_{68}H_{48}N_2O_2$: 925.37886 [M+H$^+$], found 925.37739 [M+H$^+$]. Element analysis calcd. for $C_{68}H_{48}N_2O_2$: C (88.28%), H (5.23%), N (3.03%); found: C (87.53%), H (5.53%), N (2.97%).

Compound 5: A mixture of compound **1** (0.60 g, 1.46 mmol), compound **3** (0.39 g, 1.75 mmol), $Pd_2(dba)_3$ (0.30 g, 0.32 mmol) and sodium tert-butoxide (0.20 g, 2.04 mmol) was added into a Schlenk flask, which was then evacuated and deoxygenated with nitrogen gas three times. Afterwards, dry toluene (10 mL) and tri-tert-butylphosphine (0.20 g, 0.97 mmol) were injected into the Schlenk flask, respectively, and the resulting mixture was heated to 115

°C for 24 h. After cooling down to room temperature, the solution was extracted with DCM three times. The organic layer was collected, washed with water and dried with anhydrous Na_2SO_4. After concentration using a rotary evaporator, the crude product was purified by column chromatography on the silica gel (DCM/petroleum ether = 1/4, V/V) to obtain a red solid compound (1.43 g, 74.5%.). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.18 – 7.04 (m, 13H, ArH), 7.13 – 6.97 (m, 4H, ArH), 6.86 – 6.79 (m, 4H, ArH), 6.66 – 6.60 (m, 2H, ArH), 5.44 (s, 1H, -NH), 3.84 – 3.76 (m, 2H, -OCH_2-), 1.75 – 1.65 (m, 1H, -CH), 1.52 – 1.26 (m, 8H, -CH_2-), 0.96 – 0.85 (m, 6H, -CH_3).

TPE-SQ2: A mixture of compound **5** (0.50 g, 0.91 mmol) and squaric acid (0.04 g, 0.36 mmol) was added into a Schlenk flask, which was then evacuated and deoxygenated with nitrogen gas three times. Afterwards, dry n-butanol (7 mL) and dry toluene (7 mL) were injected into the Schlenk flask, and the mixture was heated to 110 ℃ for 24 h under reflux. After cooling down to room temperature, the reaction solvent was removed by using a rotary evaporator. The crude product was purified by column chromatography on the silica gel (DCM/petroleum ether = 2/1, V/V), and then further recrystallized from DCM and petroleum ether to obtain a yellow solid compound (0.13 g, 30.0%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 7.18 – 6.80 (m, 46H, ArH), 3.84 (d, *J* = 12.0 Hz, 4H, -OCH_2-), 1.72 (s, 2H, -CH), 1.53 – 1.29 (m, 16H, -CH_2-), 0.94 (s, 12H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 177.4, 158.5, 143.5, 143.1, 141.6, 140.0, 139.0, 133.1, 131.7, 131.4, 131.3, 127.7, 127.6, 126.8, 126.6, 126.5, 123.7, 114.5, 86.1, 70.5, 49.2, 43.4, 39.4, 30.5, 29.1, 29.0, 27.2, 23.9, 23.1, 20.3, 14.1, 13.8, 11.2. HR-ESI-MS: m/z calcd. for $C_{84}H_{80}N_2O_4$: 1181.61909 [M+H^+], found 1181.61862 [M+H^+]. Element analysis calcd. for $C_{84}H_{80}N_2O_4$: C (85.39%), H (6.82%), N (2.37%); found: C (85.18%), H (7.23%), N (2.29%).

Compound **8**: Compound **5** (1.06 g, 1.81 mmol) and compound **7** (0.31 g, 1.81 mmol) were added to a 50 mL round-bottomed flask, and then ethanol (10 mL) and concentrated hydrochloric acid (1 mL) were added. The mixture was heated under reflux for 3 h. After cooling down to room temperature, the reaction solvent was removed by using a rotary

evaporator. The crude product was purified by column chromatography to obtain a green solid compound (0.77 g, 63.0%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.15 – 7.08 (m, 9H, ArH), 7.07 – 6.98 (m, 10H, ArH), 6.92 – 6.86 (m, *J* = 6.8 Hz, 2H, ArH), 6.85 – 6.79 (m, *J* = 6.8 Hz, 2H, ArH), 4.69 (q, *J* = 7.2 Hz, 2H, -OCH_2-), 3.84 (d, *J* = 5.6 Hz, 2H, -OCH_2-), 1.81 – 1.65 (m, 1H, -CH-), 1.55 – 1.37 (m, 3H, -CH_3), 1.37 – 1.23 (m, 8H, -CH_2-), 1.00 – 0.88 (m, 6H, -CH_3).

Compound **9**: compound **8** (0.5 g, 0.74 mmol) was added to a 50 mL round-bottomed flask, and then acetone (5 mL) and 6M HCl (5 mL) were sequentially added. The reaction mixture was heated under reflux for 4 h. After cooling down to room temperature, the reaction was extracted with DCM three times. The organic layer was collected, washed with water and dried with anhydrous Na_2SO_4. After concentration using a rotary evaporator, the crude product was purified by column chromatography (DCM/methanol = 50/1, V/V) to obtain a gray-green solid compound (0.21 g, 43.5%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.23 – 6.25 (br, m, 23H, ArH), 3.79 (br, s, 2H, , -OCH_2-), 1.67 (s, 1H, -CH), 1.56 – 1.21 (br, m, 8H, -CH_2-), 0.97 – 0.77 (br, s, 6H, , -CH_3).

TPE-SQ3: A mixture of compound **9** (1.0 g, 1.54 mmol) and compound **10** (0.53 g, 1.54 mmol) were added to a Dean-Stark apparatus, which was evacuated and deoxygenated with nitrogen gas three times. Afterwards, n-butanol (7 mL) and toluene (7 mL) were injected into the Dean-Stark apparatus, respectively, and the resulting mixture was heated to 140 ℃ for 24 h. After cooling down to room temperature, the reaction solvent was removed by using a rotary evaporator. The crude product was purified by column chromatography (DCM/methanol = 100/1, V/V) to obtain a purple-black solid compound (0.56 g, 42.8%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ 7.36 – 7.30 (m, 2H, ArH), 7.20 – 7.03 (m, 20H, ArH), 7.01 – 6.97 (m, 3H, ArH), 6.95 – 6.91 (m, 2H, ArH), 5.93 (s, 1H, -C=CH-), 3.95 (s, 2H, -OCH_2-), 3.88 (m, 2H, -OCH_2-), 1.82 – 1.71 (m, 9H, -CH, -CH_2-, and –$C(CH_3)_2$), 1.57 – 1.31 (m, 10H, -CH_2-), 1.04 – 0.90 (m, 9H, -CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 183.5, 174.9, 170.5, 158.8, 143.5, 143.1, 142.4, 142.2, 141.6, 140.1, 139.2, 133.2, 131.4, 131.3, 127.7, 127.6, 126.9, 126.6, 126.5, 123.9, 123.6, 122.2, 114.5, 109.3, 86.1, 70.5, 49.4, 43.4, 39.4, 30.5, 29.1,

29.0, 27.2, 23.9, 23.1, 20.3, 14.1, 13.8, 11.2. HR-ESI-MS: m/z calcd. for $C_{59}H_{60}N_2O_3$: 845.46767 [M+H$^+$], found 845.46736 [M+H$^+$]. Element analysis calcd. for $C_{59}H_{60}N_2O_3$: C (83.85%), H (7.16%), N (3.31%); found: C (83.79%), H (7.10%), N (3.41%).

Preparation of Solutions of Anions

0.5 mmol of inorganic salt (NaF, NaCl, NaBr, $NaClO_4 \cdot H_2O$, Na_2CO_3, $NaClO \cdot 5H_2O$, $NaNO_2$, $NaOAc \cdot 3H_2O$, KSCN) and H_2O_2 were dissolved in distilled water (10 mL) to afford 5×10^{-2} mol/L aqueous solution. The stock solutions were diluted to desired concentrations with distilled water when needed.

Fluorescence Titration of TPE-SQ3 with ClO^-

A solution of **TPE-SQ3** (5×10^{-6} mol/L) was prepared in THF/H_2O solution (5/95, V/V). Then 4.0 mL of the solution of **TPE-SQ3** was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. The NaClO solution was introduced in portions and fluorescent intensity changes were recorded at room temperature each time (Excitation wavelength: 529 nm).

Fluorescence Intensity Changes of TPE-SQ3 with Other Anions：

A solution of **TPE-SQ3** (5×10^{-6} mol/L) was prepared in THF/H_2O solution (5/95, V/V). Then 4.0 mL of the solution of **TPE-SQ3** was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. Different anion solutions were introduced and the changes of the fluorescence intensity were recorded at room temperature each time (Excitation wavelength: 529 nm).

Fabrication of TPE-SQ3 NPs:

The **TPE-SQ3**-loaded PEG-*b*-PCL NPs were prepared through a modified nano-precipitation method. Briefly, PEG-*b*-PCL (3 mg) and **TPE-SQ3** (5 mg) were dispersed and stirred in THF (10 mL) in a vial at room temperature for 30 min. Then, neat water (30 mL) was added dropwise into the solution under vigorous stirring via a syringe pump at a flow rate of 0.05 mL/min, and the dispersion was left stirring for another 12 h. THF was then removed by

dialysis (MWCO = 6000 Da) against neat water for 24 h to obtain NPs. We made NPs to facilitate phagocytosis into the 4T1 cells for imaging.

Cell culture:

4T1 (mouse breast cancer cells) cells were obtained from the American Type Culture Collection (Manassas, VA, USA). All cells were dispersed in FBS containing 10% DMSO and stored in -80 ^{o}C refrigerator.

Cell fluorescence imaging:

4T1 cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM) containing 10% (V/V) FBS at 37 ^{o}C in CO_2−air (5%/95%). Afterward, Cells were seeded on sterilized coverslips in 6-well plates at a density of 2.0×10^6 cells per well in 2.0 mL of complete DMEM for 24 h, and then incubated with the **TPE-SQ3**-loaded PEG-*b*-PCL NPs at a final Dox concentration of 10.0 mg/L for another 4 h. Subsequently, CLSM (Olympus FV1000, Tokyo, Japan) images were taken. Excitation wavelength: 397 nm, emission collected: 630–660 nm.

Table S1. Crystal data of **TPE-SQ1**.

Identification code	mo_191011c_0m	
Empirical formula	C68 H48 N2 O2	
Formula weight	925.08	
Temperature	220(1) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 9.2148(13) Å	a= 78.093(2)°.
	b = 9.4425(13) Å	b= 87.741(2)°.
	c = 14.798(2) Å	g = 86.000(2)°.
Volume	1256.4(3) Å^3	
Z	1	
Density (calculated)	1.223 Mg/m^3	
Absorption coefficient	0.073 mm^{-1}	
F(000)	486	
Crystal size	0.12 x 0.1 x 0.1 mm^3	
Theta range for data collection	1.407 to 25.494°.	

Index ranges	-11<=h<=11, -11<=k<=10, -17<=l<=17
Reflections collected	9170
Independent reflections	4628 [R(int) = 0.0225]
Completeness to theta = 25.242°	98.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7463 and 0.6383
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4628 / 0 / 325
Goodness-of-fit on F^2	1.053
Final R indices [I>2sigma(I)]	R1 = 0.0571, wR2 = 0.1804
R indices (all data)	R1 = 0.0692, wR2 = 0.2027
Extinction coefficient	n/a
Largest diff. peak and hole	0.811 and -0.228 e.Å^{-3}

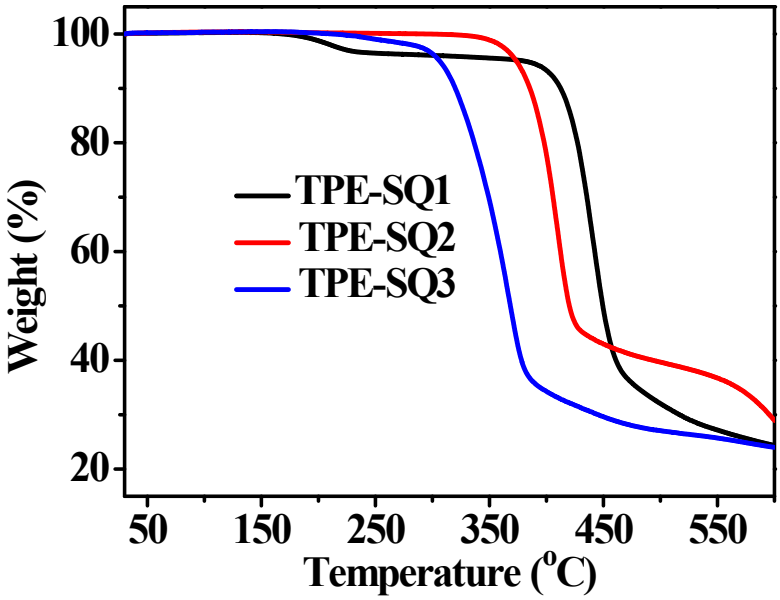

Fig. S1 Thermogravimetric analysis (TGA) of **TPE-SQ1-3** with a heating rate of 10 °C min^{-1} under N_2 atmosphere.

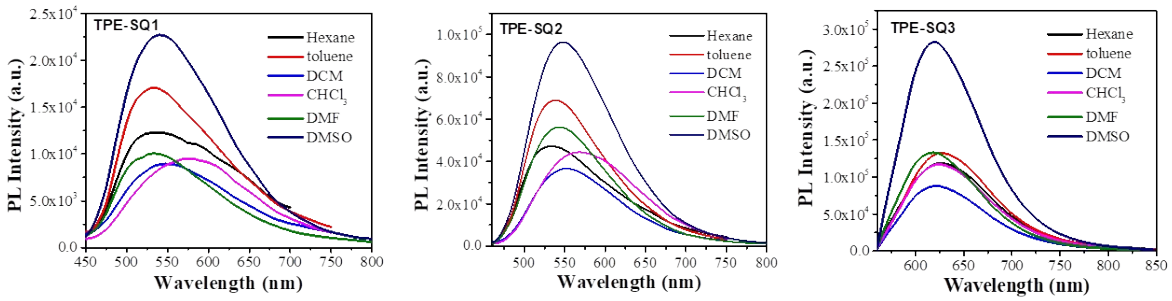

Fig. S2 Fluorescent spectra of **TPE-SQ1-3** in different solvents.

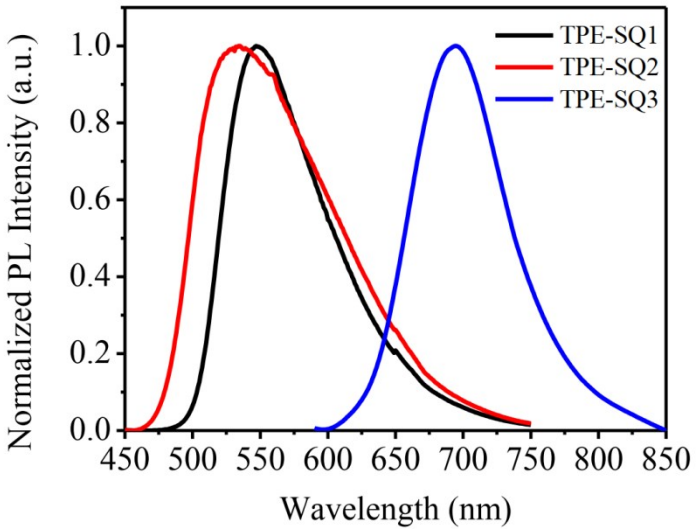

Fig. S3 Solid state emission spectra of as-prepared powders of **TPE-SQ1-3**.

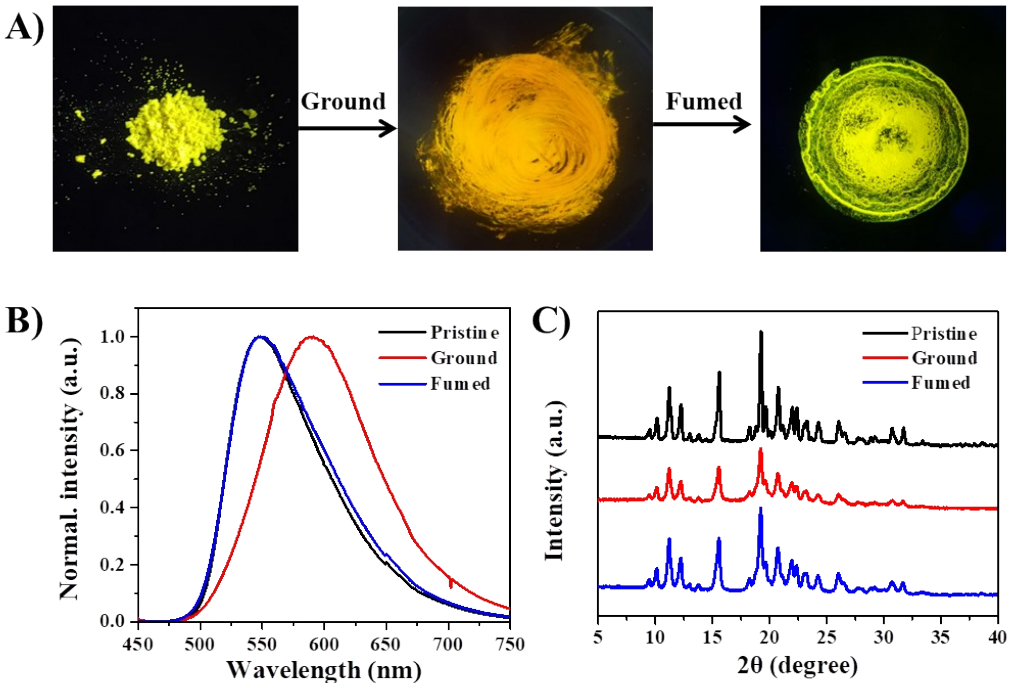

Fig. S4 (A) Mechanochromism photographs of pristine, ground and fumed **TPE-SQ1** powders under 365 nm UV lamp; Emission spectra (B) XRD patterns (C) of pristine, ground and fumed **TP1-SQ1** powders.

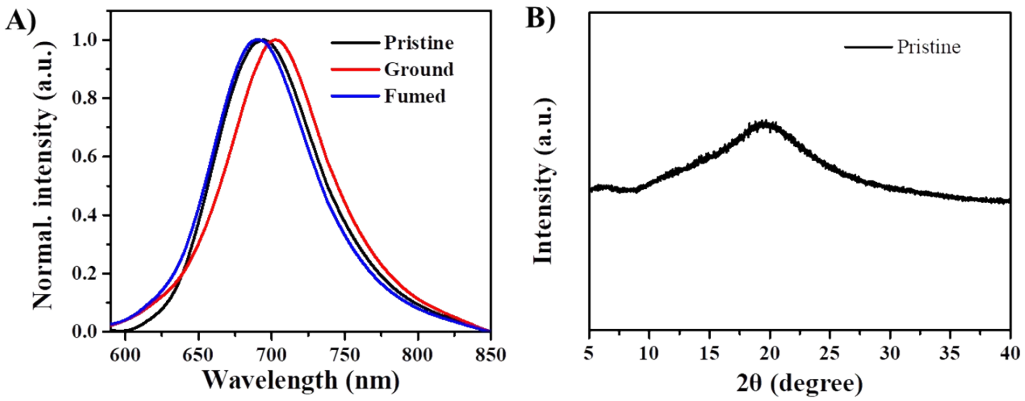

Fig. S5 Emission spectra (A) XRD patterns (B) of pristine, ground and fumed **TPE-SQ3** powders.

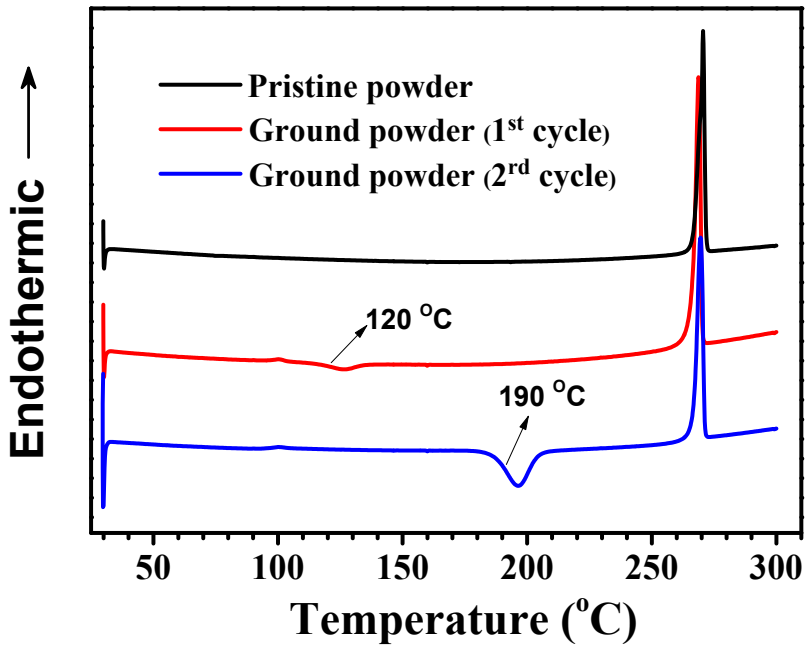

Fig. S6 DSC thermograms of pristine**,** ground powders **TPE-SQ2** recorded under a N_2 atmosphere at a heating rate of 10 ^{o}C min^{-1}.

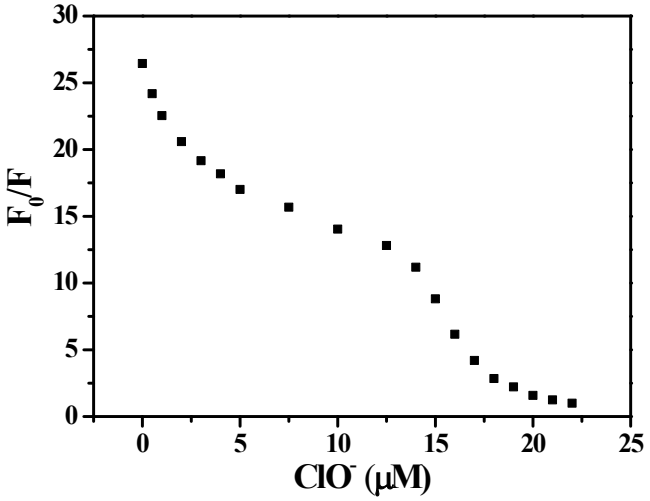

Fig. S7 Plot of fluorescent intensity change of **TPE-SQ3** at 650 nm versus different concentrations of ClO^-.

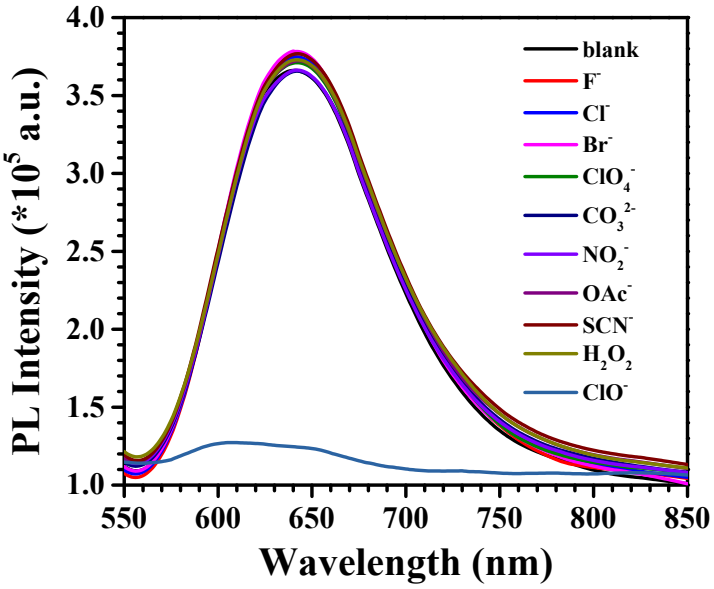

Fig. S8 Fluorescent spectra of **TPE-SQ3** (5 μM) in a 95% H_2O/THF solution with ClO^- and various competitive analytes (4 equiv.)

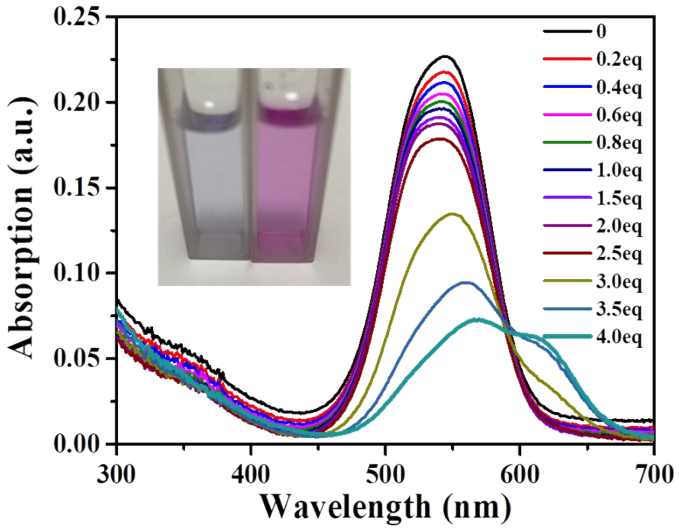

Fig. S9 Absorption titration spectra of **TPE-SQ3** (5 μM) in a 95% H_2O/THF solution with different concentrations of ClO^-. Inset: Photographs before (right) and after (left) adding ClO^-.

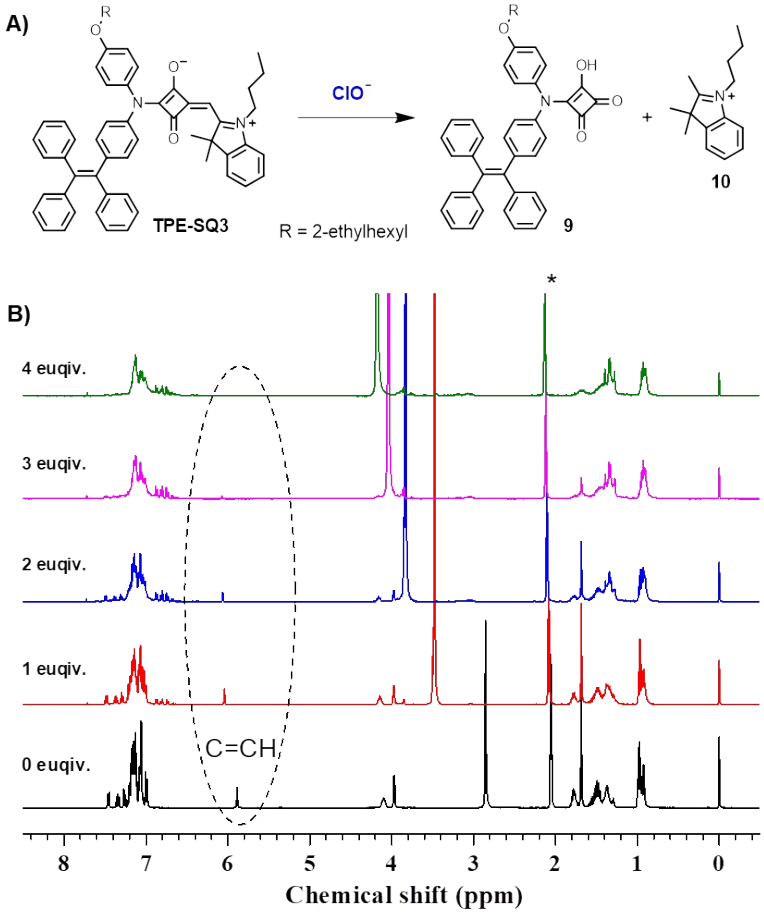

Fig. S10 The packing ^1H NMR titration spectra of **TPE-SQ3** (0.01 mM) before and after different concentrations of ClO^- conducted in acetone-d_6 (*).

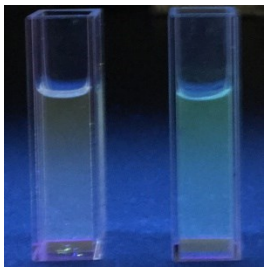

Fig. S11 Photographs of compound **9** in THF solution (left) and in THF/water mixture with a water volume fraction of 90% (right).

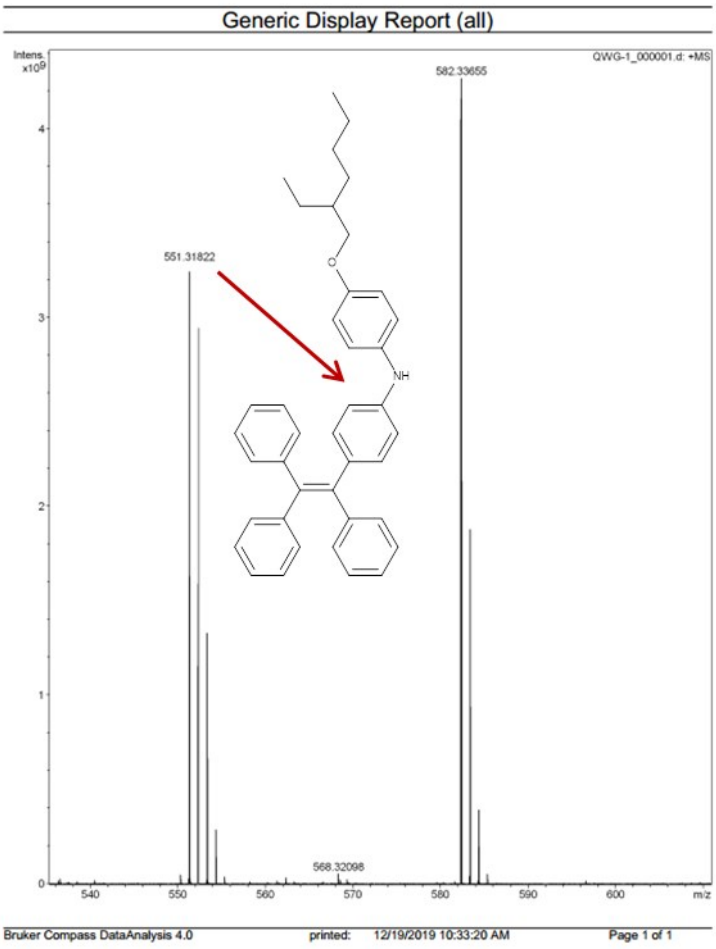

Fig. S12 ESI-MS spectrum of reaction solution of **TPE-SQ3** with ClO^-.

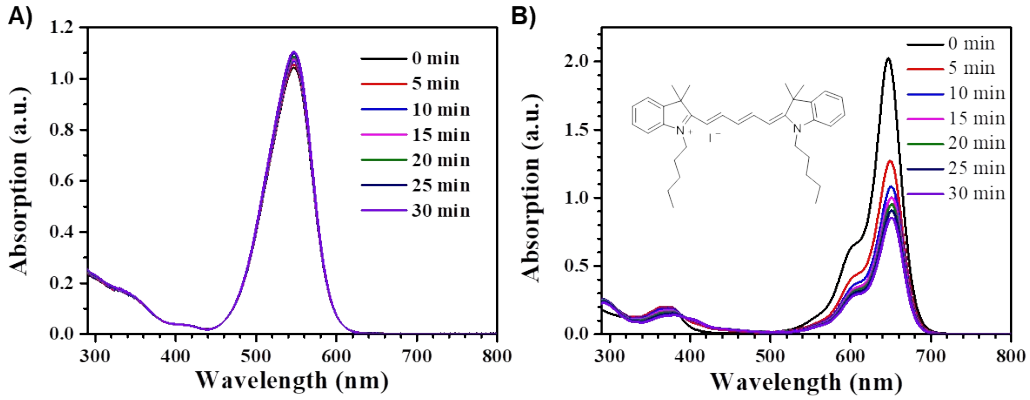

Fig. S13 The absorption spectra of **TPE-SQ3** (A) and **DPPI** (B) under light irradiation (120 mW/cm^2, 400-1000 nm) for different time in THF. Insert in **Fig. S13B** is the structure of DPPI.

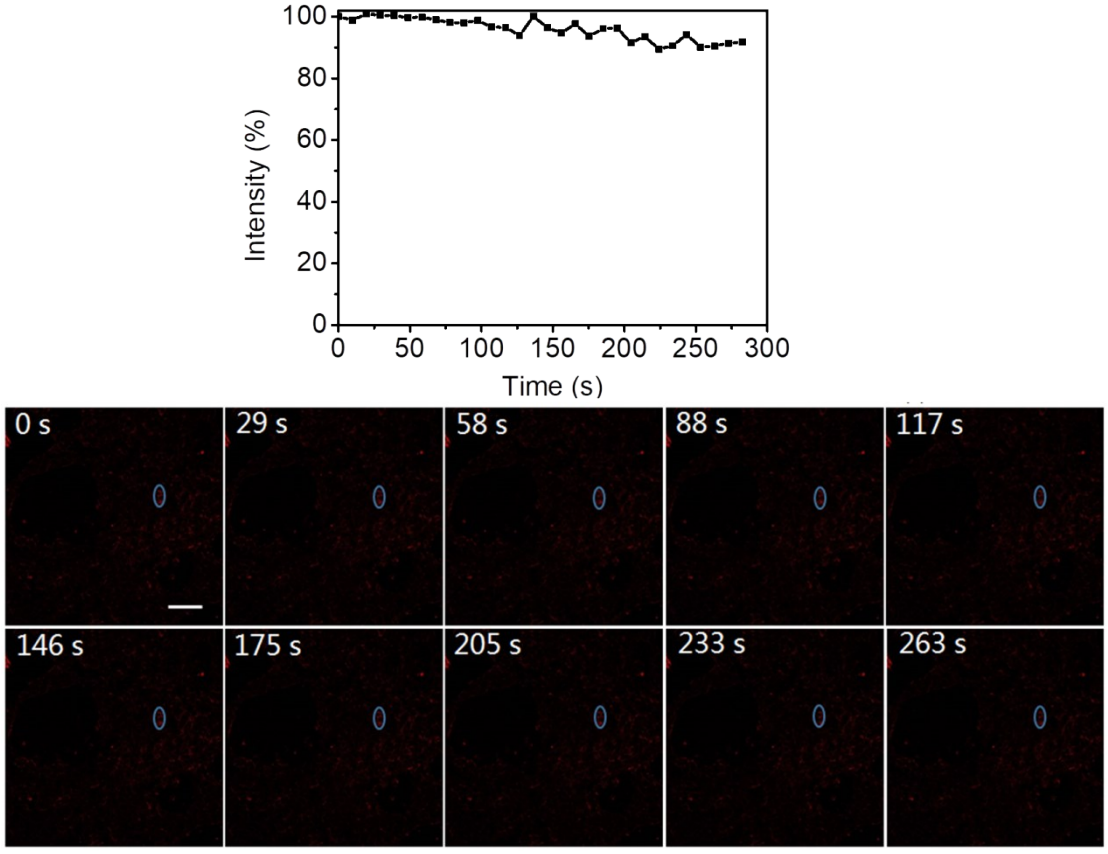

Fig. S14 (A) Dynamic fluorescent intensity changes of 4T1 cancer cells stained with **TPE-SQ3** with increasing time upon continuous laser excitation at 397 nm of CLSM scans at 10% laser power; (B) CLSM images of 4T1 cancels cells stained with **TPE-SQ3** taken at various scanning times.

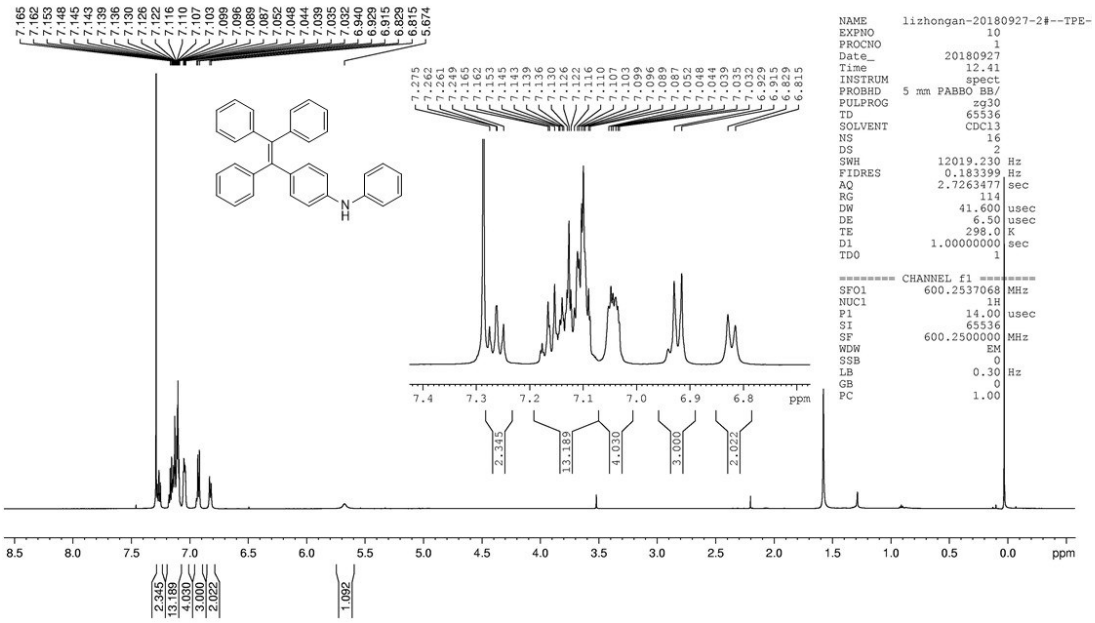

Fig. S15 ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

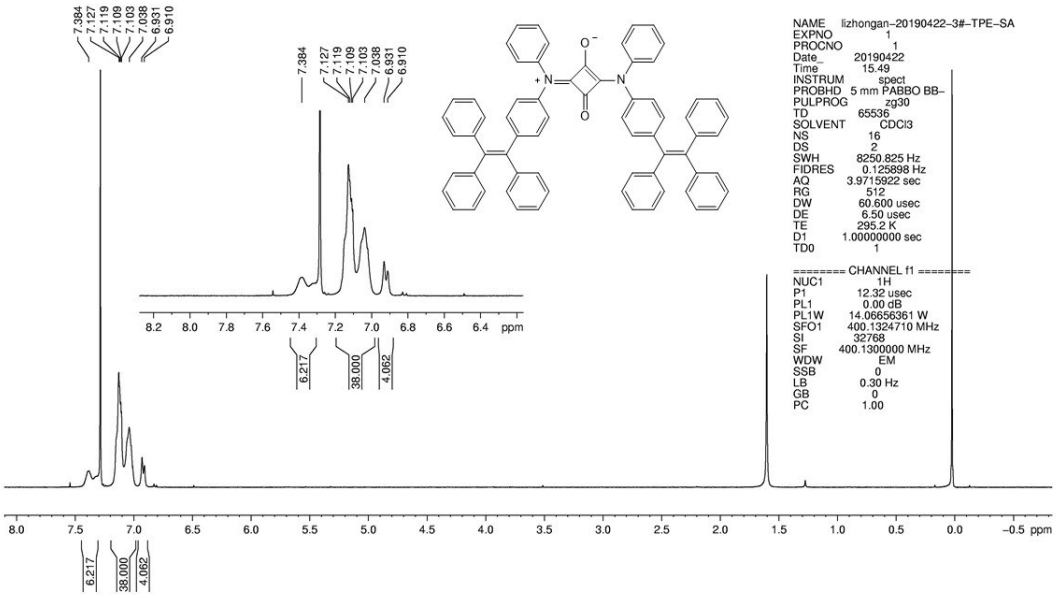

Fig. S16 ^{1}H NMR spectrum of compound **TPE-SQ1** in $CDCl_3$.

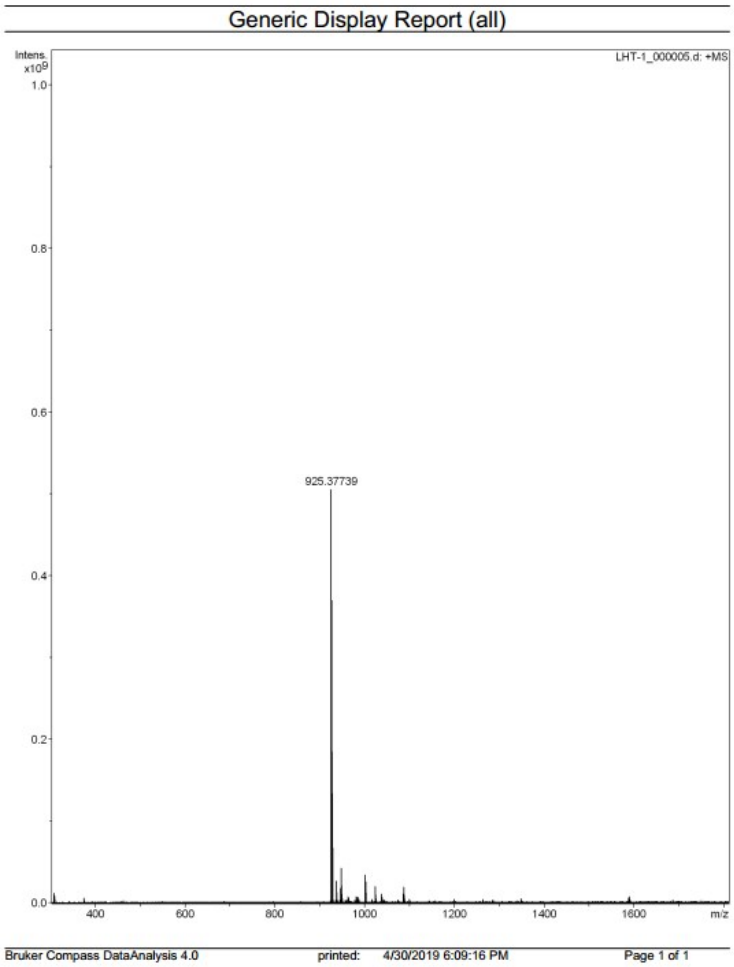

Fig. S17 HR-ESI-MS spectrum for **TPE-SQ1**.

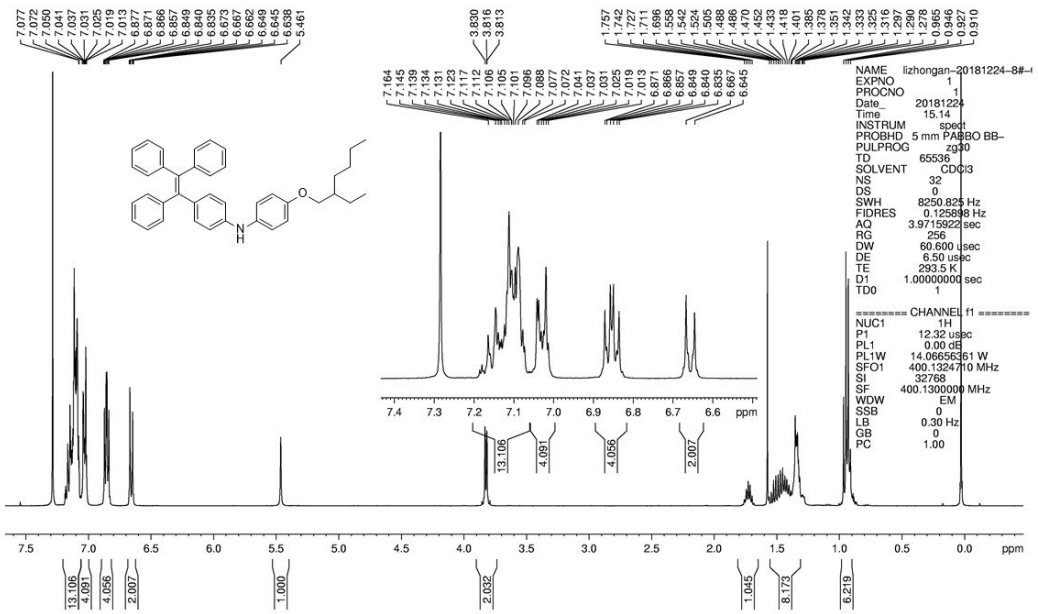

Fig. S18 ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

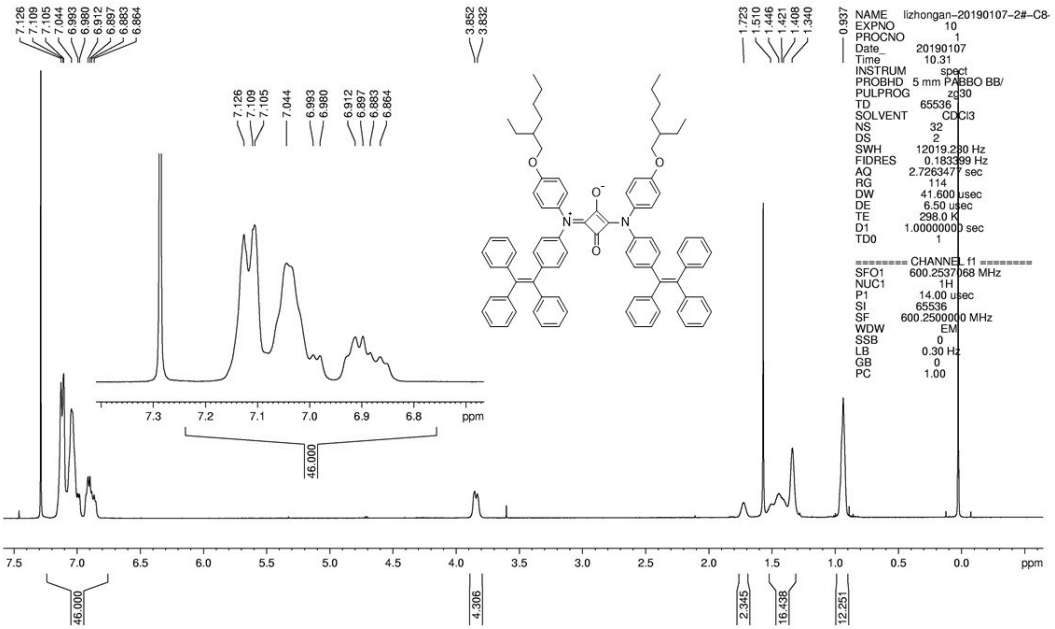

Fig. S19 ^{1}H NMR spectrum of compound **TPE-SQ2** in $CDCl_3$.

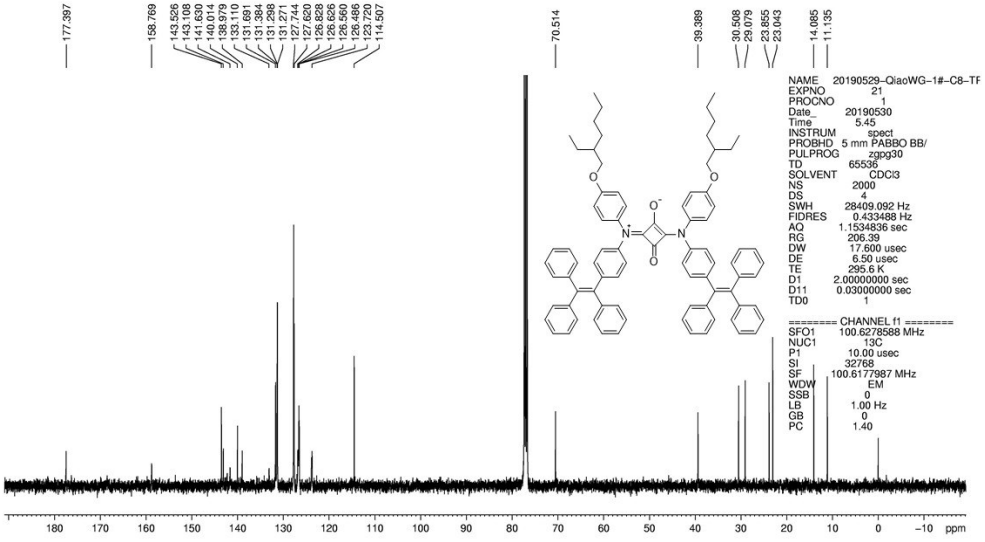

Fig. S20 ^{13}C NMR spectrum of compound **TPE-SQ2** in $CDCl_3$.

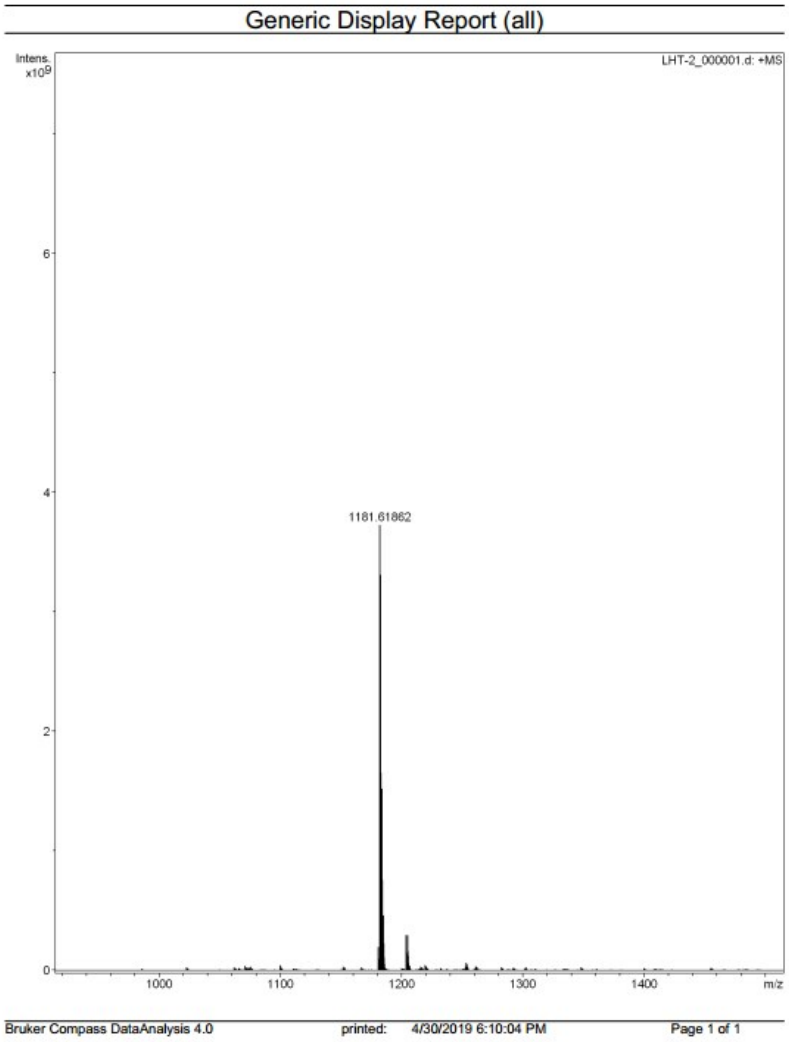

Fig. S21 HR-ESI-MS spectrum for **TPE-SQ2**.

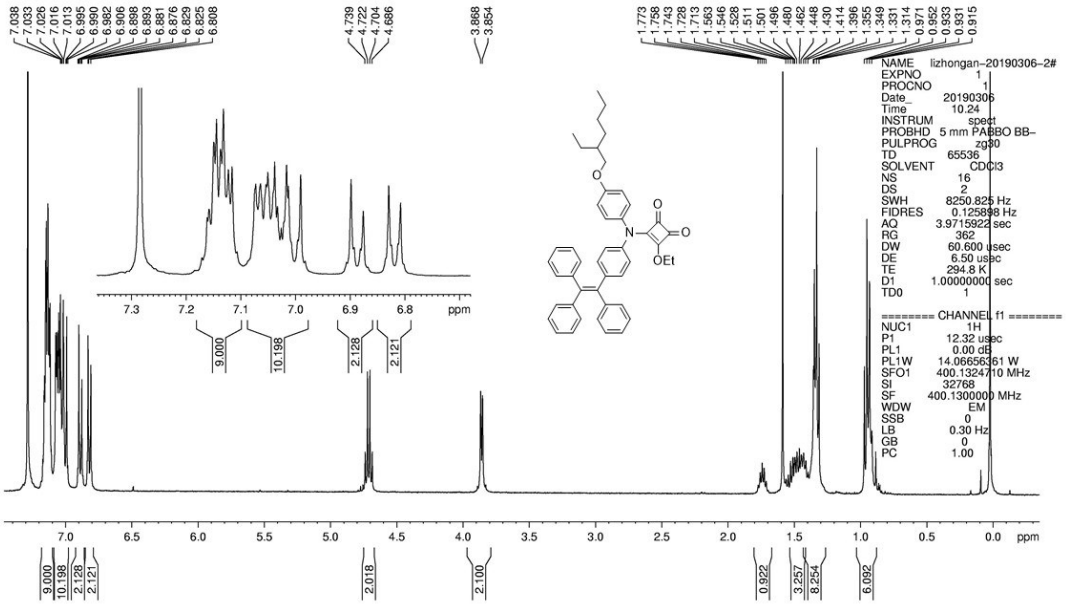

Fig. S22 ^{1}H NMR spectrum of compound **8** in $CDCl_3$.

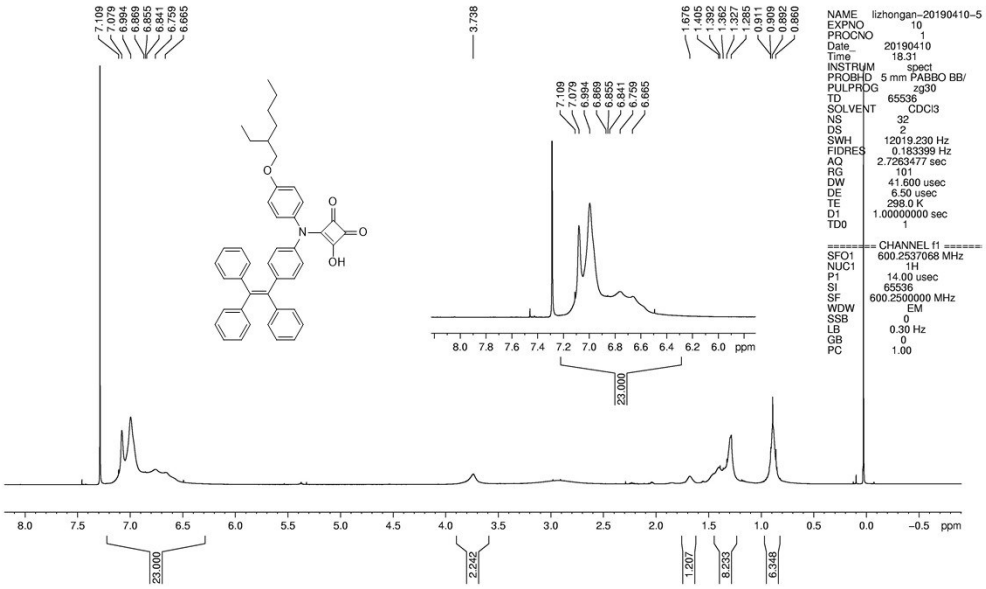

Fig. S23 ^{1}H NMR spectrum of compound **9** in $CDCl_3$.

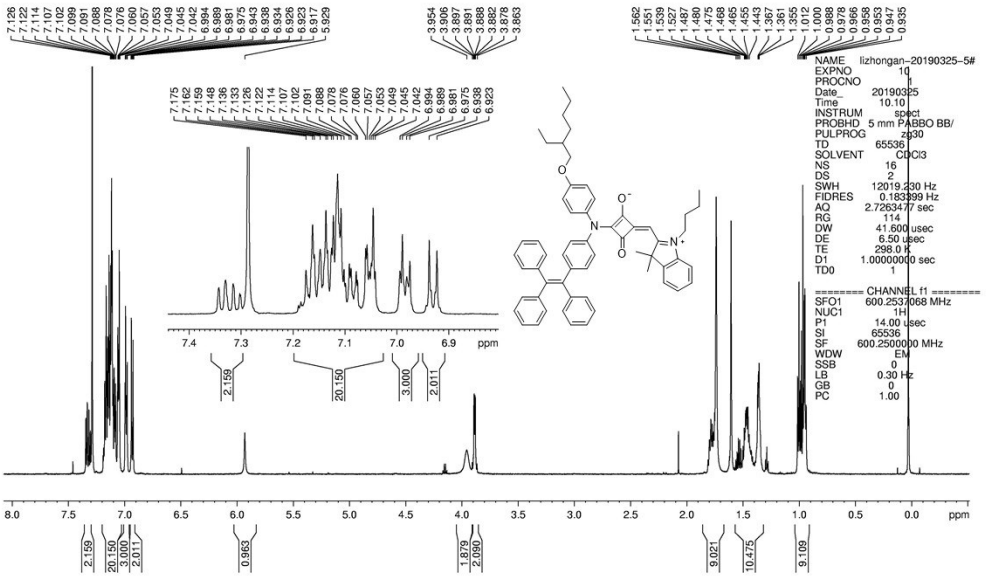

Fig. S24 ^{1}H NMR spectrum of compound **TPE-SQ3** in $CDCl_3$.

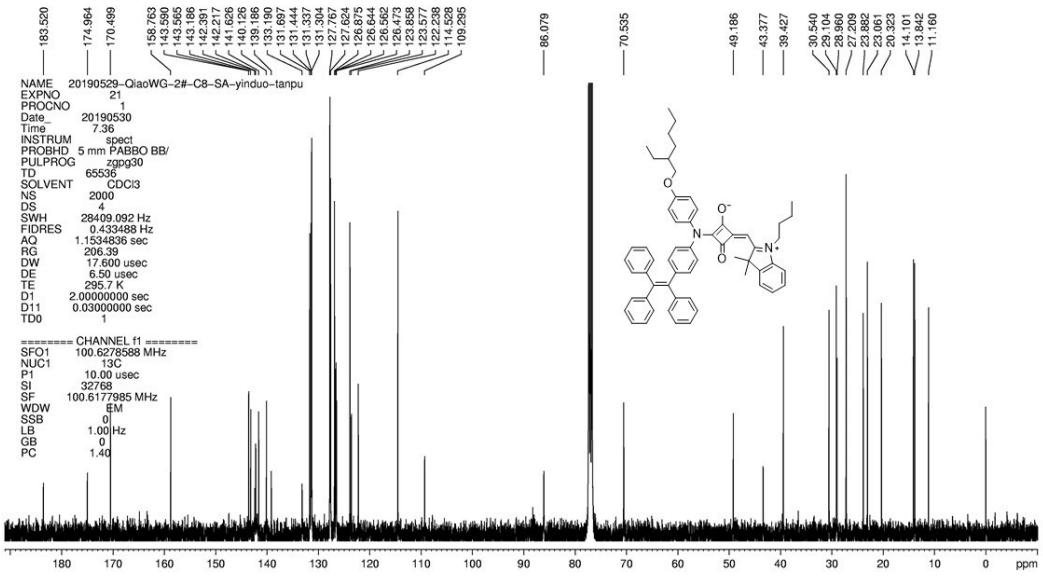

Fig. S25 ^{13}C NMR spectrum of compound **TPE-SQ3** in $CDCl_3$.

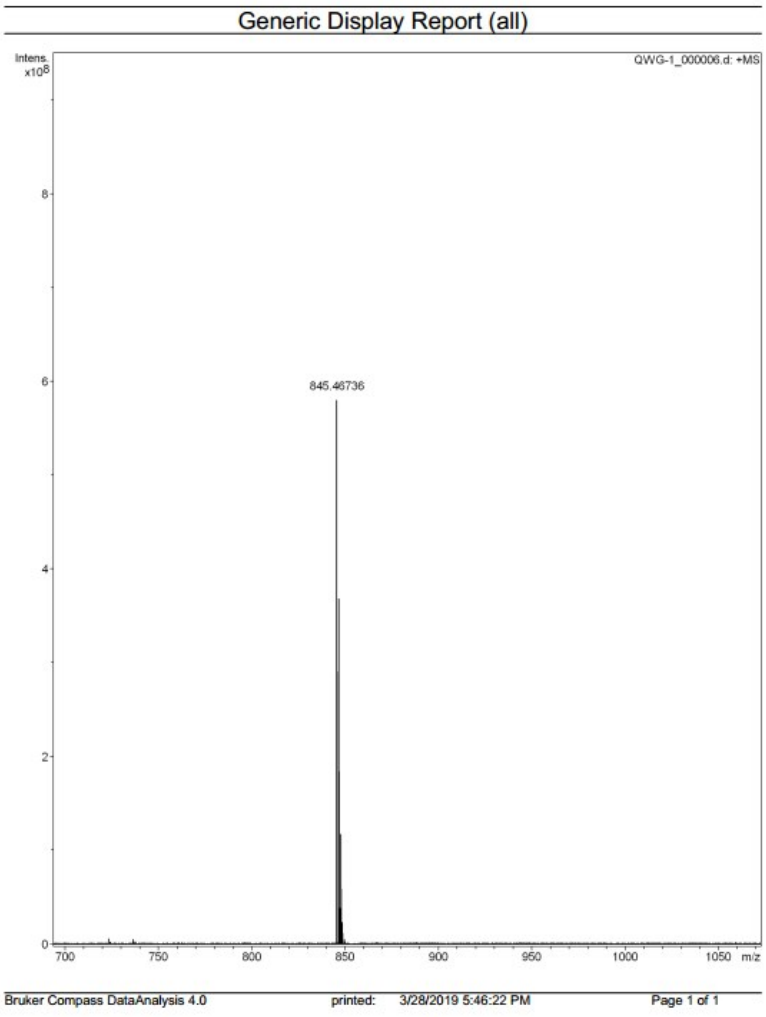

Fig. S26 HR-ESI-MS spectrum for **TPE-SQ3**.

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