Supporting Information

Cationic sulfonium functionalization renders Znsalens high fluorescence, good water solubility and tunable cell-permeability

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Content

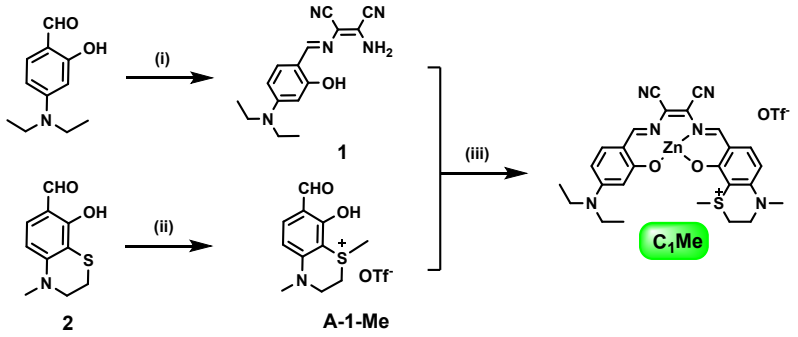
1 General experimental information

All solvents and chemicals were purchased from Alfa Aesar and J&K and used without further purification, unless specifically mentioned. Cellular imaging trackers were purchased from Invitrogen (Life Technologies). The ^{1}H NMR spectroscopic measurements were carried out using a Varian-300 NMR or a Bruker-400 NMR spectrometer, at 300 MHz or 400 MHz, respectively. Tetramethylsilane (TMS) is used as the internal reference. The ^{19}F NMR spectroscopic measurements were carried out using a Varian-300 NMR and CF_3COOH was selected as the external reference. Electrospray ionization (ESI) mass spectra were performed on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR, Bruker, USA). FT-IR spectra were taken on a Nicolet iN10 MX Fourier Transform Infrared Spectrometer. The steady-state absorption spectra were attained on an Agilent 8453 UV-*vis* spectrophotometer in 1cm path length quartz cells. Single-photon luminescence spectra were recorded using fluorescence lifetime and steady state spectrophotometer (Edinburgh Instrument FLS920). Quantum yields of one photon emission of all the synthesized compounds were measured relative to the fluorescence of Rhodamine B in ethanol. The two-photon absorption cross section of the complexes was calculated relative to Rhodamine B as standard. The two photon fluorescence data was acquired using a Tsunami femtosecond Ti: Sapphire laser (pulse width ≤100fs, 80 MHz repetition rate, tuning range 740-880 nm, Spectra Physics Inc., USA). The conductivity of sulfonium ZnSalen complexes in aqueous medium is measured by a conductivity meter (DDS-11A, Shang Hai). Dynamic light scattering experiment was performed on a ALV/DLS/SLS-5022F Laser Light Scattering Spectrometer. The sample for DLS experiments was centrifuged at 5000 rpm for 30 minutes. Confocal fluorescent images of living cells were performed using Nikon A1R-si Laser Scanning Confocal Microscope (Japan), equipped with lasers of 405/488/543/638 nm. Several lasers and channels were used to obtain images.

2 Synthesis and characterization

All the reactions were carried out under nitrogen. To monitor the reactions, thin-layer chromatography was performed and visualized by 254 nm UV-illumination.

2.1 Synthesis of C_1Me



Scheme S2-1 Synthetic route of **C_1Me**. (i) 2,3-diaminomaleonitrile, H_2SO_4, EtOH, 85 ºC, 4 h; (ii) methyl trifluoromethanesulfonate, anhydrous CH_2Cl_2, -78 to 0 ºC, dark, 4 h; (iii) $Zn(OAc)_2 \cdot 2H_2O$, EtOH, 85 ºC , 24h.

Compound **1**

A reaction mixture of 4-(diethylamino)-2-hydroxybenzaldehyde (500 mg, 2.59 mmol), 2,3-diaminomaleonitrile (280 mg, 2.59 mmol) in ethanol (50 mL) was added one drop of concentrated sulfuric acid and refluxed under nitrogen for 4 h, during which some red precipitate formed. After cooling to the room temperature, the mixture was filtered and the solid was washed in turn by ethanol, ethyl acetate and petroleum ether (10 mL each). After dried under reduced pressure, compound **1** was obtained as red brown powder (586 mg, 79 %).

^{1}H NMR (400 MHz, d^6-DMSO): δ 10.54 (s, 1H), 8.31 (s, 2H), 7.58 (d, 1H, J = 9.2 Hz), 7.31 (s, 1H), 6.27 (d, 1H, J = 9.2 Hz), 6.07 (s, 1H), 3.34 (s, 6H), 1.10 (s, 6H).

compound **2**

Synthesis of compound 2 is referenced to our previous studies.

compound **A-1-Me**

Compound **2** (100 mg, 0.48 mmol) was dissolved in 5 mL anhydrous CH_2Cl_2 in dark and methyl trifluoromethanesulfonate (274 mg, 1.67 mmol) was added at -78 ºC. The reaction was warmed slowly to 0 ºC and stirred for 4 hours, during which some white solid precipitated. Ethyl acetate (0.5 mL) and ethyl ether (20 mL) was then added to precipitate the product. The mixture was filtered and the solid was washed in turn by ethyl acetate and petroleum ether (2 mL each). After dried under reduced pressure, compound **A-1-Me** was obtained as white powder (170 mg, 95 %).

compound **C_1Me**

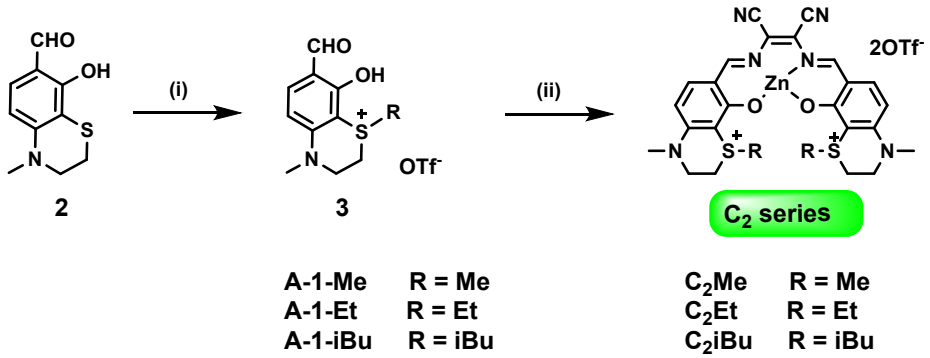
A reaction mixture of compound **1** (30mg, 0.11 mmol), compound **A-1-Me** (40 mg, 0.11 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (24.4 mg, 0.11 mmol) in ethanol was refluxed under nitrogen for 24 h, during which some purple precipitate formed. After cooling to the room temperature, the mixture was filtered and the solid was washed in turn by ethanol, ethyl acetate and petroleum ether. After dried under reduced pressure, compound **C_1Me** was obtained as purple black solid (63 mg, 84 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.33 (s, 1H), 8.12 (s, 1H), 7.60 (d, J = 9.3 Hz, 1H), 7.21 (d, J = 9.4 Hz, 1H), 6.46 (d, J = 9.3 Hz, 1H), 6.29 (dd, J = 9.2, 2.0 Hz, 1H), 5.78 (d, J = 2.1 Hz, 1H), 3.85 (m, 2H), 3.62 (m, 2H), 3.44 (q, J = 6.8 Hz, 4H), 3.23 (d, J = 17.6 Hz, 3H), 3.01 (s, 3H), 1.16 (t, J = 7.0 Hz, 6H).

HR MS (ESI$^+$, DMSO, FT-ICR): *m/z* calcd. for $C_{26}H_{27}N_6O_2SZn$ ([M-$CF_3SO_3^-$]$^+$) 551.12022, found 551.12071.

FT-IR (KBr pellete, cm^{-1}): 2214 (C≡N), 1608 (C=N).

2.2 Synthesis of C_2 series



2 3

A-1-Me	R = Me	C_2Me	R = Me
A-1-Et	R = Et	C_2Et	R = Et
A-1-iBu	R = iBu	C_2iBu	R = iBu

Scheme S2-2 Synthetic route of $\mathbf{C_2}$ series. (i) trifluoromethanesulfonate, anhydrous CH_2Cl_2, -78 to 0 ºC, dark, 16-24 h; (ii) 2,3-diaminomaleonitrile, $Zn(OAc)_2 \cdot 2H_2O$, CH_3CN, 85 ºC , 24h.

Compound **A-1-Et**

Compound **2** (150 mg, 0.72 mmol) was dissolved in 5 mL anhydrous CH_2Cl_2 in dark and ethyl trifluoromethanesulfonate (0.47 mL, 3.6 mmol) was added at -78 ºC. The reaction was warmed slowly to 0 ºC and stirred for 16 hours, during which some white solid precipitated. Ethyl acetate (0.5 mL) and ethyl ether (20mL) was then added to precipitate the product. The mixture was filtered and the solid product was recrystallized by acetone and ethyl ether. After dried under reduced pressure, compound **A-1-Et** was obtained as silver powder (125 mg, 45 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.08 (s, 1H), 9.60 (s, 1H), 7.58 (d, J = 9.1 Hz, 1H), 6.54 (d, J = 9.1 Hz, 1H), 4.25 (dt, J = 14.7, 2.9 Hz, 1H), 4.10 (dt, J = 15.9, 8.0 Hz, 2H), 3.72 (dq, J = 14.9, 7.5 Hz, 1H), 3.43 (m, 2H), 3.31 (s, 3H), 1.54 (t, J = 7.5 Hz, 3H).

Compound **A-1-iBu**

Compound **2** (150 mg, 0.72 mmol) was dissolved in 5 mL anhydrous CH_2Cl_2 in dark and isobutyl trifluoromethanesulfonate (0.5mL) was added at -78 ºC. The reaction was warmed slowly to 0 ºC and stirred for 24 hours, during which some white solid precipitated. Ethyl acetate (0.5 mL) and ethyl ether (20mL) was then added to precipitate the product. The mixture was filtered and the solid product was recrystallized by acetone and ethyl ether. After dried under reduced pressure, compound **A-1-iBu** was obtained as white powder (55 mg, 19 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.13 (s, 1H), 9.61 (s, 1H), 7.58 (d, J = 9.1 Hz, 1H), 6.54 (d, J = 9.1 Hz, 1H), 4.28 (d, J = 14.7 Hz, 1H), 4.14 (m, 2H), 3.57 (dd, J = 12.6, 7.9 Hz, 1H), 3.37 (ddd, J

= 15.3, 10.9, 5.9 Hz, 1H), 3.32 (s, 3H), 3.16 (dd, *J* = 12.6, 6.9 Hz, 1H), 2.24 (m, 1H), 1.27 (d, *J* = 6.6 Hz, 3H), 1.18 (d, *J* = 6.7 Hz, 3H).

Compound **C_2Me**

A reaction mixture of compound **A-1-Me** (50 mg, 0.13 mmol), 2,3-diaminomaleonitrile (7.2 mg, 0.067 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (16.2 mg, 0.074 mmol) in 5 mL ethanol was refluxed under nitrogen for 24 h. The system turned dark red and brown precipitate formed. After cooling to the room temperature and evaporating the solvent, the mixture was filtered and the solid was washed in turn by ethanol, ethyl acetate and petroleum ether. After dried under reduced pressure, compound **C_2Me** was obtained as purple black solid (48 mg, 81 %).

^{1}H NMR (D_2O, 400 MHz) δ 8.13 (s, 2H), 7.32 (d, 2H, *J* = 9.3Hz), 6.50 (d, 2H, *J* = 9.3Hz), 3.97 (m, 4H), 3.55 (m, 4H), 3.28 (s, 6H), 3.16 (s, 3H), 3.04 (s, 3H).

HR MS (ESI$^+$, DMSO, FT-ICR): *m/z* calcd. for $C_{27}H_{26}F_3N_6O_5S_3Zn$ ([M-$CF_3SO_3^-$]$^+$) 731.03649, found 731.03718.

FT-IR (KBr pellete, cm^{-1}): 2218 (C≡N), 1603 (C=N).

Compound **C_2Et**

Synthesis of **C_2Et** is comparable to **C_2Me**. The product was obtained as jade green powder (57%).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.35 (d, *J* = 8.1 Hz, 2H), 7.64 (dd, *J* = 9.3, 3.2 Hz, 2H), 6.51 (d, *J* = 9.4 Hz, 2H), 3.93 (d, *J* = 14.9 Hz, 2H), 3.83 (d, *J* = 6.9 Hz, 4H), 3.58 (t, *J* = 11.7 Hz, 2H), 3.43 (m, 4H), 3.24 (s, 6H), 1.56 (t, *J* = 7.4 Hz, 3H), 1.43 (t, *J* = 7.4 Hz, 3H).

HR MS (ESI$^+$, DMSO, FT-ICR): *m/z* calcd. for $C_{29}H_{30}F_3N_6O_5S_3Zn$ ([M-$CF_3SO_3^-$]$^+$) 759.06779, found 759.06877.

FT-IR (KBr pellete, cm^{-1}): 2216 (C≡N), 1601 (C=N).

Compound **C_2iBu**

A reaction mixture of compound **A-1-iBu** (40 mg, 0.096 mmol), 2,3-diaminomaleonitrile (5.2 mg, 0.048 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (11.6 mg, 0.053 mmol) in 5 mL ethanol was refluxed under nitrogen for 24 h. The system turned dark red. After cooling to the room temperature and

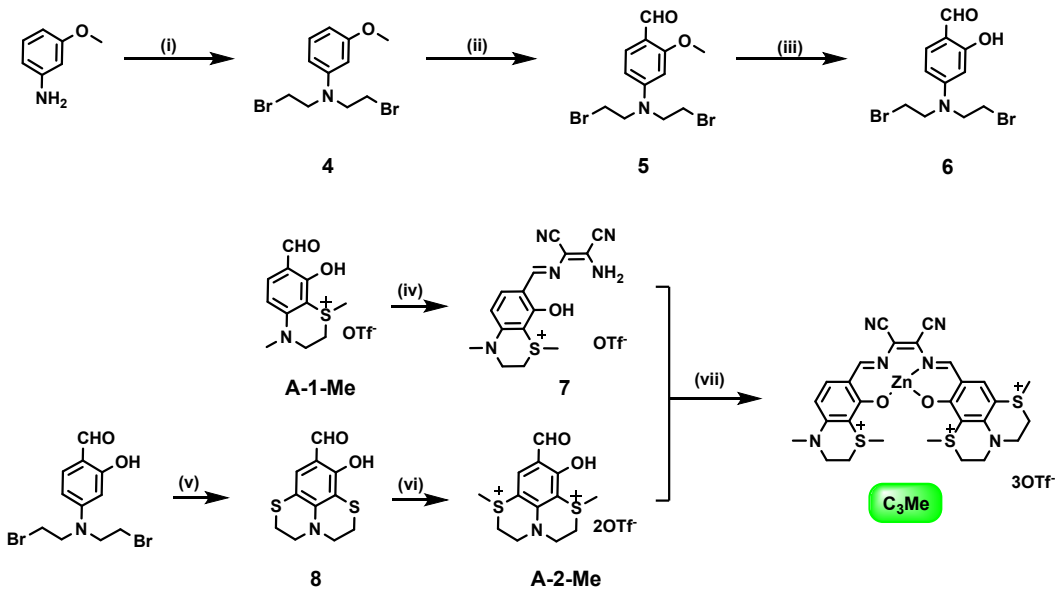
evaporating the solvent, 8 mL ethyl ether was added and red brown precipitate formed. The mixture was filtered and recrystallized by ethanol and ethyl ether to give granular brown solid. After dried under reduced pressure, compound **C_2iBu** was obtained as red brown powder (25 mg, 54 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.37 (s, 1H), 8.31 (s, 1H), 7.62 (dd, *J* = 9.4, 7.7 Hz, 2H), 6.51 (dd, *J* = 9.4, 4.4 Hz, 2H), 3.86 (m, 6H), 3.56 (m, 2H), 3.19 (m, 10H), 2.22 (m, 2H), 1.15 (m, 12H).

HR MS (ESI$^+$, DMSO, FT-ICR): *m/z* calcd. for $C_{33}H_{38}F_3N_6O_5S_3Zn$ ([M-$CF_3SO_3^-$]$^+$) 815.13039, found 815.13139.

FT-IR (KBr pellete, cm^{-1}): 2212 (C≡N), 1605 (C=N).

2.3 Synthesis of C_3Me



Scheme S2-3 Synthetic route of **C_3Me**. (i) 1,2-dibromoethane, $KHCO_3$, CH_3CN, 95 ºC, 16 h; (ii-1) $POCl_3$, DMF, 0°C to r.t., 30min; (ii-2) icy H_2O, 90min; (iii) BBr_3, DCM, -78°C to r.t., 16h; (iv) 2,3-diaminomaleonitrile, H_2SO_4, EtOH, 85 ºC, 4 h; (v) sodium methanesulfonothioate (NaMTS), $KHCO_3$, KI, CH_3CN:H_2O = 1:3, 95 ºC , 12 h; (vi) methyl trifluoromethanesulfonate, anhydrous CH_2Cl_2, -78 to 0 ºC, dark, 16 h; (viii) $Zn(OAc)_2 \cdot 2H_2O$, anhydrous CH_3CN, 85 ºC , 24h.

Compound **4**

A reaction mixture of 3-methoxyaniline (3.0 g, 24 mmol), 1, 2-dibromoethane (22.6 g, 120 mmol)

and $KHCO_3$ (6.7 g, 48 mmol) in acetonitrile (100 mL) was refluxed under nitrogen for 16 h. After filtration and evaporation, the remaining liquid residue was purified by column chromatography (elute DCM:PE = 1:12) to give compound **4** as yellow oil (4.8 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, *J* = 8.2 Hz, 1H), 6.32 (ddd, *J* = 20.1, 8.2, 2.3 Hz, 2H), 6.22 (t, *J* = 2.2 Hz, 1H), 3.78 (s, 3H), 3.74 (t, *J* = 7.6 Hz, 4H), 3.44 (t, *J* = 7.5 Hz, 4H).

Compound **5**

$POCl_3$ (0.70 mL, 7.5 mmol) was slowly added into anhydrous DMF (1.0 mL) in ice-water bath and stirred for 30 minutes. Then compound **4** (2.8 g, 7.5 mmol) was added in drops. The mixture was slowly warmed to room temperature and stirred for additional 30 min. Then the reaction was quenched by 20 mL icy water with vigorous stirring and saturated aqueous $NaHCO_3$ solution was used to tune pH to 7~8. Ethyl acetate is used as extractant, each 20 mL and the organic phase containing only one solute (monitored by TLC, PE: EA 3:1, R_f 0.3) was merged and dried by anhydrous Na_2SO_4. After evaporation, compound **5** was obtained as a yellow solid (2.2 g, 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 6.30 (d, *J* = 8.8 Hz, 1H), 6.11 (s, 1H), 3.92 (s, 3H), 3.87 (t, *J* = 7.3 Hz, 4H), 3.50 (t, *J* = 7.3 Hz, 4H).

Compound **6**

Compound **5** (1.7 g, 4.6 mmol) was dissolved in 30 mL CH_2Cl_2 under N_2, and boron tribromide (1.1 mL, 11.8 mmol) was added at -78°C. The mixture was warmed slowly to room temperature and stirred for 16 hours. Cold methanol and icy water was added to quench reaction and saturated aqueous $NaHCO_3$ solution was used to tune pH to 7~8. After extracting three times by ethyl acetate, each 25 mL, the organic layer was merged and dried by anhydrous Na_2SO_4. It was then purified by column chromatography (eluent PE: EA 10:1) to give compound **6** as a yellow solid (1.51 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.54 (s, 1H), 9.60 (s, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 6.30 (d, *J* = 8.8 Hz, 1H), 6.13 (s, 1H), 3.85 (t, *J* = 7.3 Hz, 4H), 3.49 (t, *J* = 7.3 Hz, 4H).

Compound **7**

A reaction mixture of compound **3-Me** (100 mg, 0.27 mmol), 2,3-diaminomaleonitrile (30.4 mg,

0.28 mmol) in ethanol (30 mL) was added a half drop of concentrated sulfuric acid and refluxed under nitrogen for 4 h, during which some yellow precipitate formed. After cooling to the room temperature, the mixture was filtered and the solid was washed in turn by ethanol, ethyl acetate and petroleum ether (2 mL each). After dried under reduced pressure, compound **7** was obtained as yellow solid (120 mg, 79 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ 12.34 (s, 1H), 8.42 (s, 1H), 7.85 (d, *J* = 9.2 Hz, 1H), 7.82 (s, 2H), 6.67 (d, *J* = 9.2 Hz, 1H), 3.88 (m, 2H), 3.73 (m, 2H), 3.18 (s, 3H), 3.03 (s, 3H).

Compound **8**

A mixture of compound **7** (500 mg, 1.42 mmol), sodium methanethiosulfonate (NaMTS, 380 mg, 2.8 mmol), potassium bicarbonate (170 mg, 1.7 mmol) and potassium iodide (12 mg, 0.072 mmol) in 20 mL mixed solvent of acetonitrile and water (1:3, v/v) was stirred and refluxed for 12 h. After evaporation, the residue was extracted three times by CH_2Cl_2, each 25 mL, and the organic layer was merged and dried by anhydrous Na_2SO_4. The system was further purified by column chromatography to give compound **8** as yellow solid (200 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.89 (s, 1H), 9.46 (s, 1H), 7.02 (s, 1H), 3.73 (m, 4H), 3.00 (m, 4H).

Compound **A-2-Me**

Compound **8** (120 mg, 0.47 mmol) was dissolved in 5 mL anhydrous CH_2Cl_2 in dark and methyl trifluoromethanesulfonate (2.82 mL) was added at -78 ºC. The reaction was warmed slowly to 0 ºC and stirred for 16 hours, during which some white solid precipitated. Ethyl acetate (0.5 mL) and ethyl ether (20mL) was then added to precipitate the product. The mixture was filtered and the solid product was washed by acetone and DCM. After dried under reduced pressure, compound **A-2-Me** was obtained as white powder (60 mg, 22 %).

^{1}H NMR (400 MHz, CD_3CN) δ 9.71 (s, 1H), 8.23 (s, 1H), 4.03 (m, 4H), 3.68 (m, 4H), 3.04 (d, *J* = 1.4 Hz, 6H).

Compound **C_3Me**

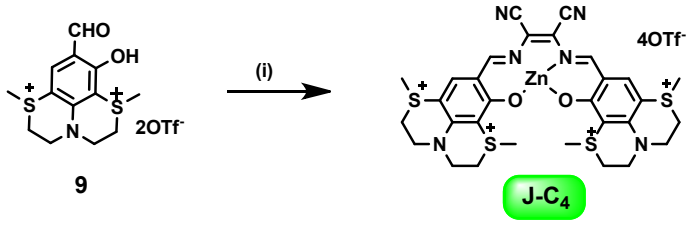
A reaction mixture of compound **7** (23.9 mg, 0.052 mmol), compound **A-2-Me** (30 mg, 0.052 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (11.9 mg, 0.054 mmol) in 10 mL acetonitrile was refluxed under nitrogen for 48 h. The system turned orange to red. After cooling to the room temperature and evaporating the solvent, 10 mL ethyl ether was added and the red brown precipitate formed was crystallized by acetonitrile and ethyl ether to give granular brown solid. After dried under reduced pressure, compound **C_3Me** was obtained as purple black powder (25 mg, 44 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.38 (d, J = 6.5 Hz, 2H), 8.28 (s, 1H), 7.67 (d, J = 9.4 Hz, 1H), 6.55 (d, J = 9.4 Hz, 1H), 3.90 (m, 12H), 3.27 (s, 6H), 3.10 (t, J = 8.3 Hz, 9H).

HR MS (ESI$^+$, DMSO, FT-ICR): *m/z* calcd. for $C_{29}H_{29}F_3N_6O_5S_4Zn$ ([M-$CF_3SO_3^-$]$^{2+}$) 395.01574, found 395.01576.

FT-IR (KBr pellete, cm^{-1}): 2218 (C≡N), 1595 (C=N).

2.4 Synthesis of C_4Me



Scheme S2-4 Synthetic route of J-C_4. (i) 2,3-diaminomaleonitrile, $Zn(OAc)_2 \cdot 2H_2O$, anhydrous CH_3CN, 85 ºC , 72 h.

Compound **C_4Me**

A reaction mixture of compound **A-2-Me** (60 mg, 0.10 mmol), 2,3- diaminomaleonitrile (5.58 mg, 0.052 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (11.9 mg, 0.054 mmol) in 10 mL anhydrous acetonitrile was refluxed under nitrogen for 72 h. The system turned orange. After cooling to the room temperature and evaporating the solvent, 10 mL ethyl ether was added and the orange to red precipitate formed was crystallized by acetonitrile and ethyl ether to give orange solid. After dried under reduced pressure, compound **C_4Me** was obtained as orange powder (47 mg, 70 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ 8.44 (d, J = 5.6 Hz, 2H), 8.33 (s, 2H), 3.85 (m, 16H), 3.10 (s,

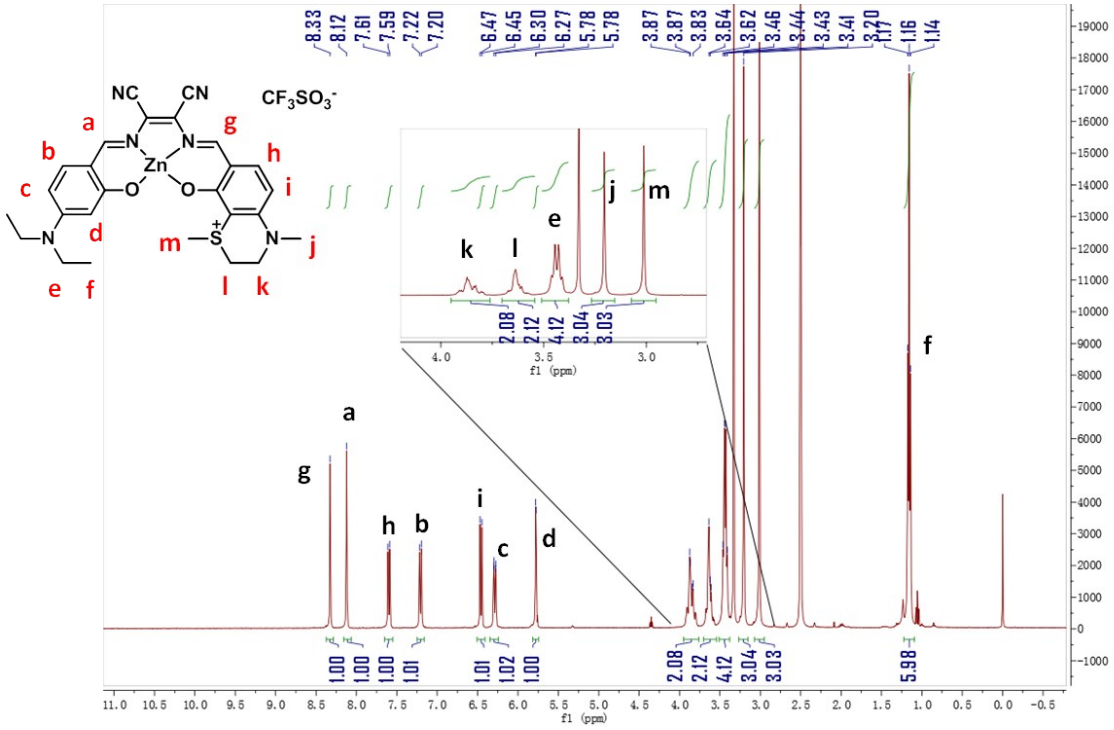
8H), 3.06 (s, 4H).

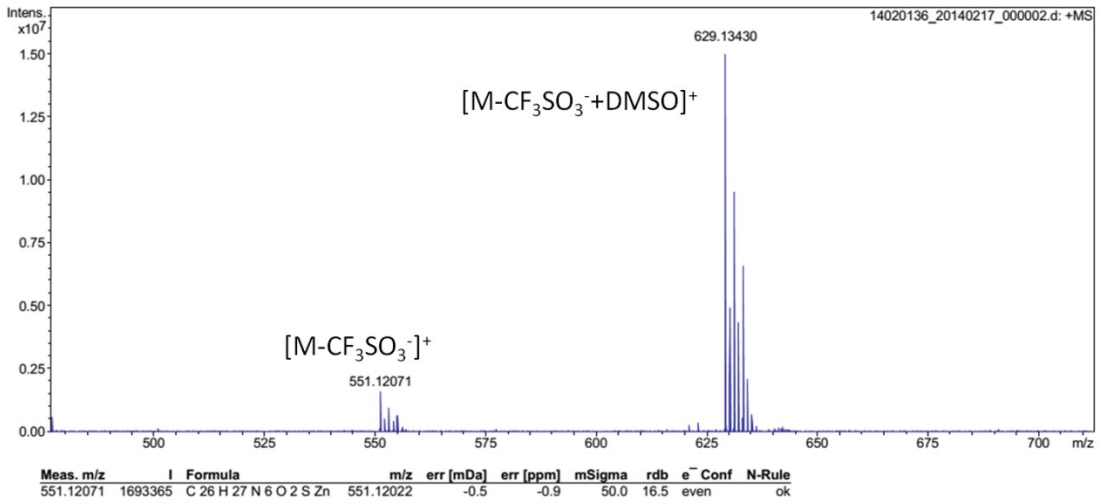
HR MS (ESI^+, DMSO, FT-ICR): *m/z* calcd. for $C_{32}H_{32}F_6N_6O_8S_6Zn$ ($[M-2CF_3SO_3^-]^{2+}$) 498.98953, found 498.99098.

FT-IR (KBr pellete, cm^{-1}): 2226 (C≡N), 1599 (C=N).

2.5 Characterization Spectra of sulfonium ZnSalens

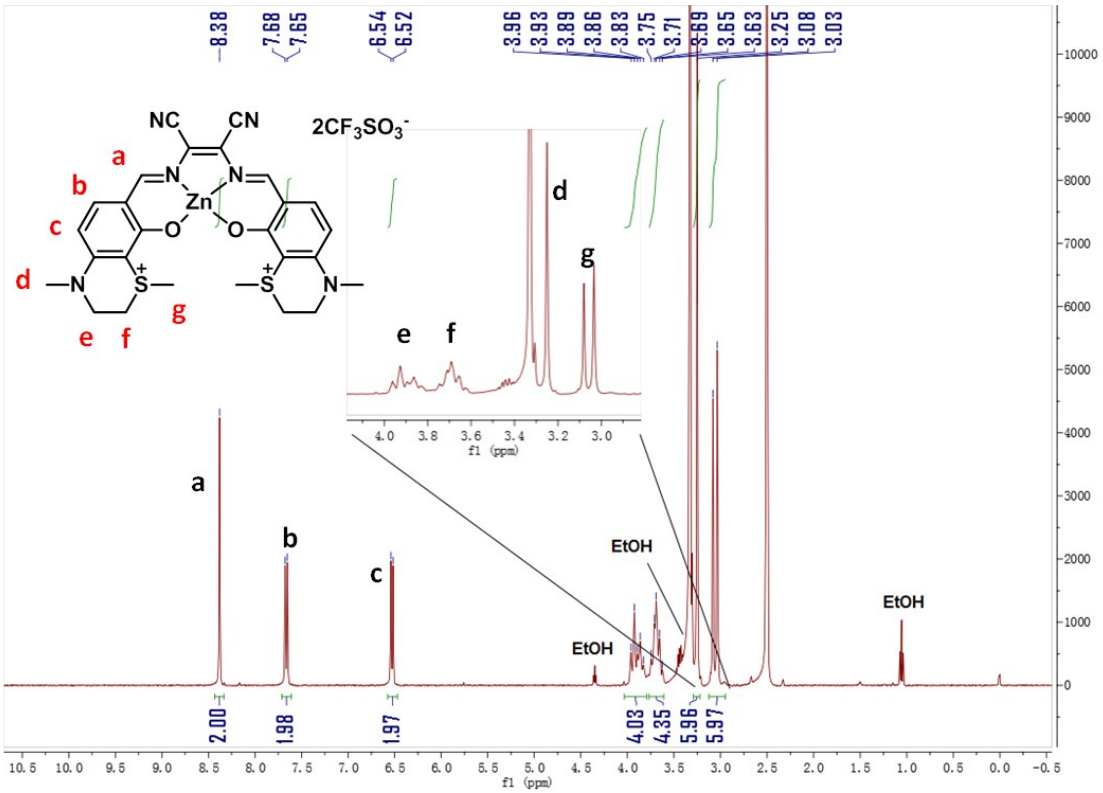
C_1Me

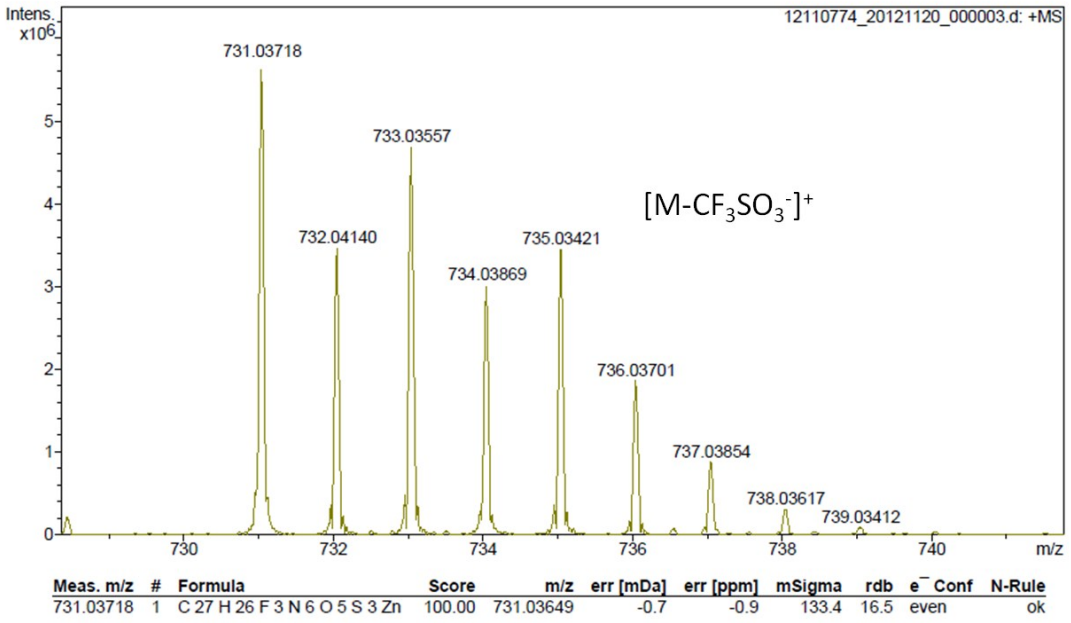




Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
551.12071	1693365	C 26 H 27 N 6 O 2 S Zn	551.12022	-0.5	-0.9	50.0	16.5	even	ok

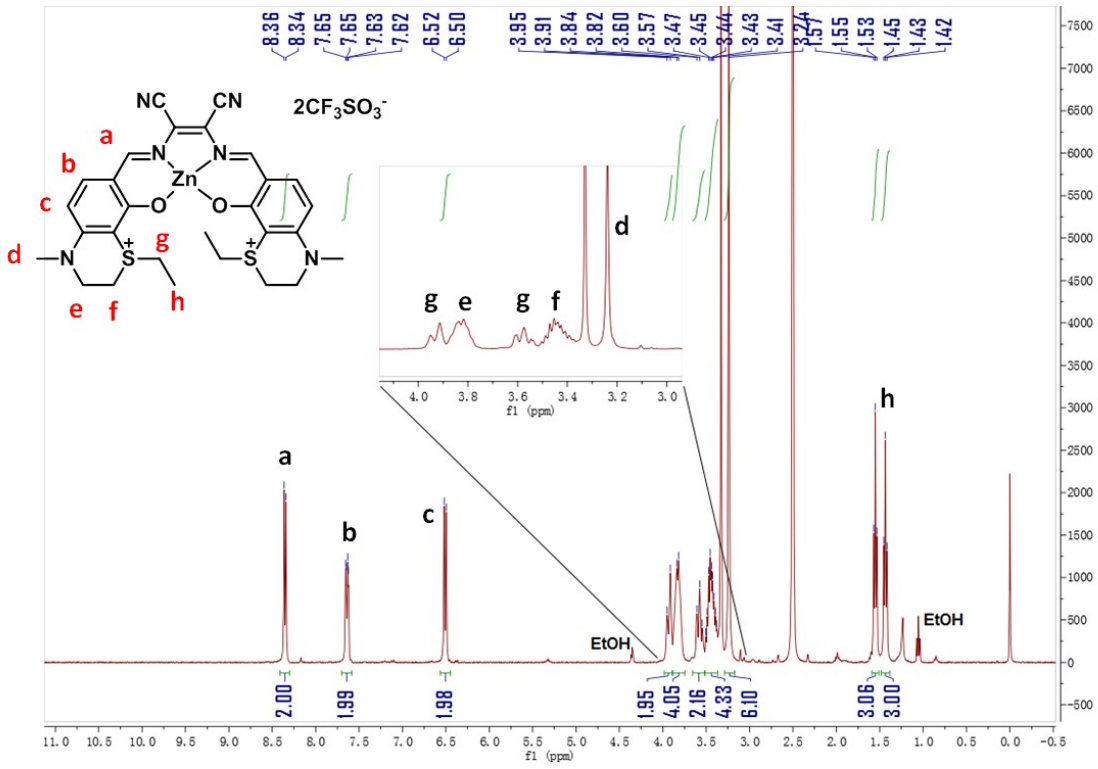
C_2Me

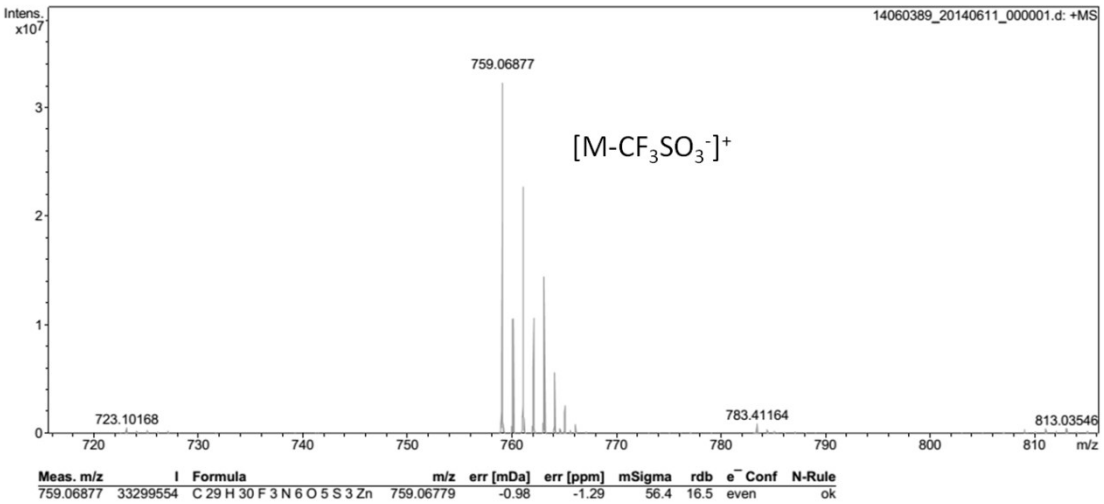




Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
731.03718	1	C 27 H 26 F 3 N 6 O 5 S 3 Zn	100.00	731.03649	-0.7	-0.9	133.4	16.5	even	ok

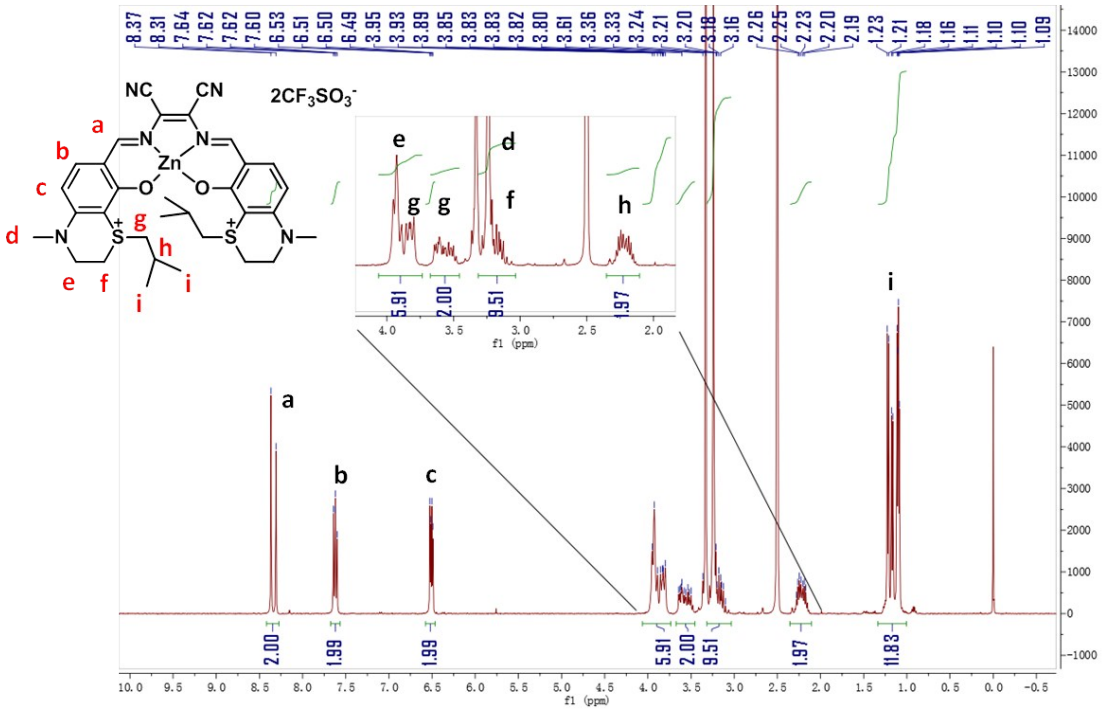
C_2Et

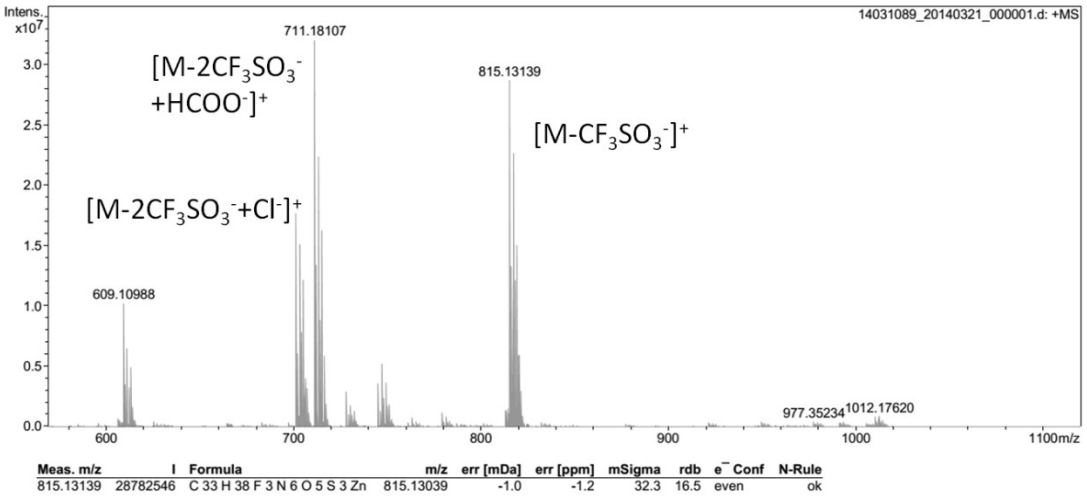




Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
759.06877	33299554	C 29 H 30 F 3 N 6 O 5 S 3 Zn	759.06779	-0.98	-1.29	56.4	16.5	even	ok

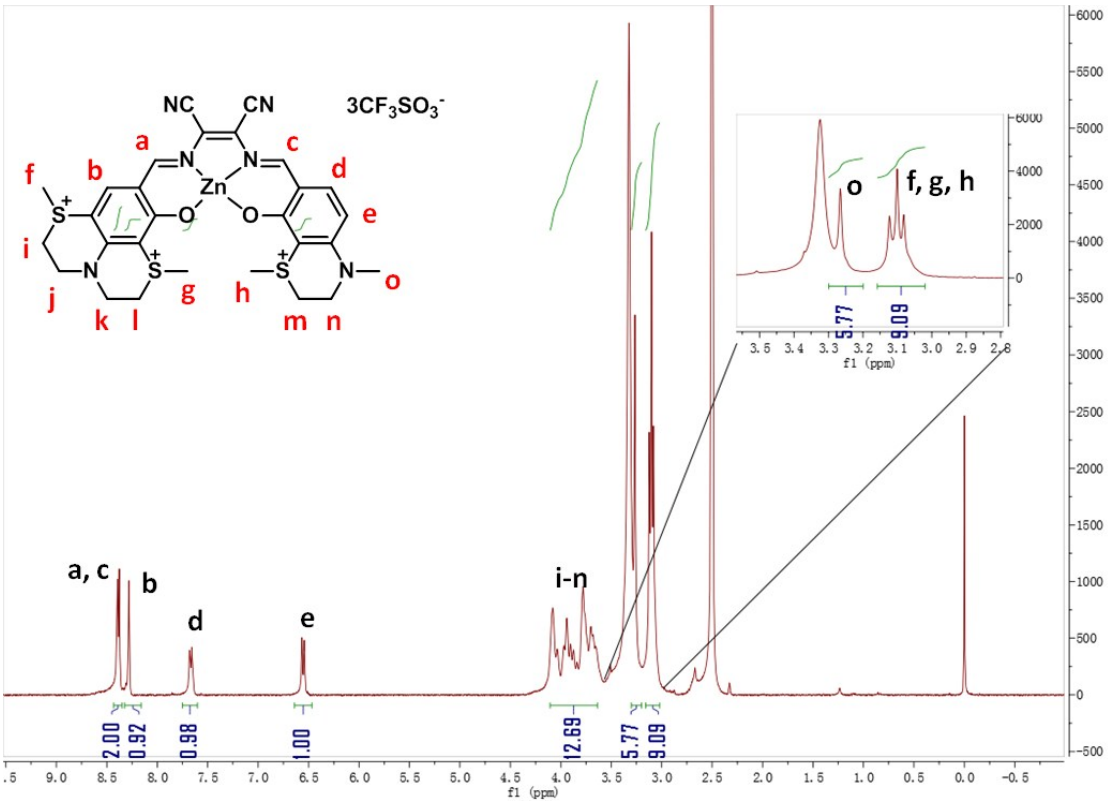
C_2iBu

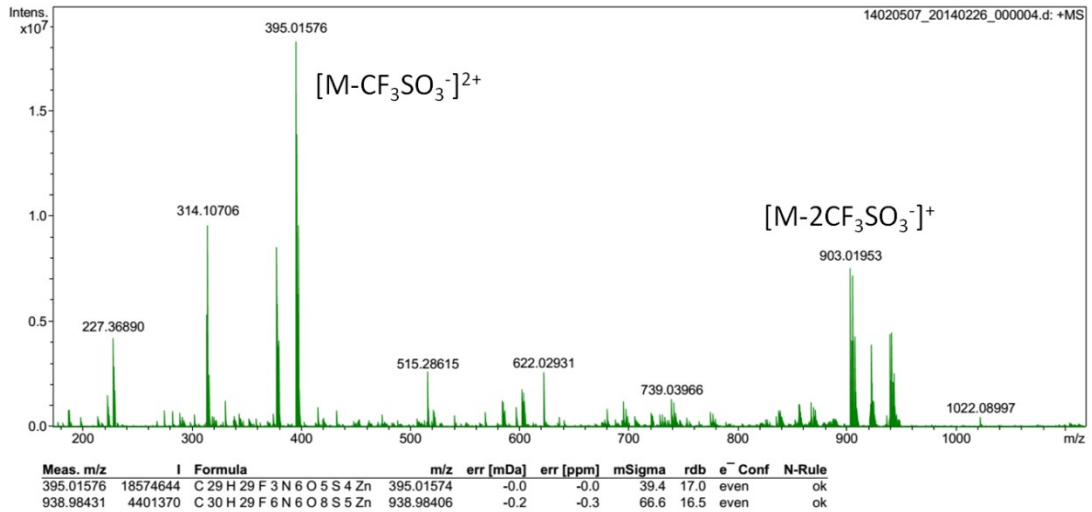




Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
815.13139	28782546	C 33 H 38 F 3 N 6 O 5 S 3 Zn	815.13039	-1.0	-1.2	32.3	16.5	even	ok

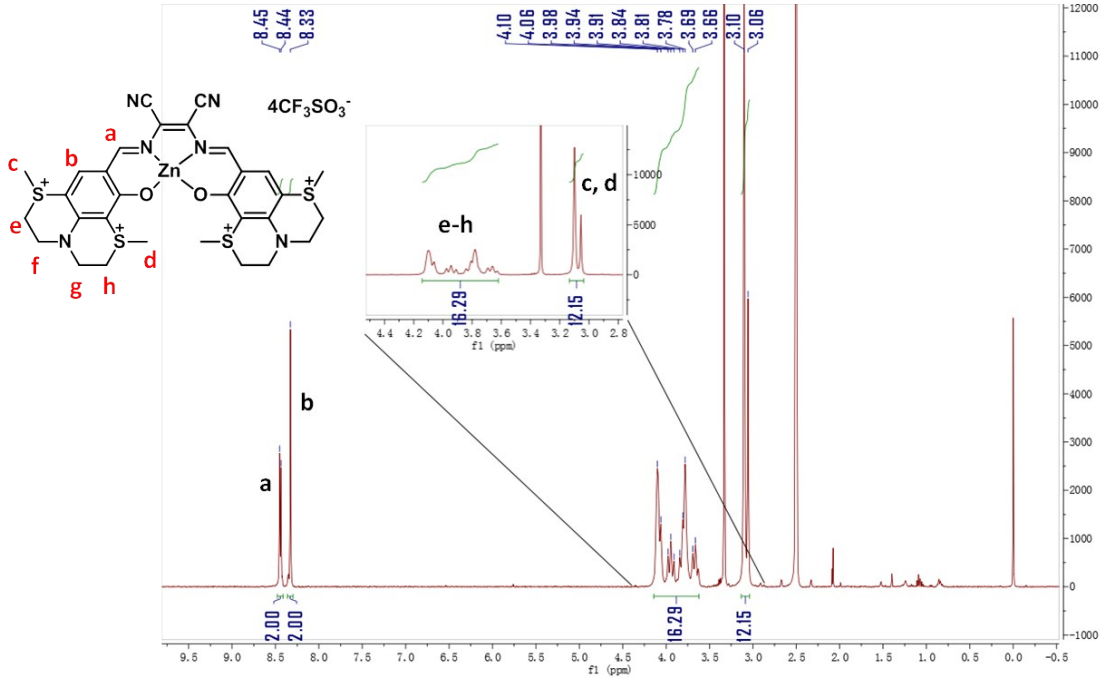
C_3Me

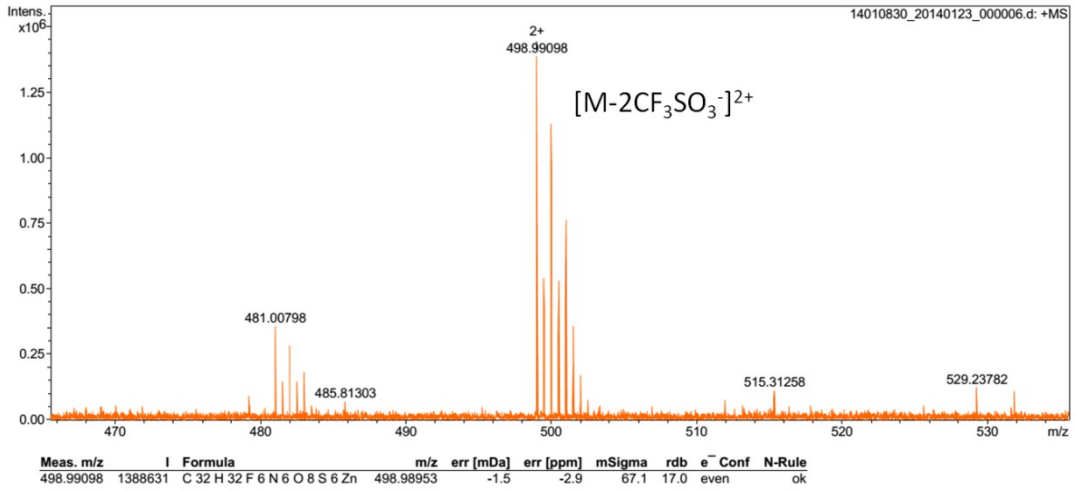




Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
395.01576	18574644	C 29 H 29 F 3 N 6 O 5 S 4 Zn	395.01574	-0.0	-0.0	39.4	17.0	even	ok
938.98431	4401370	C 30 H 29 F 6 N 6 O 8 S 5 Zn	938.98406	-0.2	-0.3	66.6	16.5	even	ok

C_4Me





Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
498.99098	1388631	C 32 H 32 F 6 N 6 O 8 S 6 Zn	498.98953	-1.5	-2.9	67.1	17.0	even	ok

2.6 Table S1. Crystal data and structure refinement parameters

Complex	**C_2Me**
molecular formula	C37 H37 F6 N9 O8 S4 Zn
formula wt. (g mol^{-1})	1043.36
temperature (K)	100.01(10)
radiation (λ, Å)	1.54184
crystal system	Triclinic
space group	*P -1*
a (Å)	13.1233(4)
b (Å)	14.4337(6)
c (Å)	14.5971(5)
β (°)	93.853(3)
Volume ($Å^3$)	2191.81(16)
Z	2
ρ_{calcd} (mg m^{-3})	1.581
μ (mm^{-1})	3.330
F(000)	1068
crystal size (mm^3)	0.25×0.25× 0.05
Theta range	3.467 to 73.198 °
reflections collected	15554
independent reflections	8538 [R(int) = 0.0372]
Completeness to theta = 67.684	100.0 %
goodness-of-fit on F^2	1.034
final R indices[R > 2σ (I)]	R1 = 0.0613 wR_2 = 0.1583
R indices (all data)	R1 = 0.0658 wR_2 = 0.1629
largest diff. peak and hole (e $Å^{-3}$)	1.208 and -1.171

3 Photophysical properties

3.1 Determination of the fluorescence quantum yield

Quantum yields of one-photon emission of synthesized ZnSalen complexes were measured with Rhodamine B (RhB, dissolved in ethanol) as reference. The one photon fluorescence measurements were performed in 1cm quartz cells with 1 μM compound in DMSO or DCM on a fluorescence lifetime and steady state spectrophotometer (Edinburgh Instrument FLS920) equipped 450 W Xenon light, slits 2.5×2.5. The values of fluorescence quantum yield, Φ (sample), were calculated according to equation as following:

$$\Phi_{sample} = \Phi_{ref} \cdot \frac{OD_{ref} \cdot I_{sample} \cdot d_{sample}^2}{OD_{sample} \cdot I_{ref} \cdot d_{ref}^2} \quad (1)$$

Φ_{ref} : The values of fluorescence quantum yield of the reference. Φ_{RhB}=0.65

I: integrated emission intensity.

OD: optical density at the excitation wavelength.

d: the refractive index of solvents. d_{DMSO} = 1.478, d_{DCM} = 1.444, d_{H_2O}=1.333, d_{EtOH} = 1.361.

3.2 Determination of the two-photon absorption cross section

The two-photon absorption spectra of sulfonium ZnSalen complexes were determined over a broad spectral region (740nm to 860nm) by the typical two-photon induced fluorescence method relative to Rhodamine B as standard. The two-photon fluorescence data were acquired using a Tsunami femtosecond Ti: Sapphire laser (pulse width ≤100 fs, 80 MHz repetition rate, tuning range 710–880 nm Spectra Physics Inc., USA). The two-photon fluorescence measurements were performed in a 1cm quartz cell with 2×10^{-5} mol/L sample dissolved in DMSO and the excitation power density is set to be 200 mW. The two-photon absorption cross section of sulfonium ZnSalens (δ_{sample}) was calculated at every 10nm wavelength from 740nm to 860nm according to equation as following:

$$\delta_{sample} = \delta_{ref} \cdot \frac{\Phi_{ref} \cdot C_{ref} \cdot I_{sample} \cdot d_{sample}}{\Phi_{sample} \cdot C_{sample} \cdot I_{ref} \cdot d_{ref}} \quad (2)$$

δ_{ref} : Two-photon absorption cross section of the reference (Rhodamine B), which was read out from the previous literature.

Φ : Quantum yield of sample and reference.

I: Integrated emission intensity.

C: Concentration of each sample.

d: The refractive index of solvents. d_{DMSO}= 1.478, d_{EtOH} = 1.361.

Table S2. Photophysical properties of sulfonium ZnSalen complexes

Compound	Solvent	λ_{max}/ nm (ε/ 10^4 M^{-1} cm^{-1})	λ_{em}/ nm	Φ	τ /ns	δ/ GM
JS	DMSO	388 (2.93), 437 (1.46), 597 (3.61)	627	0.0033	/	/
C_2Me	DMSO	379 (4.37), 430 (2.73), 576 (6.22)	616	0.65	4.25	211
	H_2O	372 (4.84), 420 (2.87), 561 (6.64)	604	0.38	/	/
C_2Et	DMSO	379 (4.35), 430 (2.75), 576 (6.62)	616	0.69	4.18	201
	H_2O	371 (4.81), 420 (2.90), 561 (6.83)	604	0.39	/	/
C_2iBu	DMSO	379 (4.37), 430 (2.81), 576 (6.92)	616	0.70	3.96	215
	H_2O	371 (4.83), 420 (2.99), 561 (7.13)	604	0.39	/	/
C_1Me	DMSO	385 (5.10), 432 (2.47), 588 (6.68)	640	0.16	1.60	202
	H_2O	377 (5.02), 427 (2.26), 572 (5.97)	626	0.012	/	/
C_3Me	DMSO	376 (3.12), 414 (1.97), 565 (4.18)	617	0.50	2.48	63
	H_2O	365 (4.59), 400 (3.04), 546 (6.78)	606	0.046	/	/
C_4Me	DMSO	364 (2.98), 383 (2.79), 405 (2.79), 541 (4.80)	589	0.56	2.47	153
	H_2O	355 (3.39), 377 (3.19), 391 (3.18), 521 (6.72)	561	0.79	/	/

4 DFT Calculation

4.1 Methods

For the theoretical study of photophysical properties of sulfonium ZnSalens, density functional theory (DFT) and time-dependent density functional theory (TD-DFT) methods were performed and the Becke's three–parameter hybrid exchange functional with Lee-Yang-Parr gradient-corrected correlation (B3LYP functional) was used with Lanl2dz pseudopotential basis set for Zn, 6-31G** for main group elements, as implemented in the Gaussian 09 package. Geometries for ssulfonium ZnSalens were fully optimized without symmetry constraints. The solvent effect was involved through the PCM approach (DMSO, ε=46.826). The vibration frequency calculations at the same level were carried out to confirm each stationary point to be either a minimum. Then we calculated the vertical excitation energies based on the optimized geometries of the ZnSalen molecules.

Table S3. DFT calculation results of sulfonium ZnSalens

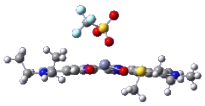
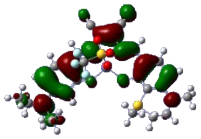
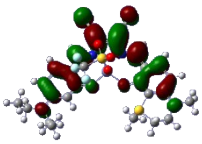
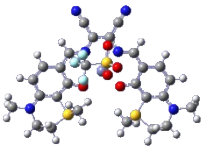
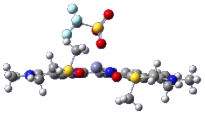
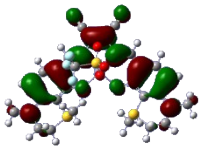
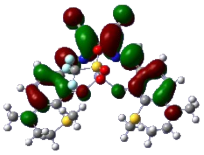
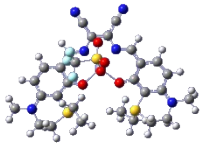
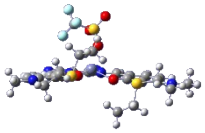
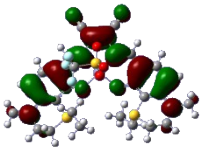
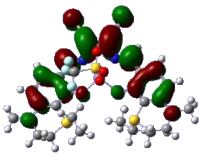
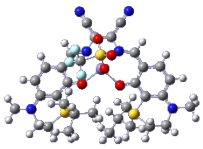
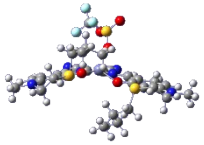
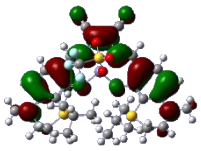
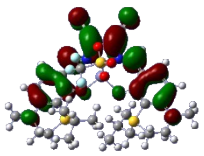
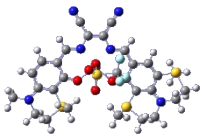
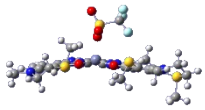
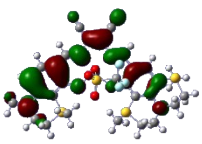
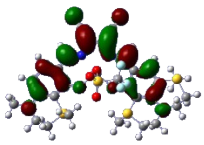
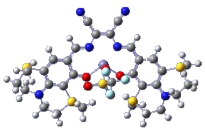
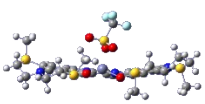
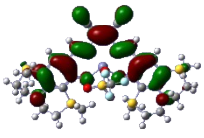
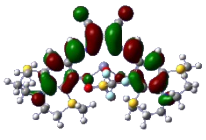
Compound	Optimized Structures		FOMOs of ZnSalen/Salophens	
			HOMO	LUMO
C_1Me			 -5.22 eV	 -2.72 eV
C_2Me			 -5.61 eV	 -3.01 eV
C_2Et			 -5.57 eV	 -2.97 eV
C_2iBu			 -5.58 eV	 -2.98 eV
C_3Me			 -5.89 eV	 -3.25 eV
C_4Me			 -12.63 eV	 -9.65 eV

Table S4. Calculated electronic transitions properties for sulfonium ZnSalens obtained from TD-DFT calculations with PCM solvation model.

Compounds	Transitions	*f*	Major contribution
C_1Me	S_0–S_1	0.9457	HOMO → LUMO (100%)
C_2Me	S_0–S_1	0.8877	HOMO → LUMO (100%)
C_2Et	S_0–S_1	0.8654	HOMO → LUMO (100%)
C_2iBu	S_0–S_1	0.8043	HOMO→LUMO (100%)
C_3Me	S_0–S_1	0.9172	HOMO→LUMO (100%)
C_4Me	S_0–S_1	0.8536	HOMO→LUMO (100%)

5 Diffusion-Ordered spectroscopy

^{1}H DOSY experiments were carried out at room temperature (27 ºC) and referenced to the residual solvent signals (D_2O: 4.79 ppm). The gradient strength was calibrated by using HDO signal at 300 K ($D=19.02\times10^{-10}$ m^2s^{-1}). The bipolar pulse pair stimulated echo pulse-sequence (Dbppste in the standard Varian pulse sequence library) was used for acquiring diffusion data with 70 ms diffusion delay (Δ), 2.0 ms of diffusion gradient length and 64 increments for gradient levels. Gradient strengths of 2% and 95% of maximum power were used to obtain spectral pairs with acquisition times of 2 s and recycle delays of 2 s. The Varian DOSY package was used for acquisition and processing (VnmrJ® version 2.2 revision C). The diffusion constant of $\mathbf{C_nR}$, D($\mathbf{C_nR}$), is averaged from the diffusion constants simulated from each of its ^{1}H NMR signal. Since their structure in solution is far from the spherical one, thus precluding a straightforward application of the Stokes–Einstein equation, the molecular mass in solution, M($\mathbf{C_nR}$), was simply estimated using Graham's law of diffusion:

$$D(C_nR) = K\left[\frac{T}{M(C_nR)}\right]^{0.5}$$

where the constant K depends on geometric factors, including the area over which the diffusion is occurring. By assuming a constant temperature and that K is the same for both species in solution, the relative diffusion rate of two species $\mathbf{C_nR}$ and the internal reference (that is, HDO) is given by:

$$\frac{M(C_nR)}{M(HDO)} = \left[\frac{D(HDO)}{D_{aver}(C_nR)}\right]^2.$$

Therefore, the diffusion rate values obtained by DOSY can be used to estimate the molecular mass of $\mathbf{C_nR}$ by the following equation[1]:

$$M(C_nR) = M(HDO)\left[\frac{D(HDO)}{D_{aver}(C_nR)}\right]^2.$$

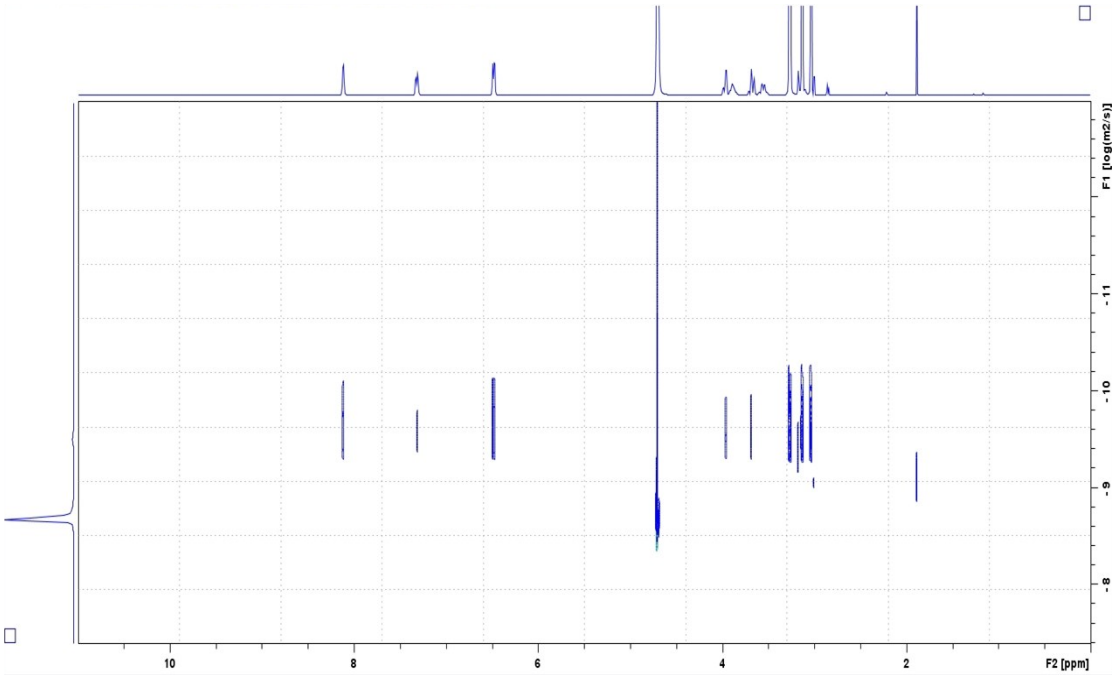


Figure S1 ^{1}H NMR DOSY Spectrum of 2 mM **C2Me** in D_2O.

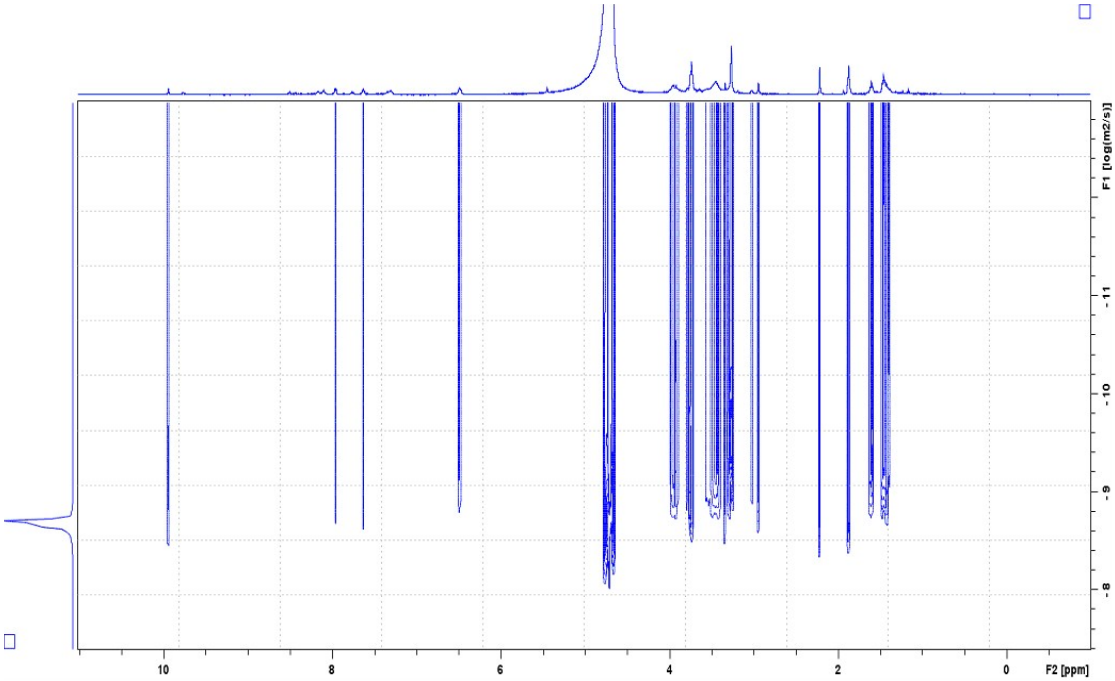


Figure S2 ^{1}H NMR DOSY Spectrum of 2 mM **C2Et** in D_2O.

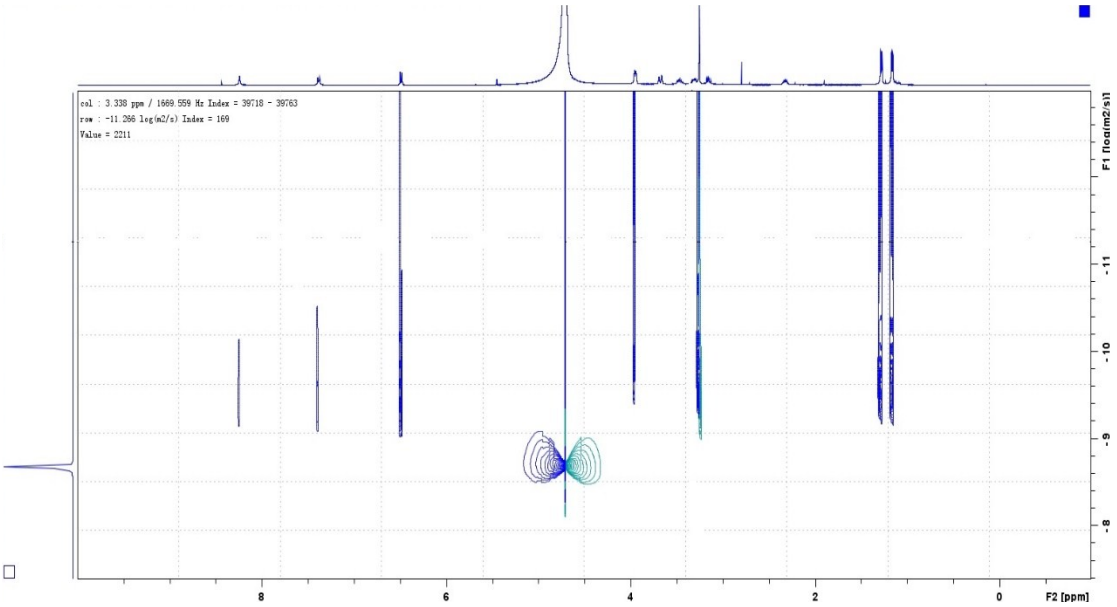


Figure S3 ^{1}H NMR DOSY Spectrum of 2 mM **C2iBu** in D_2O.

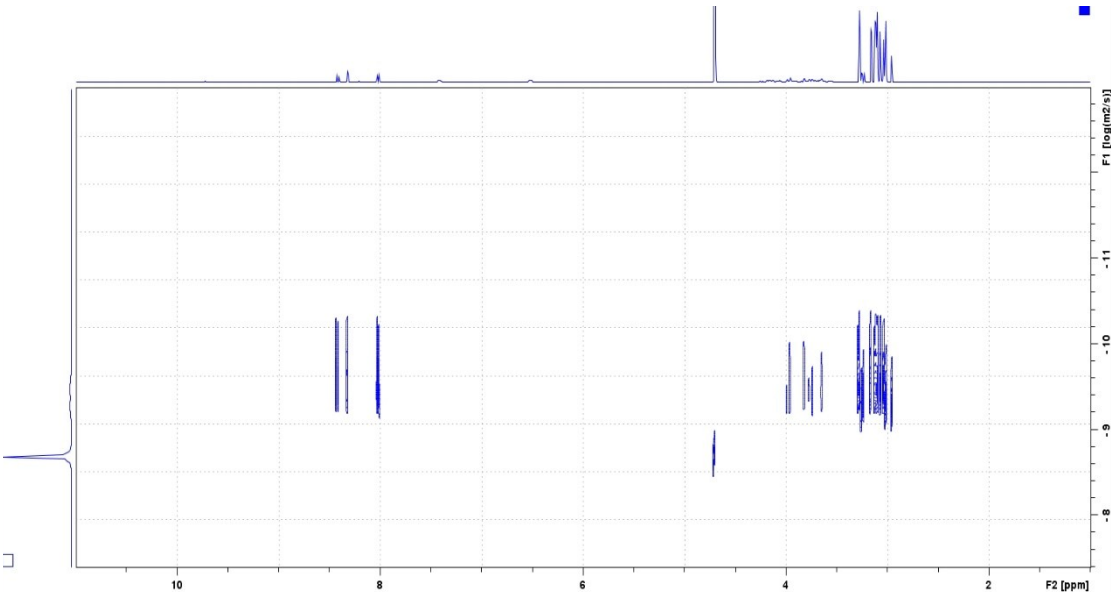


Figure S4 ^{1}H NMR DOSY Spectrum of 2 mM **C3Me** in D_2O.

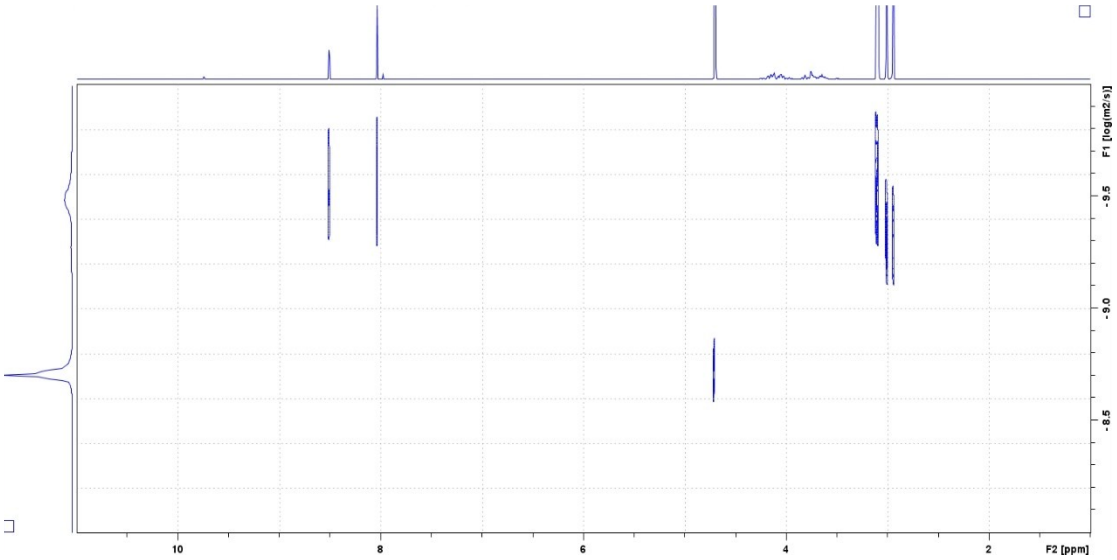


Figure S5 ^{1}H NMR DOSY Spectrum of 2 mM **C34e** in D_2O.

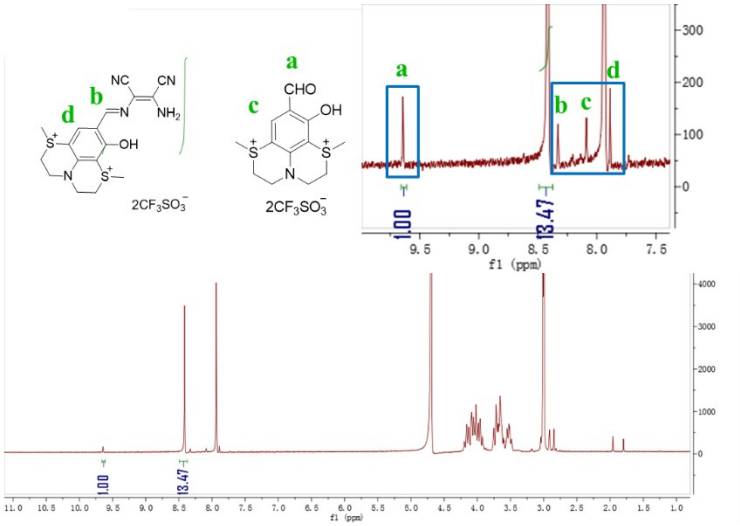
6 The octanol-water partition coefficients (log *P*)

Log *P* was determined according to Leo's methods.[2] Equal volume (2000mL) of n-octanol and water were thoroughly mixed by an oscillator and separated after 24 h. Sulfonium ZnSalens (0.50mg each) was then dissolved in 40mL of the separated n-octanol and the solution was allowed to equilibrate for further 24 h. The extinction coefficient was then calculated and 40mL of water (previously separated from the mixture) was added. The new water-octanol system was allowed to equilibrate for additional 24h. After separating, both fractions were analyzed by UV-*vis* spectra. The log *P* values were calculated by the following equation

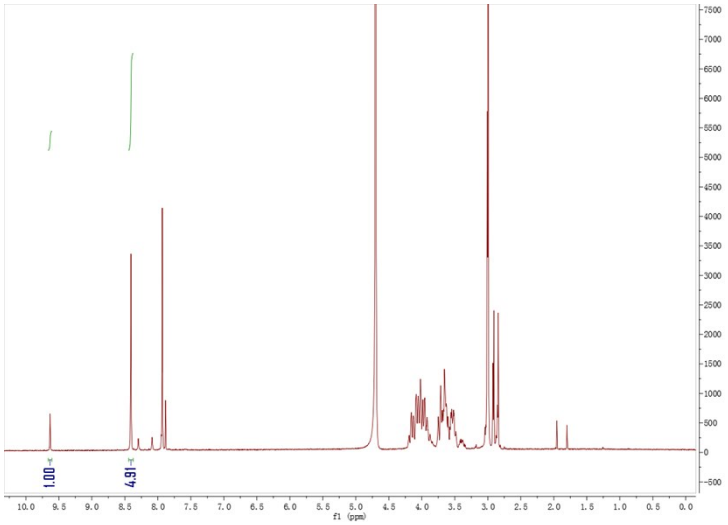
$$\log P = \log \frac{C_{octanol}}{C_{water}}$$

where $C_{octanol}$ and C_{water} refer to the concentration of ZnSalen/Salophen compound in the n-octanol and water, respectively.

7 Stability



(a)



(b)

Figure S6 Decomposition of **C_4Me** in D_2O at a concentration of 10 mM after (a) 3h and (b) 1 day.

Table S5 Stability of sulfonium ZnSalens in PBS with different pH represented by the hydrolysis degree after 6 hours[a].

Compounds	pH 5	pH 6	pH 7	pH 8	pH 9
C_1Me	0.84	0.77	0.82	0.93	0.95
C_2Me	0.97	0.99	0.98	0.95	0.91
C_2Et	0.96	0.95	0.93	0.87	0.78
C_2iBu	0.98	0.98	0.98	0.96	0.94
C_3Me	0.99	0.99	0.93	0.67	0.22
C_4Me	0.90	0.92	0.72	0.20	0.03

[a] The stability was carried out in a UV-*vis* cuvette by incubation of 20 μM sulfonium ZnSalen complex

Table S6 Reactivity of sulfonium ZnSalens in presence of different reductants and necleophiles[a].

Compound	C_1Me	C_2Me	C_3Me	C_4Me	C_2Et	C_2iBu
L-CyS	-[b]	-	-	-	-	-
L-GSH	-	-	-	-	-	-
VcNa	-	-	-	-	-	-
DTT	-	-	-	-	-	-
FAD	D[b]	D	D	D	D	D
Thiourea	-	-	-	-	-	-
L-Met	-	-	-	-	-	-
2-SH-py	-	-	-	-	-	-
DMAP	D	D	D	D	D	D
Na_2S/NaHS	-	-	D	-	-	-
KSCN	-	-	D	-	-	-
KF	-	-	D	-	-	-

[a] The reaction was carried out in a UV-*vis* cuvette by incubation of 20 μM sulfonium ZnSalen complex and 100 μM reactants in a mixed solution of DMSO/H_2O (1/1000, v/v) for 5 minutes.

[b] "-" represents for "no reaction" and "D" represents for "decomposition".

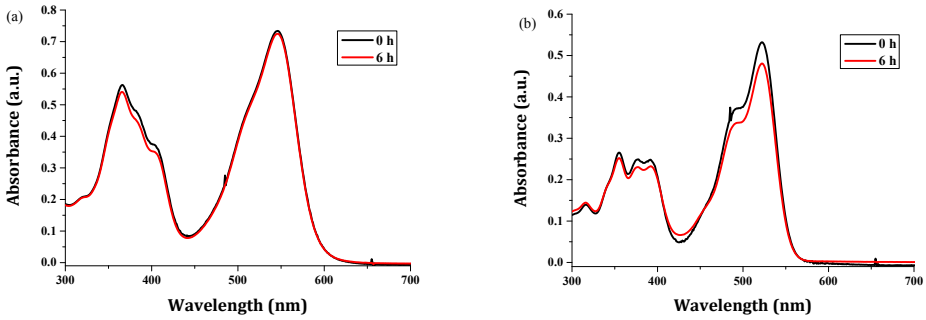


Figure S7 Stability of (a) **C_3Me** and (b) **C_4Me** under pH 5 monitored by UV-*vis* spectra. The complex was incubated in PBS buffer (pH 5) for 6 hours.

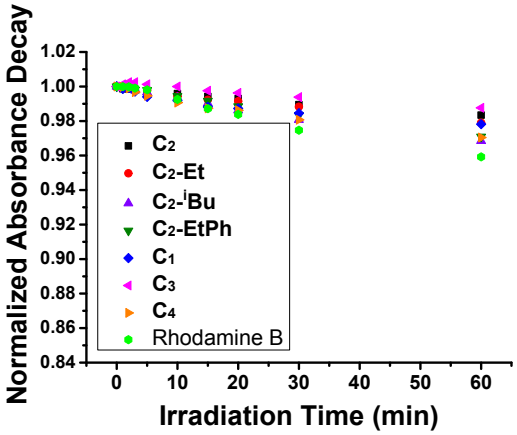


Figure S8 Photostability comparison of sulfonium ZnSalens. The complexes were irradiated by 40 mW hand-held ultraviolet lamp for 60 min and the photostability was evaluated by monitoring the absorbance maxima of UV-*vis* spectra at 0, 1, 2, 3, 5, 10, 15, 20, 30 and 60 min, respectively.

8 In vitro experiments

8.1 Cell culture

All cells were incubated in complete medium (Dulbecco's modified Eagle's Medium, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37 °C in atmosphere containing 5% CO_2.For imaging, HeLa cells were grown in poly-D-lysine-coated dishes and incubated in 2mL of complete medium for 24 h. Cells were washed with PBS, and stocked dyes (2 mM in DMSO) were added to obtain a final concentration of 2 μM. The treated cells were incubated for another hour in dark at 37 °C. A few minutes prior to confocal imaging cells were washed twice with PBS. A confocal laser scanning microscope (A1R-si, Nikon, Japan) was used to obtain images. Cells were imaged via the fluorescence mode with a 60× immersion lens with the following parameters: laser power 100%, pinhole 1.0 A.U., excitation wavelength 405nm or 488nm or 543 nm, detector slit 552-617 nm, resolution 1024×1024, and a scan speed 0.5 frames per second.

8.2 CCK-8 assay

HeLa cells were seeded in flat-bottomed 96-well plates, 10^4 cells per well, with 200 μL complete culture media in the dark for 24h. After washed with PBS for three times (200μL*3), the cells were incubated with 10 μM sulfonium ZnSalens. All stock solutions were prepared in DMSO and diluted with complete media, and the final DMSO concentrations were less than 0.1%. After cultured for 24 h, the cells were washed with PBS three times (200μL*3). 10 μL Cell Counting Kit-8 (CCK-8) solution and 90μL PBS were added per well simultaneously. After 2 hours, the absorbance at 450nm was read by 96-well plate reader. The viability of HeLa cells was calculated by the following equation:

$$CV = (As-Ab) / (Ac-Ab) \times 100\%$$

where CV stands for the viability of cells，As, Ac and Ab stand for the absorbance of cells containing ZnSalen/Salophens, cell control (0 μM ZnSalen/Salophens) and blank control (wells containing no cells or ZnSalen/Salophens).

8.3 Co-localization assay

HeLa cells were placed onto 0.1mM poly-D-lysine coated glasses in complete media and the cells were incubated for 24 h. A stock solution of sulfonium ZnSalen in chromatographic grade, anhydrous DMSO was prepared as 2 mM. The solution was diluted to a final concentration of 1 μM by complete growth medium. Stock solutions of Lyso Tracker ® Green DND-26, DiO C18(3) and MitoTracker Green were prepared as 1mM, and the stock solution was diluted to the working concentrations in complete medium (For Lyso Tracker ® Green DND-26: 72nM; for DiO C18(3): 10 mM; MitoTracker Green: 50 nM). After incubation, cells were washed with PBS buffer twice before confocal experiments. Images were taken under conditions as follows: 60× immersion lens with a resolution of 1024×1024 and a speed of 0.5 frame per second, 488 nm as excitation wavelength for lysosome or cell membrane tracker, 543 nm as excitation wavelength for ZnSalen complexes. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using Image J. The Pearson's Coefficient was calculated by Image J.

8.4 Dynamics of cellular internalization

The cellular internalization of **C_2Me** is primarily examined using confocal microscopy. HeLa cells were placed onto 0.1mM poly-D-lysine coated glasses in complete media and the cells were incubated for 24 h. HeLa cells were co-incubated with 2 μM **C_2Me** for different time (10 min; 30 min; 2 h; 6 h; 12 h). Stock solutions of Lyso Tracker ® Green DND-26, DiO C18(3) were both prepared as 1mM, and the stock solution was diluted to the working concentrations in complete medium (For Lyso Tracker ® Green DND-26: 72nM; for DiO C18(3): 10 μM). After incubation, cells were washed with PBS buffer twice before confocal experiments. Images were taken under conditions as follows: 60× immersion lens with a resolution of 1024×1024 and a speed of 0.5 frame per second, 488 nm as excitation wavelength for lysosome or cell membrane tracker, 543 nm as excitation wavelength for ZnSalen complexes. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using Image J. The Pearson's Coefficient was calculated by Image J.

8.5 Cellular uptake pathway

The cellular uptake pathway experiments were conducted according to the literature.[3-5] The cellular uptake of **C_2Me** is primarily examined using confocal microscopy. In the temperature effect assay, cells were placed at 4 ^{o}C for 15 minutes, and then incubated with 2 μM **C_2Me** for 6 hours at 4 ^{o}C or 37 ^{o}C. For endocytosis mechanism investigation, various endocytosis inhibitors including 10 mM methyl-β-cyclodextrin (MβCD) (inhibitor of caveolae-mediated endocytosis) or 450 mM sucrose

(inhibitor of clathrin-mediated endocytosis) were applied to cells for 30 minutes. Then medium containing both inhibitors and **C_2Me** was used for incubation for another 6 hours. After incubation, the cells were rinsed, and the extent of uptake was analyzed by confocal imaging and dealt with ImageJ. Images were taken under conditions as follows: 60× immersion lens with a resolution of 1024×1024 and a speed of 0.5 frame per second, 543 nm excitation wavelength and 552 to 617 nm detector slit, 100% laser power for dye. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ.

Table S7. Cytotoxicity results of 10 μM sulfonium ZnSalen complexes performed by CCK-8 assay

Compounds	Cell Viability (%)	Compounds	Cell Viability (%)
C_1Me	99 ± 4	**C_2Et**	103 ± 4
C_2Me	92 ± 8	**C_2iBu**	107 ± 4
C_3Me	102 ± 6	**C_4Me**	104 ± 1

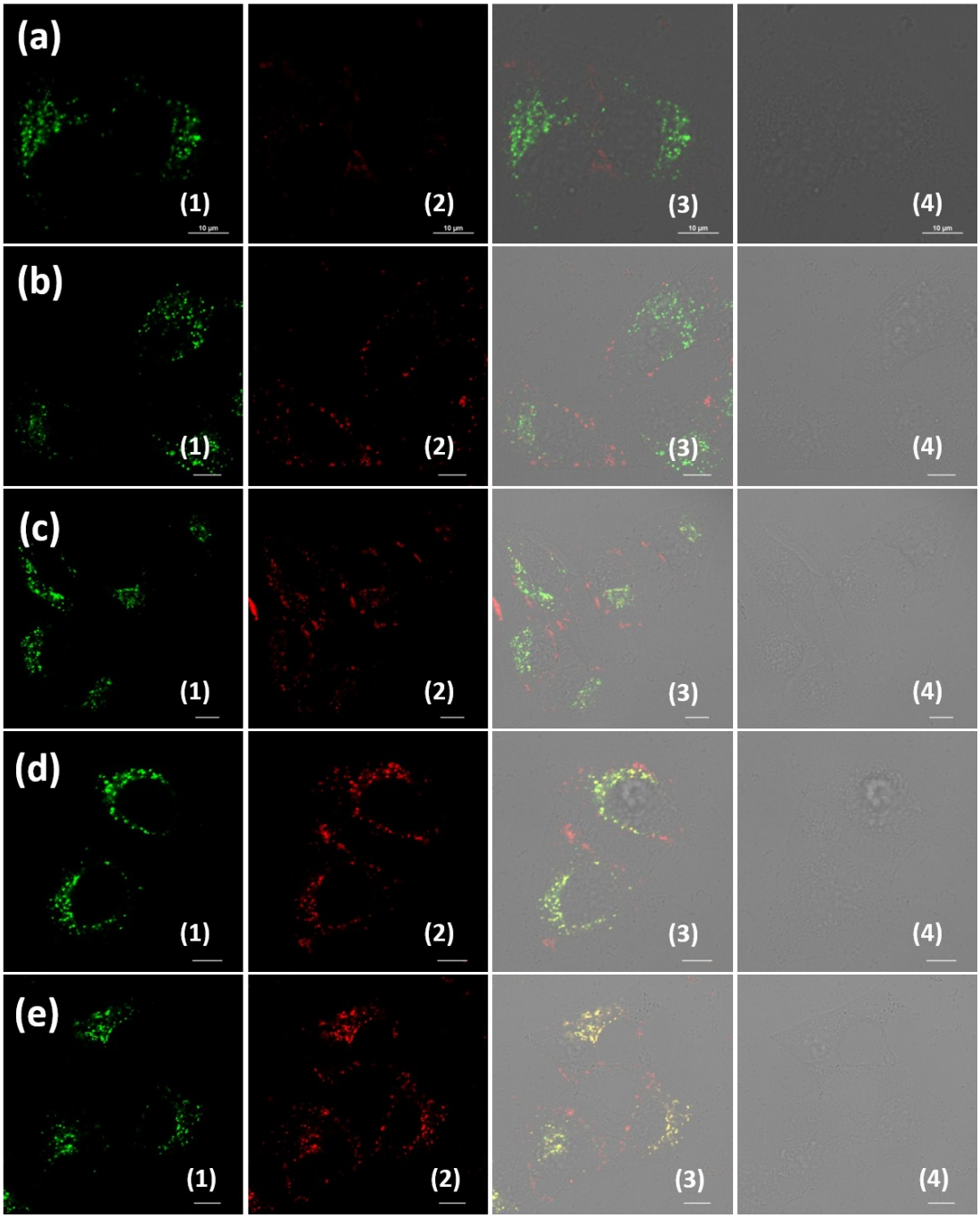


Figure S9 Confocal images of the internalization process of **C_2Et** by HeLa cells. HeLa cells were treated with A: 10 min; B: 30 min; C: 2 h; D: 6 h; E: 12 h. (a) LysoTracker® Green DND-26; (b) image of **C_2Et**; (c) merged images of (a), (b) and (d); (d) differential Interference Contrast (DIC) Image. The inset scale bar represents for 10 μm.

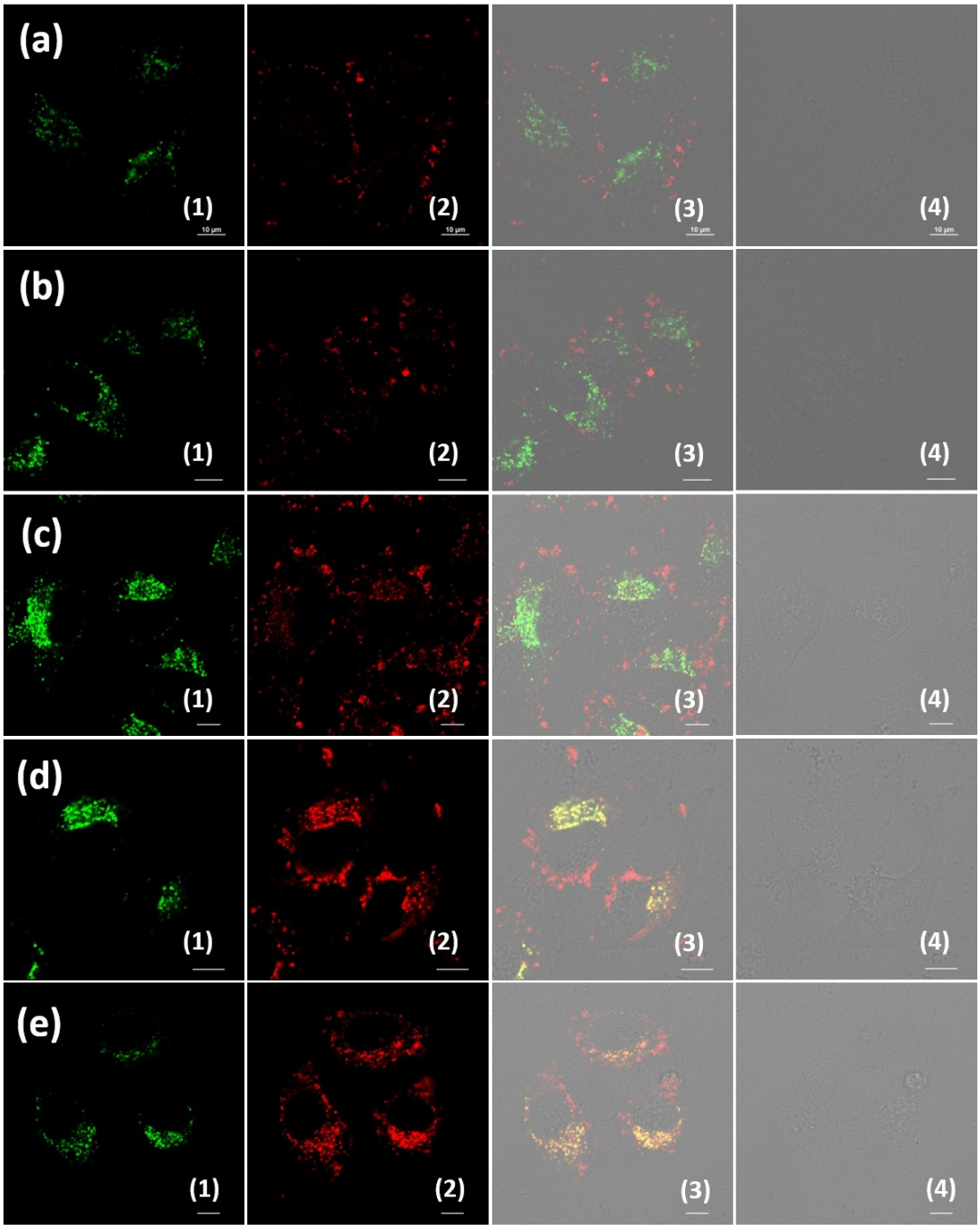


Figure S10 Confocal images of the internalization process of **C_2iBu** by HeLa cells. HeLa cells were treated with A: 10 min; B: 30 min; C: 2 h; D: 6 h; E: 12 h. (a) LysoTracker® Green DND-26; (b) image of **C_2iBu**; (c) merged images of (a), (b) and (d); (d) differential Interference Contrast (DIC) Image. The inset scale bar represents for 10 μm.

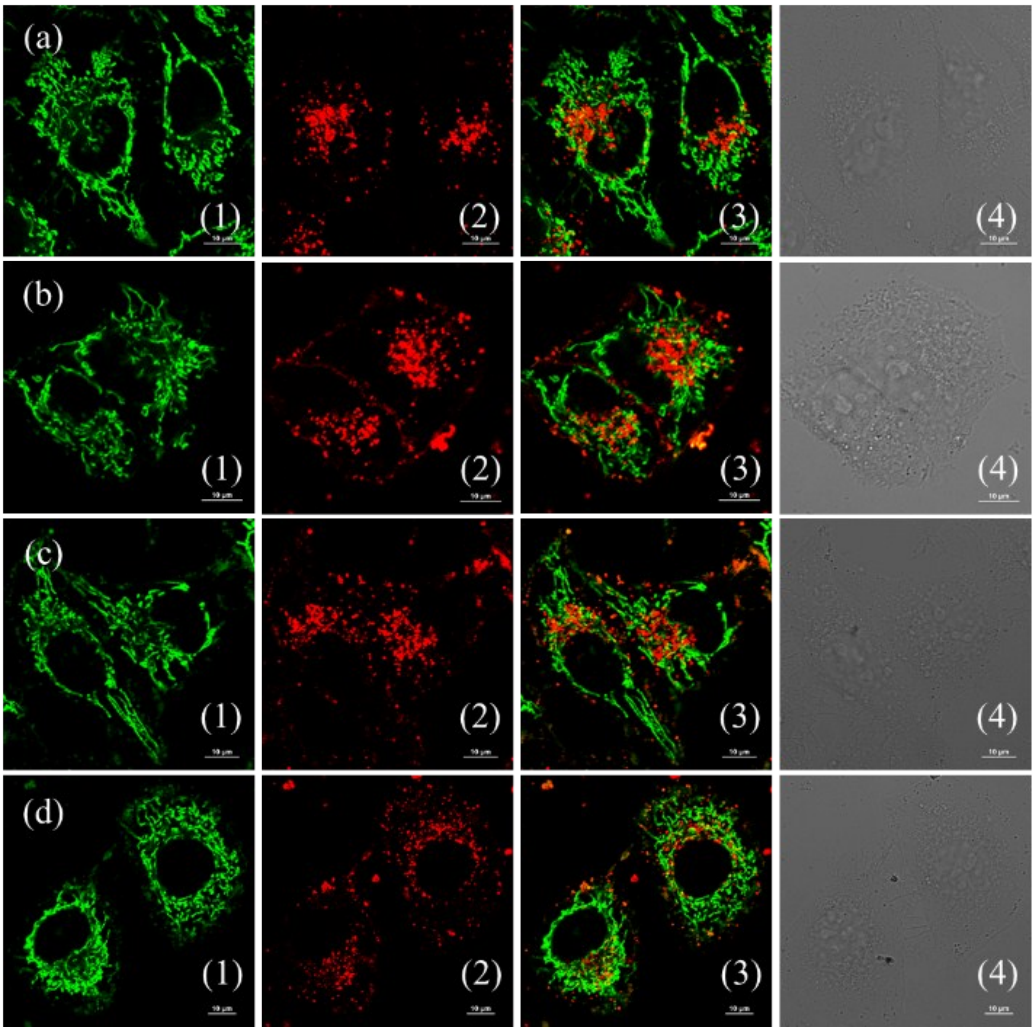


Figure S11 Imaging of sulfonium ZnSalens in HeLa cells after incubation for 6 h: (a) **C_1Me**, (b) **C_2Me**, (c) **C_2Et**, (d) **C_2iBu**. (1) Image of MitoTracker Green; (2) Image of sulfonium ZnSalens; (3) merged images of (1) and (2); (4) differential Interference Contrast (DIC) Image. The inset scale bar represents for 10 μm.

9 Reference

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