Electronic Supplementary Material (ESI) for Chemical Science

Supporting Information

Rational Design of ZnSalen as a Single and Two Photon Activatable Fluorophore in Living Cells

Jing Jing,[1] Juan-Juan Chen,[1] Yang Hai,[1] Hao Chang,[2] Jinhui Zhan,[1] Pingyong Xu,[*,2] and Jun-Long Zhang[*,1]

[1] Beijing National Laboratory for Molecular Sciences, State Key Laboratory of Rare Earth Materials Chemistry and Applications, College of Chemistry and Molecular Engineering, Peking University, Beijing, P.R. China

[2] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, P. R. China

[*]To whom corresponding should be addressed.

E-mail: zhangjunlong@pku.edu.cn, pyxu@moon.ibp.ac.cn

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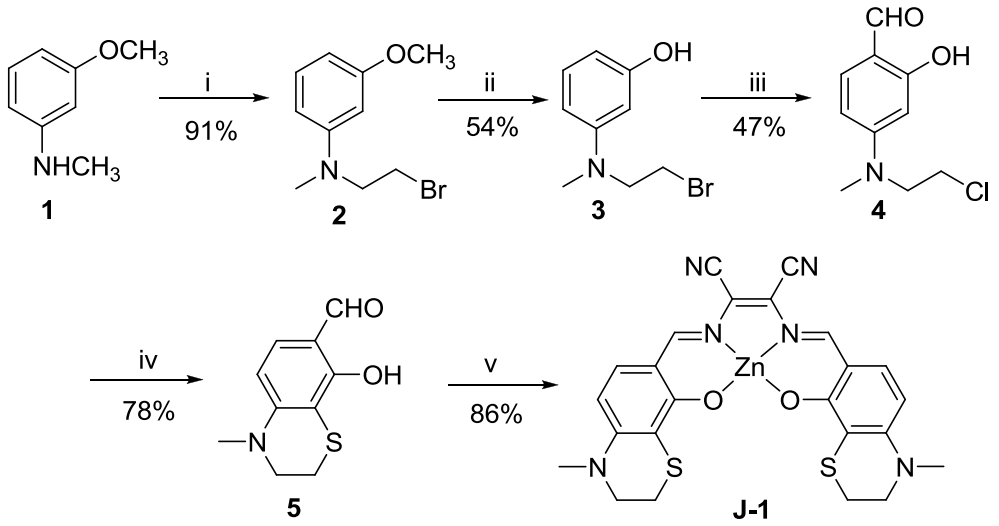
1. Experimental methods

1.1 General information

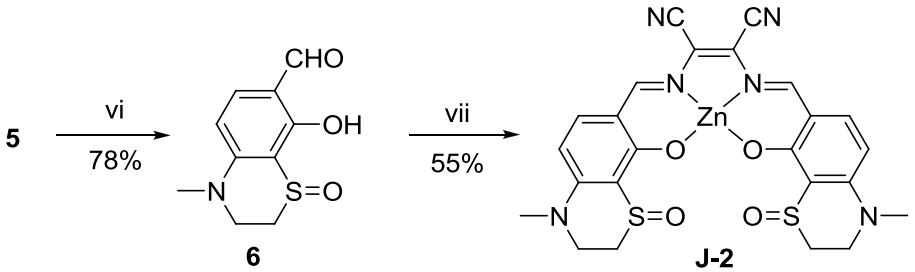
All solvents and chemicals were purchased from Alfa Aesar and J&K and used as received without further purification, unless otherwise specified. Cellular imaging trackers were purchased from Invitrogen (Life Technologies). The ^{1}H NMR spectroscopic measurements were carried out using a Varian-300 NMR spectrometer at 300 M Hz or a Bruker-400 NMR at 400 MHz with tetramethysilane (TMS) as internal reference. The ^{13}C NMR spectroscopic measurements were carried out using a 100 MHz NMR (Bruker-400, USA). Electrospray ionization (ESI) mass spectra were performed on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker, USA), positive-ion mode. FT-IR spectra were taken on a Nicolet iN10 MX Fourier Transform Infrared Spectrometer. The steady-state absorption spectra were obtained with 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 (Φ=0.65) in ethanol, and the two photon absorption cross section of the probes was calculated at each wavelength relative to RhB as standard. Several lasers were used for activation *in vitro*, a solid-state 532 nm green laser (MGL-IV-532, 20 mW), a He-Ne 633 nm red laser (25LHP151-230, polarized, 5.0 mW, EUR 869) and a 488 nm blue laser (85BCD010-230, 10 mW). 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). 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. Two photon fluorescence microscopy images were performed on a modified Olympus Fluoview FV1000MPE microscope system equipped with an excitation light laser provided by a modelocked Ti: sapphire laser, (Mai Tai, Spectra-Physics Inc., USA).

1.2 Synthesis of J-1 and J-2

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



Scheme 1. Synthetic route for preparation of **J-1**: i) $BrCH_2CH_2Br$, $KHCO_3$, CH_3CN, reflux, 12 h; ii) BBr_3, DCM, -78 °C to rt., 16h; iii-1) $POCl_3$, DMF, 0°C to rt., 12 h; iii-2) H_2O; iv) NaMTS, KBr, CH_3CN, reflux, 24 h; v) $Zn(OAc)_2{\cdot}2H_2O$, 2,3-diaminomaleonitrile, EtOH, reflux, 8 h.



Scheme 2. Synthetic route for preparation of **J-2**: vi) mCPBA, -78 °C, 1 h, vii) 2, 3-diaminomaleonitrile, $Zn(OAc)_2{\cdot}2H_2O$, EtOH, reflux, 8 h.

N-(2-bromoethyl)-3-methoxy-N-methylaniline 2[1]

A reaction mixture of 3-methoxy-N-methylaniline (**1**, 2 g, 14 mmol), 1, 2-dibromoethane (27.5 g, 140 mmol) and $KHCO_3$ (1.7 g, 17 mmol) in acetonitrile (50 mL) was refluxed under nitrogen for 12 h. After evaporation, the residue was extracted with CH_2Cl_2, washed with brine and dried with anhydrous Na_2SO_4. Then the mixture was further purified by column chromatography and gave final product as yellow oil (3.2 g, 91%).

^{1}H NMR($CDCl_3$, 300 MHz), δ (ppm): 2.96（s, 3H), 3.49 (t, 2H, J=15.3Hz), 3.69 (t, 2H, J=15.3Hz), 4.91 (s, 3H), 6.20 (m, 3H), 7.01 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 160.94, 149.50, 130.13, 105.12, 101.61, 98.84, 55.18, 54.55, 38.75, 28.36.

3-((2-bromoethyl)(methyl)amino)phenol 3[1]

N-(2-bromoethyl)-3-methoxy-N-methylaniline (**2**, 3.2 g, 13 mmol) was dissolved in 10mL CH_2Cl_2, and boron tribromide (2 mL, 20 mmol) was added at -78℃. The mixture was warmed slowly to room temperature and stirred for 16 hours. Cold methanol was added to quench reaction. After evaporation and extraction, the residue was purified by column chromatography and gave final product as yellow oil (1.6 g, 54%).

^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.98 (s, 3H), 3.44 (t, 2H, J=15.0 Hz), 3.70 (t, 2H, J=15.0 Hz), 4.94 (s, 1H), 6.26 (m, 3H), 7.09 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 156.80, 149.72, 130.35, 104.84, 104.05, 99.17, 54.48, 38.75, 28.30.

4-((2-chloroethyl)(methyl)amino)-2-hydroxybenzaldehyde 4[1]

POCl3 (1.6 mL, 10 mmol) was added slowly into anhydrous DMF (5 mL) in ice-water bath and stirred for 30 minutes. Then 3-((2-bromoethyl) (methyl) amino) phenol (**3**, 1.6 g, 7 mmol) dissolved in anhydrous DMF was added in drops. The mixture was slowly warmed to room temperature and stirred overnight. Then the reaction solution was poured into ice, stirred for a few minutes, and filtered the precipitate and the final product was obtained(700 mg, 47%).

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.14 (s, 3H), 3.67 (t, 2H, J=13.2 Hz), 3.77 (t, 2H, J=13.2 Hz), 6.14 (d, 1H, J=2.4 Hz), 6.33 (dd, 1H, J_1=8.7 Hz, J_2=2.4 Hz), 7.35 (d, 1H, J=8.7 Hz), 9.58 (s, 1H), 11.58 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 192.77, 164.19, 154.60, 135.46, 112.36, 104.41, 97.65, 53.95, 39.24, 27.44. UV-*vis* (λ_{max}=341 nm, in DCM). IR (KBr pellete, cm^{-1}): ν 1633 (s, C=O).

1, 2, 3, 4-tetrahydro-4-methyl-6-carboxaldehyde -7-methoxy-1, 4-benzothiazine 5

A mixture of 4-((2-bromoethyl)(methyl)amino)-2-hydroxybenzaldehyde (**4**, 300 mg, 1 mmol), sodium methanethiosulfonate (NaMTS, 2 g, 15 mmol) and KBr (240 mg, 2 mmol) in acetonitrile (50 mL) was stirred and refluxed for 24 h. After evaporation, the residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution followed by water. The organic phase was then dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum yielding yellow oil, which was purified by column chromatography to give yellow solid (230 mg, 78%).

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.99 (m, 2H,), 3.08 (s, 3H), 3.73 (m, 2H,), 6.32 (d, 1H, J=10.2Hz), 7.12 (d, 1H, J=10.2Hz), 9.48 (s, 1H), 12.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 192.66, 158.64, 149.93, 131.33, 111.78, 104.47, 102.59, 52.31, 40.62, 23.54. UV-*vis* (λ_{max}=343 nm, in DCM). IR (KBr pellet, cm^{-1}), ν 1641(s, C=O).

1-oxide-1, 2, 3, 4-tetrahydro-4-methyl-6-carboxaldehyde-7-methoxy-1, 4-benzothiazine 6

1, 2, 3, 4-tetrahydro-4-methyl-6-carboxaldehyde-7-methoxy-1, 4-benzothiazine (**5**, 100 mg, 0.48 mmol) was dissolved in refreshed dichloromethane at -78 ^{o}C. m-chloroperoxybenzoic acid (mCPBA, 100 mg, 0.48 mmol) was added slowly and the mixed system was stirred for an hour. After washing with $NaHCO_3$ solution and brine, the organic phase was then dried over anhydrous Na_2SO_4 and purified by column chromatography to give white solid (88 mg, 78%).

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.60 (td, 1H, J_1=27.9 Hz, J_2=3.3 Hz), 3.14 (dt, 1H, J_1=13.8 Hz, J_2=9.9 Hz), 3.25 (s, 3H), 3.53 (dt, 1H, J_1=13.8 Hz, J_2=9.9 Hz), 4.26 (td, 1H, J_1=27.9 Hz, J_2=3.3 Hz), 6.40 (d, 1H, J=9.0 Hz), 7.46 (d, 1H, J=9.0 Hz), 9.55 (s, 1H), 12.66 (s, 1H). ^{13}C NMR ($CDCl_3$, 100

MHz), δ (ppm): 192.72, 158.70, 149.99, 131.39, 111.84, 104.53, 102.65, 58.85, 47.47, 23.60. UV-*vis* (λ_{max}=344nm, in DCM). IR (KBr pellete, cm^{-1}), ν 1633 (s, C=O).

J-1

Two Zn-salens were synthesized as method described by Buey. J.[2] A mixture of **5** (120 mg, 0.6 mmol), diaminomaleonitrile (30 mg, 0.3 mmol), and $Zn(OAc)_2 \cdot 2H_2O$ (130 mg, 0.6 mmol) was dissolved in 50 mL ethanol and refluxed for 8h. Most of the solvent was removed by rotary evaporator, and the product **J-1** was obtained as dark solid after filtration (137 mg, 86%).
^{1}H NMR (d^6-DMSO, 300 MHz), δ (ppm): 2.95 (m, 4H), 3.08 (s, 6H), 3.64 (m, 4H), 6.33 (d, 2H, *J*=9.3 Hz), 7.05 (d, 2H, *J*=9.3 Hz), 8.12 (s, 2H). HR ESI$^+$-MS, $[M+H]^+$: calcd. for C24H21N6O2S2Zn, 553.04534; found, 553.04551 (-0.3 ppm). UV-*vis* (λ_{max}=597 nm, in DMSO).
IR (KBr pellete, cm^{-1}), ν 2206 (w, C≡N), 1609 (m, C=N).

J-2

Method A (by photoconversion), **J-1**(15 mg, 0.027 mmol) was dissolved in THF (500 mL) and irradiated under sunlight. Solvent was removed by rotary evaporator, and the residue was recrystallized from methanol and dichloromethane to give **J-2** (8 mg, 49%).
Method B (synthetic), A mixture of **6** (80 mg, 0.36 mmol), diaminomaleonitrile (19 mg, 0.18 mmol), and $Zn(OAc)_2 \cdot 2H_2O$ (60 mg, 0.27 mmol) was dissolved in ethanol (20 mL) and refluxed for 8 hours. Most of the solvent was removed by rotary evaporator, and the product **J-2** was obtained as dark solid after filtration (200 mg, 55%).
^{1}H NMR (d^6-DMSO, 300 MHz), δ (ppm): 2.97 (m, 4H), 3.25 (s, 6H), 3.61 (m, 4H), 6.36 (d, 2H, *J*=9.0 Hz), 7.39 (d, 2H, *J*=9.0 Hz), 8.17 (s, 2H). HR ESI$^+$-MS, $[M+H]^+$ calcd. For C24H21N6O4S2Zn, 585.03571; found, 585.03562 (-2.7 ppm). UV-*vis* (λ_{max}=586 nm, in DMSO). IR (KBr pellete, cm^{-1}), ν 2214 (w, C≡N), 1599 (m, C=N).

1.3 Quantum yield of J-1 and J-2

Quantum yields of one photon emission of **J-1** and **J-2** were measured with Rhodamine B as reference (Φ=0.65).[3] The one photon fluorescence measurements were performed in 1cm quartz cells with 1 μM compound in DMSO 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[4]:

$$\frac{\Phi_{sample}}{\Phi_{ref}} = \frac{OD_{ref} I_{sample} d^2_{sample}}{OD_{sample} I_{ref} d^2_{ref}} \quad (Equation.\ S1)$$

I: integrated emission intensity.
OD: optical density at the excitation wavelength.
d: the refractive index of solvents. d_{DMSO}=1.478; $d_{Ethanol}$=1.361

1.4 Two photon absorption of J-2

The two photon absorption spectra of **J-2** were determined over a broad spectral region 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 ≤100fs, 80 MHz repetition rate, tuning range 720–870 nm Spectra Physics Inc., USA). The two photon fluorescence measurements were performed in a 1cm quartz cell with 30 μM **J-2** in DMSO and the excitation power density is set to be 100 mW. The quadratic dependence of two photon induced fluorescence intensity on the excitation power was verified (from 34 mW to 127 mW) for excitation wavelength at 840 nm. The two photon absorption cross section of the **J-2** (δ_{sample}) was calculated at each wavelength according to equation as following[5] :

$$\frac{\delta_{sample}}{\delta_{ref}} = \frac{\phi_{ref} C_{ref} I_{sample} d_{sample}}{\phi_{sample} C_{sample} I_{ref} d_{ref}} \quad (Equation.\ S2)$$

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; $d_{Ethanol}$=1.361

1.5 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, L6 myoblasts 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 543 nm, detector slit 552-617 nm, resolution 1024×1024, and a scan speed 0.5 frame per second.

1.6 Cytotoxicity assay

The cytotoxicity of both **J-1** and **J-2** were studied using 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method which is based on the ability of mitochondrial dehydrogenases of viable cells to cleave the tetrazolium rings of MTT forming dark purple membrane impermeable crystals of formazan that could be quantified from spectral measurement in DMSO. [6] 2×10^4 per well of L6 myoblasts in 96-well plates were incubated in 200 μL of complete medium for 24 h. Then aliquots of **J-1** and **J-2** stock solutions (2 mM in DMSO) were added to obtain final concentrations of 2, 5, 8, 10 μM and the same increasing percent of DMSO, and untreated cells were used as negative controls. The treated cells were incubated for an additional 24 h in dark at 37 °C. For phototoxicity of **J-1**, cells were irradiated with halogen lamp (7.8 mW/cm^2, 20 min, 9.6 J/cm^2). Subsequently, the cells were treated with 0.5 mg/mL of MTT and incubated for an additional 4 hours (37 °C, 5%CO_2). Then the loading media was removed and the cells were dissolved in DMSO (150 μL/well), and the absorbance at 570 nm was recorded. The cell viability (%) was calculated according to the following equation:

$$Cell\ viability(\%) = \frac{OD_{570,sample}}{OD_{570,control}} \times 100 \quad (Equation.\ S3)$$

where $OD_{570,\ sample}$ represents the optical density of the wells treated with various concentrations of **J-1** and **J-2** and corresponding volume of DMSO, $OD_{570,\ control}$ represents those of the wells for controls. Three independent trials were conducted, and the averages and standard deviations are reported.

1.7 Photoactivation assay

L6 myoblasts were placed onto poly-D-lysine coated glasses in complete media and the cells were incubated for 24 h. Cells were treated with 2 μM of **J-1** for an additional hour, keeping in dark. **J-1** was sensitive to light that we scanned quickly using 20% 543nm laser when focusing the cells. Then confocal images were recorded with 100% 543 nm laser (5 mW), pinhole 1.0 A.U. To acquire irradiation time-dependent images, the cells treated with **J-1** were activated at 543nm over 5 minutes. Visual field changed until more than 30 cells were imaged. Pictures were processed with ImageJ as well as the fluorescence intensities. Average fluorescence intensities and standard deviations are reported.

1.8 Colocalization assay

L6 myoblasts 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 **J-1** in chromatographic grade, anhydrous DMSO was prepared as 2 mM. The solution was diluted to a final concentration of 2 μM by complete growth medium. Stock solutions of Lyso Tracker Green DND-26, MitoTracker Green FM, Calcein AM and Hoechst33528 were prepared as 1mM, and the stock solution was diluted to the working concentrations in complete medium (Lyso Tracker: 75 nM, Mito Tracker: 100 nM, Calcein AM: 1 μM, Hoechst 33528: 1 μM). After incubation for an hour, 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, 543 nm excitation wavelength and 552 to 617 nm detector slit, 100% laser power for **J-1**, and 80% laser power for LysoTracker (ex: 488 nm, em: 505-560 nm), MitoTracker (ex: 488 nm, em: 505-560 nm), Calcein AM (ex: 488 nm, em: 505-560 nm)and Hoechst 33528 (ex: 405nm, em: 425-460 nm). Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ. The Pearson's Coefficient was calculated by ImageJ.

1.9 Emission spectra of activated J-1 and J-2 in living cells

The emission spectra of activated **J-1** and **J-2** in living cells were recorded by Laser Scanning Confocal Microscope (A1R-si, Nikon, Japan) and probes were excited at 543 nm. Camera settings were the same as described above with the exception of spectral detector mode with a scan resolution of 2.5 nm.

1.10 Two photon confocal microscopy image

Two photon fluorescence microscopy images were performed on a modified Olympus Fluoview FV1000MPE microscope system equipped with an excitation light laser provided by a modelocked Ti: sapphire laser, (Mai Tai, Spectra-Physics Inc., USA). The microscopy settings were as follows: 60× immersion water objective, a resolution of 512×512, 840 nm excitation wavelength and 570 to 620 nm detector slit, 30 % laser power (10 mW). L6 myoblasts were treated with 5 μM of **J-1** for 24 h and washed with prewarmed PBS buffer before photoirradiation at 840 nm for 0, 5, 10, 15, and 20 min. The quantified fluorescence intensities were calculated by ImageJ, as well as the reported standard deviations.

1.11 Warm culture and imaging

C. elegans (N2 strains) were maintained at 20 ℃ on nematode growth media (NGM) agar plates seeded with OP50 *Escherichia coli* (*E. coli*) media (B-growth media) as food supplement. Then the mixture of B-growth media (50 mL) and **J-1** solution was dropped onto the NGM agar plates and dried at room temperature for worm culture. The finial concentration of **J-1** was 5 μM. The worms were transferred onto the NGM agar plates seeded with the mixture above and incubated for 24 h at 20 ℃. For imaging and photoactivation, the worms were transferred onto an agar pad on a glass slide, anesthetized with 0.1 M NaN_3, and then sealed with a glass cover slip. Other imaging operations were as the same as those described before.

2. Computational photophysics properties for J-1 and J-2

For the theoretical study of excited state photophysics of **J-1** and **J-2**, 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[7]) was used with Lanl2dz pseudopotential[8] basis set for Zn, 6-31G** for main group elements, as implemented in the Gaussian 09 package[9]. Geometries for **J-1** and **J-2** were fully optimized without symmetry constraints. The solvent effect was involved through the PCM approach[10] (DMSO, ε=46.826). The vibrational 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 two molecules.

Table S1. Calculated electronic transitions energies for **J-1** and **J-2** obtained from TD-DFT calculations with PCM solvation model.

Transitions	λ_{cal} (nm)	λ_{exp} (nm)	f	CI expansion coefficients
J-1				
S_0–S_1	617		0.0035	HOMO-1→LUMO (99%)
S_0–S_2	614		0.2709	HOMO-2→LUMO (14%), HOMO→LUMO (86%)
S_0–S_3	545	597	0.7210	HOMO-2→LUMO (85%), HOMO→LUMO (14%)
S_0–S_4	435		0.1689	HOMO-3→LUMO (84%), HOMO→LUMO+1 (12%)
S_0–S_5	385		0.0889	HOMO-1→LUMO+1 (97%)
J-2				
S_0–S_1	551	586	0.9721	HOMO→LUMO (100%)
S_0–S_2	488		0.0070	HOMO-1→LUMO (92%)
S_0–S_3	478		0.0059	HOMO-2→LUMO (99%)
S_0–S_4	432		0.1621	HOMO-3→LUMO (81%), HOMO→LUMO+1 (11%)

S_0–S_5	402	0.0387	HOMO-5→LUMO (96%)

XYZ Coordinates (angstrom)

J-1

C	-2.85131368	-0.16248362	-0.01004761
C	-5.66724349	0.21628890	0.02512076
C	-3.73775138	-1.27354999	-0.01563716
C	-3.41405345	1.17400219	-0.00263808
C	-4.83358642	1.29883075	0.00725912
C	-5.14200234	-1.12034599	0.02979769
H	-5.26245859	2.29733099	0.01445517
H	-6.73570575	0.37536145	0.05820161
C	3.41405288	1.17400153	-0.00263806
C	5.14200249	-1.12034611	0.02979358
C	4.83358599	1.29883042	0.00724263
C	2.85131319	-0.16248446	-0.01003169
C	3.73775113	-1.27355067	-0.01562575
C	5.66724343	0.21628884	0.02510200
H	5.26245801	2.29733080	0.01442665
H	6.73570606	0.37536167	0.05816869
C	-2.66958274	2.37196922	-0.00611995
H	-3.25793897	3.29469064	-0.00570989
C	0.69550706	3.68060227	-0.01434239
C	-0.69550735	3.68060262	-0.01434329
C	2.66958208	2.37196843	-0.00612227
H	3.25793824	3.29468987	-0.00572050
N	1.34989952	2.46857737	-0.01091161
N	-1.34990030	2.46857806	-0.01091522
Zn	0.00000000	0.79392087	-0.01435217
O	-1.57557233	-0.41441153	-0.01110590
O	1.57557195	-0.41441276	-0.01106789
C	-1.41255937	4.91931565	-0.01757516
N	-2.03735114	5.90283828	-0.02010702
C	1.41255950	4.91931503	-0.01757353
N	2.03735139	5.90283759	-0.02010452
N	-6.01121632	-2.18573609	0.07035618
N	6.01121732	-2.18573551	0.07035035
C	-7.45202267	-1.96056540	0.15857085
H	-7.79952448	-1.31904170	-0.65571856
H	-7.96048622	-2.92023433	0.06472536
H	-7.75009863	-1.50675000	1.11319427
C	7.45202420	-1.96056274	0.15855168

H	7.96048771	-2.92023286	0.06471842
H	7.79952056	-1.31905154	-0.65575014
H	7.75010615	-1.50673216	1.11316593
C	-5.53737487	-3.53178293	0.39557482
H	-5.30389679	-3.62087699	1.46562480
H	-6.35167748	-4.22751057	0.17856068
S	-2.89173004	-2.84837740	-0.07553737
S	2.89172964	-2.84837868	-0.07550680
C	5.53737958	-3.53178150	0.39557797
H	5.30391235	-3.62087226	1.46563059
H	6.35168023	-4.22750958	0.17855778
C	4.32253195	-3.92362997	-0.43484635
H	4.56328087	-3.88391525	-1.50026928
H	4.00891191	-4.94020210	-0.18654406
C	-4.32253579	-3.92362845	-0.43486345
H	-4.56329647	-3.88391148	-1.50028365
H	-4.00891250	-4.94020102	-0.18656703

J-2

C	2.84970100	-0.01355000	0.00198900
C	5.67206200	0.36387600	0.05604300
C	3.73981600	-1.12455500	-0.05022000
C	3.41376300	1.32425500	0.03149200
C	4.83210700	1.44268400	0.04395800
C	5.14630700	-0.97051000	0.03565900
H	5.26155600	2.44100600	0.06428100
H	6.73975700	0.52486200	0.10392100
C	-3.41370600	1.32419500	-0.03133600
C	-5.14630300	-0.97044100	-0.03545100
C	-4.83207200	1.44270000	-0.04339000
C	-2.84965200	-0.01363000	-0.00194600
C	-3.73982200	-1.12459700	0.05021700
C	-5.67208500	0.36395100	-0.05534400
H	-5.26144500	2.44105900	-0.06353300
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C	0.69509200	3.83136100	0.01085100
C	-2.66918600	2.52369500	-0.03524300
H	-3.25797000	3.44597300	-0.04557400
N	-1.35067200	2.61924200	-0.01964800
N	1.35074400	2.61930600	0.01948400
N	5.99993000	-2.03376900	0.12880800

N	-5.99983600	-2.03366200	-0.12909100
Zn	0.00002100	0.94731000	-0.00043600
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N	-2.03735500	6.05313000	-0.03590800
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C	-5.46035600	-3.35425400	-0.45571900
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H	-6.26589000	-4.08433900	-0.35882300
H	-5.12248600	-3.38270400	-1.50348300
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H	4.00900100	-4.76853600	-0.30990400
C	5.46071600	-3.35460900	0.45480900
H	5.12370900	-3.38405400	1.50282200
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3. Other supporting figures

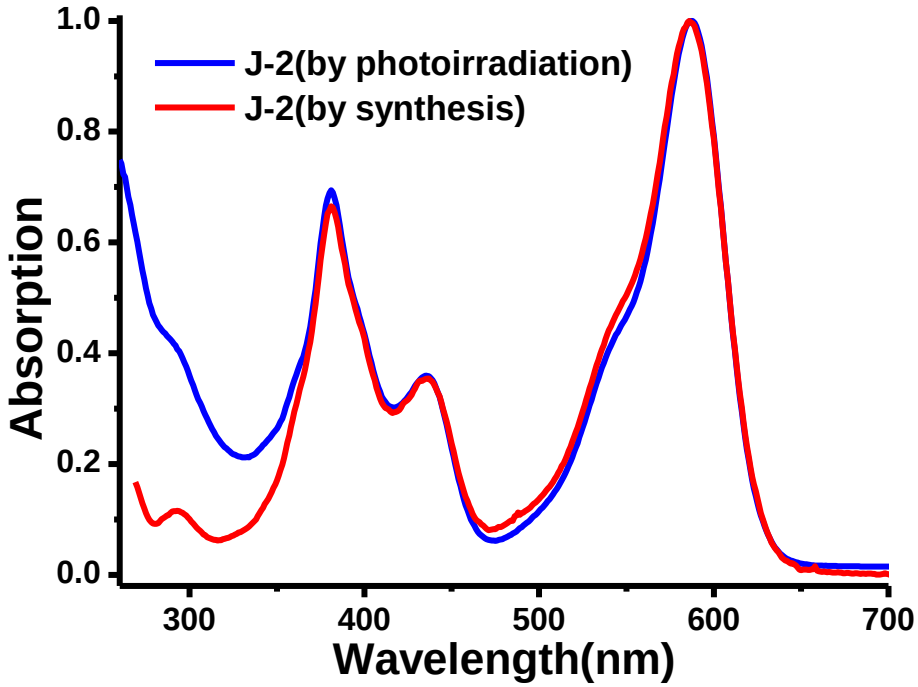


Figure S1. UV-*vis* spectra of synthetic **J-2** and photoactivated **J-1**.

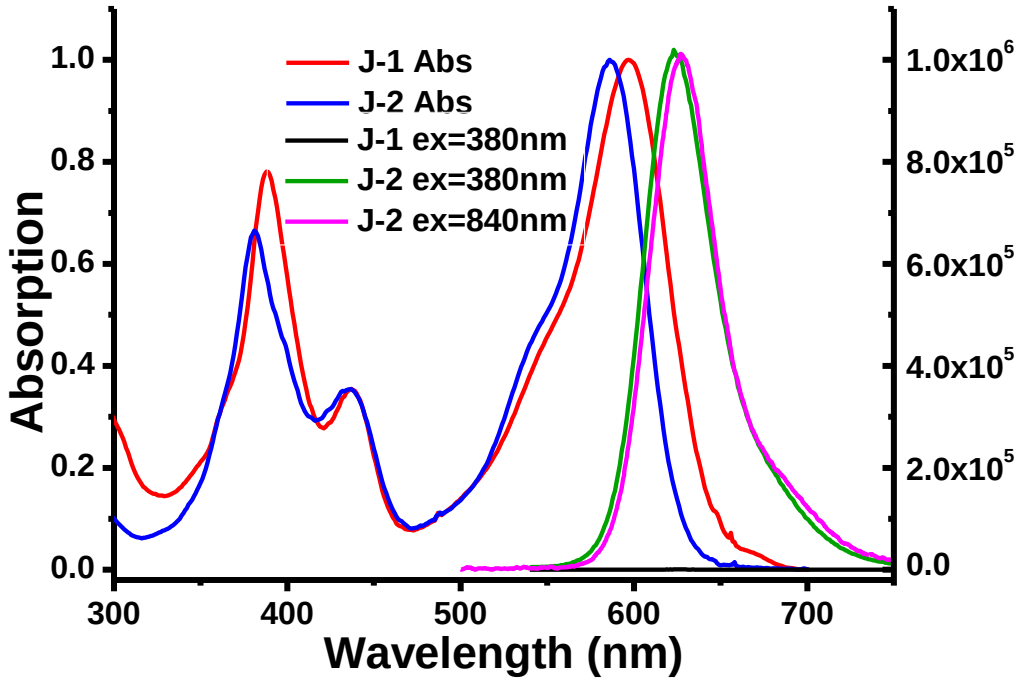


Figure S2. Absorption and emission spectra of **J-1** and **J-2**. Left: Normalized UV-*vis* spectra of **J-1** and **J-2**. Right: one- (green) and two- (violet) photon induced emission spectra of **J-2** and one photon emission of **J-1** (black).

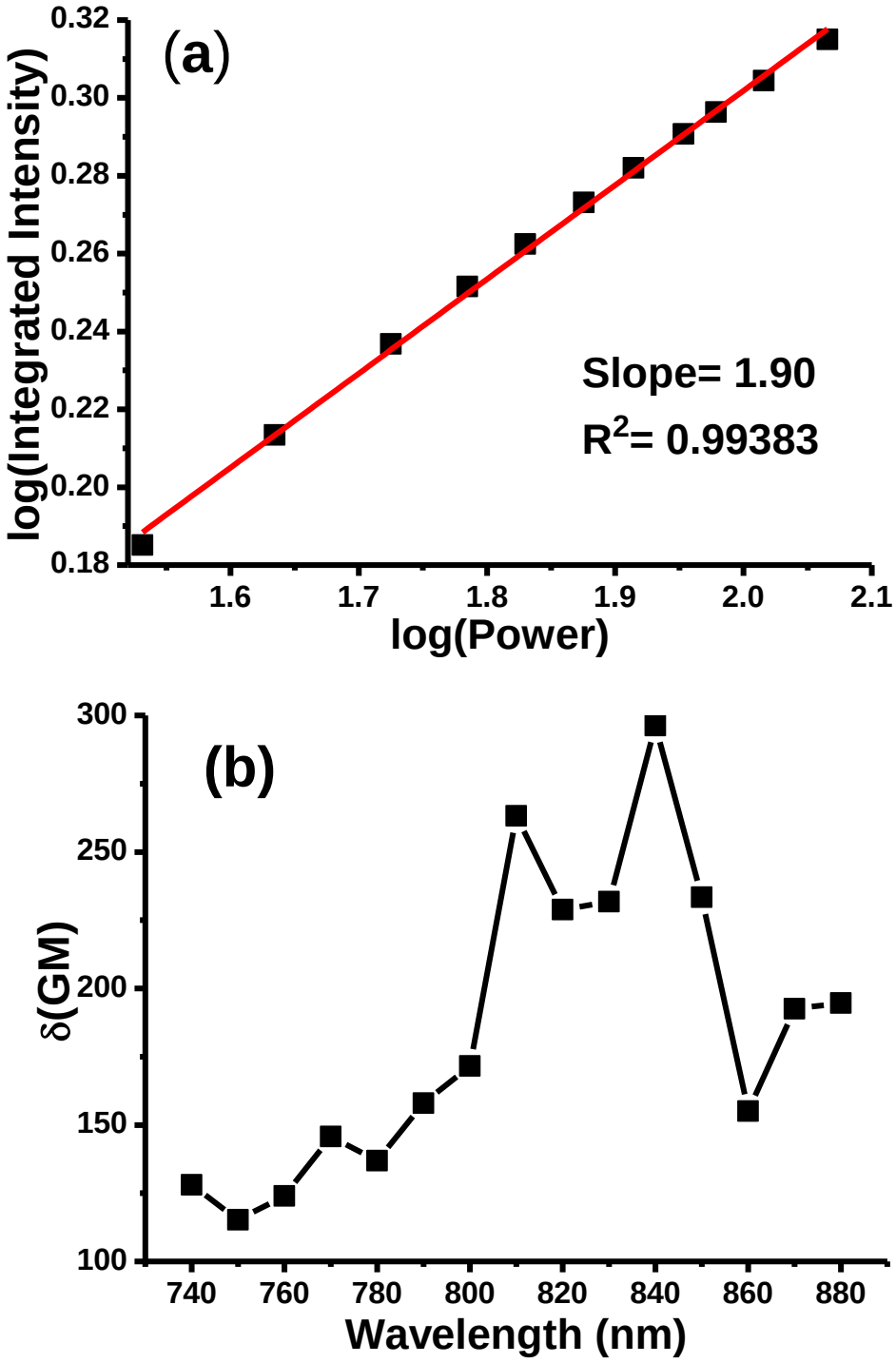


Figure S3. Two photon properties of **J-2**. (a) Power dependent response curve of **J-2** in ethanol (20 μM), carried out at 840 nm. (b) Two photon induced absorption cross-section of **J-2** with Rhodamine B as reference.

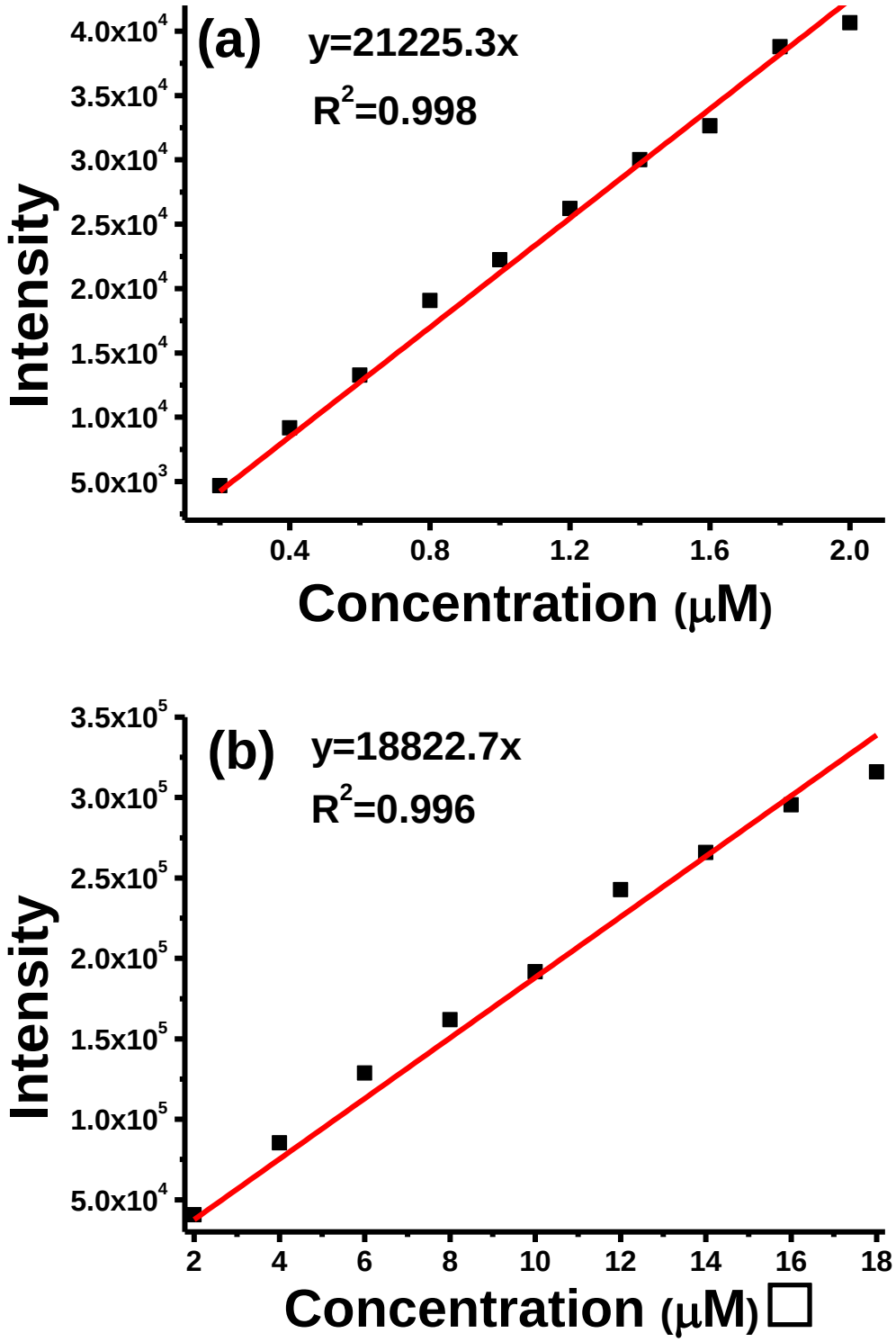


Figure S4. Standard curve for calculating activated **J-1** by relating the fluorescence intensity to concentration of J-2 in DMSO (λ_{ex}=380 nm, λ_{em}=523 nm).

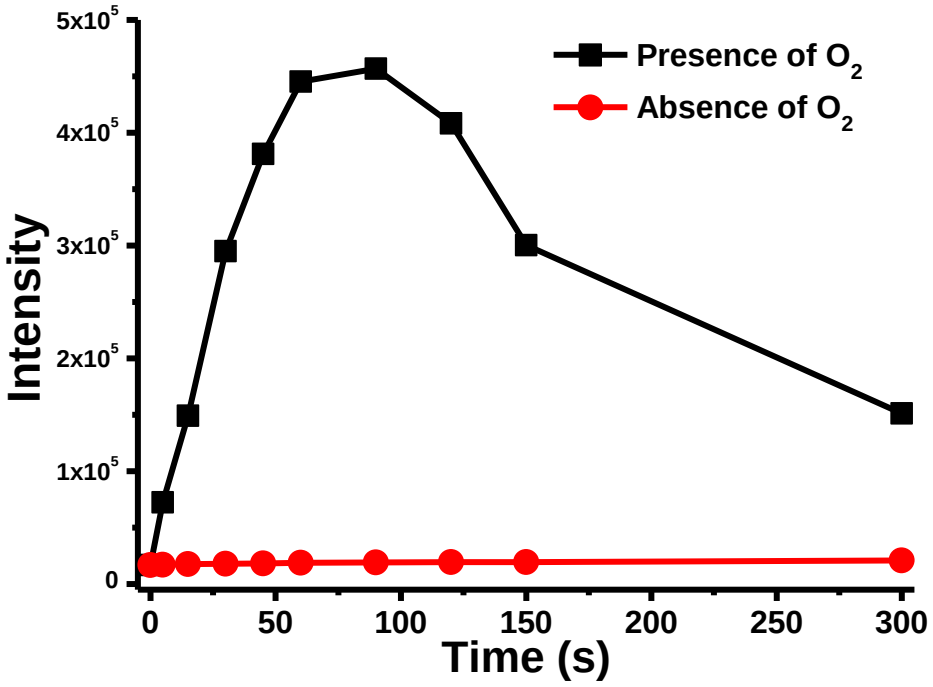


Figure S5. Oxygen effect on photoactivation of **J-1**. (30 μM of **J-1** in DMSO was irradiated over a period of time in the presence or absence of oxygen, λ_{ex}=380 nm, λ_{em}=523 nm).

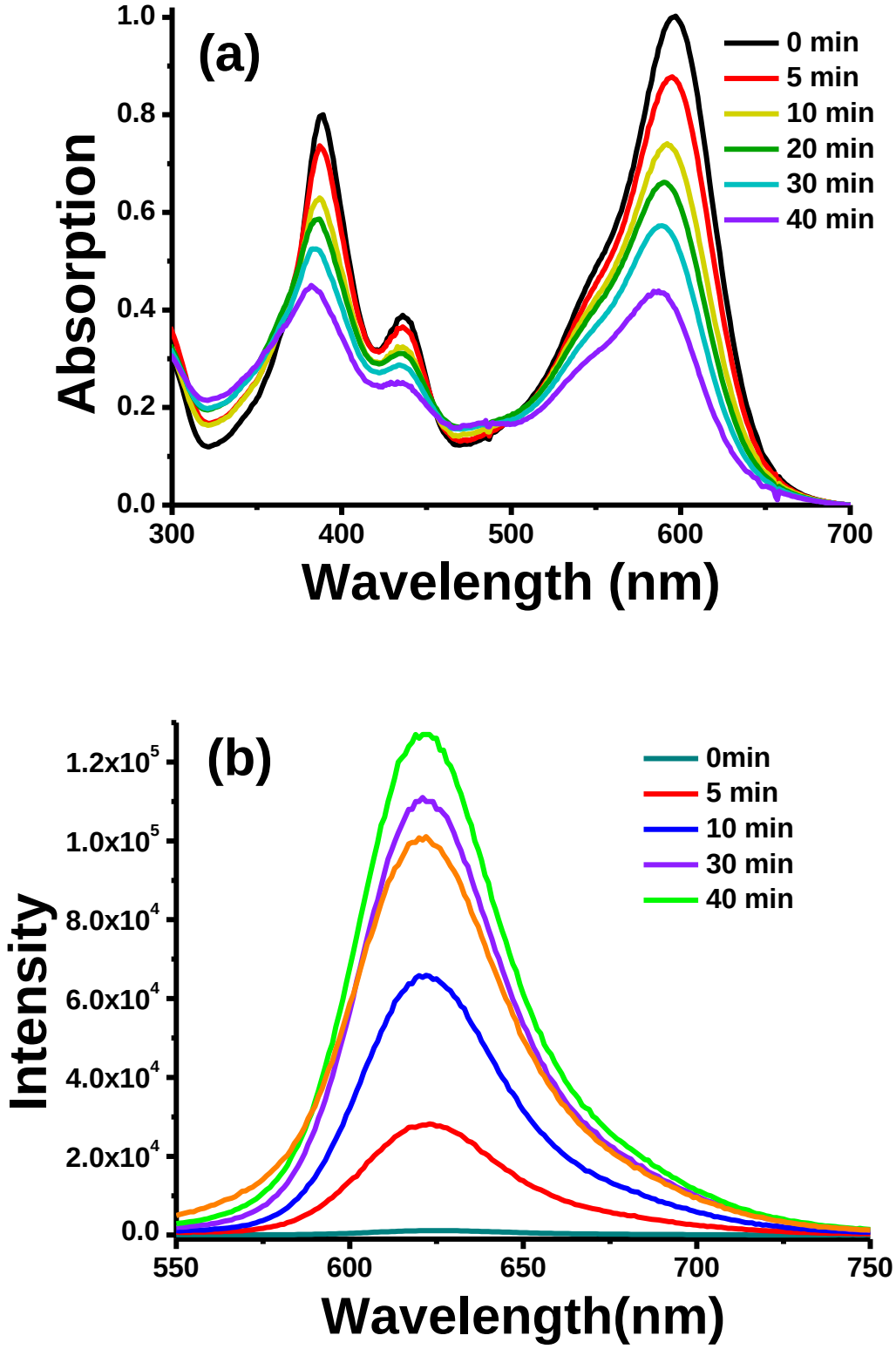


Figure S6. Spectroscopic changes of **J-1** upon two photon irradiation by 800 nm laser (300mW): (a) UV-vis absorption spectra (b) emission spectra, λ_{ex}=380 nm.

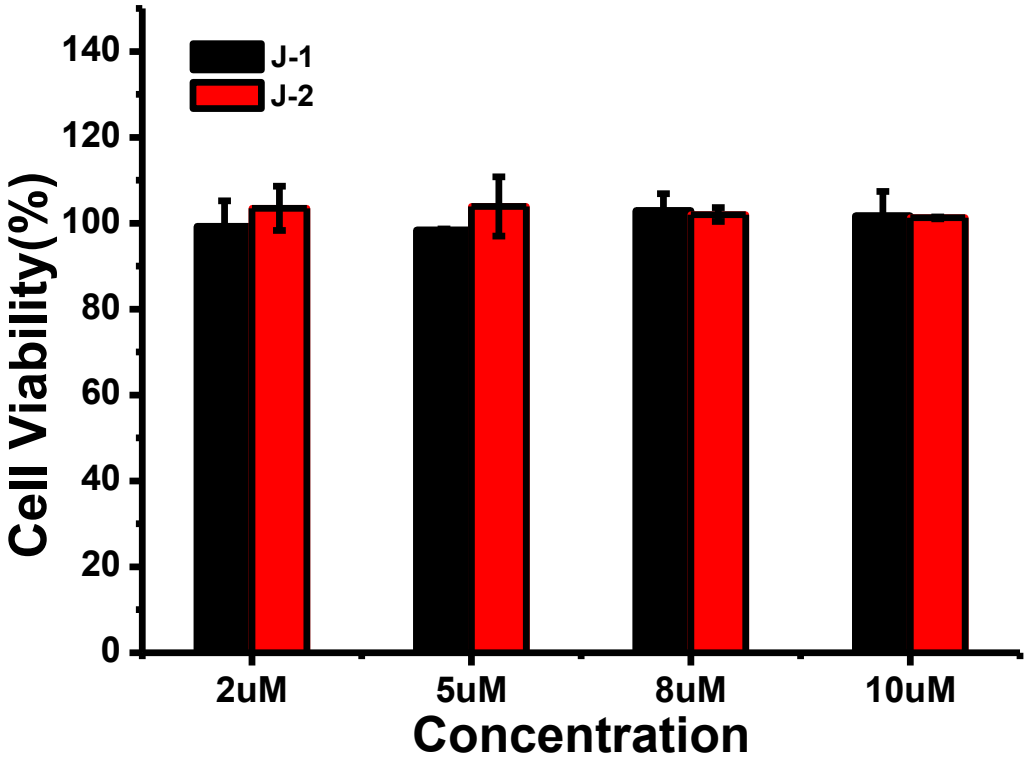


Figure S7. Viability of L6 myoblasts treated with of **J-1** and **J-2.** 2 μM, 5 μM, 8 μM or 10 μM probes were treated with L6 myoblasts for 24 hours. Cells were treated with 0.5 mg/mL of MTT and incubated for an additional 4 hours. Absorbance at 570 nm was recorded and the cell viability (%) was calculated.

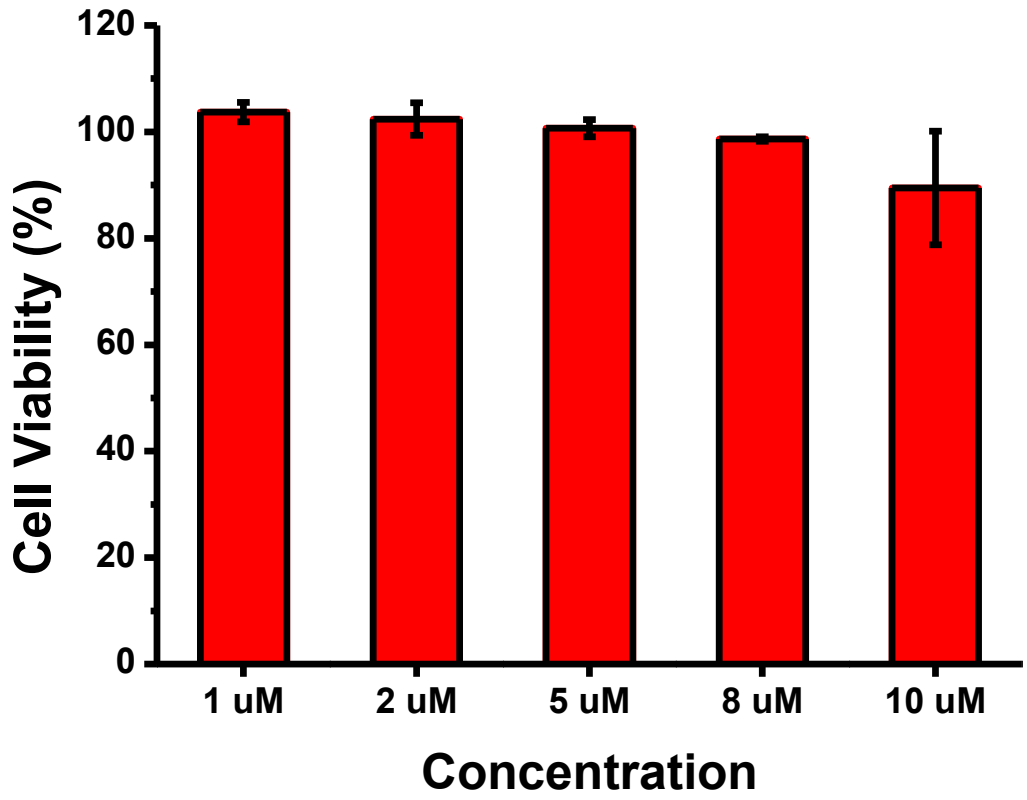


Figure S8. Phototoxicity of J-1 in L6 myoblasts. 1μM, 2 μM, 5 μM, 8 μM or 10 μM **J-1** were treated with L6 myoblasts for 24 hours and then irradiated with halogen lamp (7.8 mW/cm^2, 20 min, 9.6 J/cm^2). After 24 h, cells were treated with 0.5 mg/mL of MTT and incubated for an additional 4 hours. Absorbance at 570 nm was recorded and the cell viability (%) was calculated.

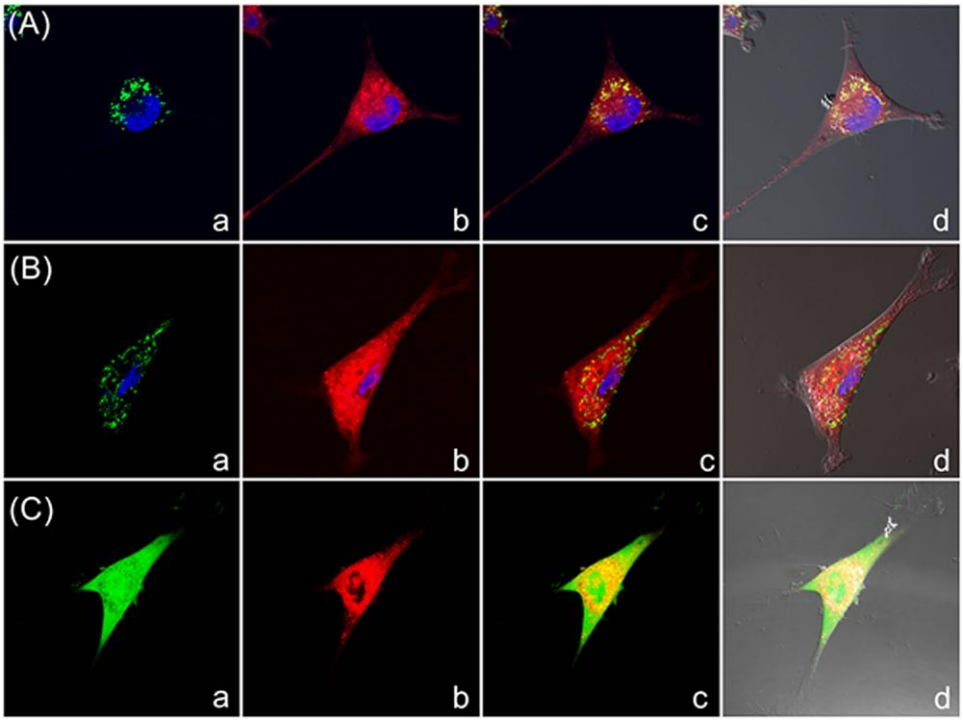


Figure S9. Colocalization assay of **J-1**. L6 myoblasts were treated with 2 μM **J-1** for an hour. LysoTracker Green DND-26, MitoTracker Green or Calcein AM was then maintained in dark at 37 ^{o}C, and corresponding confocal image was taken before washed with prewarmed PBS buffer. (a) Confocal fluorescence image of cellular trackers; (b) Confocal fluorescence image of **J-1**; (c) Merged confocal fluorescence image of **J-1** and trackers; (d) Merged image of differential interference contrast (DIC) image and (c). Pearson's Coefficients are 0.54, 0.16, and 0.78 respectively.

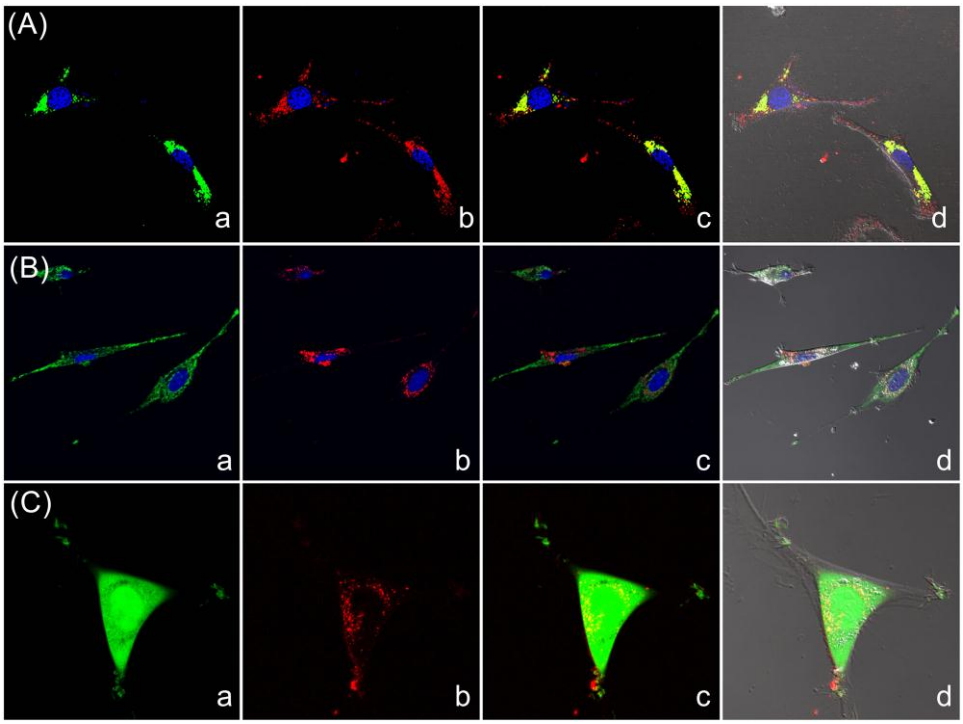


Figure S10. Colocalization assay of **J-2**. L6 myoblasts were treated with 2μM **J-2** for an hour in dark at 37°C. LysoTracker Green DND-26, MitoTracker Green or Calcein AM was then maintained in dark at 37°C, and corresponding confocal images were taken before washed with PBS buffer. (a) Confocal fluorescence image of cellular trackers; (b) Confocal fluorescence image of **J-2**; (c) Merged confocal fluorescence image of **J-2** and trackers; (d) Merged image of differential interference contrast (DIC) image and (c). Pearson's Coefficients are 0.73, 0.05, and 0.34 respectively.

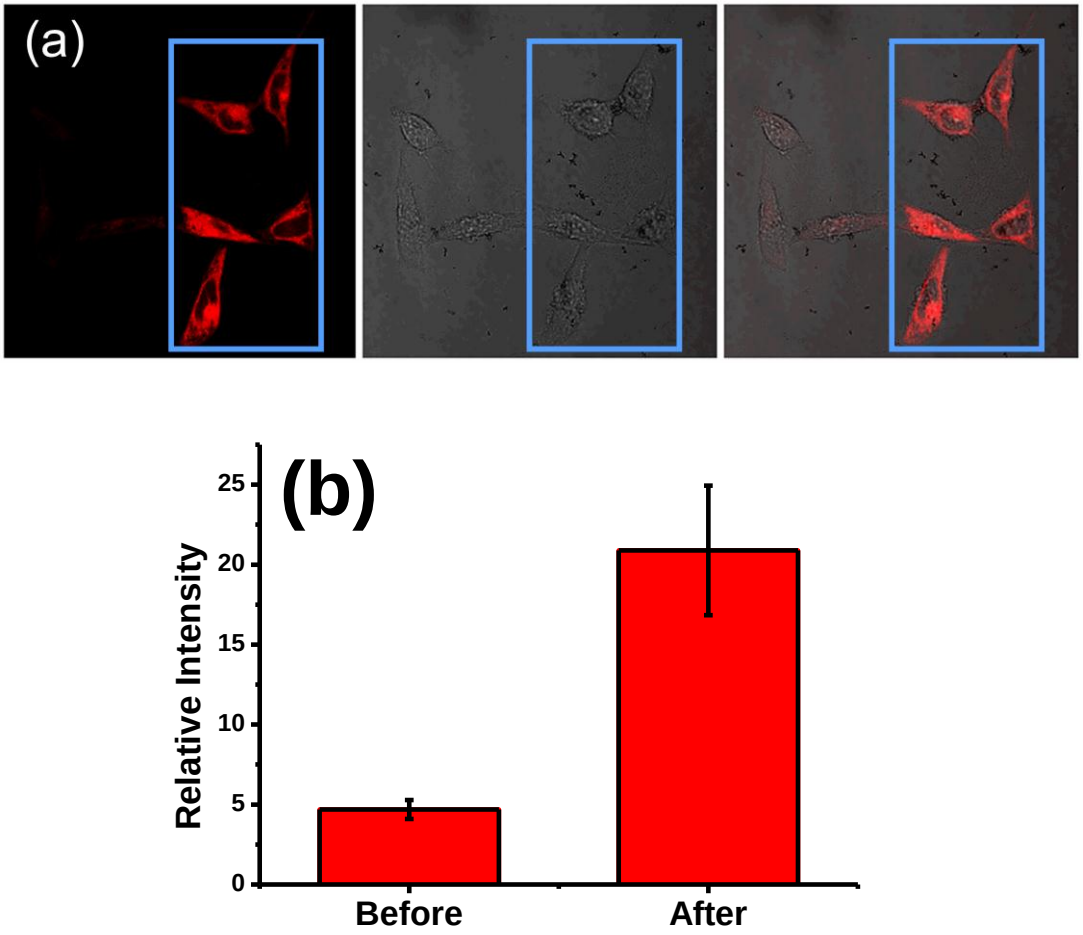


Figure S11. Spatially controlled photoactivation of **J-1** in cells. (a) Confocal images of treated L6 myoblasts. (b) Intensity comparison of cells with or without photoactivation. Cells were treated with 2 μM **J-1** for an hour and washed with PBS before imaging. Cells in blue area were exposed to 543nm for 2min before imaging. The fluorescence intensities of activated cells were quantified using Image J program and compared to those of the cells unactivated.

4. FT-IR spectra of J-1 and J-2

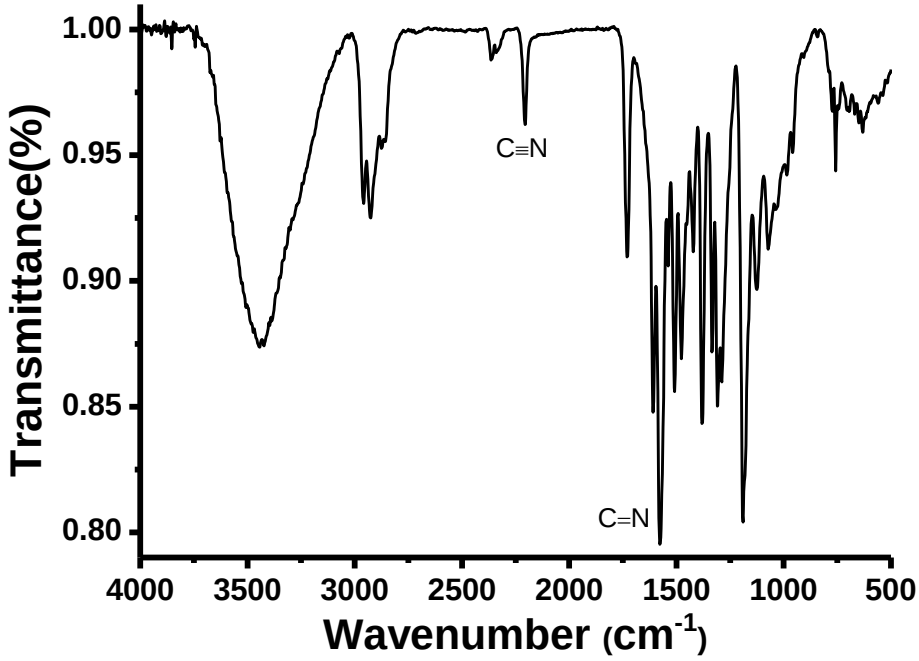


Figure S13. FT-IR spectrum (KBr pallate) of **J-1**.

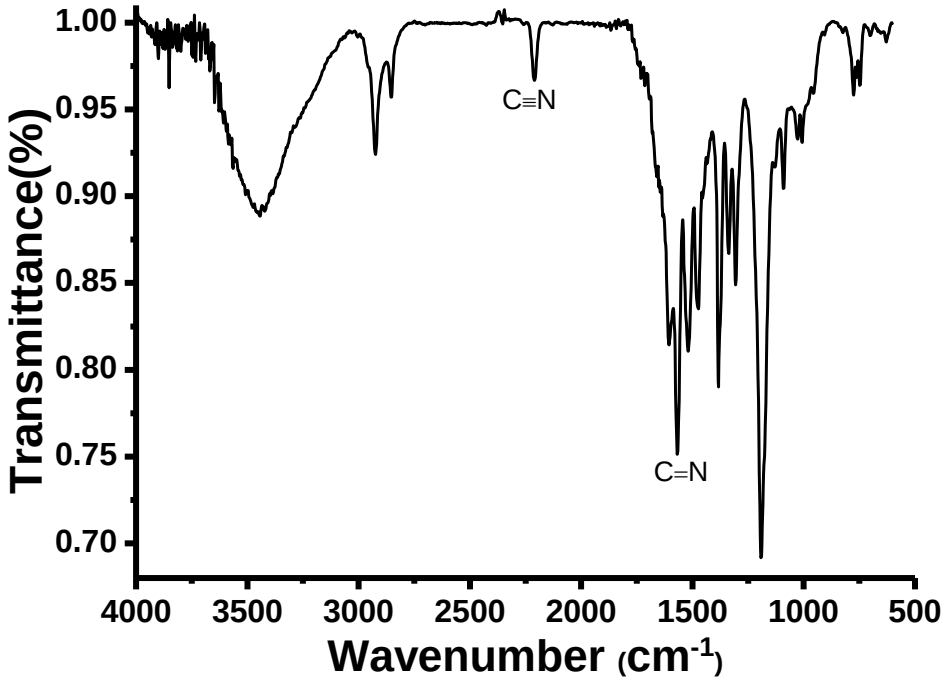


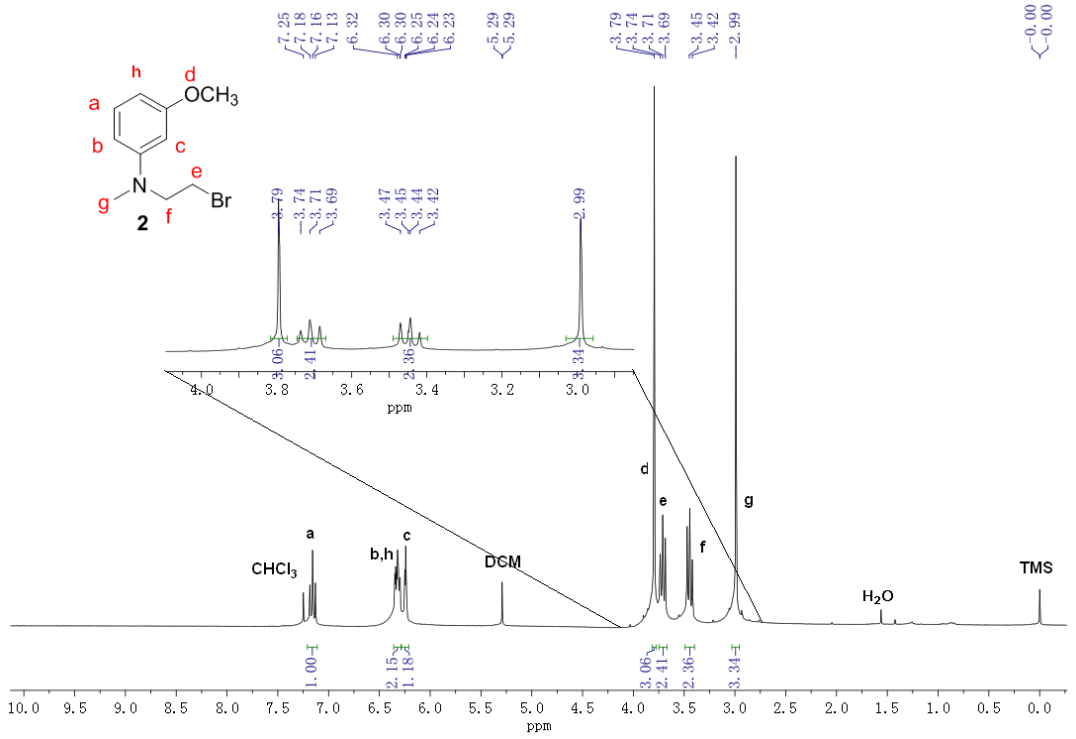
Figure S14. FT-IR spectrum (KBr pallate) of **J-2**

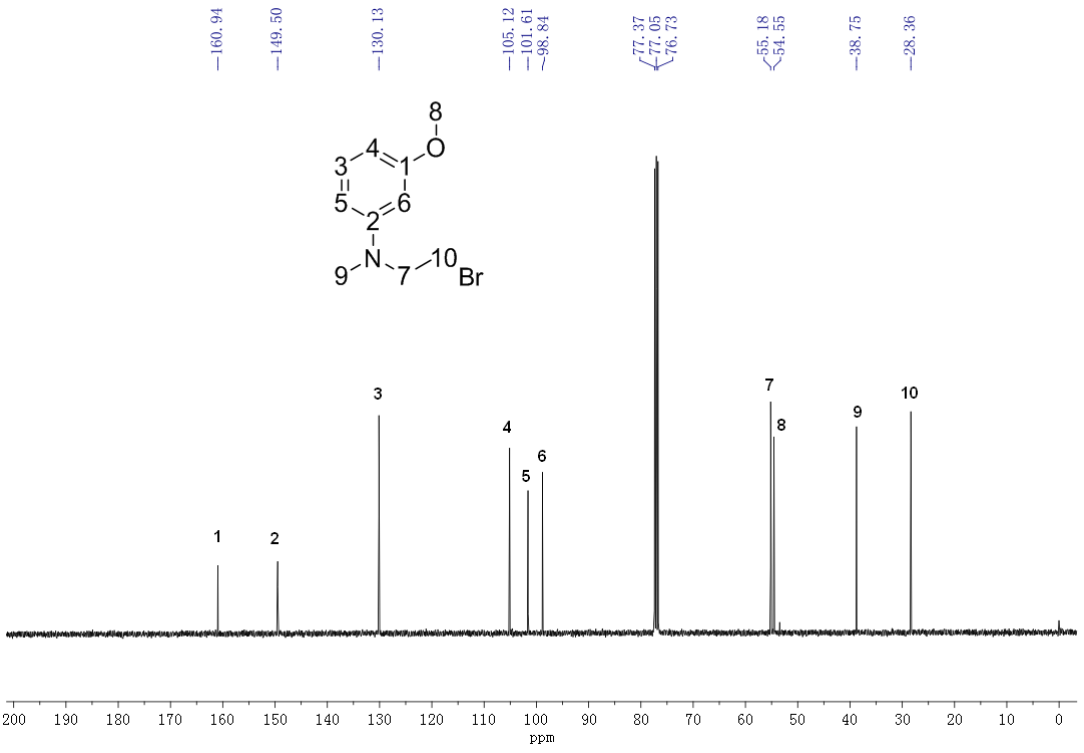
5. ^{1}H NMR and ^{13}C NMR spectra

Compound 2

^{1}H NMR($CDCl_3$, 300 MHz), δ (ppm): 2.96（s, 3H), 3.49 (t, 2H, J=15.3 Hz), 3.69 (t, 2H, J=15.3 Hz), 4.91 (s, 3H), 6.20 (m, 3H), 7.01 (m, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 160.94, 149.50, 130.13, 105.12, 101.61, 98.84, 55.18, 54.55, 38.75, 28.36.

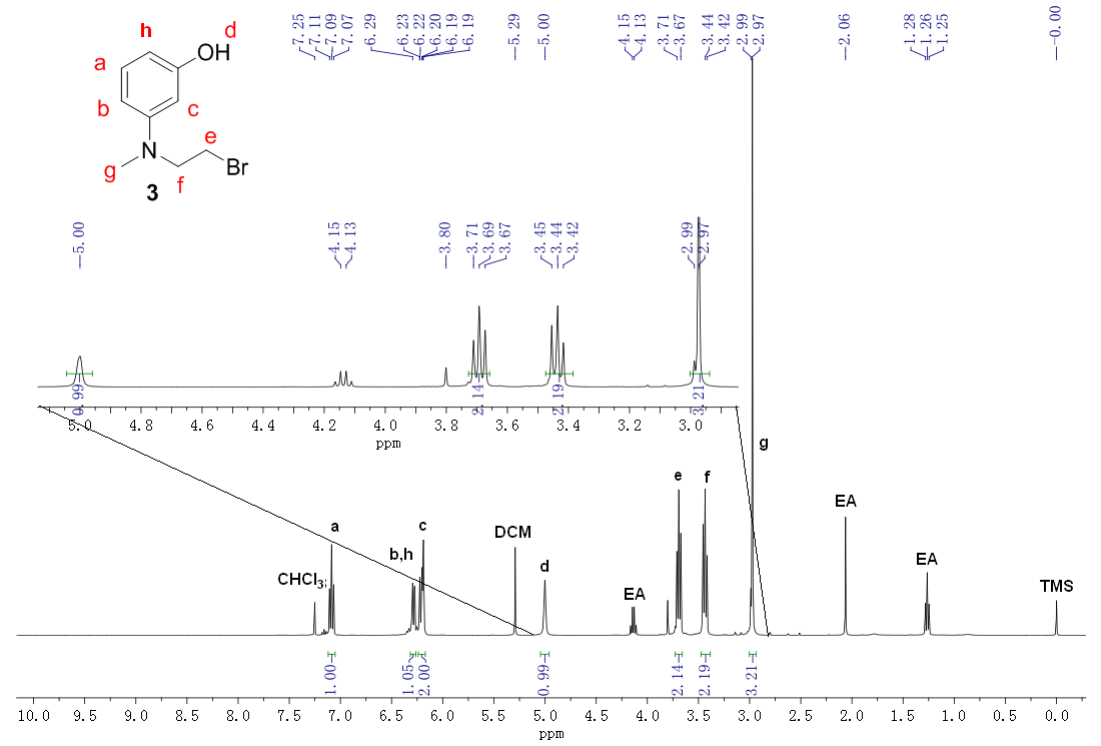


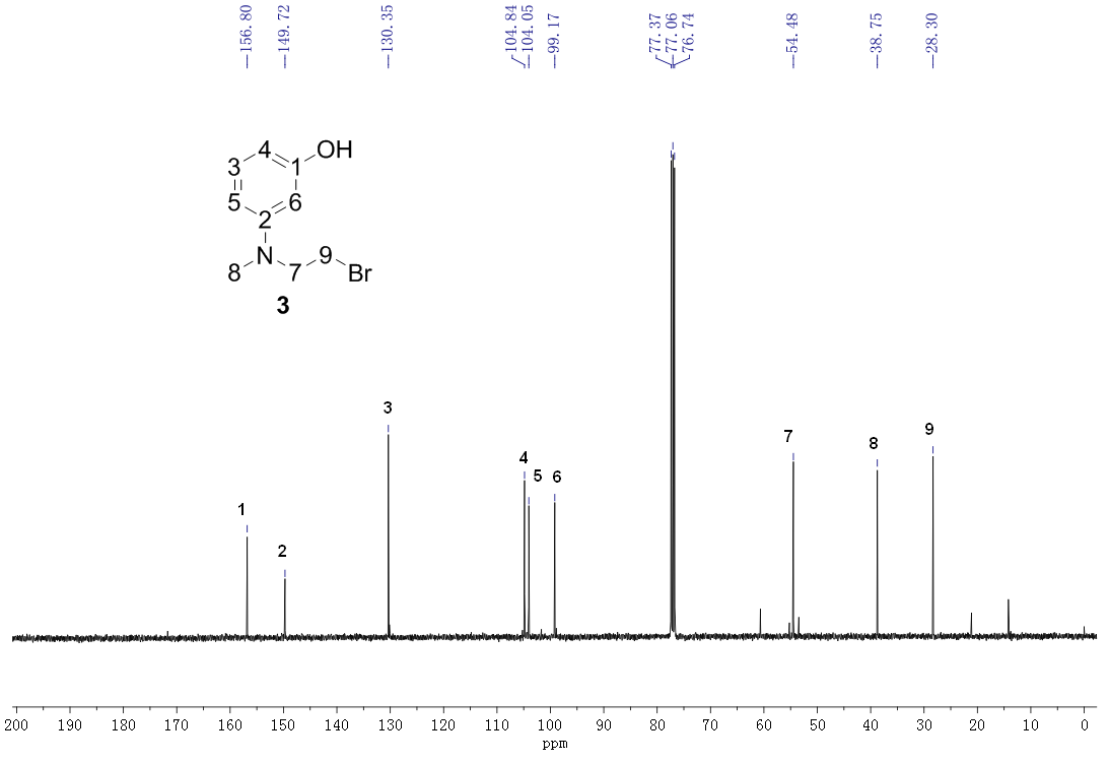


Compound 3

^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.98 (s, 3H), 3.44 (t, 2H, J=15.0 Hz), 3.70 (t, 2H, J=15.0 Hz), 4.94 (s, 1H), 6.26 (m, 3H), 7.09 (m, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 156.80, 149.72, 130.35, 104.84, 104.05, 99.17, 54.48, 38.75,28.30.

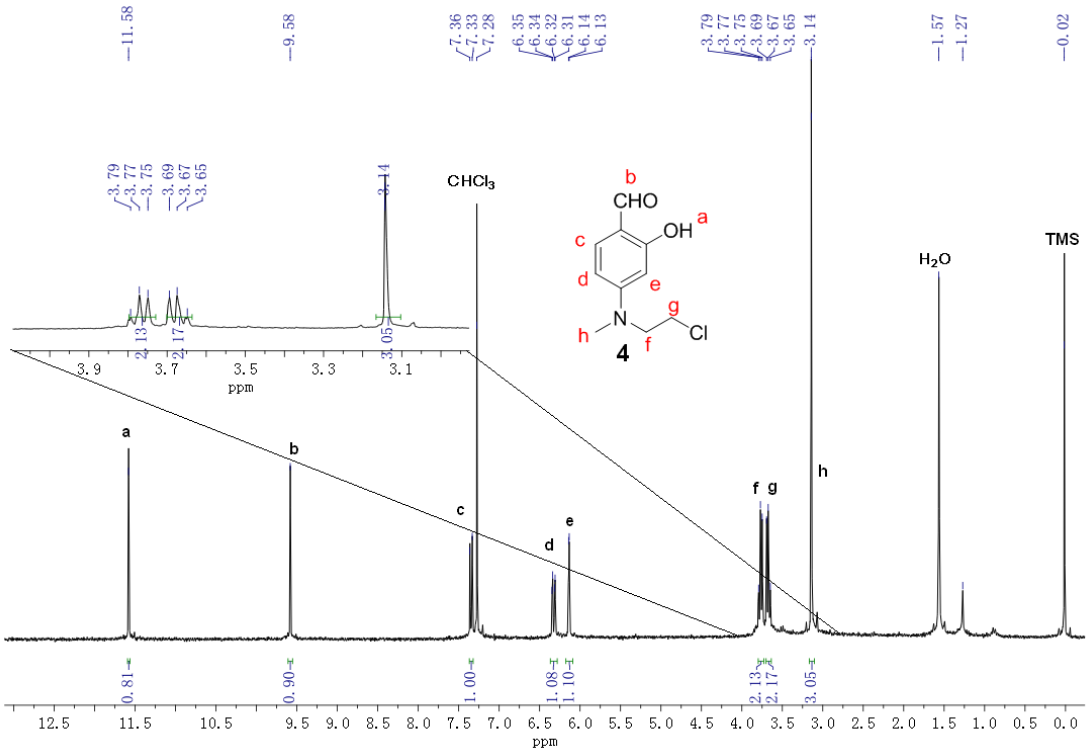


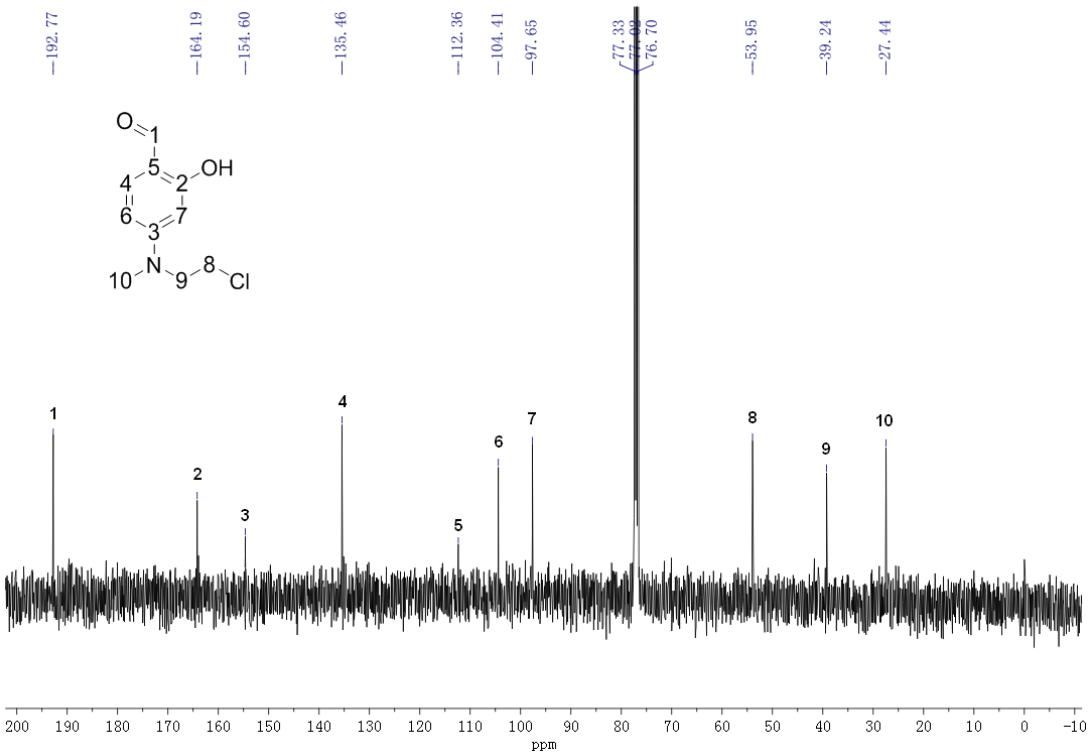


Compound 4

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 3.14 (s, 3H), 3.67 (t, 2H, J=13.2 Hz), 3.77 (t, 2H, J=13.2 Hz), 6.14 (d, 1H, J=2.4 Hz), 6.33 (dd, 1H, J_1=8.7 Hz, J_2=2.4 Hz), 7.35 (d, 1H, J=8.7 Hz), 9.58 (s, 1H), 11.58 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 192.77, 164.19, 154.60, 135.46, 112.36, 104.41, 97.65, 53.95,39.24,27.44.

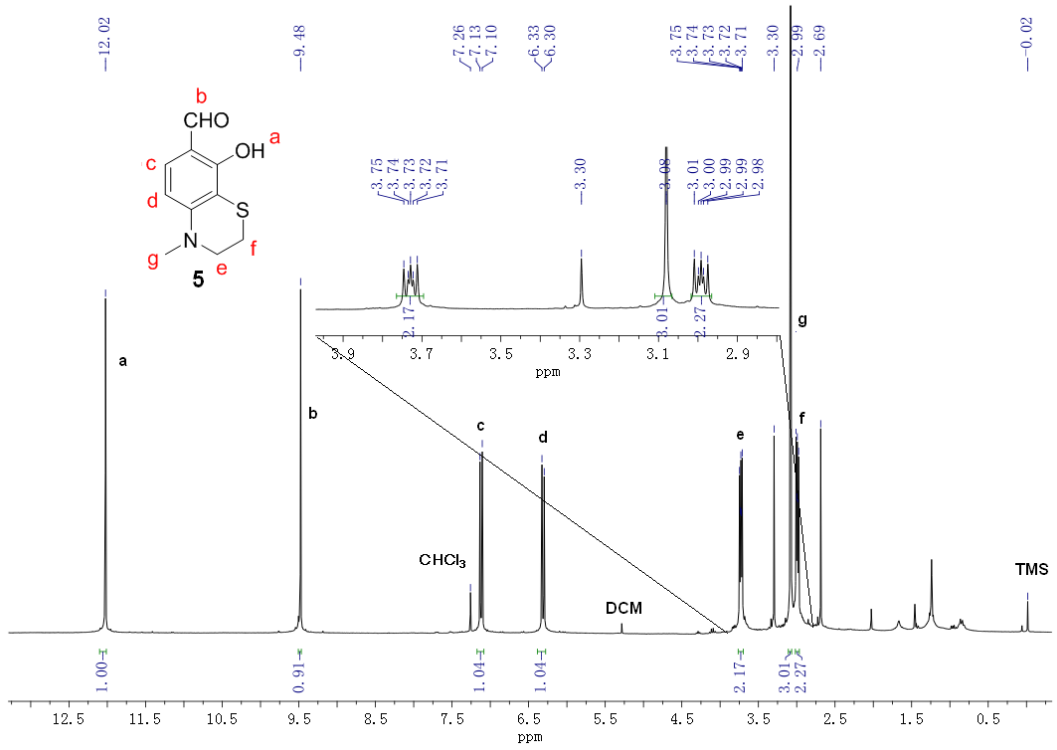


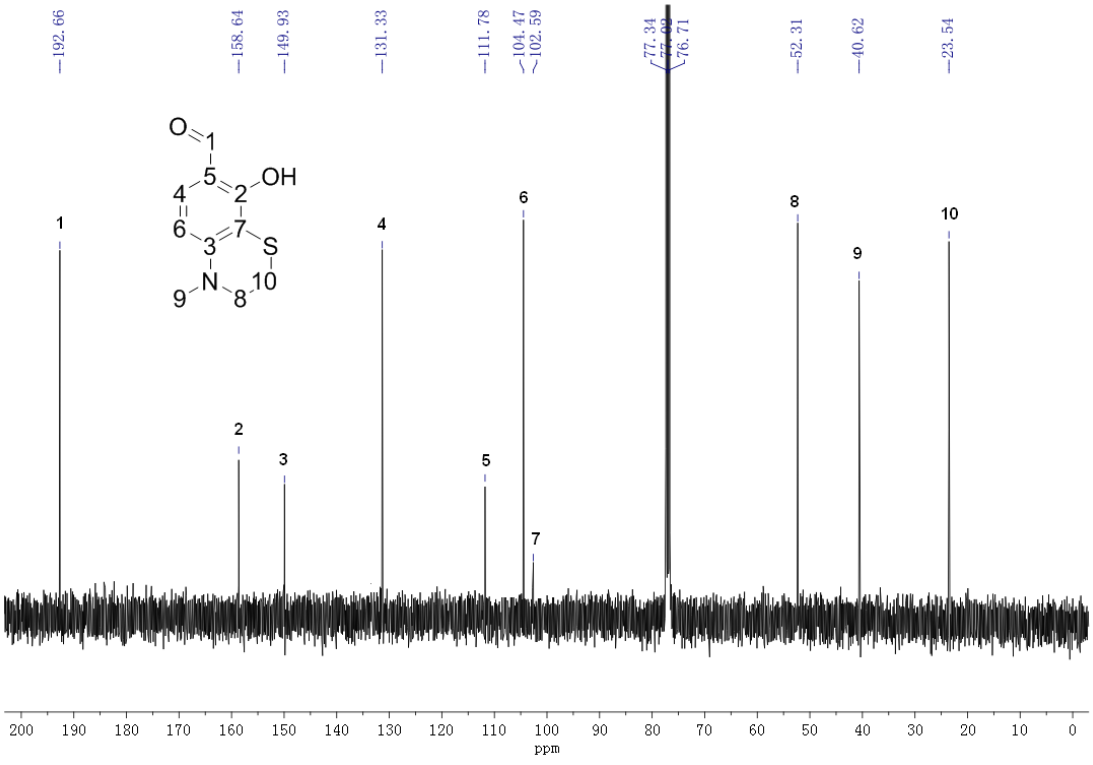


Compound 5

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.99 (m, 2H,), 3.08 (s, 3H), 3.73 (m, 2H,), 6.32 (d, 1H, *J*=10.2Hz), 7.12 (d, 1H, *J*=10.2Hz), 9.48 (s, 1H), 12.02 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 192.66, 158.64, 149.93, 131.33, 111.78, 104.47, 102.59, 52.31, 40.62,23.54.

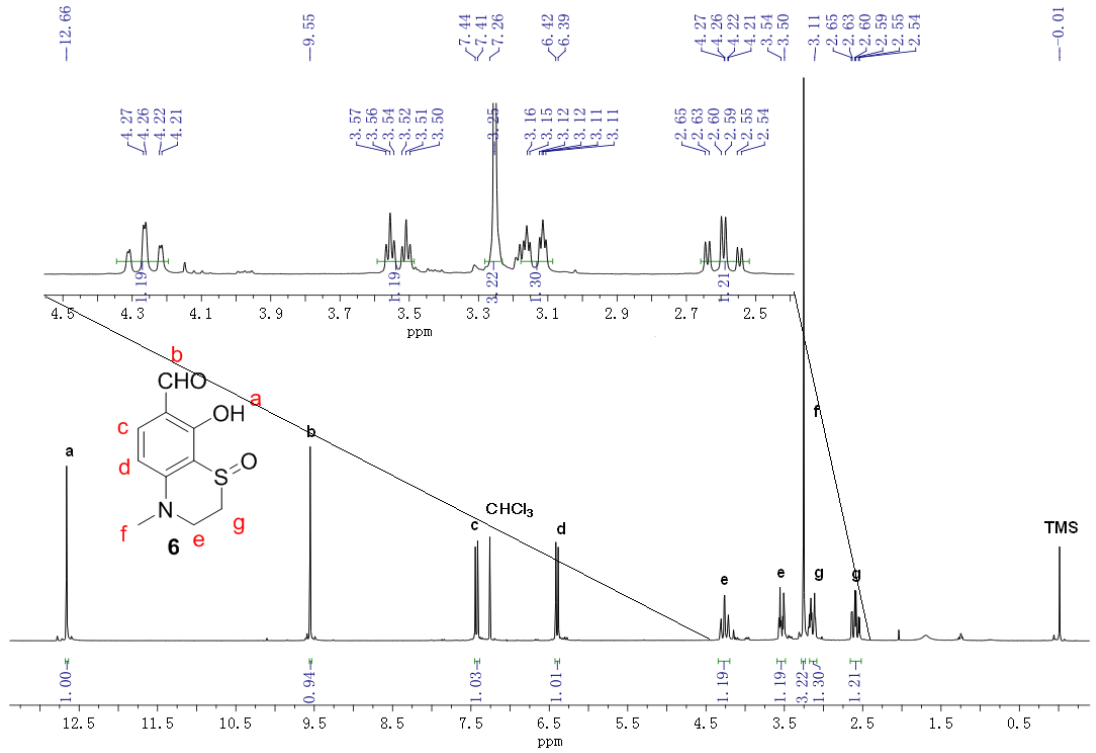


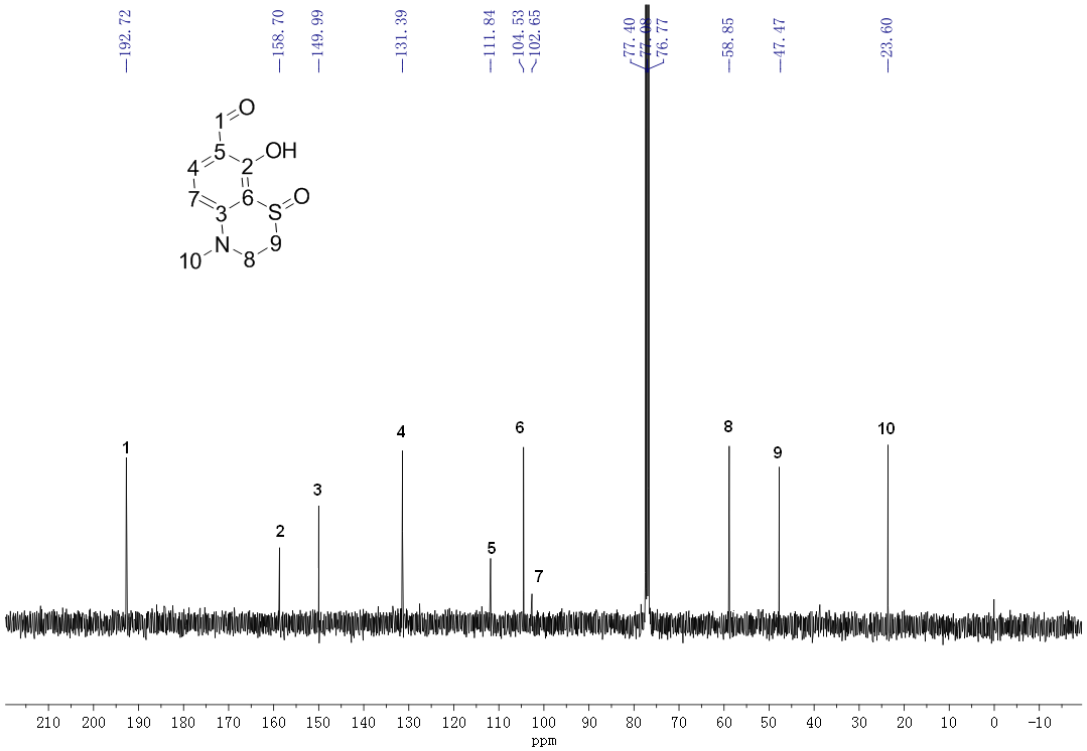


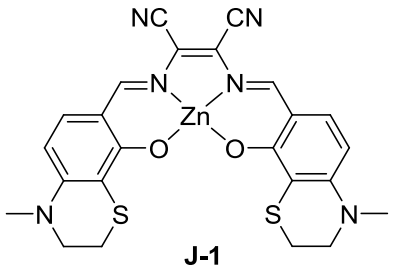
Compound 6

^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 2.60 (td, 1H, J_1=27.9 Hz, J_2=3.3 Hz), 3.14 (dt, 1H, J_1=13.8 Hz, J_2=9.9 Hz), 3.25 (s, 3H), 3.53 (dt, 1H, J_1=13.8 Hz, J_2=9.9 Hz), 4.26 (td, 1H, J_1=27.9 Hz, J_2=3.3 Hz), 6.40 (d, 1H, J=9.0 Hz), 7.46 (d, 1H, J=9.0 Hz), 9.55 (s, 1H), 12.66 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz), δ (ppm): 192.72, 158.70, 149.99, 131.39, 111.84, 104.53, 102.65, 58.85, 47.47,23.60.

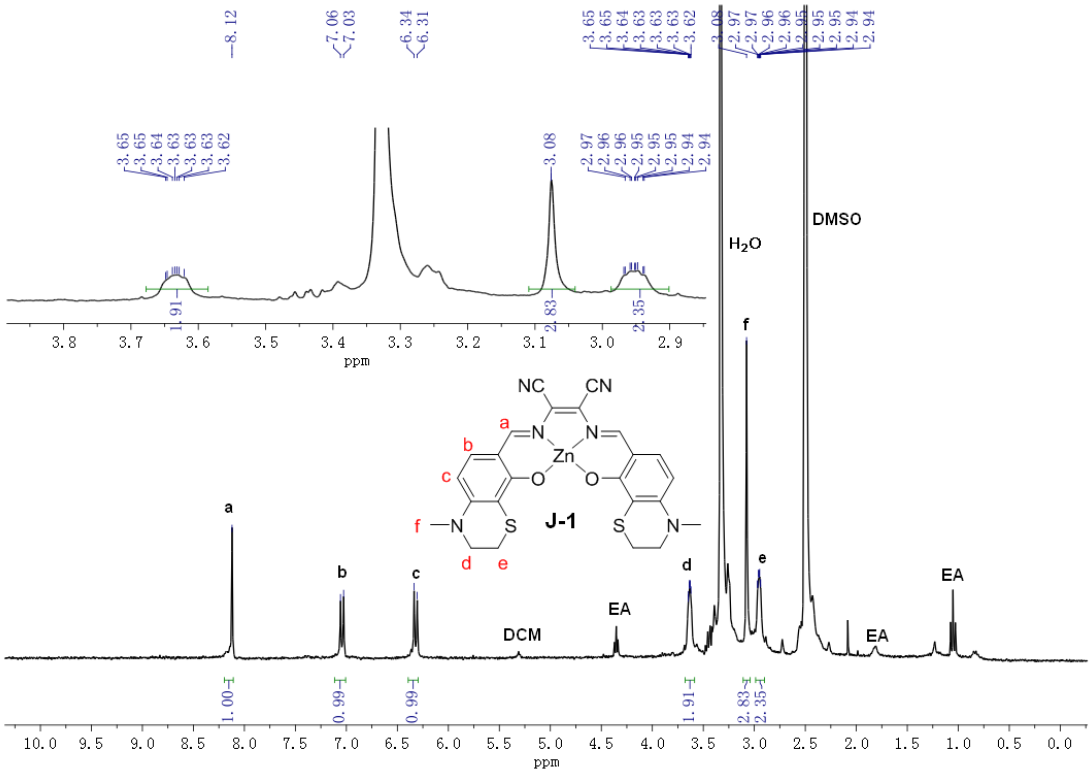


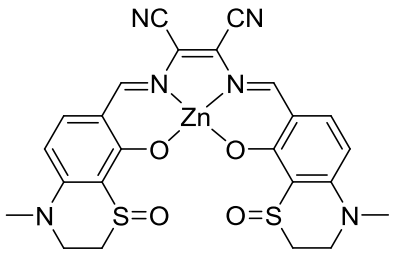




J-1

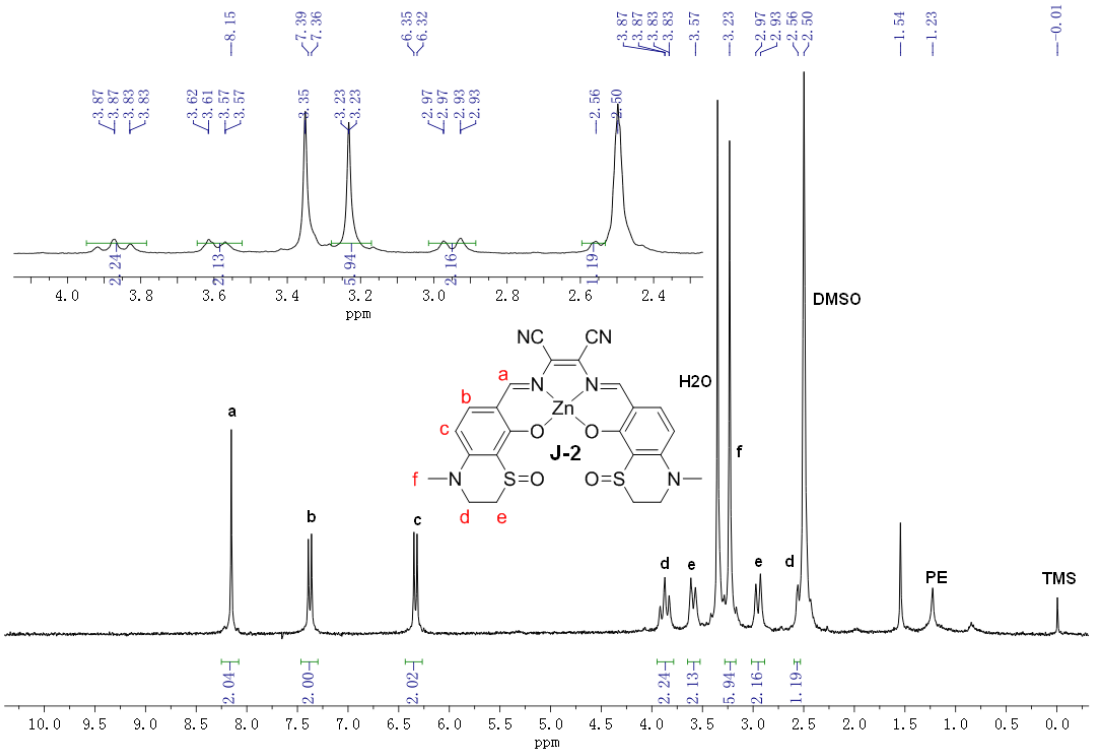
^{1}H NMR (d^6-DMSO, 300 MHz), δ (ppm): 2.95 (m, 4H), 3.08 (s, 6H), 3.64 (m, 4H), 6.33 (d, 2H, J=9.3 Hz),7.05(d,2H,J=9.3Hz),8.12(s,2H).



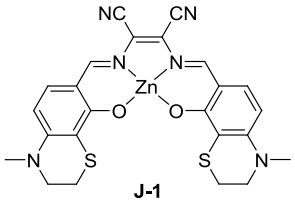


J-2

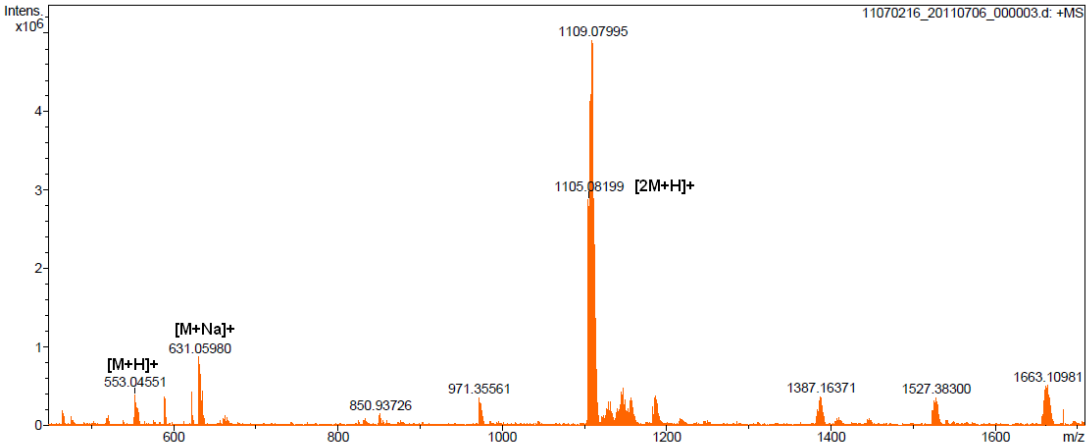
^{1}H NMR (d^{6}-DMSO, 300 MHz), δ (ppm): 2.97 (m, 4H), 3.25 (s, 6H), 3.61 (m, 4H), 6.36 (d, 2H, *J*=9.0 Hz),7.39(d,2H,*J*=9.0Hz),8.17(s,2H).



6. HR ESI-MS spectra



J-1



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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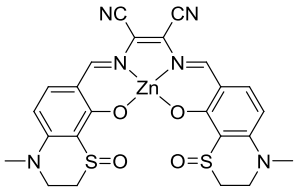
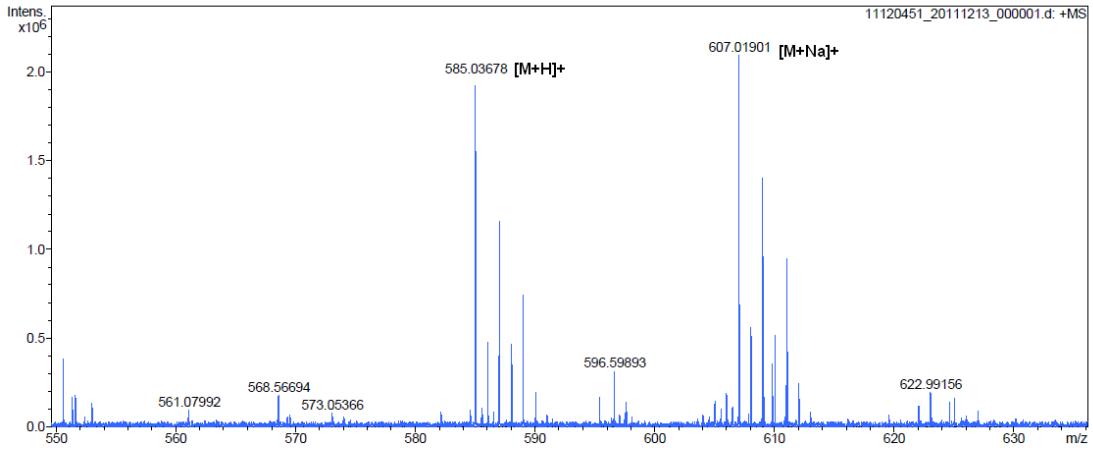
631.05980	1	C 26 H 27 N 6 O 3 S 3 Zn	100.00	631.05927	-0.5	-0.8	22.8	16.5	even	ok
1105.08199	1	C 48 H 41 N 12 O 4 S 4 Zn 2	100.00	1105.08340	1.4	1.3	13.0	34.5	even	ok



J-2



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
585.03678	1	C 24 H 21 N 6 O 4 S 2 Zn	100.00	585.03517	-1.6	-2.7	66.0	17.5	even	ok

7. References

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