Supporting Information to Accompany

"A Golgi-Localized Two-Photon Probe for Imaging Zinc Ions"

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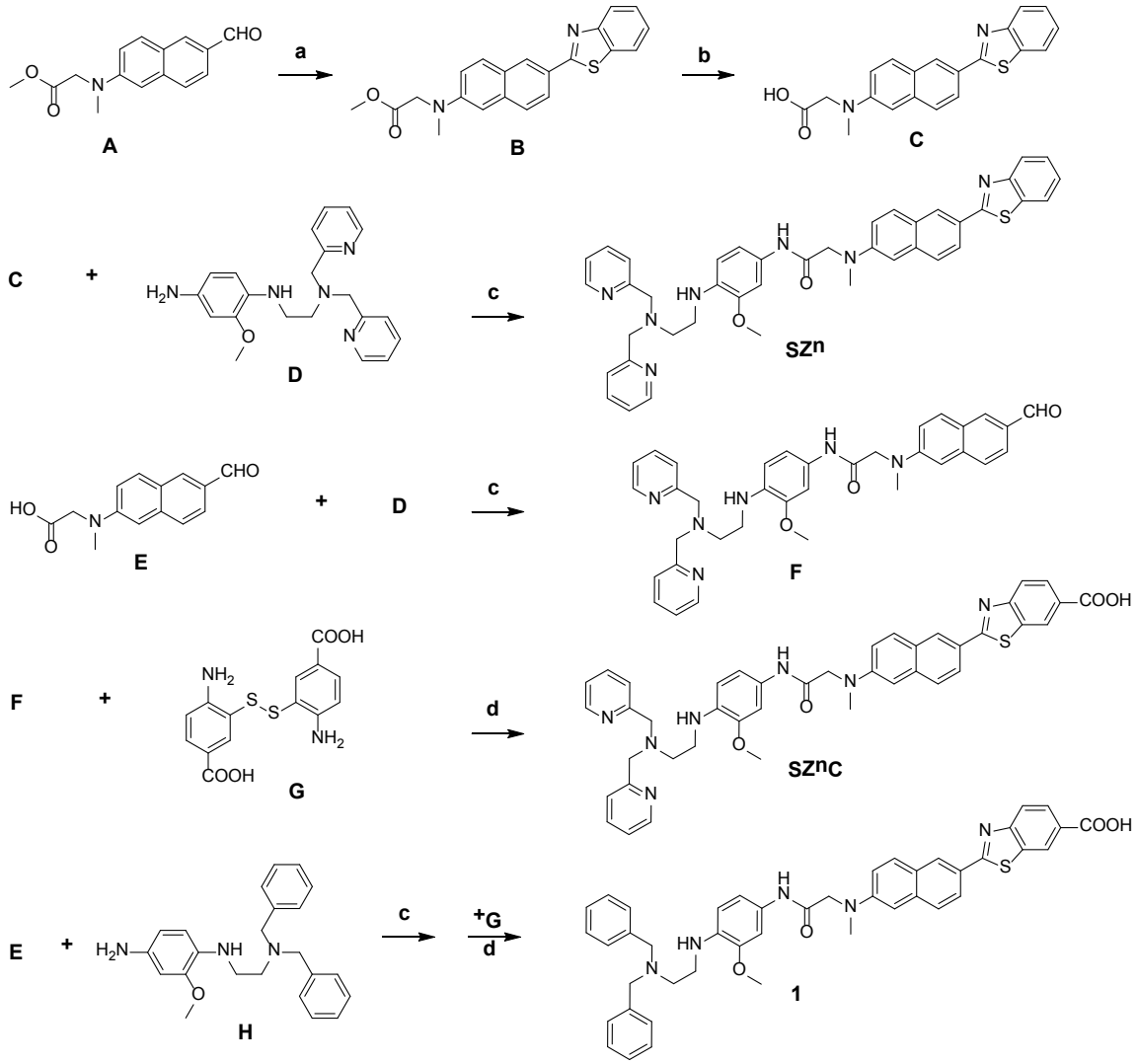
Synthesis of SZn, SZnC and 1. Compound **A**, **D**, **E**, and **G** were prepared by the literature methods[1-3]and synthesis of other compounds is described below.



Scheme S1. Reagents and conditions. (a) 2-Aminothiophenol/chlorotrimethylsilane/DMF/90ºC; (b) 1M KOH/MeOH:Dioxane (1:1); (c) DCC/HOBt/CH_2Cl_2; (d) *p*-TSA, DMF, 90ºC.

Synthesis of **B**. A mixture of **A** (0.35 g, 1.36 mmol), 2-aminothiophenol (0.21 g, 1.70 mmol), and chlorotrimethylsilane (0.44 g, 4.08 mmol) in dry DMF was heated at 80-90ºC for 12 h under N_2. DMF was distilled off and residue was dissolved in DCM. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated to get the crude. The product was recrystallized from hot methanol to get the light yellow colored solids in 65% yield. m.p. 157-159ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.10-8.05 (m, 2H), 7.91-7.89 (m, 1H), 7.83 (d, 1H, J = 8.0 Hz), 7.73 (d, 1H, J = 8.0 Hz), 7.50-7.46 (m, 1H), 7.38-7.34 (m, 1H), 7.11 (dd, 1H, J_1 = 4.0 Hz, J_2 = 4.0 Hz), 6.91 (d, 1H, J = 4.0 Hz), 4.22 (s, 2H), 3.75 (s, 3H), 3.21 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 171.17, 168.79, 154.40, 148.05, 136.52, 135.04, 130.28, 127.56, 127.54, 127.14, 126.62, 126.36, 125.11, 124.96, 122.99, 121.71, 116.14, 106.37, 54.56, 52.45, 40.03.

Synthesis of **C**. To a solution of **B** (0.30 g, 0.82 mmol) in 1:1 MeOH:dioxane (10 mL) was added 1.6 mL 1M KOH (0.092 g, 1.64 mmol) dropwise and the mixture was stirred at room temperature for 12 h . The solvent was removed under vacuum and the product was dissolved in water and reaction mixture was cooled to 0^{o}C using ice bath. 2M HCl (aq.) was added drop-wise to the above solution until pH reached to 2. The yellow colored solid was formed which was filtered off and washed with water, dried under vacuum to get the desired product in 70% yield. m.p 223-225 ^{o}C.^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 8.10 (d, 1H, J = 12.0 Hz), 8.03-7.99 (m, 2H), 7.92 (d, 1H, J = 12.0 Hz), 7.76 (d, 1H, J = 8.0 Hz), 7.53-7.49 (m, 1H), 7.43-7.39 (m, 1H),7.21 (d, 1H, J = 12.0 Hz), 6.96 (s, 1H), 4.27 (s, 2H), 3.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 171.49, 167.57, 153.50, 148.33, 135.97, 134.02, 129.64, 126.94, 126.59, 126.29, 125.83, 125.33, 124.86, 123.98, 122.26, 121.98, 116.17, 105.06, 104.99, 53.17.

Synthesis of **SZn**. Compound **C** (0.10 g, 0.29 mmol), 1,3-dicyclohexyl carbodiimide (DCC) (0.074 g, 0.36 mmol) and 1-hydroxybenzotriazole (HOBt) (0.049 g, 0.36 mmol) were dissolved in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 1 h under nitrogen atmosphere. To this mixture, compound **D** (0.137 g, 0.36 mmol) was added and the resulting mixture was stirred for 16 h. The solvent was evaporated and the mixture was dissolved in CH_3CN. The by-product urea was removed by filtration and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography using 10 % methanol in $CHCl_3$ as the eluent to give light yellow colored solid, which was further crystallized from methanol (68% yield). m.p. 189-191^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (d, 1H, J = 4.0 Hz), 8.46 (s, 1H), 8.15-8.06 (m, 3H), 7.90 (t, 2H, J = 8.0 Hz), 7.78 (d, 1H, J = 8.0 Hz), 7.63-7.59 (m, 2H), 7.50-7.47 (m, 3H), 7.38 (t, 1H, J = 8.0 Hz), 7.18 (dd, 2H, J_1 = 4.0 Hz, J_2 = 4.0 Hz,), 7.14-7.10 (m, 3H), 6.70 (d, 1H, 8.0 Hz), 6.38 (d, 1H, 8.0 Hz), 4.77 (br, 1H), 4.10 (s, 2H) 3.89 (s, 3H), 3.86 (s, 4H), 3.23 (s, 3H), 3.16 (s, 2H), 2.87 (t, 2H, J = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 168.45, 167.71, 159.24, 154.34, 149.22, 148.39, 146.32, 136.57, 136.14, 135.05, 130.59, 128.40, 127.44, 127.40, 127.33, 127.09, 126.45, 125.44, 125.12, 123.27, 123.09, 122.28, 122.25, 121.75, 116.87, 113.12, 107.78, 60.58, 59.60, 53.03, 42.04, 40.46. HRMS (FAB^{+}): m/z calculated for $[C_{41}H_{39}N_7O_2S]^{+}$: 694.2959, found: 694.2958.

Synthesis of **F**. Compound **E** (0.35 g, 1.45 mmol), DCC (0.373 g, 1.81 mmol) and HOBt (0.24 g, 1.81 mmol) were dissolved in DMF (10 mL). The reaction mixture was stirred at room temperature for 1 h under nitrogen atmosphere. To this mixture, compound **D** (0.658 g, 1.81 mmol) was added and the resulting mixture was stirred for 16 h. The solvent was evaporated and the mixture was dissolved in CH_3CN. The by-product urea was removed by filtration and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography using 4-5 % methanol in $CHCl_3$ as the eluent to give light brown colored solid in 64% yield. m.p.

145-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.51-8.49 (m, 1H), 8.19 (s, 1H), 7.98 (s, 1H), 7.91-7.86 (m, 2H), 7.73 (d, 1H, 8.0 Hz), 7.63-7.59 (m, 2H), 7.49 (d, 2H, J = 8.0 Hz), 7.19 (dd, 1H, J_1 = 2.4 Hz, J_2 = 2.4 Hz,), 7.13-7.01 (m, 2H), 7.07 (d, 1H, J = 4.0 Hz), 6.65 (dd, 1H, J_1 = 4.0 Hz, J_2 = 4.0 Hz), 6.37 (d, 1H, 8.0 Hz), 4.78 (br, 1H), 4.12 (s, 2H) 3.88 (s, 3H), 3.85 (s, 4H), 3.26 (s, 3H), 3.15 (t, 2H, J = 4.0 Hz), 2.86 (t, 2H, J = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 191.83, 167.45, 159.36, 149.62, 149.05, 146.94, 138.25, 136.56, 136.09, 134.62, 131.70, 131.39, 127.46, 126.92, 126.41, 123.89, 123.13, 122.24, 116.60, 113.33, 109.65, 107.54, 103.93, 60.59, 59.22, 55.97, 53.12, 41.57, 40.41.

Synthesis of **SZnC**. Compound **F** (0.115 g, 0.19 mmol), **G** (0.80 g, 0.24 mmol) and *p*-toluenesulfonic acid monohydrate (0.002 g, 0.01 mmol) were dissolved in DMF (10 mL) and the reaction mixture was stirred at 90 °C under nitrogen atmosphere for 18 h. After cooling to room temperature, distilled water was added. The product was collected by filtration, washed with water, and purified by crystallization from ethanol in 69% yield. m.p. 195-197 °C.^{1}H NMR (400 MHz, DMSO-d_6): δ 9.77 (s, 1H), 8.73 (s, 1H), 8.51-8.45 (m, 3H), 8.06 (s, 3H), 7.95 (d, 1H, J = 8.0 Hz), 7.79-7.71 (m, 3H), 7.48 (d, 2H, 8.0 Hz), 7.27-7.20 (m, 4H), 7.01 (s, 1H), 6.89 (d, 1H, J = 8.0 Hz), 6.35 (d, 1H, J = 8.0 Hz), 4.37 (s, 1H), 4.29 (s, 2H), 3.79 (s, 3H), 3.76 (s, 4H), 3.19 (s, 3H), 3.11 (s, 2H), 2.72 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): 171.98, 167.75, 167.47, 159.47, 157.13, 149.68, 149.27, 149.18, 146.62, 137.09, 137.05, 135.08, 134.96, 130.61, 128.96, 128.33, 128.10, 127.74, 127.41, 126.19, 126.04, 124.78, 123.28, 122.7, 122.69, 117.20, 112.65, 109.71, 105.78, 103.64, 65.67, 60.29, 56.82, 56.23, 56.11, 52.90. HRMS (FAB$^+$): m/z calculated for $[C_{42}H_{39}N_7O_4S]^+$: 738.2857, found: 738.2857.

Synthesis of **1**. The compound **H** was synthesized in similar manner as compound **D** by replacing the pyridine moiety with benzyl moiety. Further, coupling of compound **E** (0.20 g, 0.83 mmol) with compound **D** (0.39 g, 1.08 mmol) in the presence of the DCC (0.26 g, 1.24 mmol) and HOBt (0.17 g, 1.24 mmol), followed by the *p*-TSA catalyzed cyclization with compound **G** furnished compound **1** in 70 % yield. m.p. 225-227°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.76 (s, 1H), 8.72 (s, 1H), 8.50 (s, 1H), 8.05-8.02 (m, 2H), 7.96 (d, 1H, J = 9.6 Hz), 7.77 (d, 1H, 8.8 Hz), 7.34-7.27 (m, 9H), 7.24-7.19 (m, 4H), 7.00 (s, 1H), 6.89 (dd, 1H, J_1 = 1.6 Hz, J_2 = 2.0 Hz), 6.31 (d, 1H, J = 8.0 Hz), 4.75 (br, 1H), 4.28 (s, 2H), 3.79 (s, 3H), 3.54 (s, 4H), 3.18 (s, 3H), 3.06 (s, 2H), 2.59 (t, 2H, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 171.16, 166.90, 166.65, 158.62, 156.30, 148.88, 145.77, 138.97, 136.28, 134.40, 134.15, 129.80, 128.35, 128.16, 128.00, 127.53, 127.29, 127.04, 126.68, 125.38, 125.24, 123.98, 121.88, 116.41, 111.84, 108.92, 104.97, 102.78, 57.34, 55.33, 51.34, 40.49. HRMS (FAB$^+$): m/z calculated for $[C_{44}H_{41}N_5O_4S]^+$: 736.2952, found: 736.2952.

Spectroscopic measurements. Absorption spectra were recorded on a S-3100 UV-Vis spectrophotometer and fluorescence spectra were obtained with FluoroMate FS-2 fluorescence spectrophotometer with a 1 cm standard quartz cell. The fluorescence quantum yield was determined by using coumarin 307 (Φ = 0.95 in MeOH) as the reference by the literature method.[4]

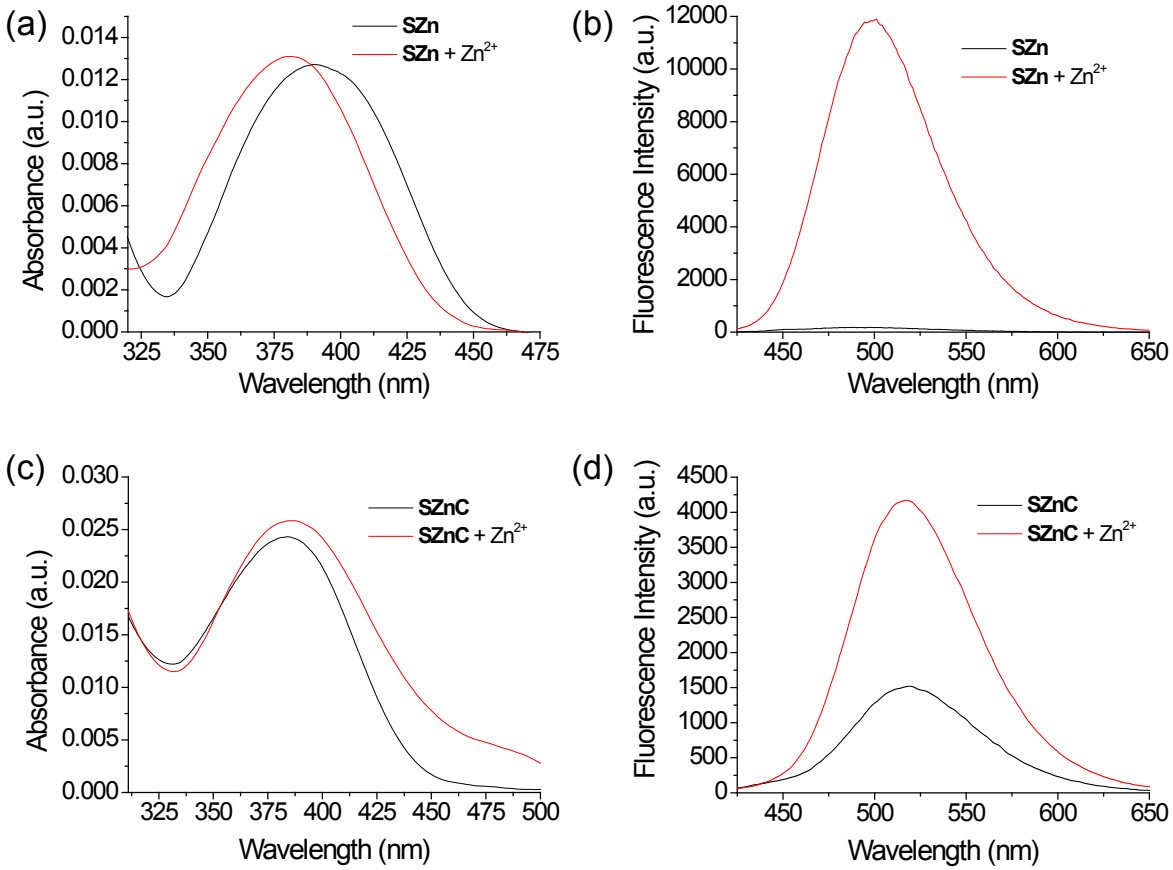


Figure S1. (a,c) One-photon absorption spectra of (a) **SZn** and (c) **SZnC** in the absence (black lines) and presence (red lines) of excess Zn^{2+} (127 nM) in MOPS buffer (30 mM MOPS, 100 mM KCl, 10 mM EGTA, pH 7.2). (b,d) One-photon fluorescence spectra of 1.0 μM (b) **SZn** and (d) **SZnC** in the absence (black lines) and presence (red lines) of excess Zn^{2+} (127 nM) in MOPS buffer. The excitation wavelengths were 390 nm for **SZn** and 384 nm for **SZnC**.

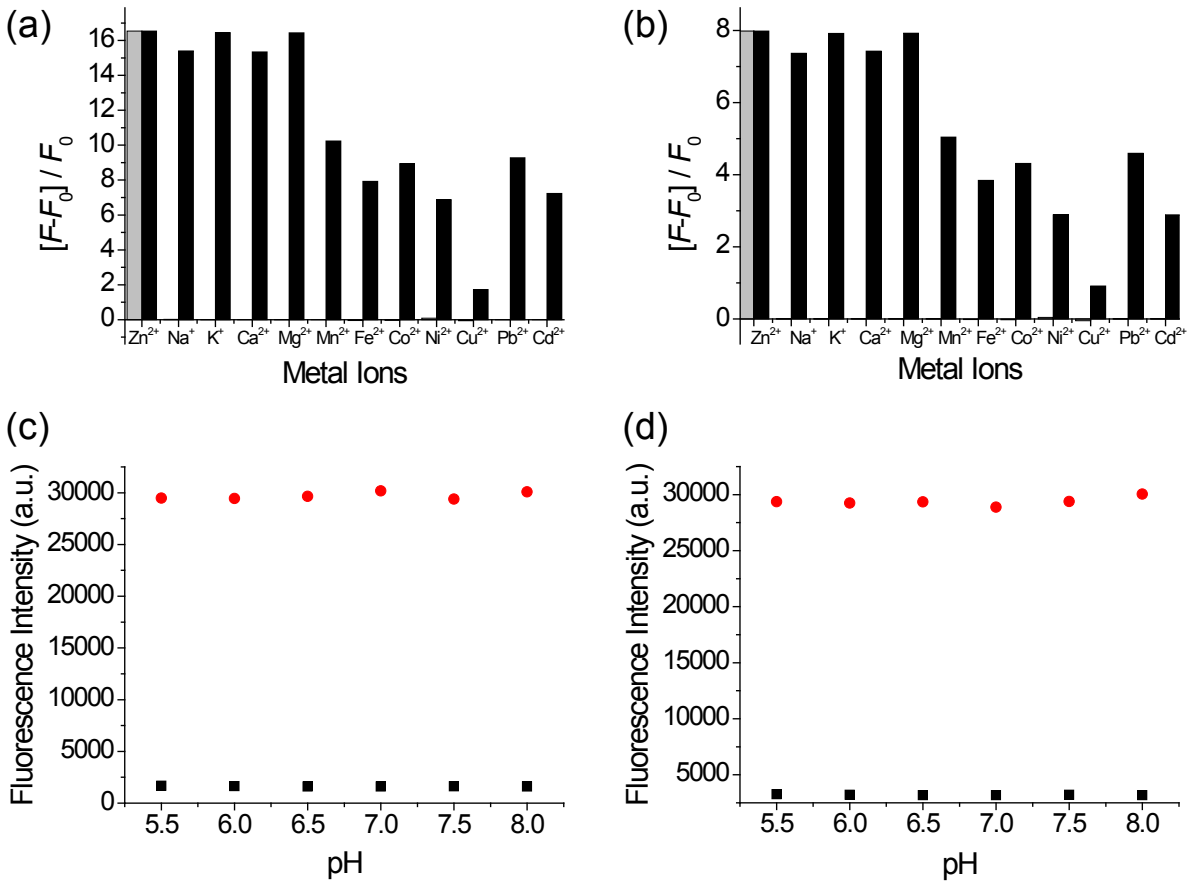


Figure S2. (a,b) The relative fluorescence intensity of 1.0 μM (a) **SZn** and (b) **SZnC** in the presence of 1.0 mM for Na^+, K^+, Ca^{2+}, Mg^{2+}; 1.0 μM for Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+} (gray bars) followed by addition of 127 nM of Zn^{2+} (black bars) in EtOH:Buffer (1:1). (c,d) Effect of pH on the one-photon fluorescence intensity of 1.0 μM (c) **SZn** and (d) **SZnC** in the absence (■) and presence (●) of excess Zn^{2+} (127 nM) in EtOH:Buffer (1:1). The excitation wavelengths were 377 nm for **SZn** and 388 nm for **SZnC**.

Determination of Apparent Dissociation Constants. A series of MOPS 3-(*N*-morpholino) propane sulfonic acid (MOPS) buffer solutions (MOPS 30 mM, 100 mM KCl, pH = 7.2) containing various amounts of $ZnSO_4$ (0 ~ 9.8 mM) and 10 mM of EGTA were prepared. The $[Zn^{2+}]_{free}$ was calculated from the app $K_{Zn\text{-}EGTA}^{app}$, $[EGTA]_{total}$, and $[Zn^{2+}]_{total}$ using Eq (1).[5,6]

$$[Zn^{2+}]_{free} = [Zn^{2+}]_{total} / (\alpha_{Zn} \times K_{Zn\text{-}EGTA}^{app} \times [EGTA]_{free}) \qquad (1)$$

Where,

$K_{Zn\text{-}EGTA}^{app} = K_{Zn\text{-}EGTA} / \alpha_{Zn}\alpha_{EGTA}$,

$\alpha_{Zn} = 1 + 10^{(pH - pK_1)} + 10^{(2pH - pK_1 - pK_2)} + 10^{(3pH - pK_1 - pK_2 - pK_3)} \ldots$,

$\alpha_{EGTA} = 1 + 10^{(pK_1 - pH + 0.11)} + 10^{(pK_1 + pK_2 - 2pH + 0.22)} + 10^{(pK_1 + pK_2 + pK_3 - 3pH + 0.33)} \ldots$,

and

$[EGTA]_{free} = [EGTA]_{total} - [Zn^{2+}]_{total}$

Thus,

$$K_{Zn\text{-}EGTA}^{app} = \frac{K_{Zn\text{-}EGTA}(1 + 10^{(pK_{Zn\text{-}EGTA} - pH)})}{(1 + 10^{(pH - pK_{Zn})})(1 + 10^{(pK_1 - pH)} + 10^{(pK_1 + pK_2 - 2pH)})}$$

The stability constant for the Zn^{2+}complex of EGTA ($K_{Zn\text{-}EGTA}$) was taken from ref. 7. Thus, for EGTA (pH 7.4, 100 mM KCl, 25 °C),

$pK_1 = 9.40$, $pK_2 = 8.79$, $pK_3 = 2.70$, $\log K_{Zn\text{-}EGTA} = 12.6$.

All protonation constants must be corrected upward by 0.11 when worked out in 0.1 M ionic strength. $[EGTA]_{total}$ was set at 10 mM, and $[Zn^{2+}]_{total}$ was varied from 0-9.8 mM.

The calculated $[Zn^{2+}]_{free}$ concentration of each solution is:

$[Zn^{2+}]_{total}$ (mM)	0.50	1.00	2.00	3.00	4.00	4.99	5.99	6.98	7.98	8.97	9.47	9.80
$[Zn^{2+}]_{free}$ (nM)	0.14	0.29	0.65	1.1	1.8	2.6	3.9	6.1	10	23	47	127

To determine the apparent dissociation constants for the Zn^{2+} complex of probes, the fluorescence titration curves (Figure 2) were obtained and fitted to Eq. 2.[8,9]

$$F = F_0 + (F_{max} - F_0)\frac{[Zn^{2+}]_{free}}{K_d + [Zn^{2+}]_{free}} \qquad (2)$$

Where F is the fluorescence intensity, F_{max} is the maximum fluorescence intensity, F_0 is the fluorescence intensity in the absence of Zn^{2+}, and $[Zn^{2+}]_{free}$ is the free Zn^{2+} concentration.

The K_d values that best fits the titration curves with Eq. 2 was calculated by using OriginPro 7.5.

Measurement of Two-Photon Cross Section. The two-photon cross section (δ) was determined by using femto second (fs) fluorescence measurement technique as described.[10] **SZn** and **SZnC** (1.0×10^{-6} M) was dissolved in 1:1 EtOH:MOPS buffer (30 mM MOPS, 100 mM KCl, 10 mM EGTA, pH 7.2) and the two-photon induced fluorescence intensity was measured at 720−880 nm by using rhodamine 6G as the reference, whose two-photon property has been well characterized in the literature.[11] The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The TPA cross section was calculated by using $\delta = \delta_r(S_s\Phi_r\phi_r c_r)/(S_r\Phi_s\phi_s c_s)$: where the subscripts *s* and *r* stand for the sample and reference molecules. The intensity of the signal collected by a CCD detector was denoted as *S*. Φ is the fluorescence quantum yield. ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as *c*. δ_r is the TPA cross section of the reference molecule.

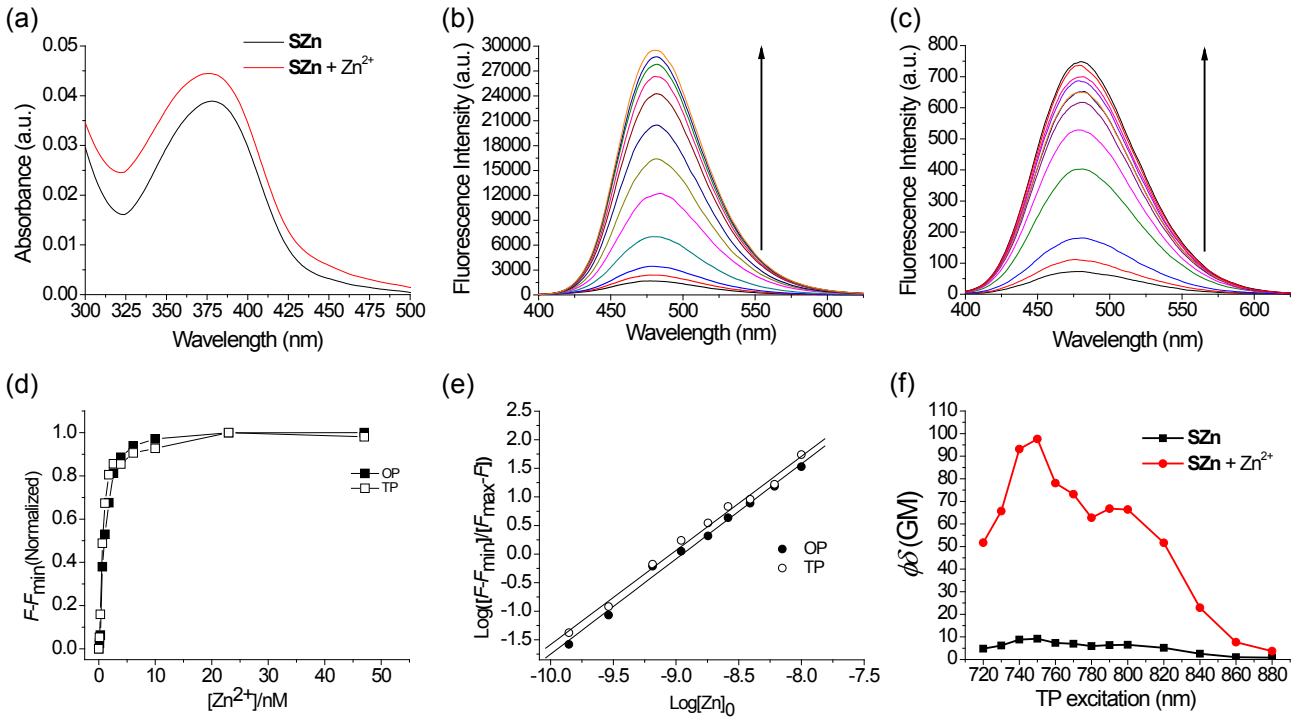


Figure S3. (a) One-photon absorption spectra of **SZn** in the absence (black line) and presence (red line) of excess Zn^{2+} (127 nM) in EtOH:Buffer (1:1). (b) One-photon and (c) two-photon fluorescence spectra of 1.0 μM **SZn** in the presence of free Zn^{2+} (0 to 127 nM) in EtOH:Buffer (1:1). (d) One-photon (■) and two-photon (□) fluorescence titration curves for the complexation of **SZn** with free Zn^{2+} (0 to 127 nM). (e) One-photon (●) and two-photon (○) Benesi-Hildebrand plots for **SZn** upon complexation with free Zn^{2+} (0 to 127 nM). (f) Two-photon action spectra of **SZn** in the absence (■) and presence (●) of excess Zn^{2+} (127 nM) in EtOH:Buffer (1:1). The excitation wavelengths were 377 nm for one-photon process and 750 nm for two-photon process.

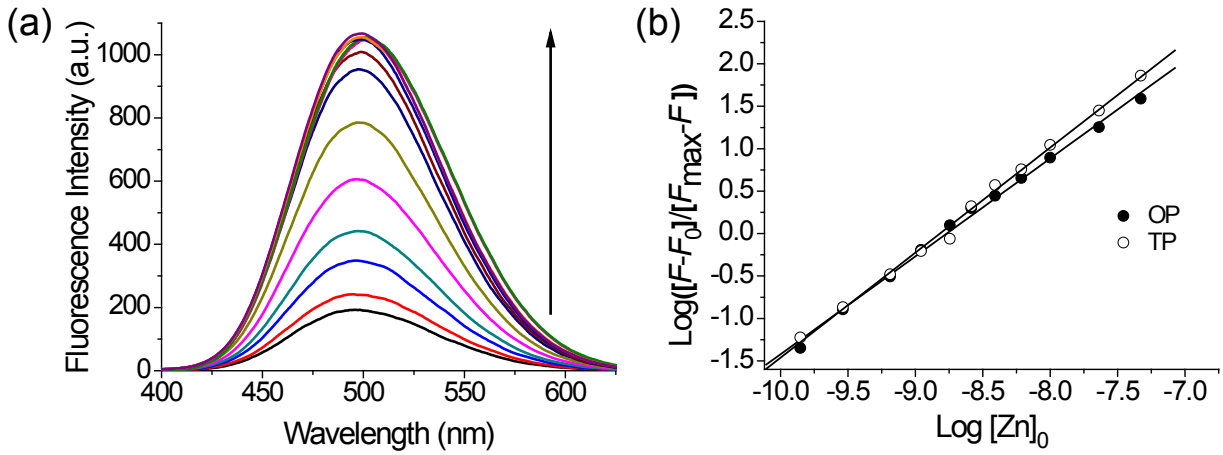


Figure S4. (a) Two-photon fluorescence spectra of 1.0 μM **SZnC** in the presence of free Zn^{2+} (0 to 127 nM) in EtOH:Buffer (1:1). (b) One-photon (●) and two-photon (○) Benesi-Hildebrand plots for **SZnC** upon complexation with free Zn^{2+} (0 to 127 nM). The excitation wavelengths were 377 nm for one-photon process and 750 nm for two-photon process.

Cell Culture. All the cells were passed and plated on glass-bottomed dishes (NEST) before imaging for two days.They were maintained in a humidified atmosphere of 5/95 (v/v) of CO_2/air at 37 °C.The cells were treated and incubated with 2 μM **SZn** and **SZnC** at 37 °C under 5 % CO_2 for 30 min, washed three times with phosphate buffered saline (PBS; Gibco), and then imaged after further incubation in colorless serum-free media for 30 min. The culture mediums for each cells are as below.

HeLa human cervical carcinoma cells (ATCC, Manassas, VA, USA): MEM (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL).

A431 cells (ATCC, Manassas, VA, USA):RPMI1640 (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL).

Raw 264.7 cells (ATCC, Manassas, VA, USA): DMEM (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL).

HepG2 cells (ATCC, Manassas, VA, USA): MEM (WelGene Inc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL).

Two-Photon Fluorescence Microscopy. Two-photon fluorescence microscopy images of probe-labeled cells and tissues were obtained with spectral confocal and multiphoton microscopes (Leica TCS SP8 MP) with ×10 dry, ×40 oil, and×100 oil objectives, numerical aperture (NA)= 0.30, 1.30, and 1.30, respectively. The two-photon fluorescence microscopy images were obtained with a DMI6000B Microscope (Leica) by exciting the probes with a mode-locked titanium-sapphire laser source (Mai Tai HP; Spectra Physics, 80 MHz pulse frequency, 100 fs pulse width)set at wavelength 750 nm and output power 2679 mW, which corresponded to approximately 1.5 mW average power in the focal plane. Live cell imaging was performed using the live cell incubator systems (Chamlide IC; Live Cell Instrument) for stable cell environment by maintaining proper temperature, humidity and pH over the long term.To obtain images at 400-600 nm range, internal PMTs were used to collect the signals in an 8 bit unsigned 512×512and 1024×1024 pixels at 400 and 200 Hz scan speed, respectively.

Colocalization experiment. Co-localization experiments were conducted by co-staining the HeLa, A431, Raw 264.7, and HepG2 cells with appropriate combinations of **SZnC** (2.0μM) and each commercial organelle markers (5.0 μM of BODIPY TR ceramide complexed to BSA for Golgi, 1.0 μM of LysoTracker Red DND-99 for Lysosome, 1.0μM MitoTracker Red FM for Mitochondria, 1.0 μM of ER-Tracker Red for Endoplasmic Reticulum) for 30 min. TPM and OPM images were obtained by collecting the emissions at 400-500 nm (**SZnC**, λ_{ex}= 750 nm)and 600-650 nm (Organelle markers, λ_{ex}= 552 nm), respectively. The background images were corrected, and the distribution of pixels in the TPM and OPM images acquired in the green and red channels, respectively, was compared by using scatter gram. The Pearson's colocalization coefficient (*A*) was calculated using LAS AF software.

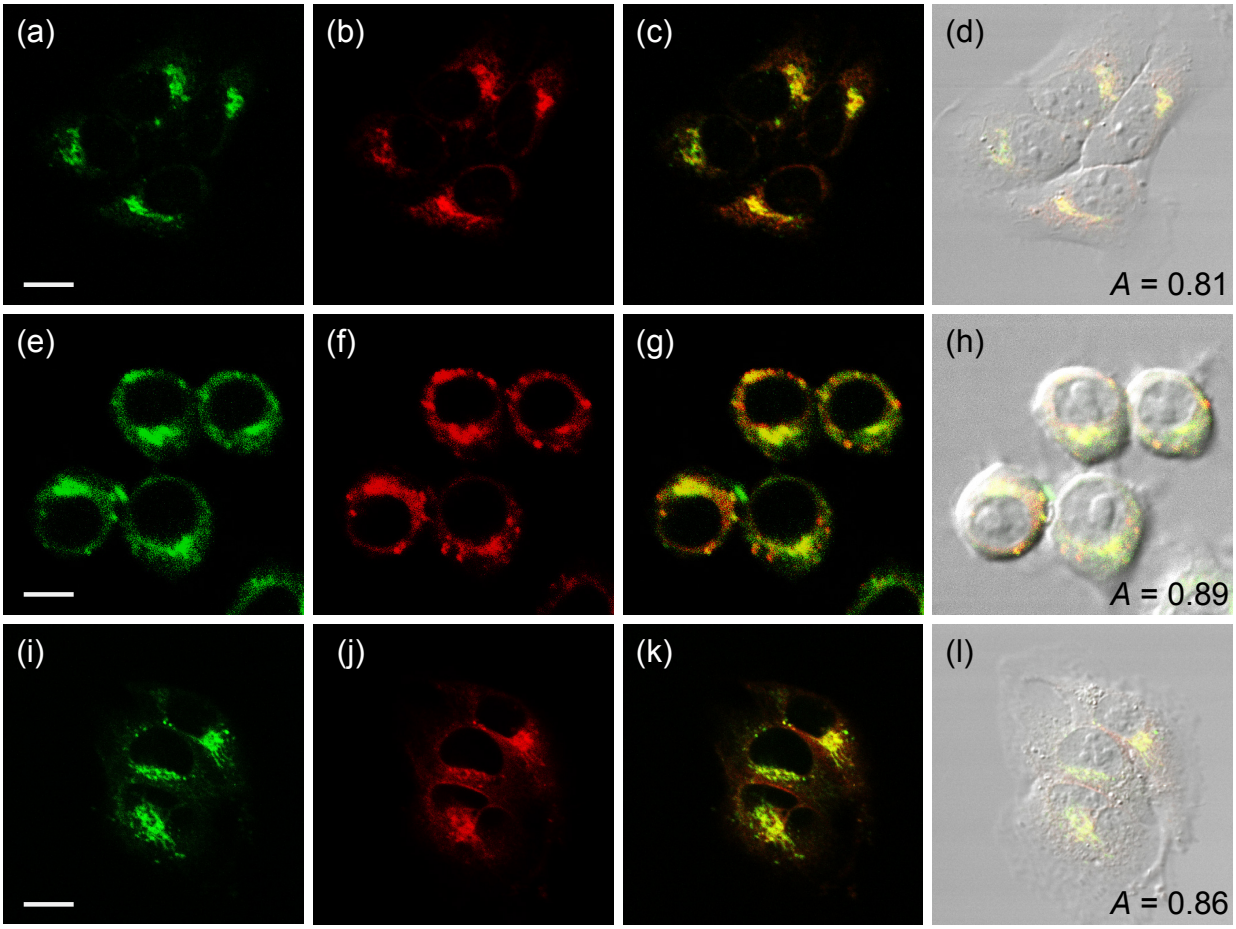


Figure S5. Co-localization experiments of **SZnC** and Golgi marker in various cell lines. (a,e,i) TPM, (b,f,j) OPM, and (c,g,k) merged images of (a-d) A431, (e-h) Raw 264.7, and (i-l) HepG2 cells co-labeled with **SZnC** (2.0 μM) and commercial Golgi marker (BODIPY TR ceramide complexed to BSA, 5.0 μM). (d,h,l) Merged images with the corresponding DIC images. Excitation wavelengths for TPM and OPM are 750 nm and 552 nm, respectively. Scale bars = (a,i) 16 and (e) 8 μm. Cells shown are representative images from replicate experiments (n = 5).

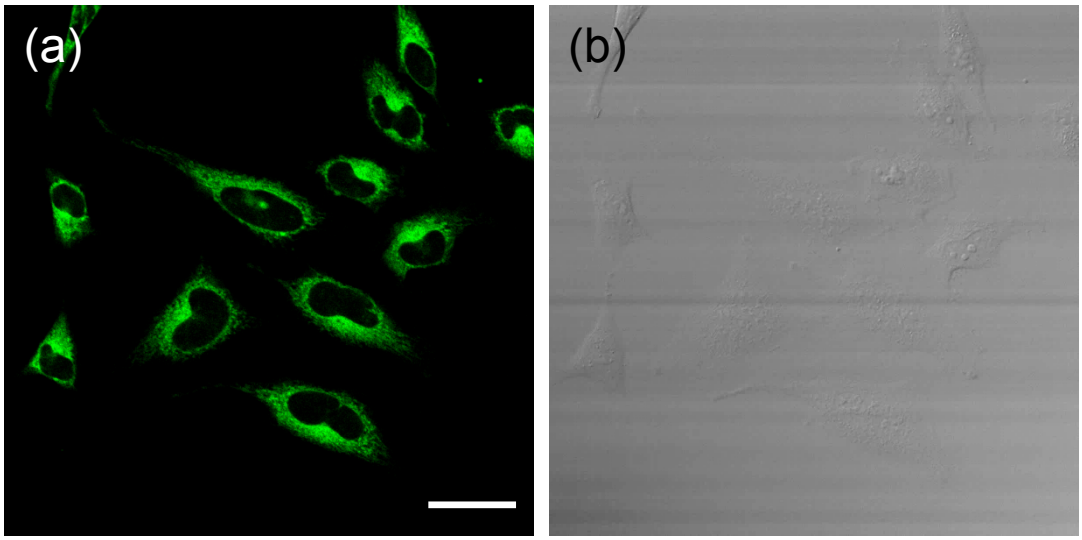


Figure S6. (a) TPM image of HeLa cells labeled with **1 (**2 μM). (b) DIC image. Excitation wavelength for TPM was 750 nm. Scale bar = 45 μm.

Cell viability. To confirm that **SZnC** couldn't affect the viability of HeLa cells in our incubation condition, MTS assay (Cell Titer 96H; Promega, Maidson, WI, USA) was used according to the manufacture's protocol. The results are shown in Figure S7.

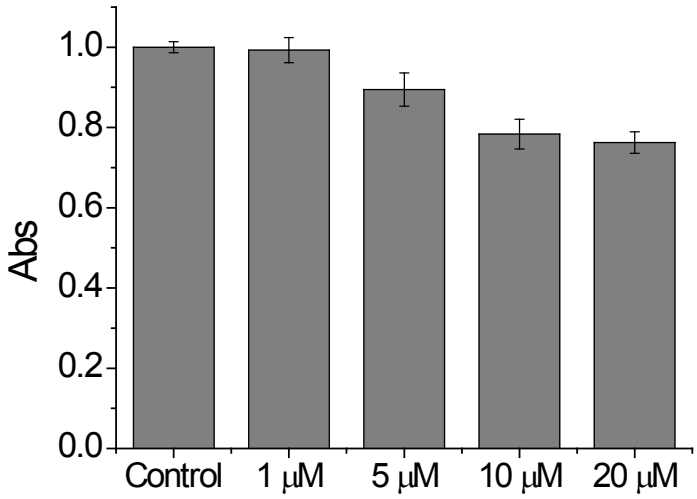


Figure S7. Viability of HeLa cells in the presence of **SZnC** as measured by using MTS assay. The cells were incubated with probe for 2 h.

Determination of Octanol-water partition coefficient (log P_{oct}). Small aliquot (20 μL) of 20 mM Probe solution in DMSO was added to a vial containing 5 mL *n*-octanol by using a micro syringe. To this solution, 5 mL of MOPS buffer (30 mM MOPS, 100 mM KCl, 10 mM EGTA, pH 7.2) was added. The resulting mixture was stirred vigorously and kept in dark for 10 min. The concentrations of probe in each layer were determined by the UV-Vis absorbance with their molar extinction coefficients as shown in Table S1. The log P_{oct} value was calculated by using log P_{oct} = log $[probe]_{oct}$ − log $[probe]_{MOPS}$: where the $[probe]_{oct}$ and $[probe]_{MOPS}$ are the concentrations of the probe in *n*-octanol and MOPS, respectively. The log P_{oct} values for **SZn**, **SZnC**, and **1** are 2.5, 2.9, and 3.1, respectively.

Table S1. Molar extinction coefficient and log P_{oct} for **SZn**, **SZnC** and **1** in *n*-octanol and MOPS.

Probe	Solvent	ε ($10^{-4}M^{-1}cm^{-1}$)	log P_{oct}
SZn	*n*-octanol	3.41	2.5 ± 0.1
	MOPS	1.58	
SZnC	*n*-octanol	3.85	2.9 ± 0.1
	MOPS	2.89	
1	*n*-octanol	2.76	3.1 ± 0.1
	MOPS	1.70	

Preparation and Staining of Fresh Rat Hippocampal Slices. Rat hippocampal slices were prepared from the hippocampi of 2-weeks-old rat (SD) according to an approved institutional review board protocol. Coronal slices were cut into 400 μm-thicks using a vibrating-blade microtome in artificial cerebrospinal fluid (ACSF; 138.6 mM NaCl, 3.5 mM KCl, 21 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 9.9 mM D-glucose, 1 mM $CaCl_2$, and 3 mM $MgCl_2$). Slices were incubated with 20 μM probe in ACSF bubbled with 95 % O_2 and 5 % CO_2 for 1 h at 37 °C. Slices were then washed three times with ACSF and transferred to glass-bottomed dishes (NEST) and observed in a spectral confocal multiphoton microscope.

Photostability. Photostability of **SZnC** was determined by monitoring the changes in TPEF intensity with time at designated position of **SZnC**-labeled (20 μM) rat hippocampal slice chosen without bias (Figure S8). The TPEF intensity remained nearly the same for one hour, indicating high photostability.

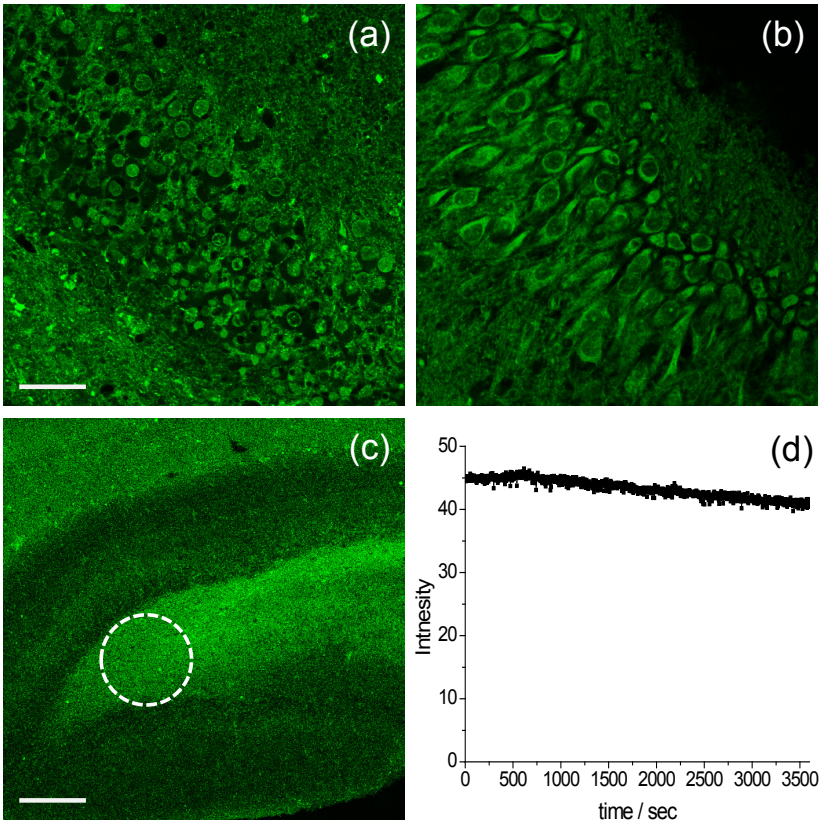


Figure S8. TPM images of a rat hippocampal slice stained with 20 μM **SZnC** for 1h. (a,b) Magnification at 40x in the pyramidal neuron layer of (a) dentate gyrus (DG) and (b) CA3 regions at a depth of approximately 120 μm. (c) Magnification at 20x in the DG regions at a depth of 120 μm. (d) The relative TPEF intensity from the white circle in (c) as a function of time. The digitized intensity was recorded with 2.00 sec intervals for the duration of one hour using *xyt* mode. The TPEF intensities were collected at 400-600 nm upon excitation at 750 nm with fs pulse. Scale bars: (a) 48 and (c) 190 μm. Images shown are representative images from replicate experiments (n = 3).

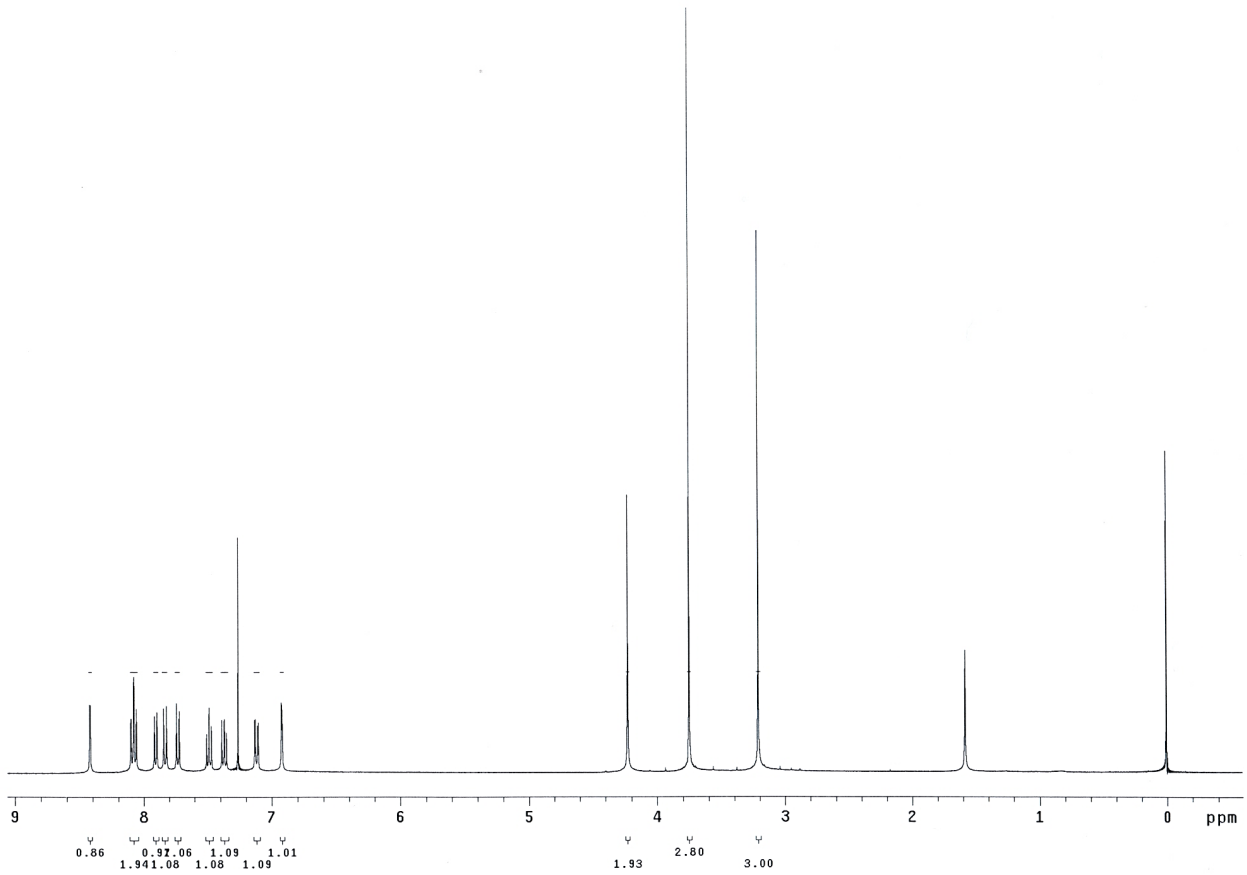


Figure S9. ^{1}H-NMR spectrum (400 MHz) of **B** in $CDCl_3$

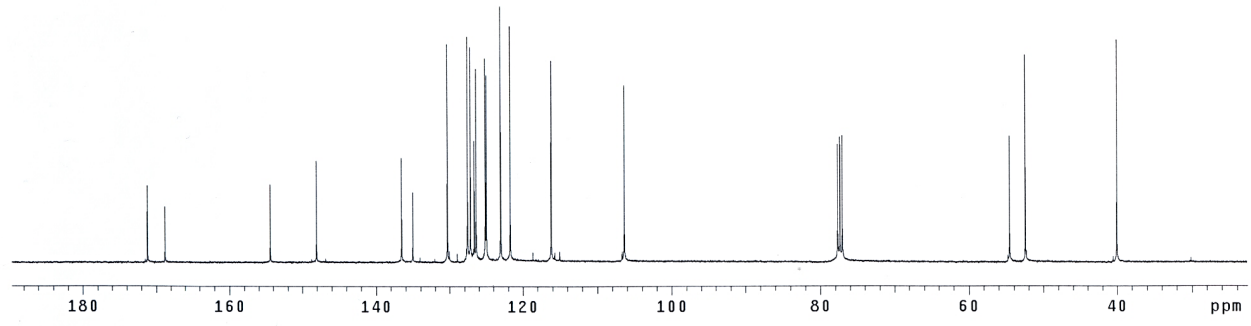


Figure S10. ^{13}C-NMR spectrum (100 MHz) of **B** in $CDCl_3$

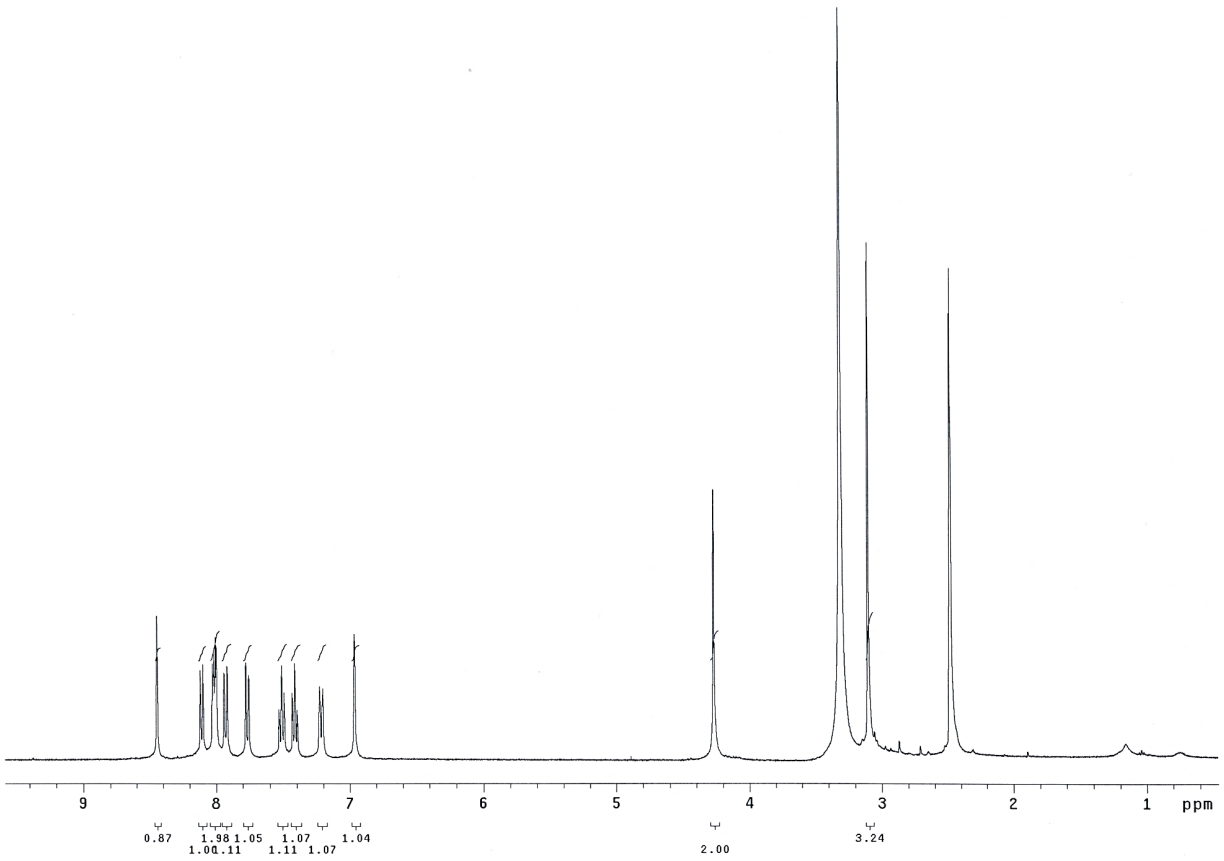


Figure S11. ^{1}H-NMR spectrum (400 MHz) of **C** in DMSO

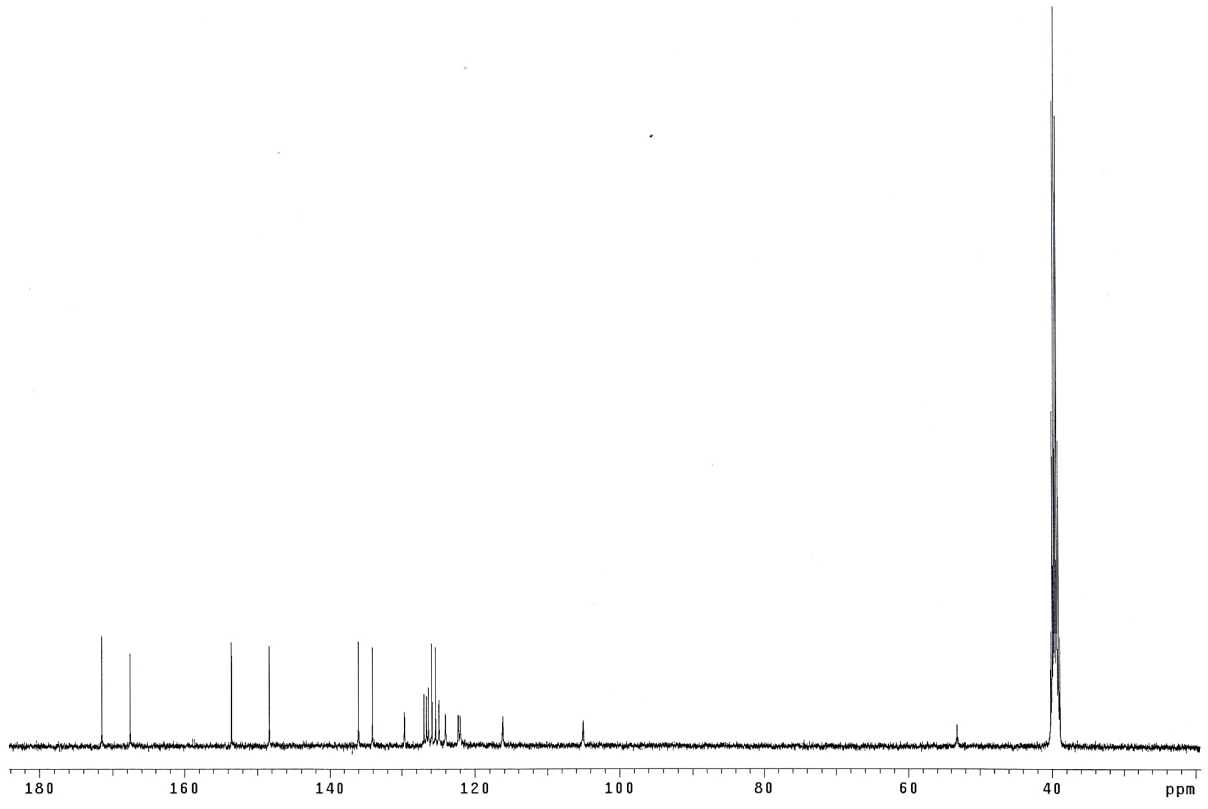


Figure S12. ^{13}C-NMR spectrum (100 MHz) of **C** in DMSO

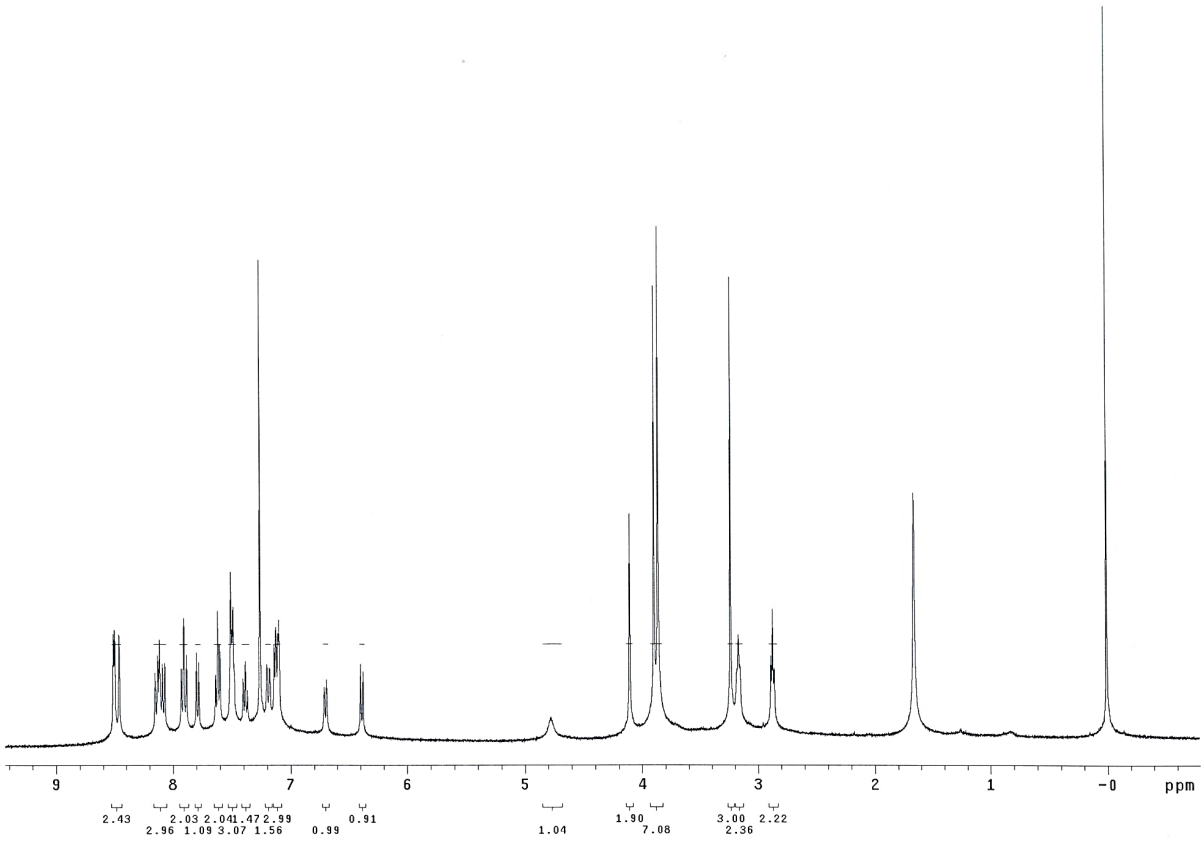


Figure S13. ^{1}H-NMR spectrum (400 MHz) of **SZn** in $CDCl_3$

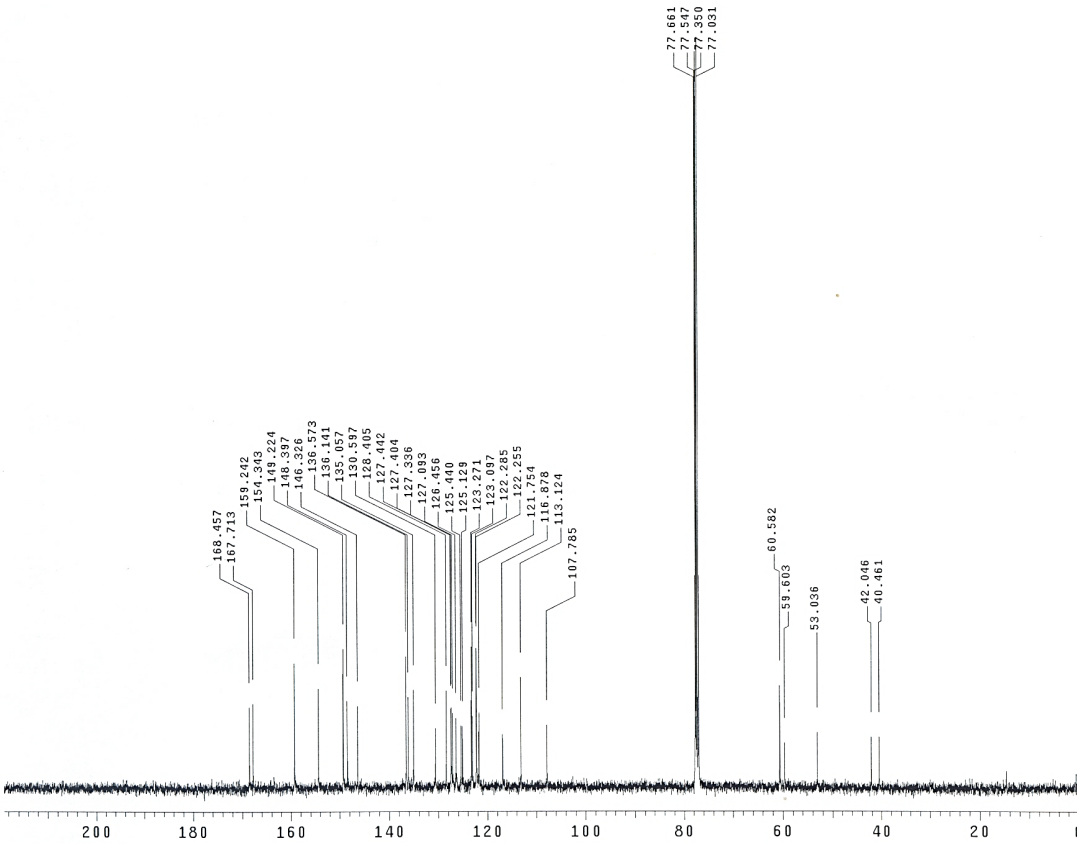


Figure S14. ^{13}C-NMR spectrum (100 MHz) of **SZn** in $CDCl_3$

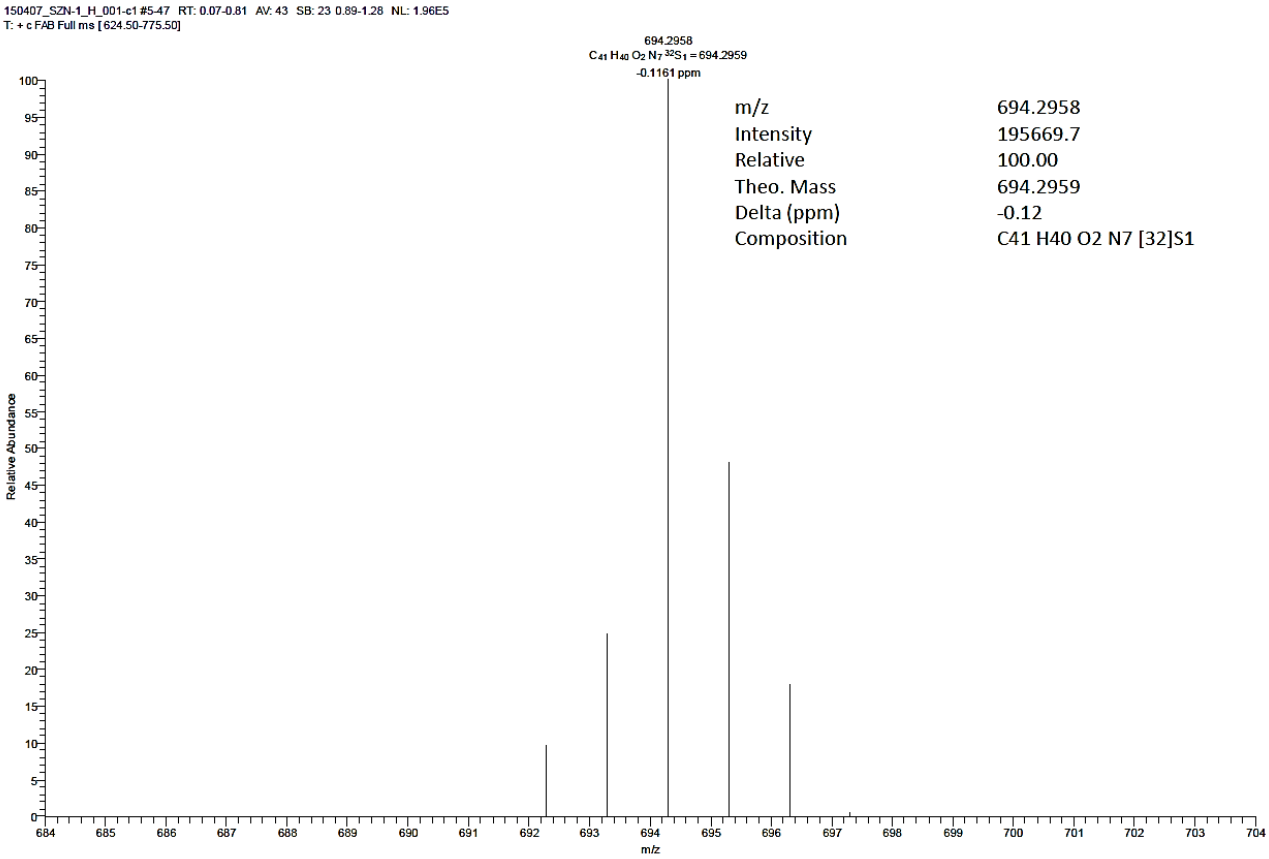


Figure S15. HRMS spectrum of **SZn**.

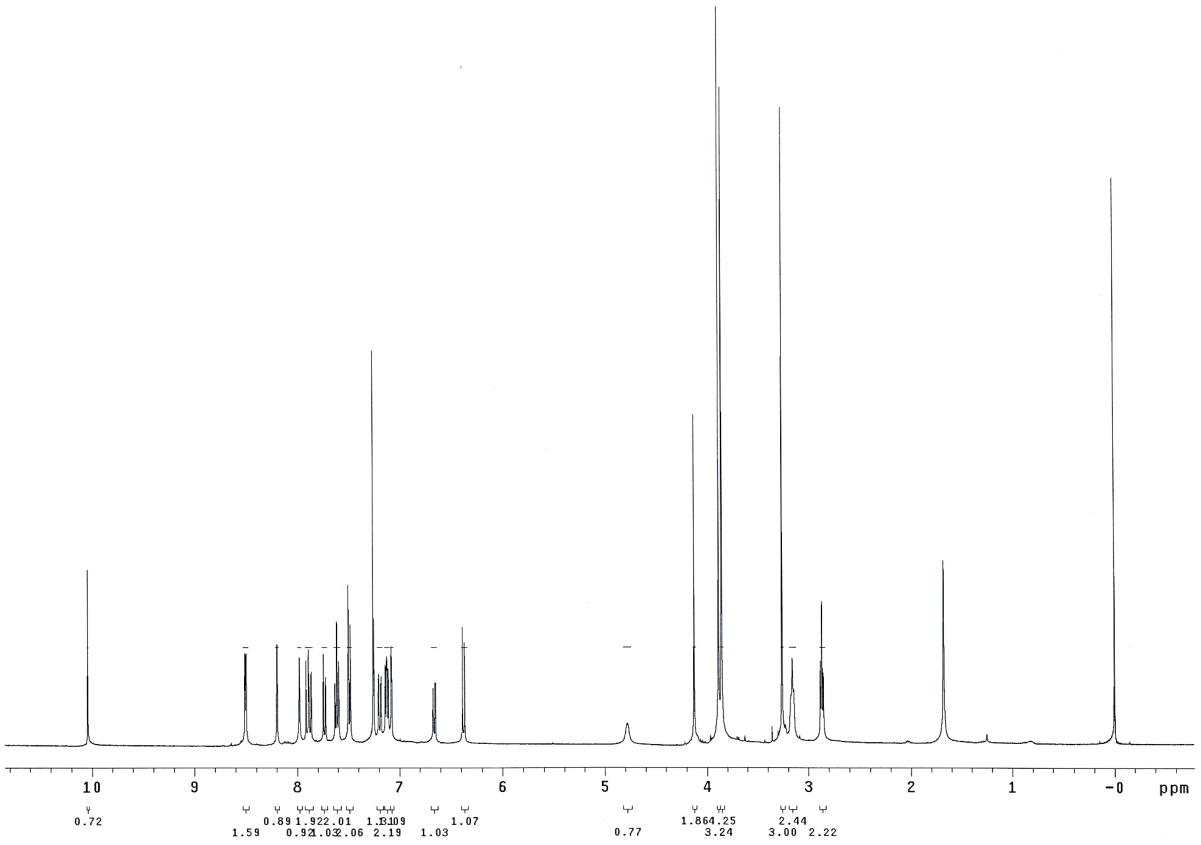


Figure S16. ^{1}H-NMR spectrum (400 MHz) of **F** in $CDCl_3$

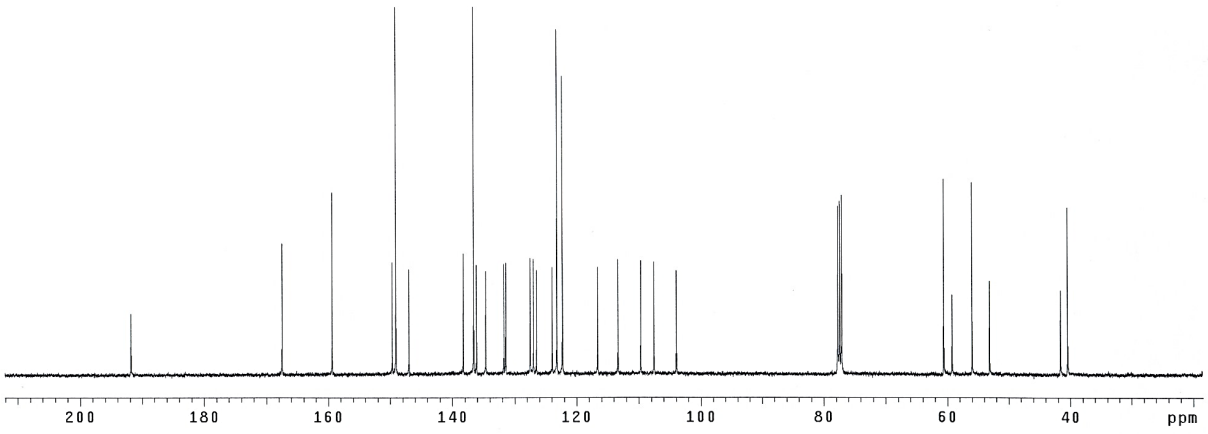


Figure S17. ^{13}C-NMR spectrum (100 MHz) of **F** in $CDCl_3$

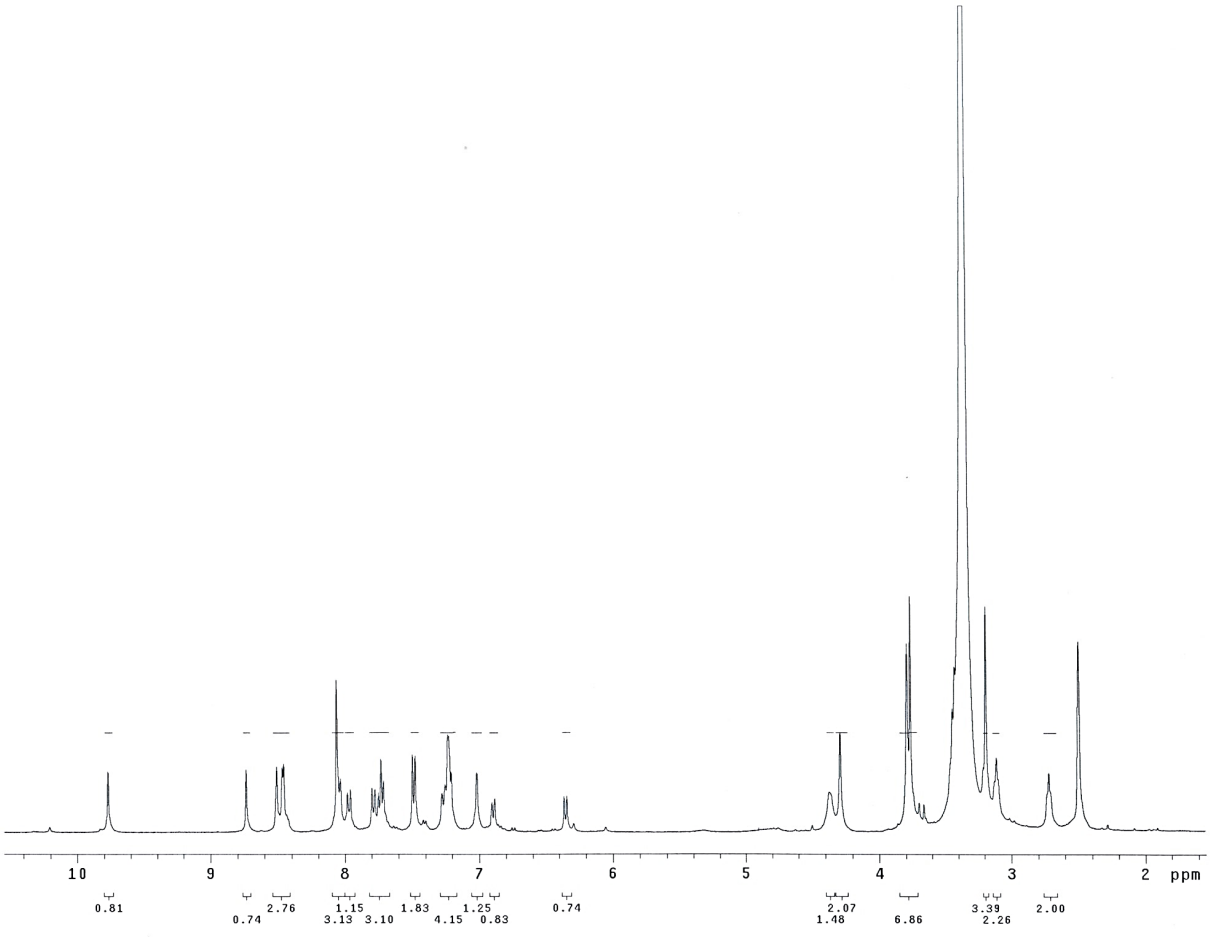


Figure S18. ^{1}H-NMR spectrum (400 MHz) of **SZnC** in DMSO

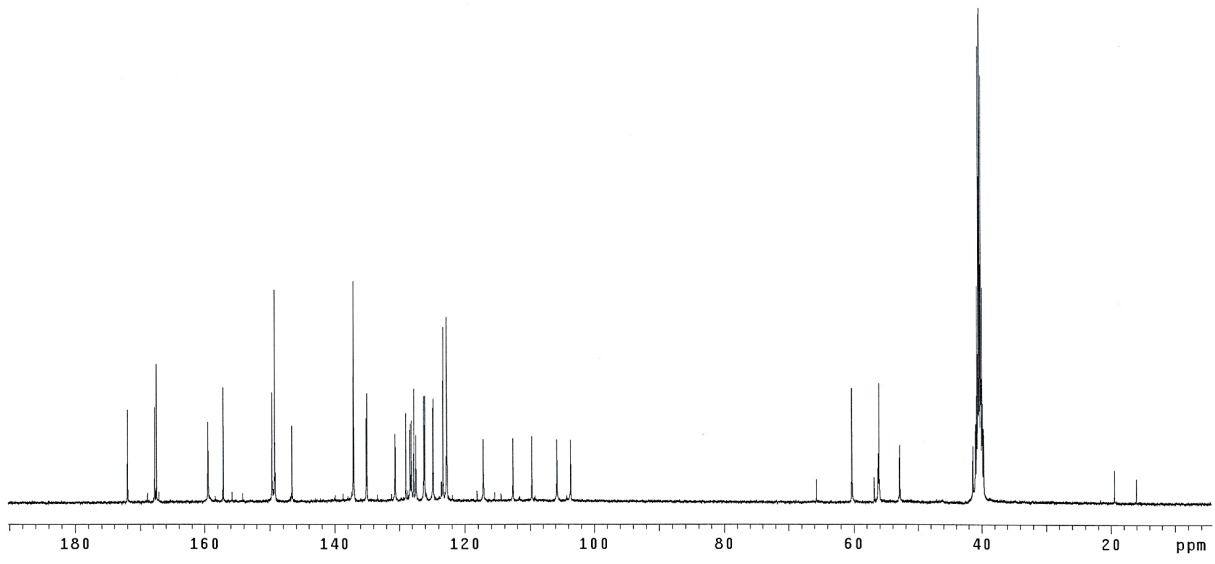


Figure S19. ^{13}C-NMR spectrum (100 MHz) of **SZnC** in DMSO

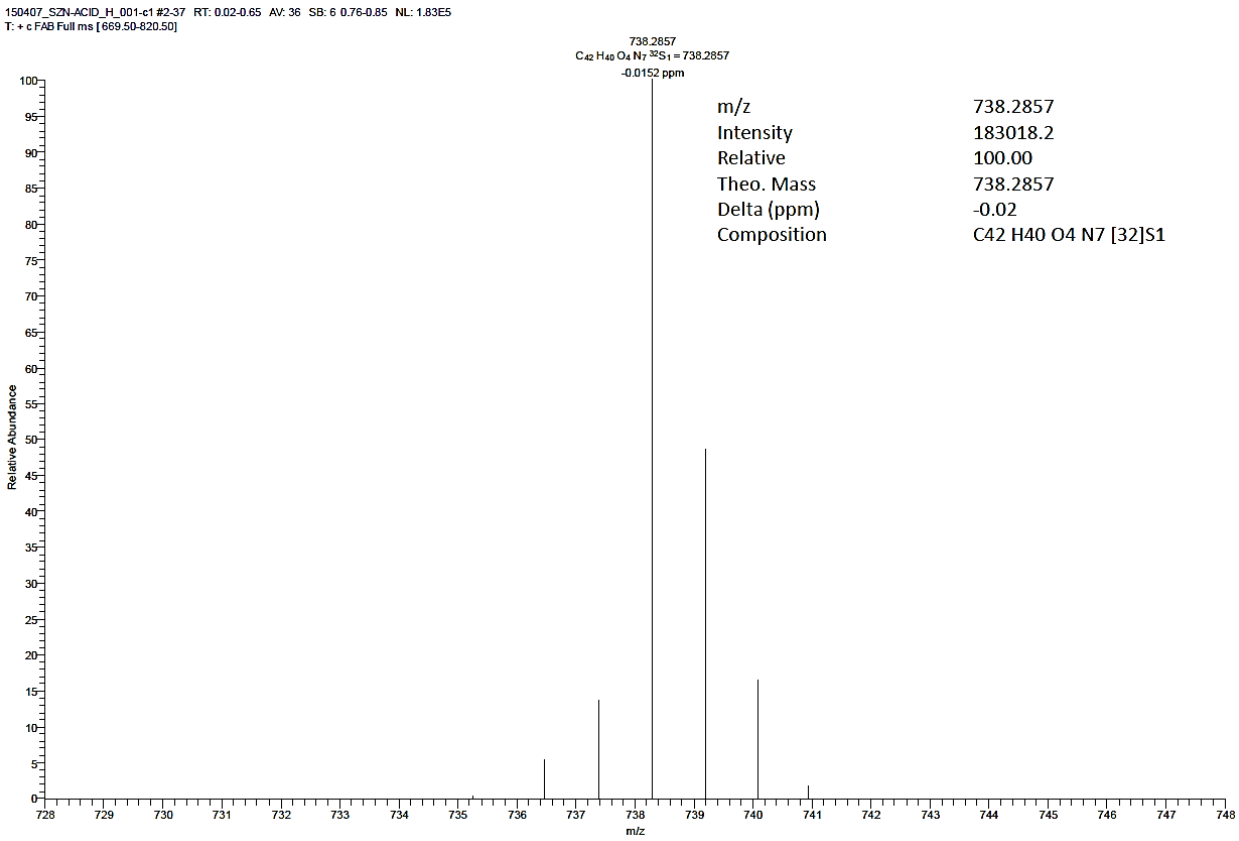


Figure S20. HRMS spectrum of **SZnC**.

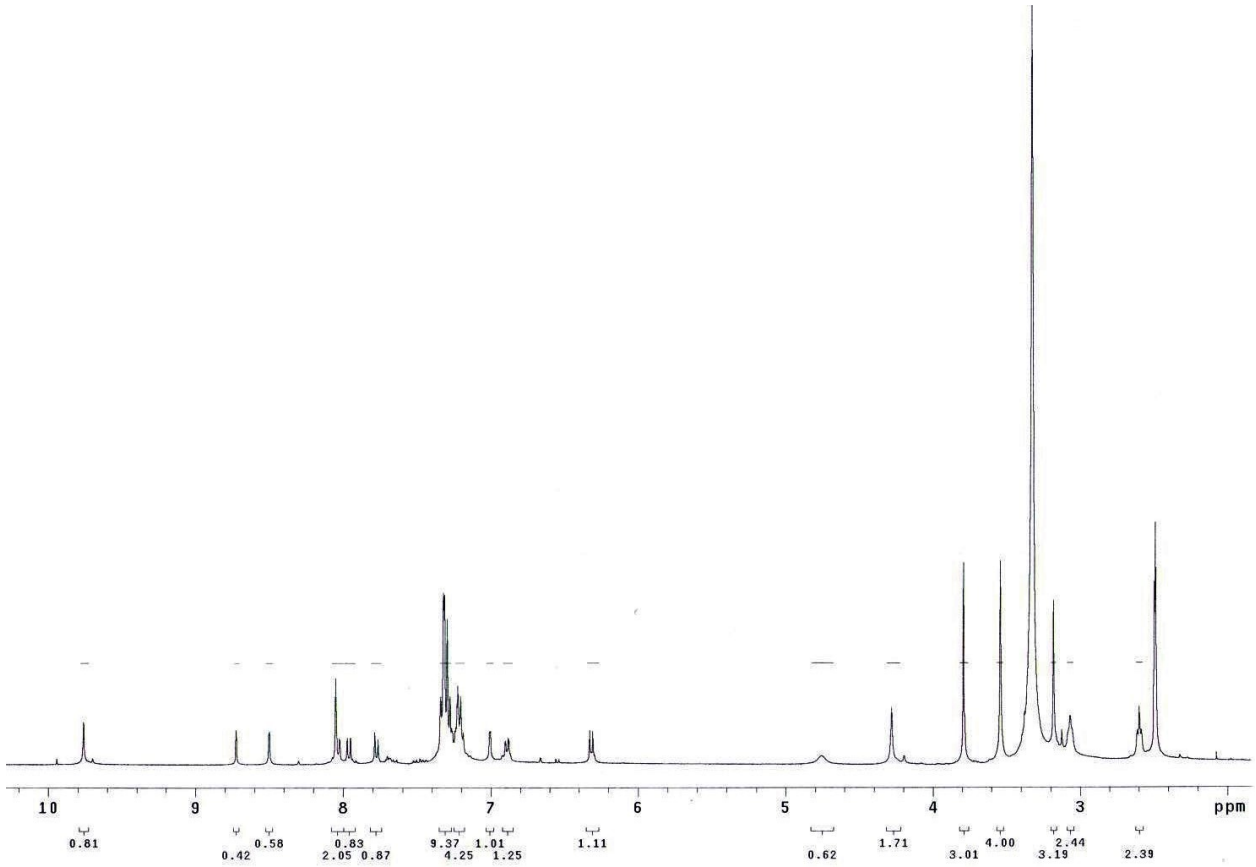


Figure S21. ^{1}H-NMR spectrum (400 MHz) of **1** in DMSO

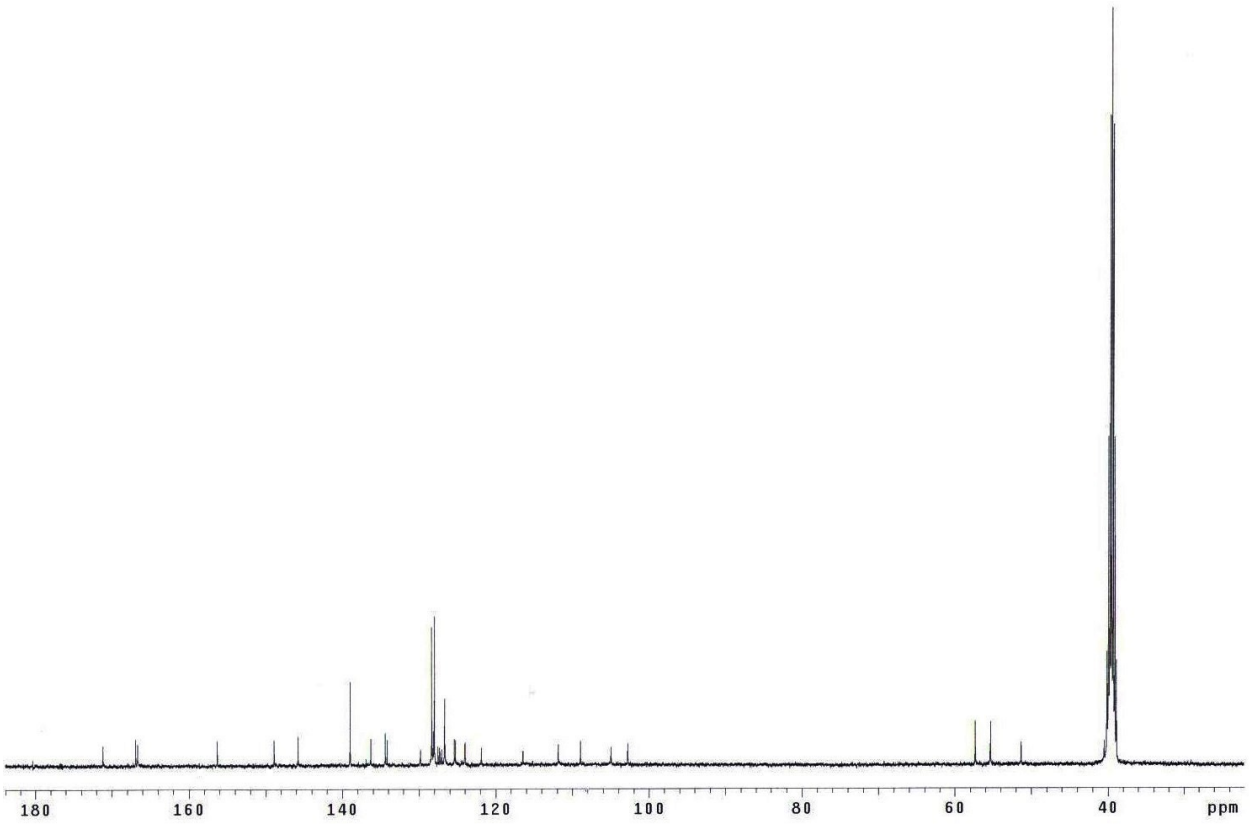


Figure S22. ^{13}C-NMR spectrum (100 MHz) of **1** in DMSO

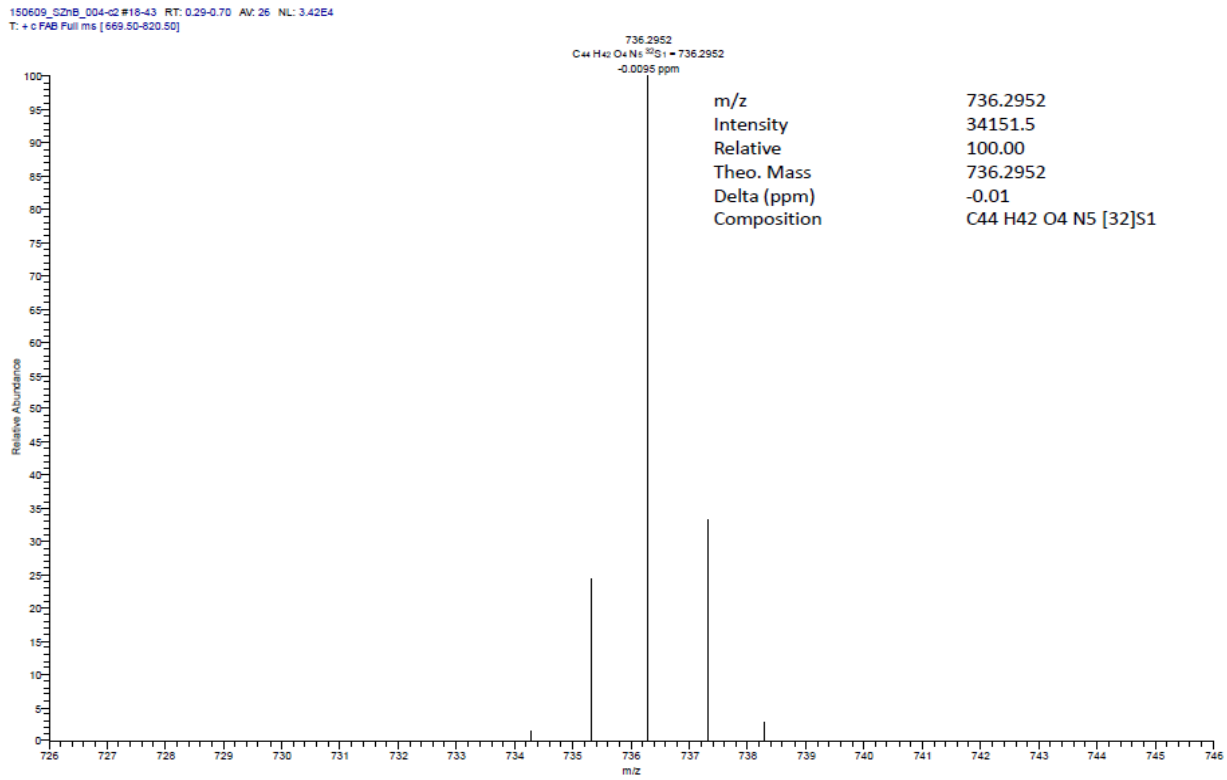


Figure S23. HRMS spectrum of **1**.

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