Supporting Information

Luminescent zinc salen complexes as single and two-photon fluorescence subcellular imaging probes

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Experimental section

1.1 General experimental information: The $^1$H, $^{13}$C NMR and $^{31}$P NMR spectroscopic measurements were performed using a Varian 300 NMR spectrometer at 300 MHz with tetramethyilsilane (TMS) as internal reference; $^1$H (referenced to TMS at $\delta = 0.0$ ppm), $^{13}$C (referenced to CDCl$_3$ at $\delta = 77.16$ ppm) and $^{31}$P (referenced to 85% H$_3$PO$_4$ aqueous solution at $\delta = 0.0$ ppm). Chemical shifts of $^1$H and $^{13}$C spectra were interpreted with the support of CS ChemDraw Ultra version 11.0. Electrospray ionization (ESI) mass spectra were recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker, USA). IR spectra were recorded on a Nicolet iN10 MX Fourier Transform Infrared Spectrometer. LysoSensor Yellow/Blue DND-160, LysoTracker Green DND-26, ER-Tracker Green and MitoTracker Green FM were purchased from Invitrogen (USA). All cells were incubated in complete medium (Dulbecco’s modified Eagle’s Medium, supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin) at 37 °C in atmosphere containing 5% CO$_2$. The steady-state absorption spectra were obtained with an Agilent 8453 UV/Vis spectrophotometer in 10 mm path length quartz cuvettes with dyes concentration ~ $1 \times 10^{-5}$ M. Single-photon luminescence spectra were recorded using a Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer. All measurements were performed at room temperature.

1.2 Photophysical properties of ZnL$_{1-10}$ complexes

The fluorescence quantum yield of porbes ZnL$_{1-10}$ in spectroscopic grade DMSO was recorded by using Rhodamine B in ethanol ($\Phi = 0.65$) as standard. The optical density of probes ZnL$_{1-10}$ and standard was less than 0.1. The values of fluorescence quantum yield, $\Phi$(sample), were calculated according to equation (1)[1]:

$$\phi_{(\text{sample})} = \phi_{(\text{ref})} \frac{OD_{(\text{ref})}}{OD_{(\text{sample})}} \frac{I_{(\text{sample})}}{I_{(\text{ref})}} \frac{n^2_{(\text{sample})}}{n^2_{(\text{ref})}}$$ (1)

where $\phi$ is the quantum yield, $I$ is integrated emission intensity, OD is optical density at the excitation wavelength, and $n$ is the refractive index of pure solvents; subscript ‘ref’ stands for reference samples, ‘sample’ stands for samples.
1.3. pH stability
The absorption spectra of probe ~1.0 μM ZnL_{3,4,7} at various pH in DMSO/buffer (0.5%, V_{DMSO}/V_{buffer}) solution (the mixed buffer comprised 20 mM sodium acetate, 10mM Mes, 10mM Mops and 10mM Tris, adjusted to the certain pH^{2}) were obtained with an Agilent 8453 UV/Vis spectrophotometer in 10 mm path length quartz cuvettes. The emission spectra was recorded by a Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer.

1.4. Stability of ZnSalens in aqueous solution:
Zn(Salen) were dissolved in DMSO/H_{2}O (v/v=5:95) solution for longer than 3 days. The electron spectra were obtained with an Agilent 8453 UV/Vis spectrophotometer in 10 mm path length quartz cuvettes.

1.5. Determination of two-photon absorption cross sections:
The two-photon absorption spectra of probes determined over a broad spectral region by the typical two-photon induced fluorescence (2PF) method relative to Rhodamine B in ethanol as standard.\[1\] The two-photon fluorescence data were acquired using a Tsunami femtosecond Ti:Sapphire laser (pulse width ≤100fs, 80 M Hz repetition rate, tuning range 720–870 nm Spectra Physics Inc., USA). Two-photon fluorescence measurements were performed in 10 mm fluorometric quartz cuvettes with ZnSalen at ~1×10^{-5} M in DMSO. The experimental fluorescence excitation and detection conditions were conducted with negligible reabsorption processes which can affect 2PA measurements. The quadratic dependence of two-photon induced fluorescence intensity on the excitation power was verified for excitation wavelength at 800 nm. The two-photon absorption cross section of the probes was calculated at each wavelength according to equation (2)^[3]:

\[
\delta_{(sample)} = \delta_{(ref)} \frac{\phi_{(ref)} C_{(ref)} I_{(sample)} n_{(sample)}}{\phi_{(sample)} C_{(sample)} I_{(ref)} n_{(ref)}},
\]

where I is the integrated fluorescence intensity, C is the concentration, n is the refractive index, and \( \Phi \) is the quantum yield, subscript ‘ref’ stands for reference samples, ‘sample’ stands for samples.
1.6. Cytotoxicity Assay:
To ascertain the cytotoxicity of Zn Salen complexes treatment for 24 h, MTT assay was performed. 2 ×10^4 per well of Hela cells in 96-well plates were incubated in 200 μL of complete medium for 24 h. Prior to ZnSalen treatment, the complete media was removed and replaced with fresh complete media. Then aliquots of ZnSalen stock solutions (1mM in DMSO) were added to obtain final concentrations of 2, 5, 10, 20, 40 μM and the same increasing percent of DMSO, and untreated cells were used as control. The treated cells were incubated for an additional 24 h in dark at 37 °C. Subsequently, the cells were then treated with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazolium bromide (MTT) and incubated for an additional 4 h(37 °C, 5%CO2). Then the loading media was removed and the cells were dissolved in DMSO (150μL/well), and the absorbance at 570 nm was recorded. The cell viability (%) was calculated according to the following equation: Cell viability % = OD_{570(sample)}/OD_{570(control)} × 100, where OD_{570(sample)} represents the optical density of the wells treated with various concentration of ZnL and corresponding volume of DMSO, OD_{570(control)} represents that of the wells for control. Three independent trials were conducted, and the averages and standard deviations are reported.

1.7. Colocalization study:
Confocal fluorescent microscopy of living cells was preformed using Olympus LSCMFV500 scanning laser microscope. HeLa or CHO cells were placed onto poly-D-lysine coated glasses in complete media and the cells were incubated for 24 h before incubating with fluorescent probes. A stock solution of fluorescent probe Znsalens dissolved in DMSO was prepared as ~1.0 mM solution. The solution was diluted to 2 μM solutions by complete growth medium, and freshly placed over the cells for a 0.5 h incubation period. Stock solutions of LysoSensor Yellow/Blue DND-160, LysoTracker Green DND-26, ER-Tracker Green and MitoTracker Green FM in DMSO were prepared as 1.0 mM solutions. And the stock solution was diluted to the working concentration in complete growth medium (LysoSensor: 1 μM, LysoTracker: 75 nM, ER-Tracker: 1μM, MitoTracker: 100 nM). After incubation time (0.5 h for ER-Tracker and MitoTracker, 5 min for LysoSensor), the cells were washed with KRBB (129 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 5 mM NaHCO₃, 10 mM HEPES, 3 mM glucose, 2.5 mM CaCl₂·2H₂O, 1.2 mM MgCl₂·6H₂O, 0.1% BSA) 3-5 times. The slips were overlaid with KRBB and examined under
confocal microscopy. The setting for confocal microscopy were as follow: 60×immersion oil objective with resolution 512×512, 543 nm excitation wavelength and >560 nm detector slit, 20% laser power for our complexes, LysoSensor (ex:405 nm, em: 505-560 nm), LysoTracker (ex: 488 nm, em: 505-560 nm), MitoTracker (ex: 488 nm, em: 505-560 nm), ER-Tracker (ex: 488 nm, em: 505-560 nm). Differential interference contrast (DIC) and fluorescent images were processed and analyzed using Olympus Fluoviewer. 2.0a Viewer software or Image J. The Pearson’s Coefficient was recorded by Image J. **Transfection with EGFP plasmids:** Adherent Hela cells were grown to about 80% confluency and then reseeded in 12-well plates; cells were transfected with 1.6 μg FVYE-EGFP plasmids, EHD1-EGFP plasmids or commercially available Gogli EGFP plasmids by using Lipfectamine™ 2000 according to manufacturer’s instruction.

### 1.8. Photostability of ZnSalens in living cells:

In order to test the photostability of $\text{ZnL}_4$ as subcellular probes in cells, the one-photon fluorescence images were collected every 7 s in the channel. (Ex: 543 nm; Em:>560 nm) for $\text{ZnL}_4$ and the channel (Ex: 488 nm; Em: 505-560 nm) for ER-Tracker Green. The fluorescence intensity of the images was recorded by Image J. In order to test the 2PFM photostability of $\text{ZnL}_4$ and $\text{ZnL}_7$, the two-photon fluorescence images were collected every 2 s for $\text{ZnL}_7$ or 3 s for $\text{ZnL}_4$ in the channel (Ex: 840 nm; Em:>560 nm).

### 1.9. Two-photon microscopy imaging:

Two-photon fluorescence microscopy (2PFM) images were collected on a modified Olympus Fluoview FV1000MPE microscope system equipped with 60×immersion water objective. The excitation light was provided by a modelocked Ti: sapphire laser, (Mai Tai, Spectra-Physics Inc., USA) tuned to 840 nm. The setting for confocal microscopy were as follow: 840 nm excitation wavelength and >560 nm detector slit, 10% laser power for our complexes.

### 1.10. Retention Time of ZnL₉ in HeLa cells:

HeLa cells were grown to about 80% confluency (about $1 \times 10^6$/well in 6-well plates) and divided into two groups. First group was incubated in dye-free complete medium for 2 h, and then was treated with 2 μM of $\text{ZnL}_9$ for 0.5 h. The other group was treated with 2 μM of $\text{ZnL}_9$ for 0.5 h, and
then was incubated in dye-free complete medium for 2 h. After incubation, the two groups of cells were trypsinized, and washed three times with PBS. Resuspended the cells in 1 mL PBS and immediately analyzed on FACS Aria II. The 405 nm laser line was used for excitation and the fluorescence signal was collected between 593 and 639 nm. 10,000 events were counted for each sample.

1.11. Emission spectra of ZnSalens in living HeLa cells:
The emission spectra of ZnL4 and ZnL7 in living cells were recorded by Laser Scanning Confocal Microscope (A1R-si, Nikon, Japan) and the dye was excited at 543 nm. The first dichroic filter set was DM 405/488/543/638 nm. The imaging procedure was the same as that described in 1.7 colocalization study.
2. Synthesis.

General: All reactions were performed under nitrogen atmosphere. Solvents were carefully dried and distilled from appropriate drying agents prior to use. Commercially available reagents were used without further purification unless otherwise stated. All reactions were monitored by thin layer chromatography (TLC).

2.1 Synthesis of ZnL1.

ZnL1 was prepared by the modified literature method.\textsuperscript{[4]} A mixture of 98.0 mg (0.51 mmol) of 4-(diethylamino)-2-hydroxybenzaldehyde, 27.5 mg (0.25 mmol) of diaminomaleonitrile, and 55.5 mg (0.25 mmol) of Zn(OAc)\textsubscript{2}·2H\textsubscript{2}O was dissolved in 50 mL of ethanol and the solution was refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 65 mg (50% yield) of ZnL1. \textsuperscript{1}H NMR (d\textsubscript{6}DMSO, 300 M Hz): \(\delta\) 1.13 (t, 12H, \(J = 6.9\) Hz), 3.40 (t, 8H, \(J = 6.3\) Hz), 5.80 (s, 2H), 6.21 (d, 2H, \(J = 9.3\) Hz), 7.16 (d, 2H, \(J = 9.3\) Hz), 8.11 (s, 2H). ESI (m/z): [M+Na]\textsuperscript{+} calcd. for C\textsubscript{26}H\textsubscript{28}N\textsubscript{6}NaO\textsubscript{2}Zn, 543.14574; found 543.14532. IR: \(\nu\) (C\textequivN) = 2208\,\text{cm}^{-1}, \(\nu\) (C=N) = 1615\,\text{cm}^{-1}.

2.2 Synthesis of ZnL2.

\textsuperscript{1}H NMR (d\textsubscript{6}DMSO, 300 M Hz): \(\delta\) 1.13 (t, 12H, \(J = 6.9\) Hz), 3.40 (t, 8H, \(J = 6.3\) Hz), 5.80 (s, 2H), 6.21 (d, 2H, \(J = 9.3\) Hz), 7.16 (d, 2H, \(J = 9.3\) Hz), 8.11 (s, 2H). ESI (m/z): [M+Na]\textsuperscript{+} calcd. for C\textsubscript{26}H\textsubscript{28}N\textsubscript{6}NaO\textsubscript{2}Zn, 543.14574; found 543.14532. IR: \(\nu\) (C\equivN) = 2208\,\text{cm}^{-1}, \(\nu\) (C=N) = 1615\,\text{cm}^{-1}.

Compound 1. 4-(dimethylamino)-2-hydroxybenzaldehyde
**Compound 1.** was synthesized with simple literature procedure\(^5\). POCl\(_3\)(0.5 mL, 1.5 eqv.) was added slowly in dry DMF at 0 °C and stirred for 30 min. Then 3-(dimethylamino)phenol (0.50 mg, 3.6 mmol) dissolved in dry DMF was added to the reaction mixture at 0 °C slowly. Then the solution was slowly warmed to room temperature and stirred overnight. The reaction mixture was poured into ice and stirred for several minutes. The crude product was extracted by CH\(_2\)Cl\(_2\) and purified by column chromatography to give yellow oil (310 mg, yield: 56%). \(^1\)H NMR (300M Hz, CDCl\(_3\)); \(\delta\) 3.08 (s, 6H), 6.09 (d, 1H, \(J = 2.4\) Hz), 6.29 (dd, 1H, \(J = 2.4\) Hz, \(J = 9.0\) Hz), 7.30 (d, 1H, \(J = 8.7\) Hz), 9.53 (s, 1H), 11.61 (s, 1H).

**Compound ZnL\(_2\)**

A mixture of 100.6 mg (0.61 mmol) of compound 1, 4-(dimethylamino)-2-hydroxybenzaldehyde, 32.6 mg (0.30 mmol) of diaminomaleonitrile, and 66.1 mg (0.30 mmol) of Zn(OAc)\(_2\)·2H\(_2\)O was dissolved in 50 mL of ethanol and the solution was refluxed overnight. The solvent was removed by rotary evaporators, and the 74 mg (53% yield) of ZnL\(_2\). \(^1\)H NMR (d\(^d\)DMSO, 300 M Hz); \(\delta\) 3.38 (s, 12H), 5.95 (d, 2H, \(J = 2.7\) Hz), 6.26 (dd, 2H, \(J = 2.4\) Hz, \(J = 9.0\) Hz), 7.12 (d, 2H, \(J = 9.3\) Hz), 8.15 (s, 2H). ESI (m/z): [M+Na]\(^+\) calcd. for C\(_{22}\)H\(_{20}\)N\(_6\)NaO\(_2\)Zn, 487.08314; found 487.08277.

IR: \(\nu\) (C≡N) = 2207cm\(^{-1}\), \(\nu\) (C = N) = 1618cm\(^{-1}\).

### 2.3 Synthesis of ZnL\(_3\).

[Scheme 3. (a) BrCH\(_2\)COOCH\(_2\)CH\(_3\), (NH\(_4\))\(_2\)HOP\(_4\), KI, CH\(_3\)CN. (b) POCl\(_3\), DMF. (c) diaminomaleonitrile, Zn(OAc)\(_2\)·2H\(_2\)O, EtOH.]

**Compound 2. Diethyl 2,2'- (3-hydroxyphenylazanediyl)diacetate**

**Compound 2.** was synthesized with simple literature procedure\(^6\). \(m\)-Aminophenol (2.53 g, 0.023 mol), ethyl bromoacetate (5.2 mL, 0.047 mol), (NH\(_4\))\(_2\)HPO\(_4\) (3.04 g, 0.023 mol), KI (1.53 g, 9.2 mmol) were added to 100 mL of acetonitrile in a 250 mL flask. The solution was refluxing under nitrogen overnight, and the solvent was removed under the reduced pressure. The brown residue
was extracted with CH₂Cl₂, and then was purified by column chromatography to give yellow oil (3.9 g, yield: 55%). ¹H NMR (300M Hz, CDCl₃): δ 7.05 (m, 1H), 6.23 (m, 2H), 6.08 (m, 1H), 4.24 (q, 4H, J = 7.2 Hz), 4.10 (s, 4H), 3.87 (s, 1H), 1.29 (t, 6H, J = 9.76 Hz).

**Compound 3. Diethyl 2,2’-(4-formyl-3-hydroxyphenylazanediyl)diacetate**

**Compound 3.** was synthesized with simple literature procedure⁴. POCl₃ (2 mL, 1.5 eqv.) was added slowly in dry DMF at 0 °C and stirred for 30 min. Then **compound 2.** diethyl 2,2’-(3-hydroxyphenylazanediyl)diacetate (2.95 g, 0.01 mol) dissolved in dry DMF was added to the reaction mixture at 0 °C slowly. The solution was slowly warmed to room temperature and stirred overnight. Then the reaction mixture was poured into ice and stirred for several minutes. The product precipitated as white solids and was filtered (1.60 g, yield: 52%). ¹H NMR (300M Hz, CDCl₃): δ 1.30 (t, 6H, J = 6.9 Hz), 4.17 (s, 4H), 4.24 (q, 4H, J = 7.2 Hz), 6.06 (d, 1H, J = 2.7 Hz), 6.22 (dd, 1H, J = 2.4 Hz, J = 6.3 Hz), 7.35 (d, 1H, J = 8.7 Hz), 9.60 (s, 1H), 11.49 (s, 1H). ESI (m/z): [M+H]⁺ calcd. for C₁₅H₂₀NO₆, 310.12851; found 310.12866.

**Compound ZnL₃**

A mixture of 100.5 mg (0.3 mmol) of **compound 3.** diethyl 2,2’-(4-formyl-3-hydroxyphenylazanediyl)diacetate, 17.5 mg (0.15 mmol) of diaminomaleonitrile, and 65.8 mg (0.15 mmol) of Zn(OAc)₂·2H₂O was dissolved in 50 mL of ethanol and the solution was refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 61 mg (54% yield) of ZnL₃. ¹H NMR (d⁶DMSO, 300 M Hz): δ 1.23 (t, 12H, J = 6.9 Hz), 4.16 (q, 8H, J = 7.2 Hz), 4.28 (s, 8H), 5.65 (s, 2H), 6.05 (dd, 2H, J = 2.4 Hz), 7.20 (d, 2H, J = 9.3 Hz), 8.18 (s, 2H). ESI (m/z): [M+Na]⁺ calcd. for C₃₅H₃₆N₆NaO₁₀Zn, 775.16766; found 775.16614. IR: ν(C≡N) = 2216 cm⁻¹, ν(C = O) = 1745 cm⁻¹, ν(C = N) = 1615 cm⁻¹.

**2.4 Synthesis of ZnL₄.**
Scheme 4. (a) 1,4-dibromobutane, KOH, CH₃CN. (b) BBr₃, CH₂Cl₂, -78°C. (c) POCl₃, DMF. (d) diaminomaleonitrile, Zn(OAc)₂·2H₂O, EtOH.

**Compound 4. 1-(3-methoxyphenyl)pyrrolidine**

1.21 g (8.8 mmol) of 3-methoxy-N-methylaniline and 1.06 g (18.9 mmol) of KOH were dissolved in 30 mL of CH₃CN, and then 10.21 g (47.3 mmol) of 1,4-dibromobutane was added to the solution slowly. The solution was refluxed under nitrogen overnight, and the solvent was removed under the reduced pressure. The brown residue was extracted with CH₂Cl₂, and then was purified by column chromatography to give yellow oil (1.01 g, Yield: 65%). ¹H NMR (300 MHz, CDCl₃): δ 1.99 (m, 4H), 3.26 (m, 4H), 3.80 (s, 3H), 6.11 (t, 1H, J = 2.4 Hz), 6.22 (m, 2H), 7.13 (t, 1H, J = 8.1 Hz). ESI (m/z): [M+H]⁺ calcd. for C₁₁H₁₆NO, 178.1; found 178.1.

**Compound 5. 3-(pyrrolidin-1-yl)phenol**

0.86 g (4.8 mmol) of compound 4, 1-(3-methoxyphenyl)pyrrolidine was dissolved in 10 mL of dichloromethane. Added 1.0 mL (10.6 mmol) of BBr₃ to the solution at -78°C, and the solution was slowly warmed to room temperature and stirred overnight. Added iced methanol to the solution in ice bath, and removed the solvent under the reduced pressure. Then purified the product by column chromatography to give colorless oil (0.56 g, yield: 71%). ¹H NMR (300 MHz, CDCl₃): δ 2.00 (m, 4H), 3.26 (m, 4H), 4.54 (s, 1H), 6.05 (t, 1H, J = 2.4 Hz), 6.22 (m, 2H), 7.13 (t, 1H, J = 8.1 Hz). ESI (m/z): [M+H]⁺ calcd. for C₁₁H₁₆NO, 178.1; found 178.1.

**Compound 6. 2-hydroxy-4-(pyrrolidin-1-yl)benzaldehyde**

POCl₃ (0.2 mL) was added slowly in dry DMF at 0 °C and stirred for 30 min. Then 162.8 mg (1.0 mmol) compound 5, 3-(pyrrolidin-1-yl)phenol dissolved in dry DMF was added to the reaction mixture at 0 °C slowly. The solution was slowly warmed to room temperature and stirred overnight. Then the reaction mixture was poured into ice and stirred for several minutes. Extracted the product with DCM and then purified product by column chromatography to give colorless oil (107 mg, yield: 56%). ¹H NMR (300 MHz, CDCl₃): δ 2.04 (m, 4H, J = 3.0 Hz), 3.38 (m, 4H), 5.97 (d, 1H, J = 2.4 Hz), 6.13 (dd, 1H, J = 6.3 Hz, J = 2.4 Hz), 7.29 (d, 2H, J = 9.0 Hz), 9.50 (s, 1H), 11.70 (s, 1H).
**Compound ZnL₄**

A mixture of 60.5 mg (0.32 mmol) of compound 6, 2-hydroxy-4-(pyrrolidin-1-yl)benzaldehyde, 17.5 mg (0.15 mmol) of diaminomaleonitrile, and 65.8 mg (0.15 mmol) of Zn(OAc)₂·2H₂O was dissolved in 50 mL of ethanol and refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 32.8 mg (42% yield) of ZnL₄. ¹H NMR (d⁶DMSO, 300 M Hz): δ 1.81 (s, 8H), 3.42 (s, 8H), 5.69(s, 2H), 6.13 (d, 2H, J = 4.4 Hz), 7.16 (d, 2H, J = 9.0 Hz), 8.12 (s, 2H). ESI (m/z): [M+Na]⁺ calcd. for C₃₄H₃₆N₆NaO₁₀Zn, 539.11444; found 539.11400. IR: ν(C≡N) = 2205cm⁻¹, ν(C= N) = 1620cm⁻¹.

**2.5 Synthesis of ZnL₅**

Scheme 5. (a) 1-bromotetradecane, KHCO₃, CH₃CN. (b) BBr₃, CH₂Cl₂, -78°C. (c) POCl₃, DMF. (d) diaminomaleonitrile, Zn(OAc)₂·2H₂O, EtOH.

**Compound 7. 3-methoxy-N-methyl-N-tetradecylaniline**

A mixture of 1.0 g (7.4 mmol) of 3-methoxy-N-methylaniline, 0.74 g (7.4 mmol) of KHCO₃ and 5.73 g (20.7 mmol) of 1-bromotetradecane were dissolved in 30 mL of CH₃CN, the solution was refluxed under nitrogen overnight, and the solvent was removed under the reduced pressure. The brown residue was extracted with CH₂Cl₂, and then was purified by column chromatography to give yellow oil (2.1 g, yield: 85%). ¹H NMR (300M Hz, CDCl₃): δ 0.88 (m, 3H), 1.26 (s, 22H), 1.56 (s, 2H), 2.91 (s, 3H), 3.27 (t, 2H, J = 7.5 Hz), 3.79 (s, 3H), 6.22 (m, 2H), 6.32 (m, 1H), 7.12 (t, 1H, J = 7.8 Hz). ¹³C NMR (300M Hz, CDCl₃): δ 160.92, 150.90, 129.90, 105.45, 100.62, 98.78, 55.22, 53.01, 38.50, 32.07, 29.80 (broad), 29.76, 29.70, 29.51, 27.33, 26.86, 22.84, 14.27. ESI (m/z): [M+H]⁺ calcd. for C₂₂H₄₀NO, 334.31044; found 334.31030.

**Compound 8. 3-(methyl(tetradecyl)amino)phenol**

2.4 g (7.2 mmol) of compound 7. 3-methoxy-N-methyl-N-tetradecylaniline was dissolved in 10
mL of dichloromethane. Added 1.0 mL (10.6 mmol) of BBr₃ in the solution at -78 °C. And the mixture was slowly warmed to room temperature and stirred overnight. Added iced methanol to the solution in ice bath, and removed the solvent under the reduced pressure. Then purified the product by column chromatography to give colorless oil (1.34 g, yield: 58%). ¹H NMR (300M Hz, CDCl₃): δ 0.88 (t, 3H, J = 6.9 Hz), 1.28 (s, 22H), 1.55 (m, 2H, J = 7.2 Hz), 2.88 (s, 3H), 3.20 (t, 2H, J = 7.2 Hz), 6.14 (m, 2H), 6.32 (dd, 1H, J = 2.1 Hz, J = 6.0 Hz), 7.06 (t, 1H, 8.1 Hz). ESI (m/z): [M+H]+ calcd. for C₂₁H₃₈NO, 320.29479; found 320.29439.

**Compound 9. 2-hydroxy-4-(methyl(tetradecyl)amino)benzaldehyde**

POCl₃ (0.3 mL) was added slowly in dry DMF at 0 °C and stirred for 30 min. Then 1.28 g (4.0 mmol) of compound 8.3-(methyl(tetradecyl)amino)phenol in dry DMF was added to the reaction mixture at 0 °C slowly. And the mixture was slowly warmed to room temperature and stirred overnight. Then the reaction mixture was poured into ice and stirred for several minutes. Extracted the product with DCM and then purified crude product by column chromatography to give colorless oil (0.68 g, yield: 49%). ¹H NMR(300M Hz, CDCl₃): δ 0.88 (t, 3H, J = 5.4 Hz), 1.26 (s, 22H), 1.55 (m, 2H), 3.03 (s, 3H), 3.36, (t, 2H, J = 7.2 Hz), 6.07 (d, 2H, J = 2.4 Hz), 6.27 (dd, 1H, J = 2.7 Hz, J = 8.8 Hz), 7.27 (d, 1H, 8.7 Hz), 9.50 (s, 1H), 11.62 (s, 1H). ¹³C NMR(300M Hz, CDCl₃): δ 192.24, 164.37, 155.57, 135.34, 111.72, 104.65, 97.18, 52.74, 38.73, 32.06, 29.76, 29.70(broad), 27.17, 22.82, 14.24.

**Compound ZnL₅**

A mixture of 370 mg (1.06 mmol) of compound 9. 2-hydroxy-4-(methyl(tetradecyl)amino)benzaldehyde 55.9 mg (0.52 mmol) of diaminomaleonitrile, and 129.7 mg (0.59 mmol) of Zn(OAc)₂·2H₂O was dissolved in 50 mL of ethanol and the solution was refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 278 mg (63% yield) of ZnL₅. ¹H NMR (d₆DMSO, 300 M Hz): δ 0.83 (t, 6H, J = 4.8 Hz), 1.22 (s, 44H), 1.53 (s, 4H), 2.91 (s, 6H), 3.57 (t, 4H, J = 7.2 Hz), 5.80 (s, 2H), 6.24 (d, 2H, J = 8.7 Hz), 7.14 (d, 2H, J = 9.3 Hz), 8.12(s, 2H). ESI (m/z): [M+Na]+ calcd. for C₄₈H₆₀N₆NaO₂Zn, 851.49004; found 851.48942. IR: υ(C≡N) = 2213¹⁺, υ(C - N) = 1605¹⁺.

**2.6 Synthesis of ZnL₆**
Scheme 6. (a) 1,3-dibromopropane, KHCO₃, CH₃CN. (b) BBr₃, CH₂Cl₂, -78 °C. (c) POCl₃, DMF. (d) diaminomaleonitrile, Zn(OAc)₂·2H₂O, EtOH.

**Compound 10. N-(3-bromopropyl)-3-methoxy-N-methylaniline**

A mixture of 2.0 g (14.6 mmol) of 3-methoxy-N-methylaniline, 15 g (74.2 mmol) of 1,3-dibromopropane and 1.54 g (15.3 mmol) of KHCO₃ were dissolved in 30 mL of CH₃CN, and the solution was refluxed under nitrogen overnight. After reaction, the solvent was removed under the reduced pressure. The brown residue was extracted with CH₂Cl₂, and then was purified by column chromatography to give yellow oil (2.04 g, Yield: 54%). ¹H NMR(300M Hz, CDCl₃): δ 2.13 (m, 2H), 2.95 (s, 3H), 3.47 (m, 4H), 3.79 (s, 3H), 6.28 (m, 2H), 6.35 (dd, 1H, J = 1.5 Hz, J = 9.2 Hz), 7.14 (t, 1H, J = 7.5 Hz). ESI(m/z): [M+H]⁺ calcd. for C₁₁H₁₇BrNO, 258.04812; found: 258.04880.

**Compound 11. 3-((3-bromopropyl)(methyl)amino)phenol**

2.10 g (8.1 mmol) of **compound 10. N-(3-bromopropyl)-3-methoxy-N-methylaniline** was dissolved in 15 mL of dichloromethane. Added 1.0 mL (10.6 mmol) of BBr₃ to the solution at -78 °C, and the solution was slowly warmed to room temperature and stirred overnight. Added iced methanol to the solution in ice bath, and removed the solvent under the reduced pressure. Then purified the product by column chromatography to give colorless oil (1.04 g, yield: 53%). ¹H NMR(300M Hz, d⁶DMSO): δ 1.97 (m, 2H), 3.06 (s, 3H), 3.51 (m, 4H), 3.70 (s, broad, 1H), 6.60-6.80 (m, 3H), 7.22 (t, 1H, J = 7.8 Hz). ESI(m/z): [M+H]⁺ calcd. for C₁₀H₁₄BrNO, 244.03244; found: 244.03315.

**Compound 12. 4-((3-chloropropyl)(methyl)amino)-2-hydroxybenzaldehyde**

POCl₃ (0.9 mL) was added slowly in dry DMF at 0 °C and stirred for 30 min. Then 1.01 g (4.1 mmol) of **compound 11. 3-(methyl(tetradecyl)amino)phenol** in dry DMF was added slowly to the reaction mixture at 0 °C. And the solution was slowly warmed to room temperature and stirred...
overnight. Then the reaction mixture was poured into ice and stirred for several minutes. Extracted the product with DCM and then purified product by column chromatography to give colorless oil (480 mg, yield: 51%). $^1$H NMR (300M Hz, CDCl$_3$): $\delta$ 2.08 (m, 2H), 3.08 (s, 3H), 3.60 (m, 4H), 6.11 (d, 1H, J = 2.4 Hz), 6.32 (dd, 1H, J = 2.4 Hz, J = 9.0 Hz), 7.30 (d, 1H, 8.7 Hz), 9.54 (s, 1H), 11.59 (s, 1H). $^{13}$C NMR(300M Hz, CDCl$_3$): $\delta$ 192.59, 164.33, 155.36, 135.47, 112.06, 104.66, 97.50, 49.62, 42.36, 39.12, 30.03. ESI(m/z): [M+H]$^+$ calcd. for C$_{11}$H$_{15}$ClNO$_2$, 228.07858; found: 228.07800.

**Compound ZnL$_6$**

A mixture of 110 mg (0.48 mmol) of compound 12, 4-((3-chloropropyl)(methyl)amino)-2-hydroxybenzaldehyde, 26.0 mg (0.24 mmol) of diaminomaleonitrile, and 53.8 mg (0.24 mmol) of Zn(OAc)$_2$·2H$_2$O was dissolved in 50 mL of ethanol and refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 68 mg (48% yield) of ZnL$_6$. $^1$H NMR (d$_6$DMSO, 300 M Hz): $\delta$ 2.00 (m, 4H), 3.02 (s, 6H), 3.54 (t, 4H, J = 6.9 Hz), 3.71 (t, 4H, J = 6.3 Hz), 5.88 (d, 2H, J = 2.1 Hz), 6.28 (dd, 2H, J = 2.1 Hz, J = 9.2 Hz), 7.19 (d, 2H, J = 9.3 Hz), 8.16(s, 2H). ESI (m/z): [M+H]$^+$ calcd. for C$_{26}$H$_{27}$N$_6$Cl$_2$O$_2$Zn, 589.08585; found 589.08407. IR: $\nu$(C≡N) = 2210cm$^{-1}$, $\nu$(C = N) = 1615cm$^{-1}$.

**2.7 Synthesis of ZnL$_7$**

Scheme 7. (a) morpholine, KHCO$_3$, CH$_3$CN. (b) diaminomaleonitrile, Zn(OAc)$_2$·2H$_2$O, EtOH.

**Compound 13. 2-hydroxy-4-(methyl(3-morpholinopropyl)amino)benzaldehyde**

A mixture of 150 mg (0.66 mmol) compound 12, 4-((3-chloropropyl)(methyl)amino)-2-hydroxybenzaldehyde, 66 mg (0.66 mmol) KHCO$_3$, 490 mg (5.62 mmol) morpholine were dissolved in 30 mL of CH$_3$CN, the solution was refluxed under nitrogen overnight, and the solvent was removed under the reduced pressure. The brown residue was extracted with CH$_2$Cl$_2$, and then
was purified by column chromatography to give yellow oil (87 mg, yield: 47%). $^1$H NMR(300M Hz , CDCl$^3$): $\delta$ 1.78 (m, 2H), 2.34 (t, 2H, $J = 6.9$ Hz), 2.43 (t, 4H, $J = 4.5$Hz), 3.04 (s, 3H), 3.48 (t, 2H, $J = 6.9$ Hz), 3.74 (t, 4H, $J = 4.5$ Hz), 6.11 (d, 1H, $J = 2.4$ Hz), 6.33 (dd, 1H, $J = 2.4$ Hz, $J = 8.7$ Hz), 7.27 (d, 1H, 8.7 Hz), 9.51 (s, 1H), 11.59 (s, 1H). $^{13}$C NMR(300M Hz, CDCl$^3$): $\delta$192.18, 164.14, 155.45, 135.17, 111.59, 104.50, 97.13, 66.93, 55.48, 53.64, 50.01, 38.49, 24.09. ESI (m/z): [M+H]$^+$ calcd. for C$_{15}$H$_{23}$N$_2$O$_3$, 279.17032; found 279.17028.

**Compound ZnL$_7$**

A mixture of 80 mg (0.29 mmol) of compound 13. 2-hydroxy-4-(methyl(3-morpholinopropyl) amino)benzaldehyde, 15.1 mg (0.14 mmol) of diaminomaleonitrile, and 40.2 mg (0.14 mmol) of Zn(OAc)$_2$·2H$_2$O was dissolved in 25 mL of ethanol and refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 48 mg (50% yield) of ZnL$_7$. $^1$H NMR (d$_6$DMSO, 300 M Hz, ): $\delta$ 1.70 (m, 4H, $J = 6.6$ Hz), 2.26 (m, 12H), 2.98 (s, 6H), 3.45 (t, 4H, $J = 6.9$ Hz), 3.58 (t, 8H, $J = 4.5$ Hz), 5.82 (d, 2H, $J = 2.4$ Hz), 6.29 (d, 2H, $J = 9.9$ Hz), 7.14 (d, 2H, $J = 9.0$ Hz), 8.11(s, 2H). ESI (m/z): [M+H]$^+$ calcd. for C$_{34}$H$_{43}$N$_8$O$_4$Zn, 691.26932; found 691.26832. IR: $\nu$ (C≡N) = 2207 cm$^{-1}$, $\nu$ (C = N) = 1614 cm$^{-1}$.

### 2.8 Synthesis of ZnL$_8$

![Scheme 8.](image)

**Compound 14. 2-hydroxy-4-((3-(4-(2-hydroxyethyl)piperazin-1-yl)propyl)(methyl) amino) benzaldehyde**

A mixture of 120 mg (0.53 mmol) of compound 12. 4-((3-chloropropyl)(methyl)amino)-2-hydroxybenzaldehyde, 60 mg (0.60 mmol) of KHCO$_3$, 290 mg (2.23 mmol) of 2-(piperazin-1-yl)-ethanol were dissolved in 30 mL of CH$_3$CN, the solution was refluxing under nitrogen overnight, and the solvent was removed under the reduced pressure. The brown residue was extracted with CH$_2$Cl$_2$, and then was purified by column chromatography to give yellow oil (68 mg, yield: 40%).
1H NMR(300M Hz, CDCl3): δ 1.78 (m, 2H), 2.61 (m, 8H), 3.04 (s, 3H), 3.33 (t, 2H, J = 6.0 Hz), 3.46 (t, 2H, J = 7.2 Hz), 3.58 (s, 1H), 3.65 (t, 2H, J = 5.7 Hz), 3.84 (t, 2H, J = 6.0 Hz), 6.14 (d, 1H, J = 2.1 Hz), 6.32 (dd, 1H, J = 2.4 Hz, J = 9.0 Hz), 7.27 (d, 1H, 8.7 Hz), 9.51 (s, 1H), 11.67 (s, 1H).

ESI (m/z): [M+H]+ calcd. for C17H28N3O3, 322.2; found 322.2.

**Compound ZnL8**

A mixture of 60 mg (0.19 mmol) of compound 14, 2-hydroxy-4-(methyl(3-morpholinopropyl)amino)benzaldehyde, 10.6 mg (0.10 mmol) of diaminomaleonitrile, and 28.3 mg (0.10 mmol) of Zn(OAc)2·2H2O was dissolved in 20 mL of ethanol and refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 32 mg (41% yield) of ZnL8. 1H NMR (d6DMSO, 300M Hz): δ 1.69 (m, 4H), 2.2-2.4 (m, 16H), 3.00 (s, 6H), 3.30 (s, 4H), 3.47 (s, 4H), 3.60 (s, 4H), 3.84 (s, 4H), 5.75 (s, 2H), 5.83 (d, 2H, J = 2.1 Hz), 6.30 (dd, 2H, J = 2.1 Hz, J = 9.3 Hz), 7.16 (d, 2H, J = 9.3 Hz), 8.13 (s, 2H). ESI (m/z): [M+H]+ calcd. for C38H53N10O4Zn, 777.35372; found 777.35300. IR: ν(C≡N) = 2208 cm−1, ν(C=N) = 1614 cm−1.

2.9 Synthesis of ZnL9

![Scheme 9](attachment://image.png)

Compound 15. 1-(3-((4-formyl-3-hydroxyphenyl)(methyl)amino)propyl)-pyridinium chloride

Added 120 mg (0.53 mmol) of compound 12, 4-((3-chloropropyl)(methyl)amino)-2-hydroxy-benzaldehyde in 30 mL of pyridine, the solution was refluxing under nitrogen for 24h, and the solvent was removed under the reduced pressure and give the crude product (150 mg, yield: 92%). 1H NMR(300M Hz, CDCl3): δ 2.46 (m, 2H), 3.12 (s, 3H), 3.75 (t, 2H, J = 6.9 Hz), 5.22 (t, 2H, J = 8.1 Hz), 6.04 (d, 1H, J = 2.1 Hz), 6.38 (dd, 1H, J = 2.4 Hz, J = 8.7 Hz), 7.31 (d, 1H, 8.7 Hz), 8.03 (t, 2H, J = 7.2 Hz), 8.41 (t, 1H, J = 7.8 Hz), 9.52 (s, 1H), 9.67 (d, 2H, J = 5.7 Hz), 11.54 (s, 1H).
ESI (m/z): [M-Cl]+ calcd. for C\textsubscript{16}H\textsubscript{19}N\textsubscript{2}O\textsubscript{2}, 271.14410; found 271.14390.

**Compound ZnL\textsubscript{9}**

A mixture of 150 mg (0.49 mmol) of compound \textbf{15}. 1-(3-((4-formyl-3-hydroxyphenyl)(methyl)-amino)propyl)-pyridinium chloride, 25 mg (0.23 mmol) of diaminomaleonitrile, and 65.3 mg (0.23 mmol) of Zn(OAc)\textsubscript{2}·2H\textsubscript{2}O was dissolved in 50 mL of ethanol and refluxed overnight. The mixture was filtered hot and the solvent was removed by rotary evaporators. Then the crude product was washed repeatedly by ethanol to give 64 mg (17% yield) of ZnL\textsubscript{9}. \textsuperscript{1}H NMR (\textit{d}\textsubscript{6}DMSO, 300 M Hz):\(\delta\) 2.23 (m, 4H), 2.98 (s, 6H), 3.53 (t, 4H, J = 7.5 Hz), 4.67 (t, 4H, J = 8.1 Hz), 5.80 (d, 2H, J = 1.8 Hz), 6.26 (dd, 2H, J = 1.8 Hz, J = 9.0 Hz), 7.16 (d, 2H, J = 9.3 Hz), 8.13 (s, 2H), 8.15 (t, 4H, J = 6.3 Hz), 8.60 (t, 2H, J = 7.2 Hz), 9.10 (d, 4H, J = 6.0 Hz). ESI (m/z): [M-Cl]+ calcd. for C\textsubscript{36}H\textsubscript{36}N\textsubscript{8}O\textsubscript{2}ZnCl, 711.19505; found 755.14393. IR: ν(C≡N) = 2209 cm\textsuperscript{-1}, ν(C = N) = 1614 cm\textsuperscript{-1}.

### 2.10 Synthesis of ZnL\textsubscript{10}

![Scheme 10. (a) triphenylphosphine, mesitylene. (b) diaminomaleonitrile, Zn(OAc)\textsubscript{2}·2H\textsubscript{2}O, EtOH.](image)

**Compound 16. (3-((4-formyl-3-hydroxyphenyl)(methyl)amino)propyl)triphenylphosphonium chloride**

A mixture of 150 mg (0.66 mmol) of compound \textbf{12}. 4-((3-chloropropyl)(methyl)-amino)-2-hydroxy-benzaldehyde and 680 mg (2.59 mmol) triphenylphosphine were dissolved in 25 mL of degased mesitylene. The solution was refluxing under nitrogen for 3 days. The crude product precipitated as black crystals and then the mixture was filtered. The product was recrystallized by EtOH. (48 mg, yield: 15%). \textsuperscript{1}H NMR (300 M Hz, CDCl\textsubscript{3}):\(\delta\) 2.02 (m, 2H), 3.14 (s, 3H), 3.73 (t, 2H, J = 7.8 Hz), 3.97 (m, 2H), 5.92 (d, 1H, J = 2.4 Hz), 6.34 (dd, 1H, J = 2.4 Hz, J = 8.7 Hz), 7.26 (d, 1H, 9.3 Hz), 7.50-7.86 (m, 15H, PPh\textsubscript{3}), 9.51 (s, 1H), 11.53 (s, 1H). ESI (m/z): [M-Cl]+ calcd. for C\textsubscript{29}H\textsubscript{29}NO\textsubscript{2}P, 454.19304; found 454.19375.
**Compound ZnL₁₀**

A mixture of 46 mg (0.09 mmol) of compound 16, (3-((4-formyl-3-hydroxyphenyl)(methyl)-amino)propyl)triphenylphosphonium chloride, 4.9 mg (0.045 mmol) of diaminomaleonitrile, and 14.7 mg (0.05 mmol) of Zn(OAc)₂·2H₂O was dissolved in 20 mL of ethanol and refluxed overnight. The solvent was removed by rotary evaporators, and the crude product was washed repeatedly by iced ethanol to give 22 mg (37% yield) of ZnL₁₀. ¹H NMR (d⁶DMSO, 300 M Hz): δ 1.80 (m, 4H), 2.98 (s, 6H), 3.44 (s, 4H), 3.57 (s, 4H), 5.77 (d, 2H, J = 2.1 Hz), 6.18 (dd, 2H, J = 2.1 Hz, J = 9.0 Hz), 7.15 (d, 2H, J = 9.9 Hz), 7.76-7.90 (m, 30H, PPh₃) 8.15 (s, 2H). ³¹P NMR (d⁶DMSO, 300 M Hz): 25.0. ESI (m/z): [M-Cl]⁺ calcd. for C₆₂H₅₆N₆O₂P₂ZnCl, 1077.29145; found 1077.29410. IR: ν(C≡N) = 2209 cm⁻¹, ν(C=N) = 1612 cm⁻¹.
Figure S1. (a) Normalized absorption and (b) emission spectra of ZnL$_{1-10}$ in DMSO.
Figure S2. (a) Normalized absorption spectra of ZnL$_{1-10}$ in DMSO/H$_2$O (v/v=5:95) solution and (b) emission spectra of ZnL$_{1-4, 6-10}$ in DMSO/H$_2$O (v/v=5:95) solution.
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<th>Probe</th>
<th>$\lambda_{\text{max}}/\lambda_{\text{max}}$</th>
<th>$\varepsilon_{\text{max}}$</th>
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<th>$\tau/\text{ns}$</th>
<th>$\delta(\text{GM})$</th>
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<td>ZnL$_4$</td>
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<td>ZnL$_5$</td>
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$^a$ All data were measured in DMSO. $^b$ $\lambda_{\text{max}}$ values of the one-photon absorption and emission spectra in nm of the absorption spectrum. $^c$ Extinction coefficient in $1 \times 10^4 \text{ M}^{-1}\cdot\text{cm}^{-1}$. $^d$ Fluorescence quantum yield, the uncertainty is ±15%. $^e$ Life time. $^f$ Two-photon absorption cross section at 840 nm.
Table S2 Photophysical data of ZnL1-10 in DMSO/H2O (v/v=5:95) solution

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<th>Probe</th>
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<th>ε\text{max}^b</th>
<th>Φ^c</th>
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<td>2.00, 2.21</td>
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\(\lambda\text{max}\) values of the one-photon absorption and emission spectra in nm of the absorption spectrum. \^b\ Extinction coefficient in \(1 \times 10^4\) M\(^{-1}\)cm\(^{-1}\). \(^c\) Fluorescence quantum yield, the uncertainty is ±15\%. \(^d\) The photophysical data of ZnL5 were not determined for the poor solubility in aqueous solution.
**Figure S3.** (a) Normalized absorption of ~1.0 μM ZnL₃ with the increase of pHs (from 2.38 to 12.32) in 50 mM mixed buffer. (b) Normalized emission spectra of ~2.0 μM ZnL₃ at different pHs in 50 mM mixed buffer.
Figure S4. (a) Normalized absorption of ~1.0 μM ZnL₄ with the increase of pHs (from 2.23 to 11.76) in 50 mM mixed buffer. (b) Normalized absorption of ~1.0 μM ZnL₄ with the decrease of pHs (from 12.78 to 3.78) in 50 mM mixed buffer. (c) Normalized emission spectra of ~2.0 μM ZnL₄ at different pHs in 50 mM mixed buffer.
Figure S5. (a) Normalized absorption of ~1.0 μM ZnL7 with the increase of pHs (from 4.05 to 9.33) in 50 mM mixed buffer. (b) Normalized absorption of ~1.0 μM ZnL7 with the decrease of pHs (from 11.04 to 4.75) in 50 mM mixed buffer. (c) Normalized emission spectra of ~2.0 μM ZnL7 at different pHs in 50 mM mixed buffer.
Figure S6. Absorption spectra of ZnL₈ in DMSO/H₂O (v/v=5:95) solution at different time.
Figure S7. Power dependence response curve of ZnL₇, carried out at 800nm.

slope = 1.87 ± 0.05
Figure S8. Two-photon induced excitation spectrum of $\text{ZnL}_4$ (a), $\text{ZnL}_7$ (b) and $\text{ZnL}_8$ (c) in DMSO ($1\times10^{-5}$ M).
Figure S9. Solvent effect of ZnL$_4$ (a) and ZnL$_7$ (c) on the emission spectra and emission spectra of ZnL$_4$ (b) and ZnL$_7$ (d) in living HeLa Cells.
Figure S10. Single-photon fluorescence colocalization images of HeLa cells incubated with \textbf{ZnL}$_1$ (2 μM, 0.5 h). (a) Bright-field image of the HeLa cells stained with \textbf{ZnL}$_1$. (b) One-photon confocal fluorescence image of \textbf{ZnL}$_1$. (c) Merged image of (a) and (b).
**Figure S11.** Single-photon fluorescence colocalization images of CHO cells incubated with ZnL₂ (2 μM, 0.5 h). (a) Bright-field image of the CHO cells stained with ZnL₂. (b) One-photon confocal fluorescence image of ZnL₂. (c) Merged image of (a) and (b).
Figure S12. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₃ (2 μM, 0.5 h) and ER-Tracker Green (1 μM, 0.5 h). (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of ER-Tracker Green. (c) Confocal fluorescence image of ZnL₃. (d) Merged image of (b) and (c). Pearson’s Coefficient: 0.73.
Figure S13. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₅ (10 μM, 0.5 h). (a) Bright-field image of the HeLa cells stained with ZnL₅. (b) One-photon confocal fluorescence image of ZnL₅. (c) Merged image of (a) and (b).
Figure S14. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₆ (2 μM, 0.5 h) and ER-Tracker Green (1 μM, 0.5 h). (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of ER-Tracker Green. (c) Confocal fluorescence image of ZnL₆. (d) Merged image of (b) and (c). Pearson’s Coefficient: 0.84.
Figure S15. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL7 (2 μM, 0.5 h) and transfected with FYVE-EGFP plasmids. (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of FYVE-EGFP. (c) Confocal fluorescence image of ZnL7. (d) Merged image of (b) and (c). Pearson’s Coefficient: 0.72.
Figure S16. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₈ (2 μM, 0.5 h) and LysoTracker Green DND-26 (75 nM, 30 min). (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of LysoTracker Green DND-26. (c) Confocal fluorescence image of ZnL₈. (d) Merged image of (b) and (c). Pearson’s Coefficient: 0.68.
**Figure S17.** Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₈ (2 μM, 0.5 h) and transfected with FYVE-EGFP plasmids. (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of FYVE-EGFP. (c) Confocal fluorescence image of ZnL₈. (d) Merged image of (b) and (c).
Figure S18. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL₈ (2 μM, 0.5 h) and transfected with Golgi-EGFP plasmids. (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of Golgi-EGFP. (c) Confocal fluorescence image of ZnL₈. (d) Merged image of (b) and (c).
Figure S19. Single-photon fluorescence colocalization images of HeLa cells incubated with \( \text{ZnL}_{10} \) (2 μM, 0.5 h) and transfected with FYVE-EGFP plasmids. (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of FYVE-EGFP. (c) Confocal fluorescence image of \( \text{ZnL}_{10} \). (d) Merged image of (b) and (c).
Figure S20. Single-photon fluorescence colocalization images of HeLa cells incubated with ZnL10 (2 μM, 0.5 h) and MitoTracker Green FM (100 nM, 0.5 h). (a) Differential interference contrast (DIC) image. (b) Confocal fluorescence image of MitoTracker Green FM. (c) Confocal fluorescence image of ZnL10. (d) Merged image of (b) and (c).
Figure S21. (a) Bright-field image of the HeLa cells stained with ZnL₄. (b) Two-photon microscopy image of the same cells with excitation at 840 nm. (c) Merged image of (a) and (b).
Figure S22. Relative cellular fluorescence intensity (RCFI) of ZnL. HeLa cells were stained with 2 μM of ZnL for 30 min and then incubated in dye-free complete medium for different time.
**Figure S23.** Photostability of ZnL₇ in HeLa cells under two-photon excitation. (a) Confocal Microscopy Images were taken at (a) 0, (b) 60, (c) 120, (d) 180 and (e) 240 s under successive irradiation. All the images are obtained under the same condition. (b) Fluorescence intensity curves of ZnL₇.
Figure S24. Photostability comparison of ZnL₄ under Single and two-photon excitation. Normalized Fluorescence intensity curves of ZnL₄.
Reference:


