Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

A Two-In-One Fluorescent Sensor With Dual Channels to Discriminate Zn^{2+} and Cd^{2+}

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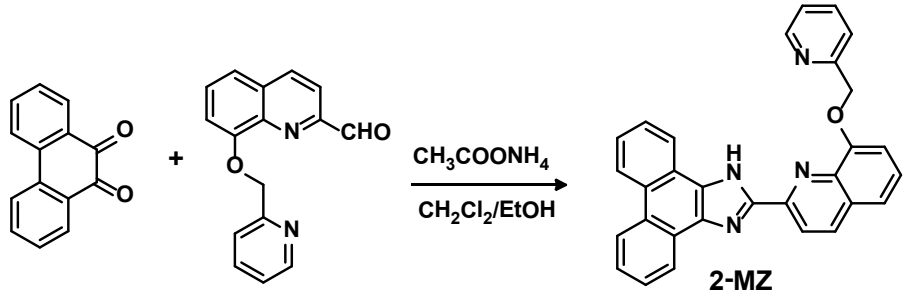
Contents

Materials and Instrument

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz) using TMS as internal standard. Mass spectra (MALDI-TOF) were obtained Autotlex III spectrometers. Steady-state emission spectra were recorded at ambient temperature on a Hitachi F-7000 Spectrophotometer and UV/Vis spectra were recorded on a Perkin-Elmer Lambda 950 UV-visible spectrophotometer.

Preparation of 2-MZ



Scheme S1 Synthesis route of 2-MZ

9, 10-phenanthrenequinone (0.21 g, 1 mmol), ammonium acetate (0.75 g, 10 mmol) were added to the solution of ethanol and dichloromethane (1:1, v/v). After refluxing for 10 min, 8-pyridymethyloxy-quinoine-2-carbaldeyde[1] (0.32 g, 1.2 mmol) and a catalyst amount of glacial acetic acid were added. The reaction mixture was held at reflux for another 3 h. After cooling to room temperature, the mixture was filtered. The solid was purified by column chromatography on silica gel to obtain 2-MZ as a yellow solid in 32% yield. ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ_H[ppm]: 13.99 (s, 1H), 8.97 (d, J = 7.8 Hz, 1H), 8.89 (dd, J = 12.0, 8.5 Hz, 2H), 8.67 (d, J = 7.9 Hz, 1H), 8.62 (dd, J = 7.4, 4.8 Hz, 2H), 8.54 (d, J = 8.7 Hz, 1H), 7.89-7.83 (m, 1H), 7.83-7.73 (m, 3H), 7.68 (dd, J = 12.3, 6.9 Hz, 2H), 7.64-7.60 (m, 1H), 7.52 (t, J = 7.9 Hz, 1H), 7.39-7.35 (m, 1H), 7.32 (d, J = 6.9 Hz, 1H), 5.64 (s, 2H). HRMS(ESI$^+$): calcd.for $C_{30}H_{20}N_4O$, $[M+H]^+$ 453.1710, found 453.1673. $[M+Na]^+$ 475.1529, found 475.1500.

Reference

1. L. Xue, H. H. Wang, X. J. Wang and H. Jiang, *Inorg. Chem.*, 2008, **47**, 4310.

Characterization of 2-MZ

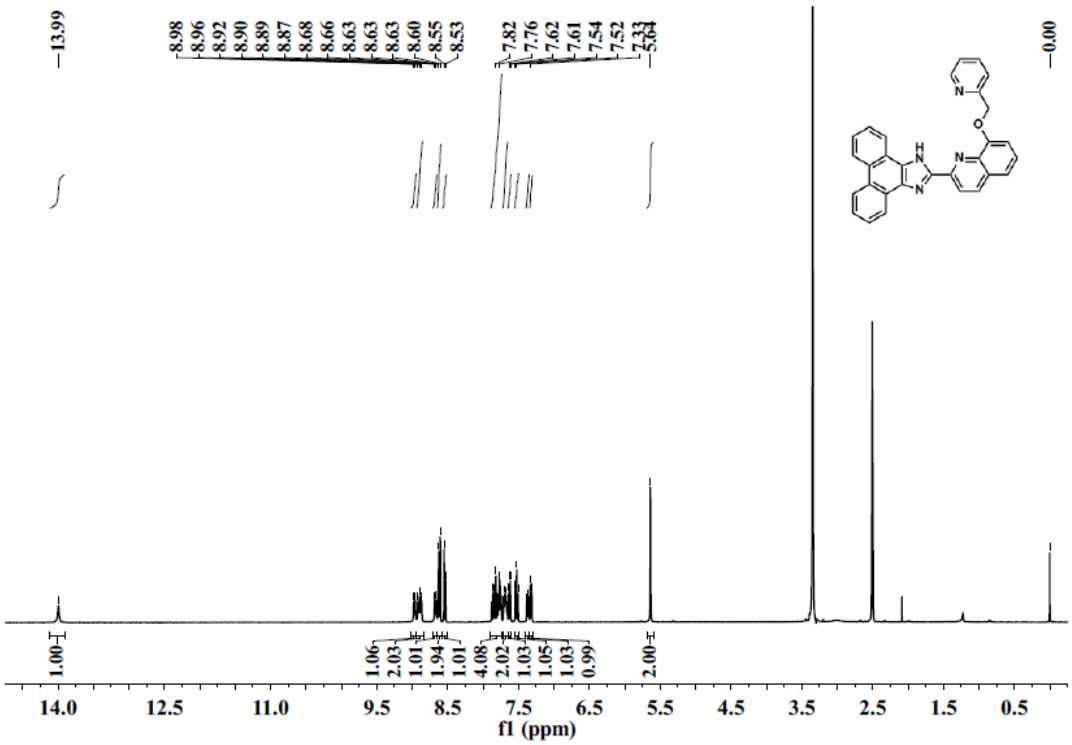


Fig. S1 ^{1}H NMR of 2-MZ in DMSO-d_6

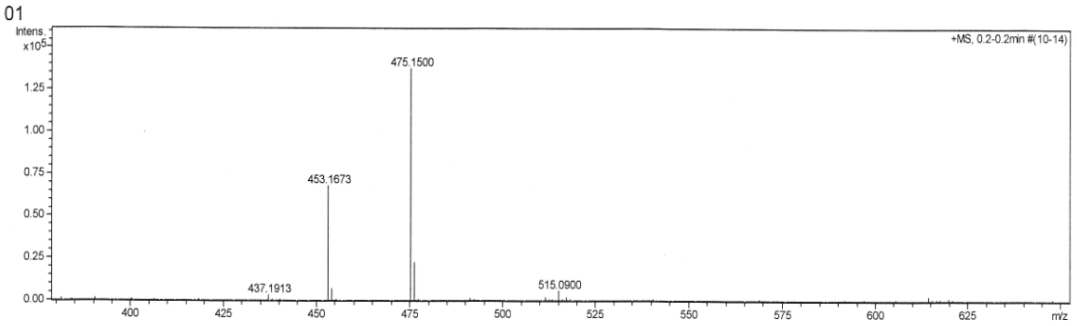


Fig. S2 ESI-MS spectrum (positive mode) of 2-MZ. Peak at m/z 453.1673 corresponds to $[2\text{-MZ} + H]^+$， m/z 475.1500 corresponds to $[2\text{-MZ} + Na]^+$

Job's plot

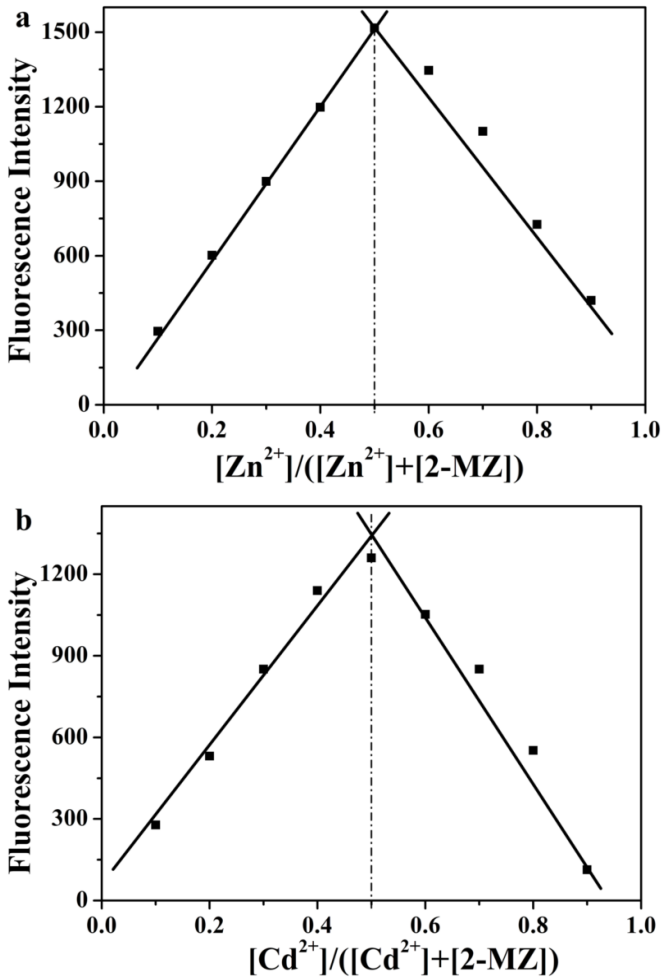
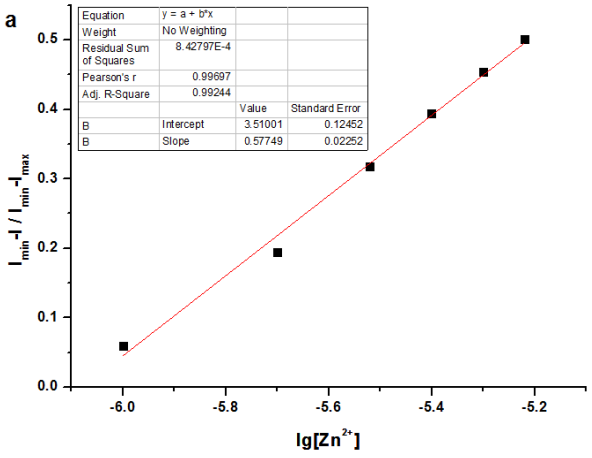


Fig S3 Job's plot for determining the stoichiometric ratio between 2-MZ and Zn^{2+} (a) or 2-MZ and Cd^{2+} (b), where the variations of fluorescence intensity as a function of molar ratio X_M. The sum of2-MZ and Zn^{2+} or Cd^{2+} concentrations is 10 μM

Value of Detection Limit



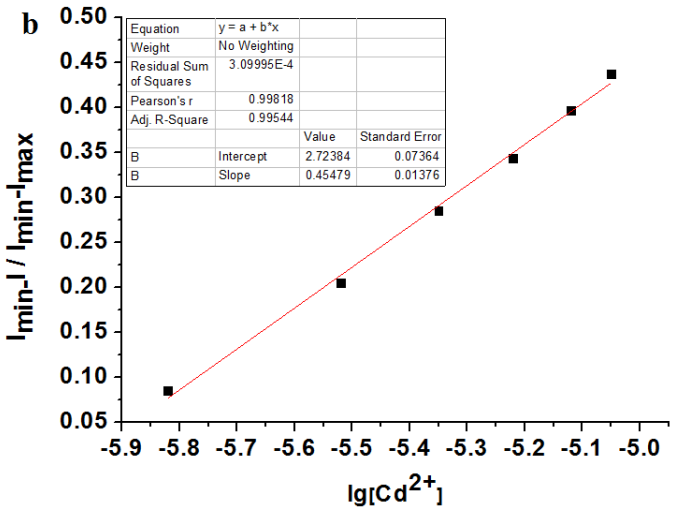


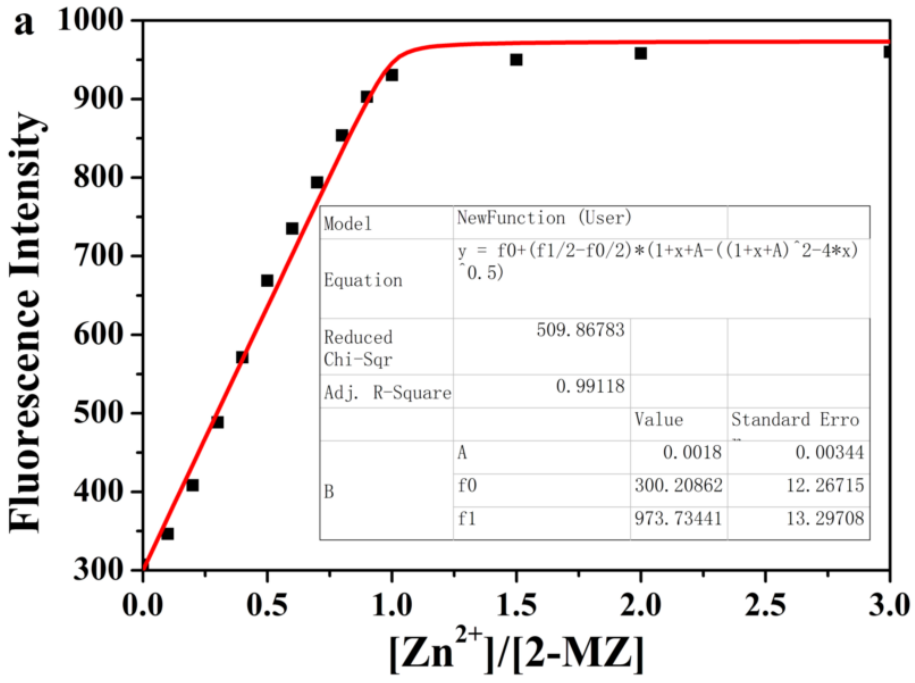
Fig S4 Fitting of fluorescence titration curve of 2-MZ and Zn^{2+} (a) or 2-MZ and Cd^{2+} (b).

Determination of K_d

The apparent dissociation constants (K_d) of 2-MZ with Zn^{2+} and Cd^{2+} were determined using the nonlinear least-squares analysis based on a 1:1 complex expression:

$$F = F_0 + \frac{F_{max} - F_0}{2}\left\{1 + \frac{C_M}{C_L} + \frac{1}{K_S C_L} - \left[\left(1 + \frac{C_M}{C_L} + \frac{1}{K_S C_L}\right)^2 - 4\frac{C_M}{C_L}\right]^{1/2}\right\}$$

Where F and F_0 are the fluorescence intensities of 2-MZ in the presence and absence of Zn^{2+}or Cd^{2+}, C_M and C_L are the concentrations of Zn^{2+} or Cd^{2+} and 2-MZ, and K_s is the stability constant.



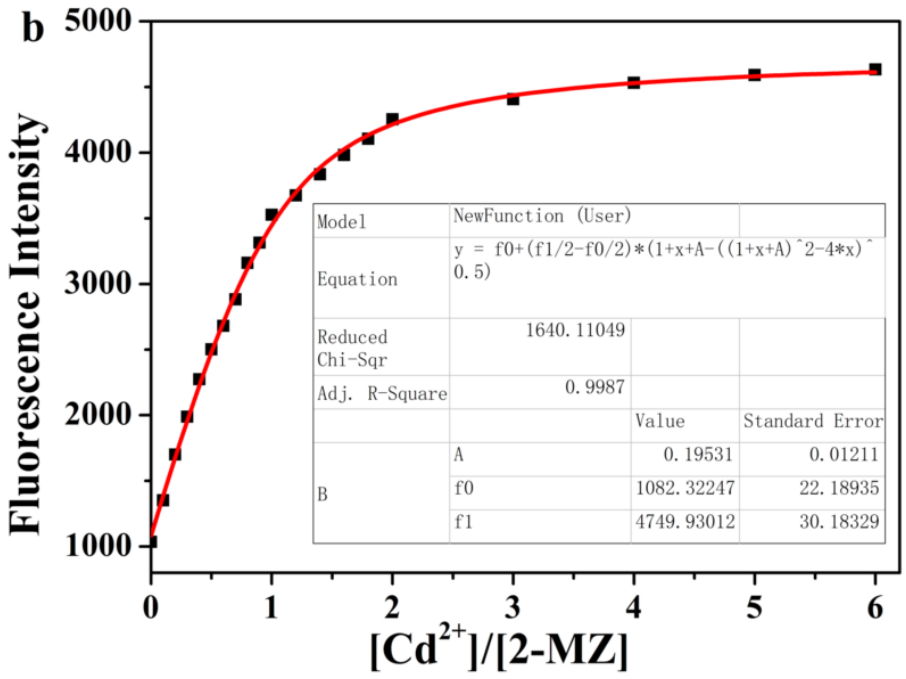


Fig. S5 (a) A nonlinear fitting curve of the fluorescence intensity of 2-MZ versus $[Zn^{2+}]/[2\text{-MZ}]$ at 525 nm. (b) A nonlinear fitting curve of the fluorescence intensity of 2-MZ versus $[Cd^{2+}]/[2\text{-MZ}]$ at 490 nm.

pH influence

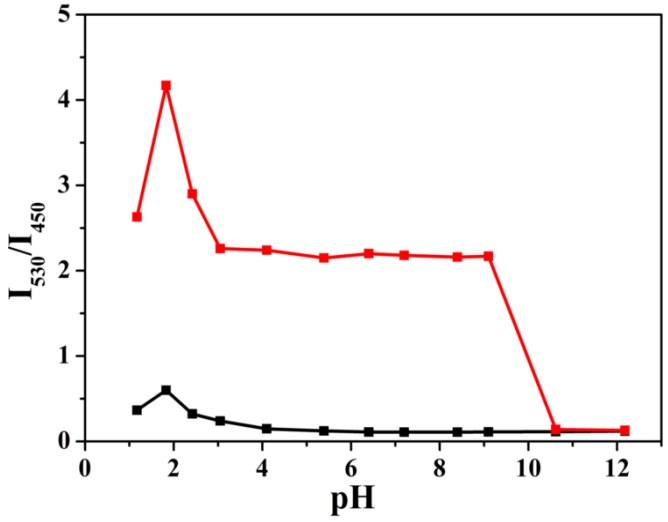


Fig. S6 Emission ratio (I_{530}/I_{450}) of 2-MZ vs. pH values in the absence (black line) and in the presence (red line) of Zn^{2+}. Excitation at 375 nm.

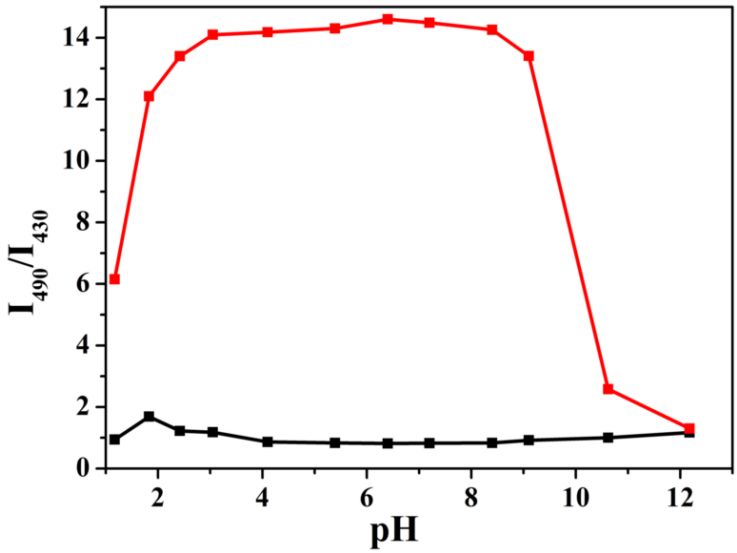


Fig. S7 Emission ratio (I_{490}/I_{430}) of 2-MZ vs. pH values in the absence (black line) and in the presence (red line) of Cd^{2+}. Excitation at 375 nm.

Excitation spectrum

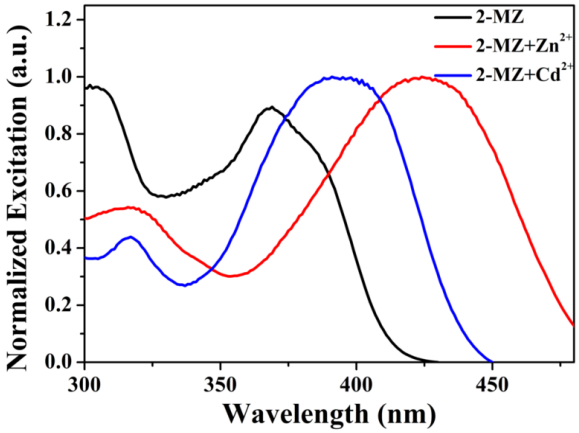


Fig. S8 (a) Excitation spectral changes of 2-MZ (10 μM) in aqueous solution (10 mM HEPES; DMF/H_2O = 1:1, v/v; pH 7.2) upon addition of Zn^{2+} and Cd^{2+}.

Spectrum changes of 2-MZ-Cd^{2+} upon addition of Zn^{2+}

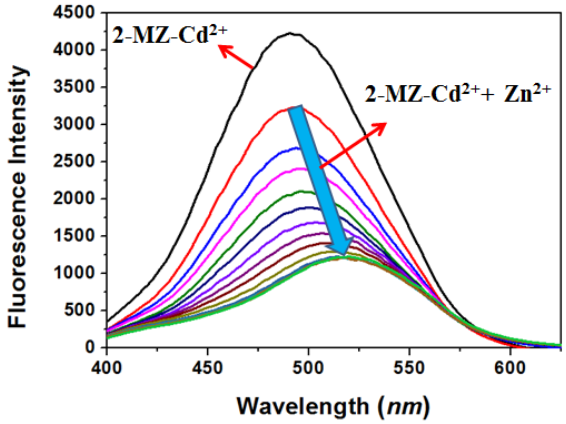


Fig. S9 Fluorescence changes of 2-MZ (10 μM) and Cd^{2+} (60 μM) in aqueous solution (10 mM HEPES; DMF/H_2O = 1:1, v/v; pH 7.2) upon addition of Zn^{2+} (0 to 50 μM).

Theoretic Calculations

All of the ab initio calculations were carried out using Gaussian 09 program package. The geometry optimizations were performed in the solvent at B3LYP/6-311+G(d), LANL2DZ level. The solvent effect of water was evaluated by using the SMD implicit solvent model.

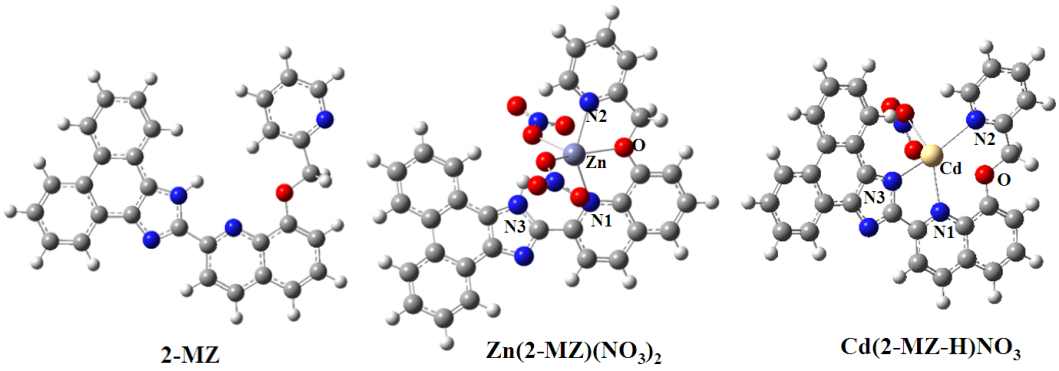


Fig. S10 B3LYP optimized geometries of 2-MZ and its complexes with Zn^{2+} and Cd^{2+}. Grey = C; Red = O; Blue = N; Slate Gray = Zn, Light Yellow = Cd.

Table S1 Selected Bond Distances (Å) for the complexes $Zn(2\text{-}MZ)(NO_3)_2$ and $Cd(2\text{-}MZ\text{-}H)NO_3$

Compound	$Zn(2\text{-}MZ)(NO_3)_2$	$Cd(2\text{-}MZ\text{-}H)NO_3$
M-N1	2.29	2.43
M-N2	2.28	2.42
M-N3	3.26	2.33
M-O	2.28	2.60

M represents the metal ion Zn or Cd

Cell culture and confocal imaging

HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum, penicillin (100 units/mL), streptomycin (100 mg/mL) and 5% CO_2 at 37 °C. The cells were cultured 3 days before dye loading onto 35-mm-diameter glass-bottomed coverslips. Then the cells were washed with PBS, bathed in serum-free DMEM containing 10 μM 2-MZ (1% DMSO) for 30 min at 37 ℃, washed with PBS three times to removethe excess cell, and bathed in DMEM (2 mL) before imaging. For imaging of Zn^{2+}, the exogenous Zn^{2+} was introduced by incubating the cells with 50 μM $ZnCl_2$ solution for 20 min. Confocal fluorescence imaging was performed on a Zeiss LSM 510 Meta microscopy system with a 40 × oil immersion objective lens. Fluorescence at two emission channels of 420-480 nm and 505-530 nm was measured at room temperature by excitation at 405 nm.