Supplementary Material (ESI) for Dalton Transactions

Supporting Information

A Ratiometric Fluorescent Probe for Oxalate based on Alkyne-Conjugated Carboxamidoquinolines in Aqueous Solution and Imaging in Living Cell

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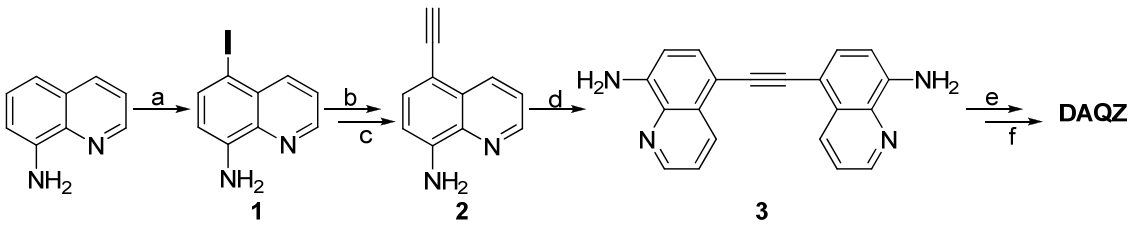
Materials. All the solvents were of analytical grades without further purification unless otherwise noted. ^{1}H-NMR spectra were measured on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$ or DMSO-d_6, TMS as internal standard). Electrospray ionization (ESI) Mass Spectroscopy was performed in a HP 1100 LC-MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and were uncorrected. All pH measurements were made with a Sartorius basic pH-Meter PB-20. Fluorescence spectra were determined by using a Varian Cary Eclipse Fluorescence Spectrometer. Absorption spectra were determined by a Varian Cary 100 UV-vis Spectrophotometer. Fluorescence quantum yields were determined by using quinine sulphate in 0.05 M H_2SO_4 (Φ = 0.564) as a reference.

Synthesis



Scheme S1 Synthesis of **DAQZ**. (a) Iodine, dioxane/pyridine, 0 ^{o}C, 77.6%; (b) Trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, CuI, THF, *i*-Pr_2NEt, room temperature; (c) K_2CO_3, MeOH, room temperature, 60%; (d)**1** and **2**, $PdCl_2(PPh_3)_2$, CuI, THF, *i*-Pr_2NEt, room temperature, 40.7%; (e) 2-chloroacetyl chloride, CH_2Cl_2, *i*-Pr_2NEt, 0 ^{o}C; (f) 2-(2-aminoethoxy)ethanol, KI, CH_3CN, *i*-Pr_2NEt, 43.5%.

Synthesis of 5-iodoquinolin-8-amine (1) [1]**:**

The 8-aminoquinoline (720 mg, 5 mmol) was dissolved in dioxane (30 mL) and pyridine (30 mL) and the solution was cooled to 0 ^{o}C. Iodine (1.90 g, 15 mmol) was added in one portion. The solution progressively took a dark brown color. After 1 h, the ice bath was removed and a supplementary portion of iodine (630 mg, 5 mmol) was added. The solution was further stirred for one hour at room temperature. A saturated solution of sodium thiosulfate was then added until the brown color disappeared. The mixture was extracted with dichloromethane and washed with water. After evaporation, the product was filtered through a short plug of silica, eluted with the dichloromethane/petroleum ether (3:1, v/v) to afford 5-iodoquinolin-8-amine. Yield: 1.05 g (77.6%), mp: 123.1~124.5 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ 8.73 (dd, J_1 = 1.2 Hz, J_2=2.4 Hz, 1H), 8.30 (dd, J_1 = 1.2 Hz, J_2=1.2 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.48-7.46 (m, 1H), 6.73 (d, J = 8.0 Hz, 1H), 5.24 (-CNH_2, s, 2H) ppm.

Synthesis of 5-ethynylquinolin-8-amine (2):

Coupling a terminal alkyne with an aryl halide is based on the reported procedure (Castro-Stephens/Sonogashira protocol)[2]. To an oven dried flask containing a magnetic stir bar was added the 5-iodoquinolin-8-amine (200 mg, 0.74 mmol), bis(triphenylphosphine)palladium(II) dichloride (25 mg, 0.036 mmol), and copper(I) iodide (14 mg, 0.074 mmol). The vessel was then sealed with a rubber septum, evacuated and backfilled with N_2 three cycles. A co-solvent of anhydrous THF (5 mL) was added followed by *i*-Pr_2NEt (600 μL). The terminal trimethylsilylacetylene (87 mg, 0.89 mmol) was added and the mixture was heated to 30 ^{o}C for 4h. The reaction vessel was cooled to room temperature and quenched with water. The organic layer was diluted with CH_2Cl_2 and washed with a saturated solution of NH_4Cl. The organic layer was

dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. The crude product (280 mg) was not further purified and characterized, then directly dissolved in MeOH (10 mL) and K_2CO_3 (828 mg, 6 mmol) was added. The mixture was stirred for 10 h and then poured into water and extracted with EtOAc. The extracts were washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*, the brown oil residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 5:1, v/v) to give 5-ethynylquinolin-8-amine as a yellow solid (100mg, 60%). mp: 126.2~128.5 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ 8.78 (d, J = 3.6 Hz, 1H), 8.56 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.49-7.45 (m, 1H), 6.83 (d, J = 7.6 Hz, 1H), 5.23 (-CNH_2, s, 2H), 3.42 (-CCH, s, 1H) ppm.

Synthesis of 5, 5'-(ethyne-1, 2-diyl)diquinolin-8-amine (3):

To an oven dried flask containing a magnetic stir bar was added the 5-iodoquinolin-8-amine (**1**) (64.8 mg, 0.24 mmol), $PdCl_2(PPh_3)_2$ (8.4 mg, 0012 mmol), and CuI (3 mg, 0.012 mmol). The vessel was then sealed with a rubber septum, evacuated and backfilled with N_2 three cycles. A co-solvent of anhydrous THF (5 mL) was added followed by *i*-Pr_2NEt (1 mL). The 5-ethynylquinolin-8-amine (**2**) (40 mg, 0.24 mmol) was added and the mixture was heated to 30 ^{o}C for 4h. The reaction vessel was cooled to room temperature and quenched with water. The organic layer was diluted with CH_2Cl_2 and washed with a saturated solution of NH_4Cl. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (ethyl acetate/petroleum ether, 3:1, v/v) to afforded 5, 5'-(ethyne-1, 2-diyl)diquinolin-8-amine as a brown yellow solid (30 mg, 40.7%). mp: 208.5~210.1 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, TMS): δ 8.80 (dd, J_1 = 2.4 Hz, J_2=1.2 Hz, 2H), 8.61 (d, J = 8.4 Hz, 2H), 7.67-7.63 (m, 4H), 6.89 (d, J = 8.0 Hz, 2H), 6.38 (-CNH_2, s, 4H) ppm.

Synthesis of the target compound DAQZ:

DAQZ was prepared according to the reported procedure[3]. In a typical experiment, 2-chloroacetyl chloride (30 mg, 0.24 mmol) was dissolved in anhydrous dichloromethane (3 mL), then added dropwise to a cooled, stirred solution of 5, 5'-(ethyne-1, 2-diyl)diquinolin-8-amine (25 mg, 0.08 mmol) and TEA (35 mg, 0.32 mmol) in anhydrous dichloromethane (30 mL) within 1 h, after stirred for 4 h at room temperature, the solvent was removed under vacuum to obtain a yellow

solid (35 mg), which was not further purified because of its poor solubility. The crude product, 2-(2-aminoethoxy)ethanol (73.6 mg, 0.70 mmol), *i*-Pr_2NEt (90.6 mg, 0.7 mmol) and KI (4 mg) were added to acetonitrile (20 mL), after stirred and refluxed for 10 h under nitrogen atmosphere, the mixture was cooled to room temperature and the solvent was removed under vacuum to obtain a yellow solid, which was purified by silica gel column chromatography using dichloromethane /methanol (35:1, v/v) as eluent to afford **DAQZ as** a yellow solid (20 mg, 43.5%). mp: 185.2~187.9 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz, TMS): δ 11.39 (-*NH*CO-, s, 2H), 8.93 (d, *J* = 5.6 Hz, 2H), 8.82 (d, *J* = 8.0 Hz, 2H), 8.77 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.63-7.61 (m, 2H), 3.79-7.76 (O-*CH_2CH_2*-OH, m, 8H), 3.66-3.64 (CO*CH_2*, NHCH$_2$*CH_2*, m, 8H), 3.02(NH*CH_2*CH_2, t, J_1 = J_2 = 5.2 Hz, 4H) ppm. ^{13}C-NMR ($CDCl_3$, 400 MHz): δ 170.95, 148.99, 138.62, 134.91, 133.63, 131.91, 128.28, 122.31, 116.18, 114.89, 91.04, 72.39, 70.65, 61.83, 53.74, 49.45 ppm. HRMS (ESI): [M+H$^+$] calcd for $C_{32}H_{37}N_6O_6$, 601.2775; found, 601.2772 (100%). FT-IR (KBr): ν_{max} 3297.75, 2915.73, 2843.31, 2348.31, 1679.44, 1521.97, 1451.67, 1367.31, 1322.32, 1114.24, 1060.81, 796.49 cm^{-1}.

Reference

[1] C. Monnereau, E. Blart and F. Odobel, *Tetrahedron Lett.* 2005, **46**, 5421.

[2] A. K. Flatt, Y. Yao, F. Maya and J. M. Tour, *J. Org. Chem.* 2004, **69**, 1752.

[3] Y. Zhang, X. Guo, W. Si, L. Jia and X. Qian, *Org. Lett.* 2008, **10**, 473.

Spectroscopic Data

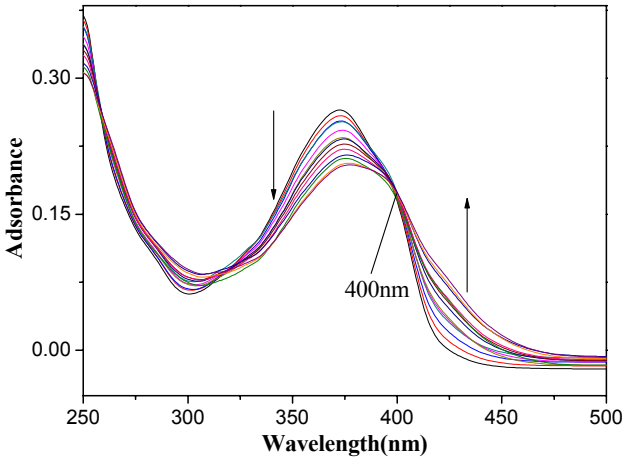


Figure S1 Absorption spectra of a solution of **DAQZ** (10 μM) in the presence of increasing Zn^{2+} concentrations (0~4 equiv) in tris-HCl (0.01 M) solution (ethanol-water, 1:9, pH=6.02).

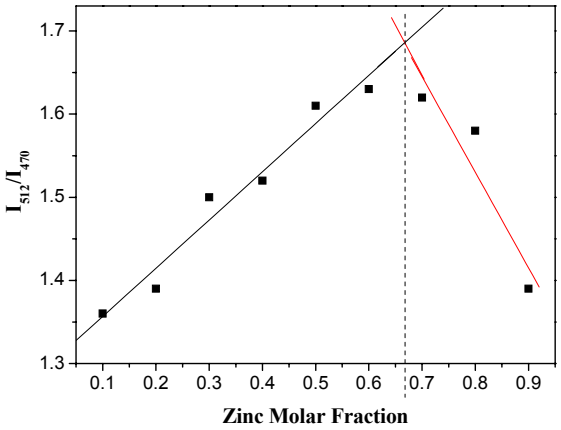


Figure S2 Job's plot for **DAQZ** in tris-HCl (0.01 M) solution (ethanol-water, 1:9, pH = 6.14). The total [DAQZ] + $[Zn^{2+}]$ = 100 μM.

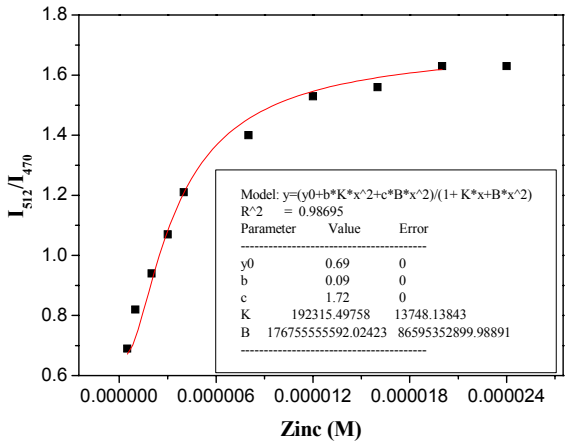


Figure S3. Fitting curve of fluorescence intensity at $I_{512\ nm}/I_{470\ nm}$ of **DAQZ** versus increasing concentrations of Zn^{2+} in tris-HCl (0.01 M) solution (ethanol-water, 1:9, pH = 6.14). The concentration of **DAQZ** was 0.2 μM.

The association constant (K_s, Formation of a 1:2 complex) was determined by a nonlinear least-squares analysis of I versus C_M using the following equation.

$$I = \frac{I_0 + C_M \Phi K_{11}[M] + Y_{lim}\beta_{21}[M]^2}{1 + K_{11}[M] + \beta_{21}[M]^2} \qquad \textbf{(1)}$$

Where $\beta_{21} = K_{11}K_{21}$, $[M] \approx C_M$ is Zn^{2+} ion concentration, I_0 or I is emission intensity in the absence or presence of Zn^{2+} ion, Φ is approximately 0.09, the quantum yield of the 1:1 **DAQZ-Zn^{2+}** complex. (B. Valeur, *Moleucular Fluorescence: Principle and Applications*, Wiley-VCH, Germany, 2002)

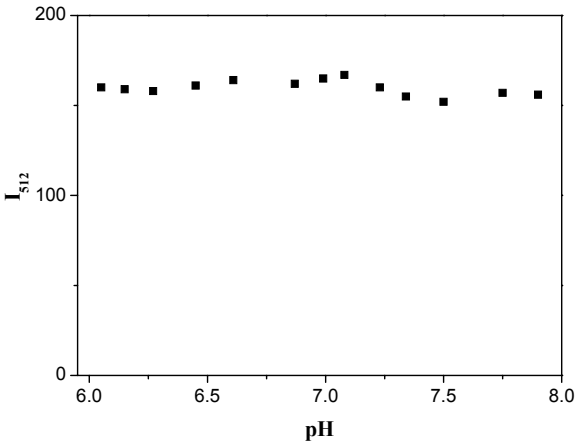


Figure S4 Influences of pH on the fluorescence of the [**DAQZ@2Zn^{2+}**] complex (10 μM) in the ethanol-water solution (1/9, V/V). The pH is modulated by adding 75% $HClO_4$ or NaOH solution.

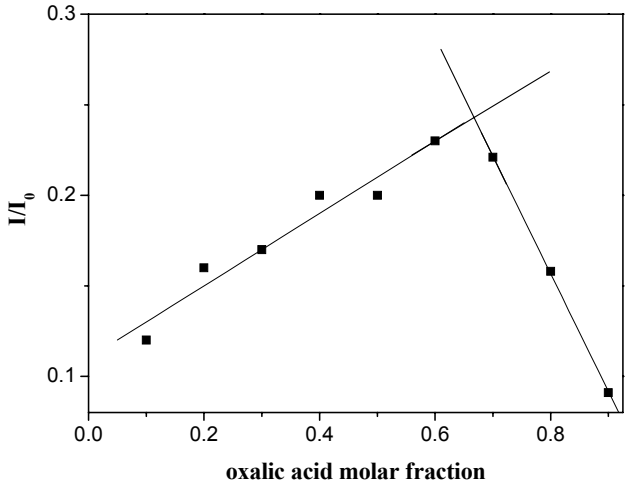


Figure S5. Job's plot for the [**DAQZ@2Zn^{2+}**] complex in tris-HCl (0.01 M) solution. The total [DAQZ@2Zn^{2+}] + [oxalic acid] = 5 μM.

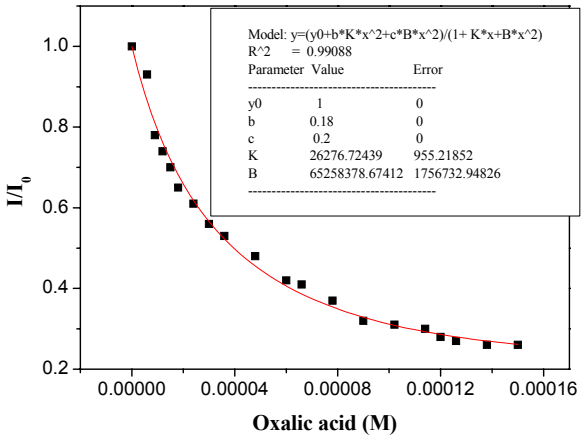


Figure S6. Fitting curve of fluorescence intensity at $I_{513\ nm}/I_{0\ 513\ nm}$ at the of the [**DAQZ@2Zn^{2+}**] complex versus increasing concentrations of oxalic acid in tris-HCl (0.01 M) solution (ethanol-water, 1:9, pH = 7.02). The concentration of the **DAQZ** was 3 μM. The fitting equation is the same as mentioned above **(equation 1)**. Here, $\beta_{21} = K_{11}K_{21}$, [M] ≈ C_M is oxalic acid concentration, I_0 or I is emission intensity in the absence or presence of oxalic acid, Φ is approximately 0.18, the quantum yield of the 1:1 [oxalic acid]- [DAQZ@2Zn^{2+}] complex.

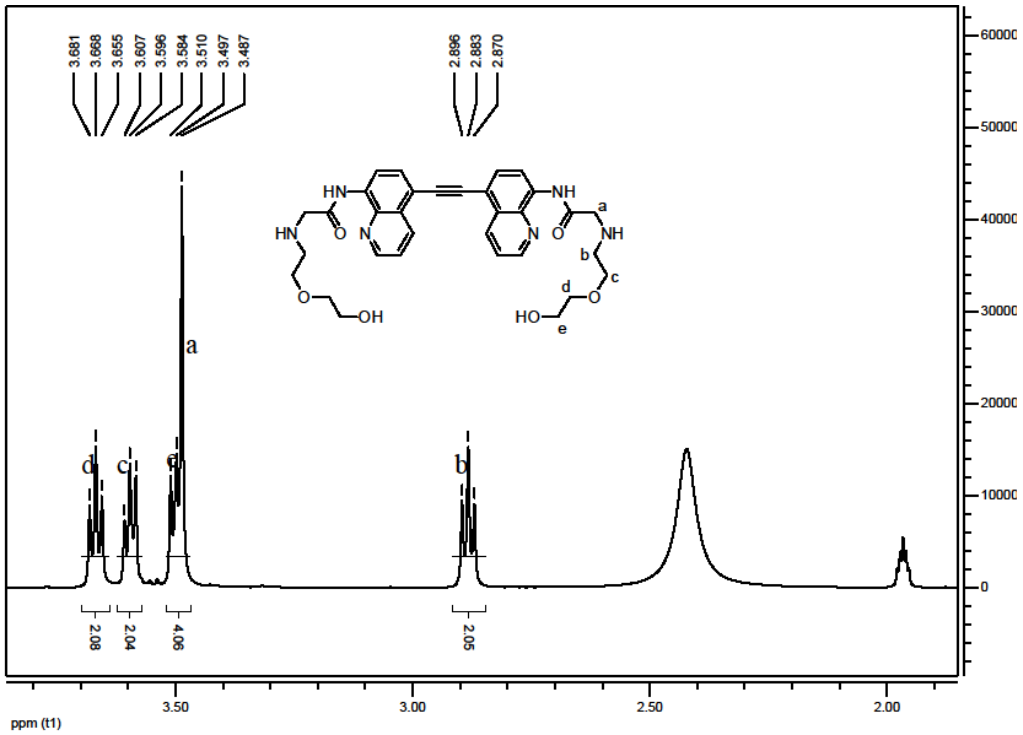


Figure S7. Partial ^{1}H-NMR spectra of **DAQZ** in CD_3CN, 0.6 M .

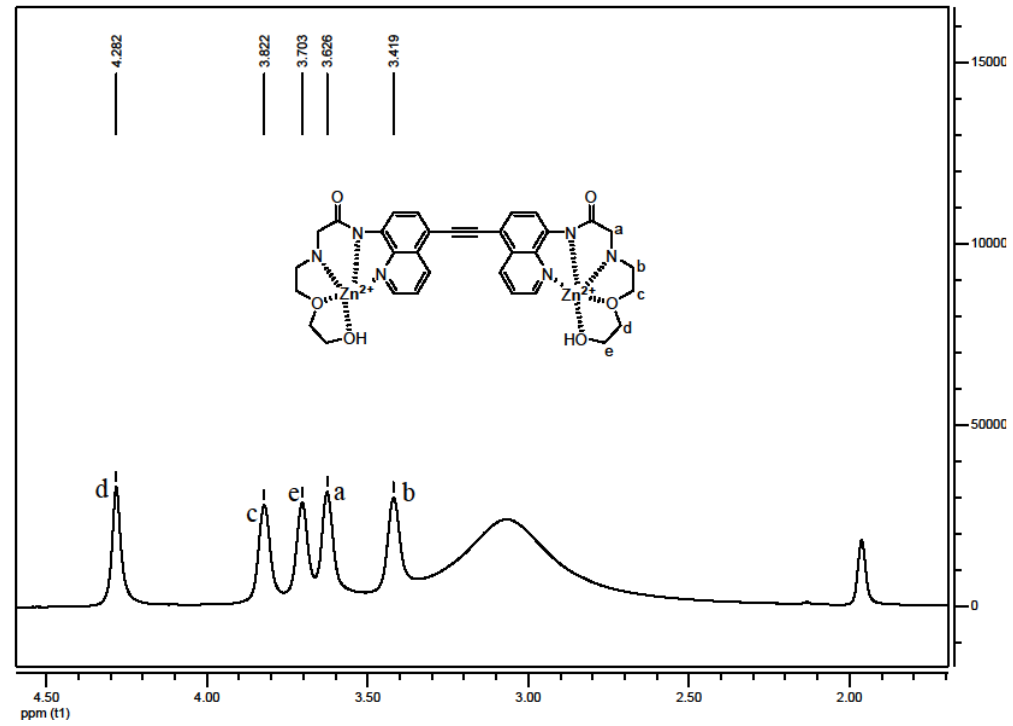


Figure S8. Partial ^{1}H-NMR spectra of **DAQZ** in CD_3CN (0.6 M) in the presence of 2 equivalent of Zn(II).

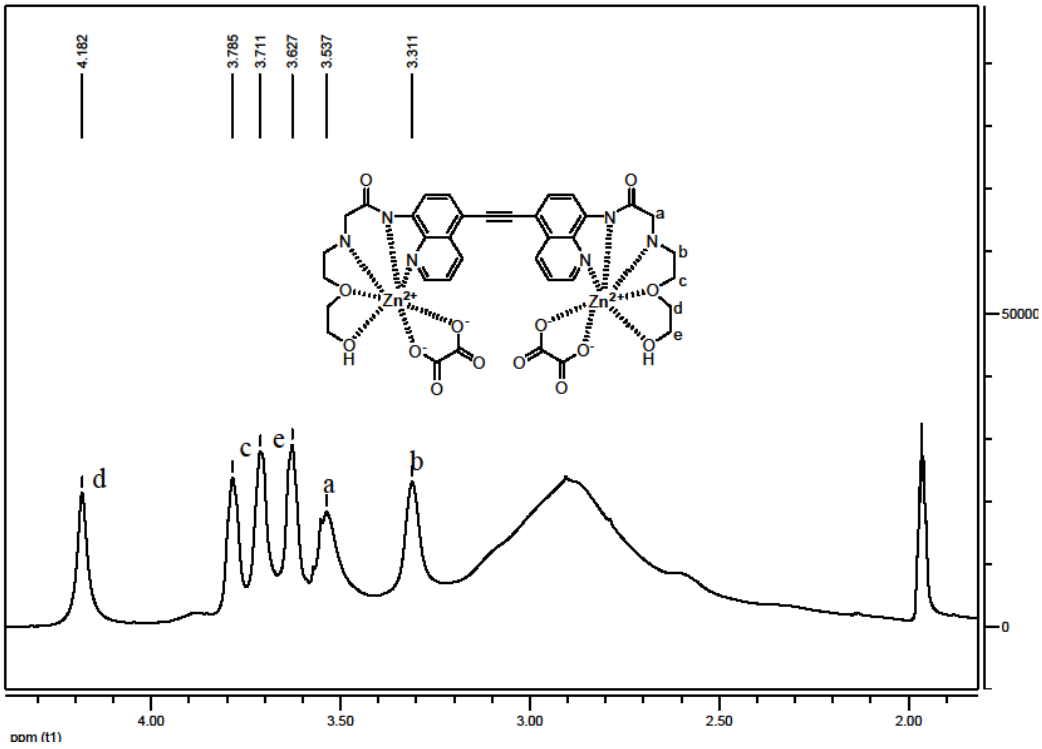


Figure S9. Partial ^{1}H-NMR spectra of [**DAQZ@2Zn^{2+}**] in CD_3CN (0.6 M) in the presence of 2 equivalent of oxalate.

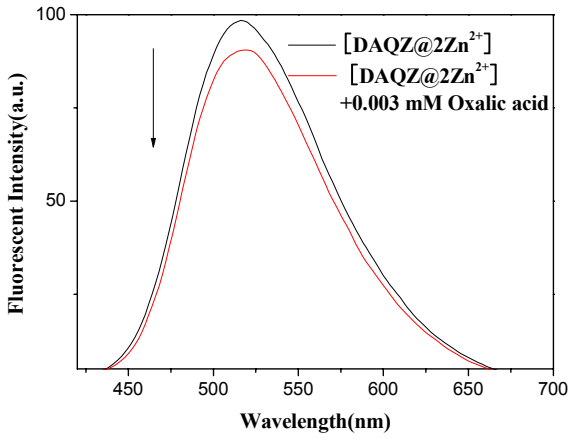


Figure S10. Fluorescence response of the [DAQZ@2Zn^{2+}] complex (3 μM) in the presence of 3 μM of oxalic acid in tris-HCl (0.01 M) solution (ethanol-water, 1:9, pH = 7.02).

Application of fluorescent sensor [DAQZ@2Zn^{2+}] complex for fluorescent images intracellular oxalic acid in HeLa cells

HeLa cells were seeded at 2×10^6 cells in a 10 cm Petri dish and were cultured in DMEM containing 10% fetal bovine serum and penicillin/streptomycin at 37 ^{o}C in 5% CO_2 and 95% air. After 24 h of cell attachment, the cells were incubated with the [**DAQZ@2Zn^{2+}**] complex ([**DAQZ**] = 4.0 μM, [**Zn^{2+}**] = 8.0 μM) for 60 min at 37 °C. The [**DAQZ@2Zn^{2+}**] complex were then removed and the cells were washed twice with PBS buffer. The [**DAQZ@2Zn^{2+}**] complex pretreated cells were then diluted with the addition of different kind of mono-, dicarboxylic acids (100 μM) and incubated for another 0.5 h at 37 °C, respectively. Then, the cells were washed with PBS buffer three times for further testing.

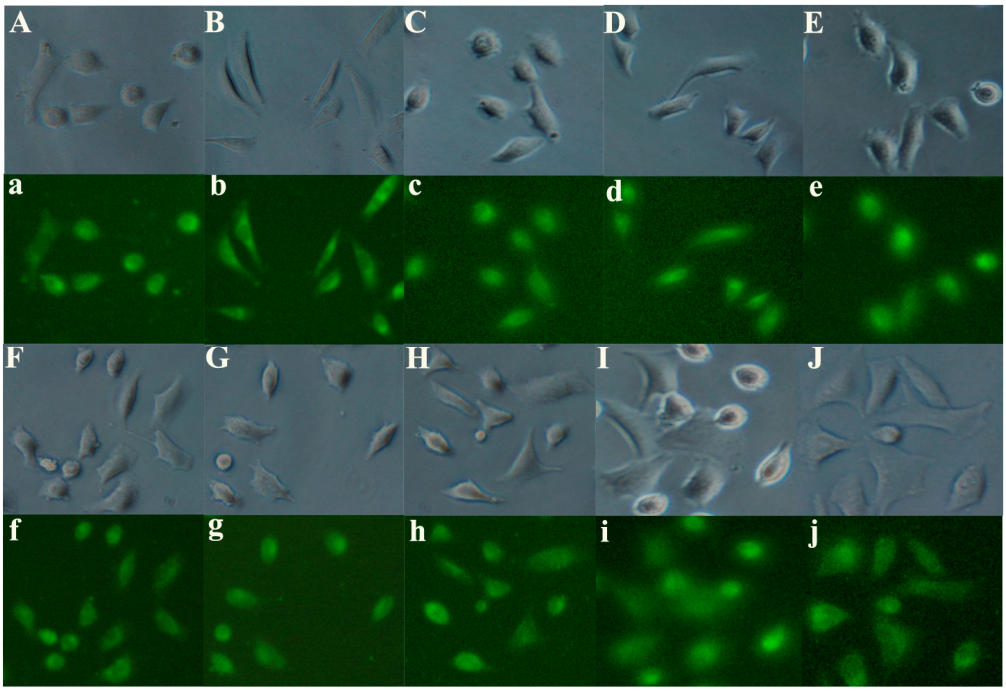


Figure S11. Fluorescence images of the [**DAQZ@2Zn^{2+}**] complex ([**DAQZ**] = 4.0 μM) induced by intracellular mono-, dicarboxylic acids in HeLa cells. (A~J) Bright-field transmission image of HeLa Cells incubated with [**DAQZ@2Zn^{2+}**] complex for 60 min, washed three times, and then further incubated with 1×10^{-4} M different kind of mono-, dicarboxylic acids for 30 min, respectively. (a~j) Fluorescence image of HeLa cells incubated with [**DAQZ@2Zn^{2+}**] complex for 60 min, washed three times, and then further incubated with 1×10^{-4} M different kinds of mono-,

dicarboxylic acids for 30 min, respectively. (A, a) formic acid, (B, b) acetic acid, (C, c) propionic acid, (D, d) butanoic acids, (E, e)malonic acid, (F, f)succinic acid, (G, g)adipic acid, (H, h)sebacic acid, (I, i)o-phthalic acid, (J, j)PO_4^{3-}.