Supporting Information

Fluorescent and Colorimetric Probe Containing Oxime-Ether for Pd^{2+} in Pure Water and Living Cells

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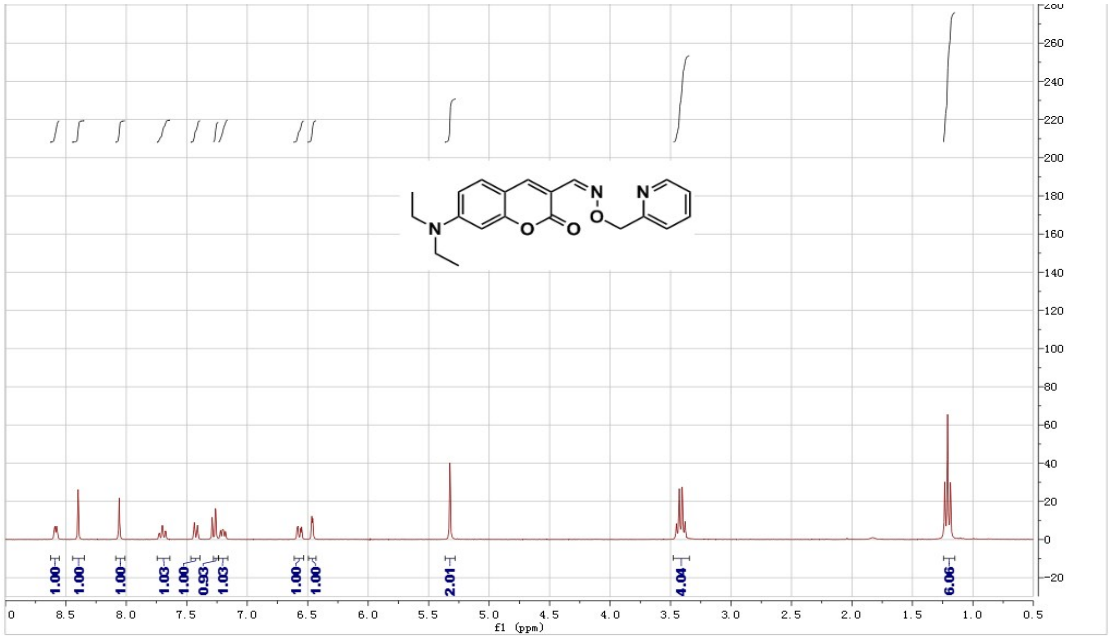
Synthesis of compound 2:

Compound **3** (0.2 g, 0.82 m mol) was dissolved in 10mL EtOH, then K_2CO_3 (0.34 g, 2.5 m mol) and hydroxylamine hydrochloride (0.17 g, 2.4mmol) were added. The mixture was stirred at room temperature for 2 hours and then poured into 50 mL water and extracted with 50 mL EtOAc. After washed with 50mL saturated brine, dried over anhydrous Na_2SO_4, the organic layer was evaporated to give 0.2 g yellow solid in 94 % yield, which was used in the next step without any characterization and purification.

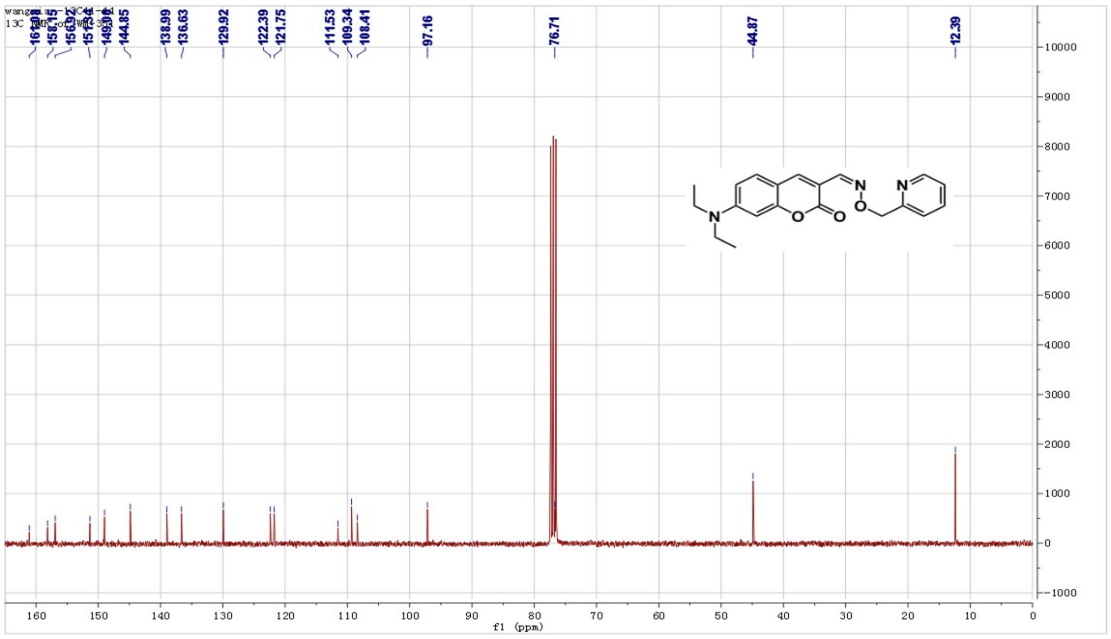
Synthesis of compound 1:

A mixture of compound **2** (0.2 g, 0.77 mmol) , K_2CO_3 (0.33 g, 2.2mmol) and 2-(chloromethyl)pyridine hydrochloride (0.12 g, 0.94 mmol) in 20 mL MeCN was heated under reflux for 3 hours. When the mixture was cooled to room temperature, it was poured into 50 water and extracted with 50 mL EtOAc. After washed with 50 mL saturated brine, dried over anhydrous Na_2SO_4, the organic layer was evaporated and the crude residue was purified by flash chromatography using CH_2Cl_2 as an eluent to give 0.16 g yellow solid in 68 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, J = 4.9 Hz, 1H), 8.42 (s, 1H), 8.08 (s, 1H), 7.73 (td, J = 7.7, 1.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.31 (dd, J = 8.8, 5.7 Hz, 1H), 7.23 (dd, J = 7.0, 5.5 Hz, 1H), 6.59 (dd, J = 8.9, 2.5 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 5.35 (s, 2H), 3.44 (q, J = 7.1 Hz, 4H), 1.23 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.08, 158.15, 156.92, 151.34, 149.00, 144.85, 138.99, 136.63, 129.92, 122.39, 121.75, 111.53, 109.34, 108.41, 97.16, 76.71, 44.87, 12.39. HRMS calcd for $C_{20}H_{21}N_3O_3$: [M + H^+] 352.1660, found 352.1656. MP: 176-177 °C.

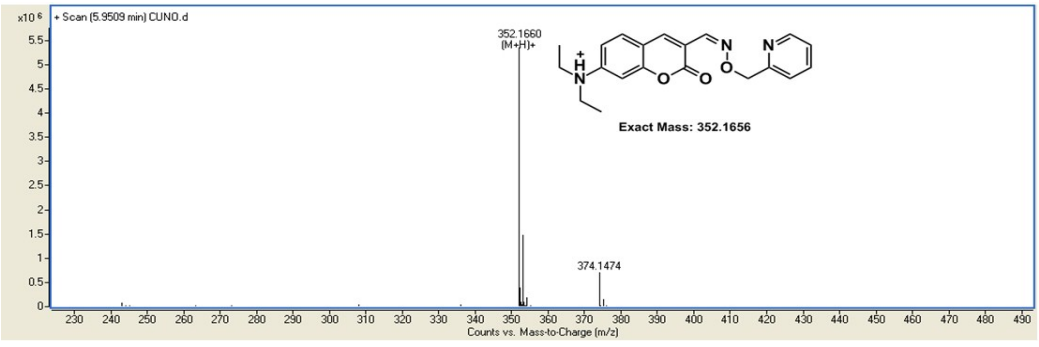
(a)


1.00
1.00
1.00
1.03
1.00
0.53
1.03
1.00
1.00
2.01
4.04
6.06
f1 (ppm)

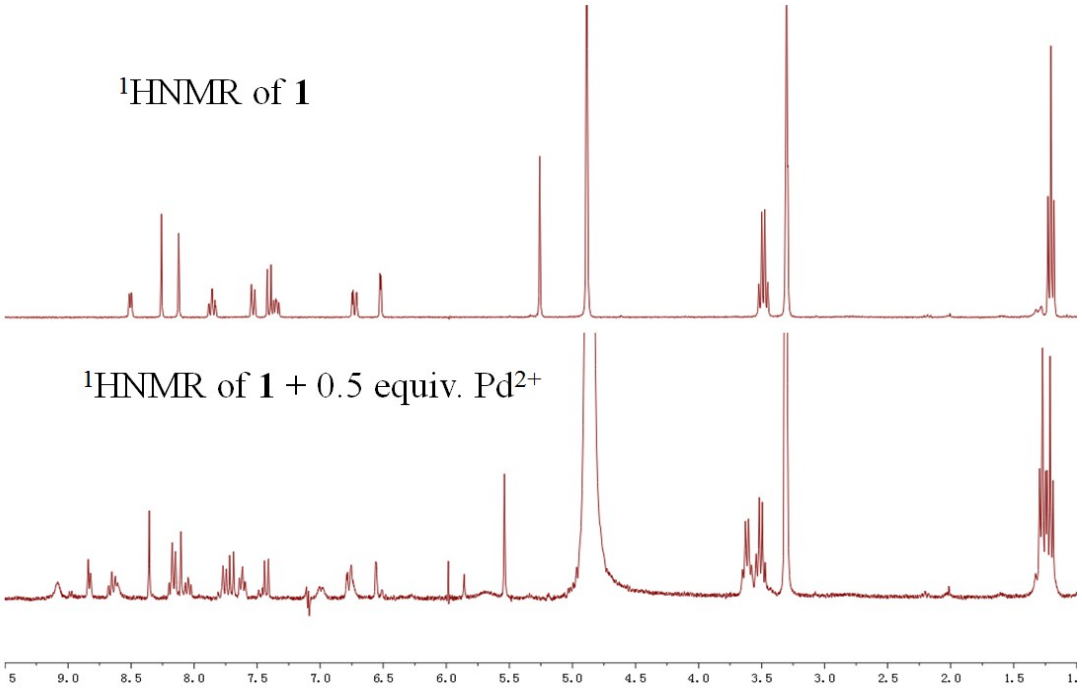
(b)


144.85
138.99
136.63
129.92
122.39
121.75
111.53
109.34
108.41
97.16
76.71
44.87
12.39
f1 (ppm)

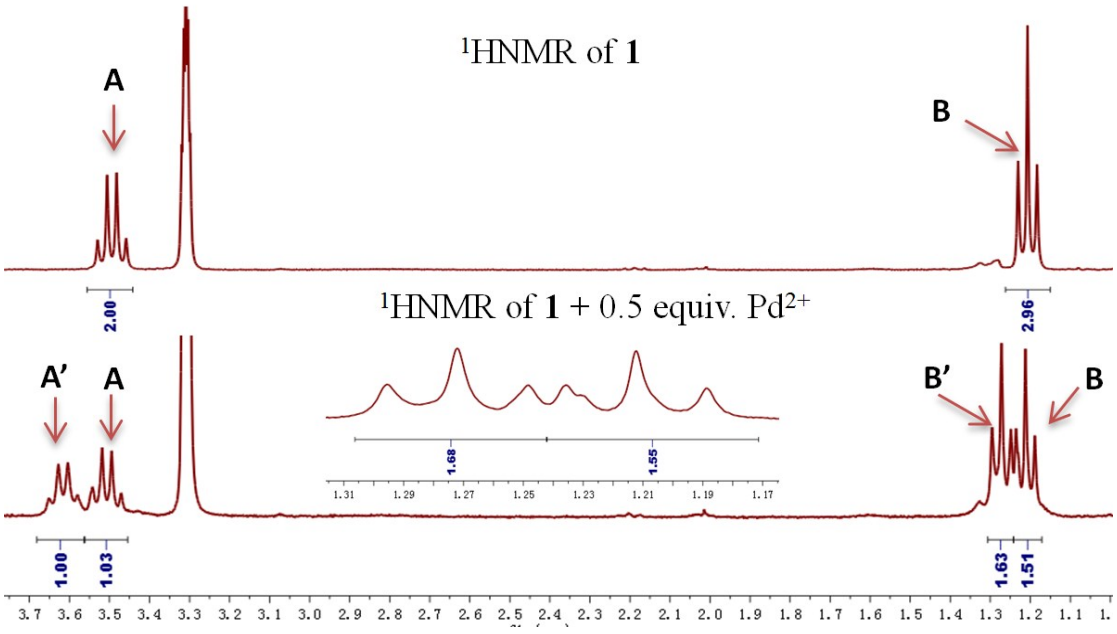
(c)


+ Scan (5.9509 min) CUNO.d
352.1660
(M+H)+
Exact Mass: 352.1656
374.1474
Counts vs. Mass-to-Charge (m/z)

(d)


¹HNMR of 1
¹HNMR of 1 + 0.5 equiv. Pd²⁺

(e)


¹HNMR of 1
A
B
2.00
2.96
¹HNMR of 1 + 0.5 equiv. Pd²⁺
A'
A
B'
B
1.68
1.55
1.00
1.03
1.63
1.51

(f)

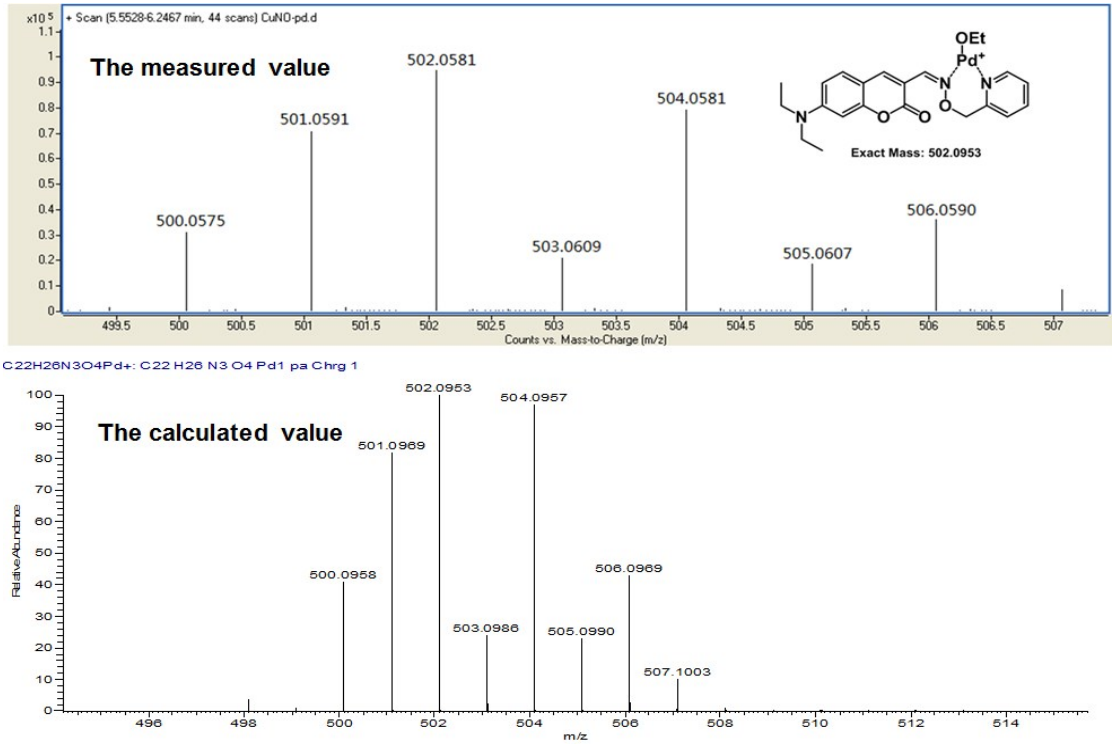


Fig. S1 ^{1}H NMR (a), ^{13}C NMR (b) and HRMS (c) spectra of **1**. ^{1}H NMR (d, e) and HRMS (f) spectra of **1** + Pd^{2+}.

In Figure (e), peak A and peak B represent a methylene group and a methyl group of **1** respectively**,** peak A' and peak B' represent a methylene group and a methyl group of 1-Pd^{2+}.

As showed in Figure (d), When the CD_3OD solution of **1** was added by 0.5 equiv. of Pd^{2+}, Compared to the CD_3OD solution of **1** without Pd^{2+}, there are more peaks in the ^{1}HNMR spectrum of **1** + 0.5 equiv. Pd^{2+}. Owing to electron withdrawing effect of metal ions, there are some peaks move downfield direction in the ^{1}HNMR spectrum of **1** + 0.5 equiv. Pd^{2+}. Finally, as showed in Figure (e), the integration results show that the molar amount of **1** and **1**-Pd^{2+} is approximately 1: 1 when 0.5 equiv. Pd^{2+} was added to a CD_3OD solution of **1.** In Figure (e), the measured and calculated HRMS spectra show that **1** and Pd^{2+} can form a 1: 1 coordination compound.

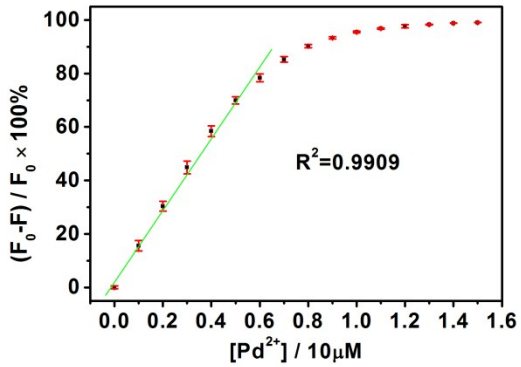


Fig. S2 The percentage decline in fluorescence intensity at 505 nm of **1** (10.0 μ M) in water solution in response to the presence of Pd^{2+} (0.0 to 1.5 equiv.), λ_{ex} = 435 nm, F_0 : the fluorescence intensity at 505 nm of **1** without Pd^{2+}, ***F*****:** the fluorescence intensity at 505 nm of **1** in response to the presence of Pd^{2+}. Data are expressed as the mean ± 2σ ("σ" represents the standard deviation of three sets of parallel experiments). The detection limit of **1** for Pd^{2+} is calculated according to the equation: LOD=3σ/S ("LOD" represents limit of detection, "σ" represents the standard deviation of the 11 blank samples, "S" represents the slope of the linear fitting, In our experiments, $\sigma = 2.568\times10^{-3}$, $S = 1.408\times10^{5}$, $LOD = 3\times2.568\times10^{-3} / 1.408\times10^{5} = 5.5\times10^{-8}$ M).

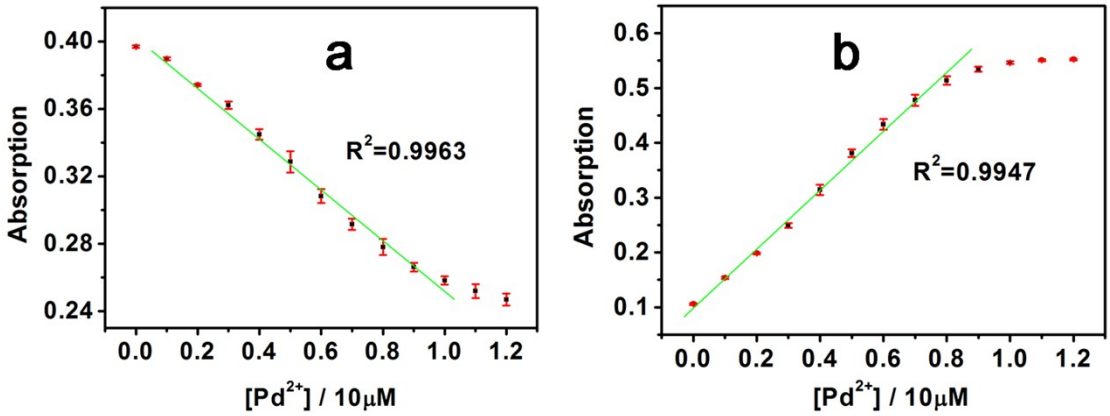


Fig. S3 (a) Changes in absorption at 435 nm of a water solution of **1** (10.0 μ M) in response to the presence of Pd^{2+} (0.0 to 1.2 equiv.). (b) Changes in absorption at 478 nm of a water solution of **1** (10.0 μ M) in response to the presence of Pd^{2+} (0.0 to 1.2 equiv.). Data are expressed as the mean ± 2σ ("σ" represents the standard deviation of three sets of parallel experiments).

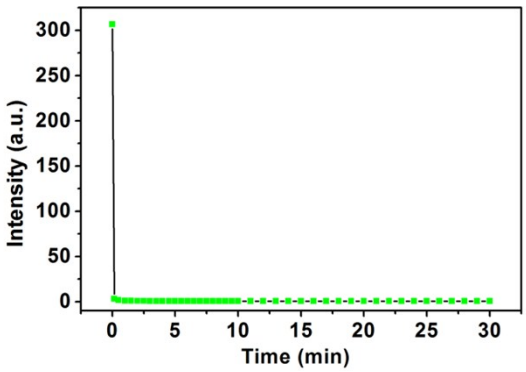


Fig. S4 Time-dependent fluorescence intensity changes at 505 nm upon addition of 1.0 equiv. of Pd^{2+} in a water solution of **1** (10.0 μ M). λ_{ex} = 435 nm.

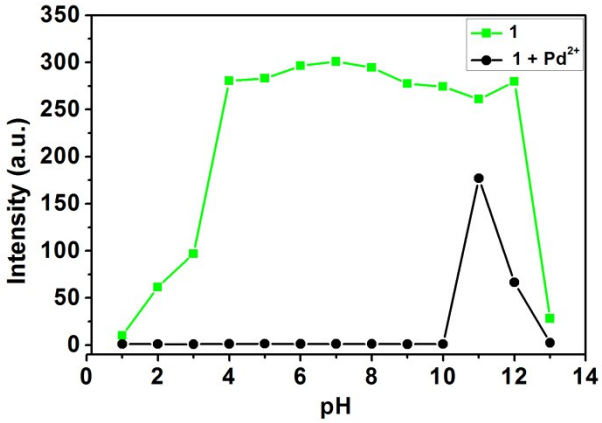


Fig. S5 Fluorescence intensity of **1** (10.0 μ M) at 505 nm in water solution under different pH (■) and further addition of 1.0 equiv. of Pd^{2+} (●) (pH value of the solution was adjusted by $HClO_4$ or NaOH), λ_{ex} = 435 nm.

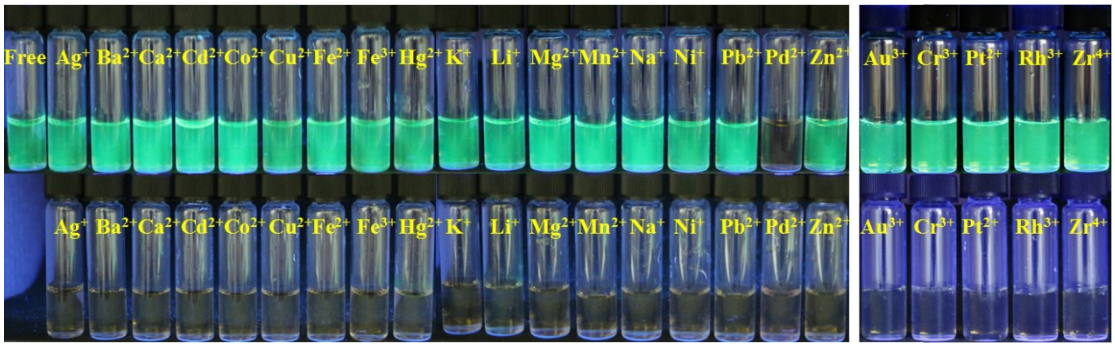


Fig. S6 Fluorescence images of **1** (10.0 μ M) in water solution after addition of various cations and those after further addition of 1.0 equiv. of Pd^{2+} (expect Pd^{2+}). Au^{3+} and Cu^{2+} were added by 2.5 equiv., other competing cations including Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{+}, Pb^{2+}, Pt^{2+}, Rh^{3+}, Zn^{2+}, Zr^{4+} were added by 5.0 equiv.

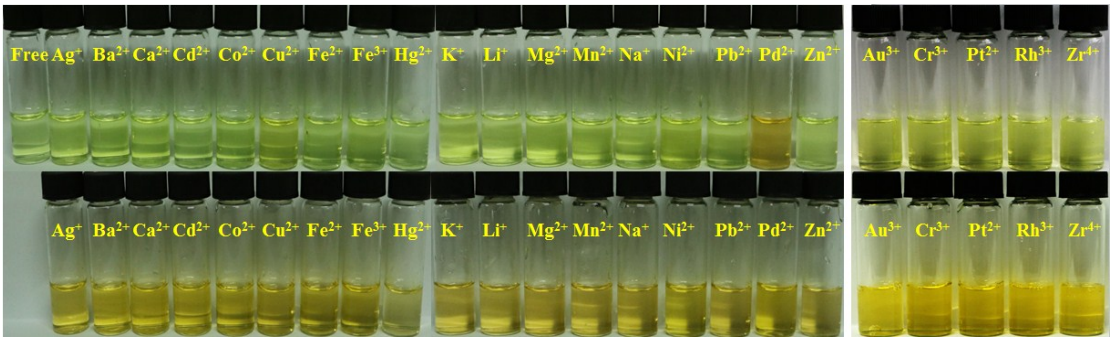


Fig. S7 Color changes of **1** (10.0 μ M) in water solution after addition of various cations and those after further addition of 1.0 equiv. of Pd^{2+} (expect Pd^{2+}). Au^{3+} and Cu^{2+} were added by 2.5 equiv., other competing cations including Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{+}, Pb^{2+}, Pt^{2+}, Rh^{3+}, Zn^{2+}, Zr^{4+} were added by 5.0 equiv.

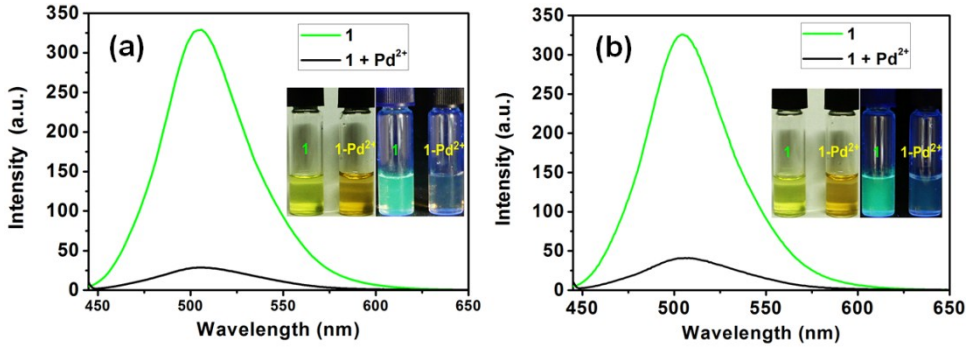


Fig. S8 Fluorescence spectra of **1** (10 μ M) in actual water samples (a: surface water, b: underground water) after addition of 2.0 equiv. of Pd^{2+}, λ_{ex} = 435 nm. Inset: fluorescence and color images of **1** (10 μM) in actual water samples and those after further addition of 2.0 equiv. of Pd^{2+}.(Surface water samples from Fuhai in The Old Summer Palace, underground water from water well in China Agricultural University)

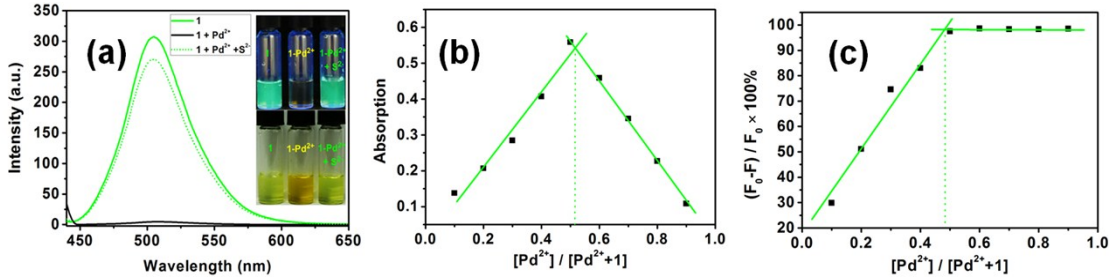


Fig. S9 (a) Fluorescence spectra of **1** (10 μ M) in water solution after addition of 1.0 equiv. of Pd^{2+} and further addition of 4.0 equiv. of S^{2-} , λ_{ex} = 435 nm. Inset: fluorescence and color images of **1**, **1** + Pd^{2+} and **1** + Pd^{2+} + S^{2-}. (b) Job's plot of **1** with Pd^{2+} obtained by UV-Vis measurements (λ = 478 nm), the total concentration of **1** and Pd^{2+} is 20 μ M. (c) Job's plot of **1** with Pd^{2+} obtained by fluorescence emission measurements, the total concentration of **1** and Pd^{2+} is 20 μ M. λ_{ex} = 435 nm, $\mathbf{F_0}$: the fluorescence intensity at 505 nm of **1** without Pd^{2+}, **F:** the fluorescence intensity at 505 nm of **1** in response to the presence of Pd^{2+}.

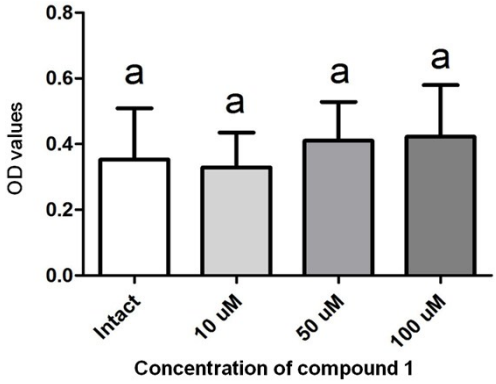


Fig. S10 Cytotoxicity assays of **1** at different concentrations (0 μ M, 10 μ M, 50 μ M, 100 μ M) for Hela cells, values are means + 2σ (n=3), $p > 0.05$. The results show that compared to the control group, the effect of **1** on the cells had no significant difference, even the concentration of **1** is 10 times the concentration of intracellular fluorescence imaging experiment.