Electronic Supplementary Information

A novel 6-quinoxalinamine-based fluorescent probe for real-time detection of palladium (II) ion in pure water and bio-imaging

Chuanliang Che, Xiangzhu Chen, Hongmei Wang, Jia-Qi Li, Yumei Xiao, Bin Fu, Zhaohai Qin*

College of Science, China Agricultural University, Beijing, 100193, P.R. China.

*Corresponding author, Tel: 010-62732958; E-mail: qinzhaohai@263.net
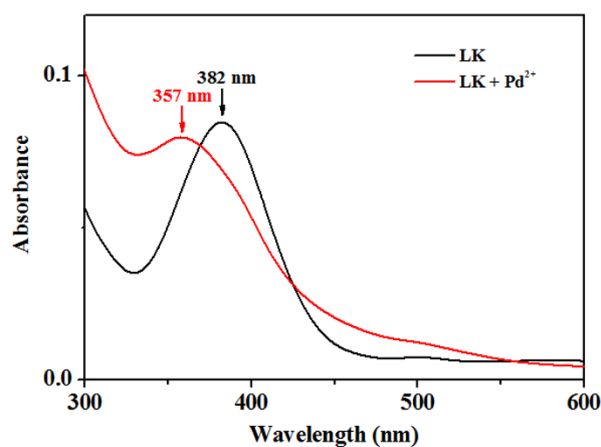
Table of Contents

1. Photophysical data of the probe **LK**

2. UV-vis spectra of **LK**

3. The synthetic details and data for characterizations of the intermediates **2, 3, 4**

4. NMR and Mass spectra of intermediate **3**

5. NMR and Mass spectra of intermediate **4**

6. NMR and Mass spectra of probe **LK**

7. Absorption spectra of **LK** in the presence of different concentrations of Pd^{2+} and under various pH

8. Fluorescence images changes of **LK** in the presence of various metal ions

9. Job's plot of **LK** with Pd^{2+}

10. Fluorescence spectra of **LK** in real water samples

11. Cytotoxicity assays of **LK** at different concentrations

12. ^{1}H NMR chemical shift of **LK** in the absence or presence of 1.0 equiv. Pd^{2+}

1. Photophysical data of the probe LK

Compound	λ_{abs} [a]	λ_{fl} [b]	$\Delta\lambda$ [c]	ε [d]	Φ [e]	LOD [f]
LK	382	540	158	15784	0.63	22

Table S1. Photophysical data for LK. [a] λmax of absorption spectra in nm. [b] λmax of fluorescence spectra in nm. [c] Stokes shift in nm. [d] Molar extinction coefficients in M^{-1} cm^{-1} at the absorption maximum. [e] Relative fluorescence quantum yield in ethanol with quinine sulfate in 0.1 M H_2SO_4 (0.66) as a reference.[1] [f] The limit of detection of **LK** in nM. LOD = 3σ/S, where σ is the standard deviation of the blank samples and S is the slope of the linear calibration plot between the relative fluorescence emission intensity and the concentration of Pd^{2+}.[2]

2. UV-vis spectra of LK

Fig. S1 UV-vis spectra of **LK** in the absence or presence of Pd^{2+}

3. The synthetic details and data for characterizations of the intermediates 2, 3, 4

3.1 Synthesis of 7-bromo-6-nitroquinoxalin-2(1H)-one (2)

The ground powder of KNO_3 (1.00 g, 10.0 mmol) was added quickly into a mixture of 7-bromoquinoxalin-2(1H)-one (2.25 g, 10.0 mmol) in H_2SO_4 (25 mL) at 0 °C. The mixture was stirred for half an hour, then the mixture was allowed to warm to room temperature, stirred for 1 h, and poured into ice (100 mL). The resulting precipitate was collected by filtration and washed with water and dried to afford a yellow solid (2.65 g, 98%). This compound was used directly in the next step without purification.

3.2 Synthesis of 7-bromo-6-nitro-2-propoxyquinoxaline (3)

7-bromo-6-nitroquinoxalin-2(1H)-one (2.65 g, 9.8 mmol) was dissolved in anhydrous DMF (100 mL) under an atmosphere of argon in round-bottom flask. The reaction was stirred in an ice-water bath for 20 min, and 60% sodium hydride in mineral oil (0.44 g, 11.0 mmol) was added in one portion, and then to the mixture was added a solution of 1-Bromopropane (0.9 mL, 10.0 mmol) in DMF (10 mL). The reaction was stirred at room temperature overnight. After quenching with brine (100 mL), it was extracted with ethyl acetate (50 mL x 3). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude product was purified by column chromatography on silica gel to afford compound **3** as a yellow solid (2.45g, 80%). ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H, 5-H), 8.52 (s, 1H, 8-H), 8.19 (s, 1H,3-H), 4.47 (t, J = 6.7 Hz, 2H, -OC$\underline{H_2}$CH$_2$CH$_3$), 1.94 − 1.86 (m, 2H, -OCH$_2$C$\underline{H_2}$CH$_3$), 1.09 (t, J = 7.4 Hz, 3H, -OCH$_2$CH$_2$C$\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.34, 141.86, 135.49, 132.28, 125.32, 124.32, 122.79, 113.63, 68.18, 20.91, 9.40; HRMS calcd for $C_{11}H_{10}BrN_3O_3$ [M+H]$^+$: 311.9978, found 311.9976.

#### 3.3 Synthesis of 7-bromo-2-propoxyquinoxalin-6-amine (4)

To a suspension of 7-bromo-6-nitro-2-propoxyquinoxaline (2.45 g, 7.9 mmol) in MeOH (500 mL) was added 5% Pd/C (0.1 g, 0.1 mmol) and the mixture was stirred overnight under H_2 (1 atm). After filtration, the filtrate was evaporated to obtain a crude product. The crude compound was purified by flash column chromatography to give compound **4** as a yellow solid (2.14g, 96 %). ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H, 3-H), 7.98 (s, 1H, 8-H), 7.27 (s, 1H, 5-H), 4.35 (t, J = 6.7 Hz, 2H, -OC$\underline{H_2}$CH$_2$CH$_3$), 3.49 (s, 2H, -NH$_2$), 1.91 − 1.79 (m, 2H, -OCH$_2$C$\underline{H_2}$CH$_3$), 1.06 (t, J = 7.4 Hz, 3H, -OCH$_2$CH$_2$C$\underline{H_3}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.22, 141.64, 139.07, 138.12, 133.34, 129.36, 114.41, 109.37, 66.92, 21.11, 9.49; HRMS calcd for $C_{11}H_{12}BrN_3O$ [M+H]$^+$: 282.0237, found 282.0236.

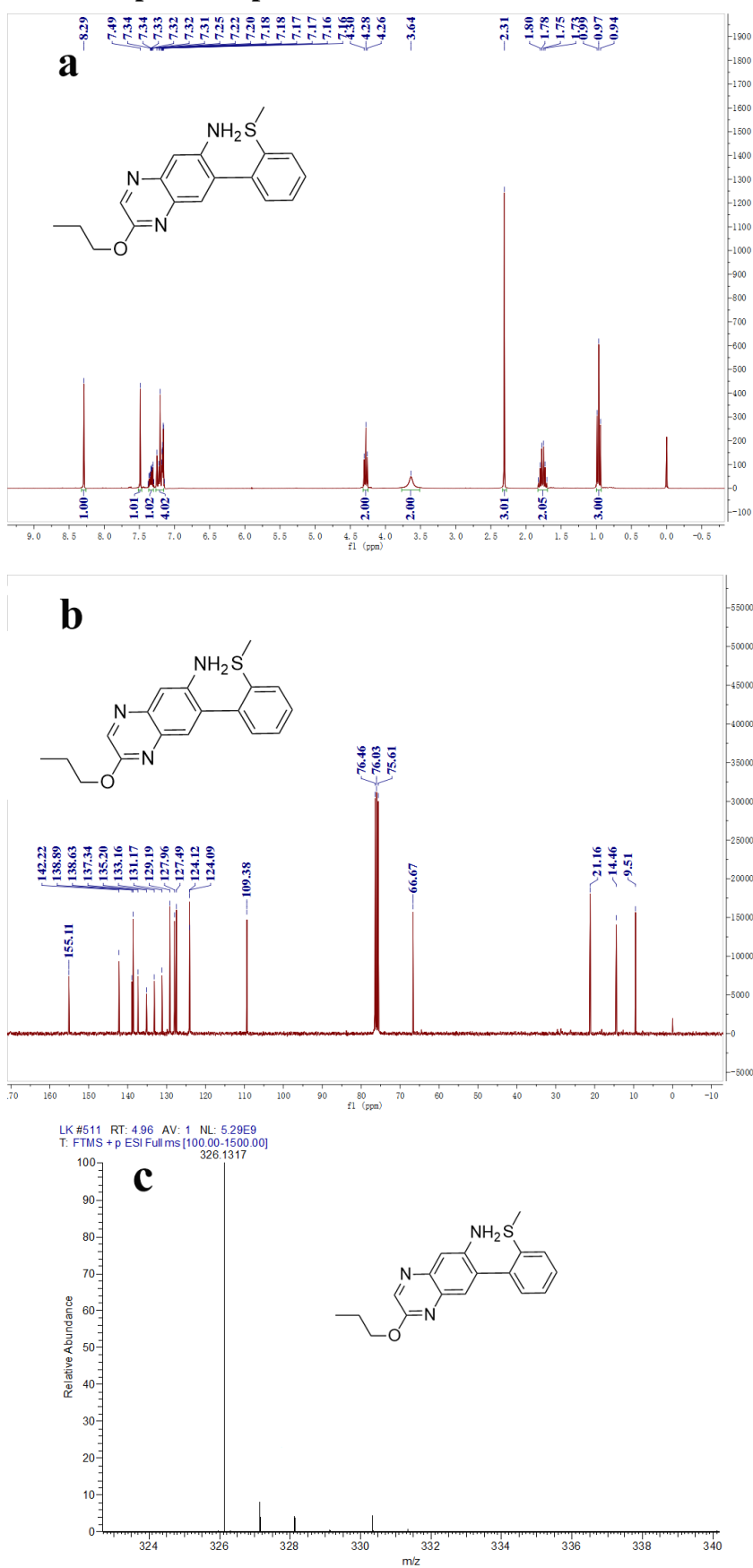# 4. NMR and Mass spectra of intermediate 3
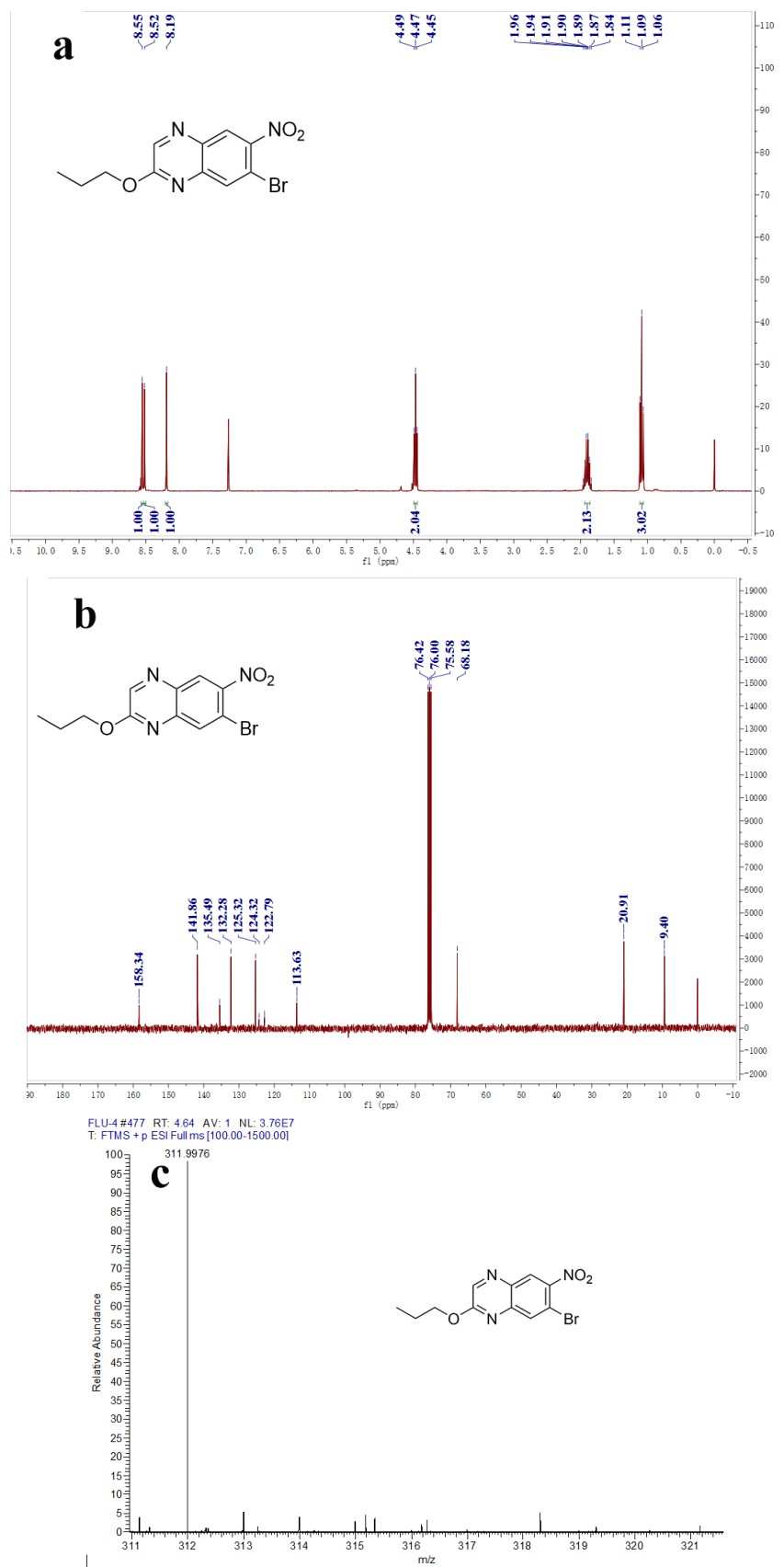
Fig. S2. ^{1}H NMR (a), ^{13}C NMR (b) and HRMS (c) spectra of compound **3**.
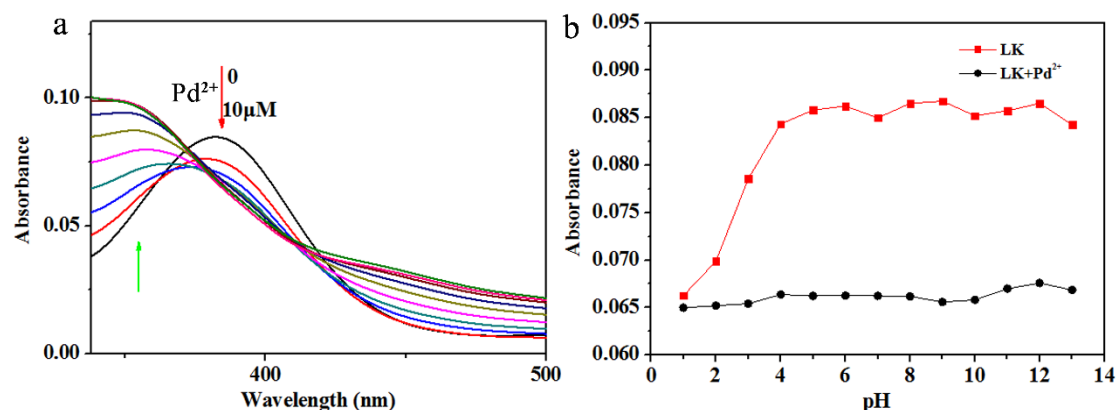
5. NMR and Mass spectra of intermediate 4
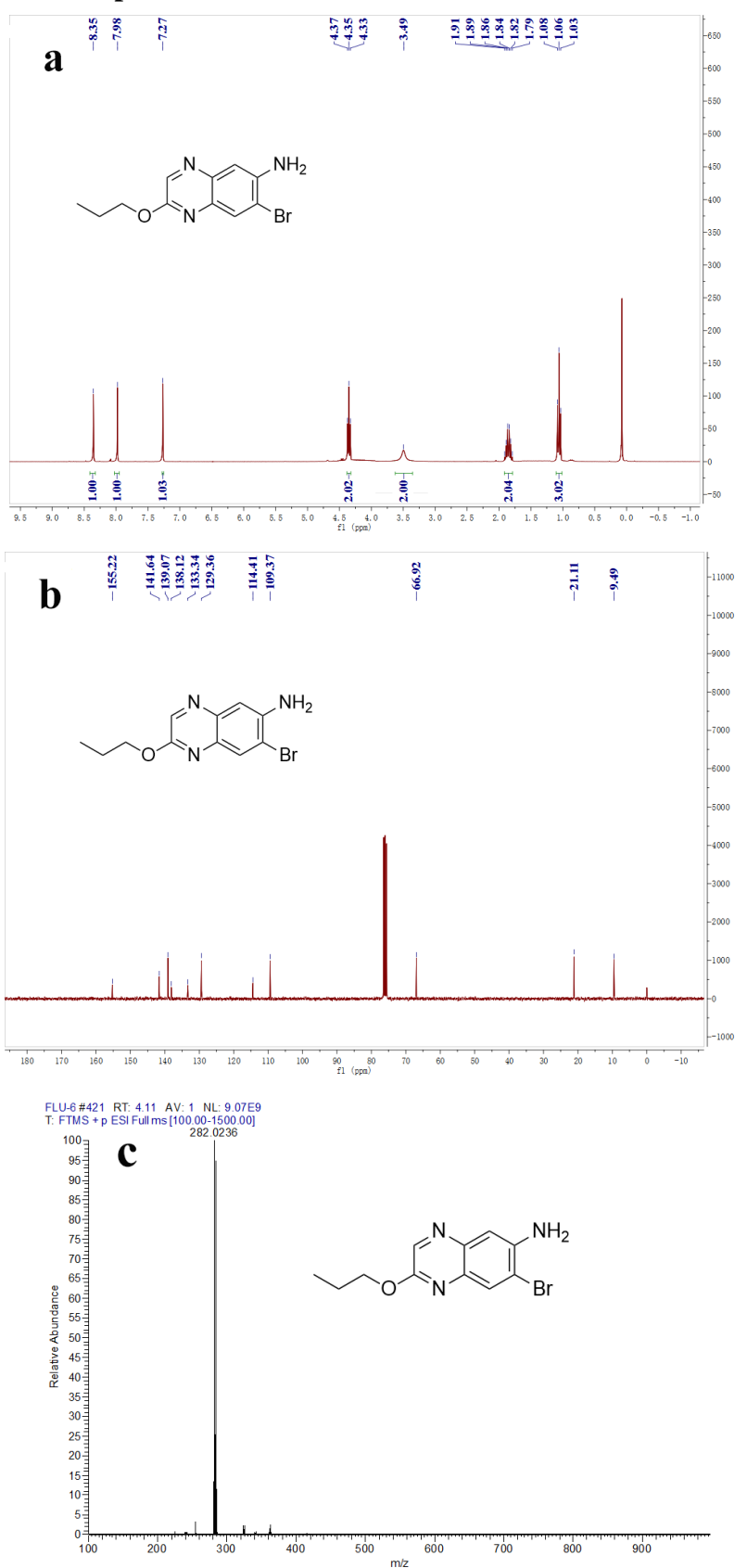
Fig. S3. ^{1}H NMR (a), ^{13}C NMR (b) and HRMS (c) spectra of compound **4**.

6. NMR and Mass spectra of probe LK

Fig. S4. ^{1}H NMR (a), ^{13}C NMR (b) and HRMS (c) spectra of probe **LK**.

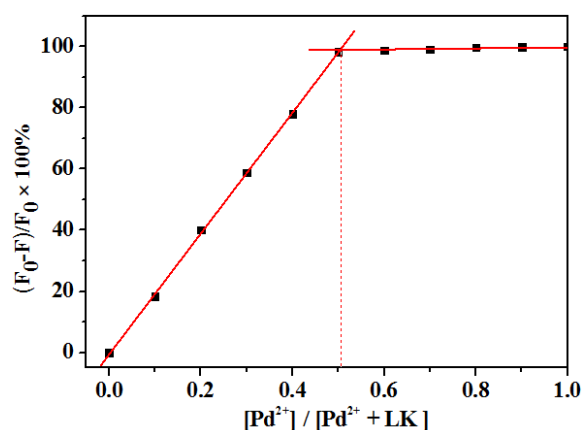# 7. Absorption spectra of LK in the presence of different concentrations of Pd²⁺ and under various pH

Fig. S5 (a) Absorption spectra of **LK** (10.0 μM) in the presence of different concentrations of Pd^{2+} (0-10 μM) in pure water. (b) Absorption spectra changes of **LK** (10.0 μM) at 382 nm under various pH in pure water.

8. Fluorescence images changes of LK in the presence of various metal ions
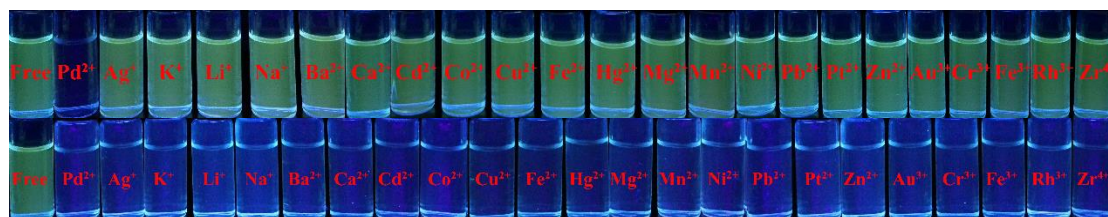
Fig. S6. Fluorescence images changes of of **LK** (10.0 μM) in the presence of various metal ions and after further addition of 2.0 equiv. of Pd^{2+}. Ag^{+}, K^{+}, Li^{+}, Na^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Pt^{2+}, Zn^{2+}, Au^{3+}, Cr^{3+}, Fe^{3+}, Rh^{3+} and Zr^{4+} were added by 5.0 equiv., separately.

9. Job's plot of LK with Pd²⁺

10. Fluorescence spectra of LK in real water samples
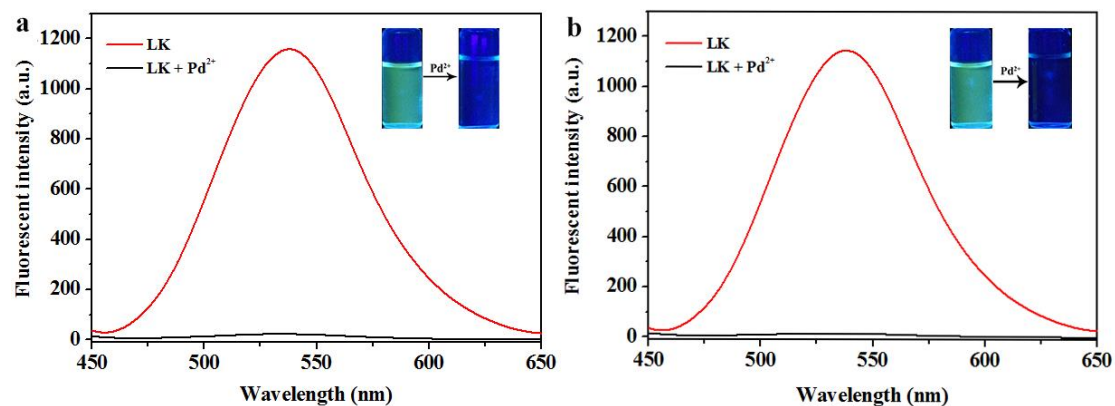
Fig. S8. Fluorescence spectra of **LK** (10 μM) in actual water samples (a: drinking water, b: tap water) after addition of 2.0 equiv. of Pd^{2+}. Inset: fluorescence of LK (10 μM) in actual water samples and those after further addition of 2.0 equiv. of Pd^{2+}.($\lambda_{ex}/\lambda_{em}$ = 382/540 nm)
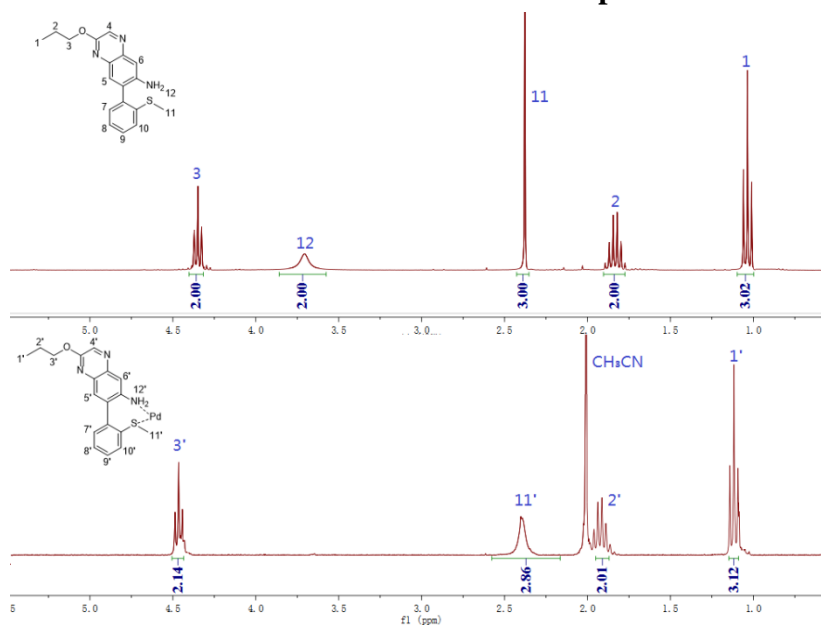
11. Cytotoxicity assays of LK at different concentrations
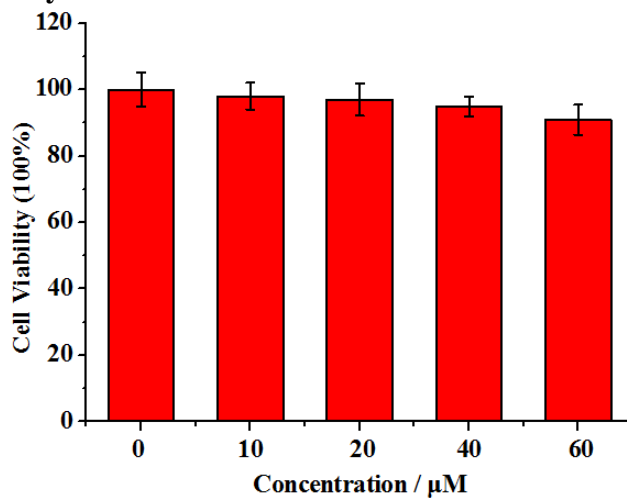
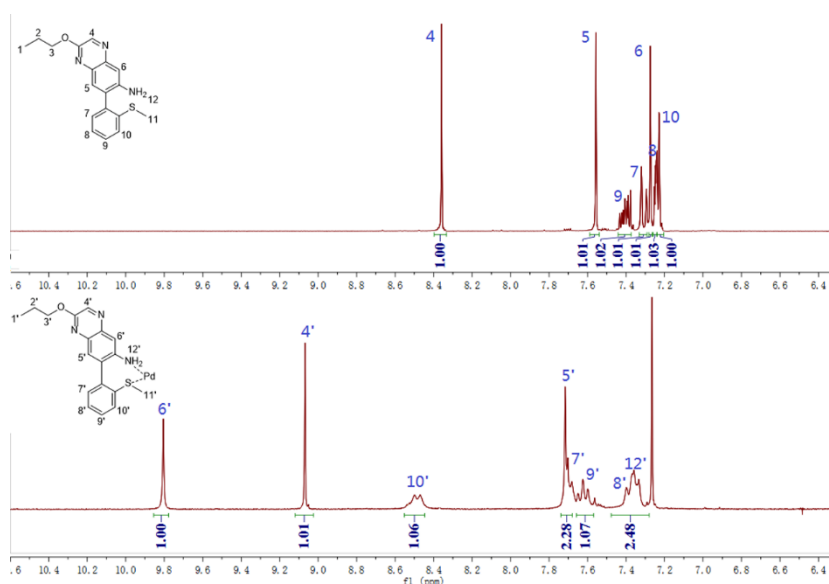**Fig. S9.** Cytotoxicity assays of **LK** at different concentrations (0 μM, 10 μM, 20 μM, 40 μM, 60 μM) for OVCAR-3 cells after 24 h. The cell viabilities were estimated through three separate MTT assays.

12. ^{1}H NMR chemical shift of LK in the absence or presence of 1.0 equiv. Pd^{2+}. [3]

Fig. S10 ^{1}H NMR chemical shift of aliphatic hydrogen of **LK** in the absence or presence of 1.0 equiv. Pd^{2+}.

Fig. S11 ^{1}H NMR chemical shift of aromatic hydrogen of **LK** in the absence or presence of 1.0 equiv. Pd^{2+}.

Reference：

[1] R.F. Chen, The fluorescence of quinine, Analytical Biochemistry, 19 (1967) 374-387.

[2] J. Luo, A simple but effective fluorescent probe for the detection of bisulfite, New Journal of Chemistry, 41 (2017) 3986-3990.

[3] Pd(CH$_3$CN)$_2$Cl$_2$ as Pd^{2+} source.