Supporting Information

for

Coumarin-Cu(II) Ensemble-based Cyanide Sensing Chemodosimeter

Hyo Sung Jung,[†] Ji Hye Han, [‡] Zee Hwan Kim,[†,§] Chulhun Kang,*[,‡] and Jong Seung Kim*[,†]

[†]*Department of Chemistry, Korea University, Seoul, 136-704, Korea,*
[‡]*The School of East-West Medical Science, Kyung Hee University, Yongin, 446-701, Korea,*
[§]*Research Institute for Natural Sciences, Korea University, Seoul, 136-701, Korea*

E-mails: kangch@khu.ac.kr; jongskim@korea.ac.kr

- **Methods**

General information and materials. All fluorescence and UV/vis absorption spectra were recorded in a Shimadzu RF-5301PC and a Shinco S-3100 spectrophotometer, respectively. NMR and mass spectra were recorded with a Varian instrument (400 MHz) and JMS-700 MStation mass spectrometer. All analytes were purchased from Aldrich and used as received. All solvents were analytical reagents from Duksan Pure Chemical Co., Ltd. All DMSO for spectra detection was HPLC reagent grade, without fluorescent impurity; H_2O was deionized.

Spectroscopic Data. Stock solutions (0.1 M) of the anion potassium salts were prepared in water. Stock solutions of **1**-Cu(II) (0.5 mM) were prepared in DMSO by mixing **1** and $Cu(ClO_4)_2$ with the ratio of 1:5. Stock solutions of compound **1** and **2** (0.5 mM) were also prepared in DMSO. For all measurements of fluorescence spectra, excitation was at 479 nm with all excitation slit widths is 1.5 nm, that of emission is 1.5 nm. UV/vis and fluorescence titration experiments were performed using 5 μM of **1** and **2** in 1.0% DMSO aqueous solution (pH 7.4, 10 mM PBS buffer) with varying concentrations of anion potassium salts at room temperature.

Cell culture. HepG2 cells, a human hepatoma cell line, were maintained and subculture every other day in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 ℃ in 5% CO_2 and 95% air environment. Cells were seeded on 24-well plates and stabilized for overnight.

Confocal microscopy. To make 500 μM **1**-Cu(II) stock solution, first, **1** was suspended in 10 equiv. $Cu(ClO_4)_2 \cdot 6H_2O$/DMSO solution and then adjusted the final concentration with DMSO. The cells were briefly washed with 1ml of PBS twice and 1 μM of **1**-Cu(II) in PBS was treated. After 40 min incubation, the remaining compound **1**-Cu(II) in the medium was removed by washing with PBS two times and the cells were placed in 1 ml of PBS solution containing an appropriate amount of KCN. The fluorescence images were taken using a confocal laser scanning microscope (Zeiss LSM 510, Zeiss, Oberko, Germany) which was equipped with a 488-nm Argon laser and a 505-nm long pass filter.

- **Synthesis**

Compounds **1** and **1**-Cu(II) were synthesized according to the procedures reported in the literature.[S1]

Compound 1. ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ 8.68 (s, 1H; CH), 8.51 (s, 1H; CH), 7.35 (d, J = 8.37 Hz, 1H; CH), 7.16 (t, J = 7.70 Hz, 1H; CH), 7.03 (s, 1H; CH), 6.99 (d, J = 8.22 Hz, 1H; CH), 6.98 (t, J = 7.62 Hz, 1H; CH), 3.33 (q, J = 5.62 Hz, 4H; CH_2), 2.91 (t, J = 6.42 Hz, 2H; CH_2), 2.78 (t, J = 4.00 Hz, 2H; CH_2), 1.99 (m, 4H). ^{13}C NMR (100 MHz, CDCl3): 163.0, 154.8, 152.9, 149.2, 147.8, 142.1, 134.5, 127.3, 126.6, 125.4, 124.3, 119.5, 118.0, 114.2, 109.0, 106.5, 50.5, 50.1, 27.7, 21.5, 20.6, 15.0. FAB-MS calc. for $C_{22}H_{20}N_2O_3$ $[M+H]^+$ 361.2, found 361.3.

Compound 1-Cu(II). ESI-MS calc. for $C_{23}H_{23}CuN_2O_4$ $[M+CH_3OH-H]^+$ 454.1, found 454.2.

S1. Jung, H. S.; Han, J. H.; Habata, Y.; Kang, C.; Kim, J. S. *Chem. Commun.* **2011**, *47*, 5142.

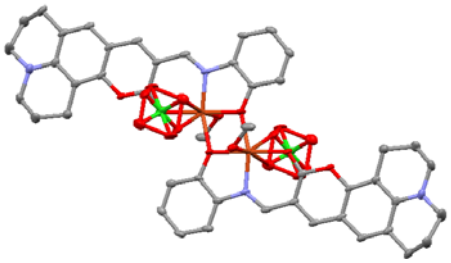

Figure S1. Crystal structure of **1**-Cu(II). Thermal ellipsoids are shown at the 50% probability level. Hydrogen atoms are omitted for clarity.

Table S1. Photophysical data of reaction mixtures [a]

The added Compounds	Absorbance λ_{max} (nm)	Emission λ_{max} (nm)	Relative quantum yield (Φ_f) [b]
1	464	514	0.67
2	464	514	0.66
1-Cu(II)	521	514	0.02
1-Cu(II) + CN^- (16 eq)	464	514	0.65

[a] After compounds (5.0 μM) were added to the 1.0% DMSO buffered solution, the photophysical data of the reaction mixture were obtained after 20 min at room temperature. [b] Φ_f: Relative Fluorescence Quantum Yield (fluorescein in 0.1N NaOH as a reference, Φ_f= 0.85).[S1]

S2. Paeker, A.; Rees, W. T. *Analyst*, **1960**, *85*, 587.

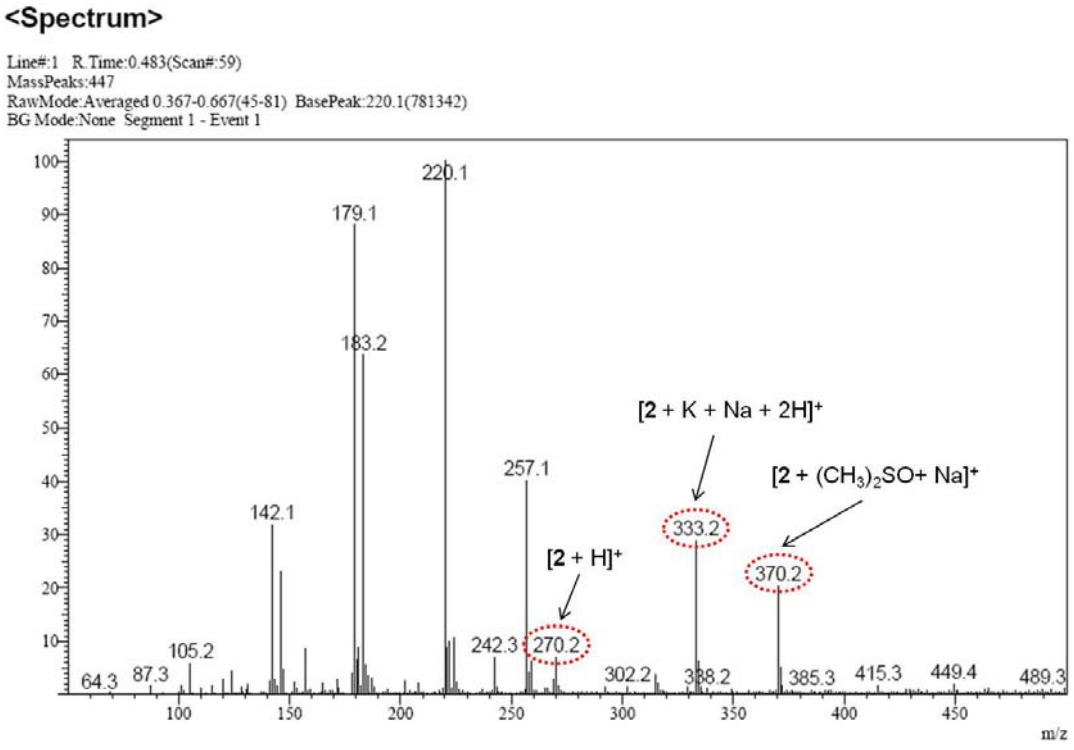

Figure S2. ESI-MS of **1**-Cu(II) with CN^- after 30 min.

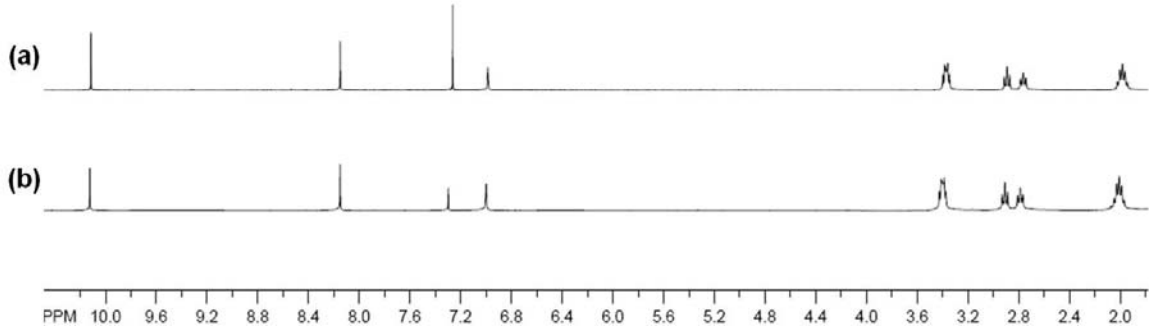

Figure S3. ^{1}H NMR spectra of (a) coumarinaldehyde **2** and (b) isolated from the mixture of **1**-Cu(II) upon addition of various anions (16 equiv) in buffered solution.

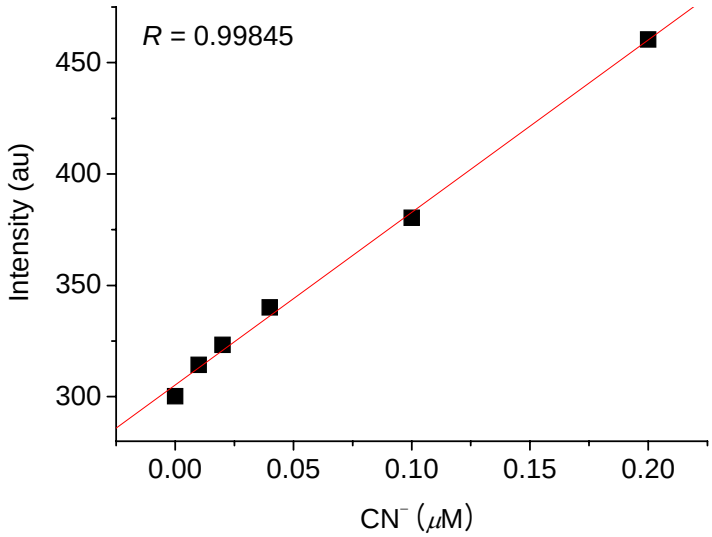

Figure S4. Fluorescence responses of **1**-Cu(II) (5.0 μM) in the presence of different concentrations of CN^- in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) with an excitation at 479 nm (slit=3/3) after 2 min. Linear correction between emmision intensity at 514 nm and CN^- concentration.

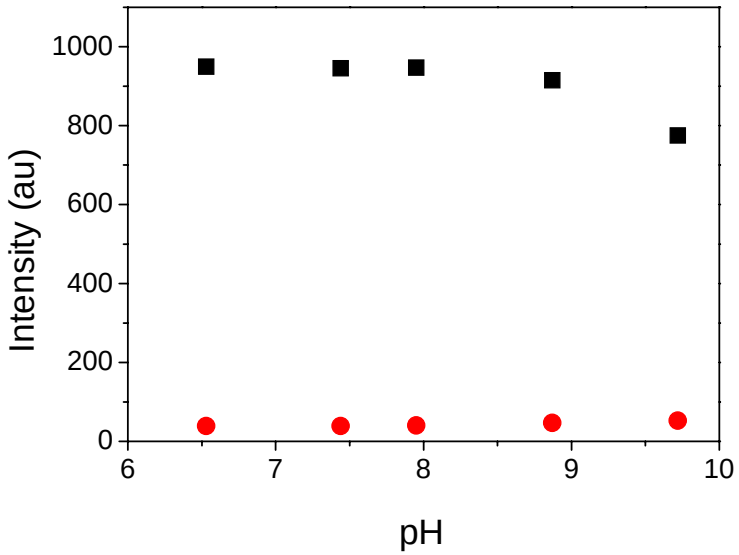

Figure S5. pH-dependent fluorescence profiles of **1**-Cu(II) (5.0 μM) and the corresponding cyanide (16 equiv) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO). Excitation at 479 nm after 20 min (slit = 1.5/1.5).

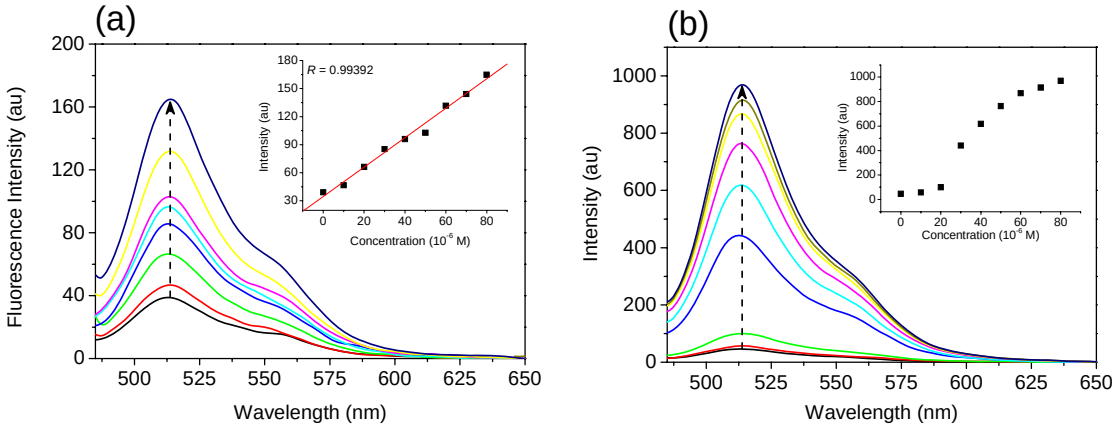

Figure S6. Fluorescence spectra of **1**-Cu(II) (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) upon addition of various concentrations of cyanide (0 – 16 equiv). Excitation at 479 nm after (a) 2 min and (b) 15 min (slit = 1.5/1.5). The inset shows the correlation between the fluorescence intensity and cyanide concentration.

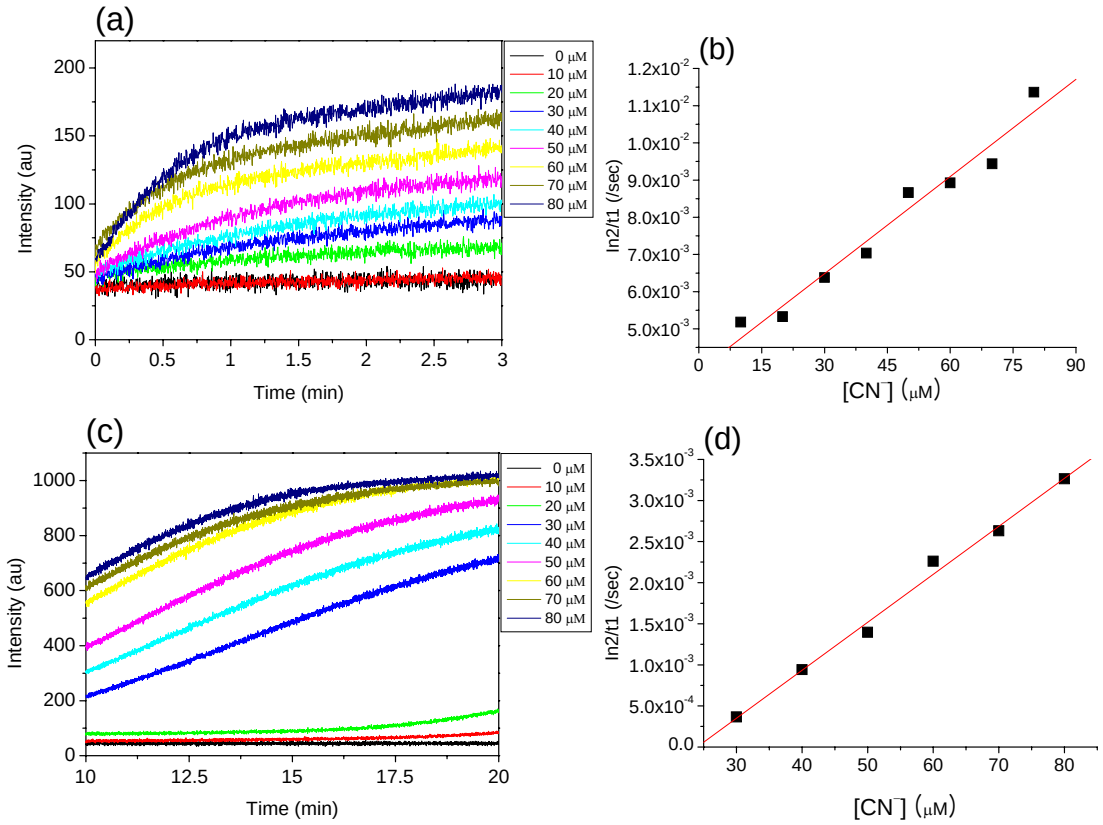

Figure S7. Time course ((a) from 0 min to 3 min and (c) from 10 min to 20 min) of the fluorescence response of **1**-Cu(II) (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) upon addition of various concentrations of cyanide (0 – 80 μM). The excitation and emission wavelength were 479 and 514 nm, respectively. A plot of ln2/t1 vs concentration of thiol containg amino acids. The life time ((b) from 0 min to 3 min and (d) from 10 min to 20 min) of compound **1**-Cu(II) (5 μM) in the presence of [CN^-] is indicated as t1 and the kinetic experiment was done in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0%DMSO).

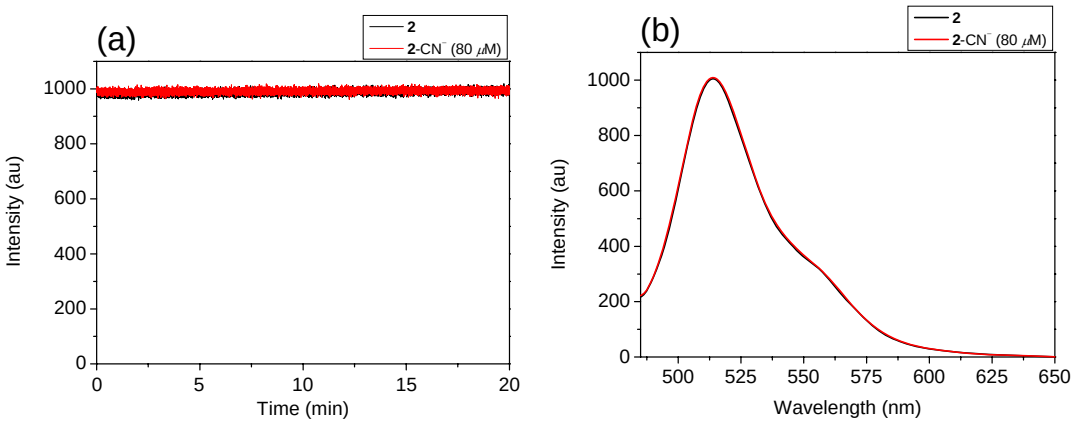

Figure S8. (a) Time course of the fluorescence response of **2** (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) in the presence of cyanide (80 μM). The excitation and emission wavelength were 479 and 514 nm, respectively. (b) Fluorescence spectra of **2** (5 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) in the presence of cyanide (80 μM) with an excitation at 479 nm after 20 min (slit=1.5/1.5).

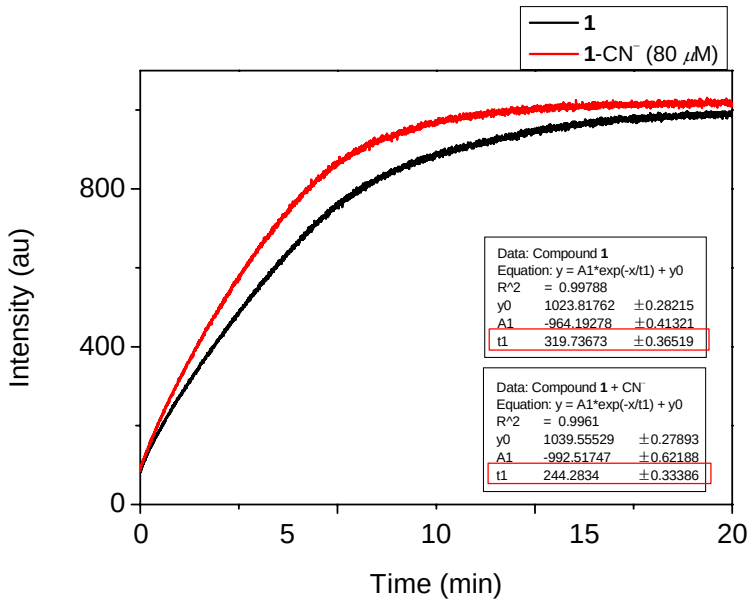

Figure S9. Time course of the fluorescence response of (a) **1**, and (b) **1**-CN^- (5.0 μM, respectively) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0 % DMSO). The excitation and emission wavelength were 479 and 514 nm, respectively. The boxes indicate kinetic analysis results based on first order decay model.

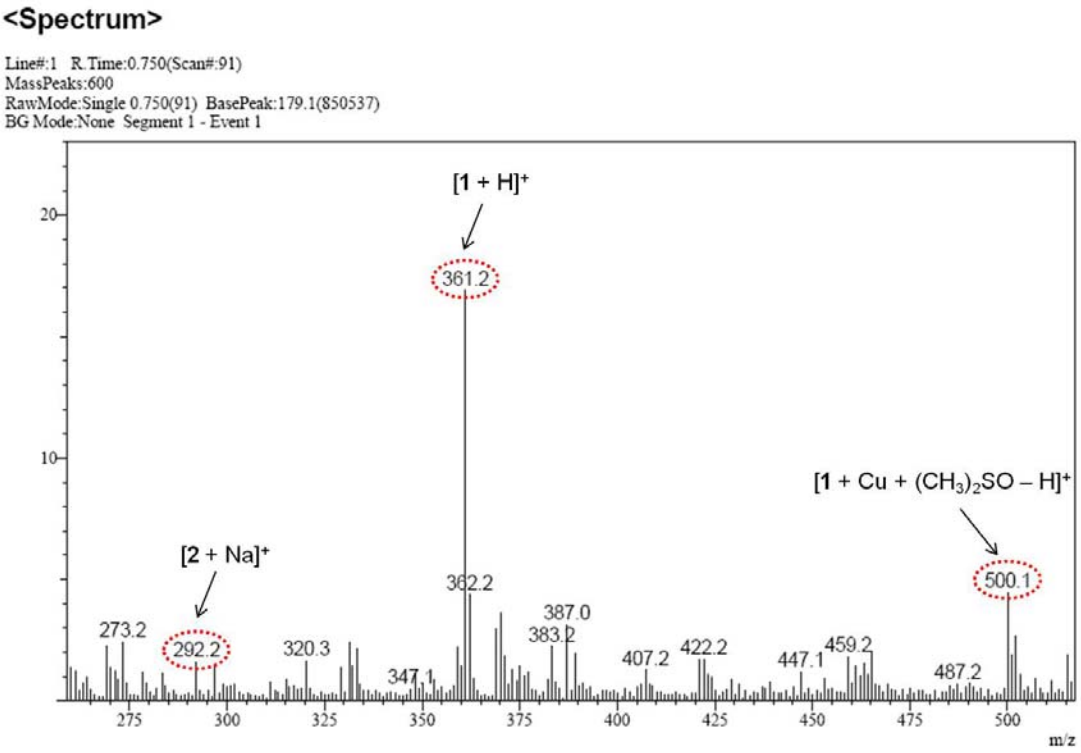

Figure S10. ESI-MS of **1**-Cu(II) with CN^- from a fast phase within 3 min.

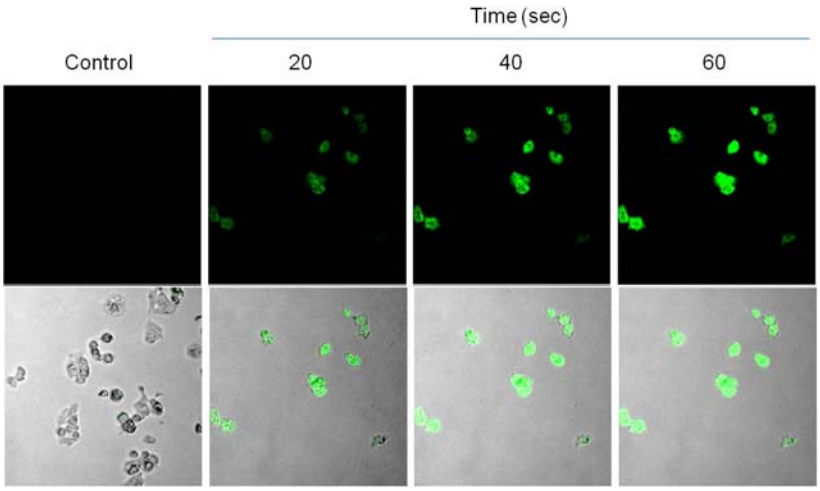

Figure S11. Confocal microscopic images of HepG2 cells in the presence of **1**-Cu(II) (1.0 μM). Time-lapse confocal imaging of HepG2 cells were taken after 250 μM KCN/PBS was treated. Bottom panels show an overlay of the image with a confocal phase contrast image.

- **Mass spectra copies**

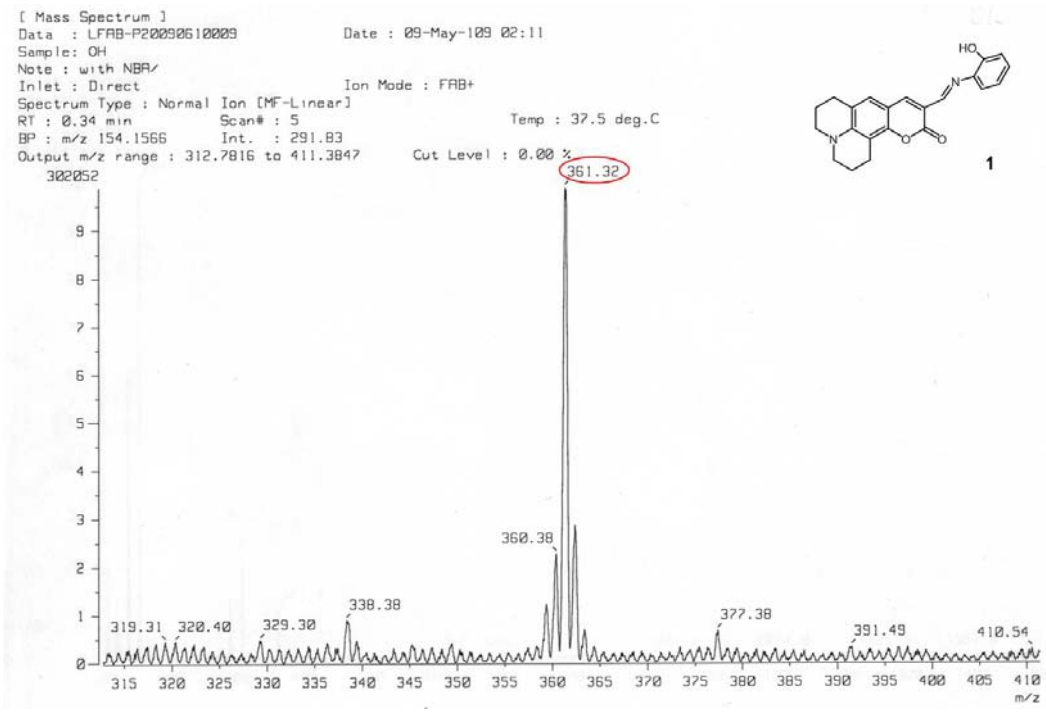

Figure S12. FAB-MS of **1**.

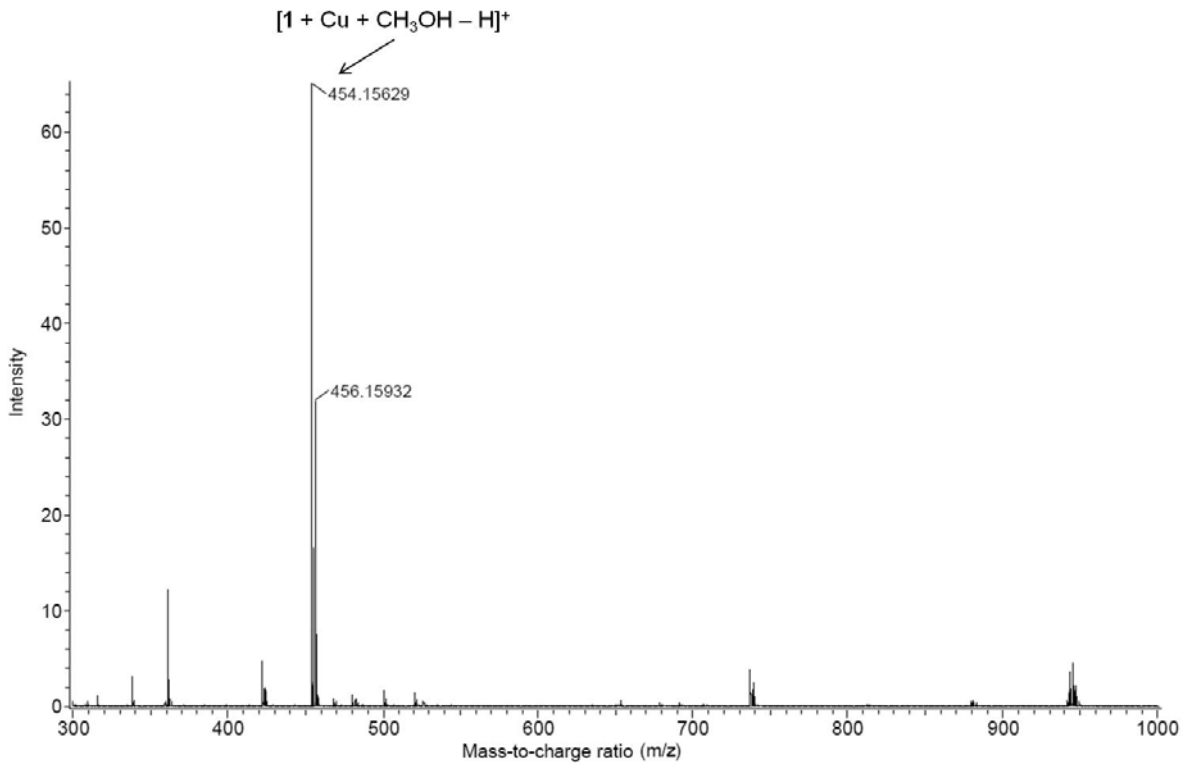

Figure S13. ESI-MS of **1**-Cu(II).

- **^{1}H NMR and ^{13}C NMR copies**

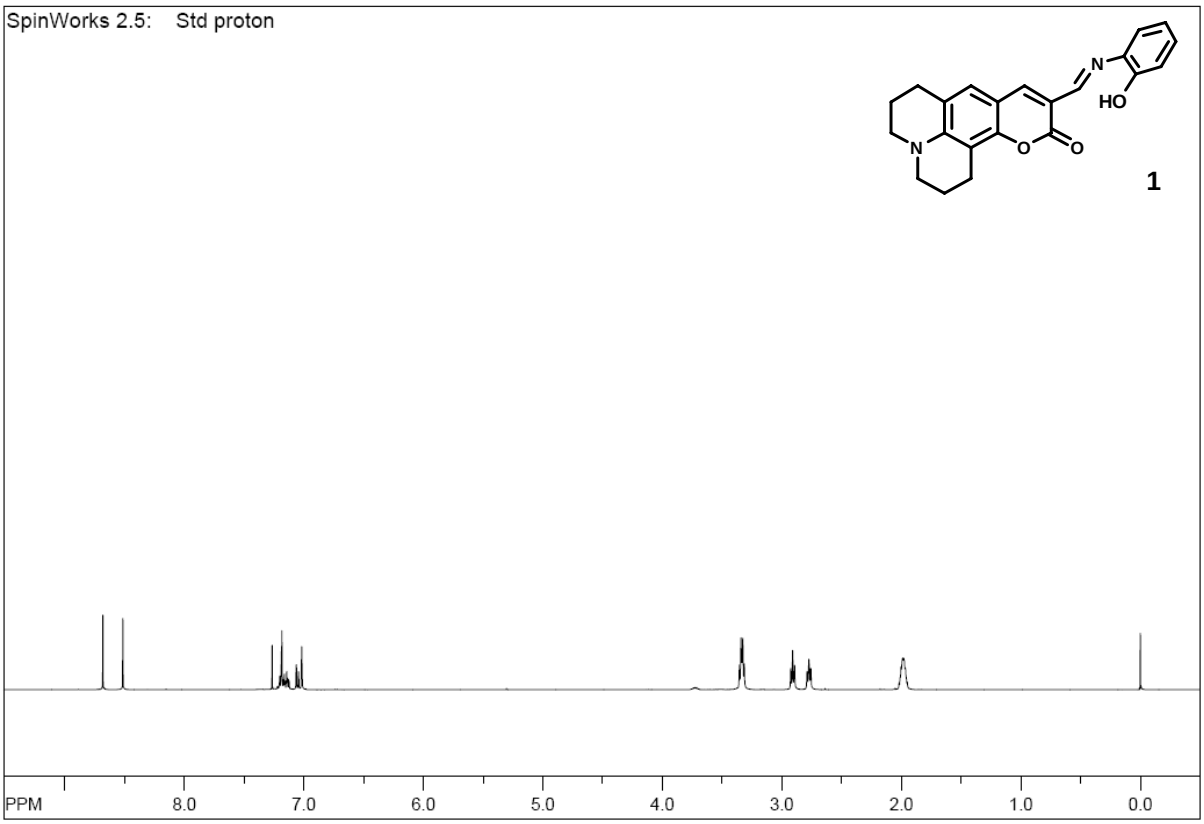

Figure S14. ^{1}H NMR spectra (400 MHz) of **1** (10 mM) in $CDCl_3$.

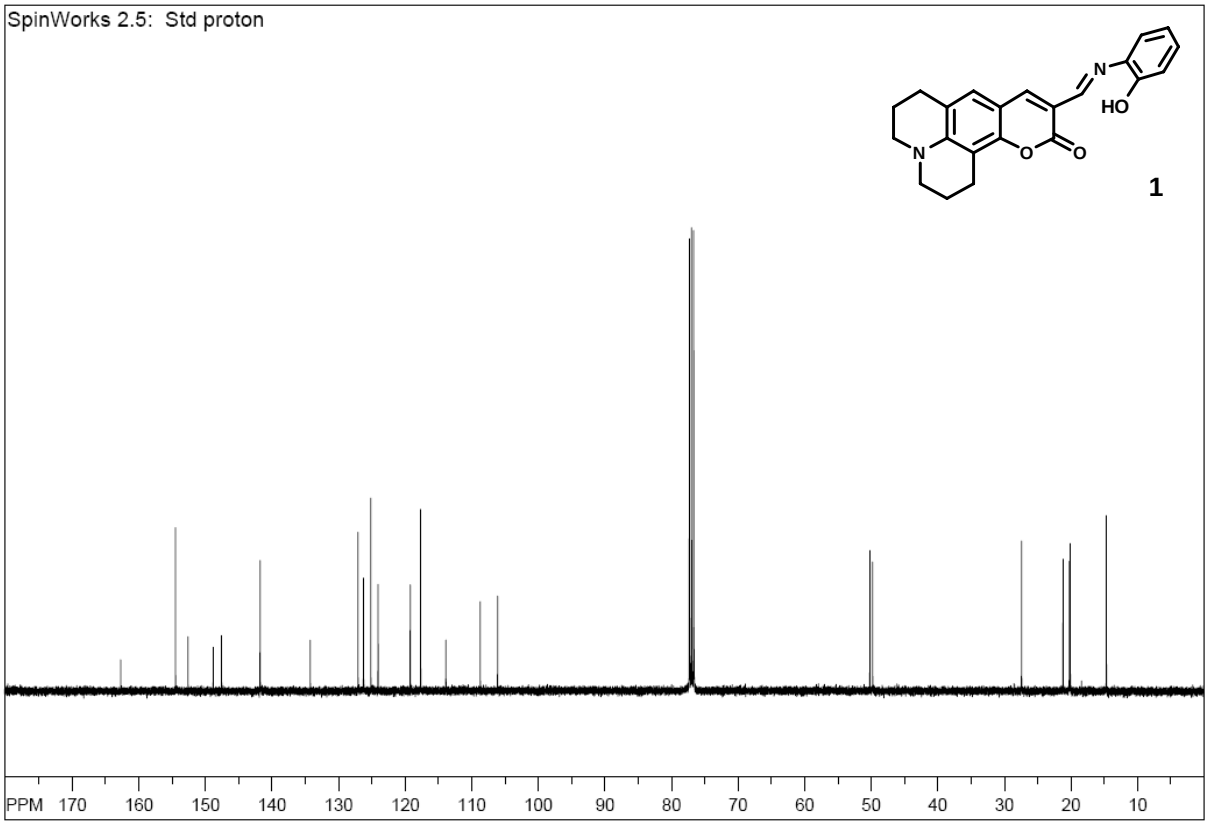

Figure S15. ^{13}C NMR spectra (100 MHz) of **1** in $CDCl_3$.