Electronic Supplementary Information

The construction of near-infrared fluorescent probe with dual advantages for imaging carbon monoxide in cells and in vivo

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Experimental section

Reagents and instruments. Boron tribromide (BBr_3), Allyl chloroformate were purchased from Aladdin (Shanghai, China). lipopolysaccharide (LPS), tricarbonylchloro(glycinato)ruthenium (III) (CORM-3), zinc protoporphyrin (ZnPP) were purchased from Sigma-Aldrich (St. Louis, USA). Unless noted, all the chemicals were of analytical reagent grade and used as received without further purification.

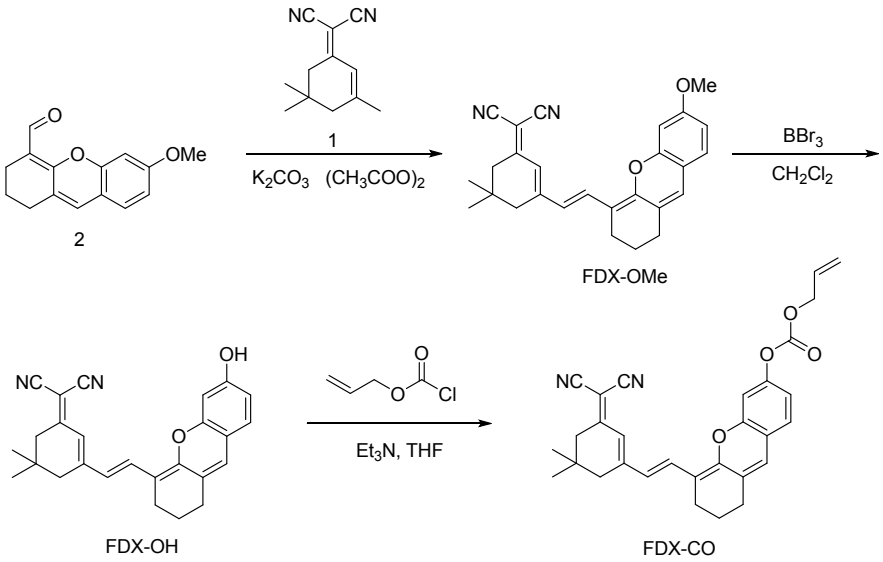
Nuclear magnetic resonance (NMR) spectra were operated on a Bruker Avance II NMR spectrometer (Germany). ^1H NMR and ^{13}C NMR were carried out at 400, 100 MHz, respectively. Mass spectra (MS) was recorded on a Bruker Autoflex MALDI-TOF mass spectrometer (Germany). Element analysis was measured on Perkin Elmer 2400 elemental analyzer (USA). The fluorescence spectra were carried out on a Hitachi F-4600 spectrophotometer (Japan). The absorption spectra were collected on an Agilent CARY 60 UV-vis spectrophotometer (USA). HPLC was obtained on an Agilent 1260 LC system with a C18 column (USA). Fluorescence imaging of cells were conducted by a Nikon confocal fluorescence microscope (Japan). Fluorescence imaging of mice was performed on an IVIS Lumina XR small animal optical in vivo imaging system (USA).

Syntheses of probe FDX-CO. The syntheses of probe FDX-CO was displayed in the following:

Compound FDX-OMe. Compound 1 [1] (0.33 g, 1.8 mmol), Compound 2 [2] (0.36 g, 1.5 mmol) and anhydrous K_2CO_3 (0.41 g, 3.0 mmol) were dissolved in anhydrous $(CH_3COO)_2$ (5 mL). The mixture was stirred at 80 °C for 14 h under nitrogen atmosphere. At the end of the reaction, the solution was concentrated and the residue was dissolved in CH_2Cl_2. After washed, dried and concentrated, the crude product was further purified by column chromatography with PE/CH_2Cl_2 (1/1, v/v) as the eluent to obtain the solid product. Yield: 0.42 g (68%) ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 15.6 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.72 (s, 1H), 6.65-6.58 (m, 2H), 6.41-6.36 (m, 2H), 3.84 (s, 3H), 2.57 (s, 2H), 2.52-2.46 (m, 6H), 1.80 (t, J = 8.0 Hz, 2H), 1.09 (s, 6H). MS (TOF): 410.3.

Compound FDX-OH. Anhydrous CH_2Cl_2 (20 mL) solution was poured into a reaction bottle. And compound FDX-OMe (0.41 g, 1.0 mmol) was dissolved in the above solution. BBr_3 (1.90 mL, 20.0 mmol) was slowly added into the above solution at 0 °C. Then, the mixture was stirred at 25 °C for another 16 hours. Lastly, the saturated $NaHCO_3$ solution was poured into the reaction solution at 0 °C. The organic layer was collected and the water layer was extracted with CH_2Cl_2 and CH_3OH. After dried and concentrated, the crude product was purified by column chromatography using CH_2Cl_2 as the eluent to obtain solid product. Yield: 0.20 g (52%). ^{1}H NMR (400 MHz, DMSO) δ 10.00 (s, 1H) 7.61 (d, J = 16.0 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.73-6.64 (m, 3H), 6.56-6.50 (m, 2H), 2.53-2.43 (m, 8H), 1.68 (t, J = 4.0 Hz, 2H), 1.02 (s, 6H). MS (TOF): 396.6

Compound FDX-CO. Triethylamine (0.03 g, 0.3 mmol) was added to the solution of compound FDX-OH (0.04 g, 0.1 mmol) in 5 mL dry CH_2Cl_2 at 0 °C under nitrogen atmosphere. After 5 min, allyl chloroformate (0.036 g, 0.3 mmol) was slowly added to the stirred solution, and the mixture was reacted at room temperature for 3 h. Then, the solvent was evaporated, and the residue was purified by silica chromatography with CH_2Cl_2 to afford a solid product. Yield: 0.04 g (83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 16.0 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 2.0 Hz, 1H), 6.84-6.82 (m, 1H), 6.73 (s, 1H), 6.38 (d, J = 12.4 Hz, 2H), 6.06-5.96 (m, 1H), 5.48-5.35 (m, 2H), 4.76 (d, J = 6.0 Hz, 2H), 2.57-2.45 (m, 8H), 1.81 (t, J = 6.0 Hz, 2H) 1.10 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 155.4, 153.2, 151.3, 150.6, 131.9, 130.9, 130.7, 126.5, 126.4, 122.3, 122.1, 120.3, 119.8, 115.9, 114.4, 113.6, 112.6, 108.4, 75.5, 69.4, 43.0, 39.2, 32.1, 29.9, 28.1, 24.6, 20.7, 1.0. MS m/z: 481.3 $[M+H]^+$. Elem. anal. (%) calcd. for $C_{30}H_{28}N_2O_4$: C, 74.98, H, 5.87; N, 5.83. Found: C, 74.36, H, 5.83, N, 5.77.



Scheme S1. The synthesized route of probe FDX-CO.

Fluorescent Detection for CO. FDX-CO solution (100 μM) was prepared in DMF and $PdCl_2$ solution (100 μM) were prepared in twice-distilled water. CORM-3 solution (100 μM, a water-soluble CO-releasing reagent) was prepared in twice-distilled water and further diluted. CORM-3 with different concentration was added into the 4 mL PBS buffer solution (0.1 M, pH 7.4) containing 10 μM FDX-CO and 10 μM $PdCl_2$ solution. The fluorescence emission spectra were recorded with the excitation at 580 nm and the emission at 690-900 nm. The excitation slit and emission slit were both set at 10 nm.

Cytotoxicity Assay. The cytotoxicity of probe FDX-CO were determined by MTT assays. HepG2 cells (1×10^4 cells/well) were cultured with different concentrations of FDX-CO (0, 5, 10, 15, 20, 25, 30 μM) in a 96-well plate. After 24 h incubation, MTT solution was added into each well and the residual MTT solution was removed. Then, DMSO was added to each well to dissolve the formazan crystals. After shaking, the absorbance of the wells were recorded by a microplate reader at 490 nm. The cell viability (%) was calculated by the following equation:

Cell viability (%) = $A/A_0 \times 100\%$

Here, A and A_0 are the absorbance of the detection group and of the control group, respectively.

Fluorescence Imaging in Living Cells. The HepG2 cells were obtained from the the State Key Laboratory of Chem/Biosensing and Chemometrics, Hunan University (Changsha, China) and cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C in a 5% CO_2 atmosphere. Then, the cells were plated on a 35 mm culture dish and allowed to adhere for 24 h.

For exogenous CO imaging experiments, the cells were divided into four groups. The first group was incubated with 10 μM FDX-CO for 30 min. The second group was incubated with 10 μM $PdCl_2$ for 30 min. In the third group, cells were pre-treated with 50 μM CORM-3 for 30 min and then were cultured with 10 μM FDX-CO and 10 μM $PdCl_2$ for 30 min. The fourth group was preincubated with 100 μM CORM-3 for 30 min before treated with 10 μM FDX-CO and 10 μM $PdCl_2$ for 30 min.

For endogenous CO imaging experiments, the cells were divided into four groups. In the first group, HepG2 cells were incubated with 10 μM FDX-CO for 30 min. For the second group, cells were pre-incubated with lipopolysaccharide (LPS, 1 μg/mL) for 12 h, and treated with 10 μM FDX-CO and 10 μM $PdCl_2$ for 30 min. The third group were pre-treated with 100 μM Heme for 10 h, and then incubated with 10 μM FDX-CO and 10 μM $PdCl_2$ for 30 min. In the fourth group, cells were pre-treated with 100 μM Heme for 2 h in presence of 20 μM ZnPP and then were cultured with 10 μM FDX-CO and 10 μM $PdCl_2$ for 30 min. All the cells were incubated at 37 °C and washed three times with PBS buffer solution. All images were obtained by confocal fluorescence microscope.

Fluorescence Imaging in Mice. Kunming (KM) mice were used and kindly kept in all the experimental process. All animal operation was carried out according to the regulations issued by the Ethical Committee of Xiangtan University. The mice were firstly intraperitoneally injected with 50 μL of 10 μM FDX-CO and 10 μM $PdCl_2$, followed by an injection of 100 μL of 10 μM CORM-3. And the mice were placed into the imaging chamber and imaged by small animal optical in vivo imaging system. The mice were anesthetized with chloral hydrate (10% in saline) and remained anesthetized throughout the image period.

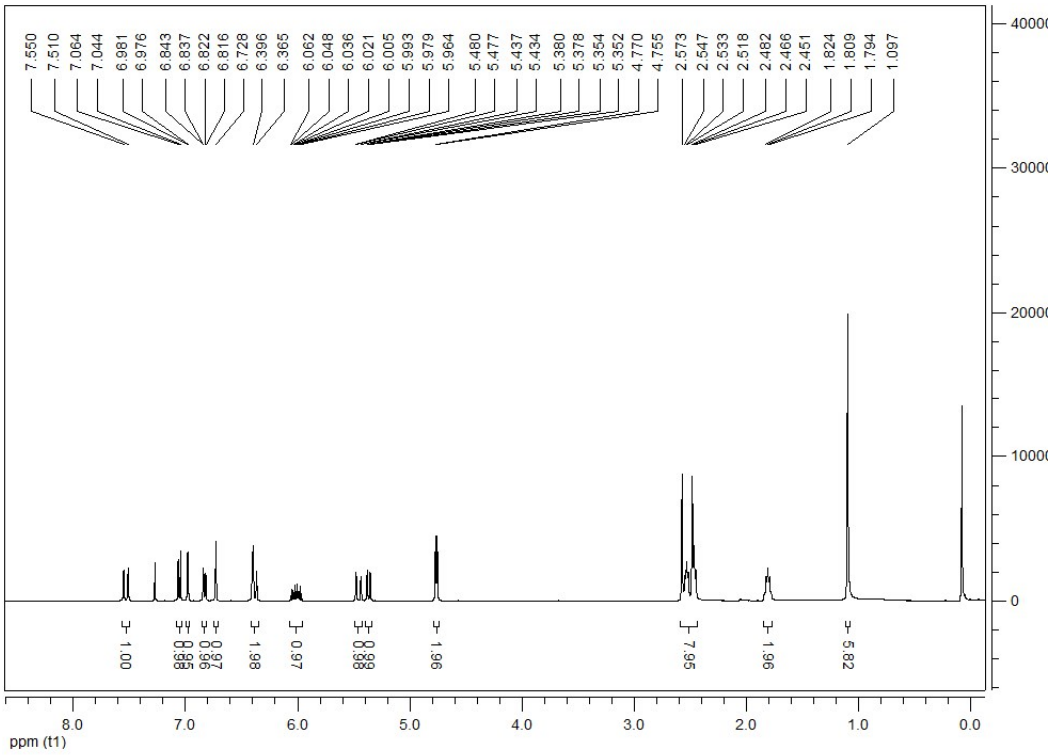


Fig. S1. ^{1}H NMR spectra of compound FDX-CO in $CDCl_3$.

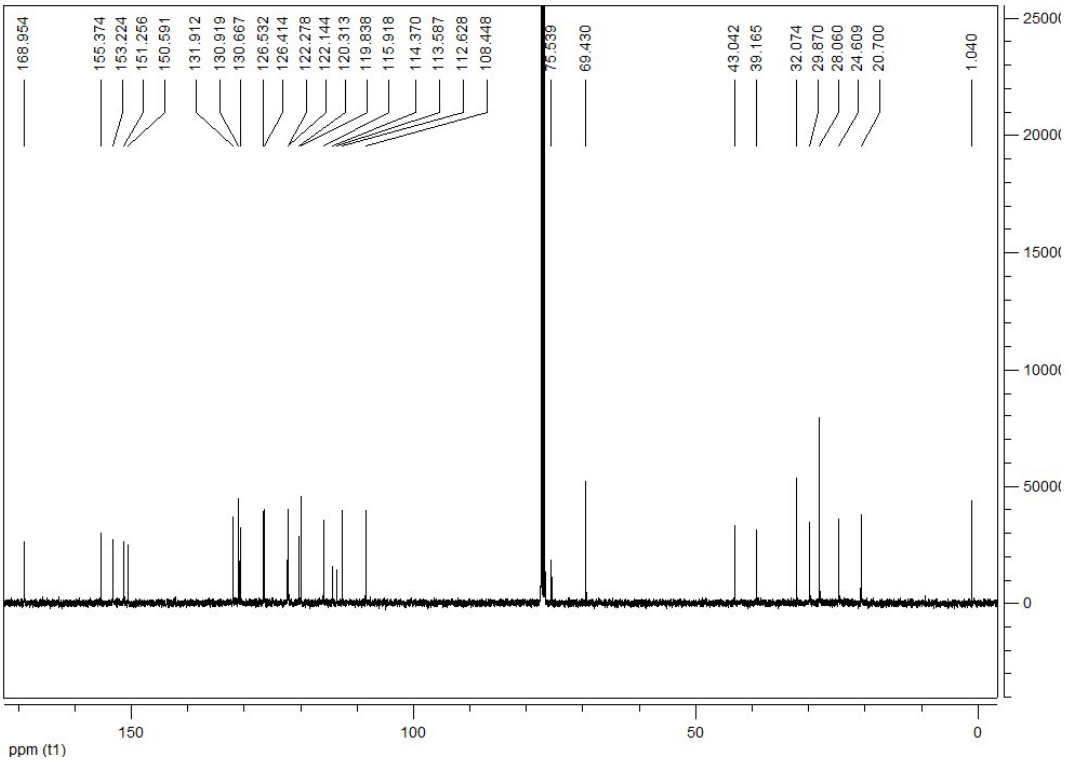


Fig. S2. ^{13}C NMR spectra of compound FDX-CO in $CDCl_3$.

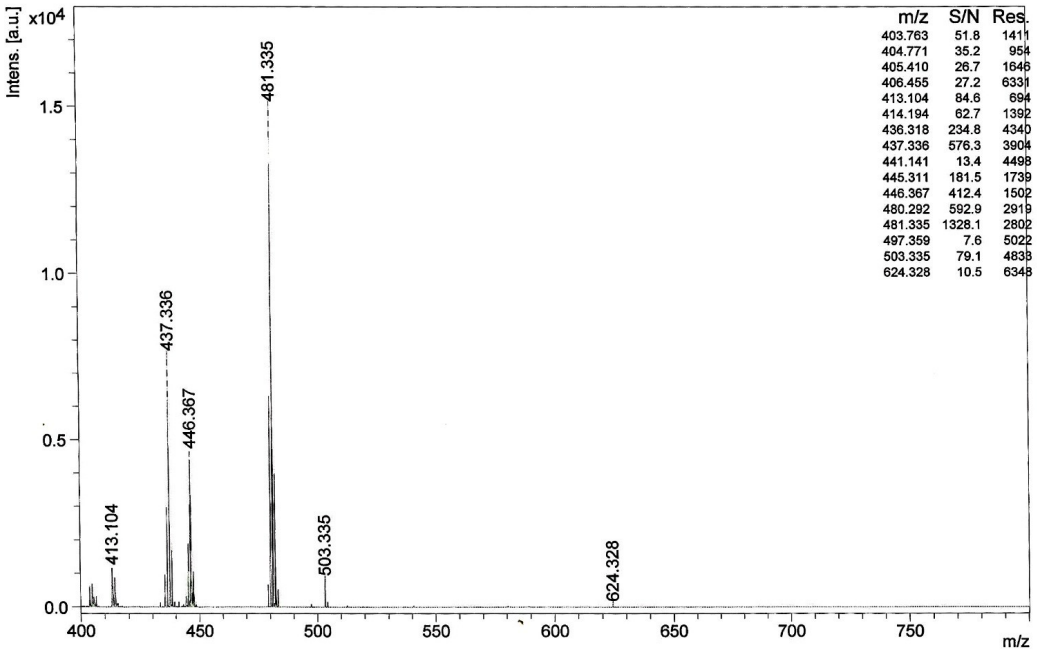


Fig. S3. Mass spectra of compound FDX-CO.

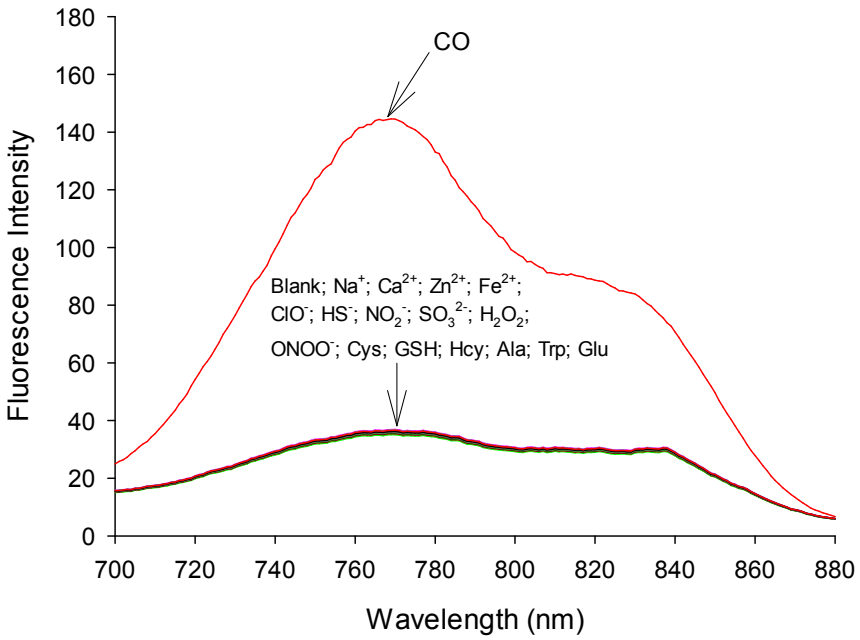


Fig. S4. Fluorescence intensity of FDX-CO (10 μM) and $PdCl_2$ (10 μM) toward different analytes: 1, Blank; 2, ClO^-; 3, H_2O_2; 4, $ONOO^-$; 5, NO_2^-; 6, HS^-; 7, SO_3^{2-}; 8, Cys; 9, Hcy; 10, GSH; 11, Ala; 12, Trp; 13, Glu; 14, Na^+; 15, Ca^{2+}; 16, Fe^{2+}; 17, Zn^{2+}; 18, CO. The concentration of CO is 30 μM and other analytes are 300 μM. λ_{ex} = 580 nm.

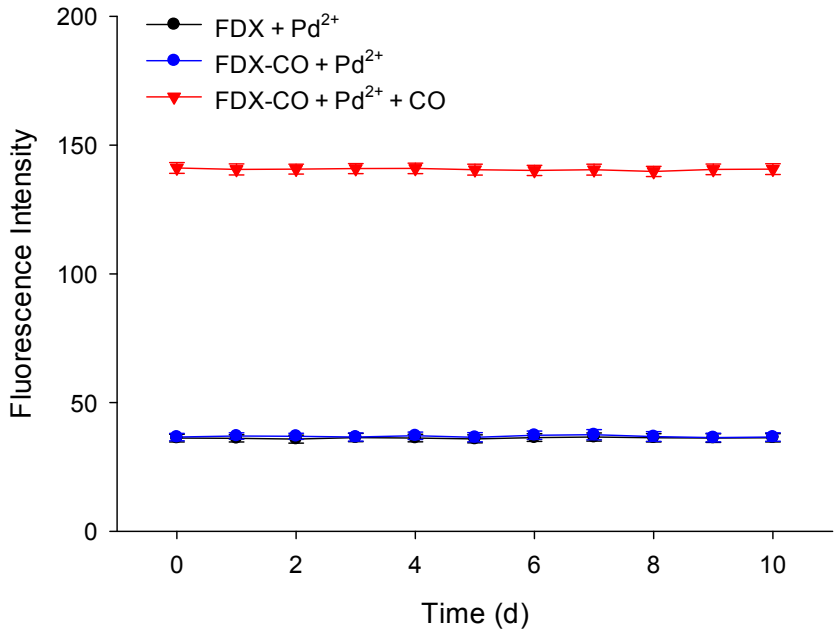


Fig. S5. The stability of probe FDX-CO (10 μM) and $PdCl_2$ (10 μM) before and after the addition of CO (30 μM). λ_{ex} = 580 nm.

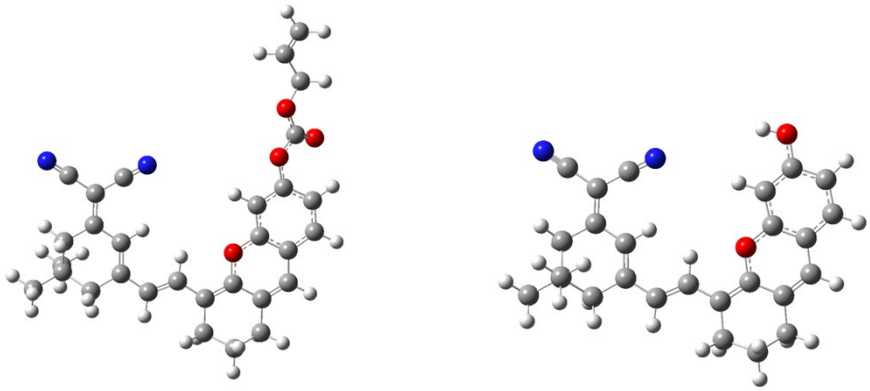


Fig. S6. The optimized structures of FDX-CO and FDX-OH.

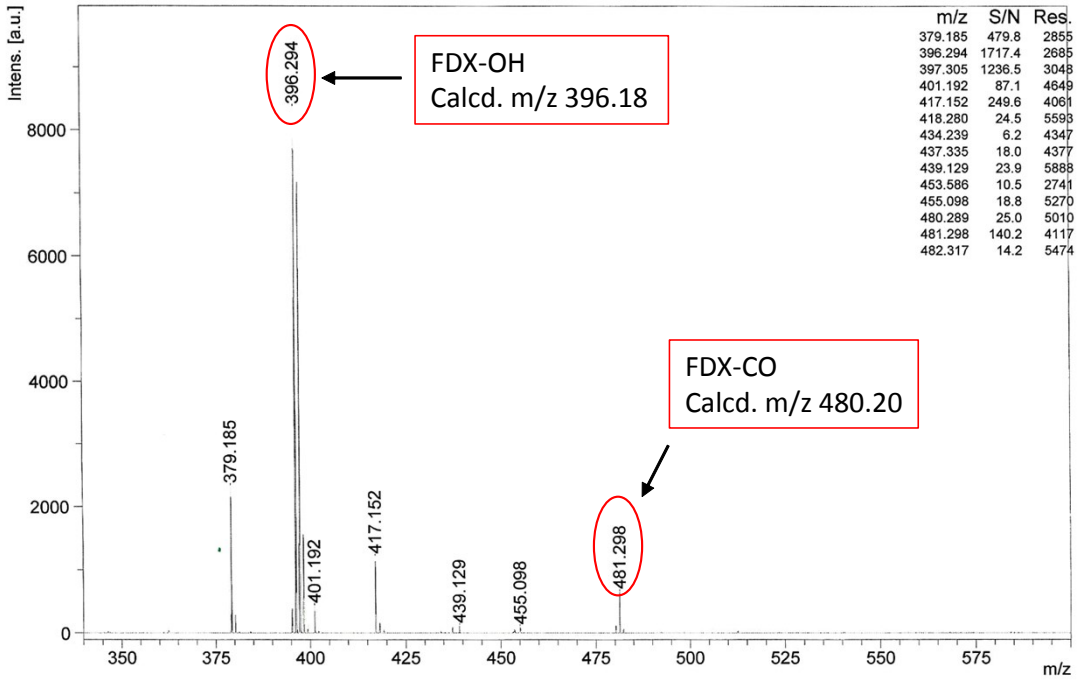


Fig. S7. Mass spectra of FDX-CO with CO.

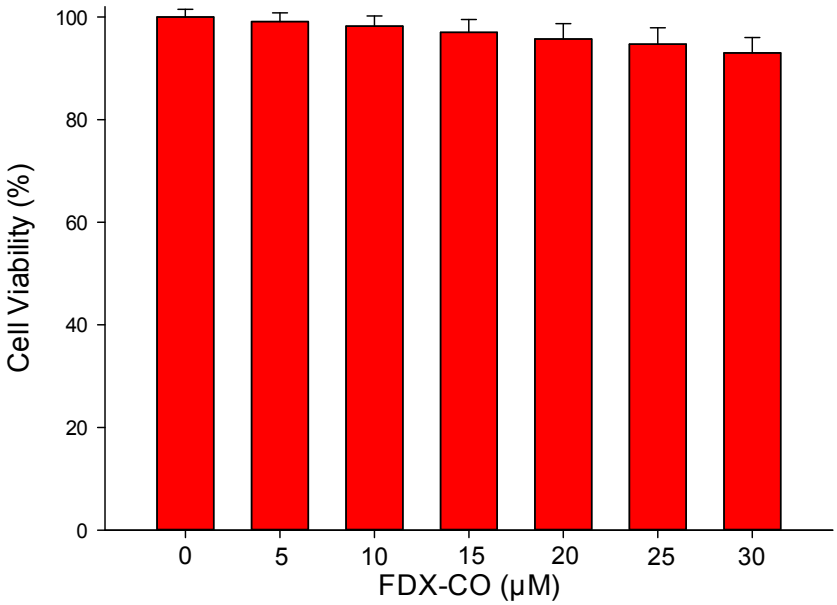


Fig. S8. MTT assay for estimating cell viability (%) of HepG2 cells treated with various concentration of FDX-CO (0-30 μM) after 24 h incubation.

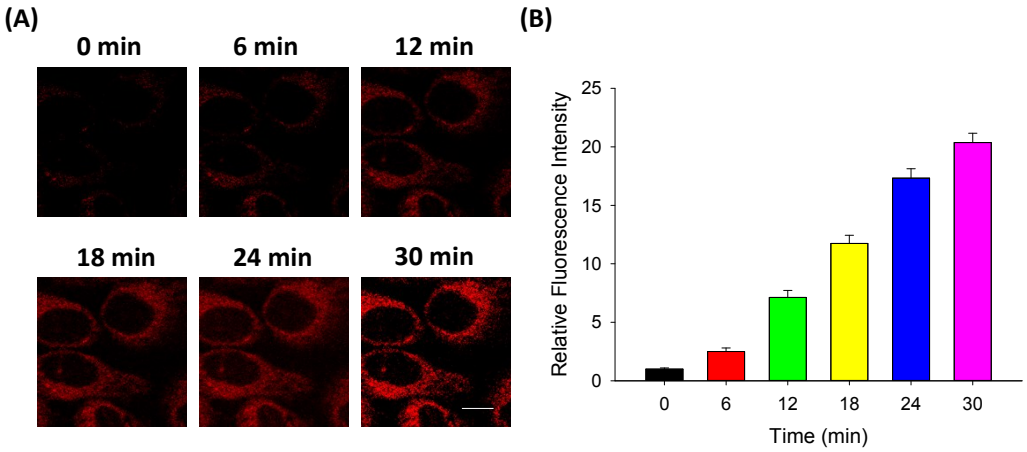


Fig. S9. The time-dependent fluorescence response to exogenous CO in HepG2 cells. (A) The cells were treated with CORM-3 (30 μM) for 30 min, and then incubated with FDX-CO (10 μM) and $PdCl_2$ (10 μM) for 0-30 min. (B) Quantification analysis of (A). λ_{ex} = 568 nm, λ_{em} = 750-850 nm. Scale bar: 10 μm.

References

1 W. Zhang, F. Liu, C. Zhang, J. G. Luo, J. Luo, W. Yu and L. Kong, *Anal. Chem.*, 2017, **89**, 12319-12326.

2 D. Y. Zhou, Y. F. Li, W. L. Jiang, Y. Tian, J. Fei and C. Y. Li, *Chem. Commun.*, 2018, **54**, 11590-11593.