Supporting Information

A Sensitive and Ratiometric Fluorescent Probe for Imaging Cytosolic H_2S Generation

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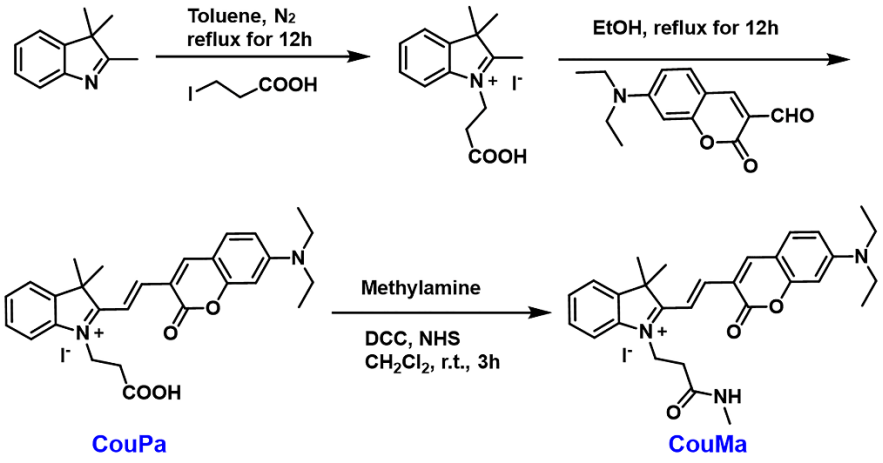
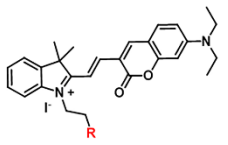
1. Materials and general methods

All reagents and solvents were purchased from commercial source including J&K chemical, Energy Chemical, Inno-chem, and Alfa Aesar, which were of analytic grade and used without purification. MTR 633 was purchased from Invitrogen. MEM media was purchased from Jiangsu Keygen BioTech Co. Ltd. ^{1}H NMR and ^{13}C NMR spectra were collected on Bruker DRX-400. HR-MS data were collected on Agilent 6540Q-TOF LC/MS mass spectrometer, and ESI-MS data were collected on Thermo Fisher ESI Mass Spectrometer. Fluorescent spectra were collected on FluoroMax-4 Spectrofluorometer with 5 nm slit for both excitation and emission. Absorption spectra were accomplished on Perkin Elmer Lambda 35 spectrophotometer. pH measurements were accomplished by a Model PHS-3C meter. Cellular confocal imaging was realized on confocal microscope Zeiss LSM710.

2. Synthesis and characterization of CouMa



Scheme S1. Synthesis route of CouMa.



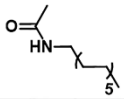
probe	R group	ClogP	subcellular distribution	Pearson's correlation coefficient with MTR	Reference
CouPa		2.67	mitochondria	0.84(MCF-7)	30
CouMa		5.79	cytosol	0.49 (MCF-7)	this article
CouDE		7.12	mitochondria	0.85 (MCF-7), 0.75 (Hep3B)	30
CouDa		11.61	mitochondria	0.84 (MCF-7)	32

Table S1. Information about derivative probes of CouPa.

Synthesis of probe CouMa

CouPa was obtained according to the procedure reported previously[1]. CouPa (1.92 mM, 1.126 g), DCC (1.92 mM, 0.396g) and NHS (1.92 mM, 0.221 g) were dissolved in CH_2Cl_2 (40 mL) and activated under 0°C for 0.5 h. After that methylamine (2.3 mM, 2 M in THF) was added and the solution was stirred at room temperature for nearly 3 h, which was monitored by TCL. When the reaction was finished, cool the solution under 4°C overnight and remove the precipitate by filtration. Solvent of filtrate was removed under reduced pressure, and the crude product was purified by silica gel chromatography with eluent CH_2Cl_2/CH_3OH (1/20, v/v) to afford CouMa as a dark blue solid. Yield, 65%.

^{1}H NMR (400 MHz, CD_3OD, δ, ppm): 8.65 (s, 1H), 8.33 (d, 1H, J = 15.7Hz), 7.95 (d, 1H, J = 15.6 Hz), 7.76-7.70 (m, 2H), 7.63-7.55 (m, 3H), 6.90 (d, 1H, J = 9.1 Hz), 6.61 (s, 1H), 4.79 (t, 2H, J = 6.6 Hz), 3.61 (q, 4H, J = 7.1 Hz), 2.86 (t, 2H, J = 6.7 Hz), 2.59 (s, 3H), 1.82 (s, 6H), 1.26(t, 6H, J = 7.1 Hz).^{13}C NMR (101 MHz, CD_3OD, δ, ppm): 183.42, 171.76, 169.12, 161.66, 159.71, 156.27, 151.99, 144.63, 142.21, 133.95, 130.39, 130.06, 123.99, 115.48, 113.82, 112.76, 111.62, 111.04, 97.94, 53.22,46.58, 44.33, 34.91, 27.33, 26.47, 12.90. HR-MS (positive mode, *m/z*): calcd. 472.2599, found 472.2595 for $[M]^+$.

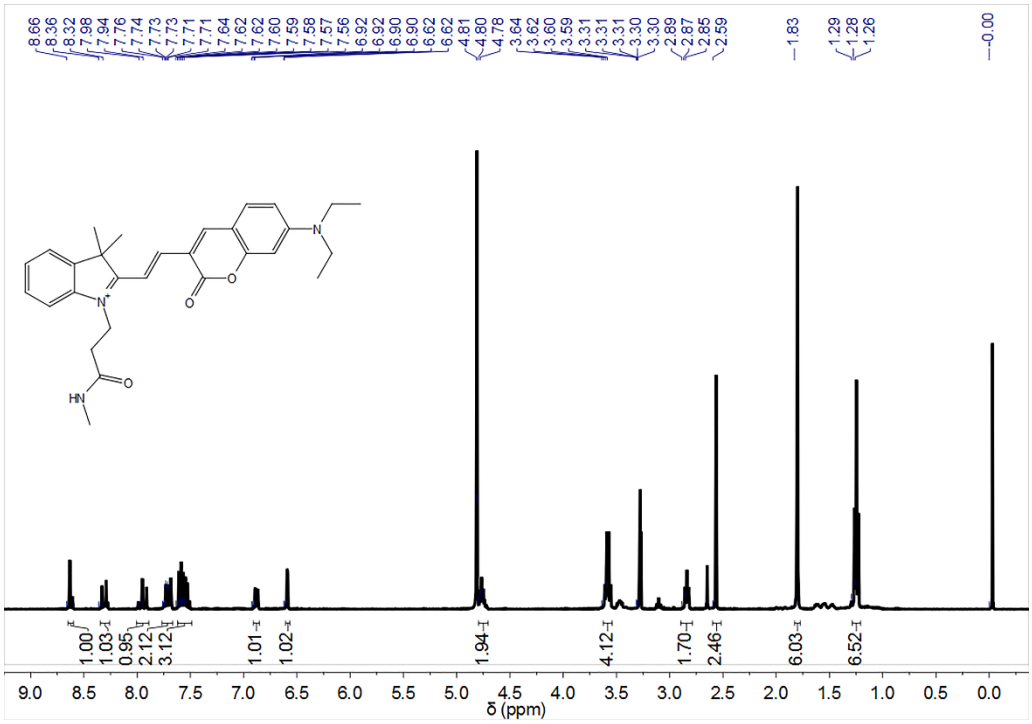


Figure S1. ^{1}H NMR spectrum of CouMa in CD_3OD.

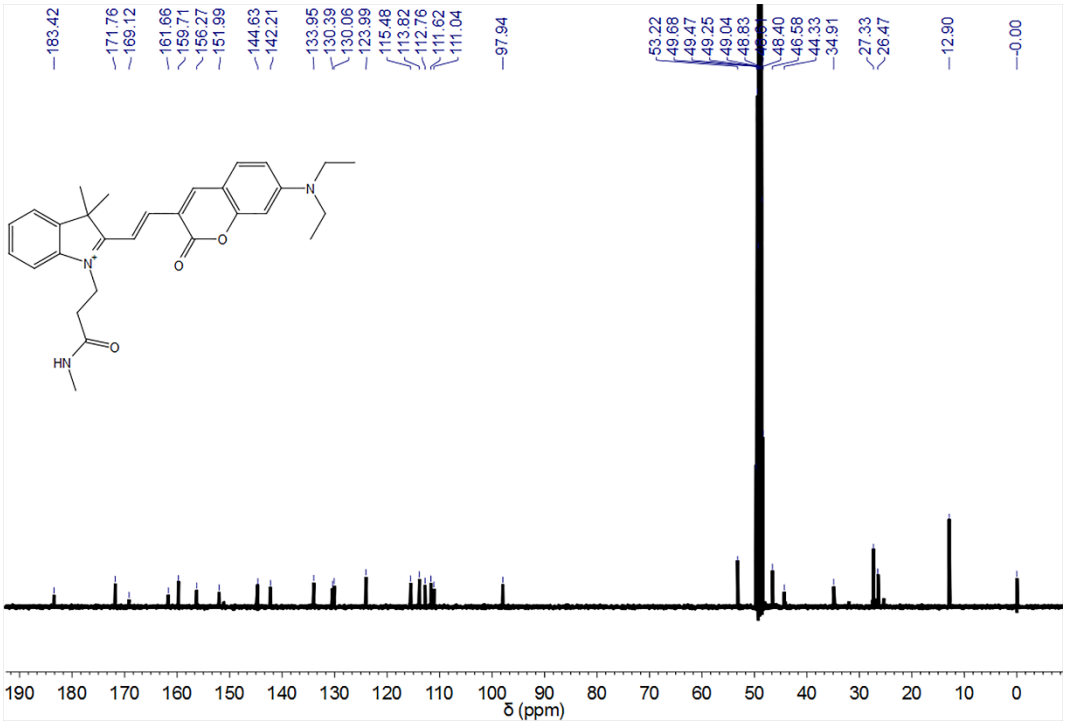


Figure S2. ^{13}C NMR spectrum of CouMa in CD_3OD.

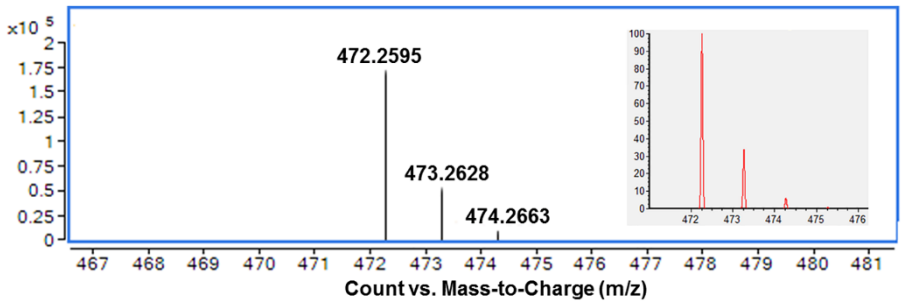


Figure S3. HR-MS spectrum of CouMa. Inset, the simulated isotopic distribution pattern of CouMa.

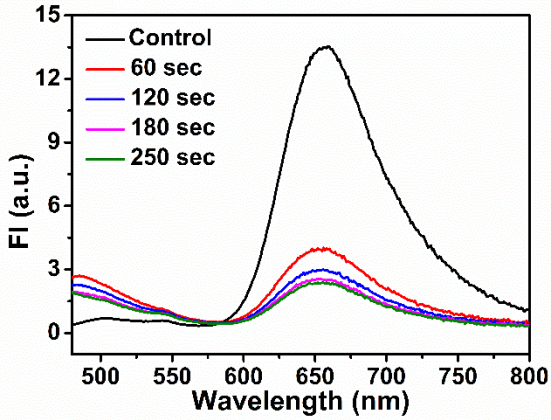


Figure S4. Time-dependent fluorescent emission spectra of CouMa (10 μM) after NaHS (200 μM) was added in HEPES buffer solution (20 mM, pH 7.4, 10% DMSO, v/v). Spectra of control was emission of CouMa before NaHS was added.

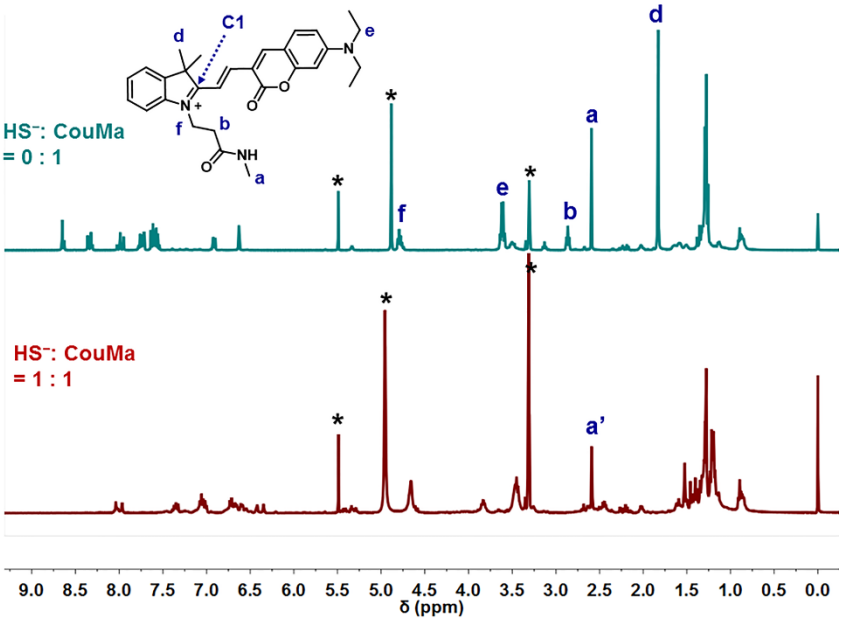


Figure S5. ^{1}H NMR spectra of CouMa (17 mM) before and after equal amount of NaHS (0.2 M in D_2O) was added. Signals of solvent (methanol, water or dichloromethane)

were signed by asterisk.

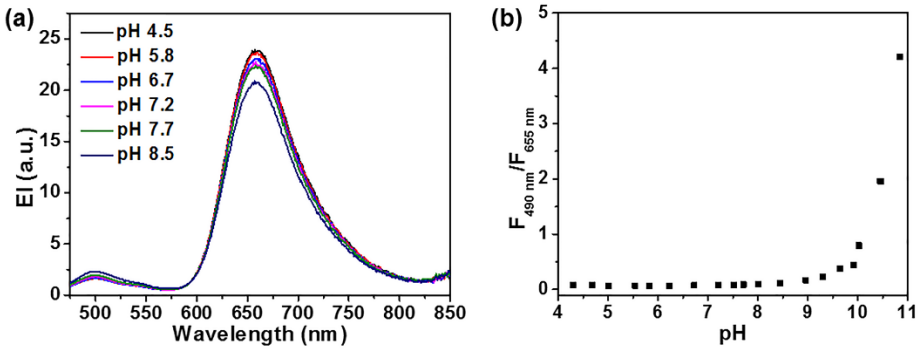


Figure S6. (a) Emission spectra of 10 μM CouMa in solutions with different pH. (b) Emission ratio $F_{490\ nm}/F_{655\ nm}$ of CouMa versus pH values. NaOH and HCl were applied to adjust pH of CouMa solution. λ_{ex} = 460 nm.

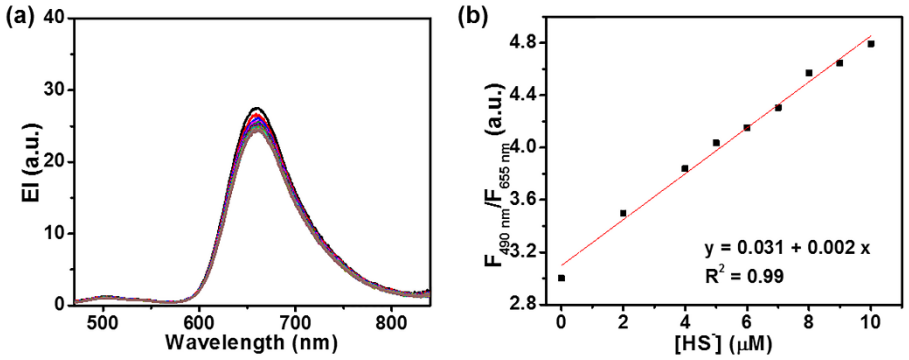


Figure S7. (a) Emission spectra of 10 μM CouMa in HEPES buffer (20 mM, pH 7.4, 10% DMSO, v/v) scanned for 20 times. (b) Linear relationship of emission ratio $F_{490\ nm}/F_{655\ nm}$ of 10 μM CouMa versus NaHS concentration (0-10 μM). λ_{ex} = 460 nm.

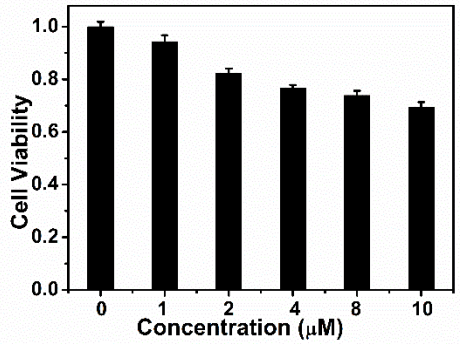


Figure S8. Cell viability of MCF-7 cells incubated with CouMa of different concentrations for 12 h.

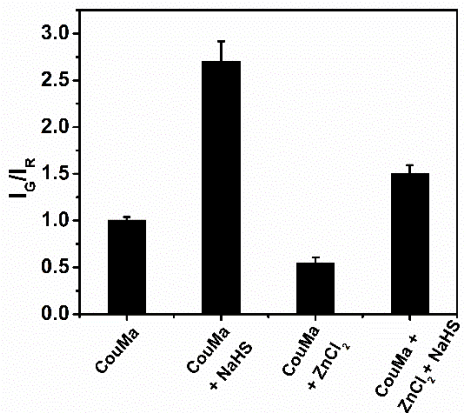


Figure S9. Quantification of ratio channel intensity of MCF-7 cells incubated under various circumstances corresponding to Figure 4. n=3.