Electronic Supplementary Information (ESI)

A spiropyran functionalized fluorescent probe for mitochondria targeting and imaging of endogenous hydrogen sulfide in living cells

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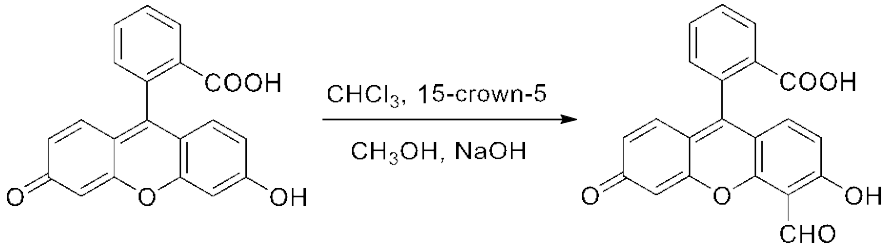
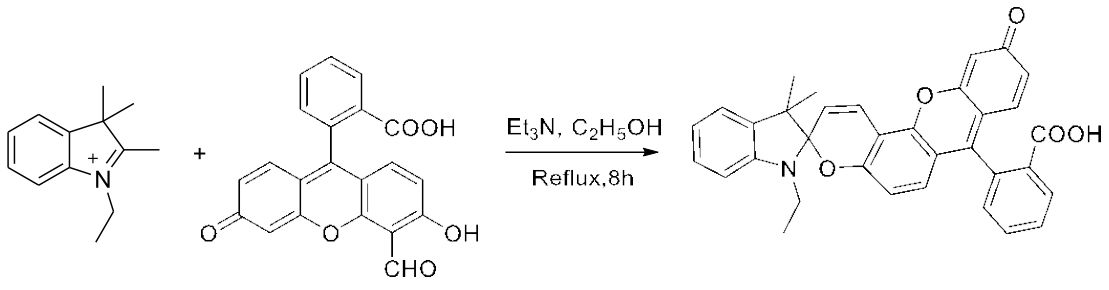
1. Synthesis of FMC



Fluorescein unsaturated monoaldehyde was synthesized as previous reported in literature.[1] Briefly, fluorescein (2.0 g, 6.02 mmol), 2.0 mL of $CHCl_3$, 0.03 mL of 15-crown-5 and 2.5 mL of MeOH were added to a solution of 10 ml of 50% NaOH solution, the reaction temperature was maintained at 55 ℃. The mixture was stirred at this temperature for 5 h. After cooling, the mixture was added with 10 M H_2SO_4, and the precipitate was filtered and dried in vacuo overnight. The precipitate was purified by chromatography on a silica gel column (15:85 EtOAc:DCM) and fluorescein monoaldehyde was obtained as a light yellow solid (608 mg, yield 28%). ^{1}H NMR(600 MHz, $CDCl_3$): δ12.18(s, 1H), 10.25 (s, 1H), 8.03(d, J=6.6 Hz, 1H), 7.70(m, J=1.1, 8.8 Hz, 1H), 7.66 (d, J=7.2 Hz, 1H), 7.62 (m, J = 1.1, 7.5 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 6.91(d, J = 8.8 Hz, 1H), 6.86(d, J = 8.8 Hz, 1H), 6.67(d, J = 8.8 Hz, 1H), 6.61(s, 1H).



FMC

Fluorescein monoaldehyde (0.40g, 1.1mmol) and 1-ethyl-2,3,3-trimethylindolenium Iodide (0.42 g, 1.3 mmol) were refluxed in ethanol (40mL) for 8 h under N_2. After that, the mixture was concentrated by evaporation and was further purified by silica gel column chromatography (15:85 EtOAc:DCM) and a light orange solid (FMC) was

obtained(309 mg, yield 52.6%). The ^{1}H NMR (600 MHz, $CDCl_3$) data are: δ 8.00(d, J=7.5Hz, 1H), 7.68(dd, J=7.5Hz, 1H), 7.43(m, J=1.8, 8.8Hz, 2H), 7.21(d, J=3.9Hz, 2H), 7.15(m, J=6.5, 1.8Hz 2H), 7.08(d, J=7.5Hz,1H), 6.83(d, J=1.8Hz 1H), 6.74(d, J=1.1Hz, 7.5Hz, 1H), 6.66(d, J=8.8Hz, 1H), 6.58(d, J=8.5Hz 1H), 6.40(s, 1H), 5.80(d, J=1.2, 7.5Hz, 1H), 3.78(m, J=7.5Hz,2H), 1.35(m, J=1.2, 7.5Hz, 3H, s, 6H). The ^{13}C NMR (600 MHz, $CDCl_3$) data are δ186.7, 169.6, 158.9, 156.7, 154.0, 153.2, 152.7, 147.4, 146.9, 144.2, 136.3, 134.9, 130.6, 130.0, 128.6, 127.6, 126.8, 126.3, 122.4, 119.5, 118.7, 113.2, 111.6,110.8, 108.6, 107.5, 97.2, 52.5, 44.2, 20.0 and 12.7. MS: m/z: calculated for $C_{34}H_{27}O_5N$: 529.21700; found: 530.19817 ($M+H^+$).

2. Determination of fluorescence quantum yield

Fluorescence quantum yield was measured by a standard method in air-equilibrated sample at room temperature. The fluorescence quantum yield was determined by using Rhodamine B (Φ=0.31 in water) as reference. [2]

$$\Phi_{sam} = \Phi_{ref} \frac{I_{sam}}{I_{ref}} \cdot \frac{A_{ref}}{A_{sam}} \cdot \left(\frac{n_{sam}}{n_{ref}}\right)^2 \quad (1)$$

Where Φ is the fluorescence quantum yield, I is the integrated emission intensity, A is the absorbance, and n is the refractive index. The subscripts sam and ref stand for sample and reference, respectively. Herein, the FMC and Rhodamine B were dissolved in ultrapure water (n =1.33) and excited under 470 nm and kept the absorbance below 0.05.

3. Supplementary figures

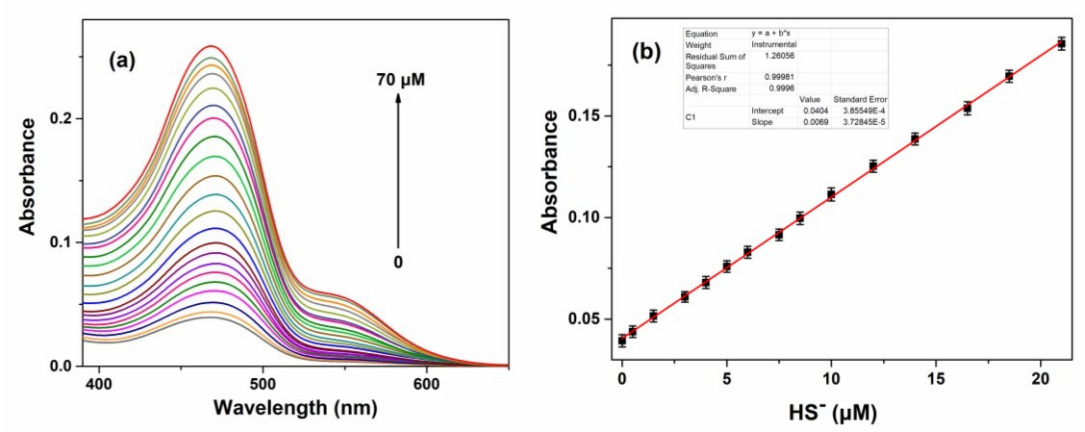


Fig. S1 (a) Absorption spectra of FMC in PBS buffer (10 mM, 7.4) with different concentrations of HS^- (0–70μM). (b) The linear region between the absorbance of FMC at 470 nm and the concentrations of HS^- in the range of 0-21μM.

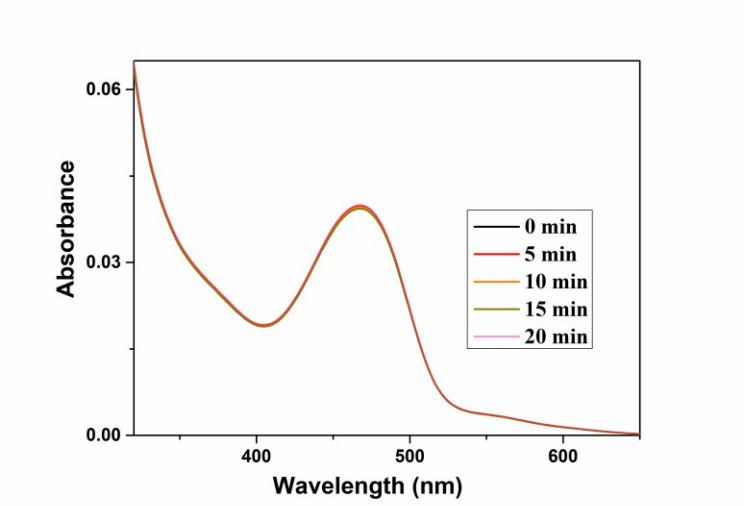


Fig.S2 Absorption spectra of FMC in PBS buffer solution (25 mM, pH 7.4) under 365 nm ultraviolet irradiation for 20 min.

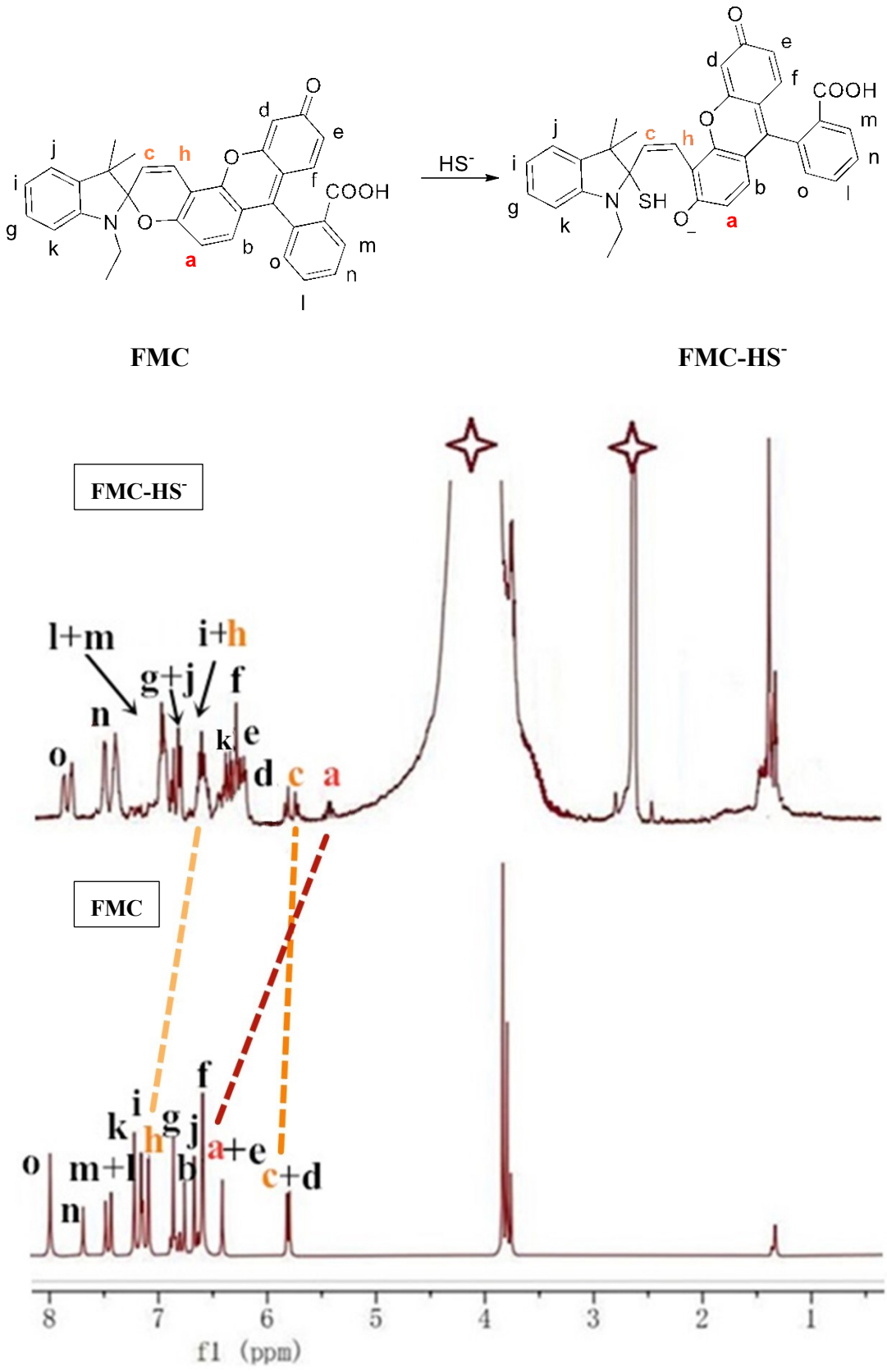


Fig. S3 ^{1}H NMR spectra of FMC and FMC-HS^{-} adduct in DMSO-*d*6. The water and solvent peaks are marked with asterisks.

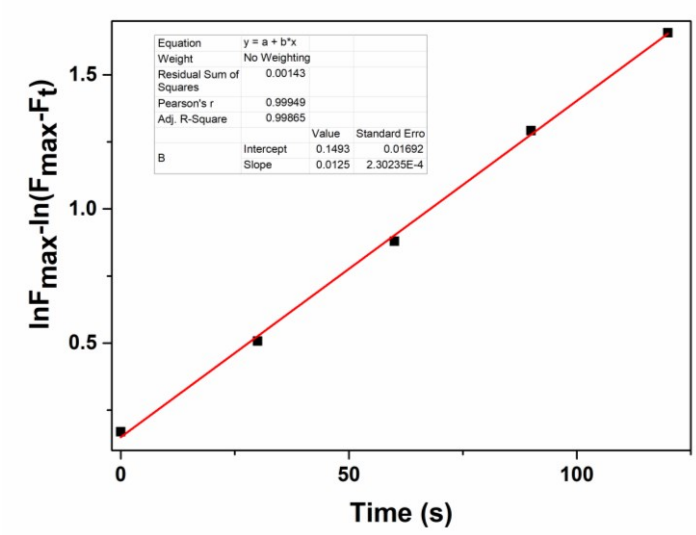


Fig.S4. Line fitting of time dependence of FMC(10 μM) with HS^- (70 μM) in PBS solution (25 mM, pH 7.4).

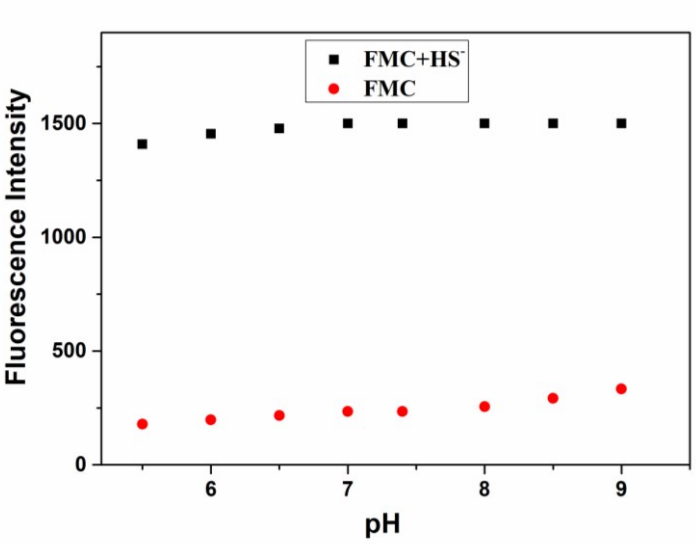


Fig.S5 Effects between the fluorescence intensities of FMC at 525 nm in the absence and the presence of HS^- and different pH from 5.5 to 9.0. The excitation wavelength is 470 nm.

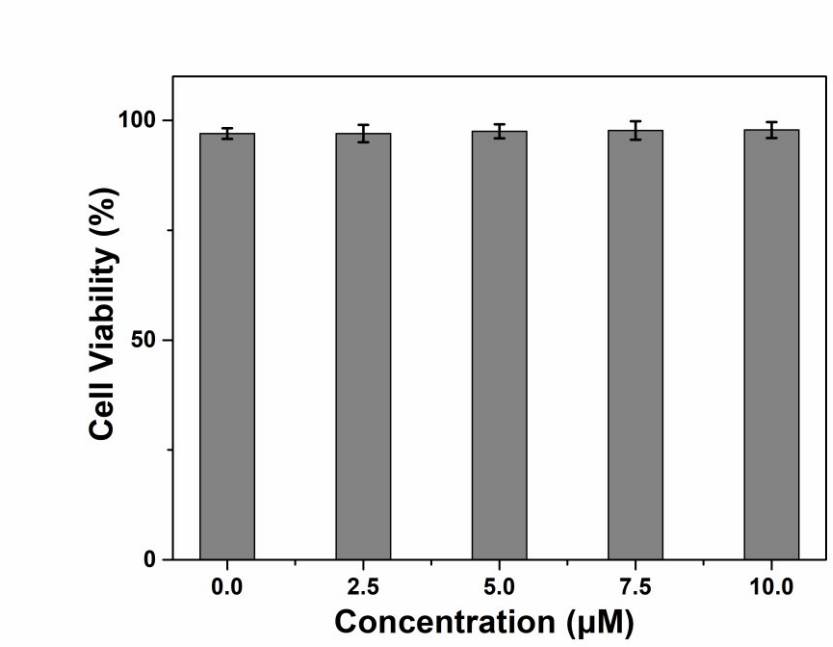


Fig.S6 Cytotoxic assay of the different concentrations of FMC on Hep-2 Cells.

4. ^{1}H NMR spectra and ^{13}C NMR spectrum and mass spectrum

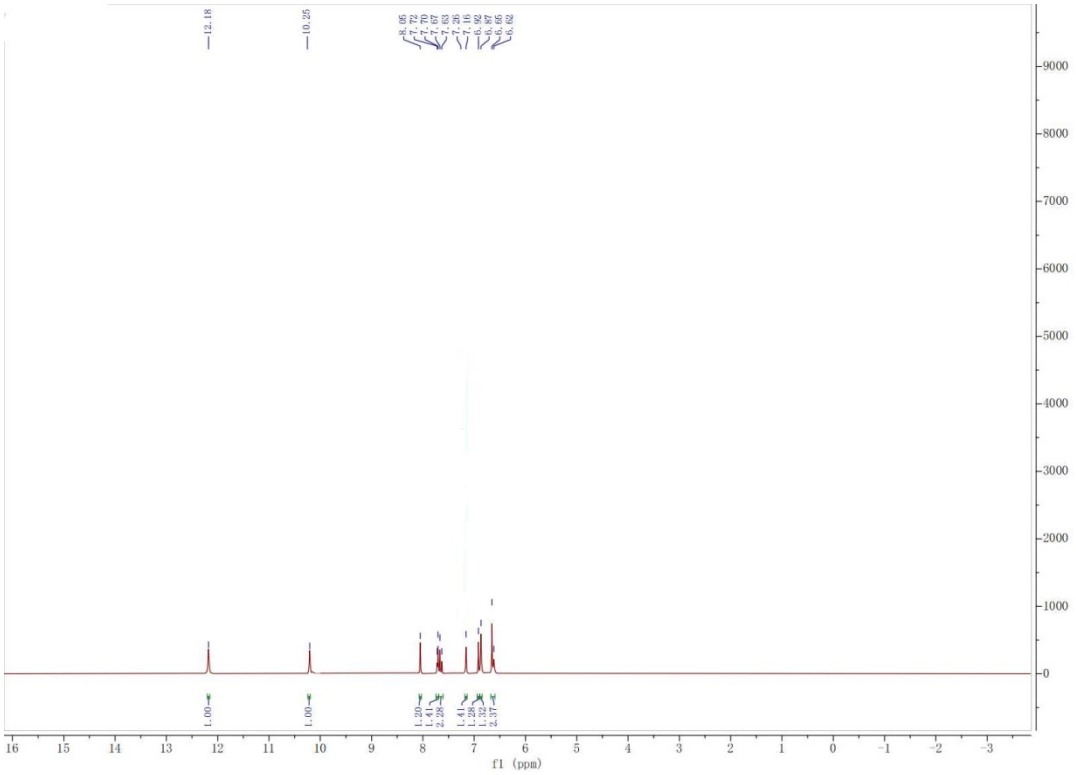


Fig. S7 ^{1}H NMR of fluorescein monoaldehyde

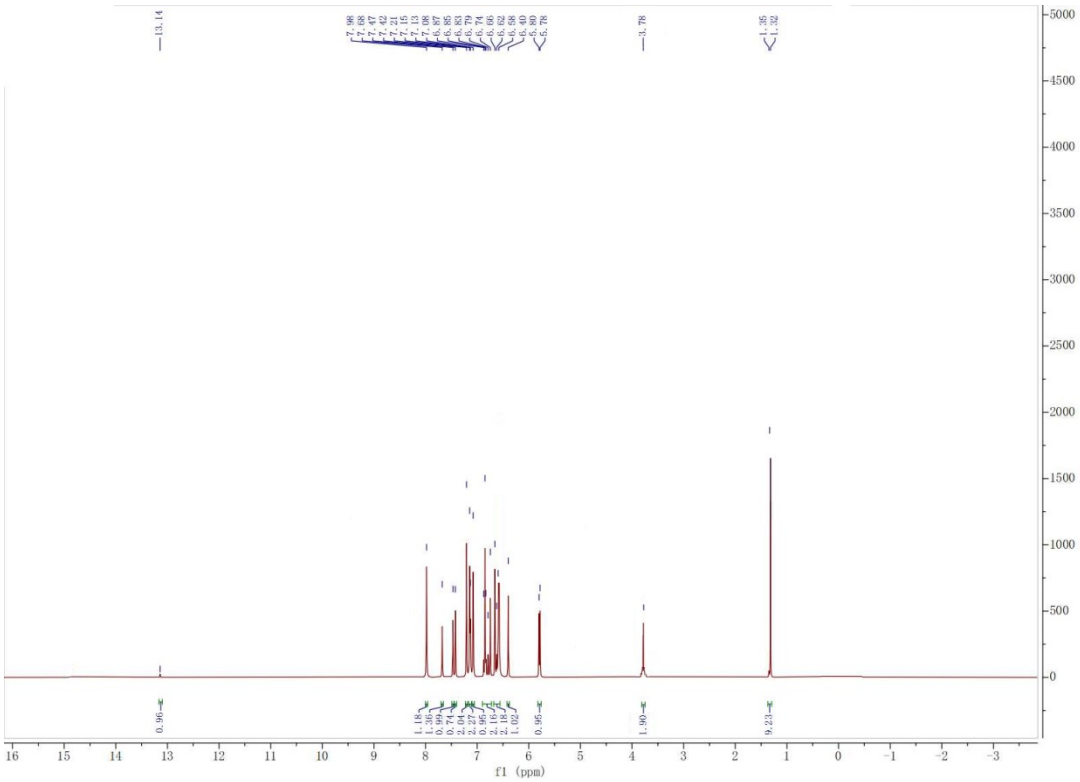


Fig. S8 ^{1}H NMR of FMC.

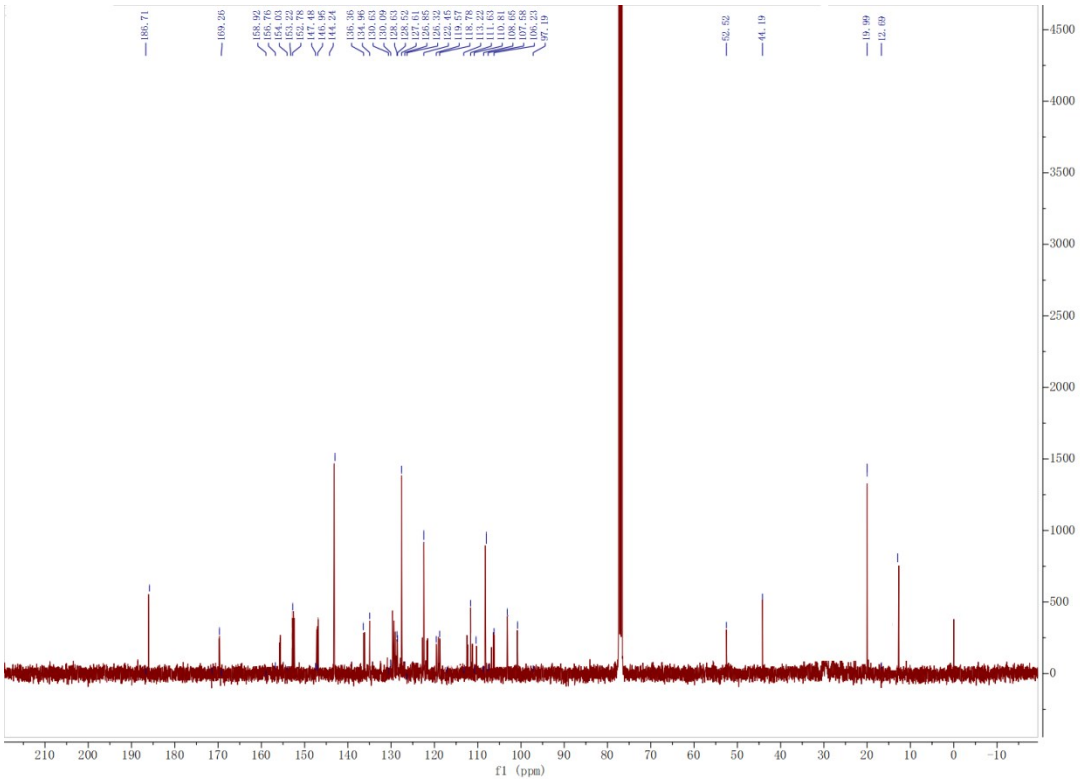


Fig. S9 ^{13}C NMR of FMC.

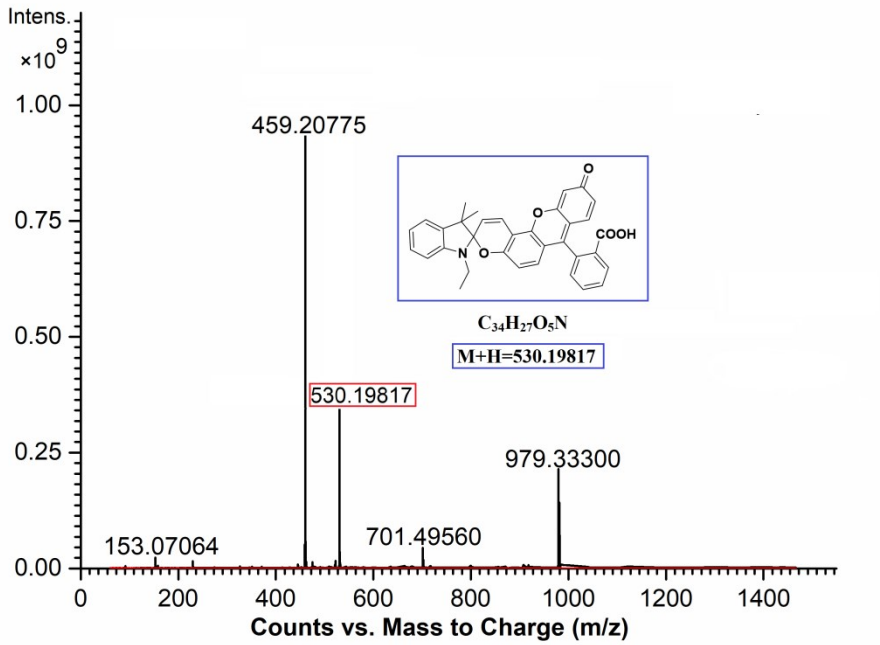


Fig. S10 Mass spectrum of FMC.

5. References

1. W. Wang, O. Rusin, X. Xu, K. K. Kim, J.O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence,F. R. Fronczek, I. M. Warner, and R. M. Strongin, *J. Am. Chem. Soc.,* 2005, **127,** 15949-15958.

2. R. F. Kubin, A. N. Fletcher, *J. Lumin.*, 1982, **27,** 455-462.