Supporting information

A H_2S-activated Ratiometric CO Photoreleaser Enabled by Excimer/Monomer Conversion

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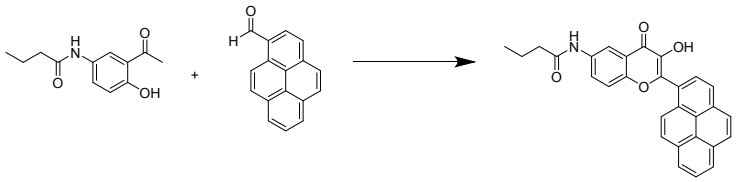
1. Materials and Instruments

All reagents for synthesis were purchased from commercial suppliers and were used without further purification unless otherwise stated. Pyrene-1-carboxaldehyde was bought from J&K Scientific Ltd. N-(3-acetyl-4-hydroxyphenyl) butyramide was purchased from Adamas Reagent, Ltd. Rat TNF-α ELISA Kit (EK0526) and Rat IL-6 ELISA Kit (EK0412) were obtained from Boster Biological Technology Co., Ltd. Endogenous carbon monoxide (CO) assay kit (A101-3) was acquired from Nanjing Jiancheng Bioengineering Institute.

PFN was dissovled in DMSO to prepare a 1 mM stock solution and the ultimate experimental content of DMSO was 1% (v/v). pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China). Absorption spectra were recorded on a UV-1700 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were obtained with a FLS-980 fluorescence spectrometer (Edinburgh Instruments Ltd., England). HPLC analysis was carried out on a Shimadzu LC-16 system equipped with SPD-16 UV-vis detector. The CO release in buffer solution and cell was irradiated with a CEL-HXF300 Xenon lamp with power 5 mW/cm^{2}at 360 nm. ^{1}H NMR and ^{13}C NMR spectra were taken on a 400 MHz spectrometer (Bruker Co., Ltd., Germany), δ values are in *ppm* relative to TMS. HRMS spectra were obtained on a maxis ultra-high resolution-TOF MS system (Bruker Co., Ltd., Germany). MTT assay was performed using a TRITURUS microplate reader. The confocal fluorescence images of cells were gained on a TCS SP8 Confocal Laser Scanning Microscope (Leica Co., Ltd., Germany).

2. Synthetic procedures and characterization details

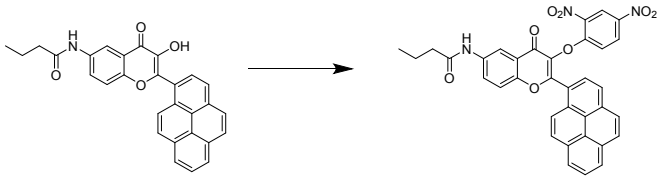
Synthesis of Compound PF



PF

The mixture of N-(3-acetyl-4-hydroxyphenyl)butyramide (0.211 g, 1.0 mM) and pyrene-1-carboxaldehyde (0.23 g, 1.0 mM) in 10 mL of EtOH was cooled to 0 °C and then added 5 mL of sodium hydroxide aqueous solution (20%). After that, the reaction solution was heated to 40 °C for 5 h. Then hydrogen peroxide (5.0 ml, 30%) was added dropwise while on ice. The mixture was stirred for another 12 h at room temperature followed by acidified to pH=6.5 with 0.5 M HCl. Finally, the suspension was filtered and resulting residue was purified by silica chromatography eluted with CH_2Cl_2/ CH_3OH (50:1, v/v) to afford compound **PF** as a yellow solid (0.30 g, 67% yield). ^{1}H NMR (400 MHz, DMSO-$_{d6}$) δ 10.26 (s, 1H), 9.28 (s, 1H), 8.62 (s, 1H), 8.46 – 8.25 (m, 7H), 8.15 (t, J = 7.6 Hz, 1H), 8.09 (d, J = 9.2 Hz, 1H), 7.94 (dd, J = 8, 4 Hz, 1H), 7.68 (d, J = 9.2 Hz, 1H), 2.38 (t, J = 8 Hz, 2H), 1.68 (sext, J = 8 Hz, 2H), 0.97 (t, J = 8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-$_{d6}$) δ 173.20, 171.91, 151.87, 148.09, 139.83, 136.54, 132.45, 131.13, 130.66, 129.15, 128.92, 128.82, 128.38, 127.72, 127.13, 126.54, 126.37, 126.09, 125.98, 125.44, 125.02, 124.21, 124.03, 122.76, 119.52, 113.43, 38.81, 19.01, 14.14. HRMS(ESI) (m/z): calculated for $C_{29}H_{21}NO_4$ $[M+H]^+$ 448.1543, found 448.1609; $[M+Na]^+$ 470.1362, found 470.1432.

Synthesis of Compound PFN



PF **PFN**

The mixture of compound **PF** (0.09 g. 0.20 mM) and anhydrous K_2CO_3 (0.08 g, 0.58 mM) in 3.0 mL of DMF was stirred at room temperature. After 10 min, 2,4-

dinitrofluorobenzene (0.05 g, 0.25 mM) was added, and the resulting mixture was stirred for another 2 h. The residue was purified by silica chromatography eluted with CH_2Cl_2/ CH_3OH (100:1, v/v) to afford compound **PFN** as an orange solid (0.10 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-$_{d6}$)) δ 10.37 (s, 1H), 8.56 (dd, *J* = 21.2, 2.5 Hz, 2H), 8.46 – 8.21 (m, 9H), 8.17 (t, *J* = 7.6 Hz, 1H), 8.10 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.85 (t, *J* = 8.4 Hz, 2H), 2.38 (t, *J* = 7.3 Hz, 2H), 1.67 (sext, *J* = 8 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-$_{d6}$) δ 172.08, 171.57, 159.22, 154.08, 152.36, 141.69, 138.20, 137.57, 136.05, 133.19, 131.02, 130.59, 129.81, 129.55, 129.33, 129.22, 127.72, 127.57, 127.37, 126.92, 126.79, 126.66, 124.99, 124.73, 123.98, 123.75, 123.38, 121.88, 119.91, 118.23, 113.64, 38.80, 18.96, 14.05. HRMS(ESI) (m/z): calculated for $C_{35}H_{23}N_3O_8$ $[M+H]^+$ 614.1557, found 614.1559; $[M+Na]^+$ 636.1377, 636.1375.

3. MTT assay

The MTT assays were carried out to test the cytotoxicity of **PFN** and **PF**. The IEC-6 cells were replanted in the 96-well micro plates to a total volume of 200 μL well^{-1}. Then, the plates were maintained at 37°C, 5% CO_2/95% air incubator for 24 hours. The cells were then treated with different concentrations **PFN** and **PF** (0, 10, 25, 50 and 100 μM) for another 12 hours. Subsequently, the culture was removed and MTT solution (5.0 mg ml-1) was added to each well. After 4 hours, the remaining MTT solution was removed and 50 μL DMSO was added into each well to dissolve the formazan crystals. The absorbance of solution was measured at 490 nm with 10 min gentle agitation using a TRITURUS microplate reader. According to the method of Huber and Koella, the IC_{50} values of **PFN** and **PF** were calculated to be 92.47 μM and 105.26 μM, respectively. To assess the cytotoxicity of photolysis products of **P**, the MTT assay was performed upon irradiation on **PF** for different periods of time (0, 3, 6, 9, 12 min) utilizing a CEL-HXF300 Xenon lamp with power 5 mW/cm^2 at 360 nm.

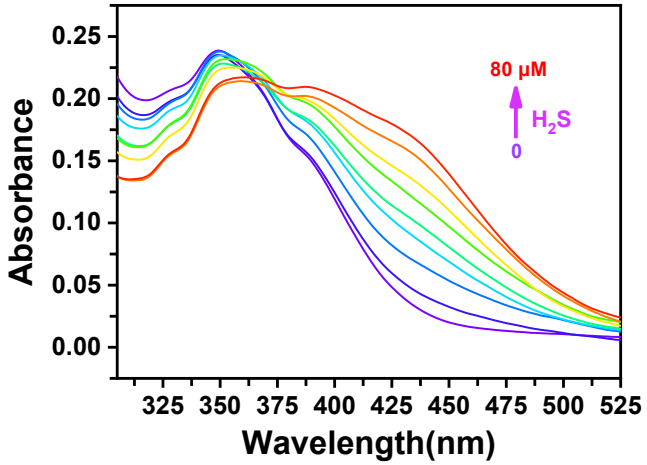


Fig. S1 Absorption spectra changes of **PFN** (10.0 μM) with increased concentration (0–80 μM) of H_2S in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB).

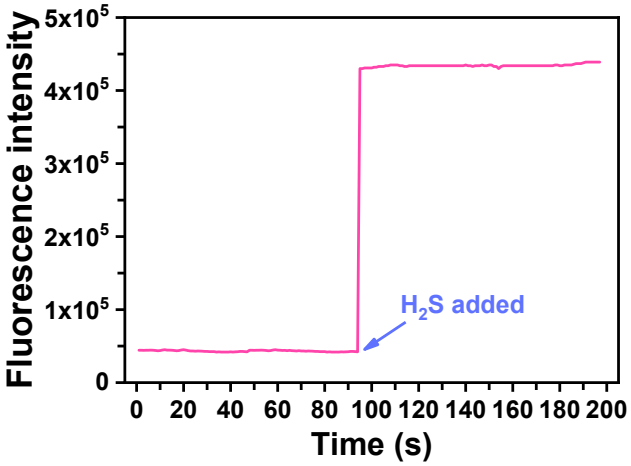


Fig. S2 Time-dependent fluorescence changes of **PFN** (10 μM) toward H_2S (25 μM) in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB) at 37 °C. $\lambda_{ex}/\lambda_{em}$ = 360 nm /490 nm. Slit width: 5 nm /5 nm.

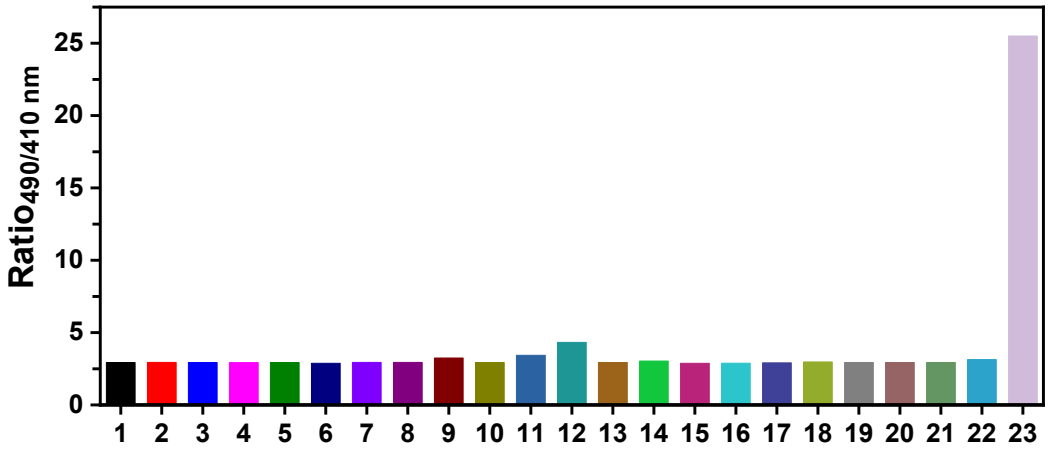


Fig. S3 The specificity of **PFN** (10 μM) towards various bioanalytes: 1. Blank; 2. H_2O_2; 3. ClO^-; 4. $^1O_2$; 5. OH; 6. $O_2^{\bullet-}$; 7. NO; 8. $ONOO^-$; 9. SO_3^{2-}; 10. Hcy; 11. Cys; 12. GSH; 13. Vc; 14. Fe^{2+}; 15. Ca^{2+}; 16. Cu^{2+}; 17. F^-; 18. Cl^-; 19. CO_3^{2-}; 20. NO_3^-; 21. HSO_3^-; 22. SO_4^{2+}; 23. H_2S (40 μM). The concentrations of GSH, Hcy, and Cys were 1.0 mM, and the other substances were 100 μM unless otherwise stated. The results were obtained in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB). λ_{ex} =360 nm. Slit width: 5 nm /5 nm.

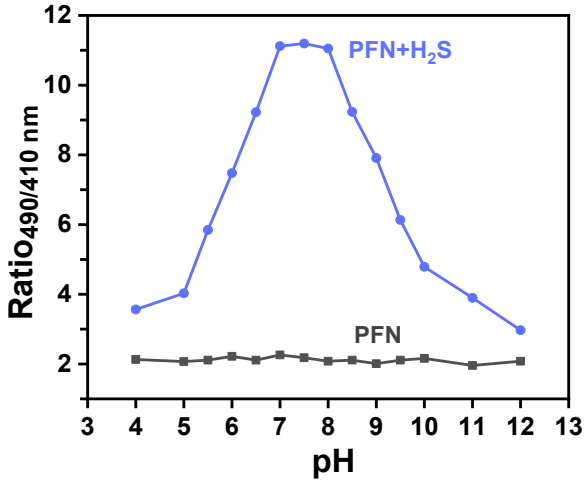


Fig. S4 The fluorescence ratiometric changes of **PFN** (10 μM) as the H_2S (15 μM) addition under different pH values (4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, 9.0, 9.5, 10, 11, 12) by using universal buffer solution (0.1 mM citric acid, 0.1 M KH_2PO_4,0.1 M $Na_2B_4O_7$, 0.1 M Tris, 0.1 M KCl, containing 300 μM CTAB). λ_{ex} = 360 nm. Slit width: 5 nm /5 nm.

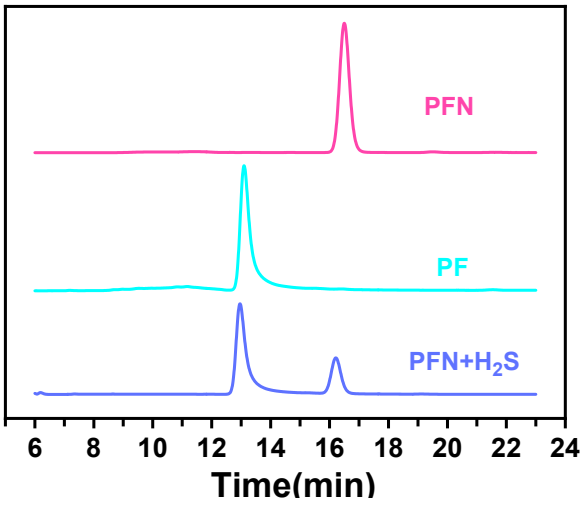


Fig. S5 HPLC chromatograms of **PFN**, **PF**, and **PFN** incubated with 10 equiv of H_2S. Chromatographic separation was performed on a ZORBAX Eclipse Plus C18 reversed-phase column (4.6 × 250 mm, 5 μm). The mobile phase was a mixture of water and methanol (10/90, v/v) delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 360 nm.

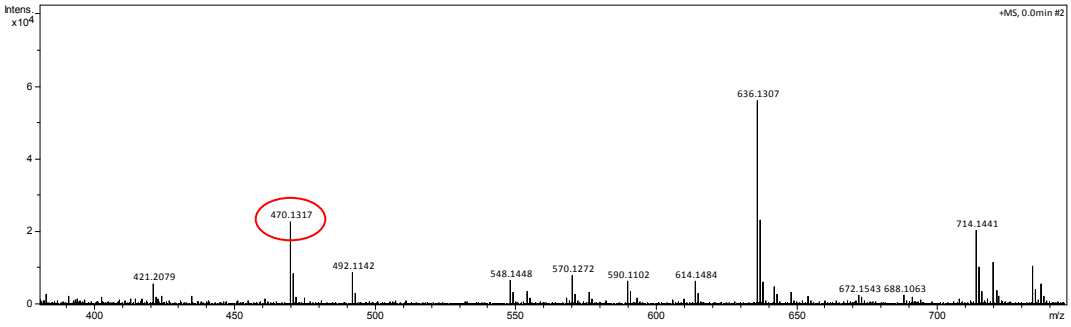


Fig. S6 HRMS spectral analysis of reaction between **PFN** and H_2S. The peak appeared at m/z 470.1317 $[M + Na]^+$ was corresponding to the reaction product **PF**.

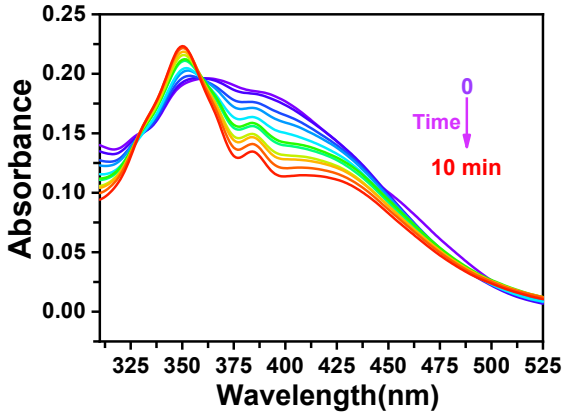


Fig. S7 Absorption spectra changes of **PFN** (10 μM) treated with H_2S (100 μM) during irradiation (λ = 365 nm, CEL-HXF300 Xenon lamp with power 5 mW/cm^2). The data was recorded every 1.0 min in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB).

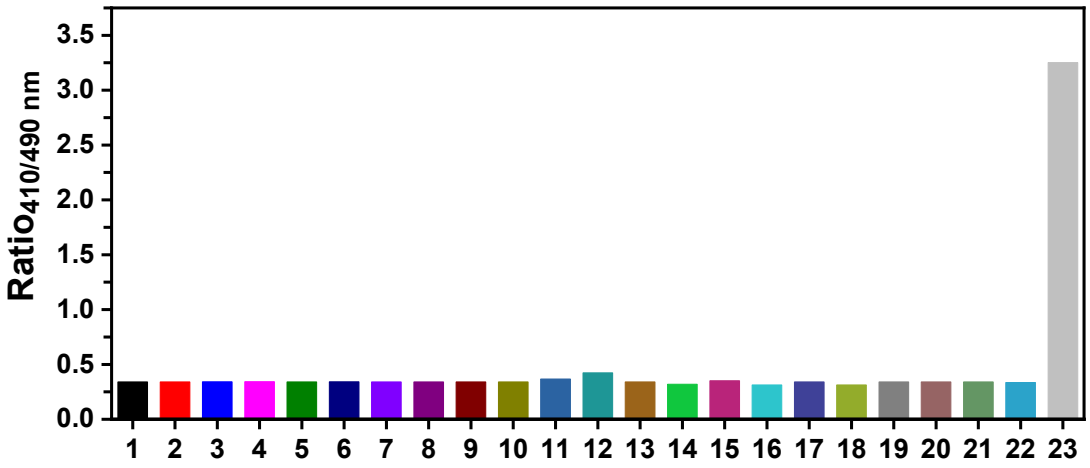


Fig. S8 The fluorescent ratio changes of **PFN** (10 μM) treated with various biological substances after 10 min irradiation (λ = 365 nm, CEL-HXF300 Xenon lamp with power 5 mW/cm^2): 1, Blank; 2, H_2O_2; 3, ClO^-; 4, $^1O_2$; 5, OH; 6, $O_2^{\bullet-}$; 7, ON; 8, $ONOO^-$; 9, SO_3^{2-}; 10,Hcy; 11, Cys; 12, GSH; 13, Vc; 14, Fe^{2+}; 15, Ca^{2+}; 16, Cu^{2+}; 17, F^-; 18, Cl^-; 19, CO_3^{2-}; 20, NO_3^-; 21, HSO_3^-; 22.SO_4^{2+}; 23, H_2S. The concentrations of GSH, Hcy, and Cys were 1.0 mM, and the other substances were 100 μM. The results were obtained in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB). λ_{ex} =360 nm. Slit width: 5 nm /5 nm.

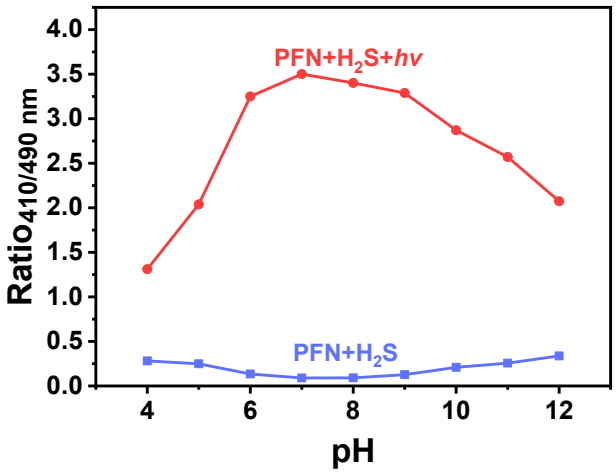


Fig. S9 The photoinduced fluorescence ratio changes of **PFN** (10 μM) with H_2S (100 μM) under different pH values (4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, 9.0, 9.5, 10, 11) by using universal buffer solution (0.1 mM citric acid, 0.1 M KH_2PO_4,0.1 M $Na_2B_4O_7$, 0.1 M Tris, 0.1 M KCl, 0.3 M CTAB). The results were collected after 10 min irradiation (λ = 365 nm, CEL-HXF300 Xenon lamp with power 5 mW/cm^2). λ_{ex} = 360 nm. Slit width: 5 nm /5 nm.

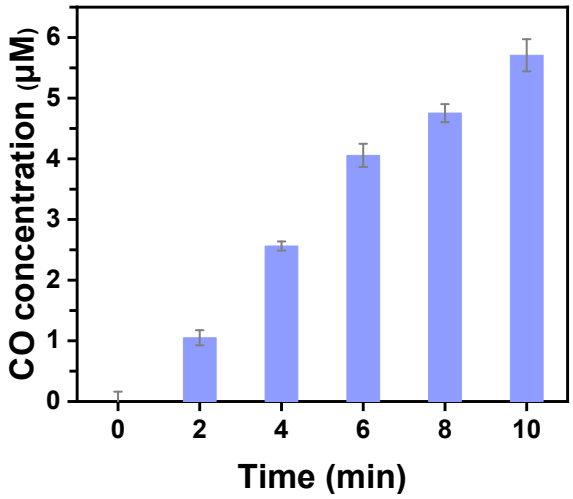


Fig. S10 The H_2S-mediated photoinduced releasing during irradiation (λ = 365 nm, CEL-HXF300 Xenon lamp with power 5 mW/cm^2). The solution of **PFN** (10 μM) with H_2S (100 μM) in PBS buffer (50 mM, pH 7.4, containing 300 μM CTAB) was irradiated for different perods of time followed by analyed with CO ELISA assay kit.

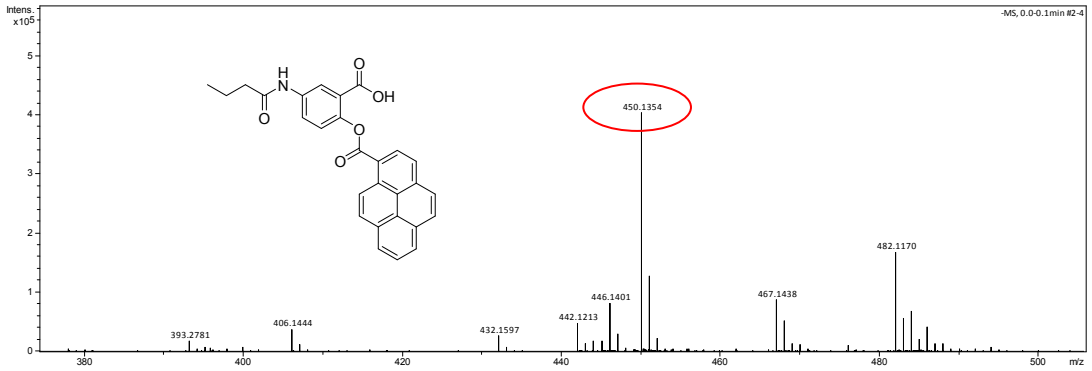


Fig. S11 HRMS spectral analysis of the photolysis of **PF**. The probe **PFN** (10 μM) was incubated with H_2S (100 μM) and then irradiated with 365 nm light (CEL-HXF300 Xenon lamp with power 5 mW/cm^2) for 10 min. After that, the mixture was characterized by HRMS spectrometry.

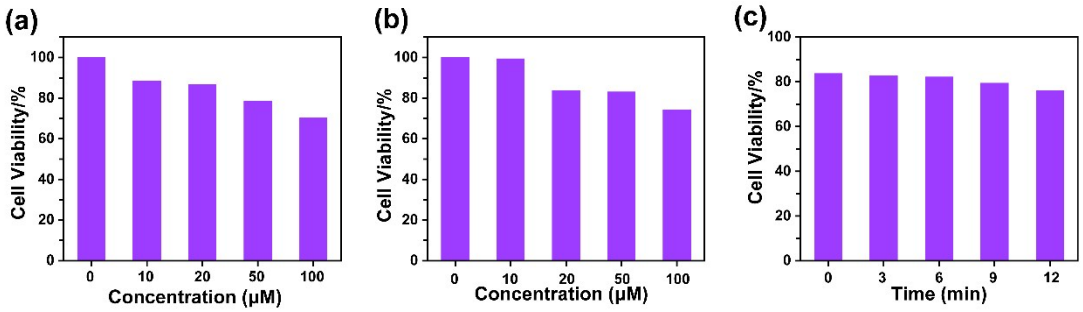


Fig. S12 MTT assays of **PFN** (a), **PF** (b) and **PF** (100 μM) under irradiation for different time periods (c) (CEL-HXF300 Xenon lamp with power 5 mW/cm^2).

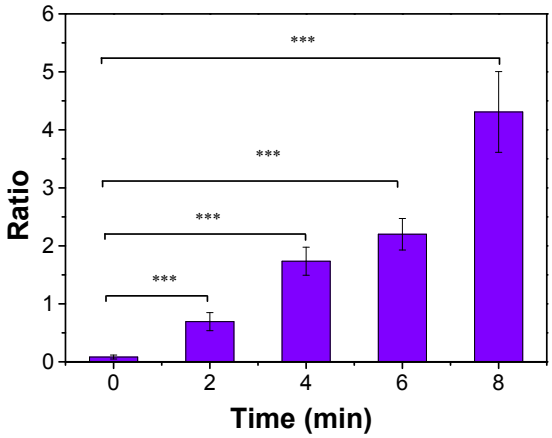


Fig. S13 The ratiometric changes of 10 μM **PFN** in 1.0 μg/mL LPS-loaded IEC-6 cells during irradiation (λ = 365 nm, CEL-HXF300 Xenon lamp with power 5 mW/cm^2). The ratio readout was derived from Fig. 5. The values are the mean±s.d. for n=3, ***p<0.001.

NMR spectra

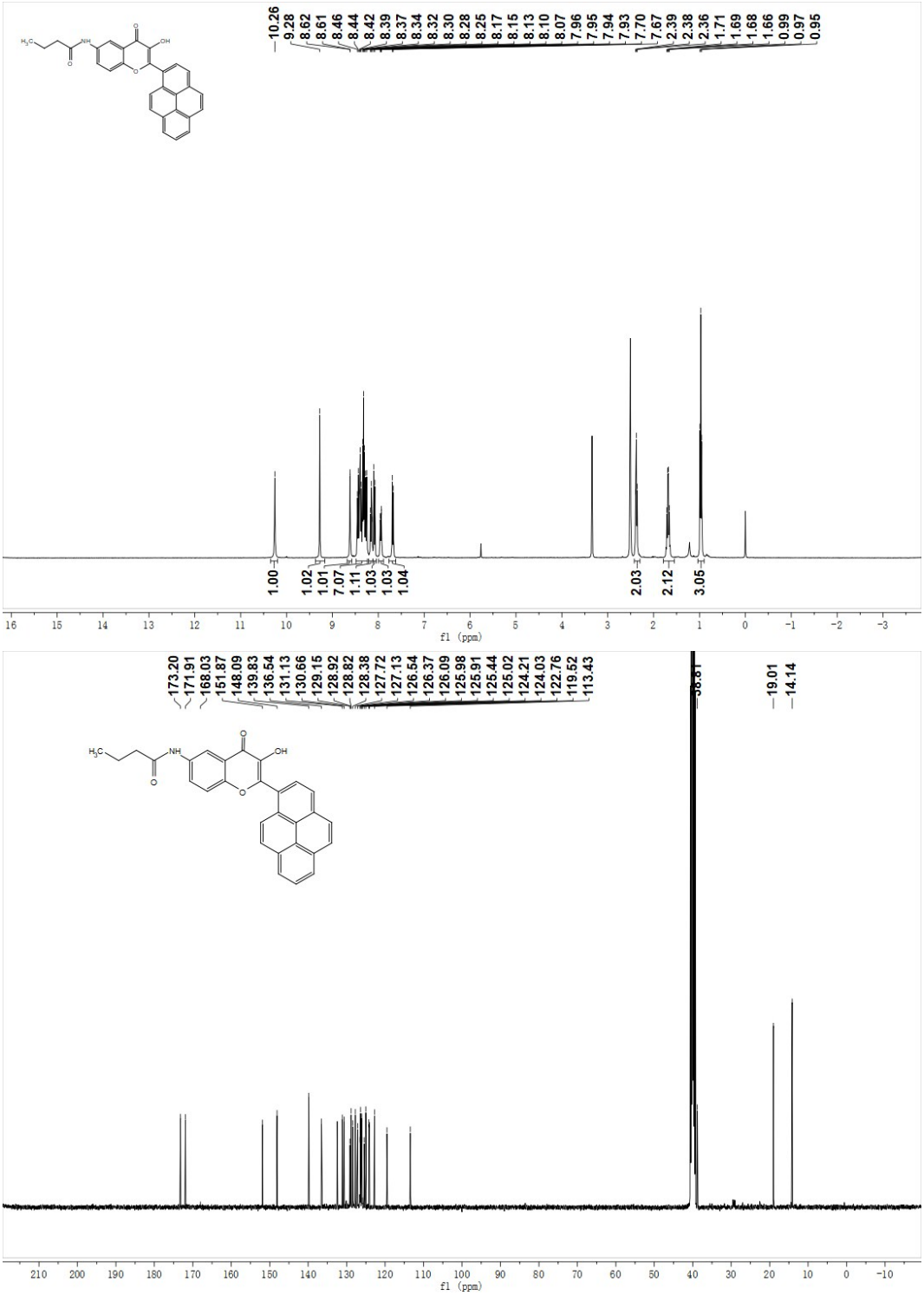


Fig. S14 ^{1}H and ^{13}C NMR spectra of compound **PF** (DMSO-d_6).

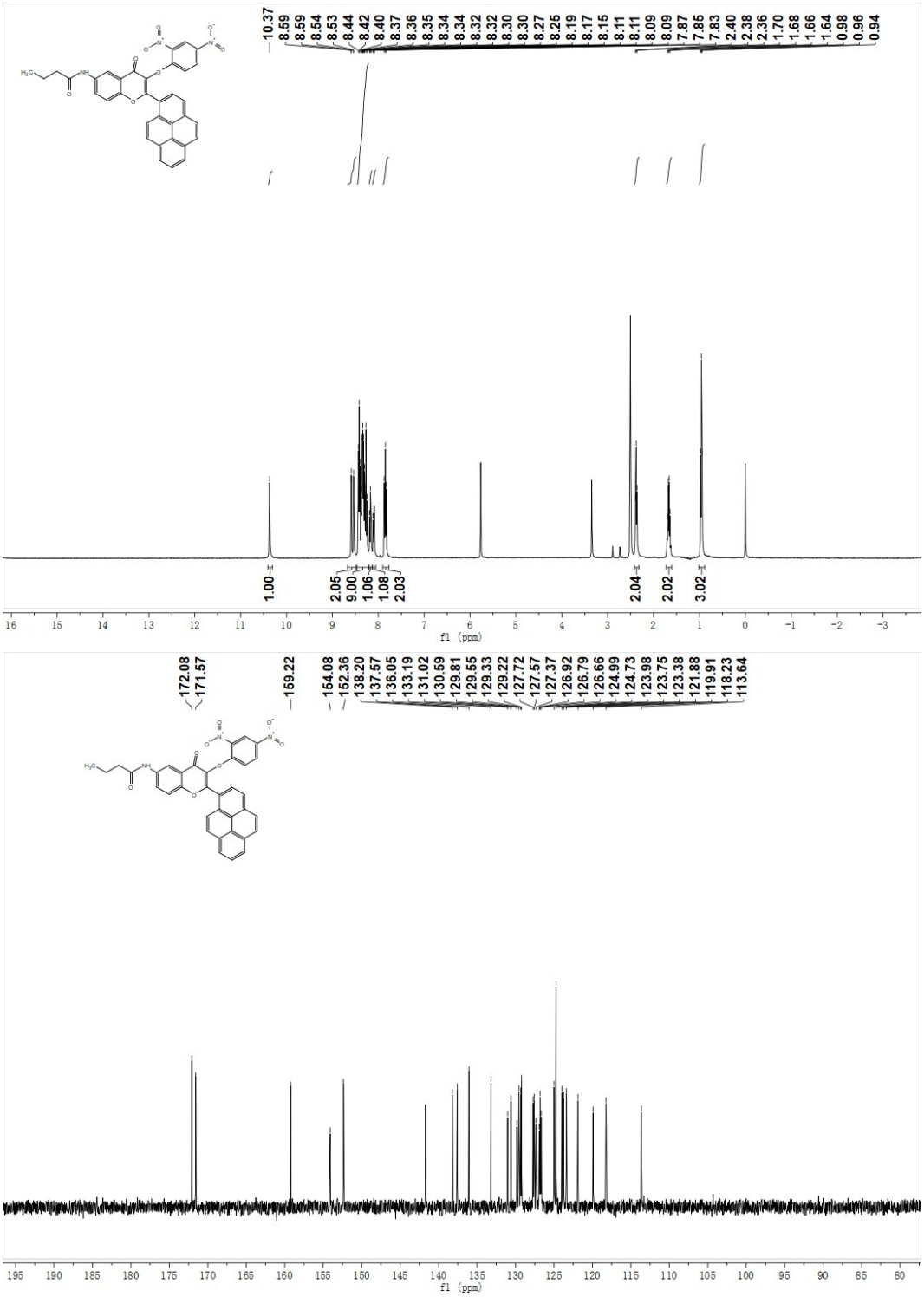


Fig. S15 ^{1}H and ^{13}C NMR spectra of compound **PFN** (DMSO-d_6).