Supporting Information

A New ESIPT-Based Fluorescent Probe for Highly Selective and Sensitive Detection of Hydrogen Sulfide and Its Application in Live-Cell Imaging

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Photophysical properties of PHS1

Table S1 Photophysical properties of the probe.

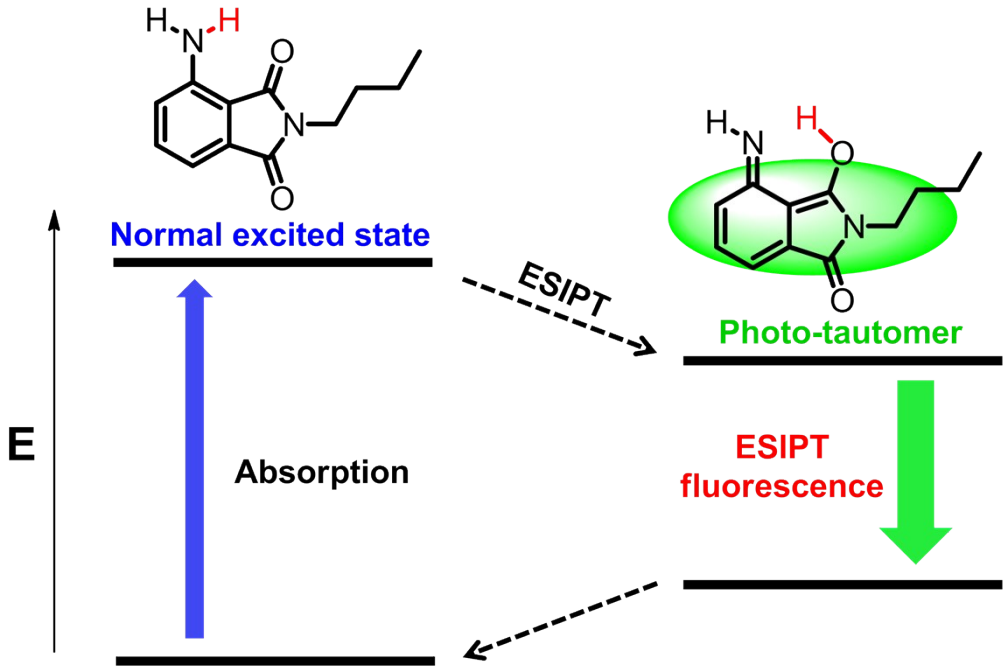
entry	$\lambda_{em\ (nm)}$	Φ^a	ε / M^{-1} cm^{-1}
PHS1	483	0.009	3277
PHS1+H_2S	483	0.104[b]	4014

(a) The quantum yield (Φ) of **PHS1** and **PHS1**-H_2S system were determined according to the literature.[1] (b) Φ was determined in the present of 2.0 equiv. of H_2S.

$$\Phi_{Sample} = \frac{\Phi_{QS} \cdot A_{QS} \cdot F_{Sample} \cdot \lambda_{exQS} \cdot \eta^2_{Sample}}{A_{Sample} \cdot F_{QS} \cdot \lambda_{exSample} \cdot \eta^2_{QS}}$$

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose fluorescein in 0.1 M NaOH as standard, which has the quantum yield of 0.95.[2]

Additional spectroscopic data



Scheme S1 ESIPT process of 3-aminophthalimide (**3**).

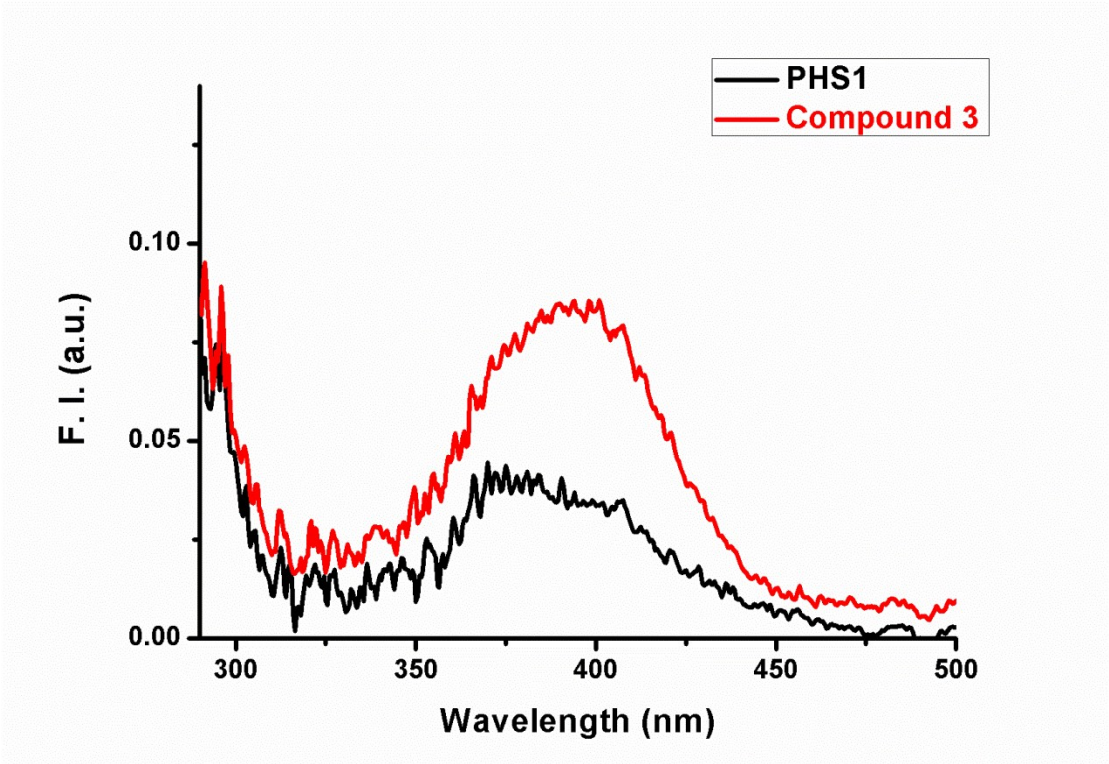


Fig. S1 The UV-vis absorption (unsmoothed curves) of **PHS1** (10.0 μM) and compound **3** (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

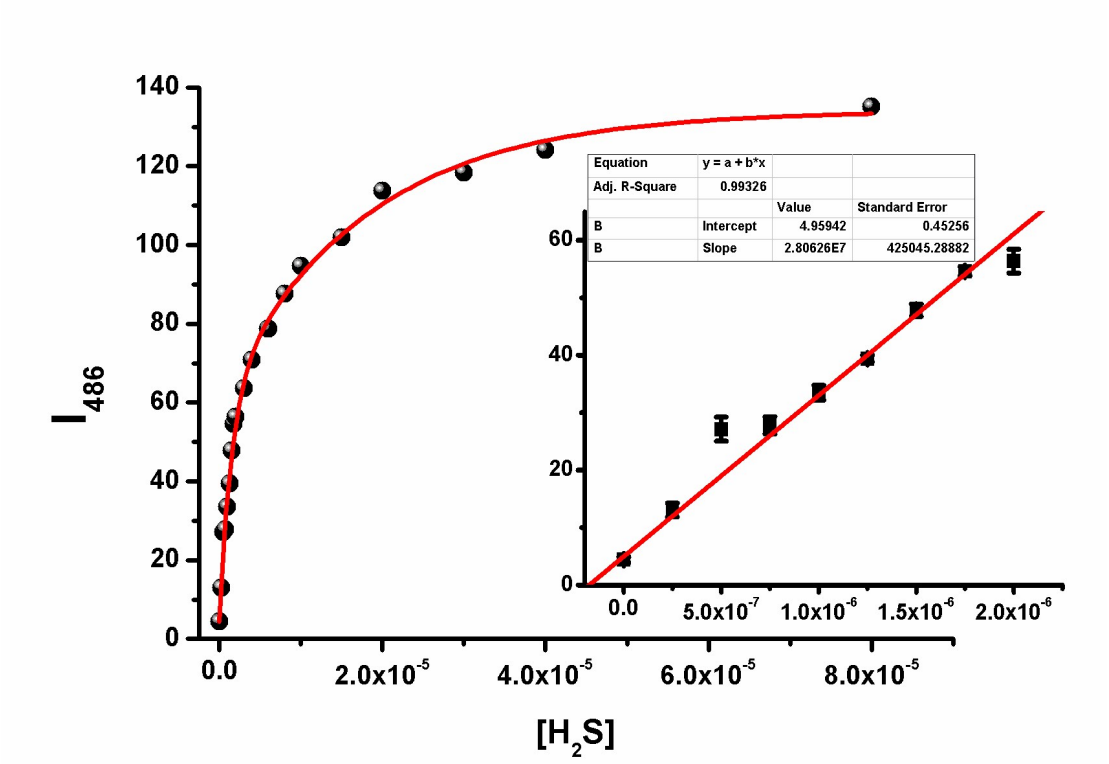
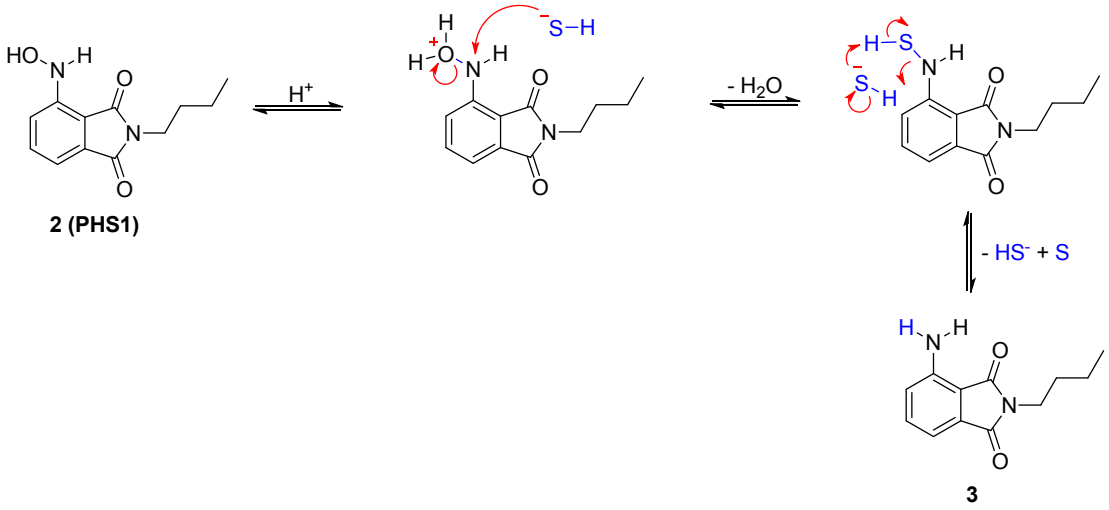


Fig. S2 Fluorescence intensity of **PHS1** (10.0 μM) at 486 nm as a function of H_2S concentration (0-80.0 μM) in PBS buffer (10.0 mM, pH 7.4, containing 50% EtOH). Inset: fluorescence intensity of **PHS1** (10.0 μM) at 486 nm as a function of H_2S concentration (0-2.0 μM) in PBS buffer (10.0 mM, pH 7.4, containing 50% EtOH). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

The detection limit (DL) of H_2S using **PHS1** was determined from the following equation: [3]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.



Scheme S2 The proposed mechanism of **PHS1**-H_2S interactions.

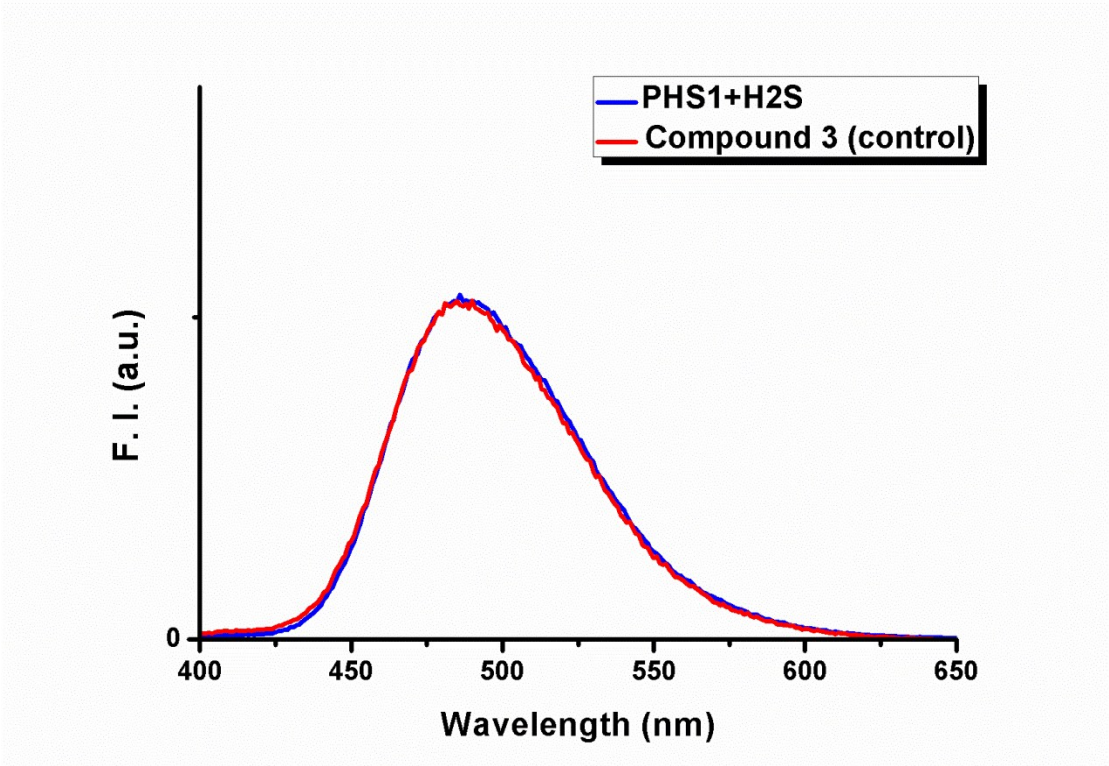


Fig. S3 The comparison of fluorescence spectra of the probe-H_2S mixture solution (**PHS1**-Na_2S mixture solution) and control (compound **3**) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 393 nm).

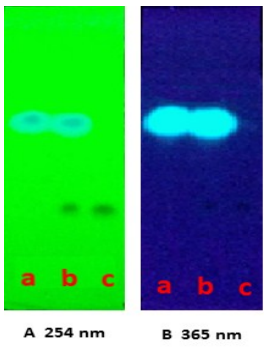


Fig. S4 Comparison of the TLC analysis of **PHS1**, **PHS1**-Na_2S system, and compound **3** (control).

The pictures of the thin layer chromatography TLC plates under different light used to compare probe **PHS1**, the reference sample of compound **3** and the reaction mixture of probe **PHS1** with Na_2S in 1:1 PBS-EtOH (v/v). (A) Under light of 254 nm, and (B) under light of 365 nm. Spots on the TLC plate are: (a) compound **3**, (b) the reaction mixture of probe **PHS1** and Na_2S, (c) probe **PHS1**. The eluent for TLC: hexane:EtOAc = 3:1 (v/v). This indicates that the reaction of probe **PHS1** with Na_2S produced compound **3**.

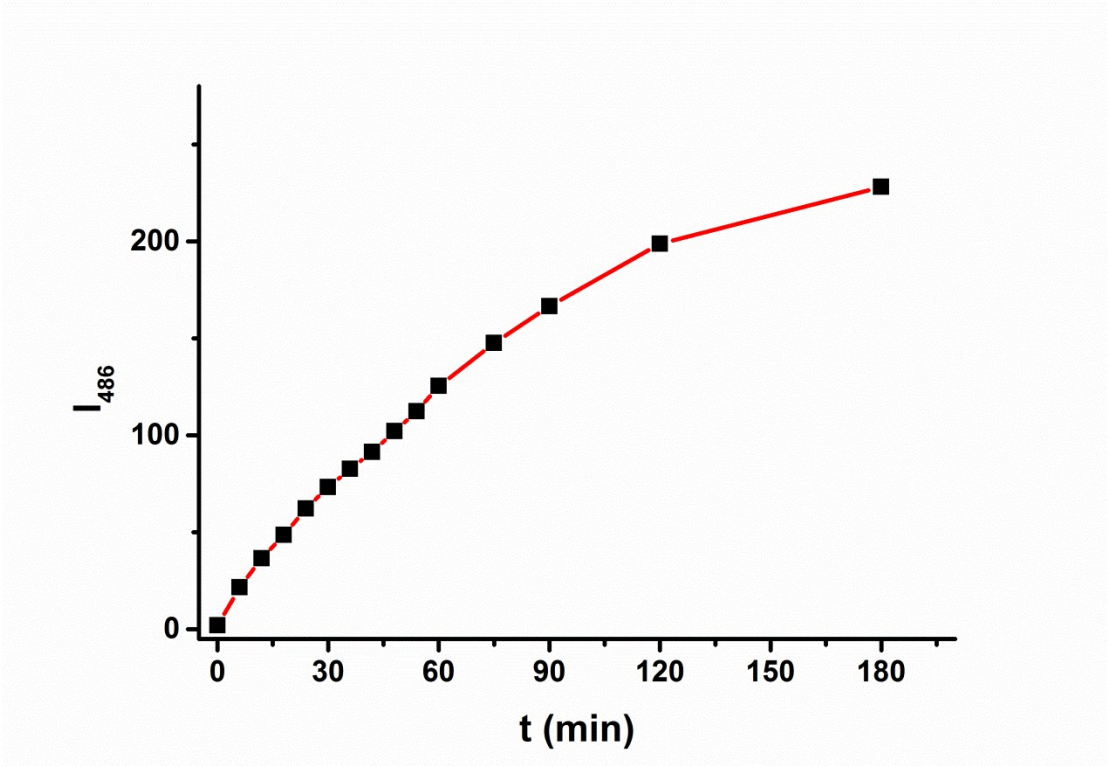


Fig. S5 Kinetics of **PHS1** (10.0 μM) in the presence of 2.0 equiv. of H_2S in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 393 nm).

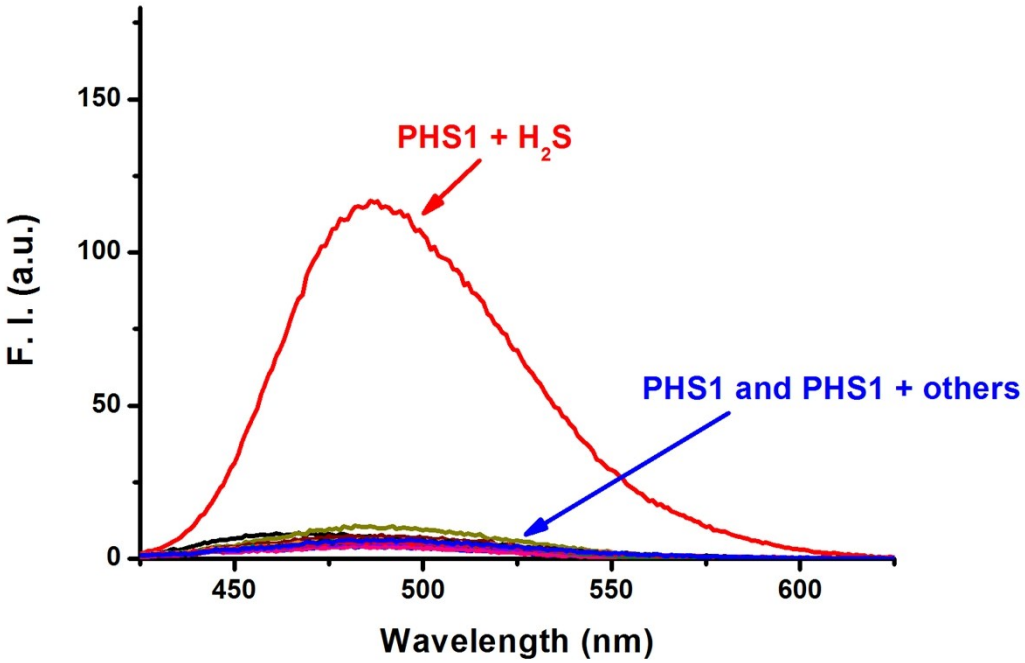


Fig. S6 Fluorescence responses of **PHS1** (10.0 μM) to various reactive sulfur species and coexisting ions (H_2S at 20.0 μM, GSH at 1.0 mM, and Cys, HSO_3^-, $S_2O_4^{2-}$, $S_2O_3^{2-}$, SO_3^{2-}, ClO^-, I^-, Fe^{3+}, F^-, Cl^-, Br^-, $H_2PO_4^-$, NO_3^- and CO_3^{2-} at 100.0 μM, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λex = 393 nm). (Data were collected after incubation of **PHS1** with each analytes for 1 h).

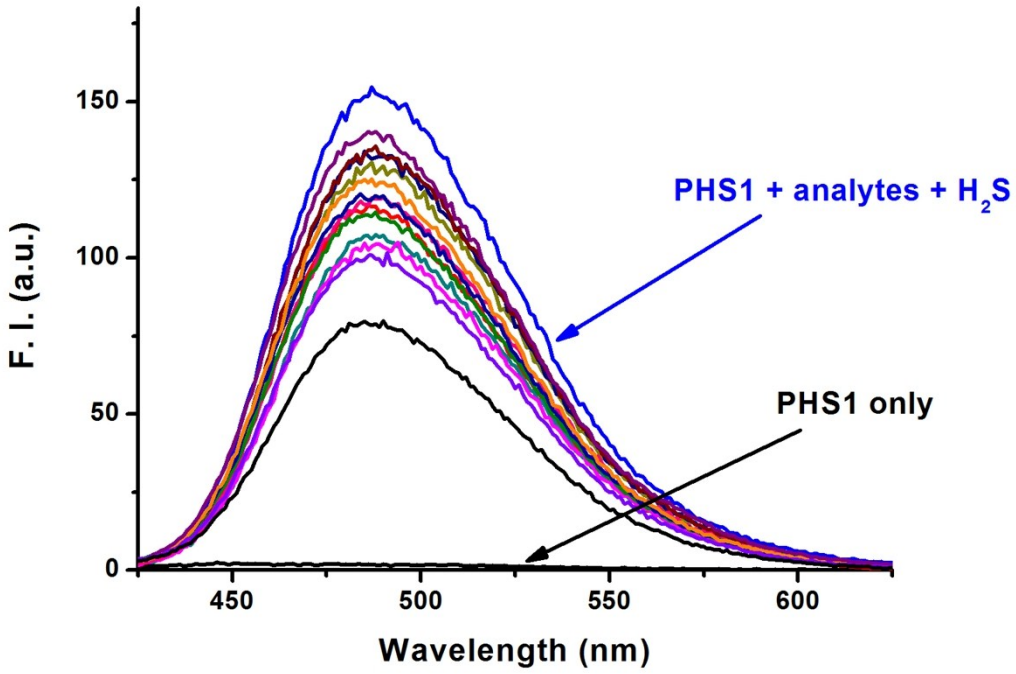


Fig. S7 Fluorescence responses of **PHS1** (10.0 μM) to H_2S (20.0 μM) in the presence of various reactive sulfur species and coexisting ions (GSH at 1.0 mM, and Cys, HSO_3^-, $S_2O_4^{2-}$, $S_2O_3^{2-}$, SO_3^{2-}, ClO^-, I^-, Fe^{3+}, F^-, Cl^-, Br^-, $H_2PO_4^-$, NO_3^- and CO_3^{2-} at 100.0 μM, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λex = 393 nm). (Data were collected after incubation of **PHS1** with each analytes for 1 h).

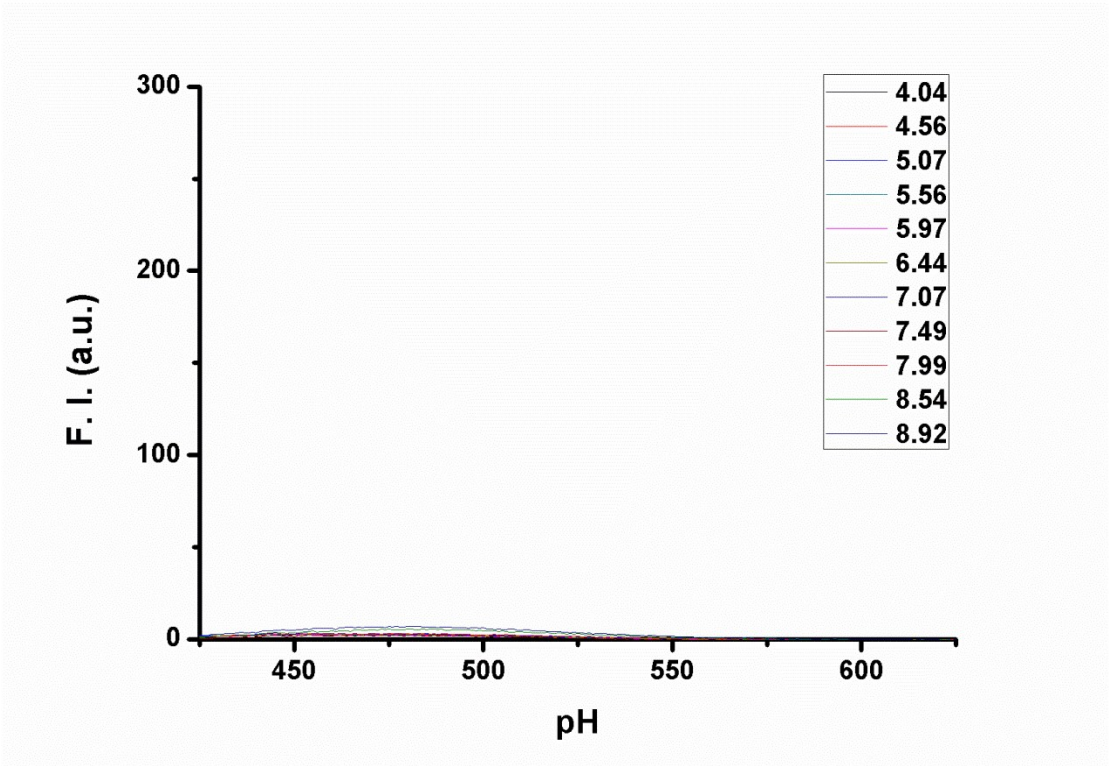


Fig. S8 Effect of the pH on the fluorescence emission of **PHS1** (10.0 μM) in buffer solution (λex = 393 nm). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

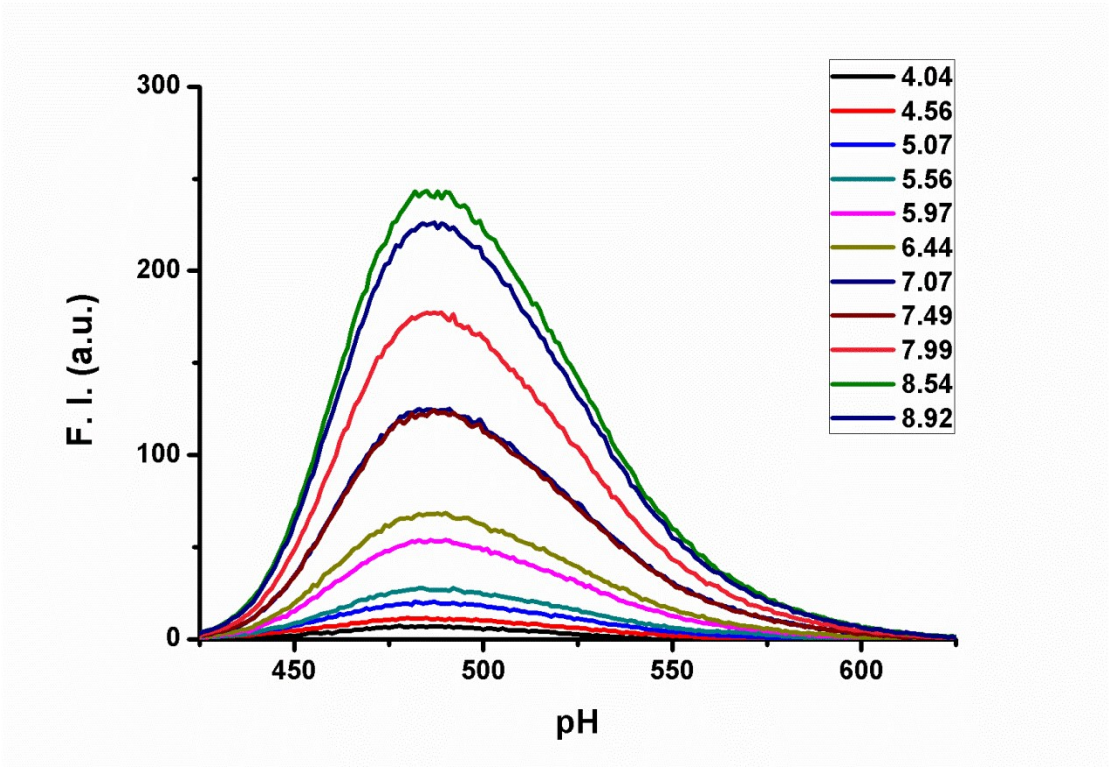


Fig. S9 Effect of the pH on the fluorescence emission of **PHS1**-H_2S system (10.0 μM of **PHS1** and 2.0 equiv. of H_2S) in buffer solution (λex = 393 nm). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

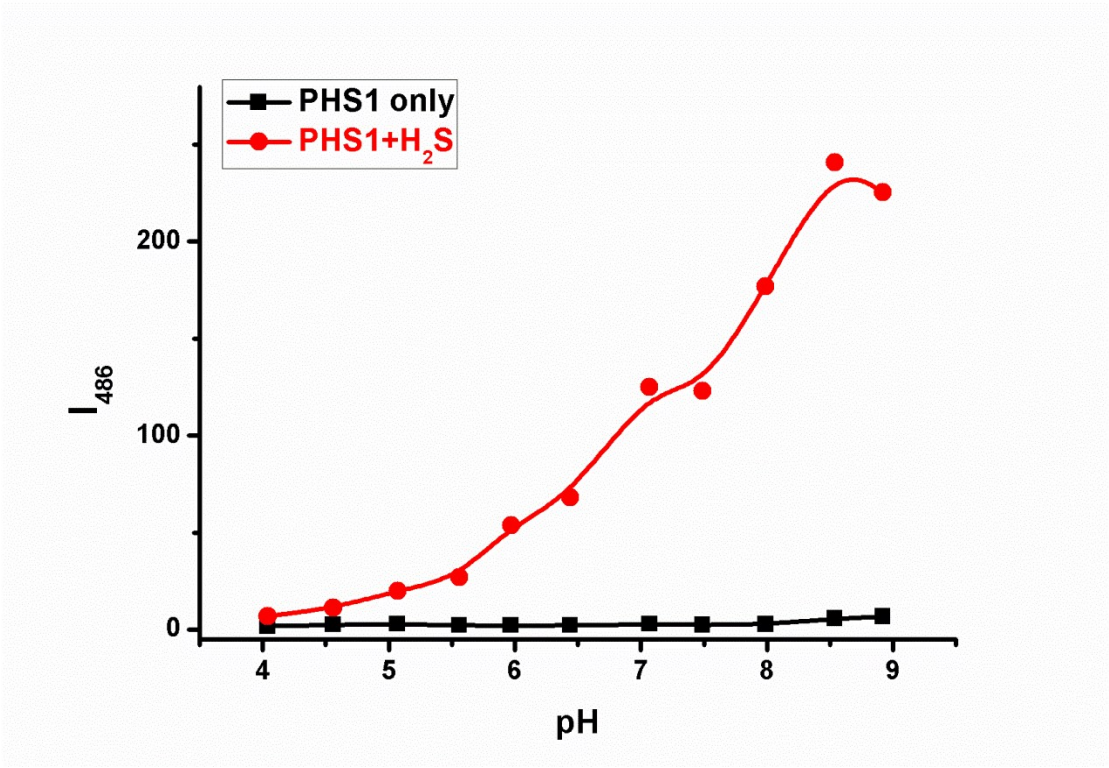


Fig. S10 Effect of the pH on the fluorescence emission of **PHS1** (10.0 μM) and **PHS1**-H_2S system (10.0 μM of **PHS1** and 2.0 equiv. of H_2S) in buffer solution (λex = 393 nm). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

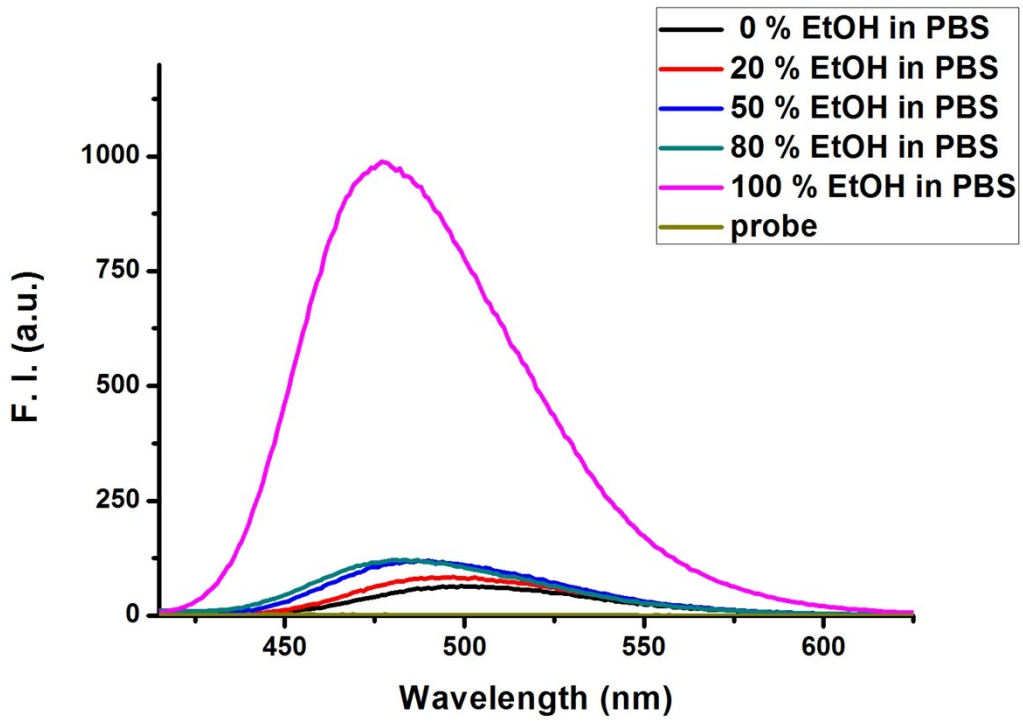


Fig. S11 Effect of different contents of EtOH in PBS solution on the fluorescence emission of **PHS1** (10.0 μM) in the presence of 2.0 equiv. of H_2S. (λex = 393 nm). (Data were collected after incubation of **PHS1** with H_2S for 1 h).

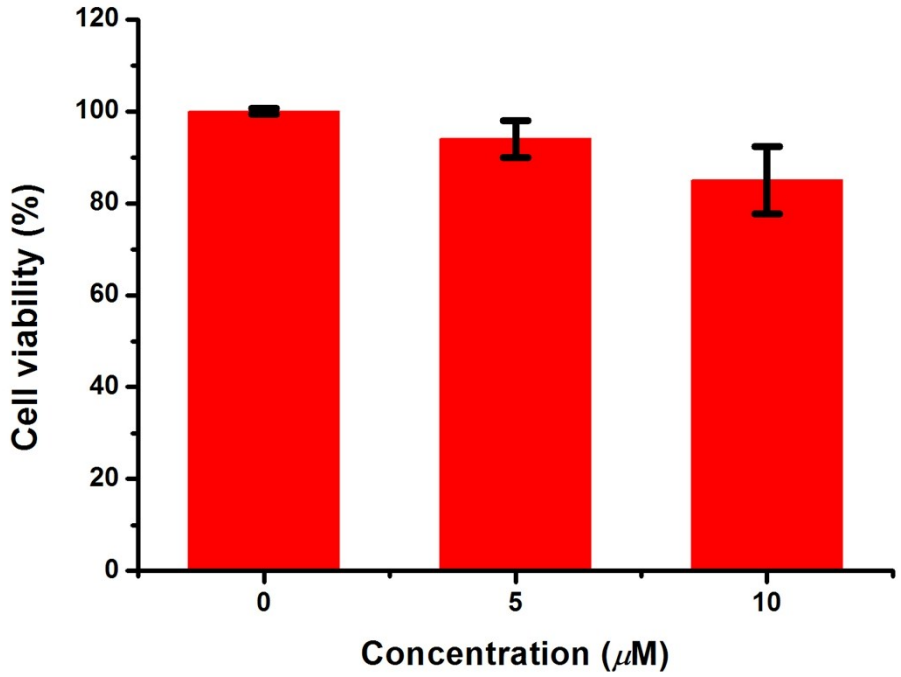
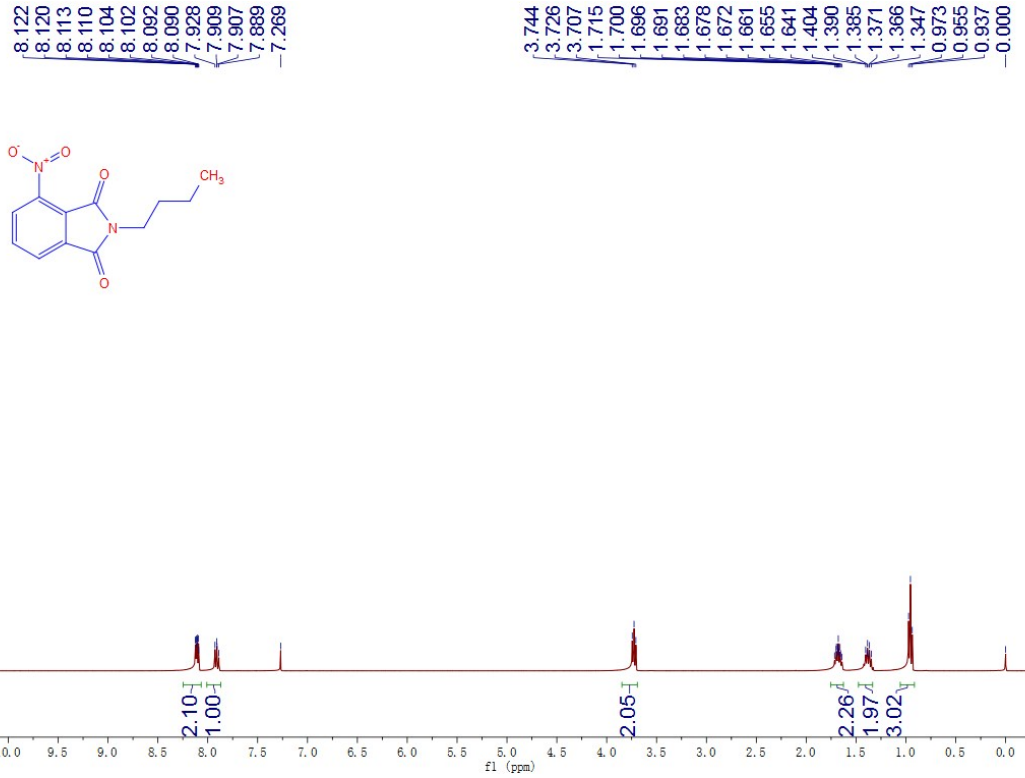


Fig. S12 Cell viability of HeLa cells treated with different concentration of **PHS1** for different time periods. No cytotoxic effect was observed for the cells incubated with **PHS1** at 10 μM even for 24 h.

The characterization data of PHS1

^{1}H NMR of **1**



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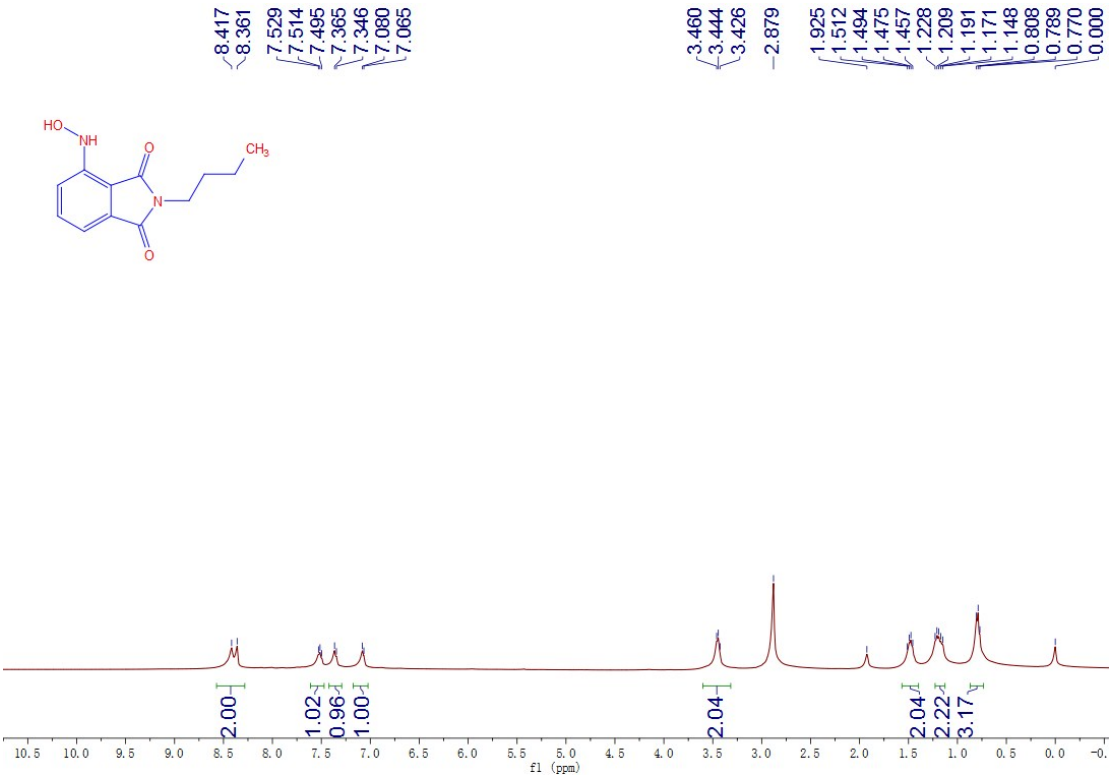
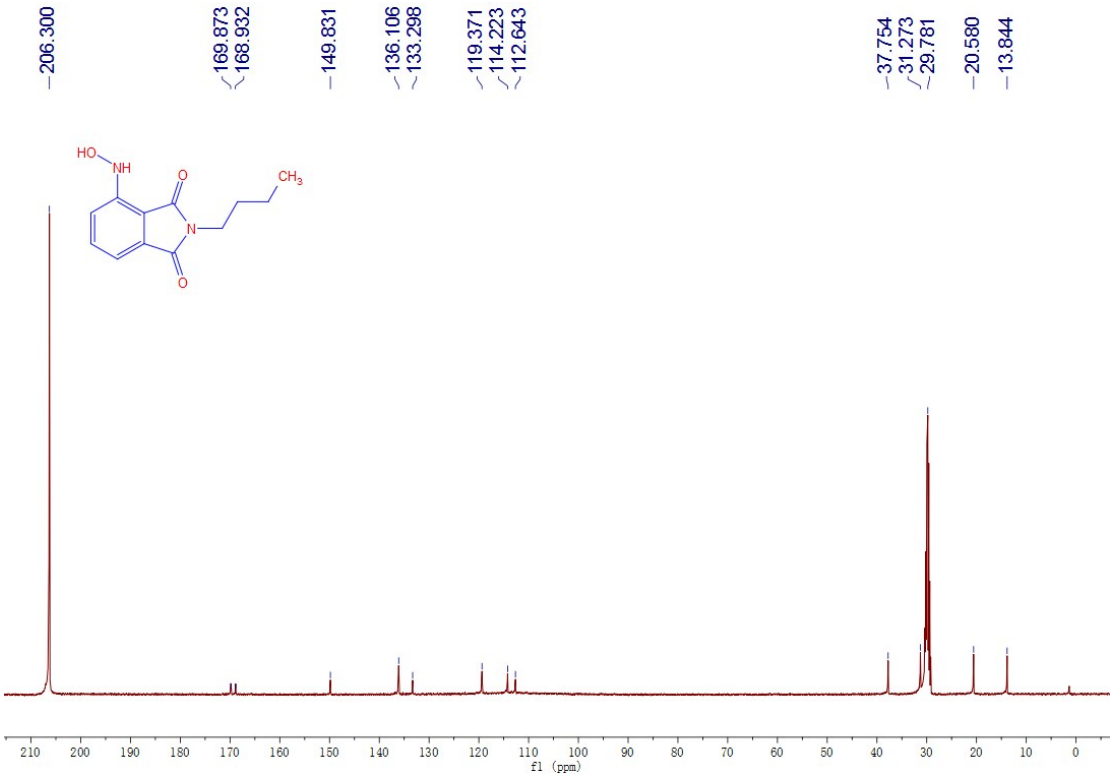
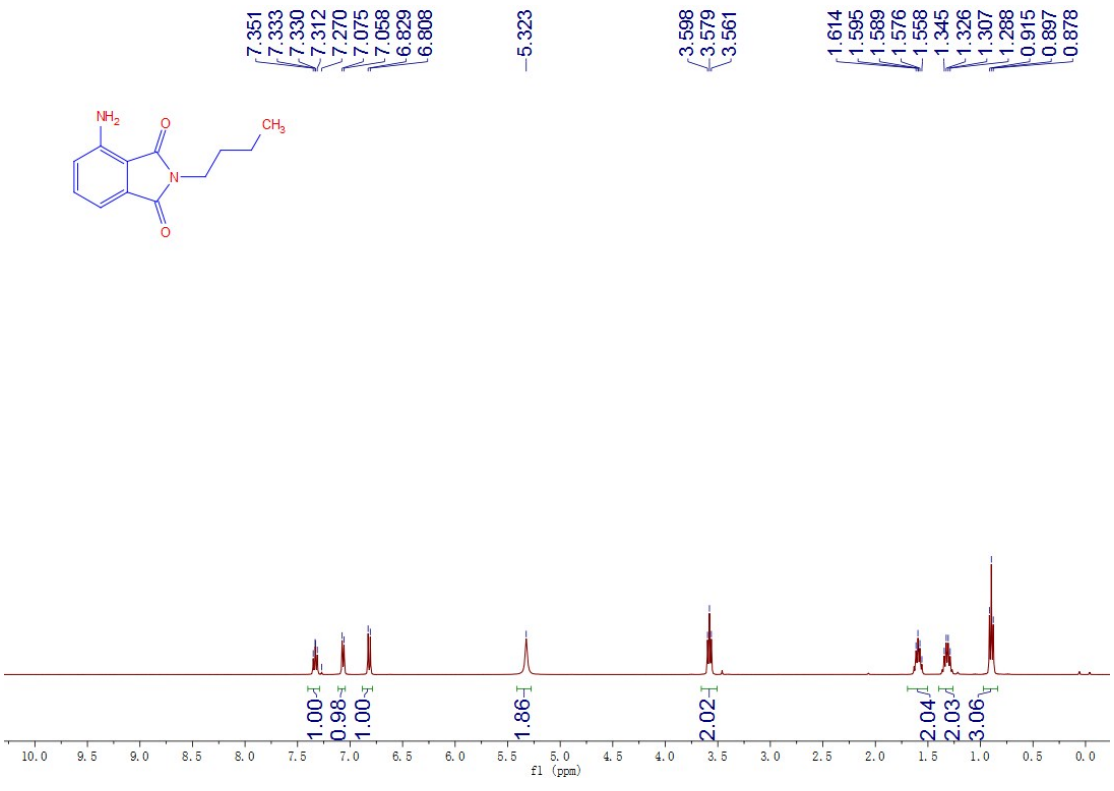


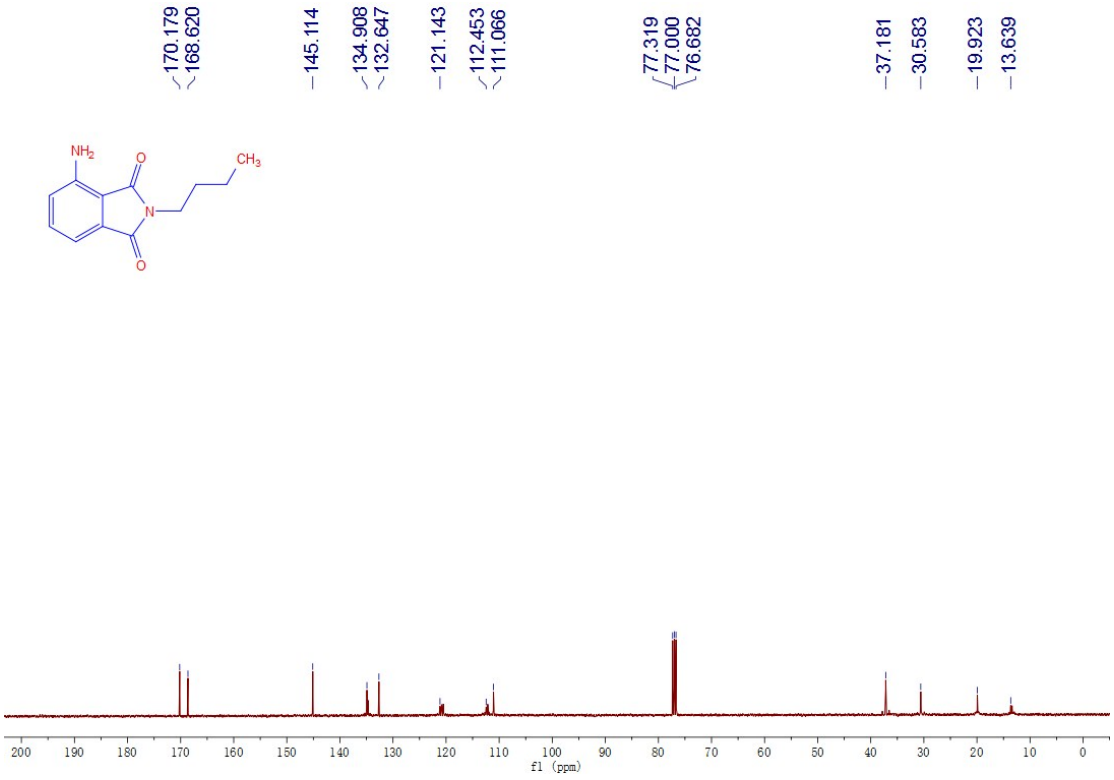
^{13}C NMR of **2**



^{1}H NMR of **3**



^{13}C NMR of **3**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134.908
132.647
121.143
112.453
111.066
77.319
77.000
76.682
37.181
30.583
19.923
13.639
NH2
O
CH3
N
O
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

References

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3 (*a*) J. T. Yeh, P. Venkatesan and S. P. Wu, *New J. Chem*., 2014, **38**, 6198-6204. (*b*) A. Roy, D. Kand, T. Saha and P. Talukdar, *Chem. Commun*., 2014, **50**, 5510-5513.