Supporting Information

The phenazine-barbituric acid colorimetric and ratiometric near-infrared fluorescent probes for sensitively differentiating biothiols and its application in the TiO_2 sensor device

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1. Experimental Section

1.1 Materials and instruments

All the reagents and reactants were purchased from Meryer Chemical Technology, Energy Chemical or Sigma-Aldrich and used as received without further purification. Compound 1 was prepared according to the previous literature protocols[1]. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bucker AM 400 MHz spectrometer with tetramethyl silane (TMS) as the internal standard. Electrospray ionization and time-of-fight analyzer (ESI-TOF) mass spectra were determinated by Waters Micromass LCT mass spectrometer. Absorption and fluorescence spectra were measured with Varian Cary 500 UV-vis spectraphotometer and Varian Cray Eclipse spectrometer, respectively.

1.2 Synthetic routes of probe PBA and PTA

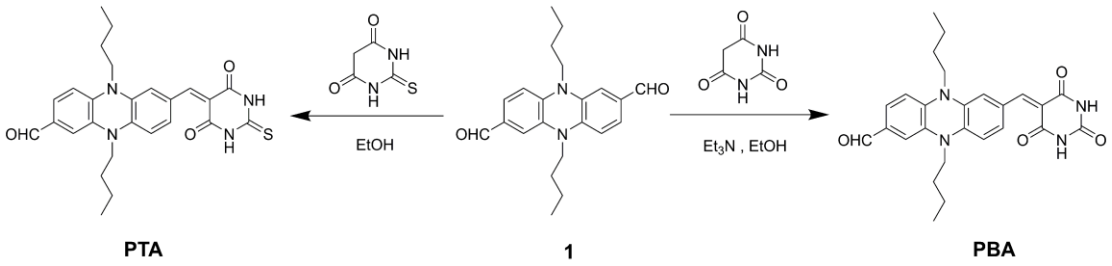


Fig. S1 Synthetic route of compound **PBA** and **PTA**

1.3 Synthesis of compound PBA

Compound **1** (100 mg, 0.28 mmol) and barbituric acid (55 mg, 0.42 mmol) were dissolved in ethanol (10 mL) under the protection of argon atmosphere. Two drops of trimethylamine were then added to the solution to catalyze the reaction. After stirred overnight at 50 °C, the color of the solution changed from orange to deep green. When removing the solvent by evaporation, the residue was purified with silica gel chromatography and eluted with dichloromethane/ethanol (20/1, v/v) to obtain compound **PBA** as a green solid (53.1 mg, 40.3%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.16 (s, 1H), 11.03 (s, 1H), 9.60 (s, 1H), 8.08 (s, 1H), 7.97 (s, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 6.52 (t, *J* = 8.0 Hz, 2H), 3.55-3.42 (m, 4H), 1.56-1.44 (m, 8H), 1.00-0.95 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):192.46, 163.99, 162.93, 153.38, 152.76, 143.95, 138.26, 131.95, 131.52, 129.91, 128.81, 128.66, 125.40, 120.37, 120.18, 120.16, 116.66, 49.77, 29.40, 20.75, 14.66. HRMS (ESI, *m/z*): $[M+H]^+$ calculated for $C_{26}H_{29}N_4O_4^+$ 461.2189, found 461.2106.

1.4 Synthesis of compound PTA

Compound **1** (100 mg, 0.28 mmol) and 4,6-dihydroxy-2-mercaptopyrimidine (62 mg, 0.43 mmol) were dissolved in ethanol under the protection of argon atmosphere. After stirred at room temperature for 3 hours, removing the solvent by evaporation. The residue was then purified with silica gel chromatography and eluted with dichloromethane/ethanol (20/1, v/v) to get the deep green solid as the target compound **PTA** (61.3 mg, 45.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.24 (s, 1H), 12.15 (s, 1H), 9.61 (s, 1H), 8.09 (s, 1H), 7.97 (s, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 12.0 Hz, 1H), 6.82 (s, 1H), 6.57-6.54 (m, 2H), 3.57 (t, *J* = 8.0 Hz, 2H), 3.49 (t, *J* = 8.0 Hz, 2H), 1.58-1.52 (m, 4H), 1.50-1.43 (m, 4H), 0.98 (t, *J* = 8.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):193.58, 175.67, 159.40, 156.08, 153.38, 143.95, 139.52, 132.03, 131.92, 129.88, 128.81, 128.66, 125.70, 120.28, 120.10, 116.66, 115.84, 50.74, 29.46, 20.80, 14.00. HRMS (ESI, *m/z*): $[M+H]^+$ calculated for $C_{26}H_{29}N_4O_3S^+$ 477.1960, found 477.1984.

1.5 Cell culture

The Hela cell lines were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U mL-1 penicillin and 100 μg mL^{-1} streptomycin and cultured in tissue culture flasks in a humidified 5% CO_2 environmental incubator at 37 °C and cultivated at 80% confluency.

1.6 The fabrication of TiO_2 sensor device

The TiO_2 films consisting of a 4 μm layer of Dyesol 90-T TiO_2 paste coated on the treated FTO conducting glass by screen-printing, followed by sintered gradually up to 500 ^{o}C and kept at this temperature for 30 min before cooling. After cooling down to the room temperature, the devices were placed into 3×10^{-4} M dye bath in CH_2Cl_2 solution for 3 hours. Then the devices were washed with anhydrous ethanol and CH_2Cl_2, respectively.

1.7 Detection in fetal bovine serum

For the quantification measurements, fetal bovine serum (FBS) was firstly dealt with saturated ammonium sulfate to remove the protein by salting out effect. The TiO_2 sensor devices were immersed to the fetal bovine serum, which have various concentrations of additional Hcy (0.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 60.0 μM), for 5 min at room temperature to obtain the linear relation between the film absorbance at 660 nm and the Hcy concentration.

In order to perform the recovery detection, different concentrations of NaHS (7.00, 13.00, 18.00 and 25.00 μM) were firstly spiked into the solution, followed by addition of the sensor devices and reaction for 5 min. After washed with ethanol, the film absorption spectra of the devices were then determined with the spectrometer. With the film absorbance at 660 nm in the spectra, the Hcy concentrations in serum could be determined from the linear plotting in Fig. 5C. After three parallel test, the measured concentration was finally confirmed with the average value. And the recovery value represented the ratio of the measured concentration and the actual concentration.

1.7 Determination of fluorescent quantum yields

The relative fluorescence quantum yields of **PBA** were respectively determined before and after the reaction with Cys. And the fluorescence of Rhodamine B (Φ_f = 0.97) in ethanol was chosen as a standard.[2]

2. Time-dependent absorption spectra of PBA towards Cys

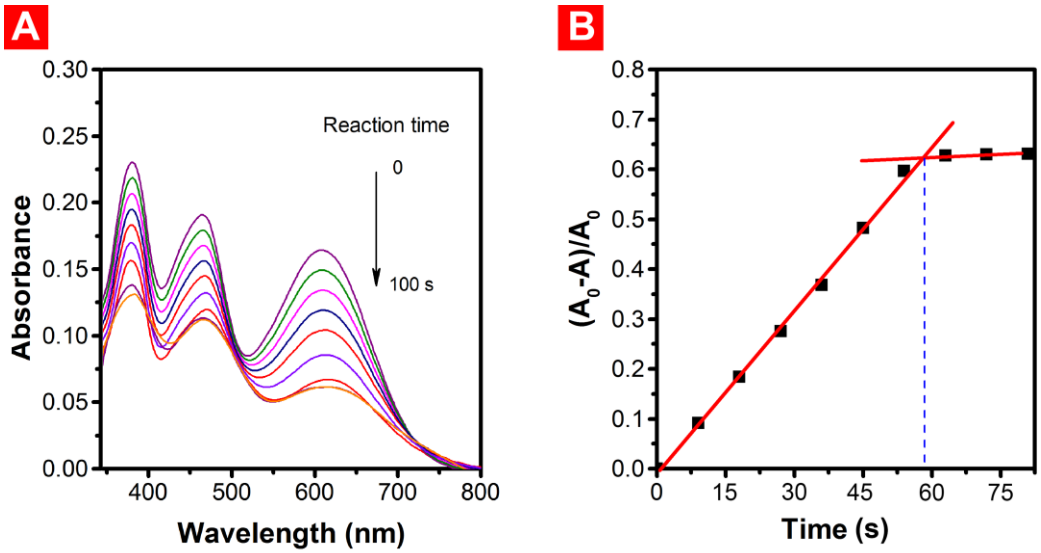


Fig. S2 (A) Time-dependent (0-100 s) absorption spectra of **PBA** (10 μM) towards Cys (80 μM) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 °C. (B) The curve of time-dependent absorbance responses towards NaHS. A_0 represented the initial absorbance at 610 nm. A represented the absorbance at 610 nm.

3. Time-dependent fluorescence spectra of PBA towards Cys

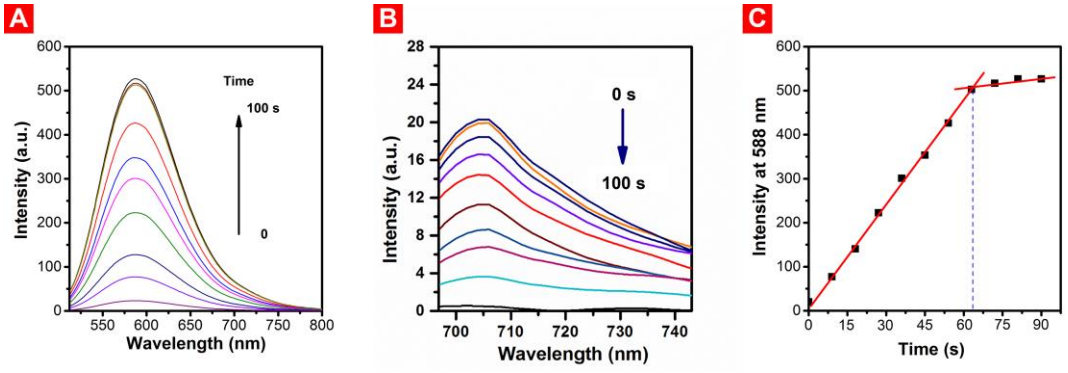


Fig. S3 (A) Time-dependent fluorescence spectra responses (0-100 s) of **PBA** (10 μM) towards 80 μM Cys with the excitation wavelength at 465 nm. (B) Time-dependent NIR fluorescence responses (0-100 s) of **PBA** (10 μM) towards 80 μM Cys with the excitation wavelength at 610 nm. (C) Linear correction between the fluorescence intensity at 588 nm towards the low concentration of Cys.

4. Concentration-dependent fluorescence spectra of PTA towards Cys

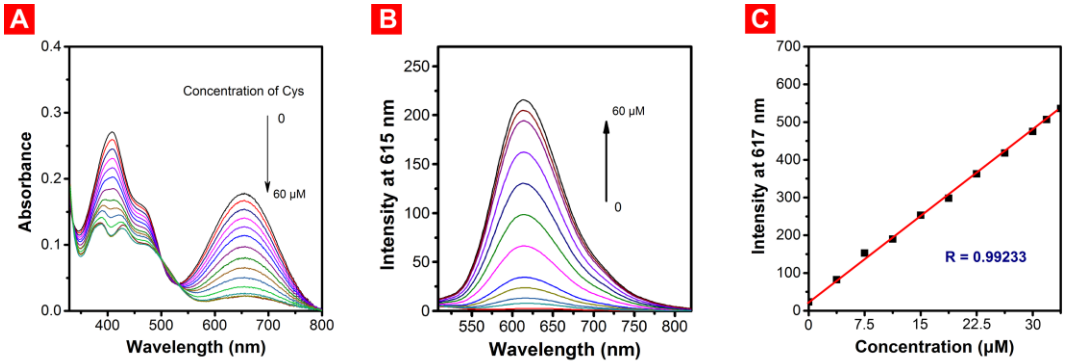


Fig. S4 (A) Concentration-dependent absorption spectra of **PTA** (10 μM) towards different concentrations of Cys (0-60 μM). (B) Concentration-dependent fluorescence spectra responses of **PTA** (10 μM) in the presence of various concentrations of Cys (0-60 μM) with the excitation wavelength at 450 nm. (D) Linear correction between the fluorescence intensity at 617 nm towards the low concentration of Cys (0-30 μM).

5. The selectivity of probe PBA

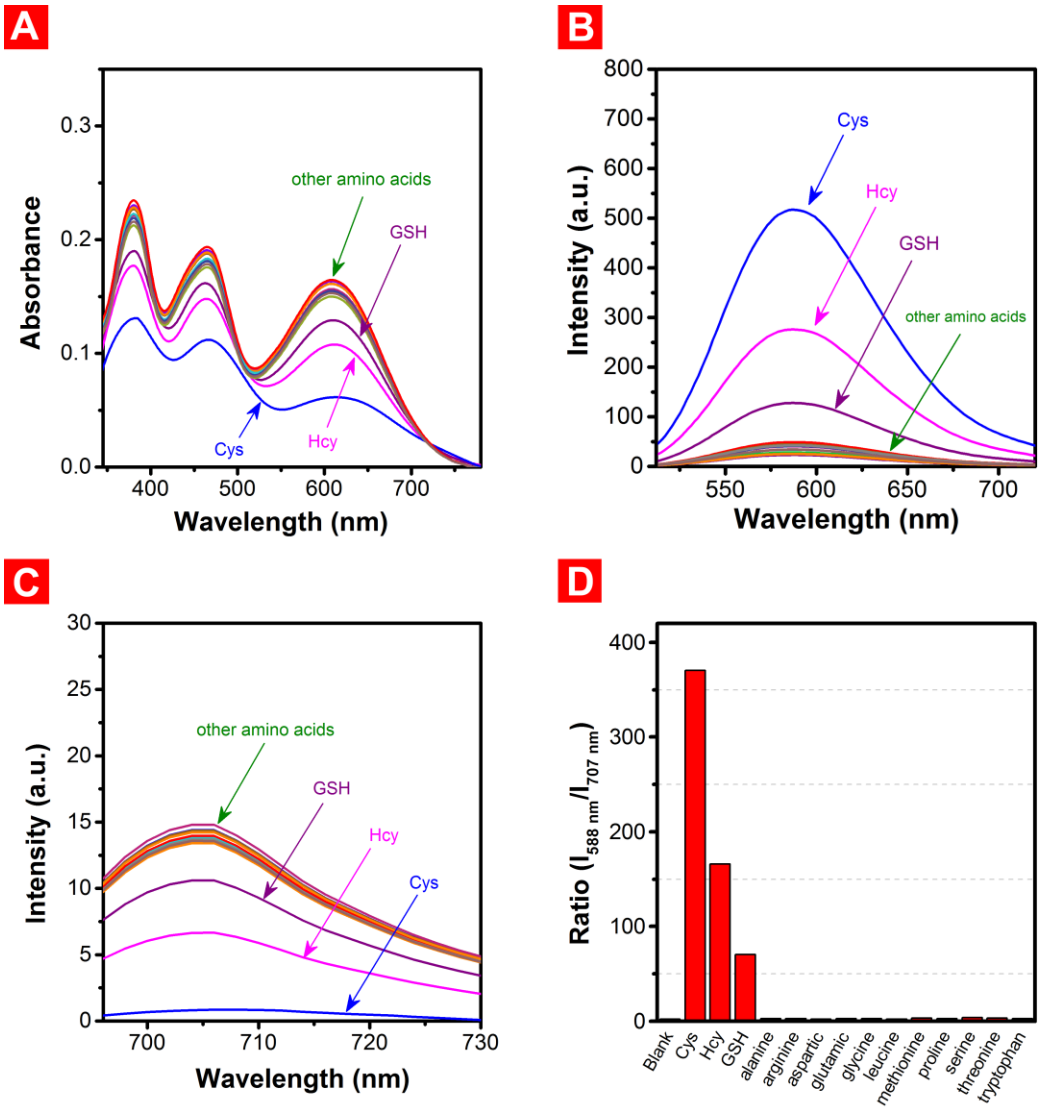


Fig. S5 The absorption (A) and fluorescence responses (B and C) of **PBA** (10 μM) upon addition of Cys (100 μM) and other kinds of amino acids (1 mM). The excitation wavelengths were 465 nm (B) and 610 nm (C), respectively. The bars (D) represented the fluorescence intensity ratio ($I_{588\ nm}$/ $I_{707\ nm}$) of **PBA** in the presence of various amino acids.

6. The selectivity of probe PTA

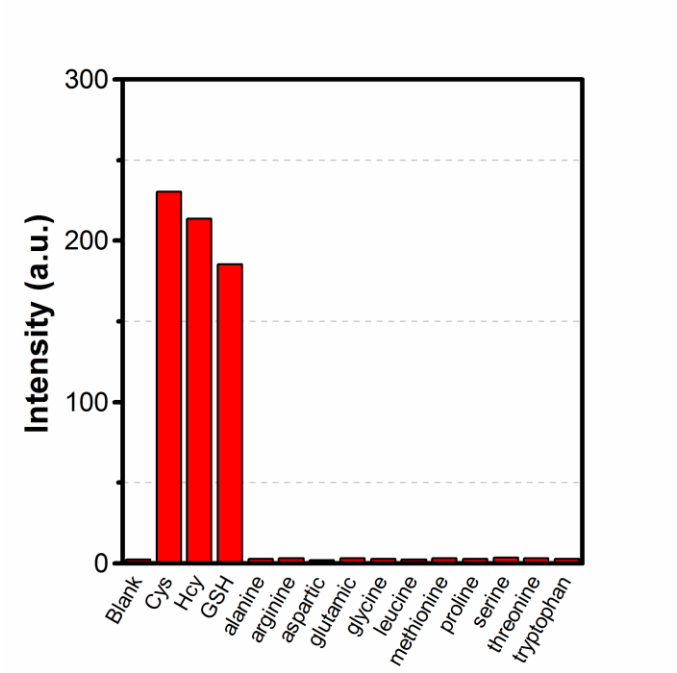


Fig. S6 The fluorescence responses of **PTA** (10 μM) upon addition of Cys (600 μM) and other kinds of amino acids (600 μM). The excitation wavelengths were 450 nm. The bars represented the fluorescence intensity at 617 nm of **PTA** in the presence of various amino acids.

7. Detection mechanism in sensing Cys

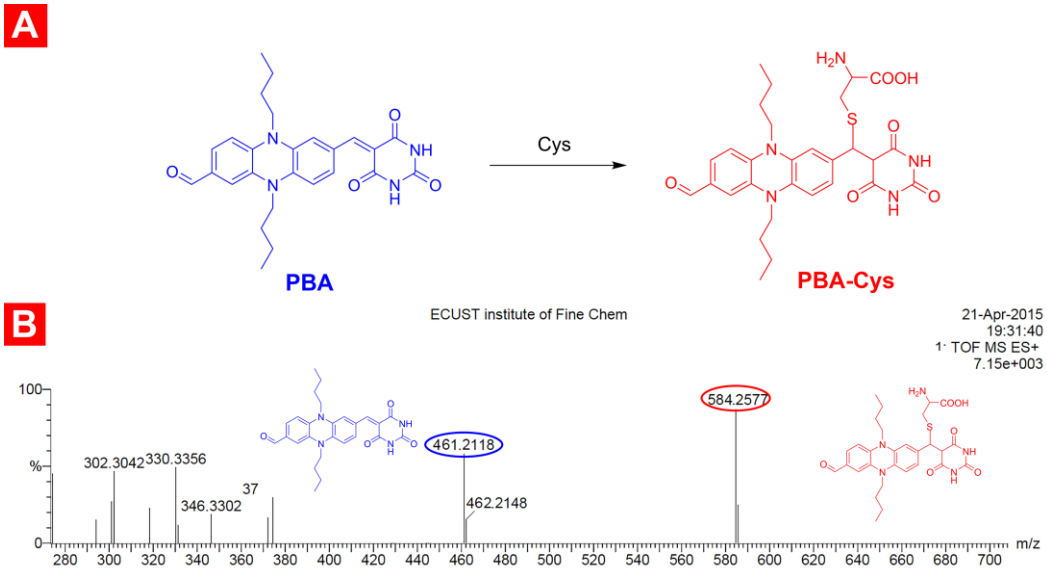


Fig. S7 (A) The proposed sensing mechanism between **PBA** and Cys. (B) The MS titration spectroscopy of **PBA** (1 mM) upon addition of Cys (0.5 mM).

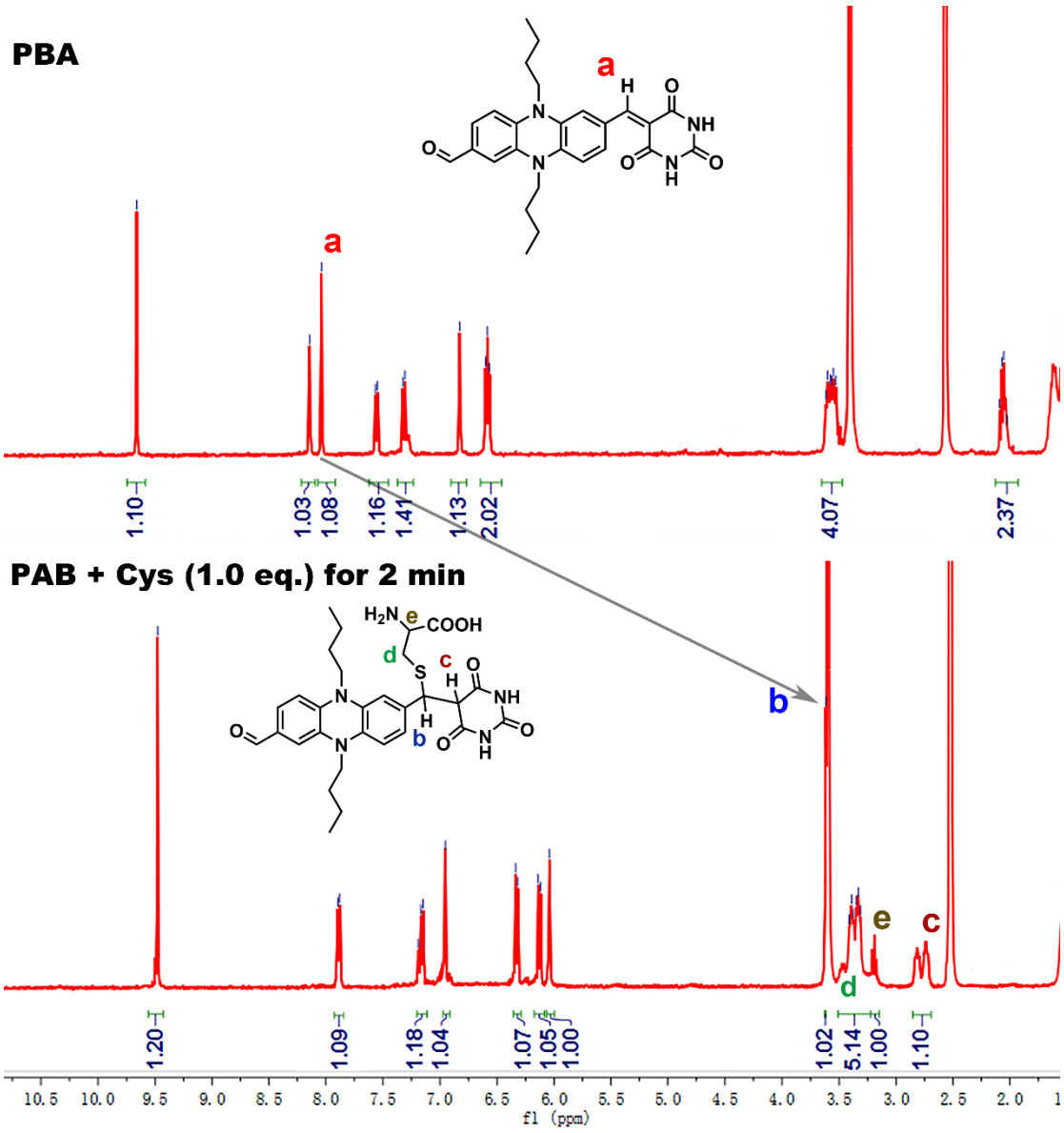


Fig. S8 The ^{1}H NMR titration spectroscopy of **PBA** (1 mM) before and after the reaction with Cys (1 mM).

8. Optical responses of PBA under different pH values

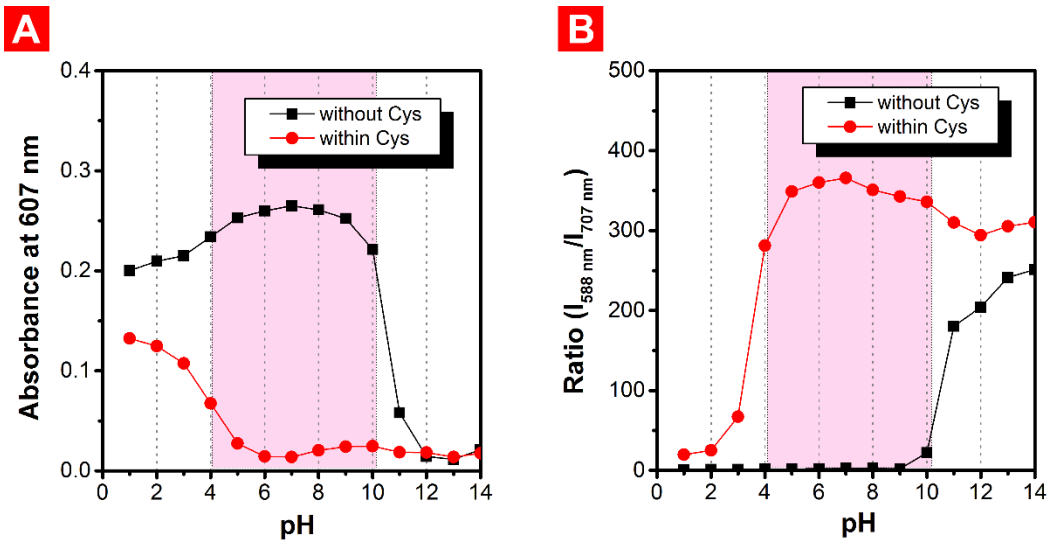


Fig. S9 (A) The absorbance at 607 nm of **PBA** (10 μM) before (black squares) and after (red dots) reaction with Cys (80 μM) at different pH values (1-14). (B) The fluorescent intensity ratio ($I_{588\ nm}/I_{707\ nm}$) of **PBA** (10 μM) in the absence (black squares) and presence (red dots) of Cys (80 μM) at different pH values (1-14).

9. Optical responses of PTA under different pH values

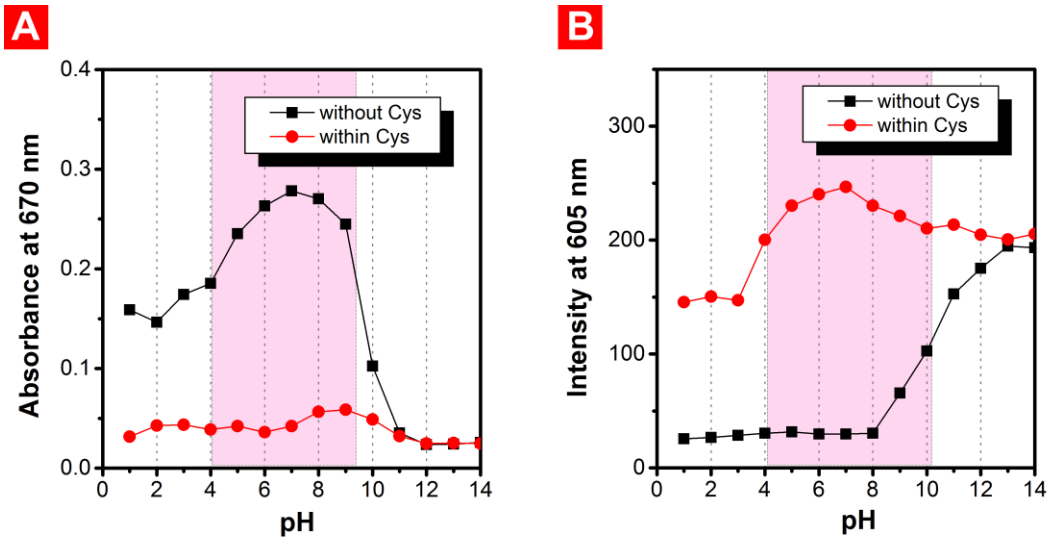


Fig. S10 (A) The absorbance at 670 nm of **PTA** (10 μM) before (black squares) and after (red dots) reaction with Cys (40 μM) at different pH values (1-14). (B) The fluorescent intensity at 605 nm of **PBA** (10 μM) in the absence (black squares) and presence (red dots) of Cys (40 μM) at different pH values (1-14).

10. The photostability of PBA

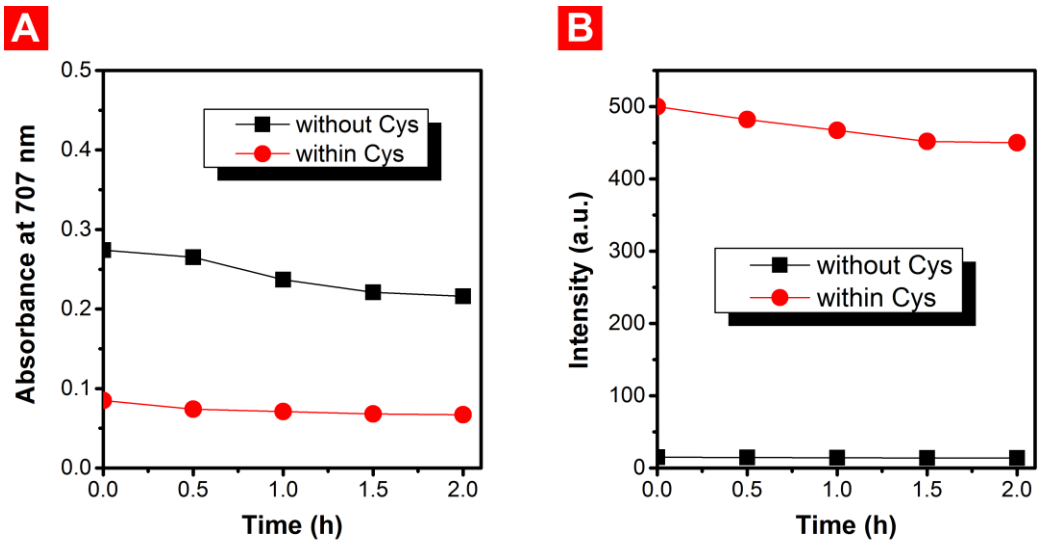


Fig. S11 The photostability of **PBA** (10 μM) and its additive product were studied under 200 W/ m^2 light irradiation for 0-2 hours.

11. Optical responses of PBA and PTA towards Hcy

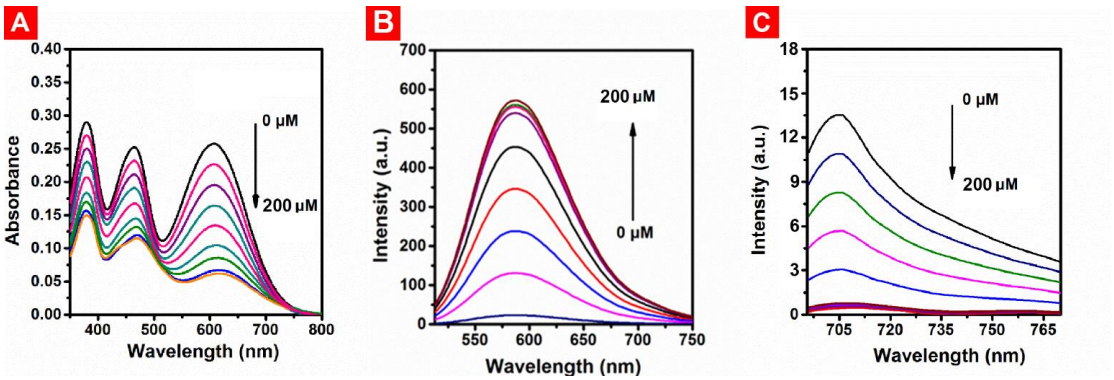


Fig. S12 (A) Concentration-dependent absorption spectra of **PBA** (10 μM) towards different concentrations of Hcy (0-200 μM). (B) Concentration-dependent fluorescence spectra responses of **PBA** (10 μM) in the presence of various concentrations of Hcy (0-200 μM) with the excitation wavelength at 465 nm. (C) Concentration-dependent NIR fluorescence responses of **PBA** (10 μM) in the presence of various concentrations of Hcy (0-200 μM) with the excitation wavelength at 610 nm.

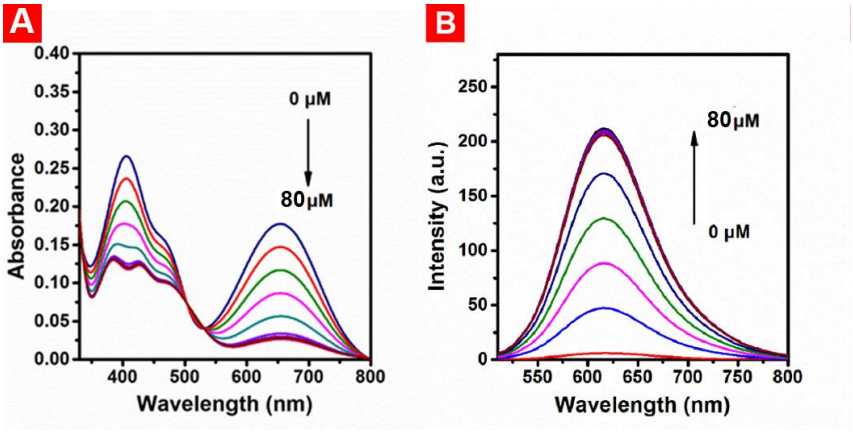


Fig. S13 (A) Concentration-dependent absorption spectra of **PTA** (10 μM) towards different concentrations of Hcy (0-80 μM). (B) Concentration-dependent fluorescence spectra responses of **PBA** (10 μM) in the presence of various concentrations of Hcy (0-80 μM) with the excitation wavelength at 450 nm.

12. Characterization of new compounds

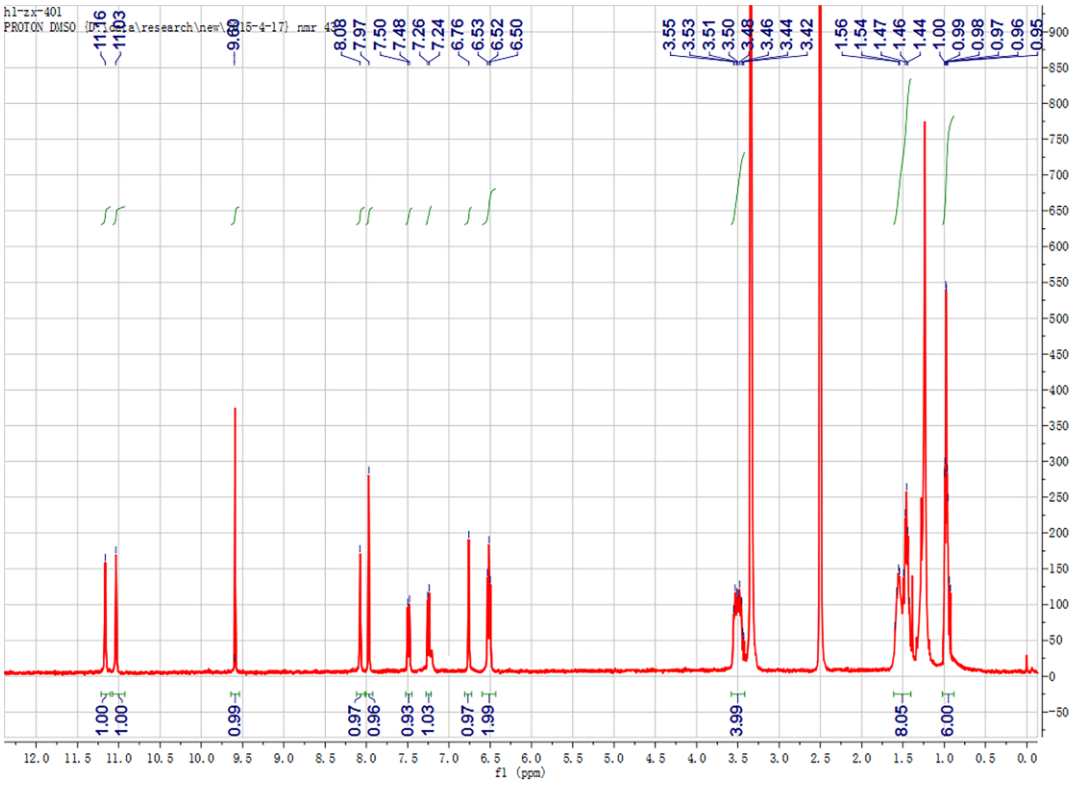


Fig. S14 ^{1}H NMR spectrum of **PBA** in DMSO-d_6

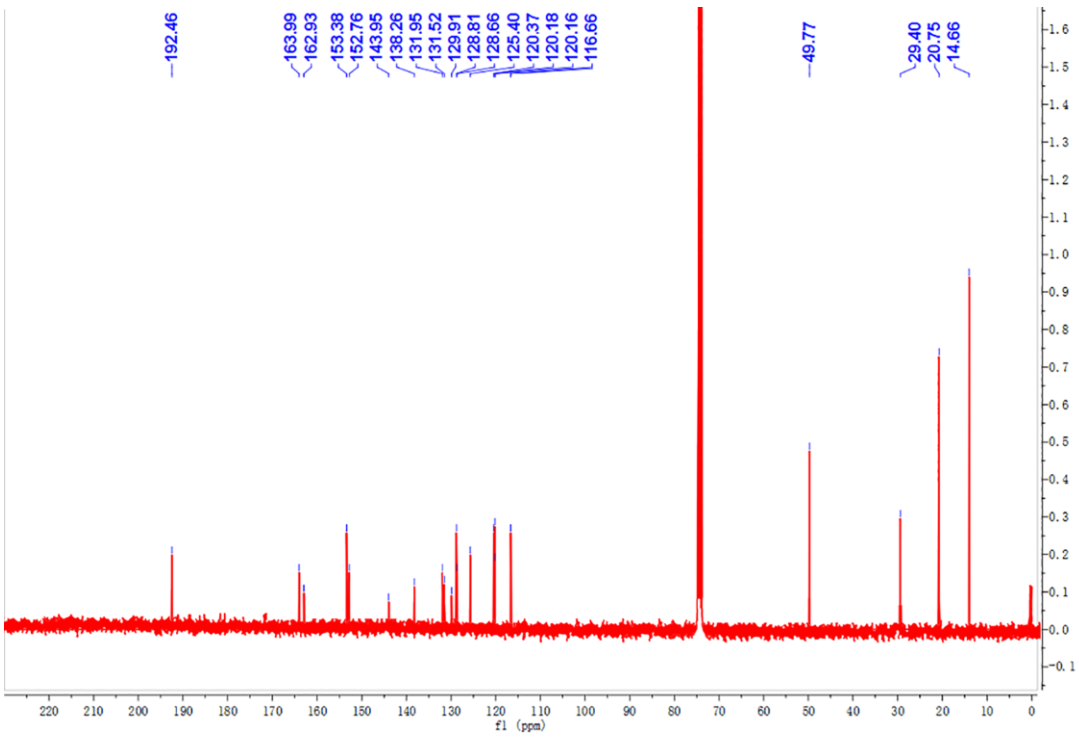


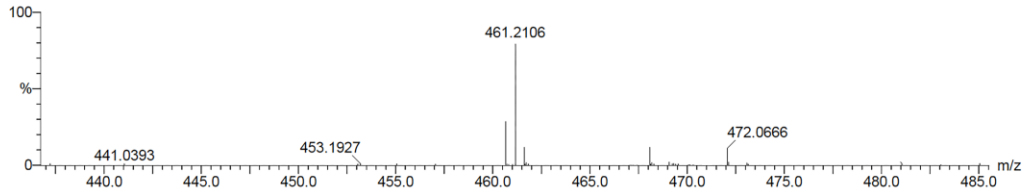
Fig. S15 ^{13}C NMR spectrum of **PBA** in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-26 H: 0-29 N: 0-4 O: 0-4

JL-HUA ECUST institute of Fine Chem 19-Mar-2015 16:13:26
HL-ZX-462 20 (0.692) Cm (16:24) 1: TOF MS ES+ 5.70e+004



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.2106	461.2189	0.9	1.9	7.5	138.4	0.0	C26 H29 N4 O4

Fig. S16 HRMS of **PBA**

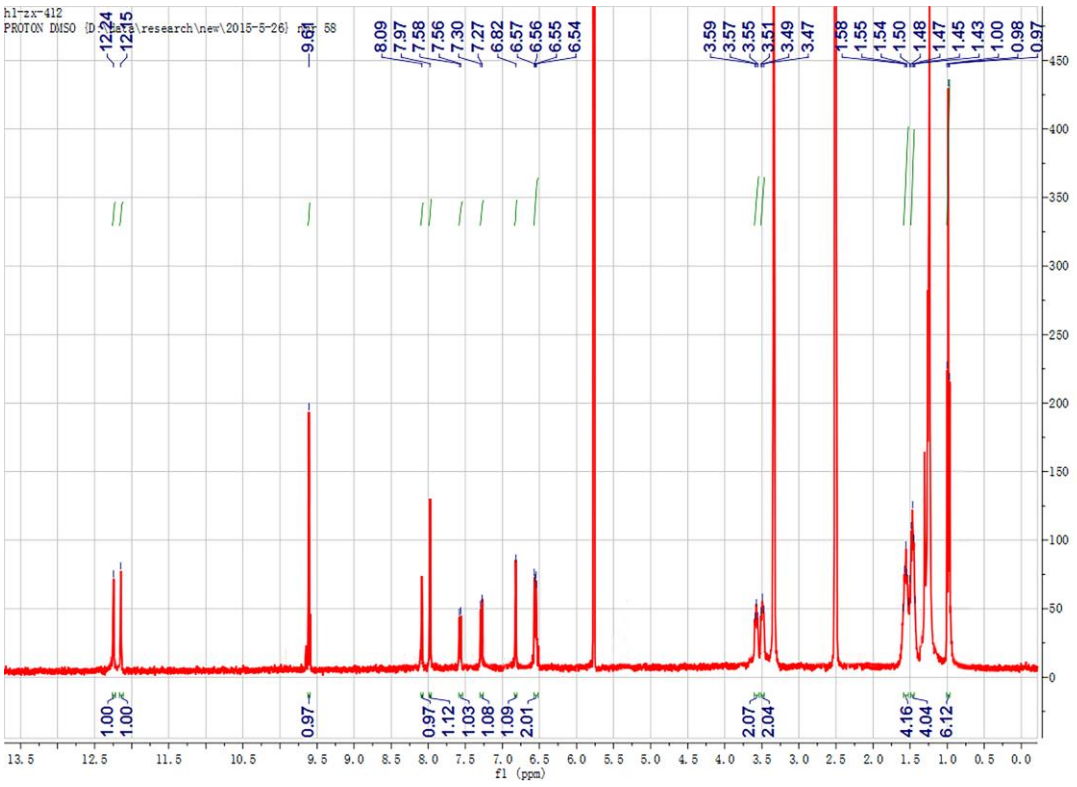


Fig. S17 ^{1}H NMR spectrum of **PTA** in DMSO-d_6

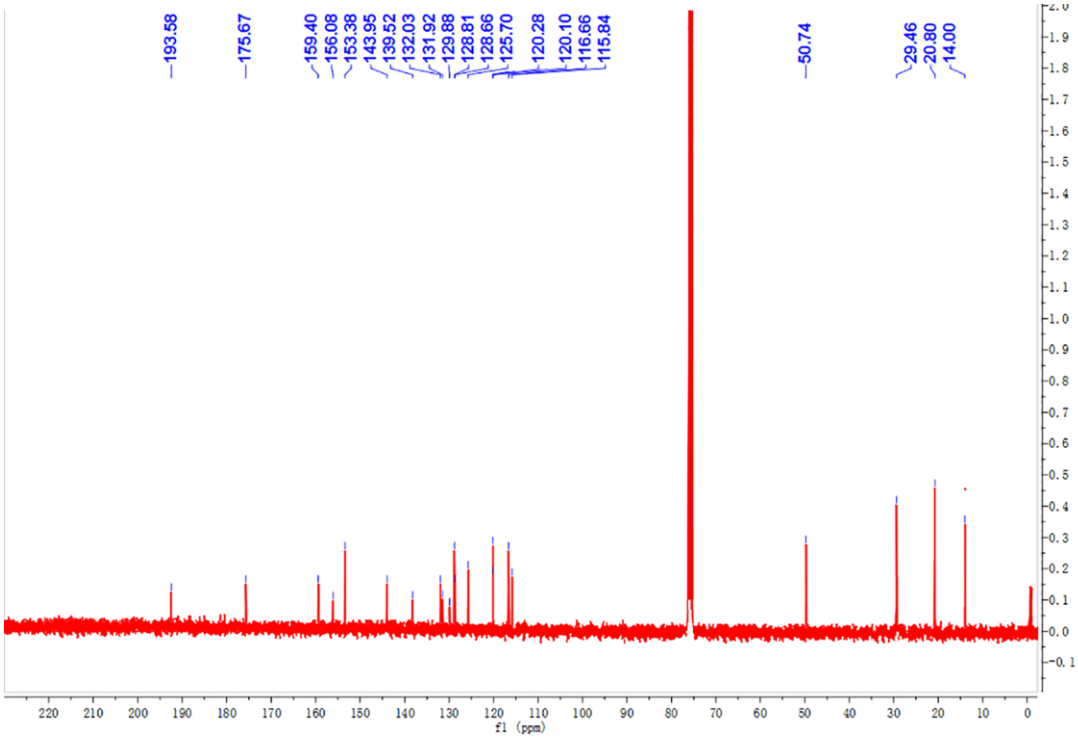


Fig. S18 ^{13}C NMR spectrum of **PTA** in $CDCl_3$

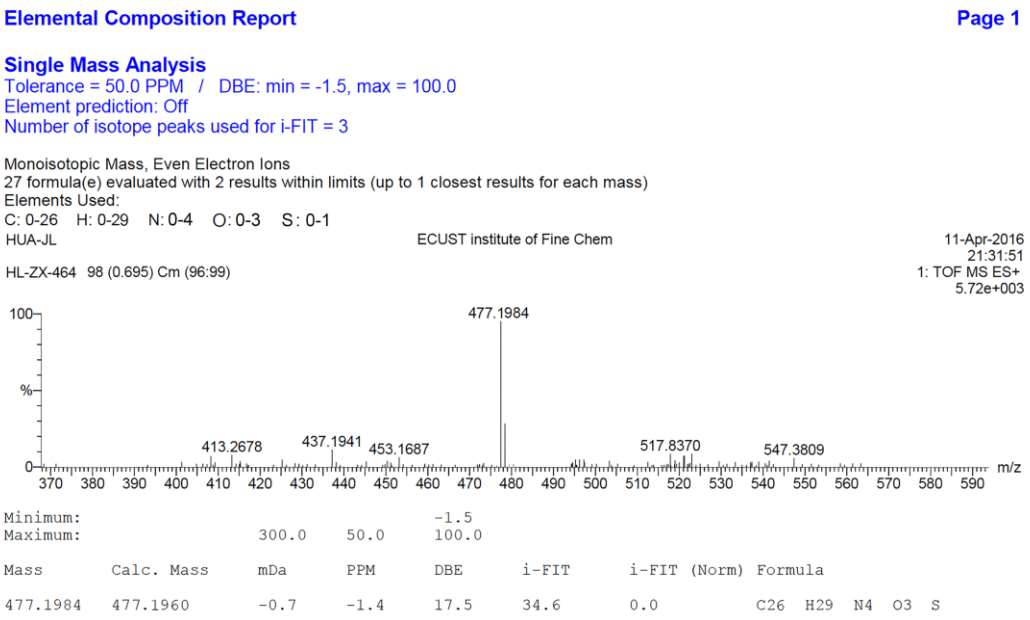


Fig. S19 HRMS of **PTA**

13. References

1. L. Yang, X. Li, Y. Qu, W. S. Qu, X. Zhang, Y. D. Hang, H. Ågren and J. L. Hua, Red turn-on fluorescent phenazine-cyanine chemodosimeters for cyanide anion in aqueous solution and its application for cell imaging, *Sens. Actuators, B*, 2014, **203**, 833-847.

2. E. Wang, L Pang, Y. Zhou, J. Zhang, F. Yu, H. Qiao and X. Pang, A high performance Schiff-base fluorescent probe for monitoring Au^{3+} in zebrafish based on BODIPY, *Biosens. Bioelectron.*, 2016, **77**, 812-817.