Supplementary Information

A novel NIR fluorescent probe for highly sensitive detection of HNO and its application in bioimaging

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Experimental

Apparatus and chemicals

Absorption spectra were accurately measured on a Lambda 35 UV/VIS spectrometer (Perkin Elmer). Fluorescence spectra were measured on the F-4500 FL Spectrophotometer and the EX Slit and EM Slit were both set at 10.0 nm. The pH was measured by a Model PHs-3C meter (Shanghai, China). ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were measured on a Bruker DTX-400 spectrometer using TMS as internal reference. HR-MS (high-resolution mass spectrometry) spectra were collected using the Q-Tof HR-MS spectrometer (Waters Micromass).

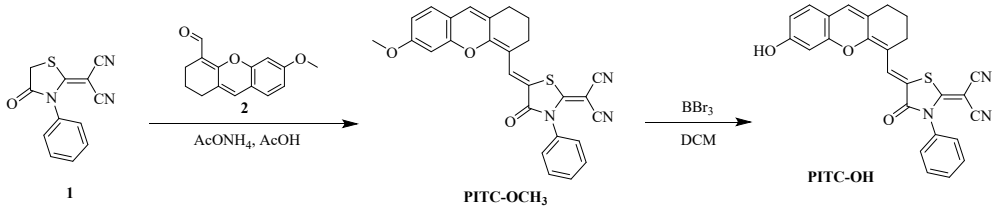
All the reagents were purchased from reagent companies without further purification and directly used in the experiment. The deionized water was purified by Milli-Q. The concentrations of all interference ions are as 10.00 mmol/L, including F^-, Cl^-, Br^-, I^-, CO_3^{2-}, HCO_3^-, HPO_4^{2-}, PO_4^{3-}, NO_3^-, NO_2^-, SO_4^{2-}, AcO^-, HSO_3^-, Na^+, K^+, HS^-, Hcy, GSH, Cys, $^1O_2$, ClO^-, H_2O_2, NO, $ONOO^-$. The preparation of interference anions is to weigh the corresponding sodium or potassium salt of the desired mass.

Synthesis

The synthetic procedure of probe **PITC-HNO** was shown in Scheme **1**. Compound **1** and **2** were synthesized by reported methods[1, 2].

Synthesis of Compound PITC-OCH$_3$ Compound **1** (241 mg, 1.0 mmol), ammonium acetate (154 mg, 2.0 mmol) and compound **2** (312 mg, 1.3 mol) were dissolved in 15 mL of glacial acetic acid. The mixture was refluxed and stirred at 118 ℃ for 24 h in nitrogen atmosphere. The reaction solution was cooled to room temperature, 2 mL of water was added to precipitate and the solution was filtered to obtain a crude product. After the crude product was dried in vacuum, dichloromethane was used as the eluent for column chromatography to obtain 347 mg of silver gray solid PITC-OCH_3 with 75% yield.

Synthesis of Compound PITC-OH At 0 ℃, BBr_3 (0.48 mL, 5 mmol) was slowly added dropwise to a solution of PITC-OCH_3 (335 mg, 0.5 mmol) in anhydrous dichloromethane (10 mL). After a period of reaction, the solution was stirred at room temperature for 14 hours. Saturated $NaHCO_3$ solution (30 mL) was added to quench the reaction, then the aqueous layer was extracted with CH_2Cl_2, the organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to obtain 210 mg of crude green product. The crude product was directly used to synthesize **PITC-HNO** without separation and purification.



Scheme 1 Synthetic route of PITC-OH

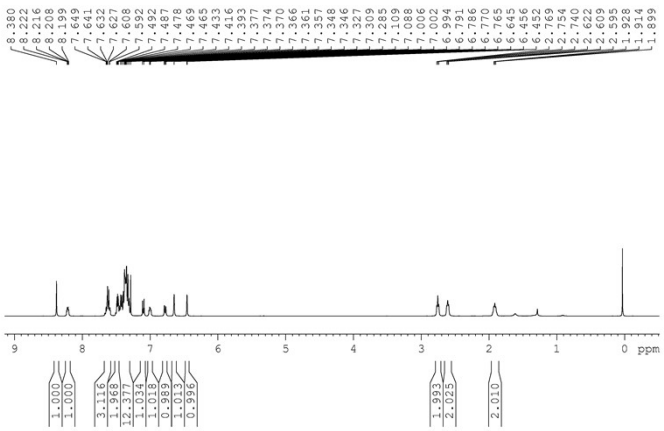


Fig.S1 ^{1}H NMR spectra of **PITC-HNO** in $CDCl_3$.

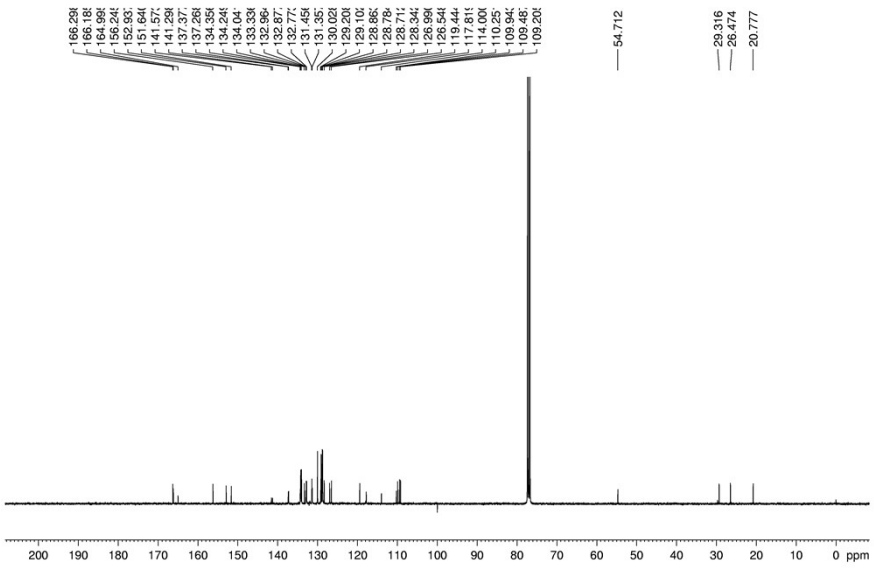


Fig. S2 ^{13}C NMR spectra of **PITC-HNO** in $CDCl_3$

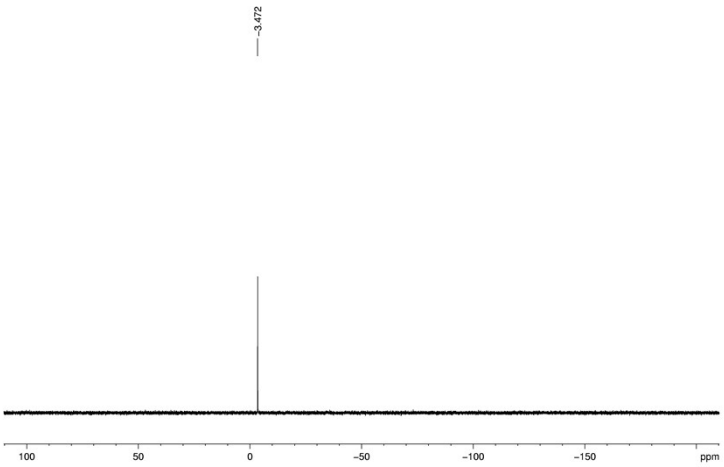


Fig.S3 ^{31}P NMR spectra of **PITC-HNO** in $CDCl_3$.

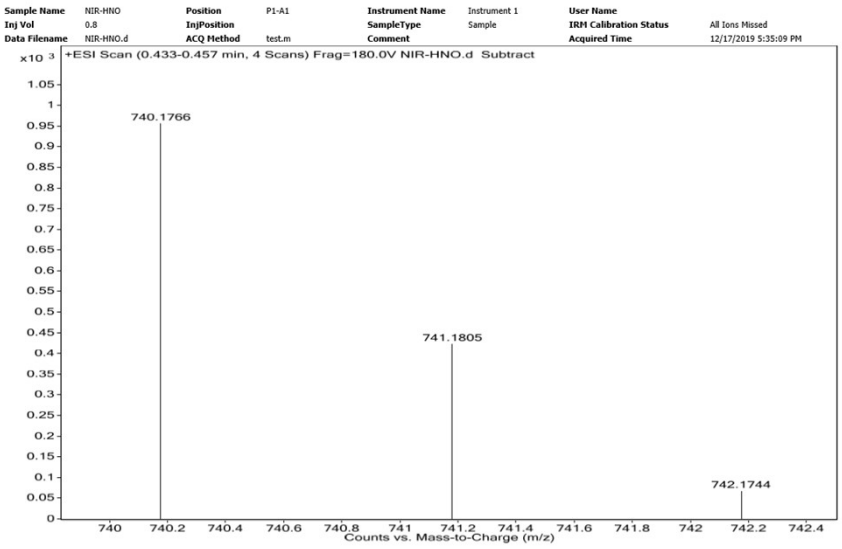


Fig.S4 ESI-HRMS spectra of **PITC-HNO**.

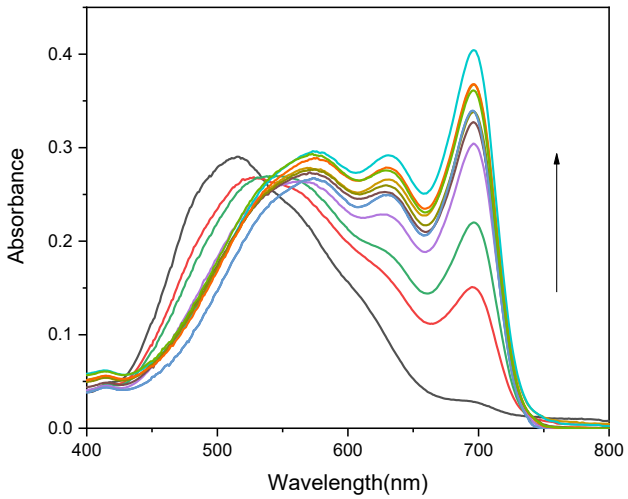


Fig.S5 The change of UV spectra of the **PITC-HNO** (10 μM) solution after adding HNO (0–15 eq.) in PBS buffer solution (pH = 7.40, containing 40% DMF).

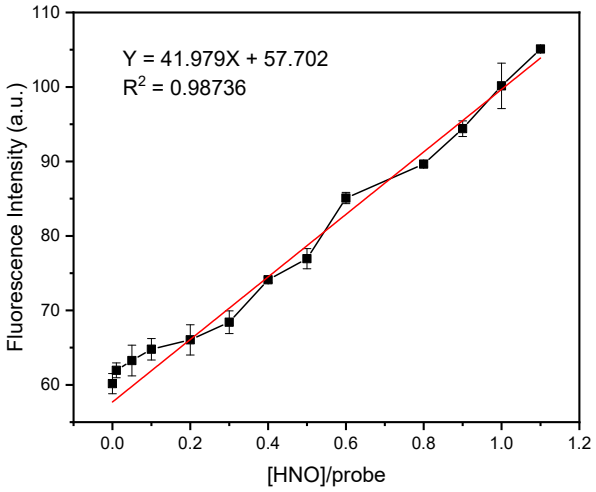


Fig. S6 The linear fitting of fluorescence intensity against concentrations of HNO (0–1.1 eq.) at 714 nm. λ_{ex} = 640 nm.

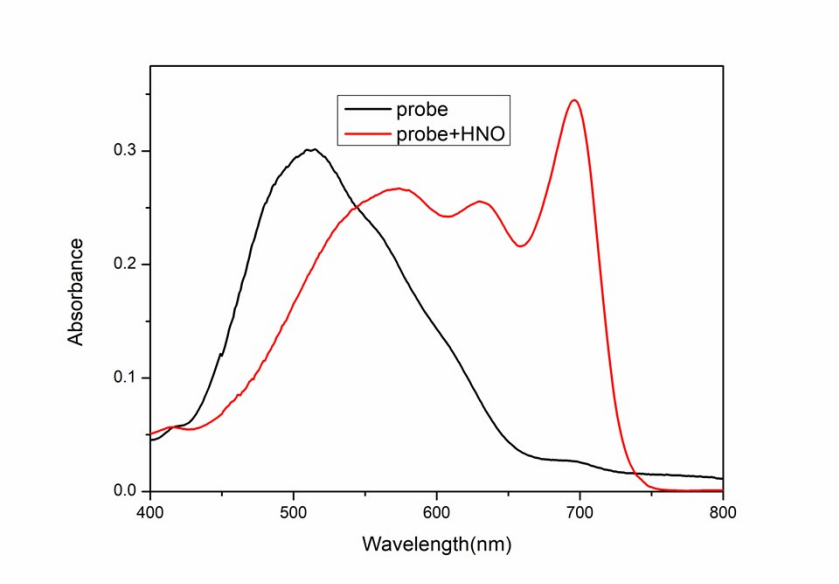


Fig.S7 The change of absorption spectra of **PITC-HNO** before and after response to HNO.

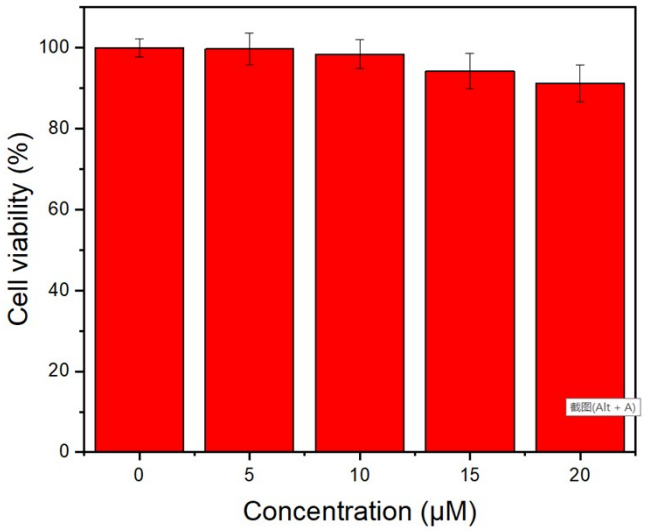


Fig.S8 The cytotoxicity test of different concentrations **PITC-HNO** in living HeLa cells for 24 h.

References

1. S. Gong, E. Zhou, J. Hong and G. Feng, *Anal Chem*, 2019, **91**, 13136-13142.
2. J. Mao, N. He, Z. Ning, Q. Zhang, F. Guo, L. Chen, W. Wu, J. Hua and H. Tian, *Angew Chem Int Ed Engl*, 2012, **51**, 9873-9876.