Supporting Information

π-Conjugated thiolate amplified spectrophotometry nitrite assay with improved sensitivity and accuracy

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Experimental Section

Chemicals. Sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), sodium dihydrogen phosphate (NaH_2PO_4), sodium sulfate (Na_2SO_4), sodium periodate ($NaIO_4$), sodium perchlorate ($NaClO_4$), sodium fluoride (NaF), sodium chloride (NaCl), sodium bromide (NaBr), sodium thiosulfate ($Na_2S_2O_3$), and sodium acetate (NaAcO) were obtained from Tianjin Fuchen Chemical Reagent Co., Ltd. (Tianjin, China). Sodium hypochlorite (NaClO) were acquired XiLong Scientific. Hydrochloric acid (HCl, 36%, V/V) and Hydrogen peroxide (H_2O_2, 30% wt) were supplied by Beijing Chemical Reagent Company (Beijing, China). Glutathione (GSH) and 4-amino-6-hydroxy-2-mercaptopyrimidine (AHMP) were purchased from Tokyo Chemical Industry. (Tokyo, Japan). 2-Thiobarbituric acid (TBA) was obtained from Alfa Aesar (Heysham, U.K.). Tiopronin (TPN) was supplied form Shanghai Yuanye Biological (Shanghai, China). Thioacetamide was provided from J&k Scientific Ltd. (Beijing, China). All reagents were analytical grade and used without further purification. Deionized water was from

a Millipore water purification system (18.2 MU cm, Milli Q, Millipore, Barnstead, CA, USA).

Characterizations. The UV−vis absorption spectra were collected with a UV−3600 spectrophotometer (Shimadzu, Japan). Fourier-transform infrared (FT−IR) spectra were obtained using a Nicolet 6700 FT−IR spectrometer (Thermo, U.S.A.). The cyclic voltammetry electrochemical measurements were conducted by a CHI660E electrochemical workstation (Shanghai Chenhua Instruments, China). The ESI-MS analyses was performed with a Xevo G2 Q-Tof mass spectrometer (Waters, USA)

UV-vis absorption measurements. A series of sodium nitrite, TBA, GSH, AHMP and TPN stock solutions were prepared for use, the reaction was conducted under acidic conditions (pH = 3.0). Separated TBA, GSH, AHMP, TPN was mixed with sodium nitrite solution and the corresponding absorption spectrum was measured.

Electrospray ionization mass spectrometry (ESI-MS) measurement. Typically, 10 mM TBA was reacted with sodium nitrite and the resulted solution was freeze-dried. A 5.7 mg of powder in 10 mL of methanol with 100-fold dilution was centrifuged at 1000 r/min for 5 minutes, and the supernatant was taken for mass spectrometry measurement. The mass spectrum of $RSNO_{TBA}$ was performed with a negative mode. The capillary electrospray voltage was 2.2 kV, and the sample cone was 30 V. The solvent system consisted of 25% water (A) and 75% acetonitrile (B) with a flow rate of 100 μL·min^{-1}.

Cyclic voltammetry measurements. A conventional three-electrode system was used for cyclic voltammetry determination. The reference electrode saturated was Ag/AgCl electrode, platinum wire as the counter electrode and the working electrode was glassy carbon electrode (GCE). The electrolyte was prepared with 0.1 M PBS (pH=7.4). The RSNO modified GCE was prepared by dropping the mixture of

thiolate and $NaNO_2$ on the surface of electrode, the scanning voltage ranged from -1.0 V to 1.0 V. For $RSNO_{GSH}$, the scanning voltage ranged from -1.7 V to 1.6 V. While for $RSNO_{TPN}$, the scanning voltage ranged from -1.9 V to 1.7 V. All the surface of electrode was dried *via* ultrapure N_2 blow, the scan rate was 100 mV/s.
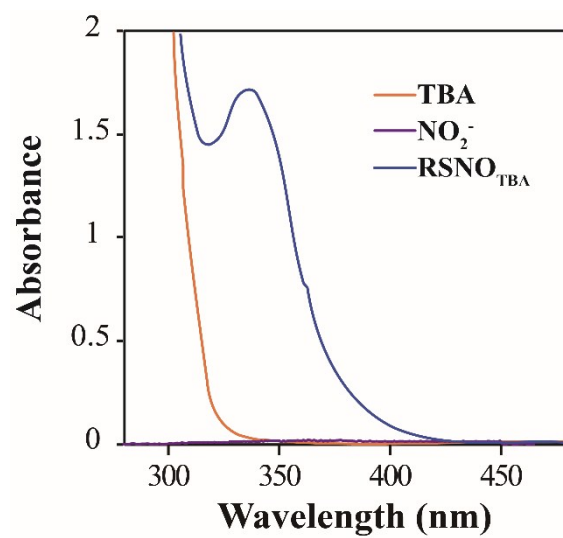
Fig. S1 UV-vis absorption spectra of TBA (1.0 mM, orange line), $NaNO_2$ (200 μM, purple line), $RSNO_{TBA}$ (200 μM, blue line).
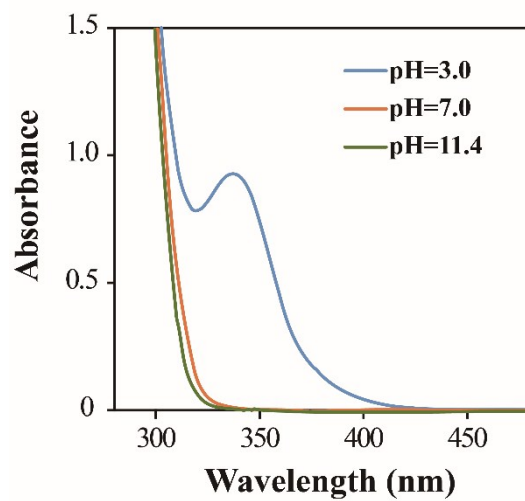
Fig. S2 UV-vis absorption spectra of TBA-NaNO$_2$ solutions under different pH conditions.
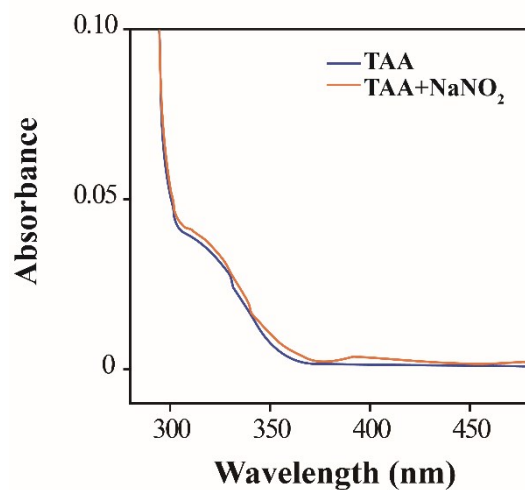
Fig. S3 UV-vis absorption spectra of thioacetamide (TAA, 1 mM, blue line) and TAA-NaNO$_2$ (orange line, NaNO$_2$: 200 μM) solution.
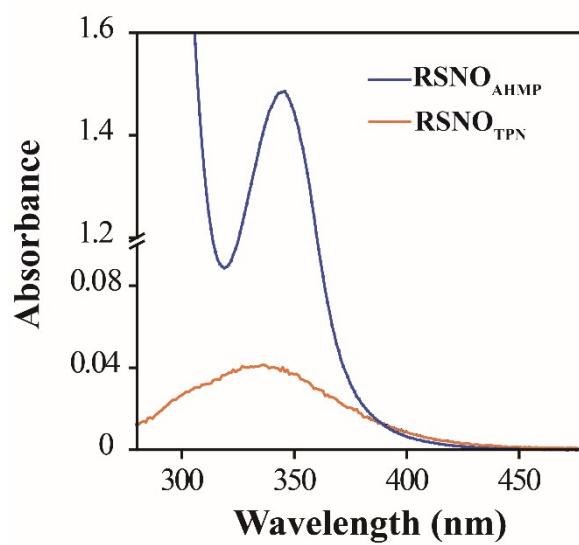
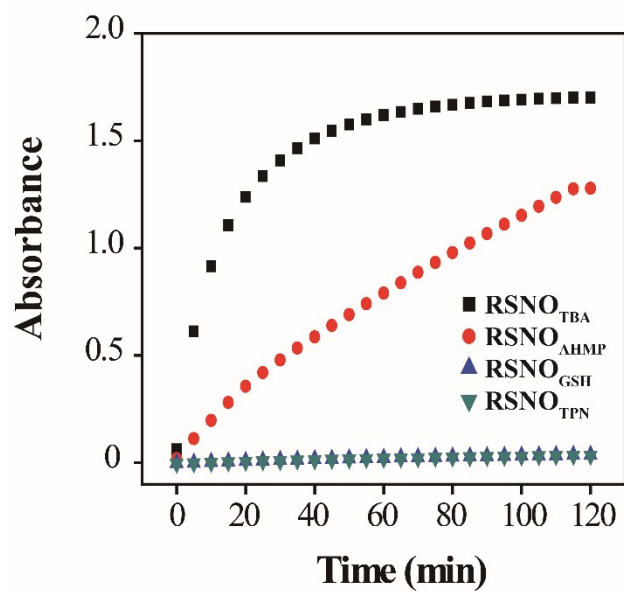**Fig. S4** UV-vis absorption spectra of 200 μM RSNO$_{AHMP}$ (blue line) and RSNO$_{TPN}$ (orange line).

Fig. S5 Time-dependent absorbance at corresponding maximum absorption wavelength of four RSNOs. The concentrations of 200 µM $NaNO_2$ and TBA, AHMP, GSH and TPN are 1, 1, 1, and 1 mM, respectively.
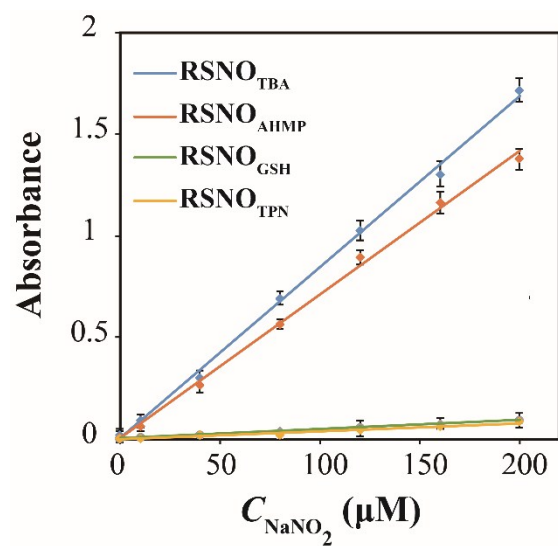
Fig. S6 Absorbance of various RSNOs at corresponding maximum absorption wavelengths versus the concentration of nitrite from 0 to 200 µM. The concentrations of GSH, TPN, TBA and AHMP are 5, 5, 1, and 1 mM, respectively.
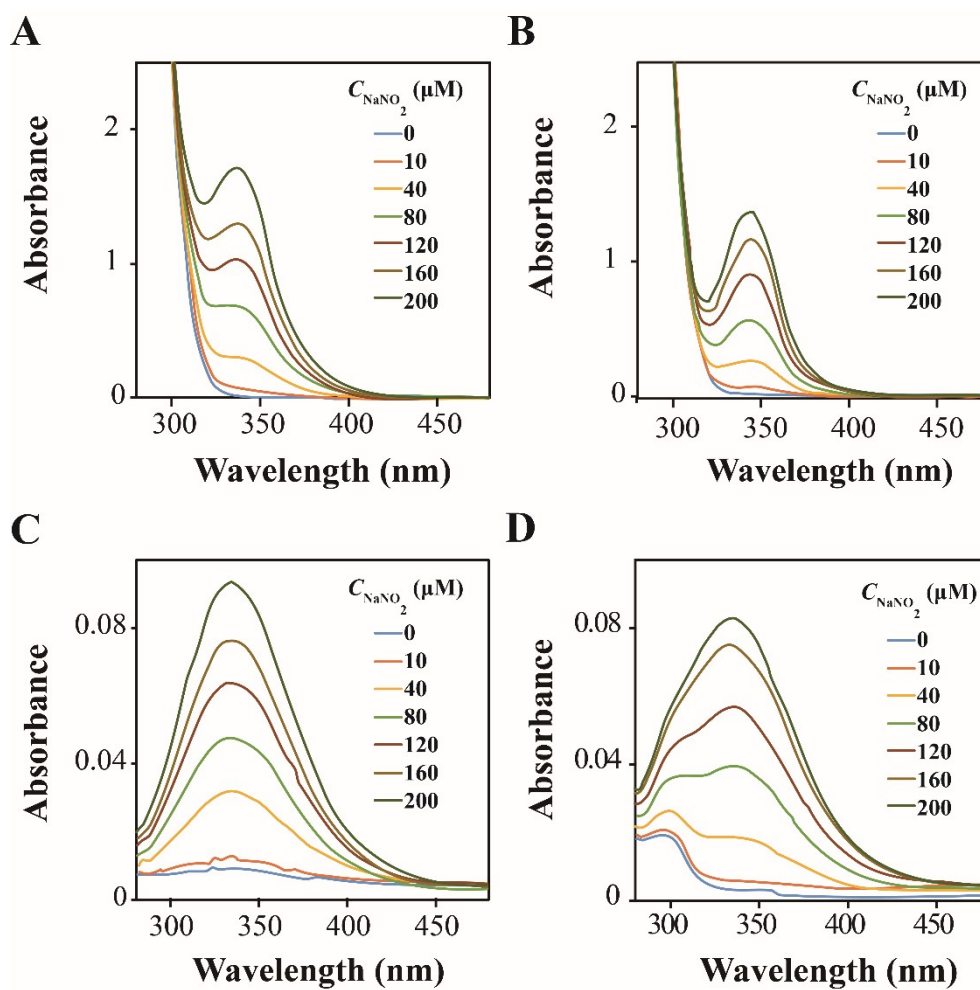
Fig. S7 UV-vis absorption spectra of RSNO$_{TBA}$ (A), RSNO$_{AHMP}$ (B), RSNO$_{GSH}$ (C), and RSNO$_{TPN}$ (D) *versus* the concentration of nitrite from 0 to 100 μM. The concentrations of TBA, AHMP, GSH and TPN are 1, 1, 5, and 5 mM, respectively.
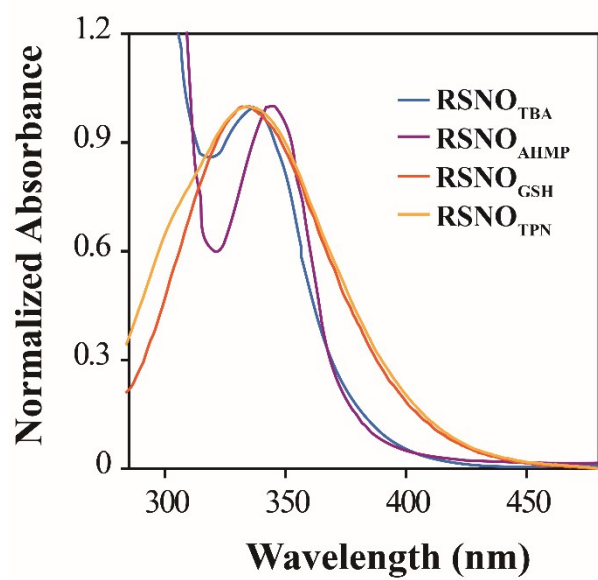
Fig. S8 Normalized UV-vis absorption spectra of $RSNO_{TBA}$, $RSNO_{AHMP}$, $RSNO_{GSH}$, and $RSNO_{TPN}$.
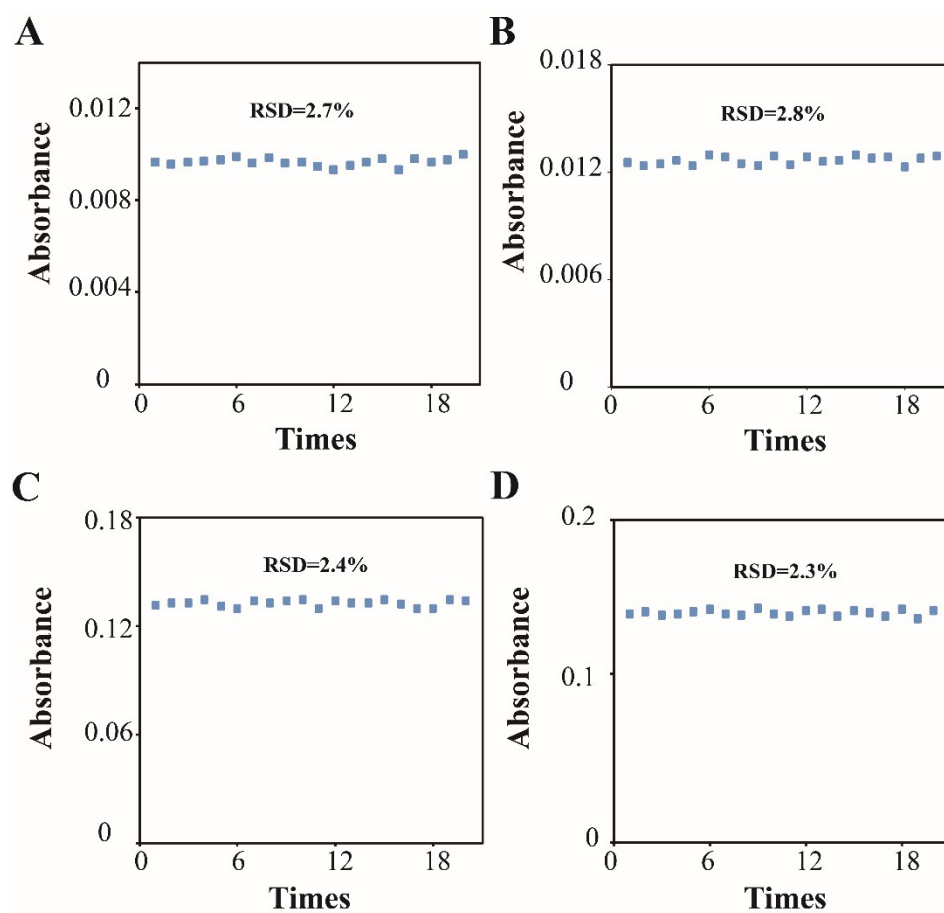
Fig. S9 The absorbance signals of thiol compounds-NaNO$_2$ solutions for 20 repeated measurements. (A) 5 mM TPN and 20 μM NaNO$_2$, (B) 5 mM GSH and 20 μM NaNO$_2$, (C) 1 mM AHMP and 20 μM NaNO$_2$, and (D) 1 mM TBA and 20 μM NaNO$_2$.

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Table S1. The calculated energy gaps, potentials and molar absorption coefficients of S-nitrosothiol compounds.

Samples	E_{OX} /V	E_{HOMO} /eV	E_{Red} /V	E_{LUMO} /eV	E_g /eV	ε (cm^{-1}·moL^{-1})
RSNO$_{TBA}$	0.05	-4.76	-0.12	-4.59	0.17	1.8×10^4
RSNO$_{AHMP}$	0.05	-4.76	-0.19	-4.52	0.24	1.3×10^4
RSNO$_{GSH}$	1.36	-6.02	-1.64	-3.08	3.00	9.1×10^2
RSNO$_{TPN}$	1.51	-6.22	-1.71	-3.00	3.22	8.2×10^2

Table S2. Comparison of this work with some established nitrite detection methods.

Method	Material	Detection limit	Linear range	Ref
Colorimetric detection	μPAD	1μM	10-150μM	1
	GNRs	0.5μM	1-15μM	2
	TMB	1μM	0.5-30μM	3
	AuNPs-rGO	0.1μM	1-20μM	4
	AuNPs	1μM	0-200μM	5
Fluorescence Detection	N-CNDs	1μM	0-1mM	6
	Fe3O4@Rh 6G	0.8μM	1-50μM	7
	Azo-based probe	0.6 μM	0.3−3.0 μM	8
Electrochemical detection	ZnP−C_{60}	1.44μM	2-164μM	9
UV resonance Raman spectra	WWTPs	<14μM	7-3500μM	10

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