Electronic Supplementary Information

A colorimetric and fluorescence lighting-up sensor based on ICT coupled with PET for rapid, specific and sensitive detection of nitrite in food

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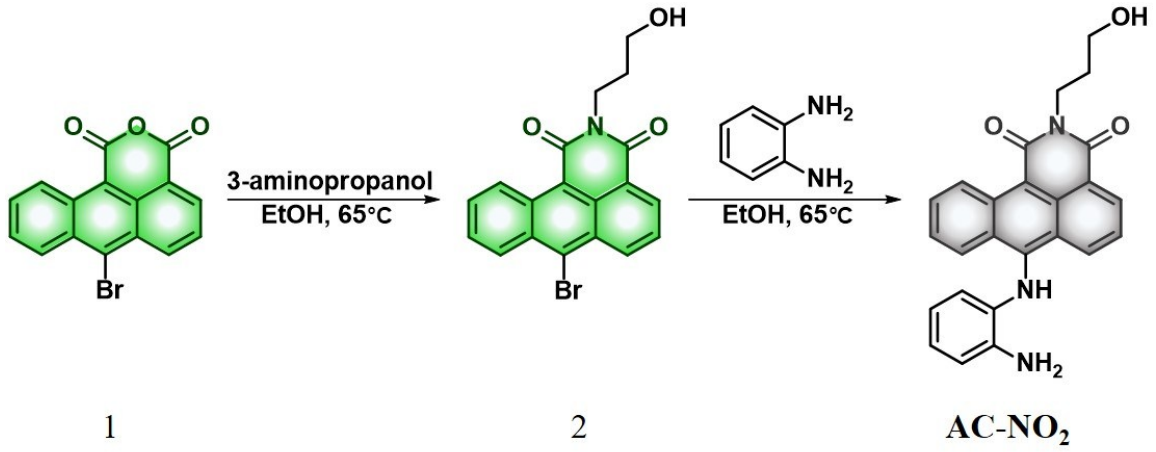
1. Experimental details

1. 1 General

All reagents and solvents in this work were purchased from commercial suppliers and used directly without further purification. 1,2-Phenylenediamine and oxalic dichloride were purchased from Sigma-Aldrich, and 9-bromoanthracene and 3-amino-1-propanol were purchased from TCI. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AV-400 spectrometer. High-resolution mass spectra (HRMS) were recorded using a HP-1100 LC-MS spectrometer. Fluorescence and UV-vis absorption spectra were measured with a Hitachi FL-7000 fluorometer and a Hitachi UV-3310 spectrometer, respectively. The solutions with different pH values were adjusted by adding very small amounts of NaOH or HCl solution into twice-distilled water using a PHS-3C pH meter. The fluorescence quantum yields were determined by a relative method with rhodamine B (Φ = 0.65 in ethanol) and fluorescein (Φ = 0.90 in 0.1 M NaOH) as the standards.



Scheme S1 Synthetic route to the sensor **AC-NO_2**.

1.2 Synthesis of compound 2

Compound 1 (997 mg, 3.06 mmol) was added to a three-necked round bottom flask containing 30 mL anhydrous EtOH, while stirring 3-aminopropanol (252 mg, 3.36 mmol) was added slowly under N_2 atmosphere and the reaction was stirred at 65 °C for 6 h. After reaction was complete, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/EtOH = 80/1 (*v*/*v*) as the eluent to afford compound 2 as a brownish

yellow solid (376 mg, 32% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.78 (d, J = 9.0 Hz, 1H), 8.64 (dd, J_1 = 8.7 Hz, J_2 = 0.8 Hz, 1H), 8.55 (dd, J_1 = 7.0 Hz, J_2 = 0.8 Hz, 1H), 8.43 (d, J = 8.6 Hz, 1H), 7.88–7.79 (m, 2H), 7.78–7.69 (m, 1H), 4.58 (t, J = 5.1 Hz, 1H), 4.18–4.07 (m, 2H), 3.56 (dd, J_1 = 11.1 Hz, J_2 = 6.0 Hz, 2H), 1.89–1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.95, 162.12, 134.13, 133.38, 133.09, 132.69, 131.20, 130.47, 128.45, 127.70, 127.60, 126.42, 122.23, 114.97, 59.07, 37.99, 30.92. HRMS (EI): m/z calcd for $[C_{19}H_{15}BrNO_3]^+$ 384.0230 ($[M+H]^+$), found 384.0235.

1.3 Synthesis of sensor AC-NO_2

A mixture of compound 2 (100 mg, 0.26 mmol) and 1,2-phenylenediamine (53 mg, 0.49 mmol) in 20 ml anhydrous EtOH was stirred at 80 °C for 10 h. Then the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH = 40/1 as the eluent to afford the **AC-NO_2** as a dark purple solid (49 mg, 46% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.98 (d, J = 9.0 Hz, 1H), 8.82 (s, 1H), 8.68–8.50 (m, 2H), 8.35 (d, J = 8.7 Hz, 1H), 7.79 (t, J = 7.8 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 6.97–6.79 (m, 2H), 6.45–6.27 (m, 2H), 5.42 (s, 2H), 4.55 (t, J = 5.1 Hz, 1H), 4.20 (t, J = 7.4 Hz, 2H), 3.54 (dd, J_1 = 11.3 Hz, J_2 = 6.0 Hz, 2H), 1.91–1.77 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.77, 163.00, 149.23, 141.72, 134.96, 133.33, 131.79, 131.68, 131.03, 129.87, 126.24, 125.42, 125.03, 124.41, 123.10, 123.06, 122.60, 121.74, 119.99, 116.68, 115.55, 105.60, 59.13, 37.40, 31.24. HRMS: m/z calcd for $[C_{25}H_{22}N_3O_3]^+$ 412.1656 ($[M+H]^+$), found 412.1667.

1.4 Spectral responses of the sensor AC-NO_2 towards various analytes

A stock solution of **AC-NO_2** (1 mM) was prepared in EtOH (HPLC grade). Stock solutions of analytes (1–5 mM) were prepared in twice-distilled water: $Na_2S_2O_3$, $NaNO_3$, NaI, NaClO, Na_2SO_4, NaBr, $NaHSO_3$, NaHS, H_2O_2, CH_3COONa, $NaHCO_3$, $HgCl_2$, $ZnCl_2$, $FeCl_2$, $CaCl_2$, $CuCl_2$, $FeCl_3$, Hcy, Cys, GSH, $AlCl_3$, TBHP, CH_3COOH, CH_3CH_2COOH. The sensor **AC-NO_2** was diluted to 10 μM with a HCl solution containing 20% EtOH for spectral measurements. EtOH was used to dissolve the sensor in all the measurements. All spectroscopic experiments were carried out at room temperature.

1.5 Determination of the detection limit

The detection limit of **AC-NO_2** sensor for NO_2^- was determined from fluorescence titration based on the signal to noise ratio (S/N =3). To determine the noise value, the fluorescence intensity of **AC-NO_2** at 522 nm was measured five times and the average deviation of blank measurement was determined. Then, the fluorescence intensity of **AC-NO_2** solution (2.0 μM) was recorded after addition of various low concentrations of NO_2^- for 4 min. The concentration of NO_2^- that caused a 3-fold fluorescence enhancement (F_{522}) relative to the average deviation of blank measurements was determined to be the detection limit.

1.6 Preparation of paper-based sensor

Filter paper was cut into strips with the size of 0.5 × 2 cm, and immersed in an ethanol solution containing 1.0 mM probe for 5 min. These paper strips were taken out and sprayed with hydrochloric acid (pH = 1) and then dried in the shade.

1.7 Determination of NO_2^- by using paper-based AC-NO_2 sensor

Paper-based **AC-NO_2** sensors were placed on a clean glass plate. The test solutions were dripped onto these paper-based strips, after 4 min. these paper-based stripes were placed in a dark box, and the photographs were taken under daylight and UV light (365 nm), respectively.

1.8 Determination of NO_2^- with spectrophotometry

2.5 g homogeneous food sample was placed in a 250 mL flask with stopper. 6.25 mL sodium tetraborate decahydrate solution (50 g/L) and 75 mL twice-distilled water at 70 ^oC were added to this flask. The mixtures were stirred homogeneously and heated by a water bath at 100 ^oC for 15 min. The flask was cooled down to room temperature and the solution was transferred to a 100 mL pycnometer flask, then 2.5 mL $K_4Fe(CN)_6$ at a concentration of 106 g/L and 2.5 mL $Zn(AcO)_2$ at a concentration of 220 g/L were added to precipitate proteins from the solution. Distilled water was

added to reach the level (100 mL) of the pycnometer flask, shaken to make it homogeneous, and suspended for 30 min. The supernatant fat at the upper layer was removed, and the remaining solutions were filtered to remove some solid. Finally, the filtrate was collected for testing.

Aliquots (40 mL) of food sample solution mentioned above were placed in 50 mL colorimetric tube with stopper, and various amounts of NO_2^- (0, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0, 12.5 μg) were added. Then, 2 mL of 4-anilinesulfonic acid was added to the above solution, mixed homogeneously and suspended for 5 min. 1 mL of naphthylethylenediamine dihydrochloride (2 g/L) was added to the above solution, mixed homogeneously and suspended for another 15 min. Finally, the absorbance at 538 nm were measured with a Hitachi UV-3310 spectrometer for three times. The results were shown as the mean ± standard deviation (n = 3). The absorbance at 538 nm were plotted as a function of NO_2^- concentration to generate a calibration curve.

2. Determination of NO_2^- in food by the sensor AC-NO_2

Sauerkraut, radish strip, luncheon meat and ham sausage were purchased from a local supermarket. Nitrite in these food samples were extracted according to reported literature procedures.[1-3] The detailed procedure is described as below: Food sample (5.0 g) was prepared into a homogenate by a pulverizer. 1g of food sample and 20 ml of twice-distilled water were placed in a beaker and sonicated for 30 min at 60 °C. The food suspension was cooled down to room temperature and the supernatant was filtered with an organic membrane (0.22 μm, Nylon-66). Then, filtrates were adjusted to pH 1.0 with 1M HCl. The filtrate solution of sauerkraut, radish strip were diluted twice with HCl solution (pH = 1) before measurement; the filtrate solution of luncheon meat and ham sausage were diluted five times with HCl solution (pH = 1) before measurement. Finally, absolute ethanol was added to these solutions to yield an EtOH/HCl solution (v/v = 1/4, pH =1) for testing. Aliquots of these food samples were also spiked with NO_2^- at different concentrations (0, 5.0, 10.0, 15.0, 25.0 μM). The resulting samples were further incubated with the sensor **AC-NO_2** (10 μM) at room temperature for 4 min. Then the fluorescence spectra were recorded with the FL-7000 fluorometer. The results were shown as the mean ± standard deviation (n = 3).

Table S1 Determination of $NaNO_2$ by using sensor **AC-NO_2** and a spectrophotometric method

Sample	Added amount (μM)	Found amount (μM)	Recovery (%)	Spectrophotometric method (μM)
Sauerkraut	0	3.7 ± 0.31	-	3.98 ± 0.21
	5	8.07 ± 0.21	87.4	8.37 ± 0.18
	10	13.95 ± 0.36	102.5	14.65 ± 0.23
	20	25.42 ± 0.42	108.6	25.82 ± 0.31
Radish strips	0	1.75 ± 0.24	-	1.78 ± 0.18
	5	5.92 ± 0.35	83.4	5.79 ± 0.25
	10	11.36 ± 0.50	96.1	11.62 ± 0.30
	20	22.53 ± 0.48	103.9	22.81 ± 0.37
Luncheon meat	0	4.44 ± 0.39	-	4.94 ± 0.30
	5	8.65 ± 0.37	84.2	9.13 ± 0.28
	10	13.28 ± 0.62	88.4	13.98 ± 0.33
	20	24.87 ± 0.58	102.1	25.72 ± 0.38
Ham sausage	0	4.09 ± 0.33	-	4.89 ± 0.22
	5	8.92 ± 0.27	96.6	9.48 ± 0.17
	10	14.31 ± 0.43	102.2	14.91 ± 0.23
	20	25.55 ± 0.50	107.3	26.25 ± 0.29

3. The linear relationship between absorbance and the concentration of NO_2^-

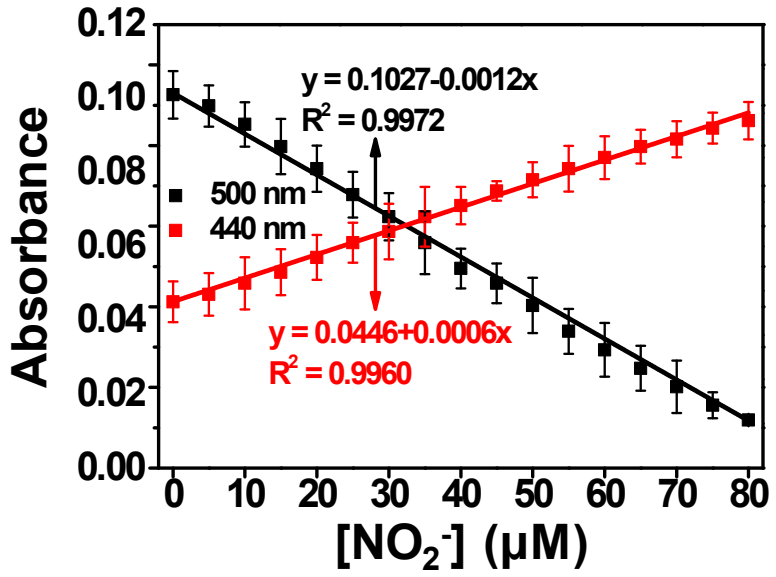


Fig. S1 The linear relationship between the absorbance of **AC-NO_2** (10 μM) and the concentration of NO_2^- (0–80 μM) in an EtOH/HCl solution (*v*/*v* = 1/4, pH = 1).

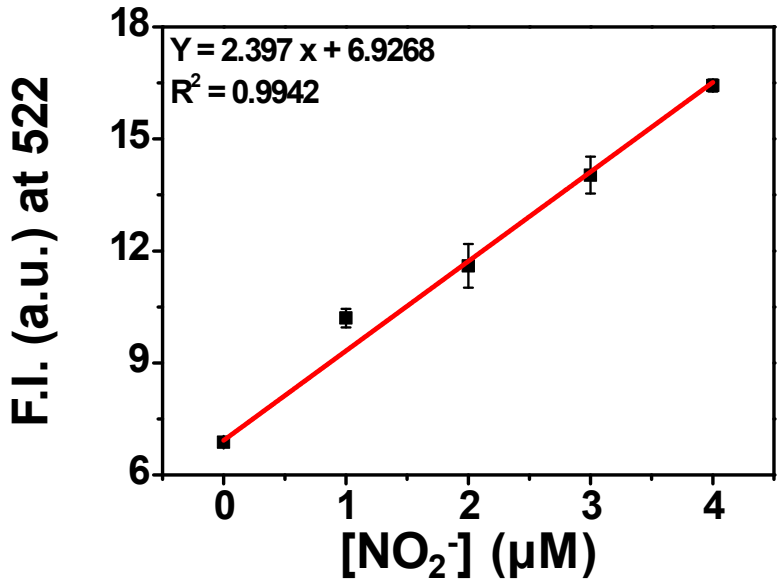


Fig. S2 Linear relationship between the fluorescence intensity of **AC-NO_2** (2 μM) and the concentration of NO_2^- (0–4 μM) in an EtOH/ HCl solution (*v*/*v* = 1/4, pH = 1). Conditions: λ_{ex} = 440 nm; λ_{em} = 522 nm.

4. pH-dependent fluorescence response of the sensor towards NO_2^-

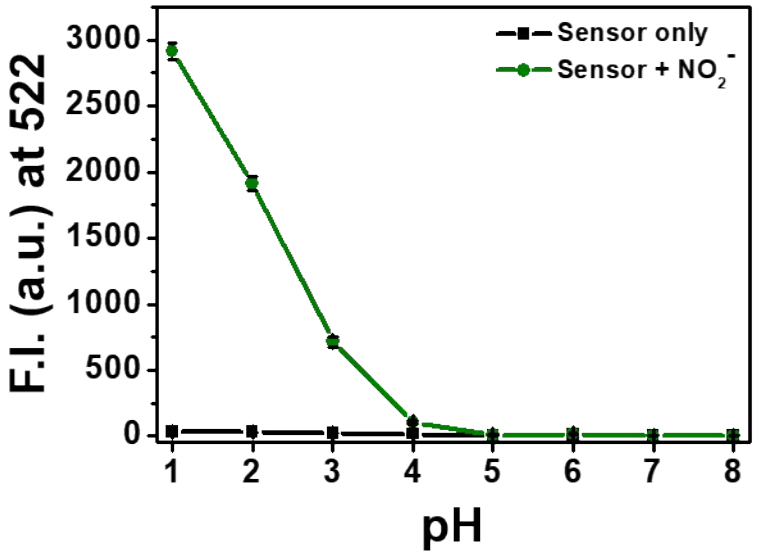


Fig. S3 The pH-dependent fluorescence response of **AC-NO_2** (10 μM) towards NO_2^- . Conditions: λ_{ex} = 440 nm; λ_{em} = 522 nm.

5. ^{1}H NMR, ^{13}C NMR and MS spectra

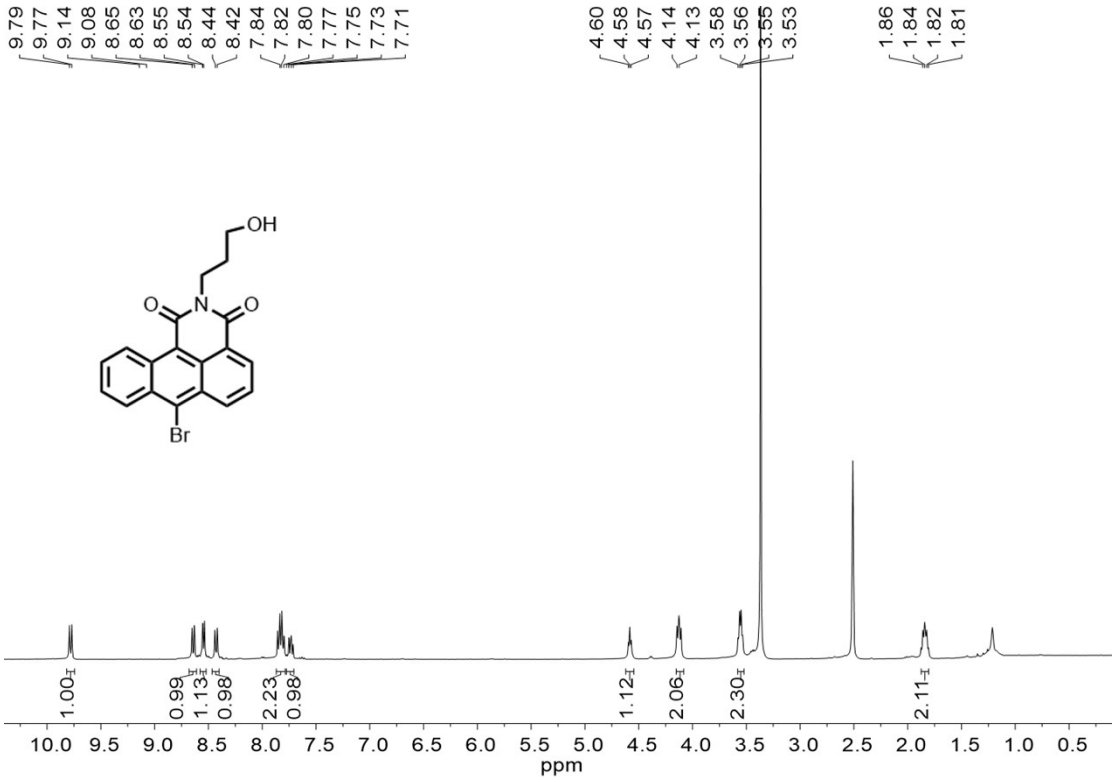


Fig. S4 ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

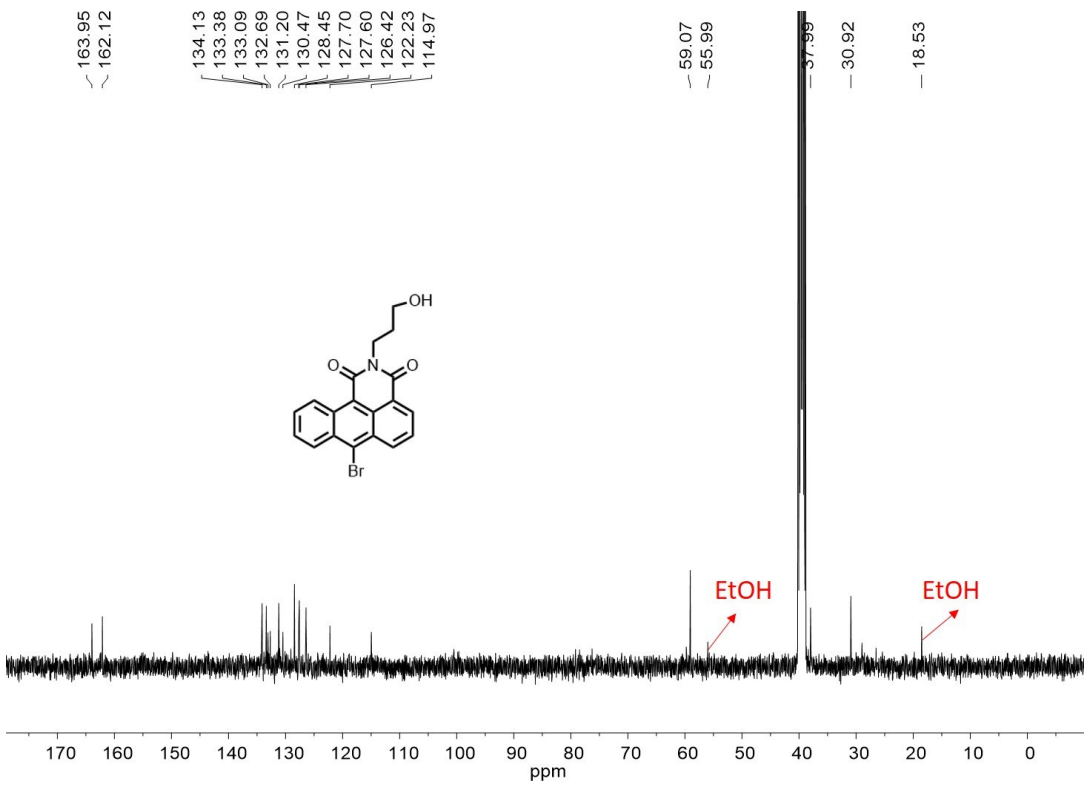


Fig. S5 ^{13}C NMR spectrum of compound **2** in DMSO-d_6.

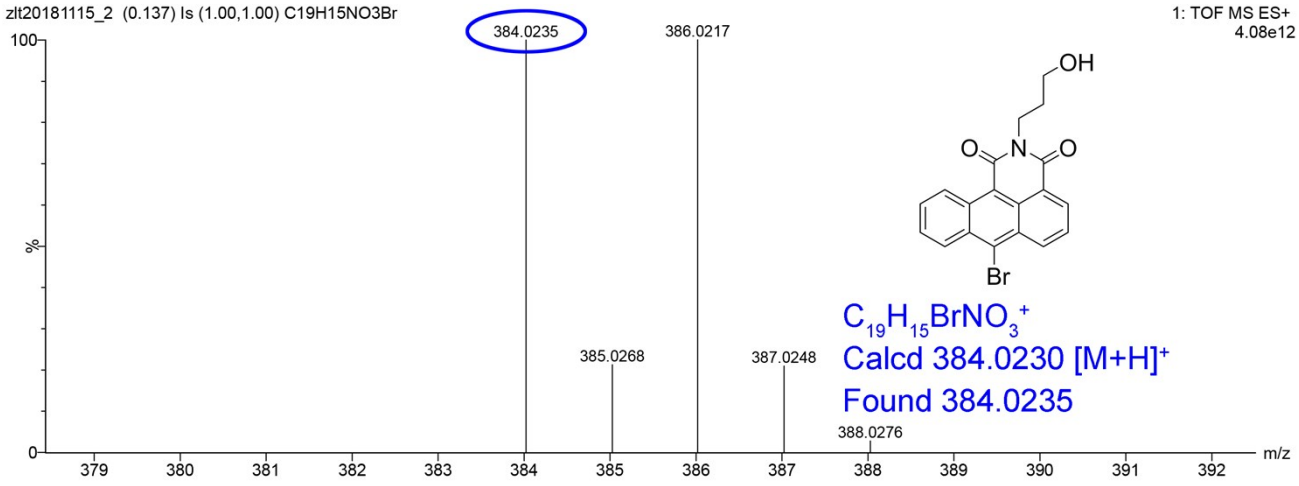


Fig. S6 HR-MS spectrum of compound **2**.

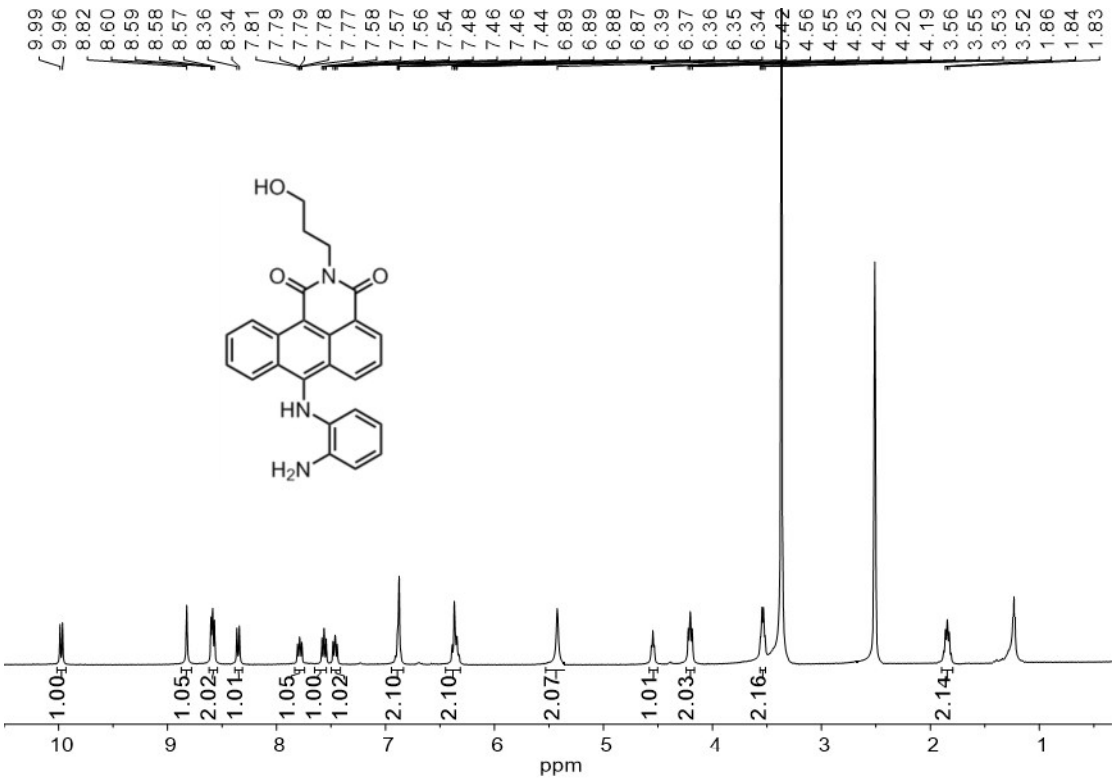


Fig. S7 ^{1}H NMR spectrum of **AC-NO$_2$** in DMSO-d_6.

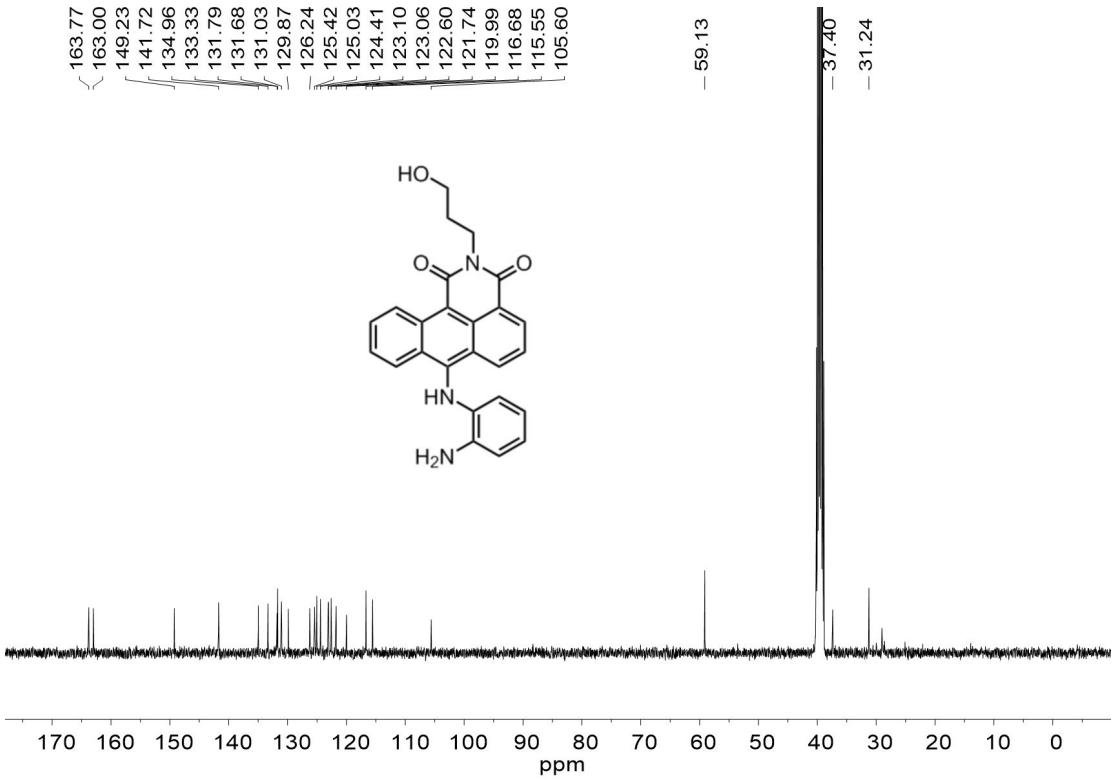


Fig. S8 ^{13}C NMR spectrum of **AC-NO$_2$** in DMSO-d_6.

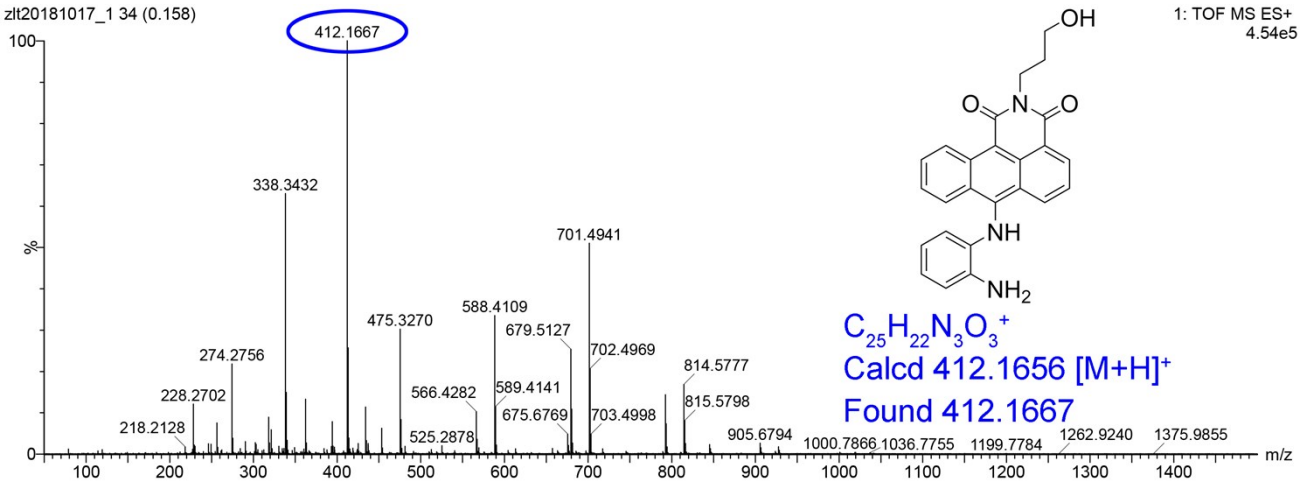


Fig. S9 HR-MS spectrum of **AC-NO$_2$**.

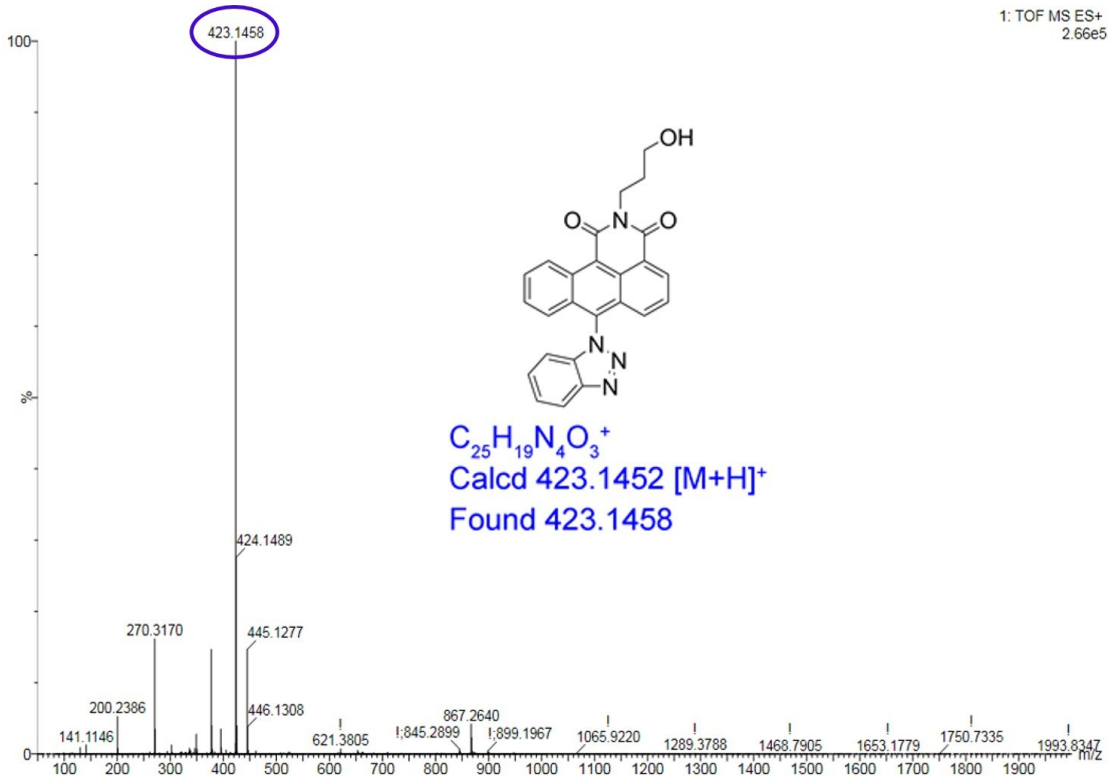


Fig. S10 HR-MS spectrum of **AC-TAz** formed from the reaction of **AC-NO$_2$** and NO_2^-.

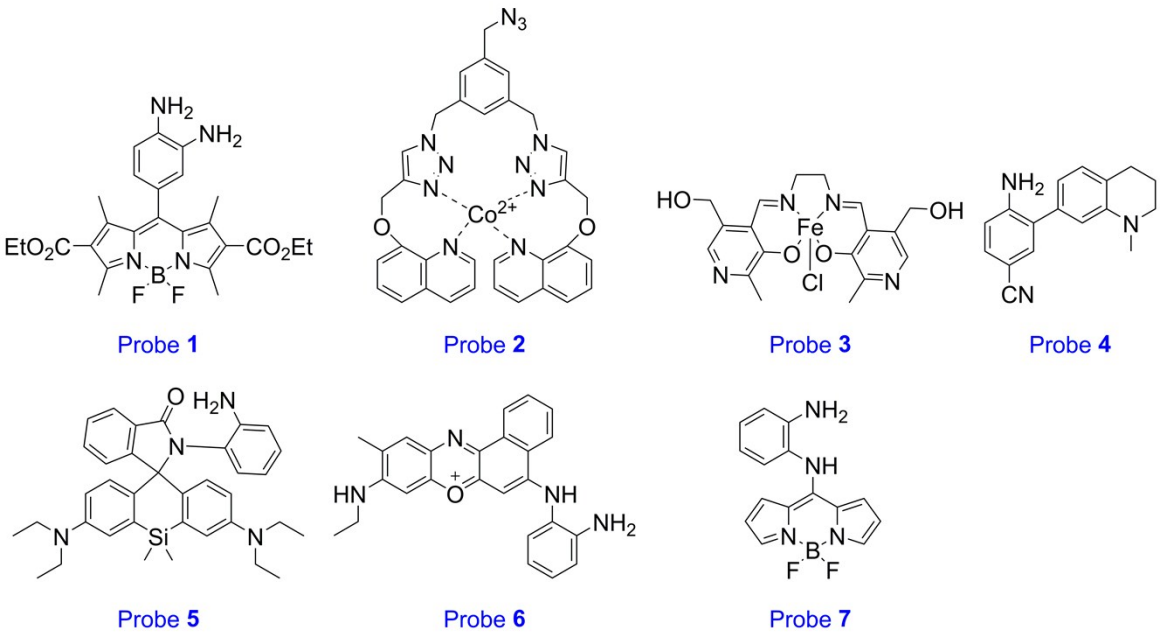


Fig. S11. Chemical structures of fluorescence "turn-on" probes for the determination of NO_2^-.

Table S2. Detection performance of fluorescence "turn-on" probes for NO_2^-.

Probe	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**AC-NO_2**
Emission	505 nm	395 nm	375 nm	555 nm	685 nm	638 nm	532 nm	522 nm
Colorimetric detection	No	No	No	No	No	Yes	Yes	Yes
Response time	15 min	Not mentioned	Not mentioned	~60 s	~15 min	< 20 min	30 min	< 4 min
Selectivity	Good	Good	Low	Not mentioned	Good	Good	High	High
Limit of detection (LOD)	0.21 nM	Not mentioned	Not mentioned	2.6 nM	9.4 nM	45 nM	15 nM	84 nM
Paper strip fabrication	No	No	No	No	Yes	No	No	Yes
Application in real food samples	Yes	No	No	No	No	No	No	Yes
Reference	[4]	[5]	[6]	[7]	[8]	[9]	[10]	**This work**

References

[1] H. H. Ren, Y. Fan, B. Wang and L. -P. Yu, *J. Agric. Food Chem.*, 2018, **66**, 8851-8858.

[2] G. Yildiz, N. Oztekin, A. Orbay and F. Senkal, *Food Chem.*, 2014, **152**, 245-250.

[3] J. J. Liu, Y. L. Chen, L. L. Wang, M. Na, H. L. Chen and X. G. Chen, *J. Agric. Food Chem.*, 2019, 67, 3826-3836.

[4] K.-J. Huang, H. Wang, Y.-H. Guo, R.-L. Fan, and H.-S. Zhang, *Talanta*, 2006, **69**, 73-78.

[5] A. K. Mahapatrac, G. Hazra, S. K. Mukhopadhyay, and A. R. Mukhopadhyay, *Tetrahedron Lett.*, 2013, **54**, 1164-1168.

[6] M. Strianese, S. Milione, V. Bertolasic, and C. Pellecchia, *Inorg. Chem.*, 2013, **52**, 11778- 11786.

[7] Y. Shen, Q. Zhang. X. Qian, and Y. Yang, *Anal. Chem.*, 2015, **87**, 1274-1280.

[8] M. Cai, X. Chai, X. Wang, and T. Wang, *J. Fluoresc.*, 2017, **27**, 1365-1371.

[9] Y.-H. Zhan, R. Sun, W.-J. Zhu, Y.-J. Xu, and J.-F. Ge, *Sensor. Actuat. B-Chem.*, 2017, **240**, 1283-1290.

[10] J. Zhang, F. Pan, Y. Jin, N. Wang, J. He, W. Zhang, and W. Zhao, *Dyes Pigm.*, 2018, **155**, 276-283.