Supplementary Information

Bright sensitive ratiometric fluorescent probe enabling endogenous FA imaging and mechanistic exploration of indirect oxidative damage of FA in various living systems

Kun Dou\textsuperscript{a}, Guang Chen\textsuperscript{a,b,c,*}, Fabiao Yu\textsuperscript{b}, Yuxia Liu\textsuperscript{a}, Lingxin Chen\textsuperscript{b}, Ziping Cao\textsuperscript{a}, Tao Chen\textsuperscript{c}, Yulin Li\textsuperscript{c} and Jinmao You\textsuperscript{a,b,*}

\textsuperscript{a}The Key Laboratory of Life-Organic Analysis; Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, China.

\textsuperscript{b}Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China.

\textsuperscript{c}Key Laboratory of Tibetan Medicine Research & Qinghai Key Laboratory of Qinghai-Tibet Plateau Biological Resources, Northwest Institute of Plateau Biology, Chinese Academy of Science, Xining 810001, Qinghai, PR China.

\textsuperscript{*}Phone: +86-537-4458301. Fax: +86-537-4458301

E-mail: chenandguang@163.com; jmyou6304@163.com
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1. Materials and reagents

Unless otherwise stated, all reagents and materials were purchased from commercial accompany and used without further purification. Terephthalaldehyde, 9, 10-phenanthroquinone, allylboronic acid pinacol ester and 2,2-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH), Vitamin E were purchased from Sigma-Aldrich Co. (Steinheim, Germany). DMSO, DMF, CH$_3$OH, petroleum ether and ethyl acetate were analytical grade without further purification. Analytes including formaldehyde, glyoxal, acetaldehyde, methylglyoxal, 4-methoxybenzaldehyde, 4-nitrobenzaldehyde, acetone, benzaldehyde, thiol-containing compounds (GSH, Cys and Hcy), ROS (H$_2$O$_2$ and ClO$^-$), RSS (HS$, HSO_3^-$) were prepared in distilled water. Water used in all experiments was the double-distilled water.

2. Instruments

UV-visible spectra were collected on Cary 300 Bio UV-vis spectrophotometer (VARIAN, USA), and fluorescence spectra were detected with Hitachi F-7000 fluorescence spectrophotometer (HITACHI, Japan). $^1$HNMR, $^{13}$CNMR spectra were measured with the Bruker ascend 500 (500.1 MHz,$^1$H; 125.8 MHz,$^{13}$C) instrument operating at the denoted spectrometer frequency given in mega Hertz(MHz). Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane (TMS) as an external standard for $^1$H and $^{13}$C-NMR spectra and calibrated against the solvent residual peak. ESI-MS and MS data were recorded on Agilent 1100 Series LC-MSD Trap-SL (ion trap) mass spectrometer. Fluorescent confocal images for cells by a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens ($\times$40) and zebrafish and tissue were acquired by Carl Zeiss LSM880 with a laser scanning microscope with an objective lens ($\times$20) and ($\times$ 40), respectively (Carl Zeiss, Germany). TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 300-400), both of which were obtained from the JianYou Chemical Company (Yan Tai, China). All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China). Flow cytometry assay were carried by Novocyte2040R (ACEA, USA).

3. Synthesis and Characterization

3.1 Synthesis of compound: 4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde (PIBE)

To a solution of glacial acetic acid (25 mL) in 50-mL round-bottom flask, 1,4-phthalaldehyde (600 mg, 4.5 mmol), 9,10-phenanthroquinone (310 mg, 1.5 mmol) and ammonium acetate (2.15 g, 30
mmol) were added. The mixture was heated to reflux for 30 min and then cooled to room temperature. The precipitate was collected by filtration, washed with acetone, and purified by column chromatography on silica gel using petroleum ether /ethyl acetate (v/v= 3:1). The yellow solid was obtained (395.9 mg, 79.3%). $^1$H NMR (DMSO-d$_6$, 500MHz), $\delta$ (ppm): 10.14 (s, 1H), 8.86 (d, $J = 8.5$ Hz, 2H), 8.62 (d, $J = 4.5$ Hz, 2H), 8.15 (q, $J = 8.5$ Hz, 2H), 7.79 (q, $J = 7.5$ Hz, 2H), 7.68 (d, $J = 7.0$ Hz, 2H). $^{13}$C NMR (DMSO-d$_6$, 125MHz), $\delta$ (ppm): (193.42, 151.33, 130.75, 130.20, 127.91, 127.71, 126.97, 126.22, 125.98, 124.94, 124.10, 122.35, 121.97. MS m/z calcd for C$_{22}$H$_{14}$N$_2$O 322.37, [M+H]$^+$ found 322.8.

3. 2 Synthesis of probe 4-(1H-phenanthro[9,10-d]imidazol-2-yl) phenyl) but-3-en-1-amine (PIPBA)

A solution of PIBE (0.322 g, 1 mmol) in anhydrous CH$_3$OH (20 ml) was cooled in an ice bath. Under vigorous stirring, 6 ml of NH$_3$ solution (7.0 N in CH$_3$OH, 42 mmol) was added and after 30 min, allylboronic acid pinacol ester (0.48 mL, 2.5 mmol) was added. The resulting mixture was warmed to ambient temperature and stirred overnight. The solvent was removed under reduced pressure, and the crude product was further purified by silica column chromatography using petroleum ether / ethyl acetate (1:1, v/v) as eluent to afford PIPBA (163 mg, 44%) as a light yellow solid. $^1$H NMR (DMSO-d$_6$, 500MHz), $\delta$ (ppm): 8.851 (d, $J = 8.0$ Hz, 2H), 8.839 (s, 2H –NH$_2$), 8.400 (d, $J = 8.0$ Hz, 2H), 8.045 (d, $J = 8.0$ Hz, 2H), 7.761 (t, $J = 15.0$ Hz, 2H), 7.651 (m, $J = 31.5$ Hz, 2H), 5.815 (m, $J = 35.0$ Hz, 1H), 5.075 (t, $J = 29.5$ Hz, 2H), 4.061 (t, $J = 13.0$ Hz, 1H), 2.468 (t, $J = 13.0$ Hz, 2H). $^{13}$C NMR (DMSO-d$_6$, 125 MHz), $\delta$ (ppm): (149.75, 147.15, 136.07, 129.41, 128.04, 127.58, 127.50, 126.59, 125.61, 124.31, 122.64, 117.67, 55.34, 43.76. MS m/z calcd for C$_{22}$H$_{14}$N$_2$O 363.17 [M+H]$^+$, found 363.9.

3.3 Synthesis of HSO$_3^-$ donor (BTSA)

Benzylamine (0.321g, 3mmol) was dissolved in 10 ml dry pyridine. To the solution, 0.2 ml of Et$_3$N was added. The solution was cooled to 0 °C. Then, a solution of 2,4-dinitrobenzenesulfonyl chloride (0.792g, 3mmol) in dry pyridine (5 mL) was slowly added. After stirring for 15min, the mixture was heated to room temperature and stirred for another 3h. The resultant mixture was poured into 20 ml water and filtered. The crude product was recrystallized in CH$_3$OH to get yellow solid, N-benzyl-2,4-dinitrophenylsulfonamide (BTSA). ESI-MS m/z calcd for C$_{13}$H$_{11}$N$_3$O$_5$ [M+H]$^+$, 338.04, found 338.1. $^1$H NMR (CD$_3$Cl, 500MHz), $\delta$ (ppm): 8.86 (d, $J = 2.0$ Hz, 1H), 8.364 (s, J, 1H), 8.120 (d, J = 8.5 Hz, 2H).
S5 Hz, H), 7.107 (d, J = 7.5 Hz, 3H), 6.996 (d, J = 6.5 Hz, 2H), 3.709 (s, 2H). 13C NMR (CD3Cl, 125MHz), δ (ppm): (148.6, 146.82, 138.32, 136.13, 131.24, 127.75, 127.69, 126.09, 126.01, 119.70, 44.35).

4 Optical experiments

Absorption spectra were obtained with 1.0-cm glass cells. Fluorescence emission spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. Fluorescent spectra of probe to FA were recorded at the excitation wavelength of 350 nm, with the excitation / emission slit width of 5 nm/5nm. All the optical properties were evaluated under the physiological conditions using 5 μM of probe PIPBA in DMSO/PBS buffer solution (v/v: 50/50; pH = 7.4, 20 mM). For spectroscopic measurement, DMSO/PBS buffer was used to dilute stock solution of PIPBA to 5 μM.

5 Cell Culture and Imaging

In this work, HeLa cells were obtained from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), which were incubated in DMEM (Dulbecco’s Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100U/mL, 100 μg/mL, Invitrogen) at 37 °C under a humidified atmosphere containing 5% CO₂. Before imaging, cells were placed at 25-Petri dishes and allowed to adhere for 24 hours.

The imaging assay of FA inside the cells included four stages of experiments. First stage, experiments were performed to confirm the capability of probe for imaging exogenous FA in HeLa cells. 5 μM PIPBA was used to incubate the cells for 30 min, and then FA in different concentrations (0 μM, 150 μM, 400 μM, 750 μM, 1 mM) was added for 2 h of incubation. The tested cells were analyzed by confocal imaging. Second stage, the capability of probe for imaging endogenous FA were evaluated. To imaging the endogenous FA, Hela cells were pretreated with the FA scavenger HSO₃⁻ (200 μM) for 30 min, which was followed by 2 h of incubation with 5 μM PIPBA. Then the cells were washed twice by PBS to remove the residue and then analyzed by confocal imaging to report the endogenous FA. To confirm the results, the tested cells were further incubated with FA (500 μM) and analyzed by confocal imaging. Third stage, the intracellular quantification of endogenous FA was achieved by collecting the fluorescence ratios. Fourth stage, to explore the biological role of FA in presence of free radicals, imaging assay was performed. HeLa cells were divided to several groups: group a was incubated with PIPBA (5 μM); group b was incubated with FA (0.8 mM) for 2h and then with PIPBA (5 μM) for 2 h; group c was treated with 1 mM of 2,2-
azobis-[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH) for 2 h and then with probe PIPBA (5 μM) for 2 h; group d were treated successively with formaldehyde (0.8 mM) and AIPH (1 mM) for 2 h, and then PIPBA (5 μM) for 2 h; and as a control, HSO₃⁻-pretreated cells were incubated with PIPBA (5 μM) for 2 h and then with formaldehyde (0.8 mM) and AIPH (1 mM) for additional 2 h.

Fluorescence imaging was performed by a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (×40). Confocal imaging: green channel (λex = 405 nm, λem = 480–580 nm), blue channel (λex = 405 nm, λem = 420–480 nm), ratio imaging (I_{green}/I_{blue}).

6 Flow Cytometry analysis

The cells were cultured at 2.0 × 10⁵ cells/well in 6-well plates, and then treated with probes as described in the confocal imaging. After harvest, cells were washed and suspended in fresh complete medium and analyzed by flow Cytometry (Novocye2040R, ACEA, USA). Excitation wavelength was 488 nm. The collected wavelengths were 500-560 nm (FITC).

7 Confocal imaging of FA in zebrafish

In this study, zebrafishes were provided by HuanTe biological corporation (Hangzhou, China), and animal experiments were performed in full compliance with international ethical guidelines. Seven days old of larva were incubated in E3 media (15 mM NaCl, 0.5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, 0.15 mM KH₂PO₄, 0.05 mM Na₂HPO₄ and 0.7 mM NaHCO₃; pH 7.5). The tested larva were pretreated with or without HSO₃⁻ (500 μM) for 1 h, then 5 μM probe added to the media for another 3 h of incubation. Then confocal imaging was performed to explore the endogenous FA in zebrafish. To further confirm the obtained results, above zebrafish that was pretreated by HSO₃⁻ was retreated with various concentration of exogenous FA and then analyzed by confocal imaging. The confocal imaging was operated by Carl Zeiss LSM880 with a laser scanning microscope with an objective lens (× 10).

8 Confocal imaging of FA in renal tissue of living mouse

The imaging assay of FA in renal tissue included three stages of experiments. Kunming mice (male, 25 g) were kindly handled during the experiments in full compliance with international ethical guidelines. First stage, we carried out the in vitro experiments to validate the fluorescent ratio response of PIPBA toward FA in renal tissue. The fresh kidney harvested from mice were cut into about 400 μm in size and were pretreated as following: 1) with PIPBA (5 μM) for 2 h; 2) with HSO₃⁻ (200 μM) for 30 min and then PIPBA (5 μM) for 2 h; 3) with PIPBA (5 μM) for 30 min, and
then with various levels of exogenous FA (250 μM; 750 μM) for 2 h. Second stage, we performed the in vivo experiments to evaluate the capability of PIPBA for reporting the endogenous FA in living renal tissue. Kunming mice (male, 25 g) were fasted for 12 h as blanks. The blank mice were injected intraperitoneally with 500 μL of saline (0.9%) containing 500 μM of BTSA (N-benzyl-2,4-dini-trophenylsulfonamide) to scavenge the endogenous FA (Figure S13-S14). After 2 h, these mice were injected with 40 μL of probe PIPBA (25 Mm and then incubated for another 2 h. Correspondingly, the blank mice were injected with the saline (0.9%). The tested mice were anesthetized by intraperitoneal injection of chloral hydrate (4%; 3 mL/kg) and laparotomized to expose the kidney. Saline was used to wash blood off, and the slice (400 μm) was cut from the intravital kidney for confocal imaging. Third stage, experiments were performed to explore the biological role of FA in the indirect oxidative damage mechanism. Blank mice were injected intraperitoneally with saline (0.9%) every other day for 2 weeks as the control. To obtain the FA-induced renal damage, blank mice were intraperitoneally with FA (10 mg/Kg) diluted by saline every other day for 2 weeks. To explore the protective effect of vitamin E against the toxicity of FA in kidney, the blank mice were intraperitoneally injected with FA (10 mg/Kg) and vitamin E (20 mg/Kg; 40 mg/Kg), respectively and alternatively every other day for 2 weeks. The mice were anesthetized by intraperitoneal injection of chloral hydrate (4%; 3 mL/kg) and vivisected to expose the kidney. Saline (0.9%) was used to wash blood off and the slices (400 μm) were cut from the kidney for the confocal imaging. The confocal imaging was collected Carl Zeiss LSM880 with a laser scanning microscope with an objective lens (× 20). The excitation wavelength was set as 405 nm, and the fluorescence images were collected with the two channels: green channel (λem =480–580 nm) and blue channel (λem =420–480 nm). Meanwhile, the morphological studies on the renal tissue of mouse was performed to monitor the damage using the Olympus IX73-DP80 polarizing microscope (20 × objective) (OLYMPUS, Japan). All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China. Approval Number: No.BZ2014-102R.

9 MTT assays

MTT assay was performed to test the cytotoxicity of probe PIPBA. Hela cells were incubated in 10% Fetal Bovine Serum (FBS, Invitrogen) with a humidified incubator containing 5% CO₂ gas at
37°C for 24 h. Then, the cells were treated by various concentrations of probe (1: 0 μM, 2: 5 μM, 3: 10 μM, 4: 15 μM, 5: 20 μM, 6: 25 μM) for 24 h. Next, 25 μL of methylthiazolyl tetrazolium (MTT) (5 mg mL⁻¹) was added to each of the wells. The resultant cells were incubated for 4 h. The cytotoxicity tests were performed using MTT assays in compliance with ISO standard 10993–5. All experiments were performed in 9 replicates. The cell viability was expressed by the average values ± standard deviation (SD).

10 Morphological studies on the renal tissue of mouse

For the study of renal histology, 400 μm-thick paraffin kidney sections were used for examination with an Olympus IX73- DP80 polarizing microscope using a 20× objective (OLYMPUS, Japan). All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China. Approval Number: No.BZ2014-102R.

11 Quantum yield calculation

The fluorescence quantum yields were determined by comparing the integrated area of the corrected emission spectrum of samples with a reference. In this work, fluorescence quantum yields for probe PIPBA, PIBE were determined in the reference of fluorescein (Φ = 0.98, 0.1 M NaOH) [1]. The quantum yields were calculated using the expression: Φ_{sample} = Φ_{standard} \times \frac{A_{standard} \times F_{sample}}{A_{sample} \times F_{standard}}, where Φ_{sample} and Φ_{standard} are the fluorescence quantum yields of the sample and the standard, respectively; F_{sample} and F_{standard} are the integrated fluorescence intensities of sample and standard spectra, respectively; A_{sample} and A_{standard} are the optical densities at the excitation wavelength of the sample and the standard, respectively.

12 Optimization of sensing condition

Before the application of PIPBA, the sensing conditions were optimized. First, the kinetic profiles for fluorescence intensities ratio (I_{520nm}/I_{440nm}) of PIPBA (5 μM) versus FA were obtained (Fig. 1C, Fig. S5). In addition, temperature-dependent fluorescence was tested (Fig. S6), showing that the desirable response of PIPBA to FA could be achieved at the physiological temperature (37 °C). Therefore, PIPBA exhibited the potential for the detection of FA in biological sample.
the biological applicability, the pH-dependent fluorescence response of probe **PIPBA** in absence and presence of formaldehyde were investigated (Fig. S7), showing that the pH range of 5.0-8.0 was favorable to provide the sensitive response of fluorescence ratio.

### 13 Determination of detection limits

Detection limit for formaldehyde was calculated by the formula: detection limit = 3 SD/ k, where k is the slope of the curve equation and SD represents the standard deviation for the fluorescence intensity ratio responses of probe to formaldehyde. \( \frac{I_{520}}{I_{440}} = 2.821 \times [FA] + 0.0719 \) \( (R^2=0.9949) \), LOD= \( 3 \times 0.00078 / 2.821 = 0.84 \) μM.

### 14 Theoretical calculations

Theoretical calculations were performed to further understand the sensing mechanism. Based on the density functional theory (DFT) at B3LYP level with the standard 6-31g (d,p) basis set, the geometry for molecular ground-state was optimized. The energy levels for highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were estimated. The single-point energy calculation of the excited states using B3LYP/6-311+G (d, p) basis set were adopted to illustrate the sensing mechanism. All data were collected using the Gaussian 09 software.

### 15 Supporting Scheme and Figures

#### 15.1 Scheme S1 Synthesis route of probe

![Scheme S1](image)
Fig. S1. Characterization of probe PIPBA: (A) $^1$H NMR spectrum (DMSO-d$_6$); (B) $^{13}$C NMR spectrum (DMSO-d$_6$) and (C) Mass Spectra: m/z 363.9 [M+H]$^+$. 
15.3 Fig. S2. HPLC-UV analysis for probe PIPBA and product PIBE.

Fig. S2. HPLC-UV analysis for probe and product PIBE. (A): PIPBA (100 μM), (B): PIPBA (100 μM) in presence of FA (5 mM); 15min (DMSO/PBS buffer solution v:v=50/50, pH = 7.4, 20 mM), (C): PIBE (50 μM).

15.4 Fig. S3. Mass spectra for product of reaction of probe PIPBA with formaldehyde

Fig. S3. Mass spectra for product from the reaction of probe PIPBA with formaldehyde
15.5 Fig. S4. The characterization of product PIBE from the reaction of PIPBA with formaldehyde

Fig. S4. (A) $^1$H NMR spectrum (DMSO-d$_6$); (B) $^{13}$C NMR spectrum (DMSO-d$_6$) and (C) Mass Spectra: m/z 322.8 [M+H]$^+$. 
15.6 Fig. S5. The $^1$H NMR of PIBE and product from the reaction of PIPBA with FA

(A)

(B)

Fig. S5. (A): The $^1$H NMR of probe PIBE, (B): The $^1$H NMR of the product from the reaction of PIPBA with formaldehyde.

15.7 Fig. S6. Time-dependent fluorescence intensity of PIPBA toward FA

(A)

(B)

Fig. S6. (A) Kinetic studies: The pseudo-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the pseudo-first-order equation: $\ln\left(\frac{F_0 - F_t}{F_0}\right) = -k't$, where $F_t$ and $F_{\text{min}}$ are the fluorescence intensities at 440 nm at time $t$ and the time 0 obtained after the reaction was complete. $k'$ is the pseudo-first order rate constant. $k = 0.089 \text{ min}^{-1}$
for 500 \( \mu M \) formaldehyde [▲], and 0.041 min\(^{-1}\) for 200 \( \mu M \) formaldehyde [■]. (B): Kinetic studies: The pseudo-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the pseudo-first-order equation: \( \ln([F_{\text{max}} - F_t] / F_{\text{max}}) = - k't, \) where \( F_t \) and \( F_{\text{max}} \) are the fluorescence intensities at 520 nm at time \( t \) and the maximum value obtained after the reaction was complete. \( k' \) is the pseudo-first order rate constant. \( k = 0.078 \text{ min}^{-1} \) for 500\( \mu M \) formaldehyde [▲], and 0.028 \text{ min}^{-1} \) for 200 \( \mu M \) formaldehyde [■].

15.8 Fig. S7. Temperature-dependent fluorescence ratio of probe PIPBA toward FA

Fig. S7. The effect of temperature on the fluorescence intensity ratio of the probe (5 \( \mu M \)) in presence of formaldehyde (500 \( \mu M \)) (DMSO/PBS buffer solution (50/50, \( v : v \), pH = 7.4, 20 mM), \( \lambda_{\text{ex}}=350 \text{ nm} \).
15.9 Fig. S8. Effect of pH Values to probe and probe derivatization

![Graph showing the effect of pH on the fluorescence intensity ratio of the probe (5 μM) in the absence (△) or presence (○) of formaldehyde (500 μM) (DMSO/PBS buffer solution (50/50, v : v, pH = 7.4, 20 mM), λex=350 nm.](image)

15.10 Fig. S9. MTT assay for probe in HeLa cells.

![Bar graph showing the effect of PIPBA with various concentration (0-25 μM) on the viability of HeLa cells (1: control, 2: 5 μM, 3: 10 μM, 4: 15 μM, 5: 20μM, 6: 25 μM). The viability of the cells in the absence of the probe is defined as 100 %, and the data are the mean standard deviation of five separate measurements.](image)
Fig. S10. The intact cells were pretreated with formaldehyde (500 μM) for 2 h and then were scanned with confocal imaging when PIPBA (5 μM) was added (time ranging from 0 to 300 min). Green channel (λex=405 nm, λem =480–580 nm) and blue channel (λex=405 nm, λem =420–480 nm). Scare bar: 20 μm.
Fig. S11. Investigation of the fluorescence intensity at different depths of samples with the confocal imaging of formaldehyde in kidney. The kidney slices were obtained from the blank mice and were incubated successively with formaldehyde (400 μM) for 30 min and probe PIPBA (5 μM) for 2 h. Fluorescence intensity were collected from different depths (0~110 μm) of kidney slice via the confocal imaging through the two channels: green channel (λex=405 nm, λem =480–580nm), blue channel (λex=405 nm, λem =420–480nm). **A**: Blue channel; **B**: green channel; **C**: Merged imaging of **A** and **B**; **D**: the ratio (I_{green}/I_{blue}) imaging. Scale bar: 50 μm.
Fig. S12. Investigation on the potential interferences

**Fig. S12.** Confocal imaging of formaldehyde in living system. 

a): Fluorescence image of living cells; 
b): Cells were incubated with 5 μM PIPBA for 2 h; 
c): Fluorescence image of zebrafish; 
d): zebrafish was incubated with 5 μM PIPBA for 3 h. 
e) Fluorescence image of kidney slides; 
f): kidney slices were incubated with 5 μM PIPBA for 2 h. 
Fluorescence images: green channel (λex=405 nm, λem =480–580 nm), blue channel (λex=405 nm, λem =420–480 nm), Scale bar: 50 μm for tissue and cells, 200 μm for zebrafish.
15.14 Fig. S13. The synthesis route of BTSA

Fig. S13. Synthesis route of BTSA (N-benzyl-2,4-dinitrophensulfonamide)

15.15 Fig. S14. The $^1$H NMR and $^{13}$C NMR of BTSA
15.16 Fig. S15. The generation of HSO$_3^-$ induced by BTSA

![Chemical reaction diagram](image)

15.17 Fig. S16. Fluorescent investigation of the scavenging effect of BTSA on formaldehyde

**Fig. S16.** Fluorescence spectra of probe with formaldehyde scavenger. **PIPBA** (5 μM); **PIPBA** (5 μM) + Cys (1 mM); **PIPBA** (5 μM) + formaldehyde (1 mM); **PIPBA** (5 μM) + Cys (1 mM) + formaldehyde (1 mM); **PIPBA** (5 μM) + Cys (1 mM) + formaldehyde (1 mM) + BTSA (1mM); **PIPBA** (5 μM) + HSO$_3^-$ (0.25 mM) + formaldehyde (1 mM). All experiments were conducted in DMSO/PBS buffer solution (50/50, v/v, pH = 7.4, 20 mM, Ex=350 nm).