Electronic supplementary information (ESI) for

A novel off-on fluorescent probe for sensitive imaging of mitochondria-specific nitroreductase activity in living tumor cells

Bo Huang, Wen Chen, Yong-Qing Kuang, Wei Liu, Xian-Jun Liu, Li-Juan Tang*, Jian-Hui Jiang*

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Hunan University, Changsha, Hunan 410082, P. R. China

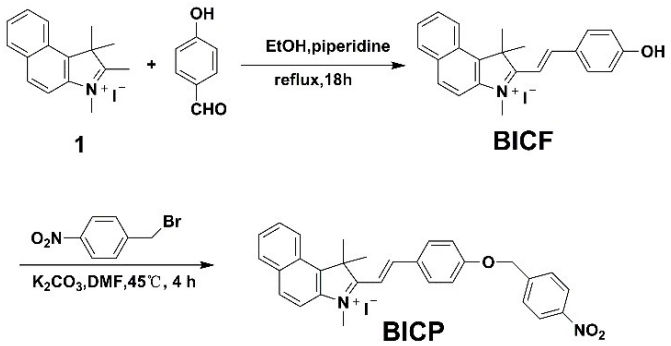
Corresponding authors:

*E-mail: tanglijuan@hnu.edu.cn

*E-mail: jianhuijiang@hnu.edu.cn

Fax: +86-731-88821916

1. Synthetic procedures



Scheme S1. Synthetic procedure of the BICP.

2. ^{1}H NMR, ^{13}C NMR and mass spectrometry (MS)

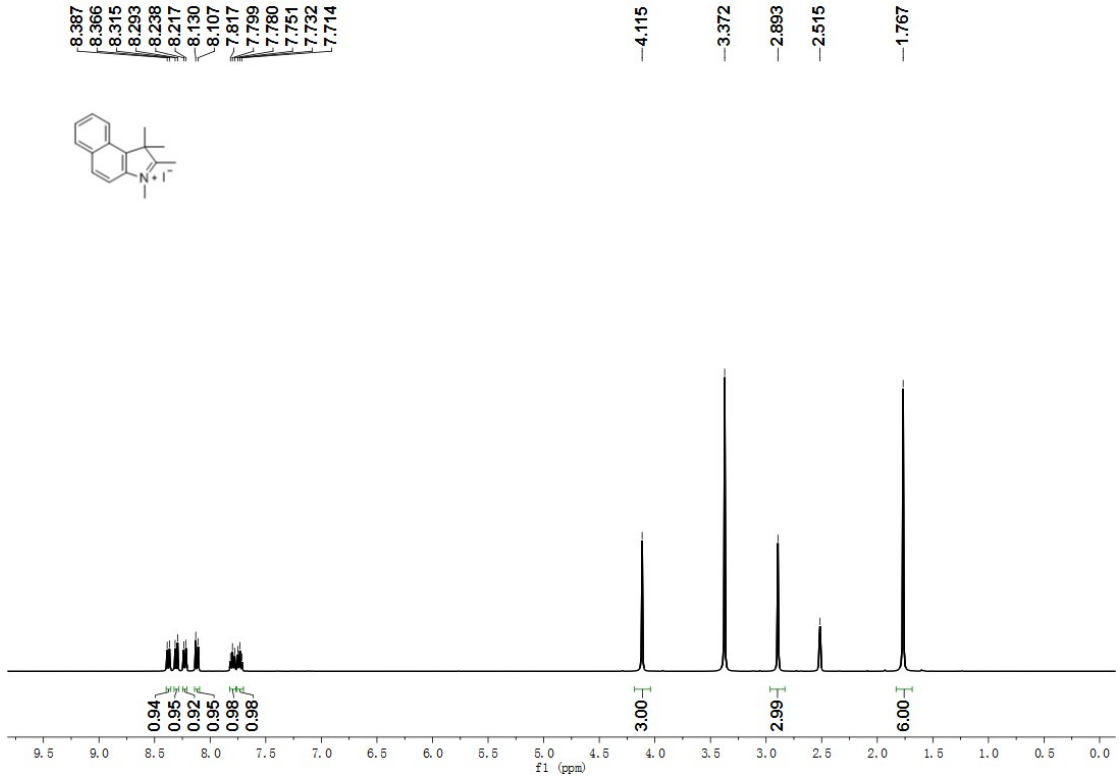


Fig. S1. ^{1}H-NMR spectra of Compound **1** in DMSO-d_6.

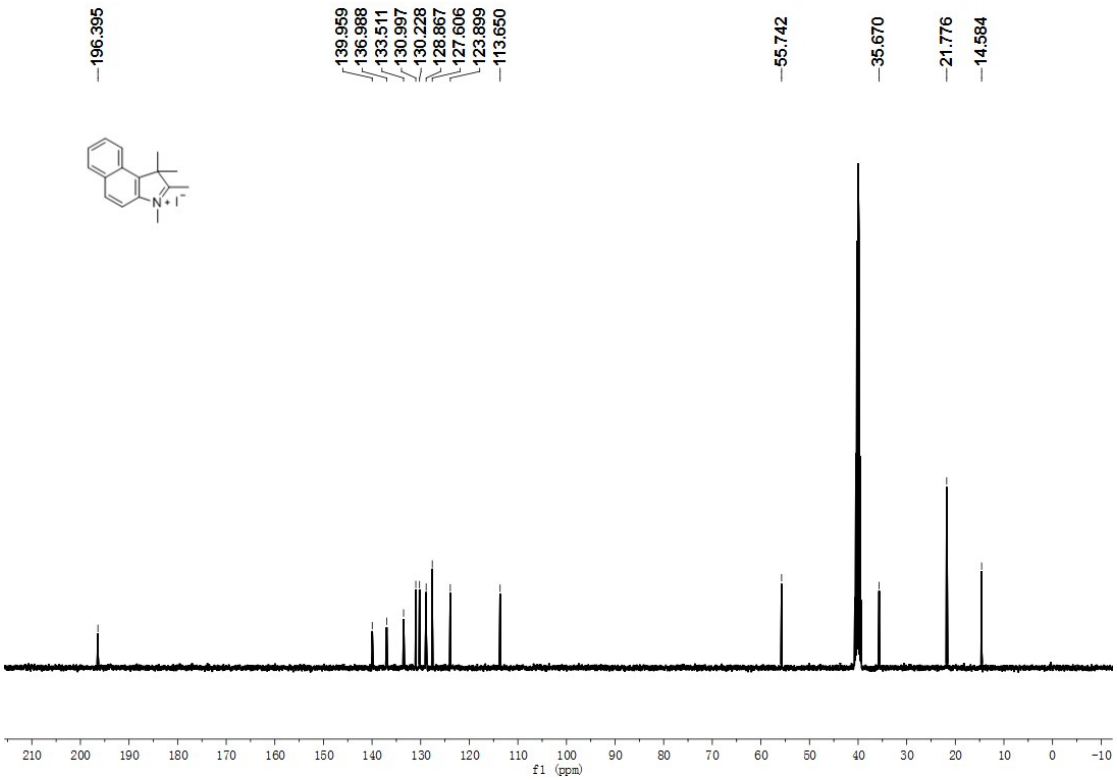


Fig. S2. ^{13}C-NMR spectra of Compound **1** in DMSO-d_6.

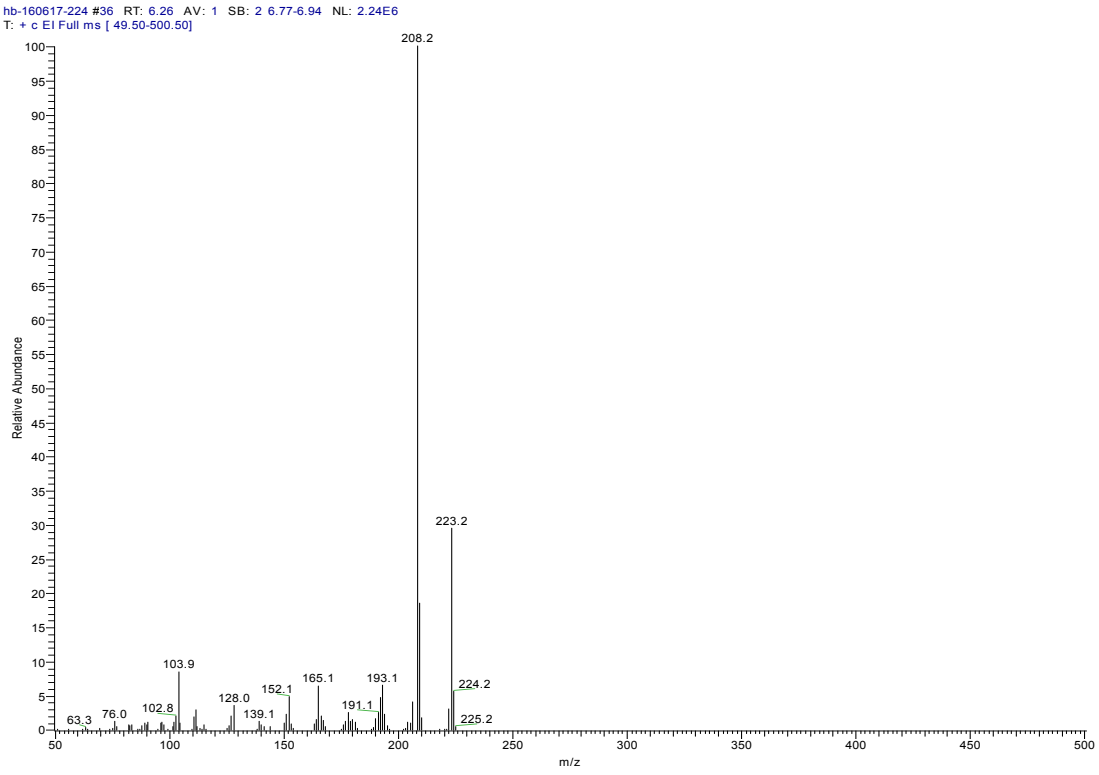


Fig. S3. MS (EI) spectra of Compound **1** in methanol.

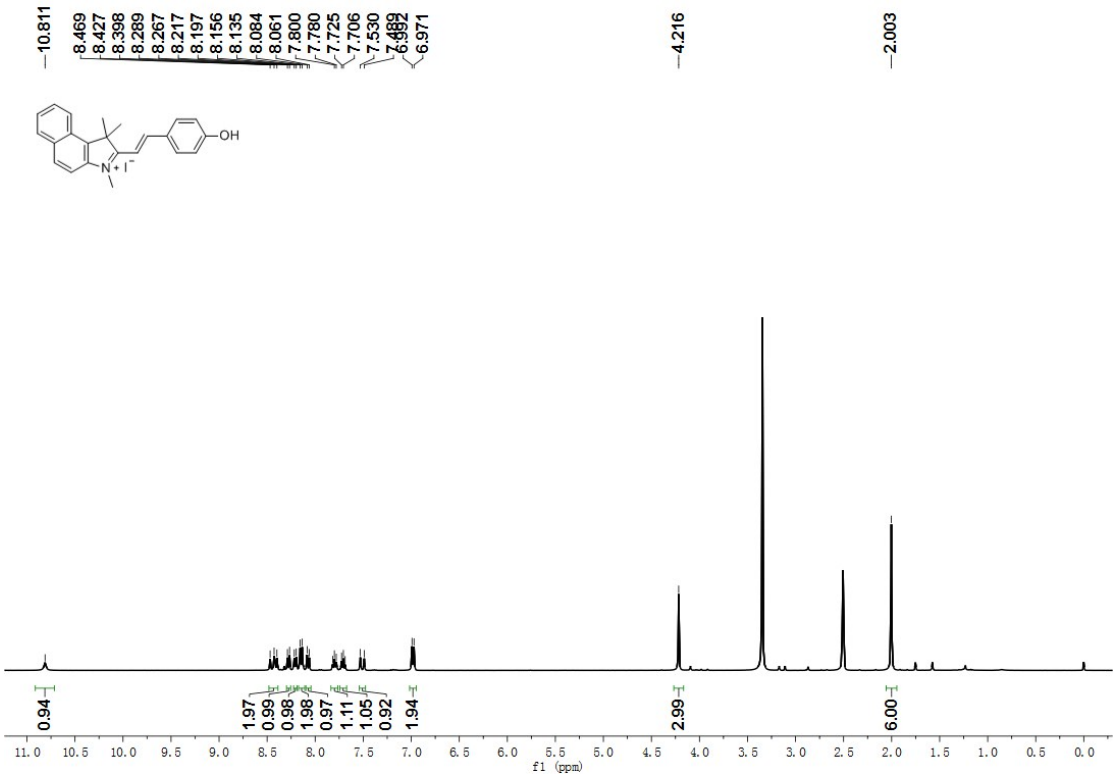


Fig. S4. ^{1}H-NMR spectra of BICF in DMSO-d_6

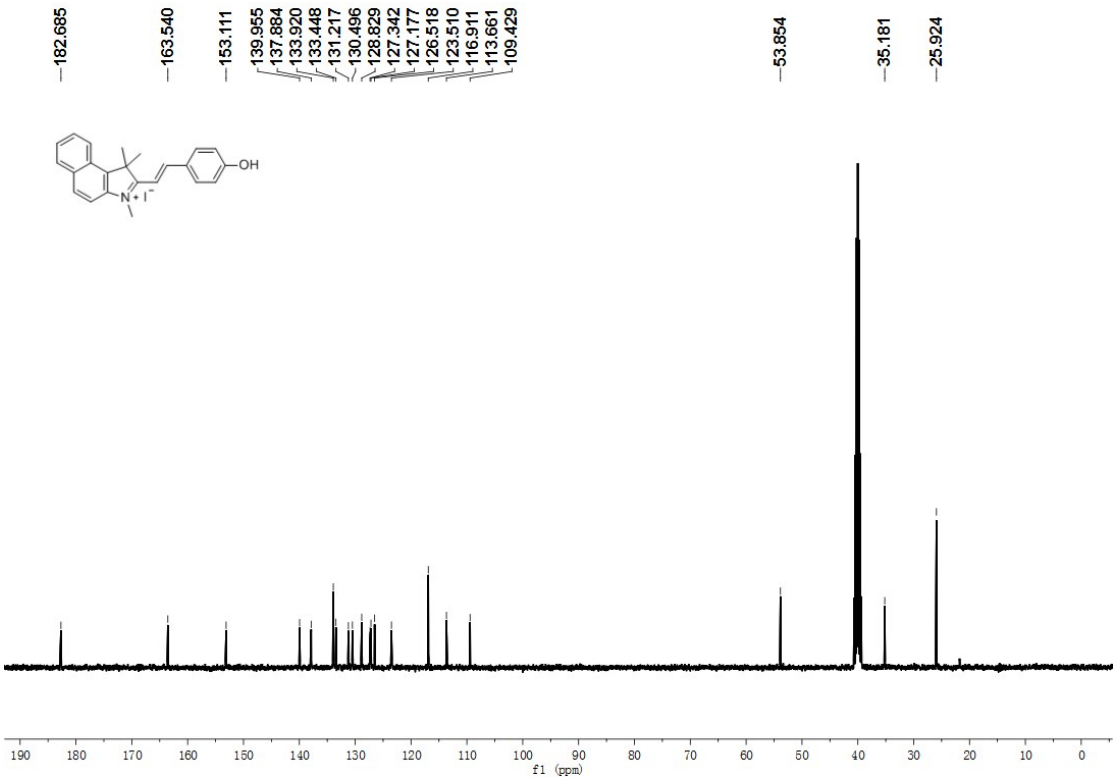


Fig. S5. ^{13}C-NMR spectra of BICF in DMSO-d_6.

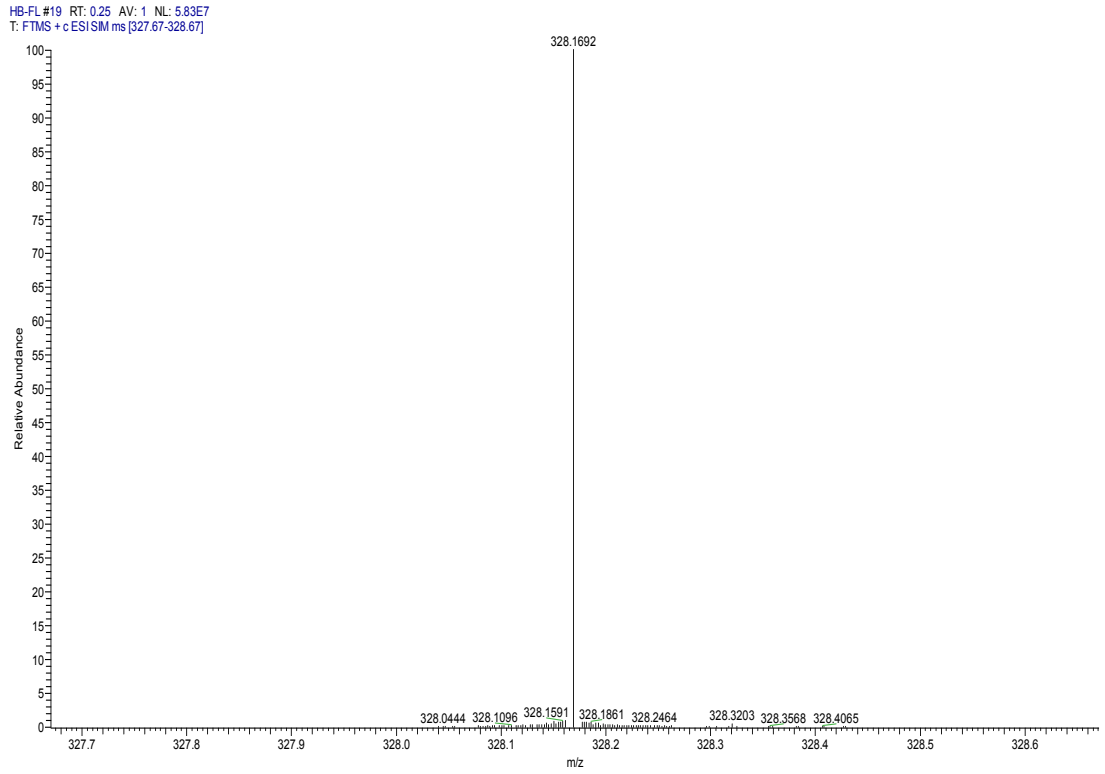


Fig. S6. HRMS (ESI) spectra of BICF in methanol.

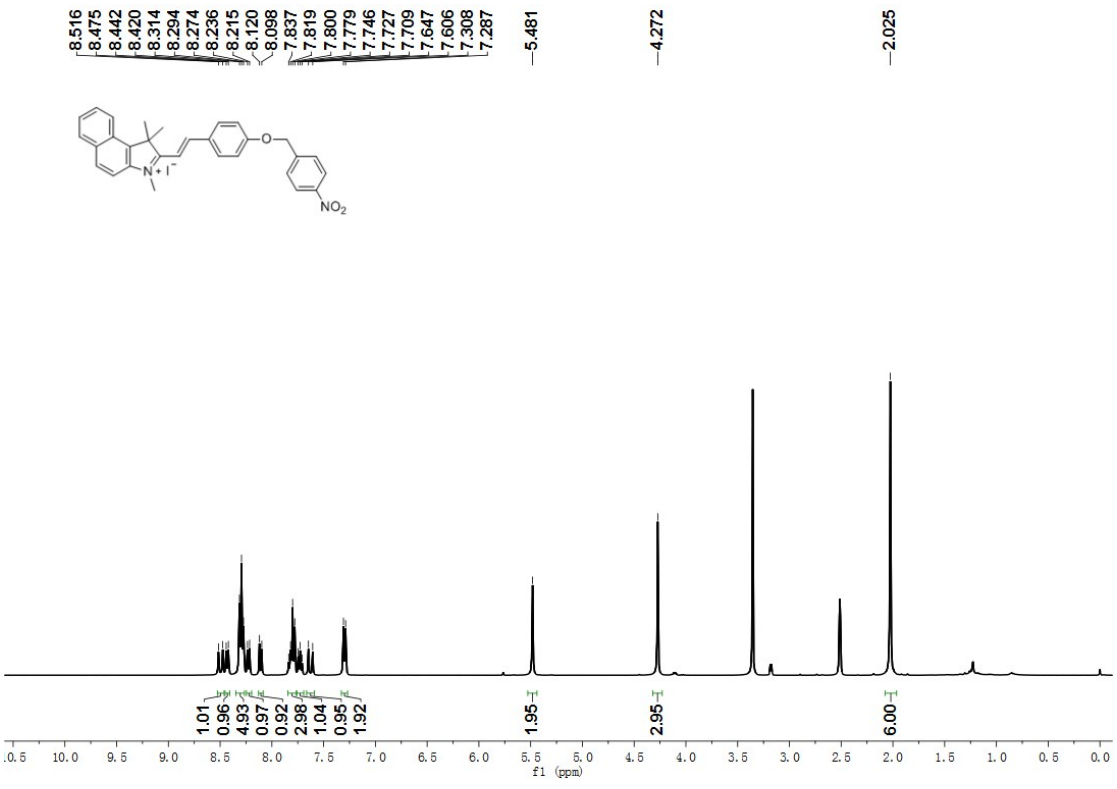


Fig. S7. ^{1}H-NMR spectra of BICP in DMSO-d_6.

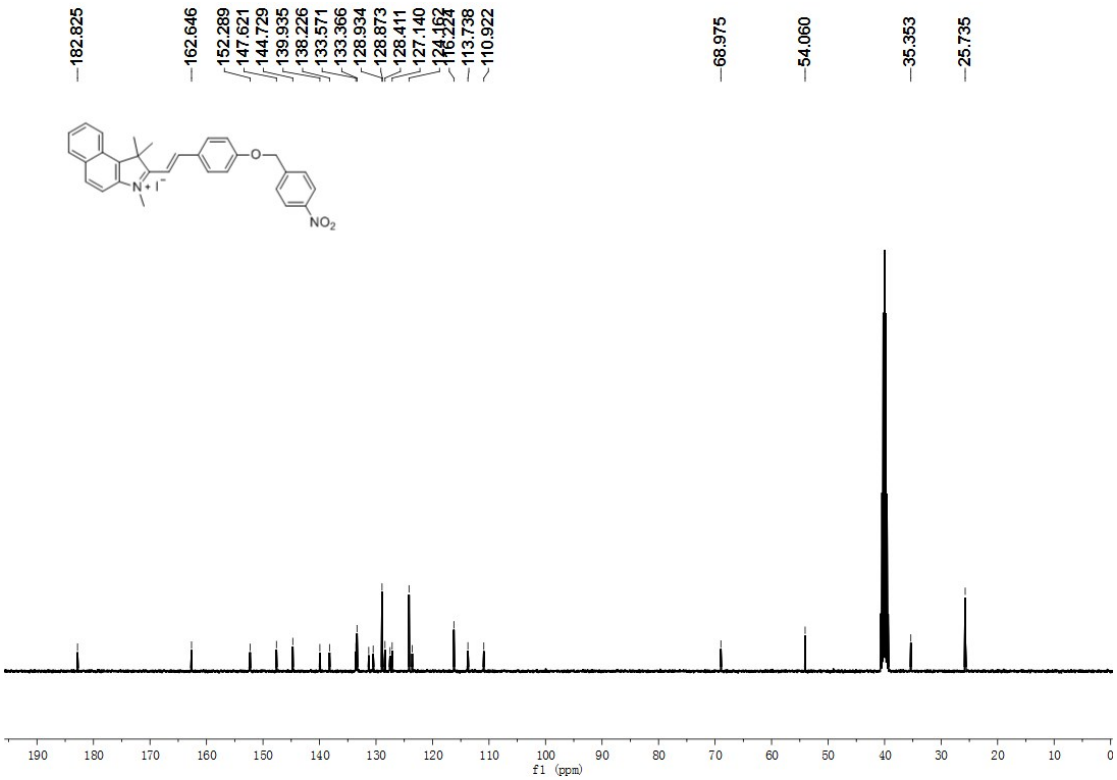


Fig. S8. ^{13}C-NMR spectra of BICP in DMSO-d_6.

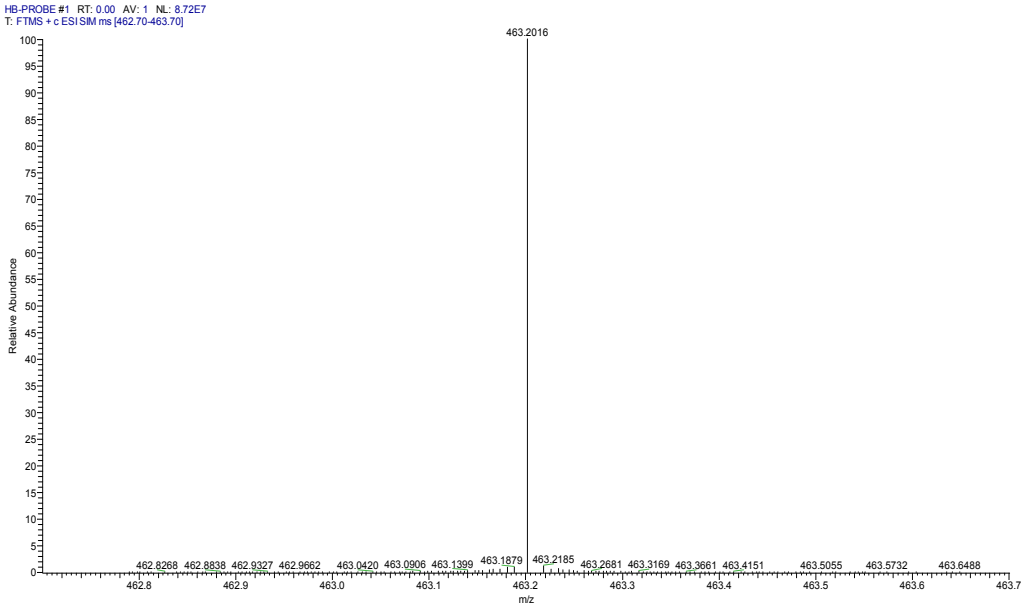


Fig. S9. HRMS (ESI) spectra of BICP in methanol.

3. UV spectroscopic data

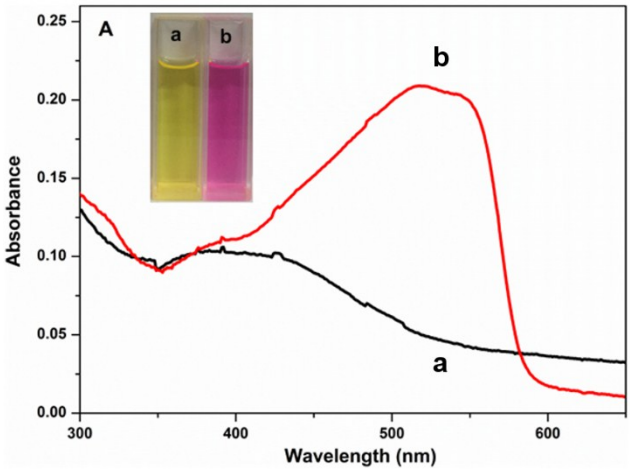


Fig. S10. Absorption spectra of BICP (10 μM) (a) before and (b) after reaction with NTR (5 μg mL^{-1}), in the presence of 500 μM NADH in 10 mM PBS (pH 7.4, containing 5% DMSO) at 37 °C for 30 min. Inset: the color changes of BICP in the absence or presence of NTR.

4. Photograph for the assay of selectivity

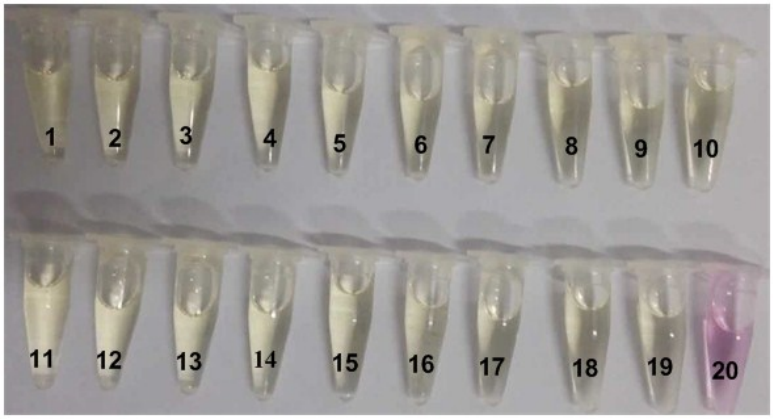


Fig. S11. Photograph for the assay of selectivity. Color changes of BICP (10 μM) in the presence of NADH (500 μM) to various species: (1) control, (2) glutathione (GSH, 5 mM), (3) cysteine (Cys, 1 mM), (4) homocysteine (Hcy, 1mM), (5) NaClO (10 μM), (6) dithiothreitol (DTT, 1 mM), (7) KCl (50 mM), (8) NaCl (10 mM), (9) $MgCl_2$ (2.5 mM), (10) $CaCl_2$ (2.5 mM), (11) NaHS (10 μM), (12) glycine (Gly, 1 mM), (13) tryptophan (Try, 1 mM), (14) vitamin C (1 mM), (15) Na_2SO_3 (10 mM), (16) H_2O_2 (1 mM), (17) tyrosine (Tyr, 1 mM), (18) glucose (10 mM), (19) arginine (Arg, 1 mM), and (20) NTR (5 μg mL^{-1}).

5. Real-time fluorescence records and kinetic parameters

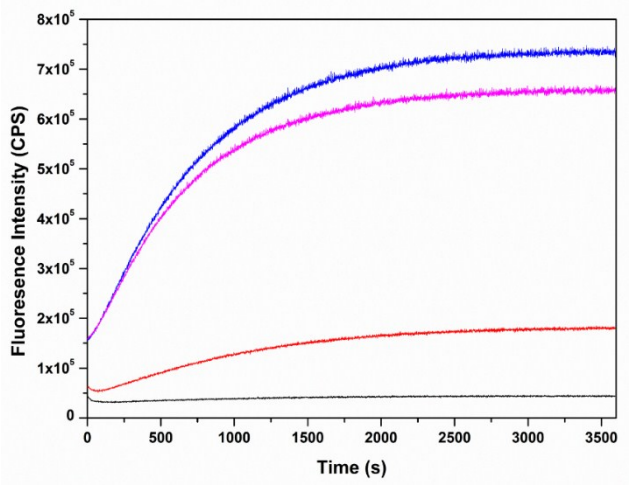


Fig. S12. A plot of fluorescence intensity of BICP (10 μM) *vs.* the reaction time in the presence of varied concentrations of NTR (from bottom to top): 0 (control, black line), 0.25 μg mL^{-1} (red line), 5 μg mL^{-1} (pink line) and 10 μg mL^{-1} (blue line). The measurements were performed at 25 °C in 10 mM PBS (pH 7.4) with $\lambda_{ex/em}$ = 523/570 nm.

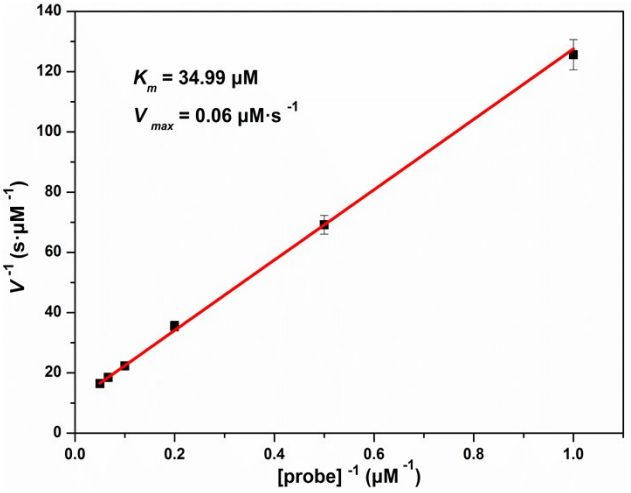


Fig. S13. Lineweaver-Burk double reciprocal plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}$[probe] / (K_m + [probe]), where V is the reaction rate, [probe] is BICP concentration (substrate), and K_m is the Michaelis constant. Conditions: 3 μg mL^{-1} NTR, 1-20 μM BICP probe. All measurements were performed at 37 °C, $\lambda_{ex/em}$ = 523/570 nm. Reaction at each probe concentration was repeated three times, and the error bars represent standard deviations. Points were fitted using a linear regression model (correlation coefficient R^2 = 0.9992).

6. Mechanism research

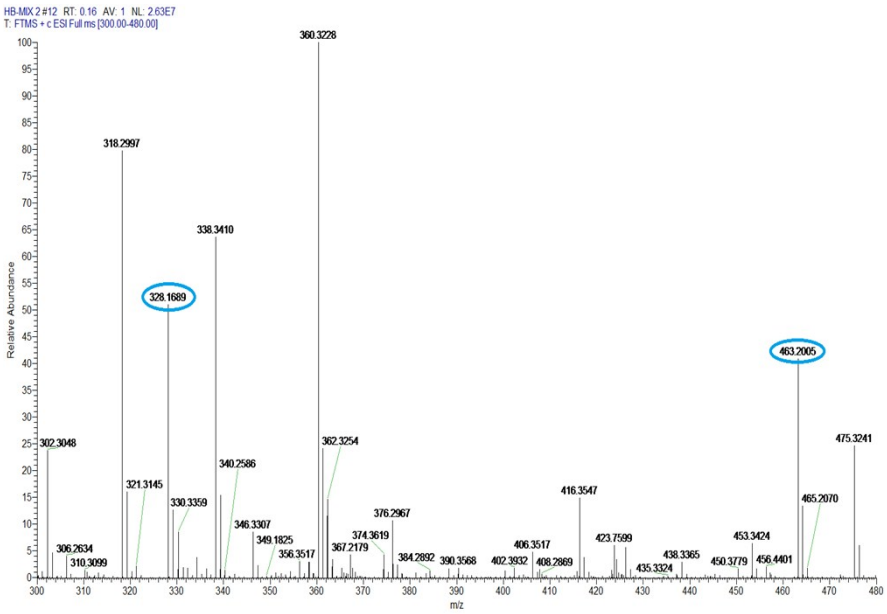


Fig. S14. HRMS (ESI) spectra of the reaction solution of BICP (100 μM) with NTR (1 μg mL^{-1}).

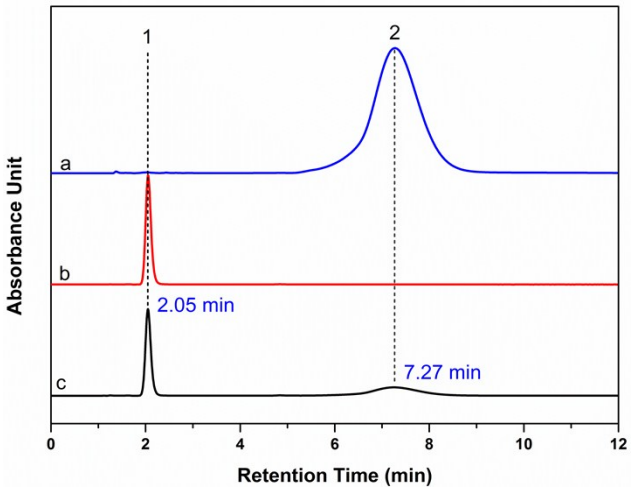


Fig. S15. Chromatograms of different reaction systems. (a) 100 μM BICP; (b) 100 μM BICF; (c) the reaction products of 100 μM BICP with 1 μg mL^{-1} NTR in the presence of 500 μm NADH. The assignments of the peaks: (1) 2.05 min, BICF; (2) 7.27 min, BICP. Detection: UV-vis (440 nm) detector. Mobile phase: methanol-water = 90:10 (v/v). Flow rate: 1 mL/min; T: 31 °C.

7. Effects of pH and temperature

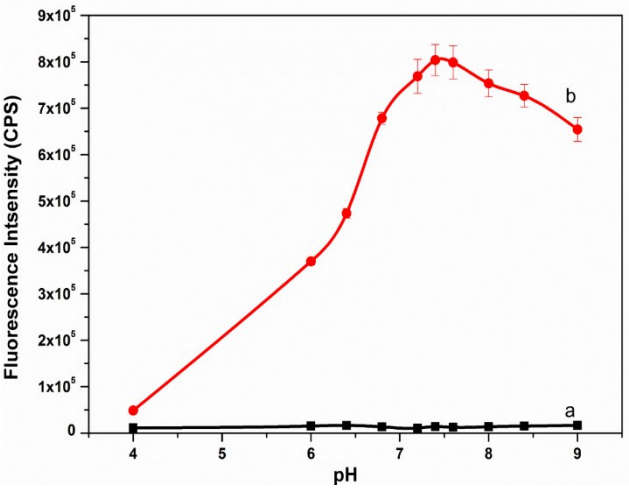


Fig. S16. Effect of pH on the fluorescence intensity of 10 μM BICP (a) before and (b) after reaction with 5 μg mL^{-1} NTR at 37 °C. Reaction at each pH value was repeated three times, and the error bars represent standard deviations. $\lambda_{ex/em}$ = 523/570 nm.

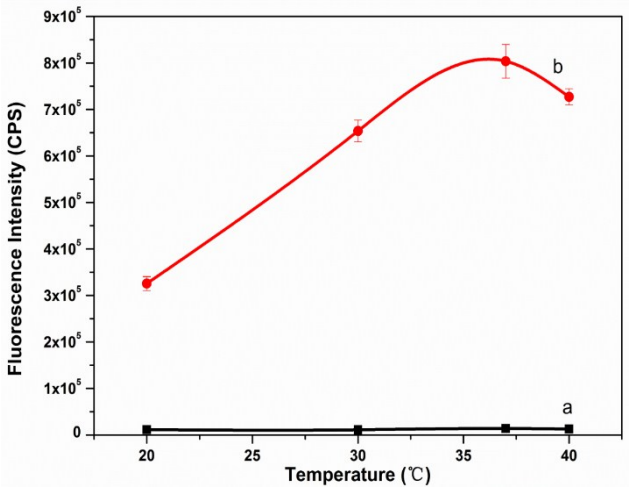


Fig. S17. Effect of temperature on the fluorescence intensity of 10 μM BICP (a) before and (b) after reaction with 5 μg mL^{-1} NTR. Reaction at each temperature was repeated three times, and the error bars represent standard deviations. $\lambda_{ex/em}$ = 523/570 nm.

8. Cell toxicity of BICP

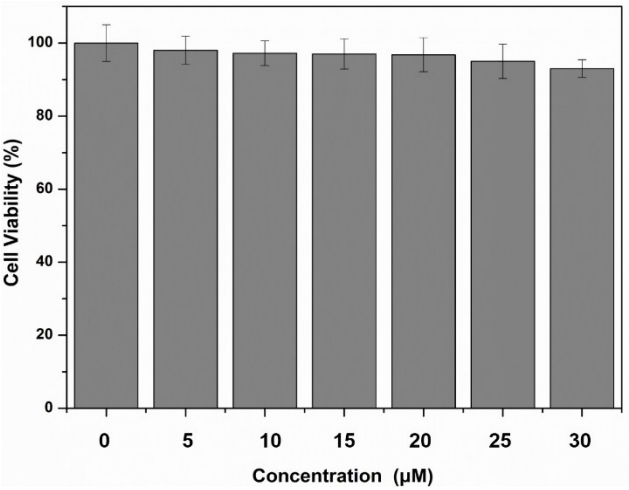


Fig. S18. Cell viabilities (%) estimated by MTT proliferation tests versus incubation concentrations of BICP. HeLa cells were incubated with 0-30 μM BICP at 37 ℃ for 24 h**.** The viability of the cells without BICP is defined as 100%.

9. Effects of dicoumarin on hypoxia-induced NTR in living cells

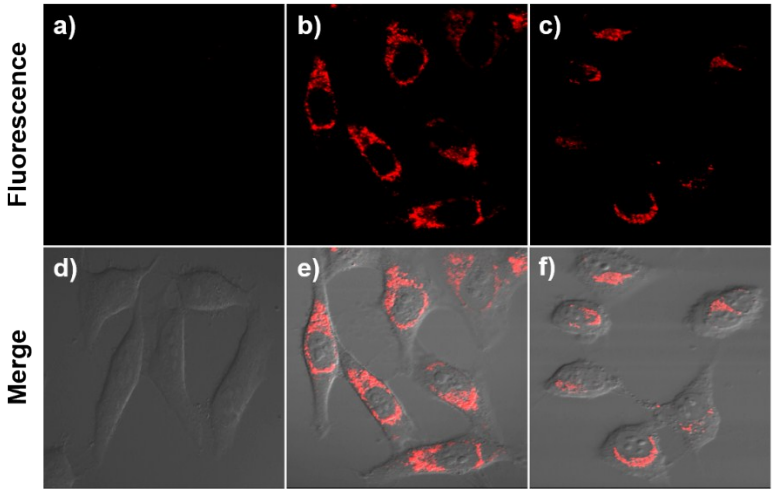


Fig. S19. Confocal fluorescence images of HeLa cells under the hypoxic condition of 1% O_2 for 4 h (λ_{ex} = 559 nm, λ_{em} = 565-600 nm). (a) HeLa cells only; (b) Cells were incubated with 10 μM BICP for 30 min; (c) Cells were incubated with 0.1 mM dicoumarin and 10 μM BICP for 30 min. (d-f) The merged fluorescence imaging and corresponding differential interference contrast (DIC) images.

10. Colocalization assays

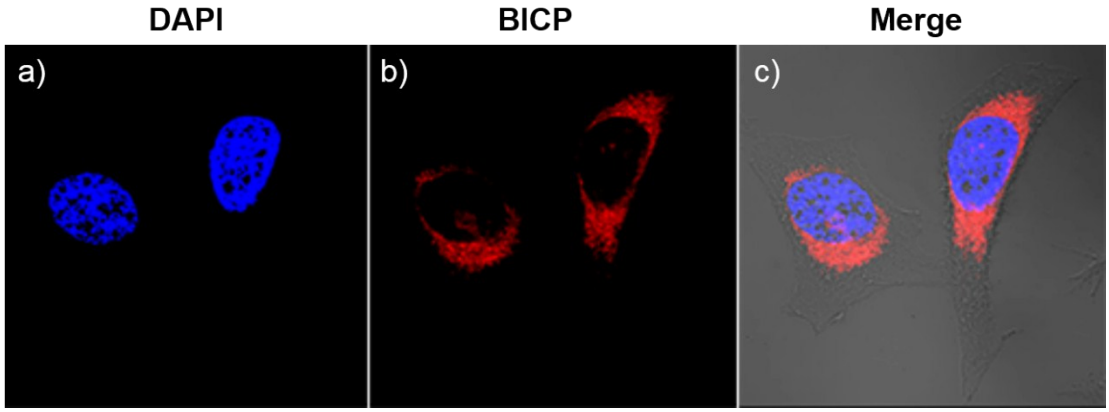


Fig. S20. Co-localization of BICP and DAPI in HeLa cells. (a) Fluorescence image of DAPI (λ_{ex} = 405 nm, λ_{em} = 440-480 nm). (b) Fluorescence image of BICP (λ_{ex} = 559 nm, λ_{em} = 565-600 nm). (c) Merged image of images a, b and differential interference contrast (DIC) images.

11. Flow cytometric assay

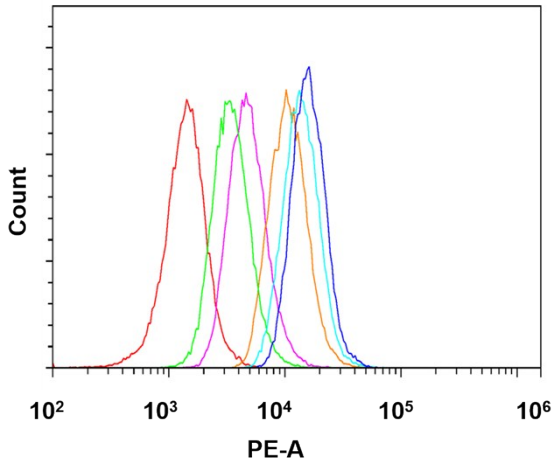


Fig. S21. Flow cytometric assay for HeLa cells. Red curve: HeLa cells only; Green curve: HeLa cells were treated with 10 μM BICP for 30 min at 37 °C and then kept under normoxic (20% O_2) conditions or 4 h.; Pink curve: HeLa cells were treated with 10 μM BICP for 30 min at 37 °C and then kept under 15% O_2 conditions for 4 h; Orange curve: HeLa cells were treated with 10 μM BICP for 30 min at 37 °C and then kept under hypoxic conditions 10% O_2 for 4 h; Light blue curve: HeLa cells were treated with 10 μM BICP for 30 min at 37 °C and then kept under 5% O_2 for 4 h.; Dark blue curve: HeLa cells were treated with 10 μM BICP for 30 min at 37 °C and then kept under hypoxic conditions 1% O_2 for 4 h.