Electronic Supplementary Material (ESI) for Analyst.

Supplemental Information

A Two-photon Fluorescent Probe for Nitroreductase imaging in Living Cells, Tissues and Zebrafish with Hypoxia Condition

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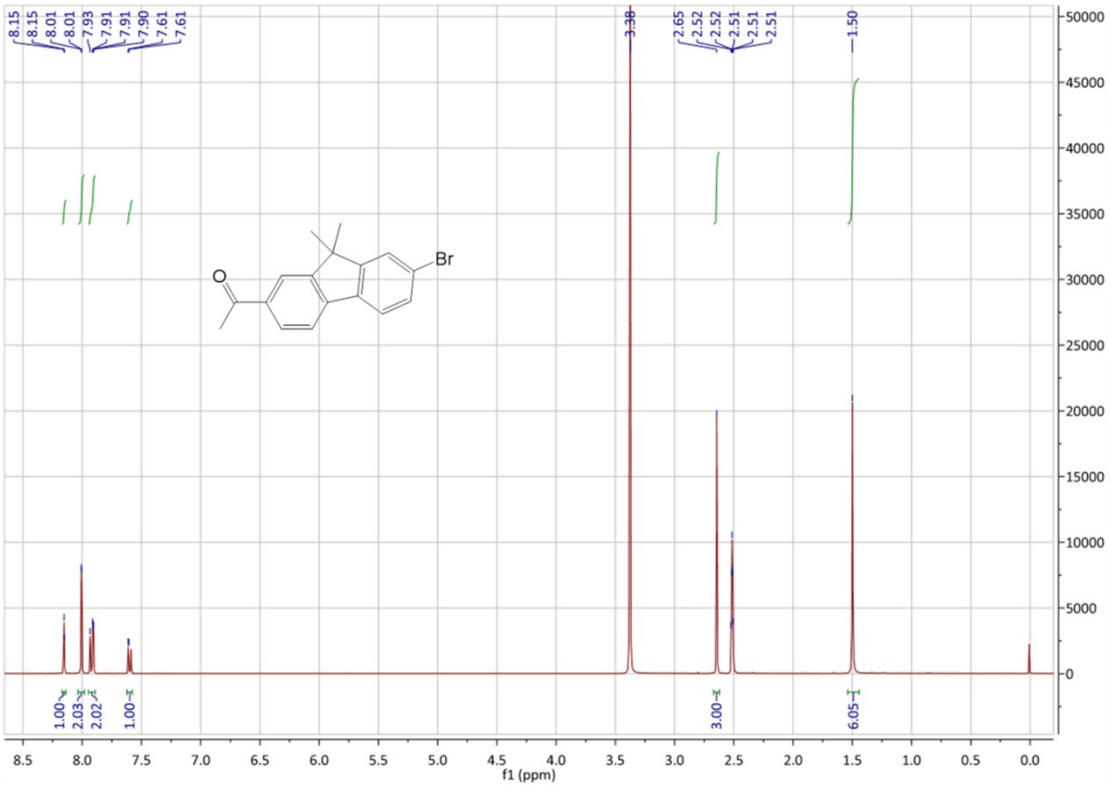

Figure S 1 ^{1}H-NMR spectrum of **2** (400 MHz, d_6-DMSO)

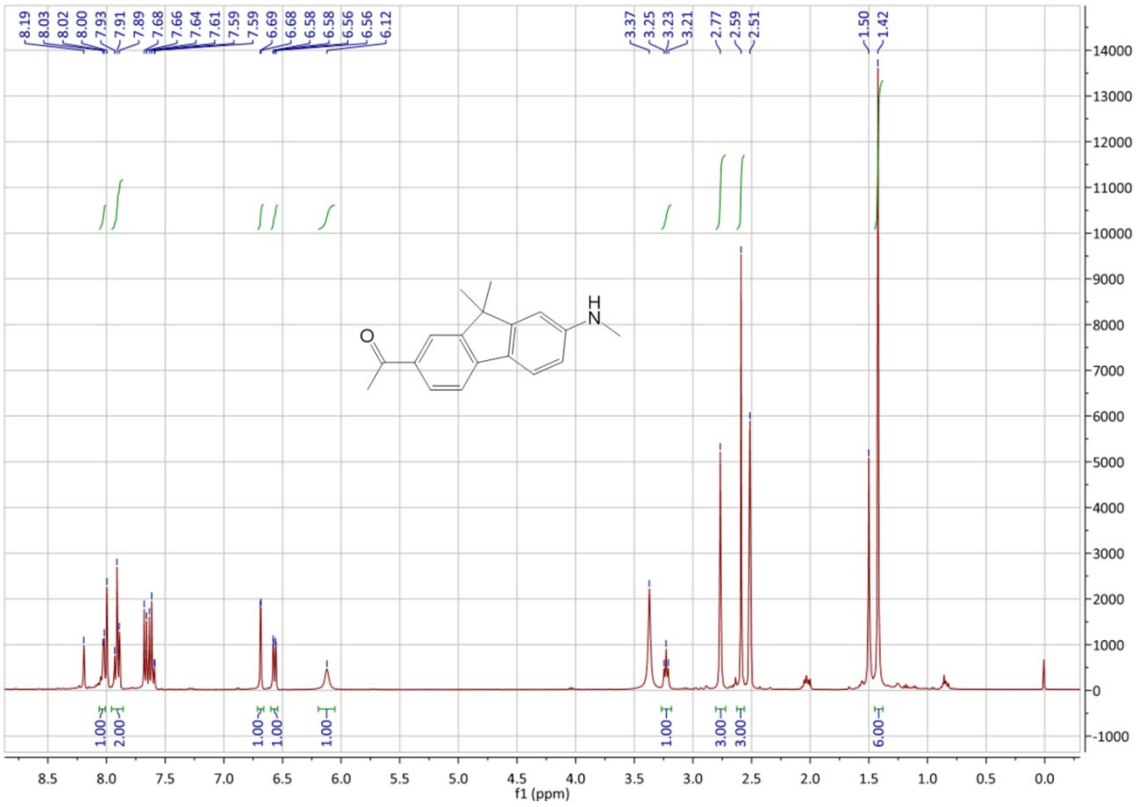

Figure S 2 ^{1}H-NMR spectrum of **1** (400 MHz, d_6-DMSO)

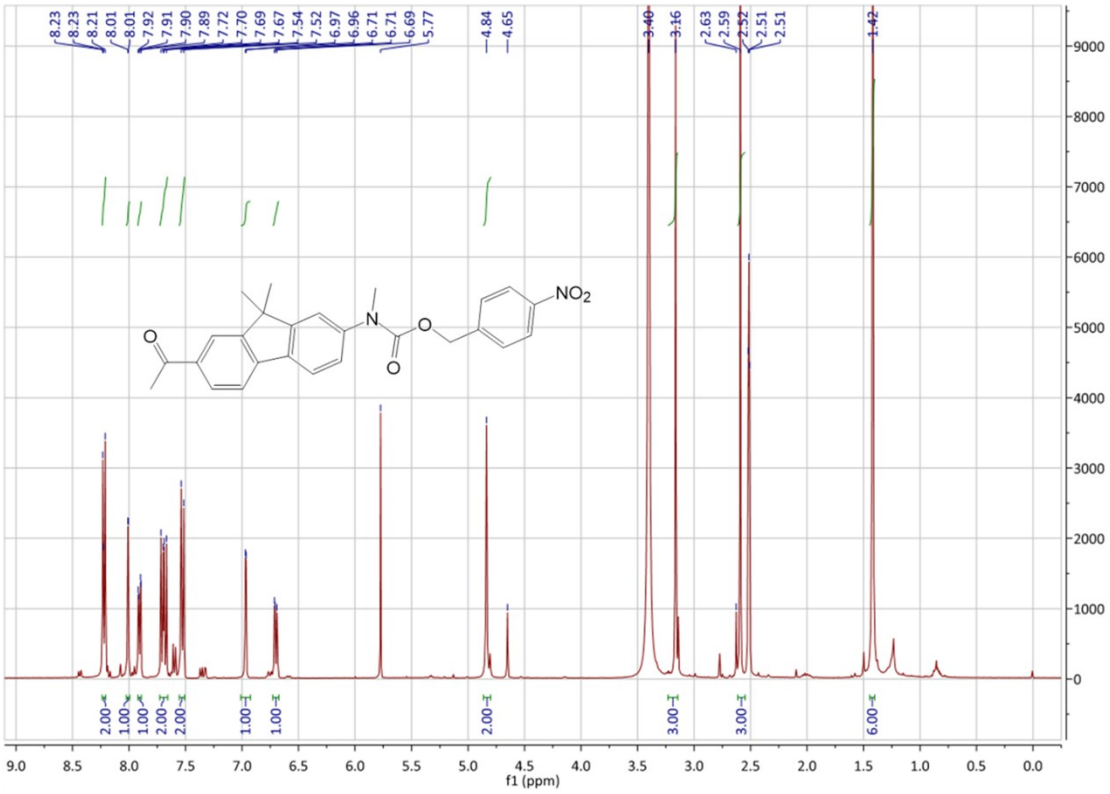

Figure S 3 ^{1}H-NMR spectrum of **FNTR** (400 MHz, d_6-DMSO)

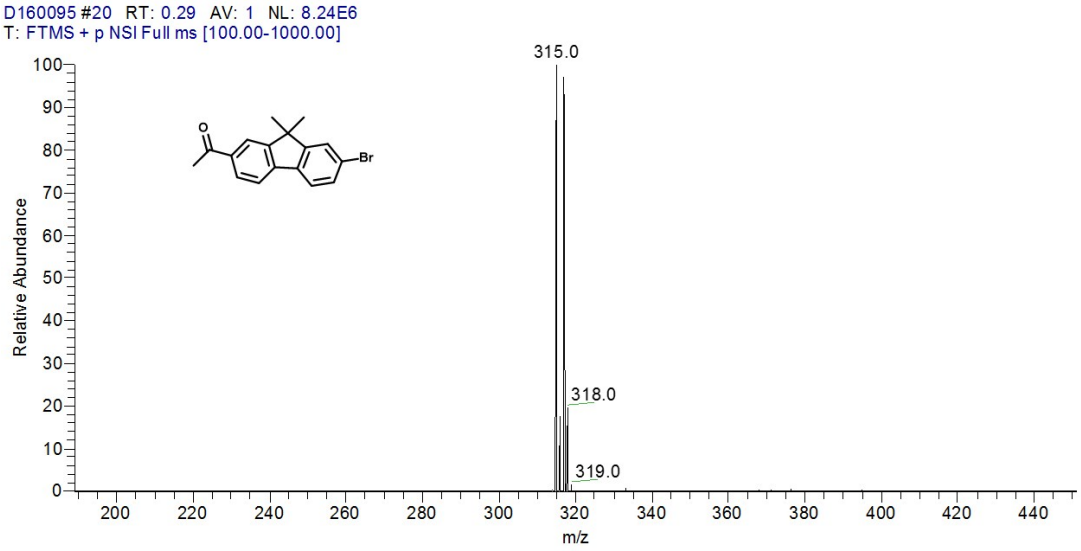

Figure S 4 HRMS (MALDI) spectrum of **2**

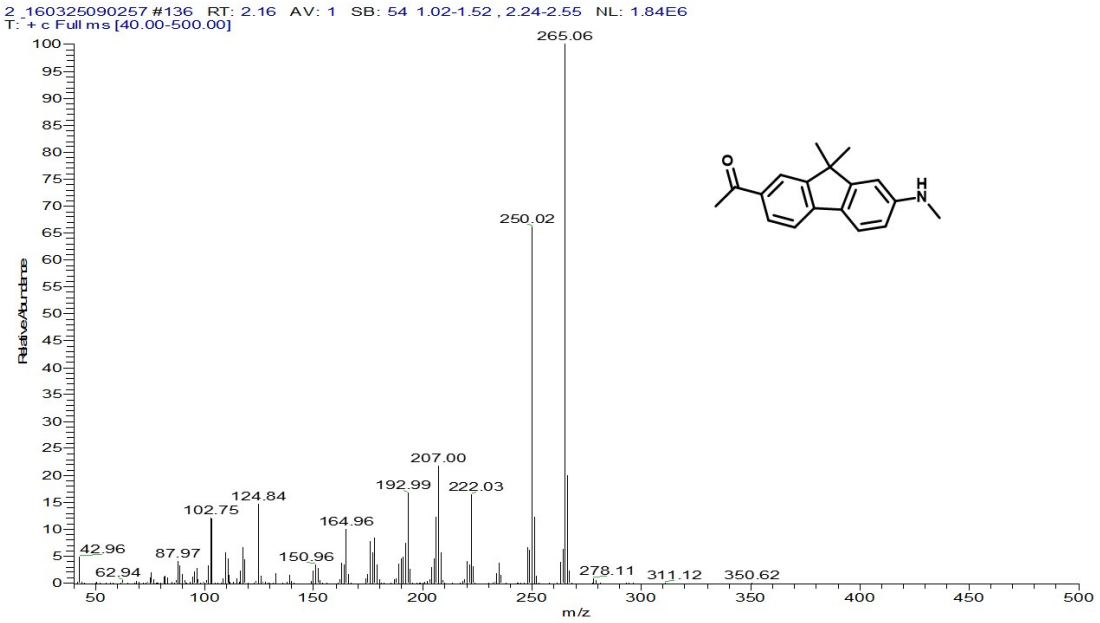

Figure S 5 HRMS (MALDI) spectrum of **1**

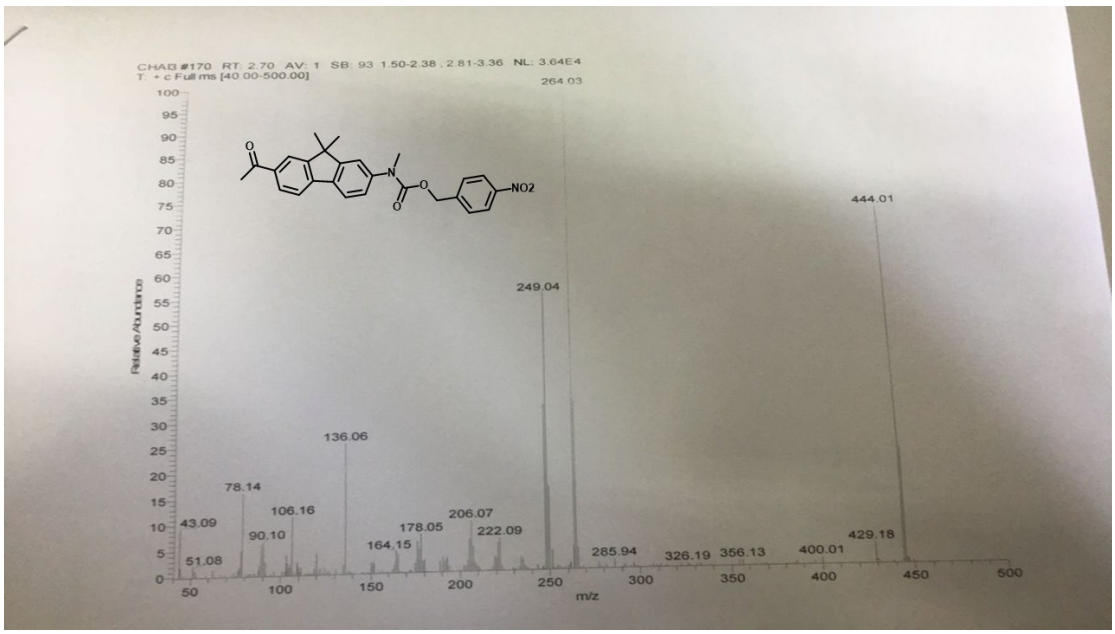

Figure S 6 HRMS (MALDI) spectrum of **FNTR**

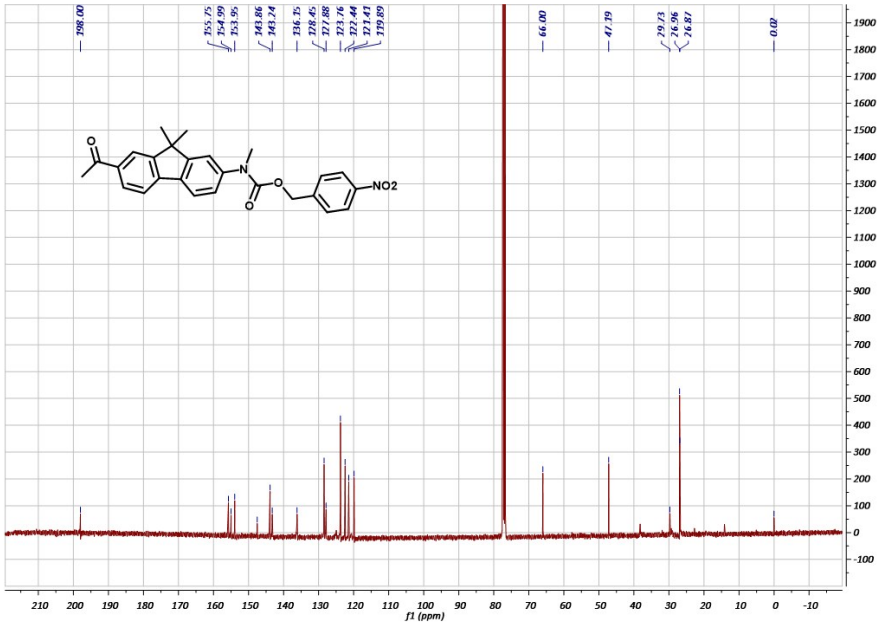

Figure S 7 ^{13}C-NMR spectrum of **FNTR** (100 MHz, d_6-DMSO)

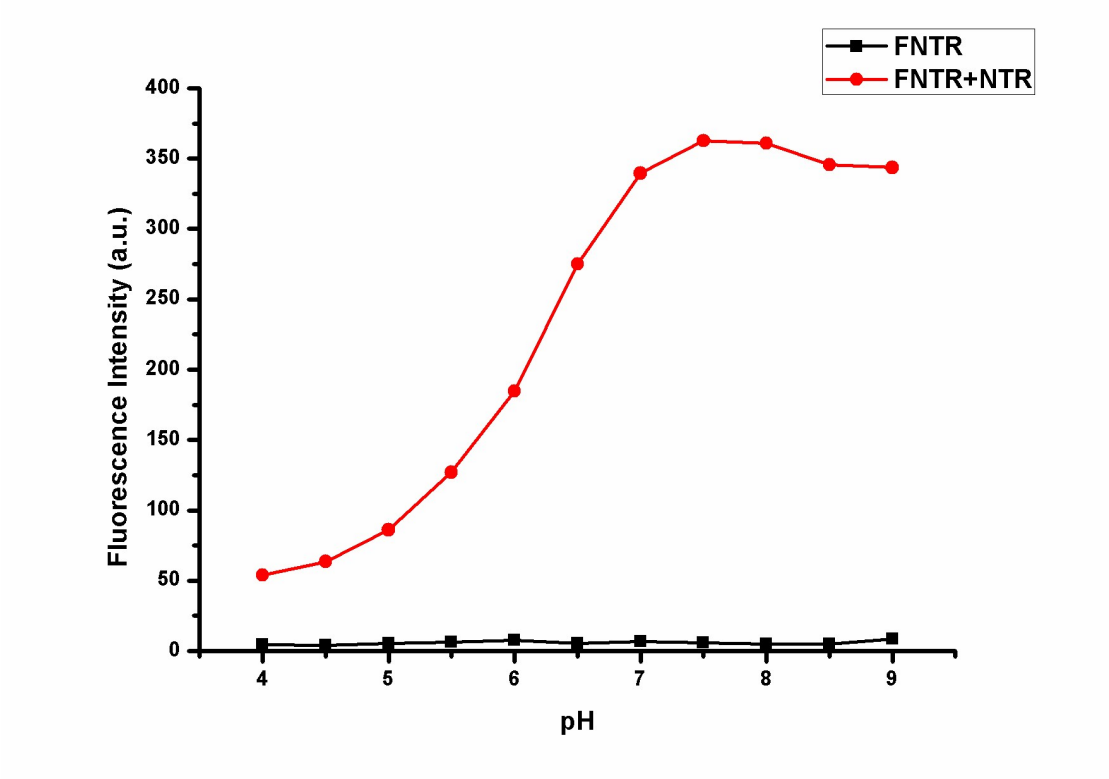

Figure S 8 Effects of pH on the fluorescence (λex/em = 410/560 nm) of **FNTR** (5 μM) and its reaction product with nitroreductase (1 μg/mL) in the presence of 500 μM NADH.

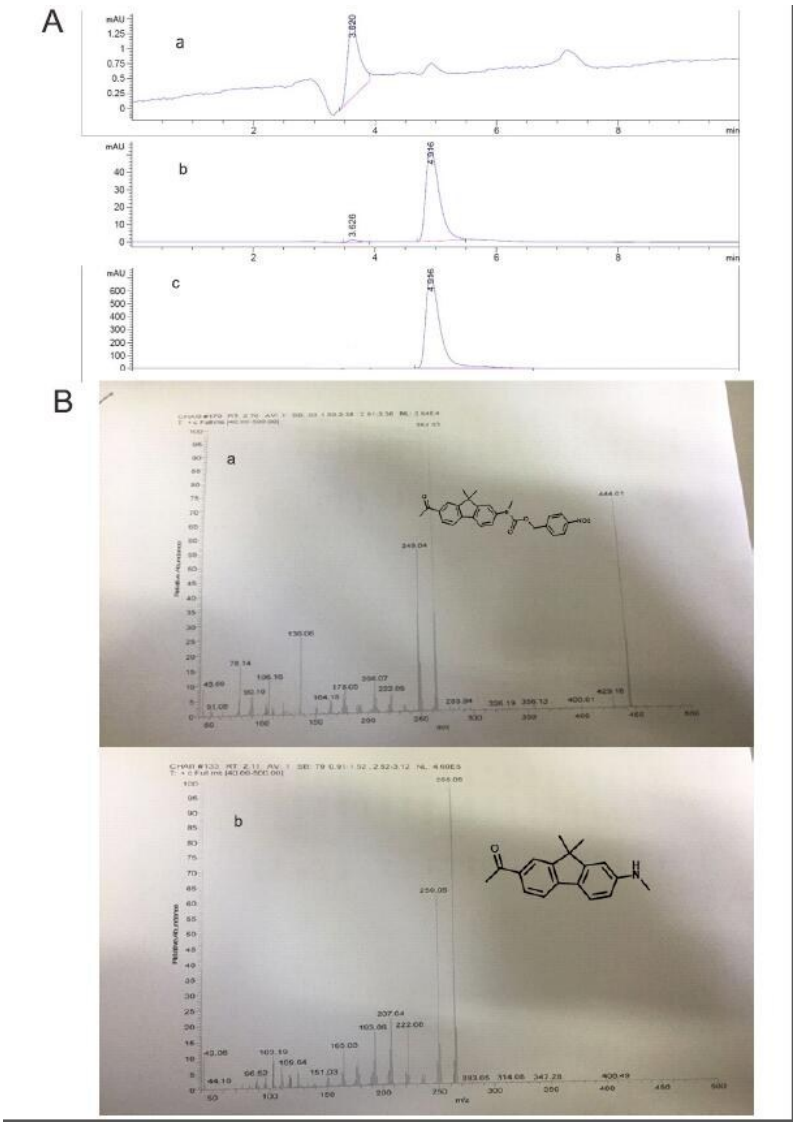

Figure S 9 (A) Chromatograms of different reaction systems. (A) 10 μM **FNTR** (a); 10 μM **1** (b); the reaction products of 10 μM **FNTR** with 20 μg/mL nitroreductase in the presence of 100 μM NADH (c). Detection: UV-vis (365 nm) detector. Flow rate: 0.3 mL/min. T: 20 °C. Injection volume: 100 μL. Mobile phase: acetonitrile–water, 50:50 (v/v). (B) The Chromatograms-mass of the production with the standard substances of **FNTR** (a) and **1** (b)

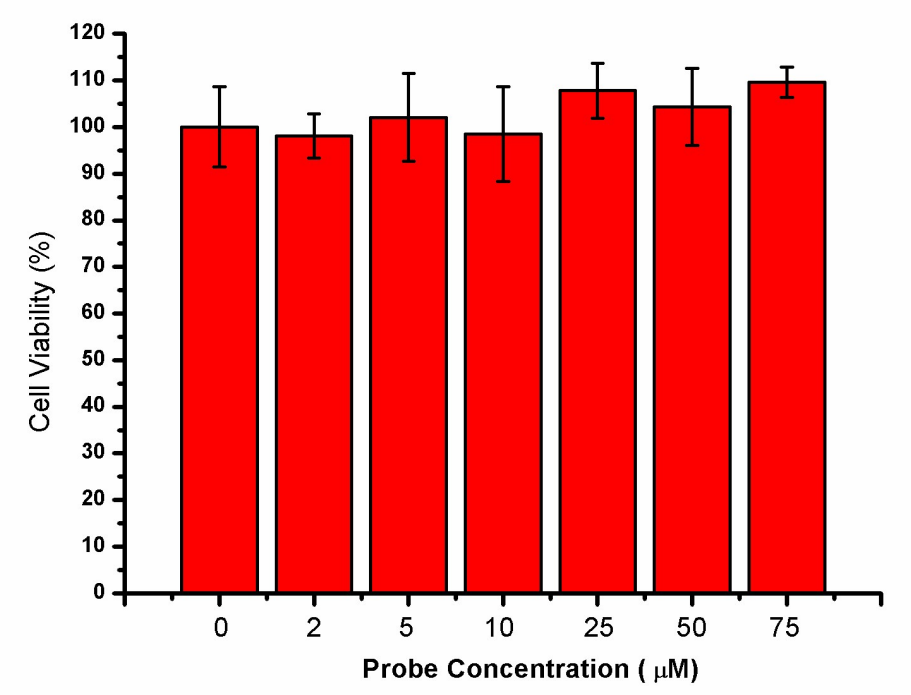

Figure S 10. Cell survival rate of control groups (without **FNTR**) and experimental group (with 2,, 5, 10, 25, 50, 75 μM of **FNTR**). All groups contain 1 % DMSO in 100 μL DMEM)

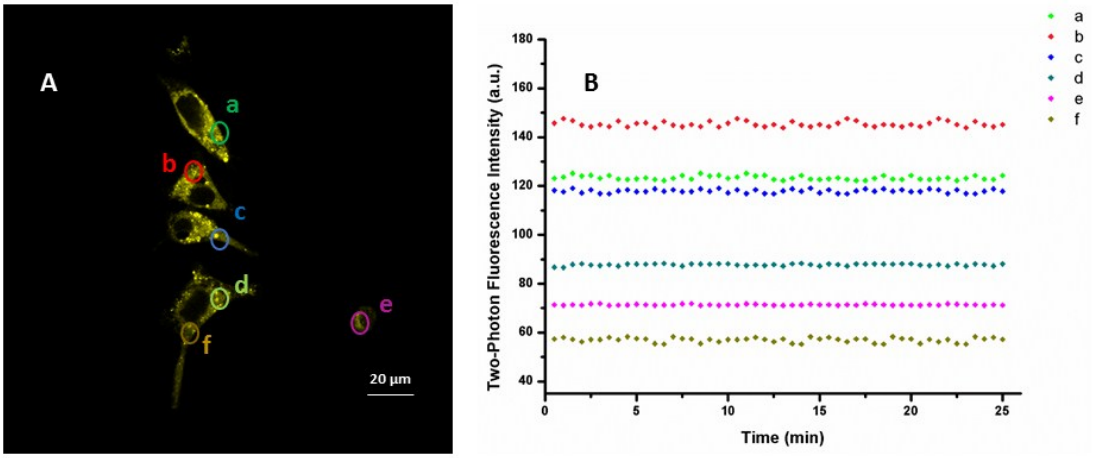

Figure S 11. (A) TPM images of HeLa cells labeled with 200 μM $CoCl_2$ for 48 h and further incubated with **FNTR** for 30 min separately. (B) Two-photon fluorescence intensity from circle a-f as a function of time. The two-photon fluorescence intensity was collected with 15 sec intervals for the duration of 25 min under *xyt* mode. Scale bar: 20 μm

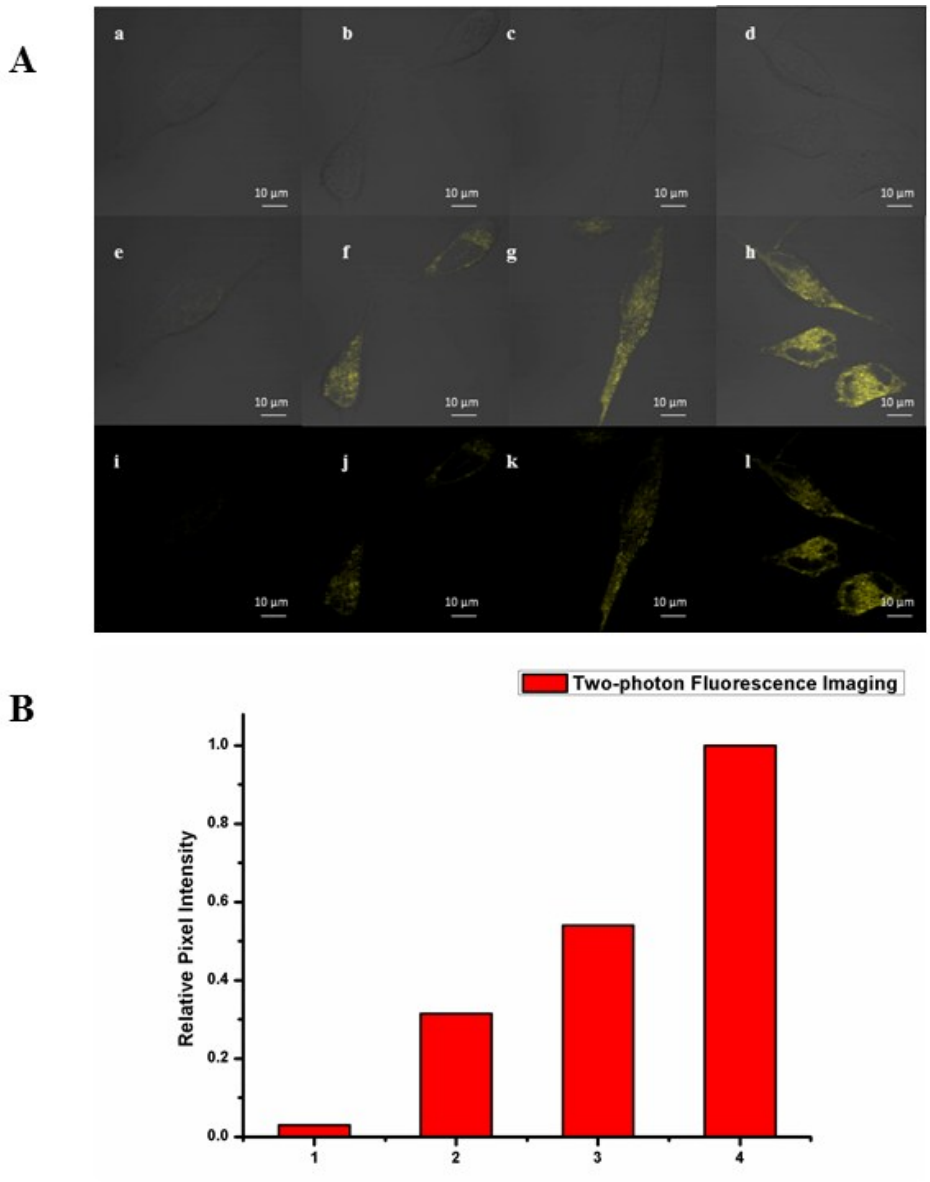

Fig. S 12 (A) TPM images of HeLa cells under normoxic, 200 μM $CoCl_2$ for 4, 24, 48 h, respectively. (a-d) The differential interference contrast (DIC) images. (e-h) Merged images of TPM and DIC. (i-l) TP fluorescence images of HeLa cells under normoxic and different hypoxic conditions. λ_{ex}= 750 nm; yellow channel: λ_{em} = 480-680 nm. Scale bar: 10 μm. (B) Relative pixel fluorescence intensity of the HeLa cells images. These cells were incubated normoxic (0 μM $CoCl_2$) and 200 μM $CoCl_2$ for 4, 24 and 48 h, respectively. And then incubated with 10 μM **FNTR** for 30 min. The strongest pixel

signal intensity from the images of HeLa cells under 200 μM $CoCl_2$ for 48 h is defined as 1.0.

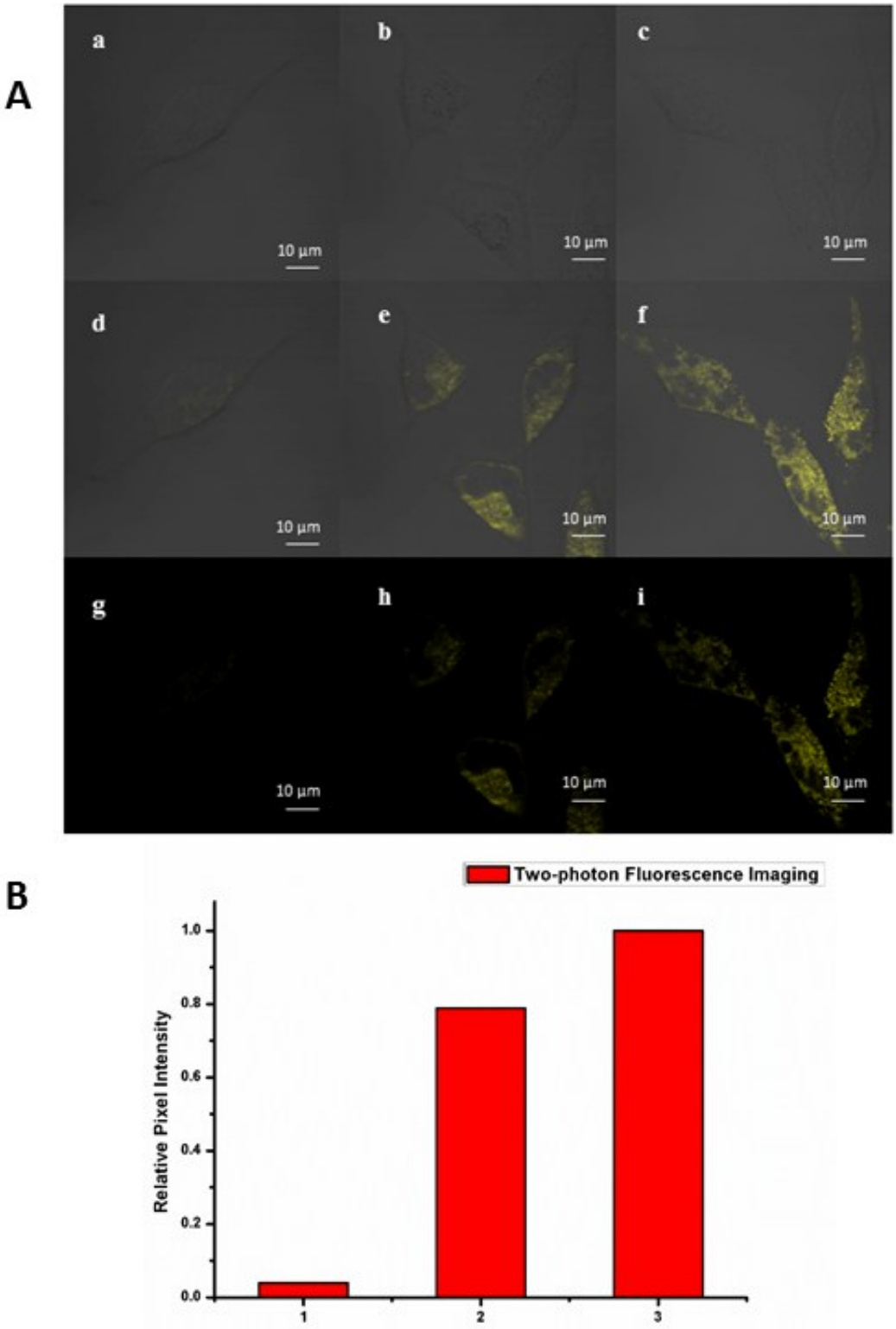

Fig. S 13 (A) TPM images of HeLa cells under normoxic, 400 μM $CoCl_2$ for 4, 24 h, (a-c) The differential interference contrast (DIC) images. (d-f) Merged

images of TPM and DIC. (g-i) TP fluorescence images of HeLa cells under normoxic and different hypoxic conditions. λ_{ex}= 750 nm; yellow channel: λ_{em} = 480-680 nm. Scale bar: 10 μm. (B) Relative pixel fluorescence intensity of the HeLa cells images. These cells were incubated normoxic (0 μM $CoCl_2$) and 400 μM $CoCl_2$ for 4, 24 h, respectively. And then incubated with 10 μM **FNTR** for 30 min. The strongest pixel signal intensity from the images of HeLa cells under 400 μM $CoCl_2$ for 24 h is defined as 1.0.

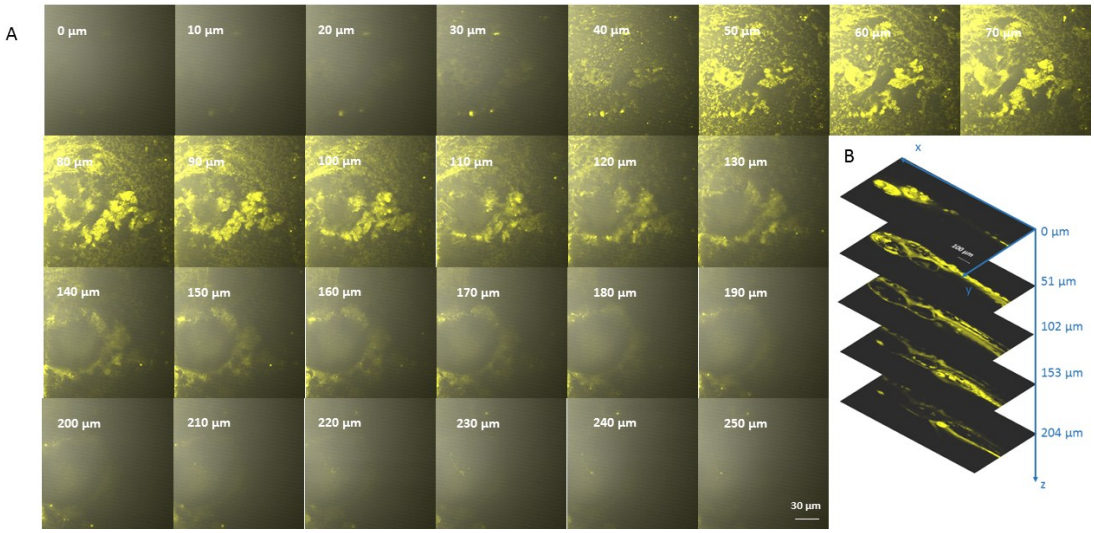

Fig. S 14 Separate images at different *z*-axis depth of the **FNTR**-labeled rat liver tissue and zebrafish . The fluorescence intensity was reflected by color. Scale bar: 30 μm and 100 μm, respectively.