Supporting Information for

Efficient two-photon fluorescent probe for measuring γ-glutamyltranspeptidase activity during oxidative stress process in tumor cells and tissues

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Table S1. Determination of GGT Level in Tissue Samples.

Sample	GGT Level/ U L^{-1}
normal liver tissue	7.36 ± 4.18
liver cancer tissue	83.56 ± 25.56

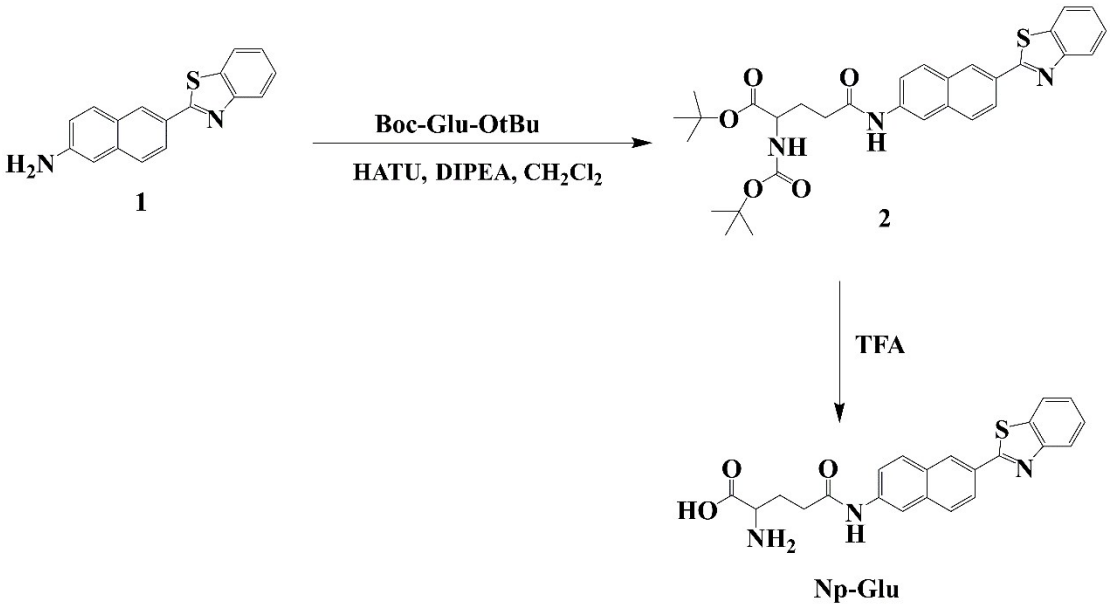

Fig. S1. Synthesis route of probe **Np-Glu**.

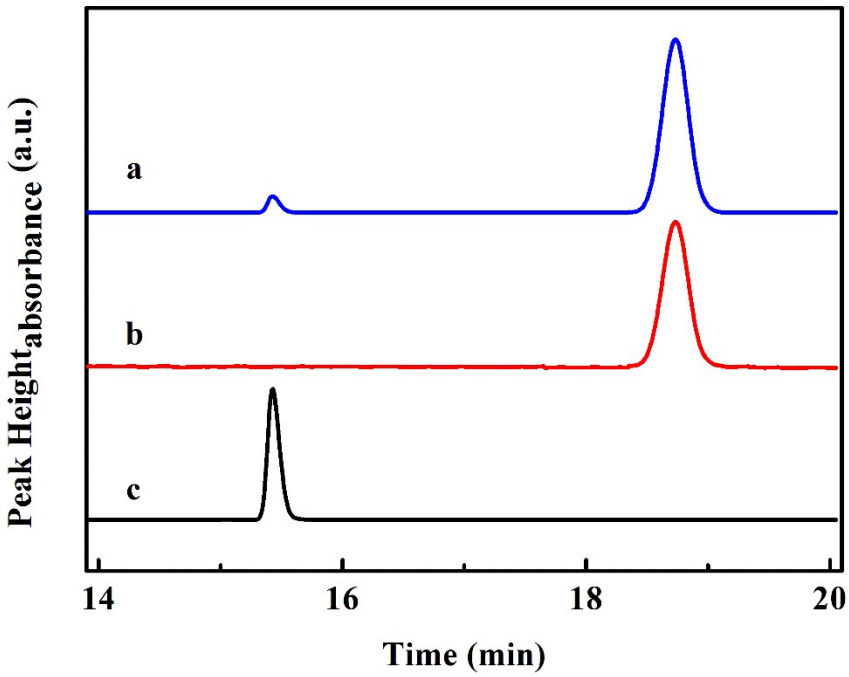

Fig. S2. Chromatograms of different reaction systems. (a) the reaction solution of 10 μM **Np-Glu** with 100 U L^{-1} GGT; (b) 10 μM compound **1**; (c) 10 μM **Np-Glu**. Detection: UV-vis (325 nm) detector. Flow rate: 3 mL/min. T: 25 °C. Injection volume: 100 μL. Mobile phase: water-acetonitrile, 70:30 (v/v) to 30:70 for 30 min.

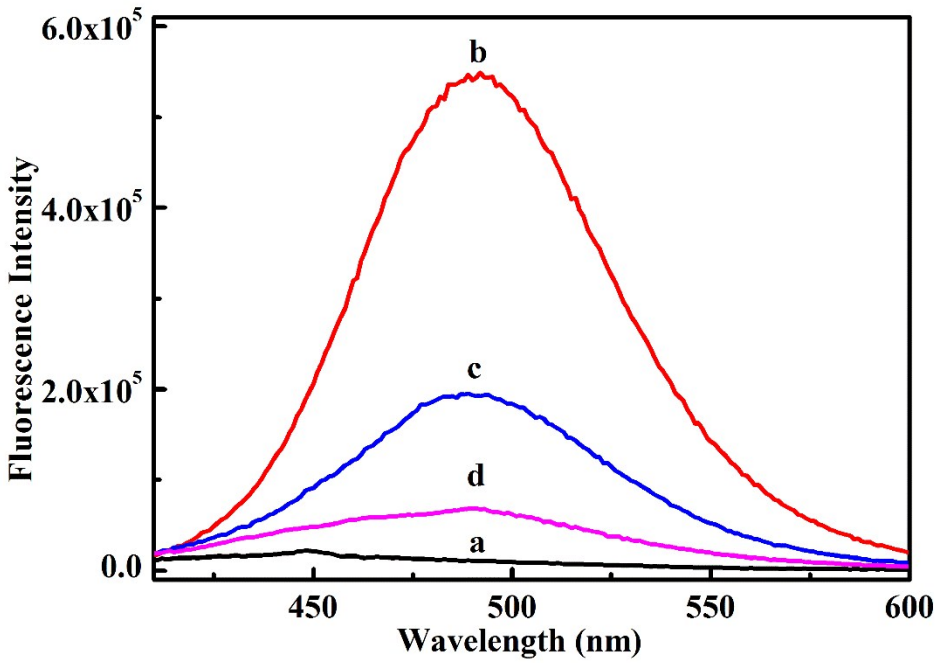

Fig. S3. Fluorescence emission spectra of different reaction systems. (a) **Np-Glu** (10μM) in PBS buffer (control); (b) system (a) + GGT (20 U L^{-1}); (c) system (b) + DON (1 mM); (d) system (b) + DON (3 mM). λ_{ex} = 390 nm.

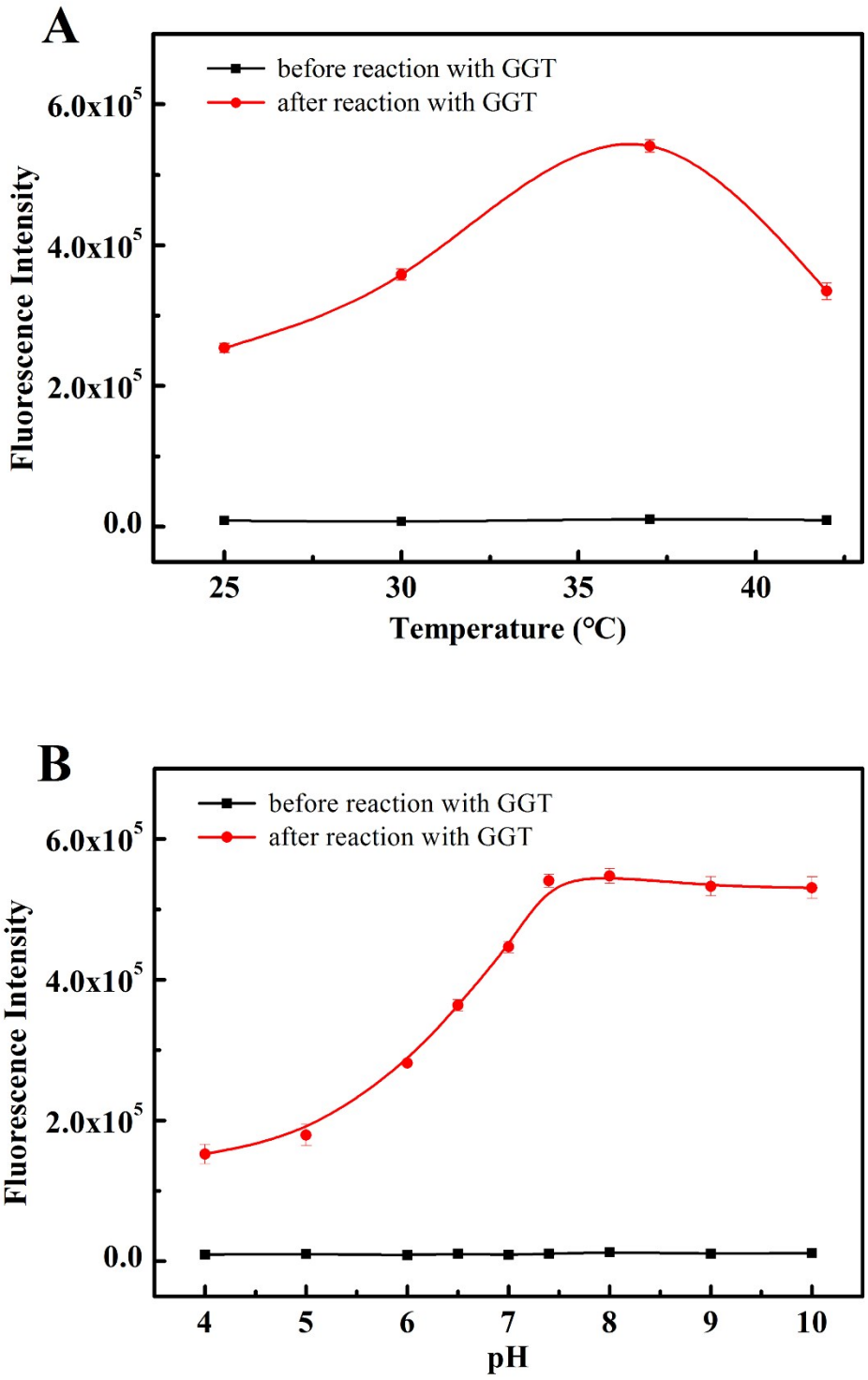

Fig. S4. Effects of (A) temperature and (B) pH on the fluorescence ($\lambda_{ex/em}$ = 390/490 nm) of **Np-Glu** (10 μM) reacting with GGT (20 U L^{-1}). The results are the mean standard deviation of three separate measurements.

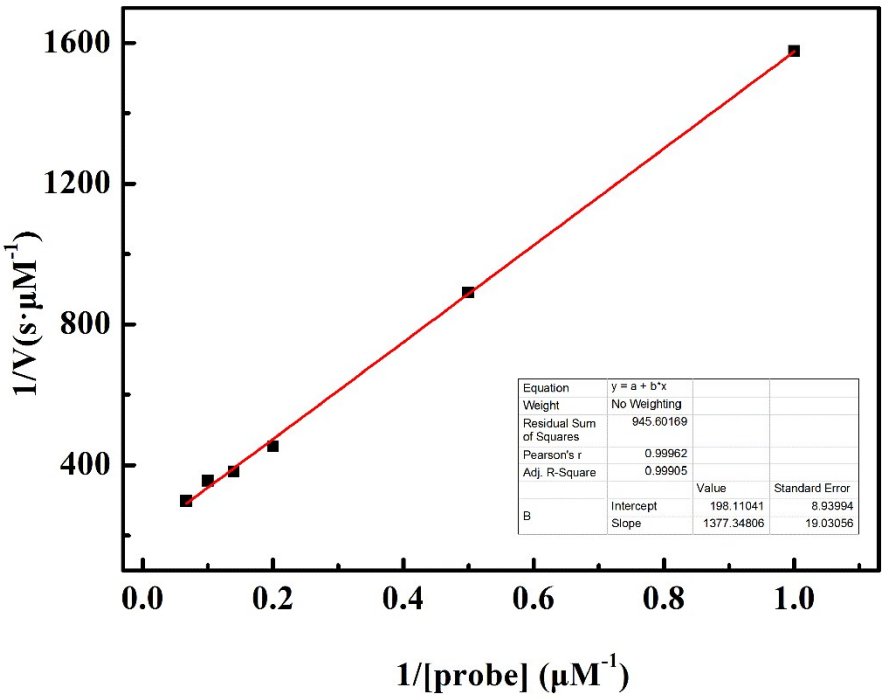

Fig. S5. Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V\text{max}\ [\text{probe}] / (K_m + [\text{probe}])$, where V is the reaction rate, [probe] is the probe concentration (substrate), and K_m is the Michaelis constant. Conditions 20 U L^{-1} GGT, 1, 2, 5, 7, 10, and 15 μM of **Np-Glu**, $\lambda_{ex/em}$ = 390/490 nm. Points were fitted using a linear regression model (correlation coefficient R = 0.999).

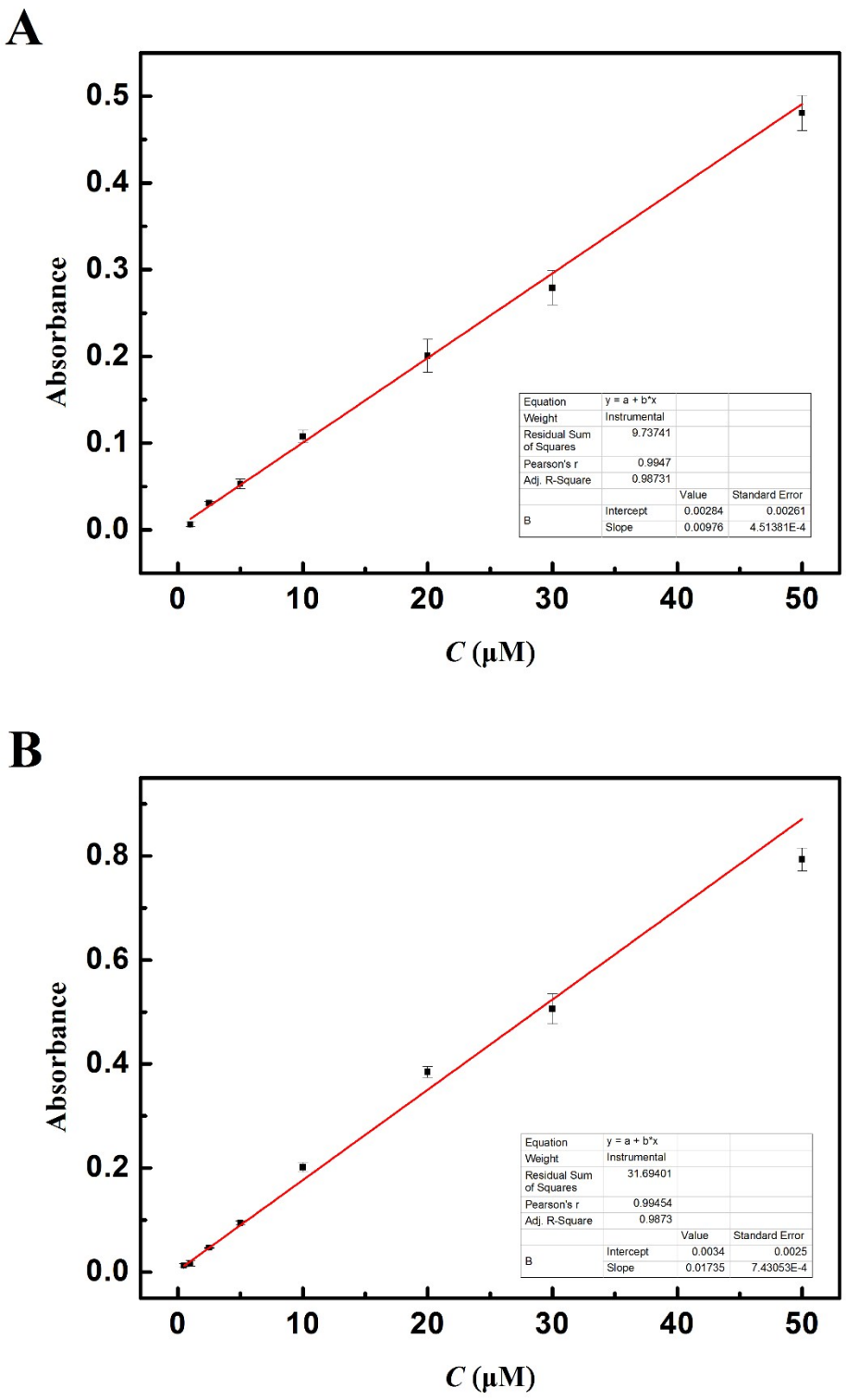

Fig. S6. The solubility data of **Np-Glu** (A) and compound **1** (B) in buffer solution used for cell incubation.

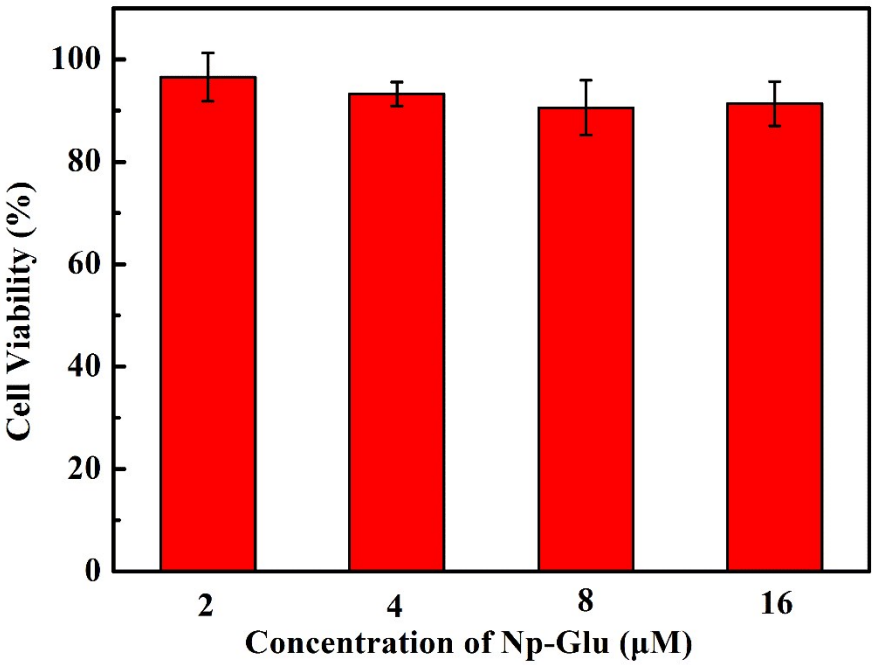

Fig. S7. Effects of **Np-Glu** with varied concentrations (2, 4, 8, 16 μM) on the viability of HepG2 cells. The viability of the cells without **Np-Glu** is defined as 100%. The results are the mean ± standard deviation of five separate measurements.

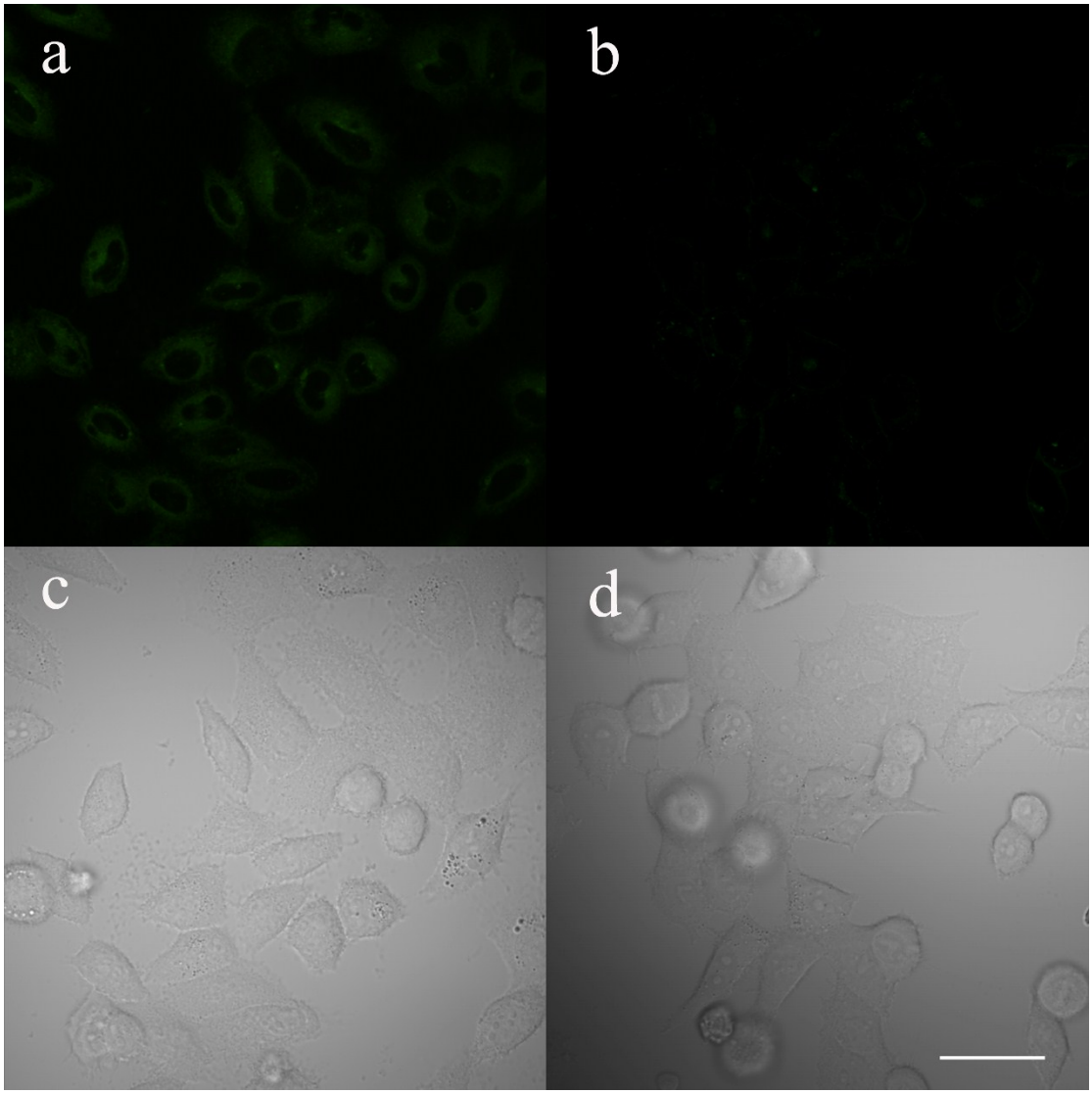

Fig. S8. TP fluorescence images (a) and DIC images (c) of HepG2 cells incubated with 5 μM **Np-Glu**. TP fluorescence images (b) and DIC images (d) of HepG2 cells incubated with 5 μM **Np-Glu** and 3 mM DON. Scale bar: 40 μm

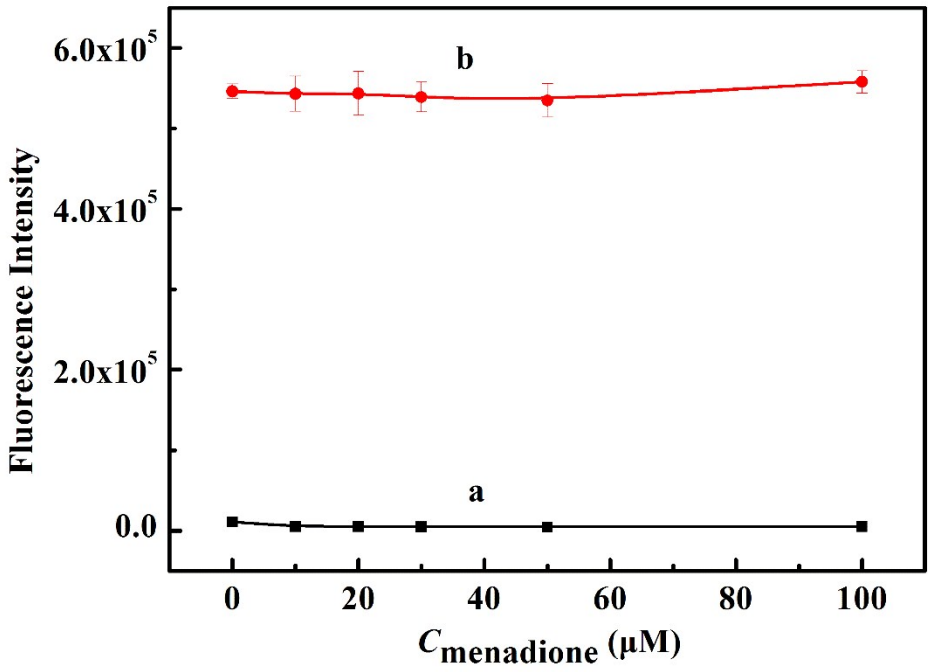

Fig. S9. Effects of menadione at varied concentrations (0, 10, 20, 30, 50 and 100 μM, respectively) on the fluorescence ($\lambda_{ex/em}$ = 390/490 nm) of **Np-Glu** with and without GGT. (a) menadione at varied concentrations was mixed with **Np-Glu** (10 μM) at 37 °C for 45 min. (b) GGT (20 U L^{-1}) was pretreated with menadione at varied concentrations for 6 h at 37 °C, and then incubated with **Np-Glu** (10 μM) for 45 min. The results are the mean ± standard deviation of three separate measurements.

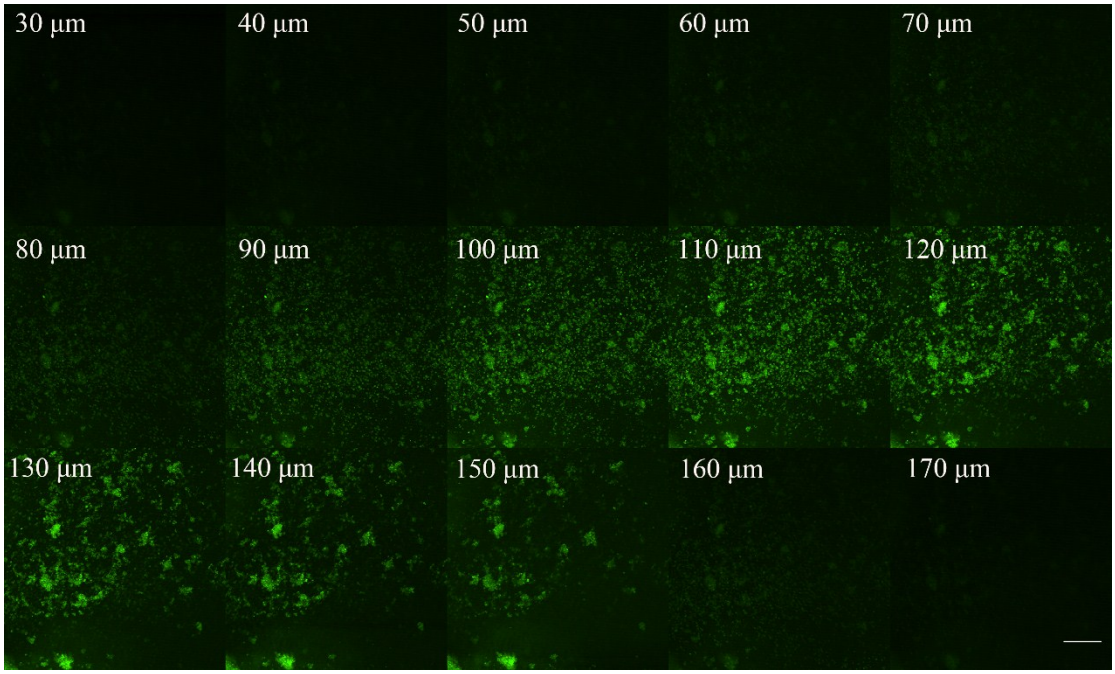

Fig. S10. Depth of TP fluorescence images of **Np-Glu** (5 μM) in tissues. Scale bar: 200 μm.

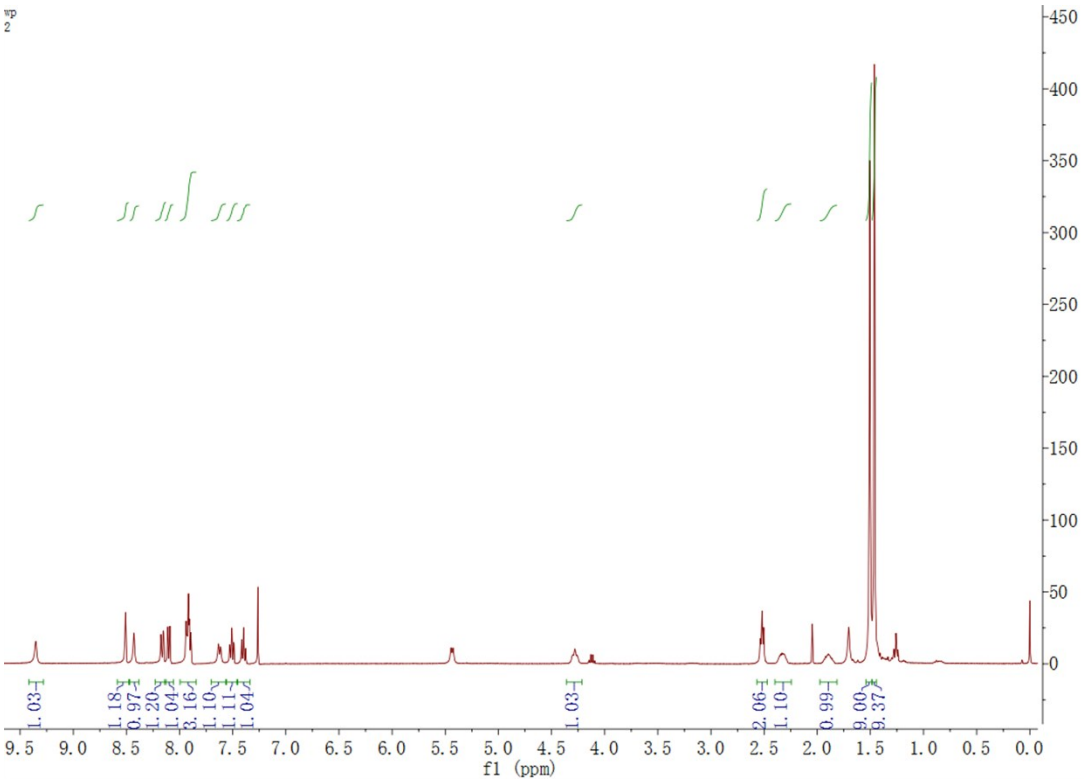

Fig. S11. ^{1}H NMR spectrum of the compound **2**.

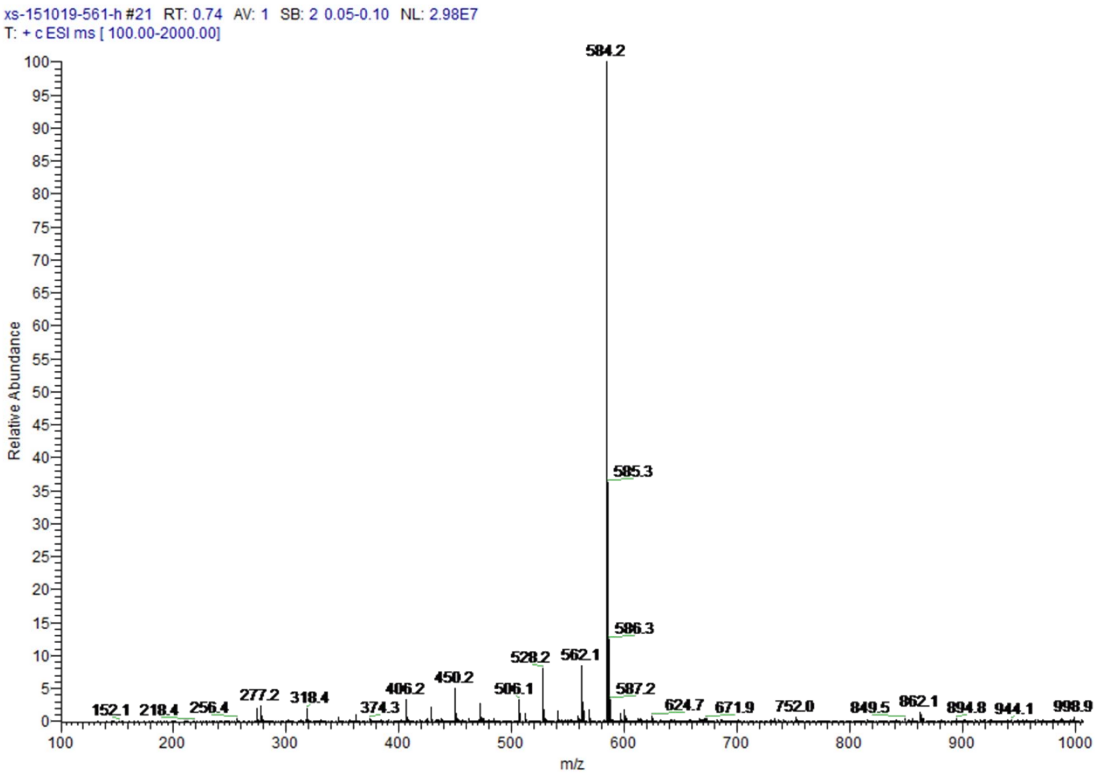

Fig. S12. ESI-MS spectrum of the compound **2**.

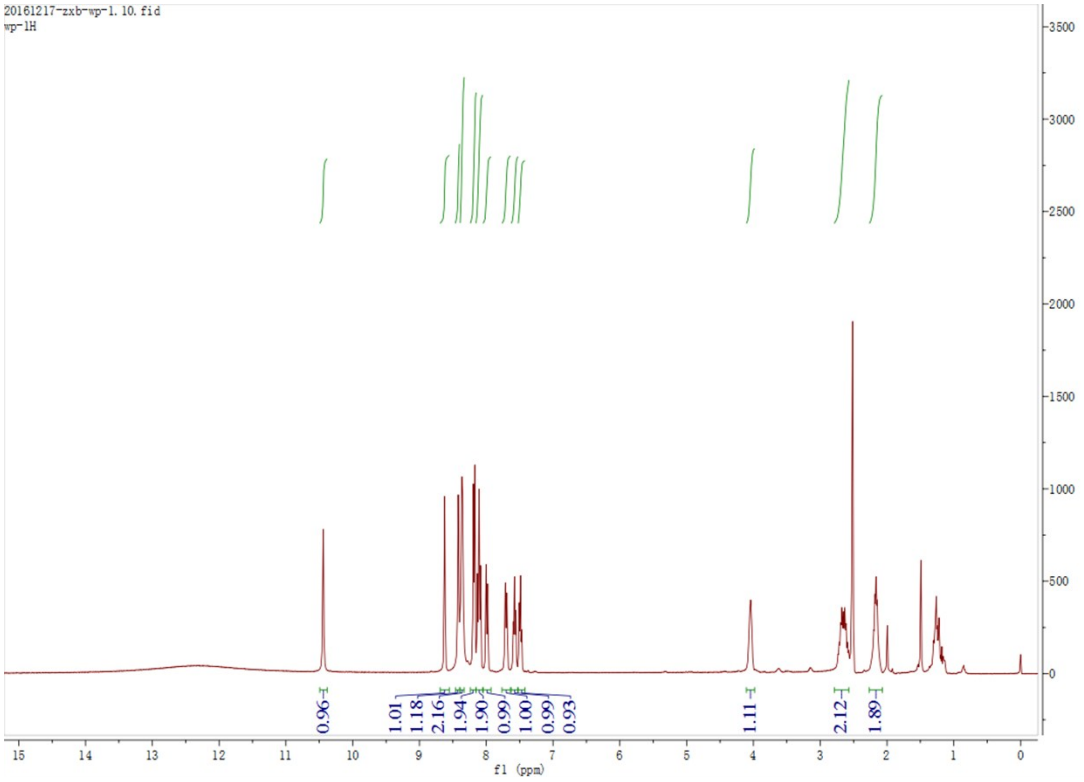

Fig. S13. ^{1}H NMR spectrum of **Np-Glu**.

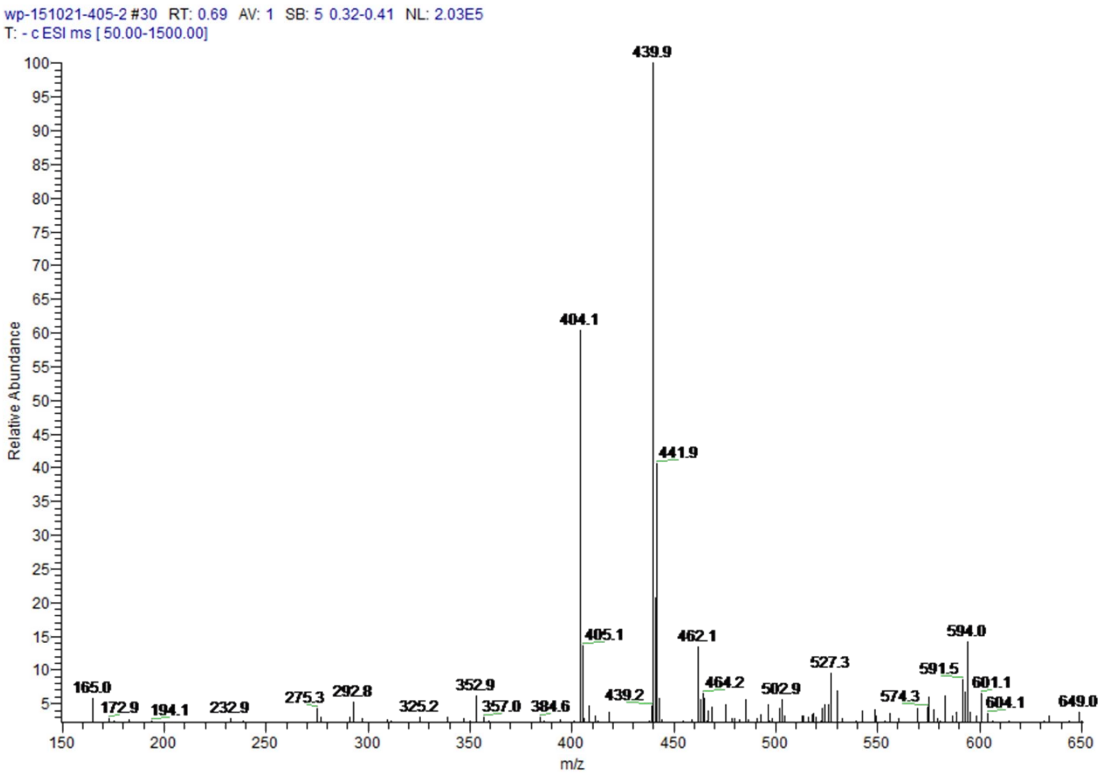

Fig. S14. ESI-MS spectrum of **Np-Glu**.

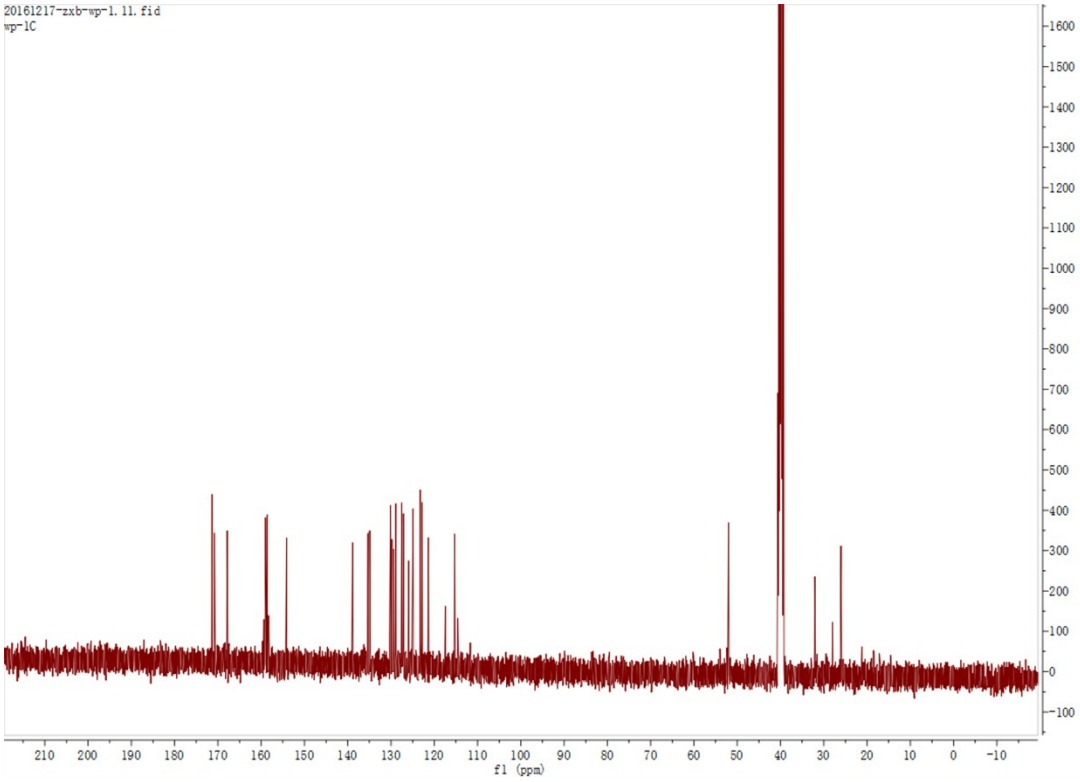

Fig. S15. ^{13}C NMR spectrum of **Np-Glu**.