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Supporting Information

A novel "AIE + ESIPT" near-infrared nanoprobe for the imaging of γ-glutamyl transpeptidase in living cells and the application in precision medicine

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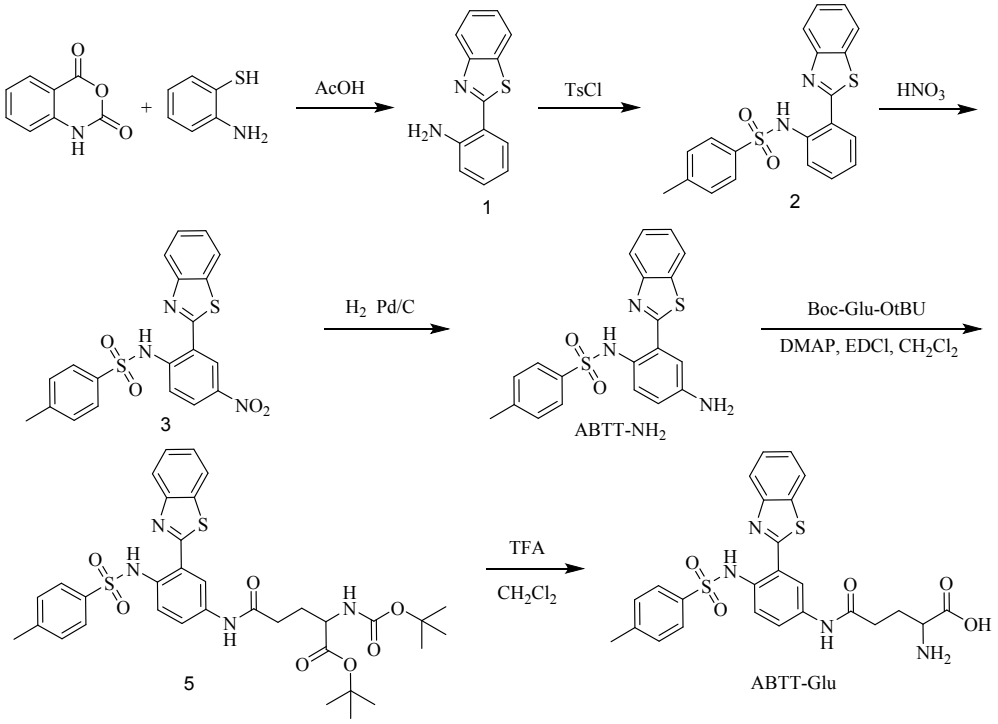

Scheme S1. Synthesis process for **ABTT-Glu**.

2-(benzo[*d*]thiazol-2-yl)aniline (1)

First, isatoic anhydride (1.0 g, 6.13 mmol) and 2-aminothiophenol (0.85 mL, 7.97 mmol) were dissolved in acetic acid (20 mL) and refluxed at 45 °C for 2 h. Then, the reaction was quenched with water and the pH was adjusted to neutral. Filtered to obtain the crude product and washed with saturated $NaHCO_3$ solution three times. Crude product was extracted by DCM and the solvent was removed under reduced pressure. The crude product was purified by chromatography on a silica gel (eluent: PE/EA=10/1, v/v) to give 1.1 g (4.90 mmol, 80.1% yield) compound **1** as a brownish yellow solid.

***N*-(2-(benzo[*d*]thiazol-2-yl)phenyl)-4-methylbenzenesulfonamide (2)**

Compound **1** (240 mg, 1 mmol) and tosyl chloride (400 mg, 2 mmol) were mixed in pyridine (2.5 mL) and stirred at room temperature for 3 h. Aqueous HCl was added and the mixture was filtered under reduced pressure to obtain crude product. Then, crude product was extracted by DCM and the solvent was removed under reduced pressure. The crude product was purified on silica gel (eluent: PE /DCM=5/1, v/v) to give 384

mg (0.95 mmol, 95.1% yield) of compound **2** as a pale yellow solid.

***N*-(2-(benzo[*d*]thiazol-2-yl)-4-nitrophenyl)-4-methylbenzenesulfonamide (3)**

Compound **2** (190 mg, 0.50 mmol) and 10 mL of acetic acid were mixed and heated at 50 °C. At the same time, 0.1 mL of nitric acid was dropped slowly to the reaction and stirred for 2 h. Aqueous NaOH was added and the mixture was filtered under reduced pressure to obtain crude product. Crude product was washed with saturated $NaHCO_3$ solution three times and dried for further purification. Then, crude product was extracted by DCM and the solvent was removed under reduced pressure. The crude product was purified on silica gel (eluent: PE /DCM=5/1-2/1, v/v) to provide 108 mg (0.25 mmol, 50.8% yield) of the compound **3** as a tan solid.

***N*-(4-amino-2-(benzo[*d*]thiazol-2-yl) phenyl)-4-methylbenzenesulfonamide (ABTT-NH_2)**

First, compound **3** (108 mg, 0.25 mmol) was dissolved in THF (10 mL) under nitrogen, 10 % Pd/C (10 mg). The resulting solution was stirred at room temperature for 30 min. After removing solids by filtration, the solution was extracted with dichloromethane and the solvent was removed under reduced pressure. Then, residual crude product was purified on silica gel (eluent: PE /DCM=1/1-1/3, v/v) to provide 42 mg (0.11 mmol, 44.0% yield) of ABTT-NH_2 as a yellow solid.

All of the above compounds had been confirmed as the literature [1-2].

2.HPLC and ESI-MS analysis of ABTT-Glu mediated reactions.

The HPLC chromatograms of **ABTT-Glu**, ABTT-NH_2, and the reaction products of **ABTT-Glu**, ABTT-NH_2 and the reaction products of GGT and **ABTT-Glu** were performed with a C18 column (150 nm × 4.6 mm), using the following conditions: binary gradient elution with acetonitrile/H_2O=3/7-8/2 (v/v); flow rate: 6 mL/min; detection wavelength: 365 nm. To further verified the reaction mechanism, ESI-MS was recommended to analyze the products of **ABTT-Glu** after reaction with GGT in positive mode.

3. The investigation toward AIE effect of compound ABTT-NH_2.

First, ABTT-NH_2 was dissolved in MeOH (2 mmol/L) and diluted with different amounts of methanol and water. Samples were prepared as ABTT-NH_2 (10 μM) in mixed solution composed with different ratios of water/methanol. Then, the fluorescence spectra were recorded, and the fluorescence intensities at 650 nm were plotted against different water/methanol VOL%.

4. The effect of pH and temperature for the detection of probe ABTT-Glu toward GGT.

To evaluate the effect of pH and temperature for the detection of **ABTT-Glu** toward GGT, firstly, we mixed probe **ABTT-Glu** with GGT and incubated at different temperature (25 °C, 30 °C, 35 °C, 37 °C, 40 °C, 45 °C) for 1 h. Then, the fluorescence spectra were recorded. Furthermore, probe **ABTT-Glu** and GGT were mixed in buffers of different pH values (4.0, 5.0, 6.0, 6.8, 7.0, 7.4, 8.0, 9.0, 10.0) and incubated at 37 °C for 1 h. And, the fluorescence spectra were recorded. Fluorescence intensities at 650 nm were plotted against different pH and temperature values. The effect of pH and temperature on the stability of ABTT-Glu was also studied under the same conditions.

5. Kinetic Studies

A series of concentrations of **ABTT-Glu** (1, 2, 5, 10, 20 μM) were mixed with GGT (80 U/L) and incubated at 37 °C for 1 h in ultrapure water (pH=7.0), containing 0.5% DMF. Then, the fluorescence spectra were recorded for further analysis. The data points were in accordance with the Michaelis-Menten curve. According to the Michaelis Menten equation, the initial reaction velocity (μM S^{-1}) and kinetic parameters could be calculated (Michaelis Menten equation: $V = V_{max}*[S] (K_m + [S])$, where V is initial velocity, and [S] is substrate concentration).

6. Determination of the fluorescence quantum yield

Fluorescence quantum yields were determined by using quinine bisulfate (0.55 in 1.0

N H_2SO_4) as a standard according to a published method [3,4]. For ABTT-Glu, ABTT-NH_2 and quinine bisulfate, the absorbance spectra were measured. The quantum yield was calculated according to the equation:

$$\Phi_S = \Phi_R \times \frac{A_R \times F_S}{A_S \times F_R}$$

where Φ is the fluorescence quantum yield, A is the optical densities at the excitation wavelength and F is the corresponding relative integrated fluorescence intensities. Subscripts S and R refer to the test sample and the reference, respectively.

7. References

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8. Figures S1-S19

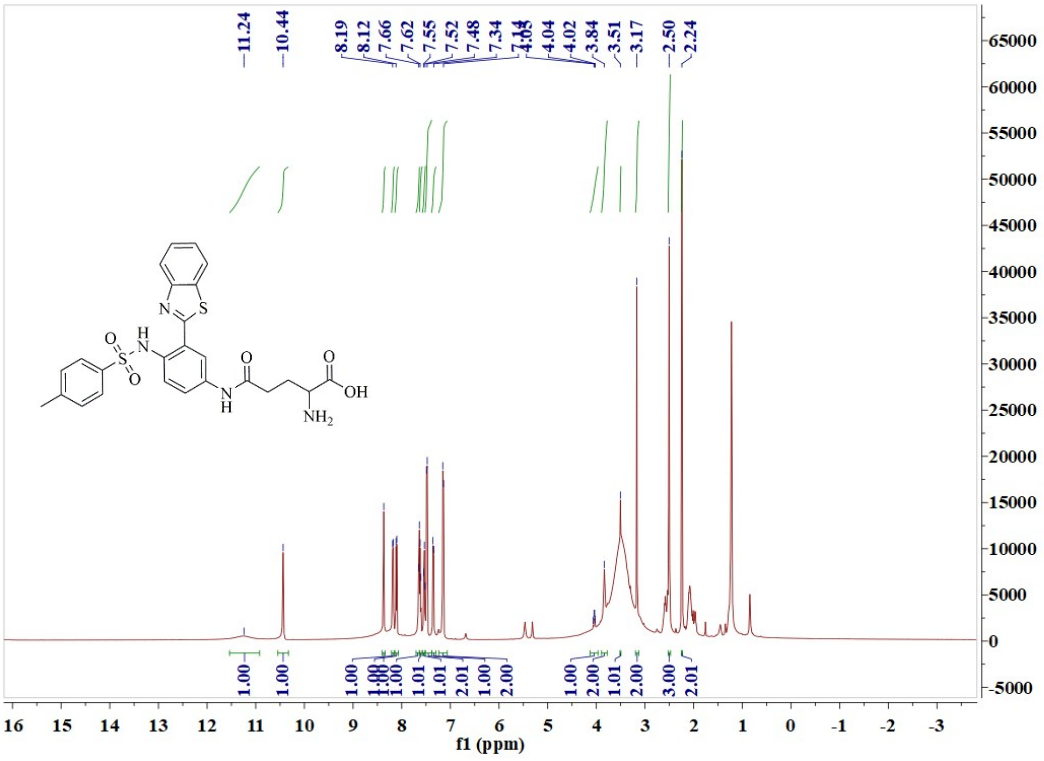

Fig.S1 ^{1}H NMR spectrum of **ABTT-Glu** in d6-DMSO.

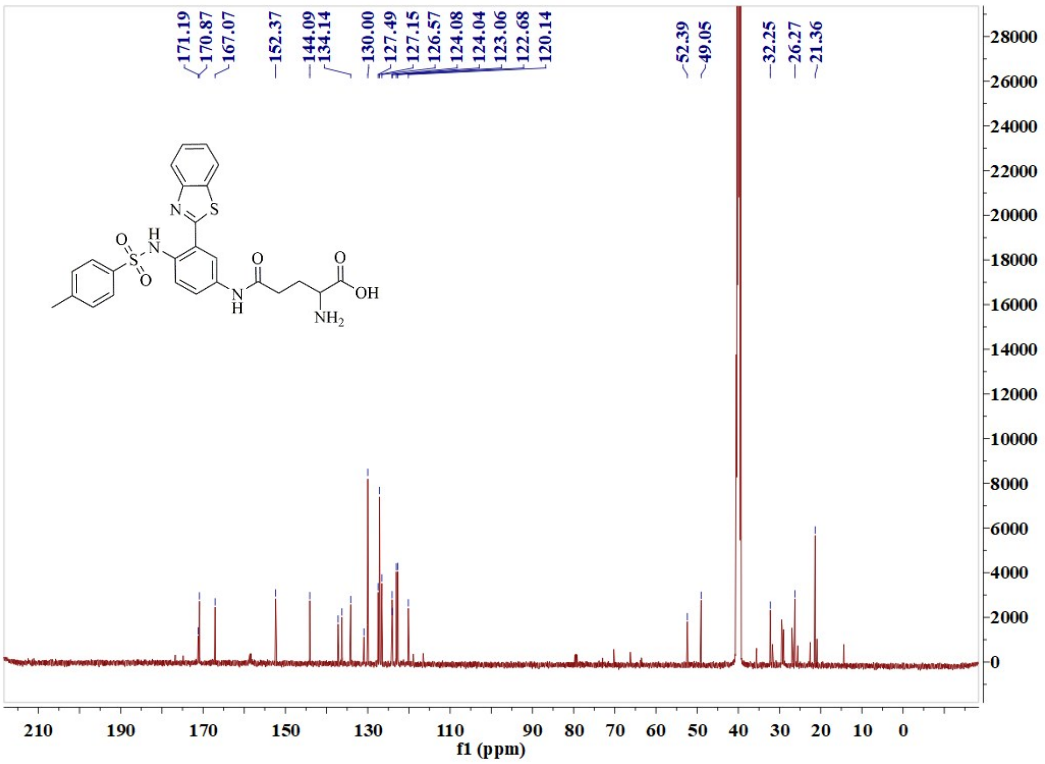

Fig.S2 ^{13}C NMR spectrum of **ABTT-Glu** in d6-DMSO.

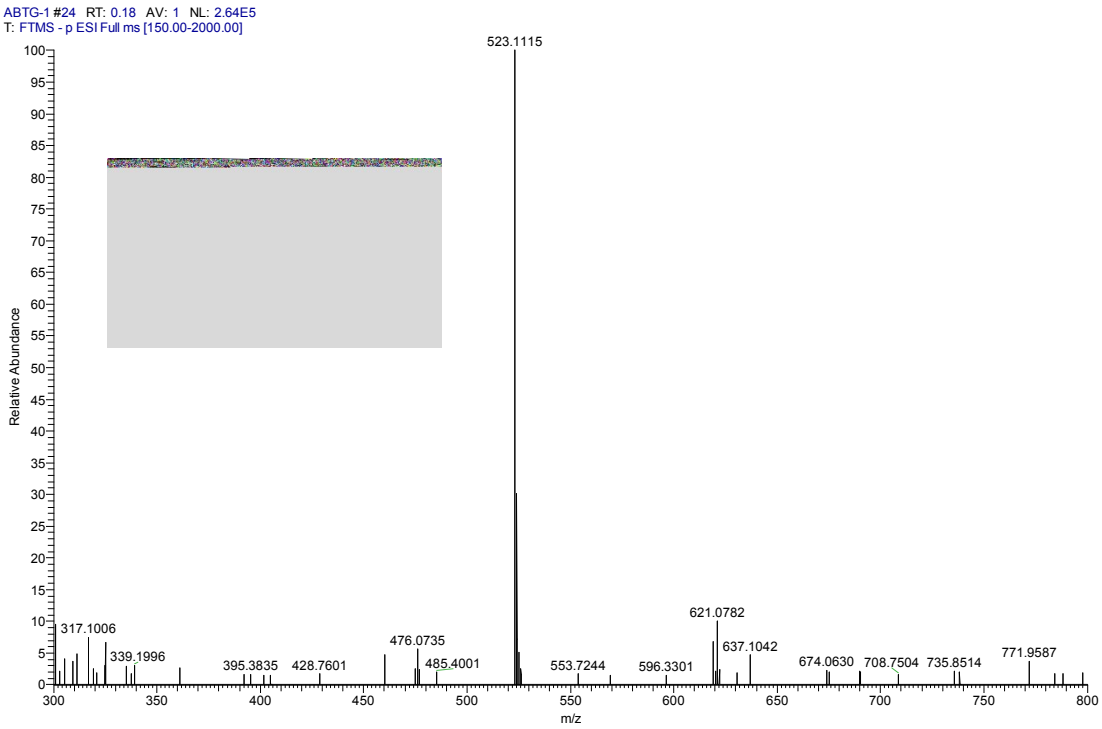

Fig. S3 ESI spectrum of **ABTT-Glu** in methanol.

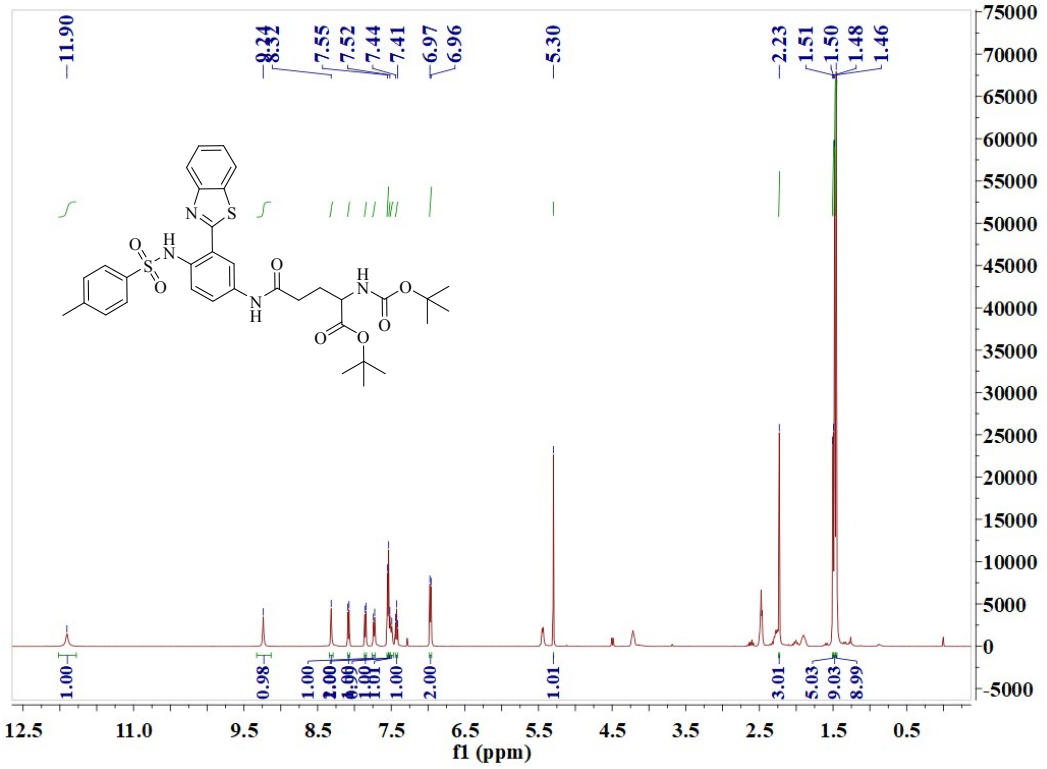

Fig.S4 ^{1}H NMR spectrum of ABTT-Glu-BOC in $CDCl_3$.

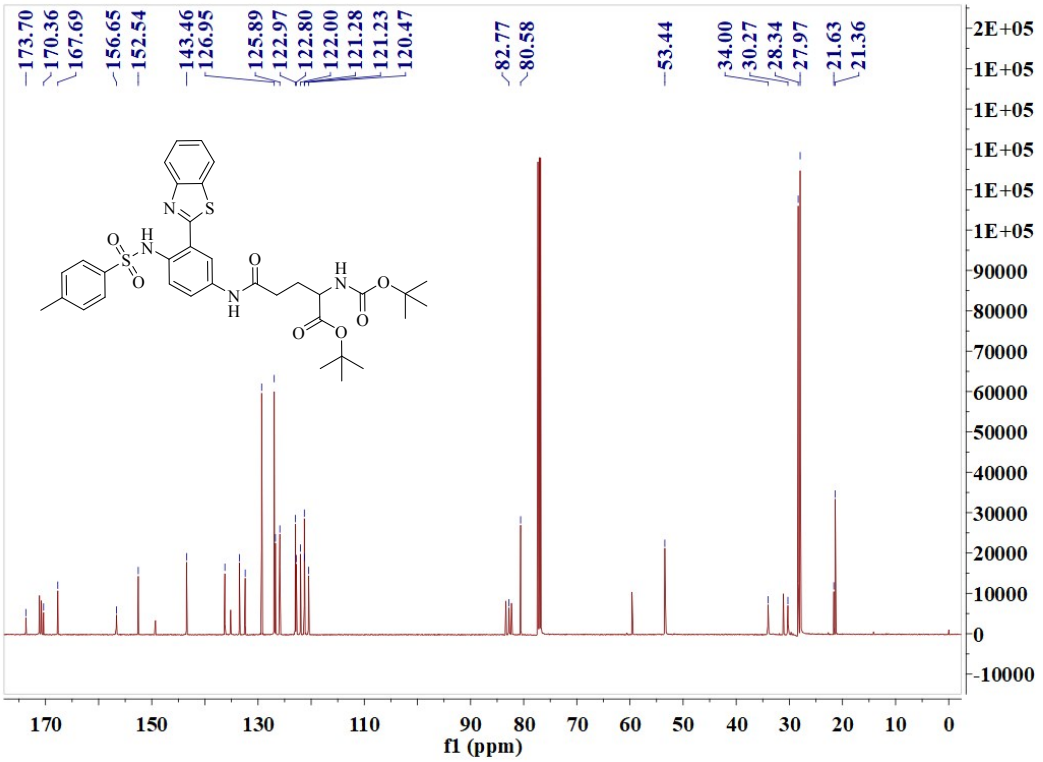

Fig.S5 ^{13}C NMR spectrum of ABTT-Glu-BOC in $CDCl_3$.

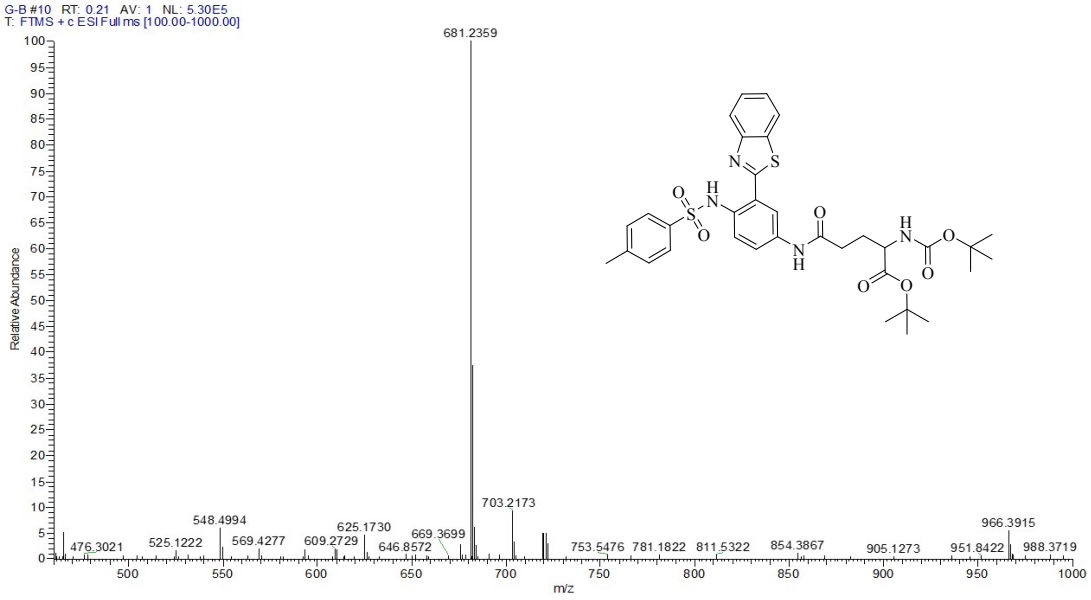

Fig. S6 ESI spectrum of ABTT-Glu-BOC in methanol.

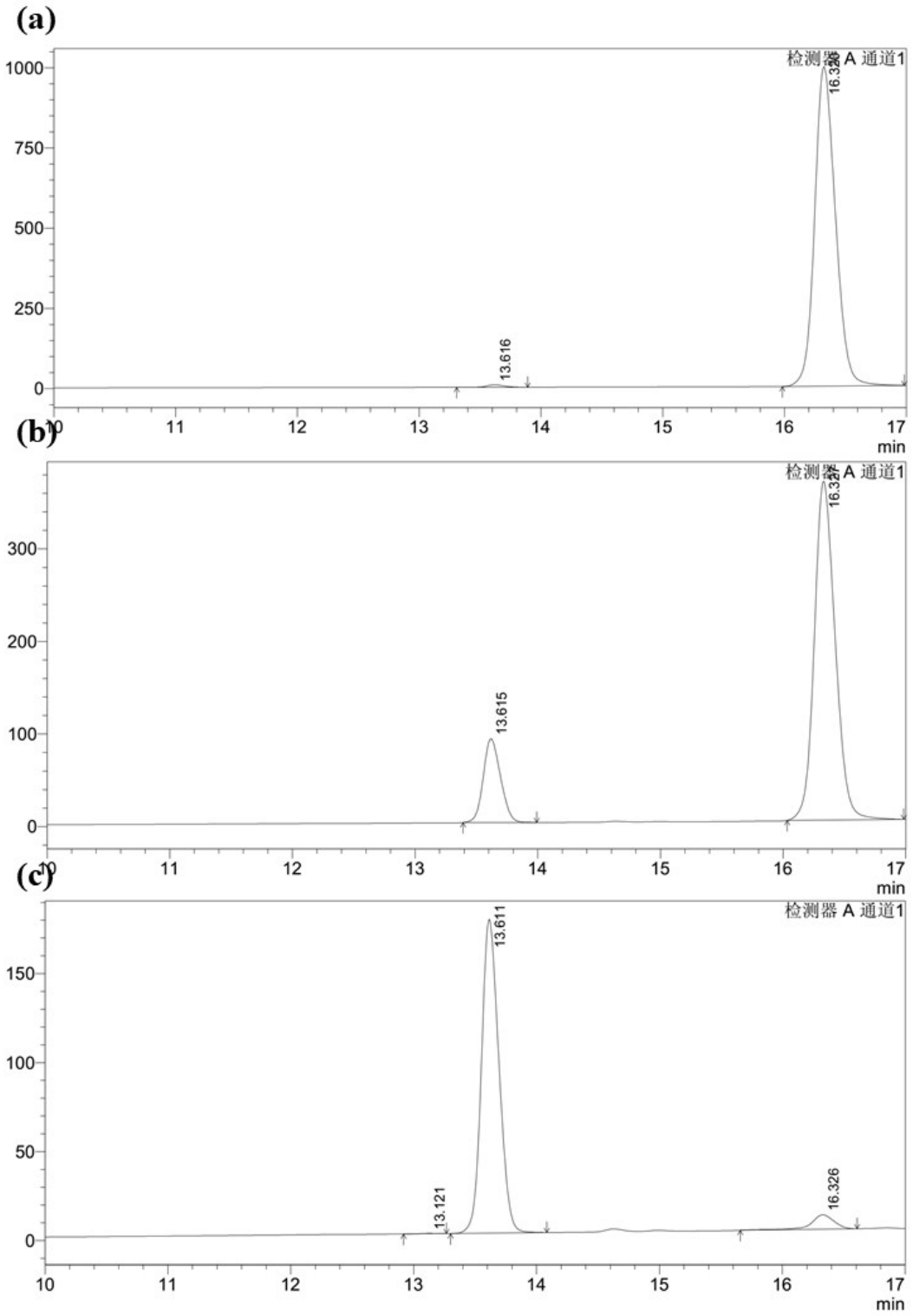

Fig. S7 HPLC profiles of (a) ABTT-NH_2, (b) **ABTT-Glu** incubated with GGT for 1 h at 37 °C, (c) **ABTT-Glu**. Detection wavelength=365 nm.

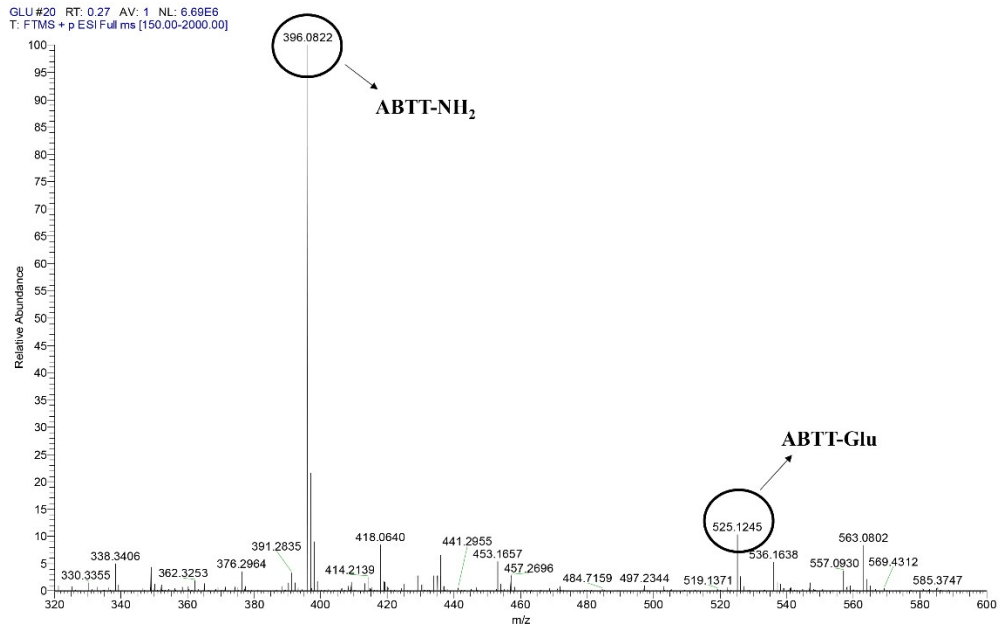

Fig.S8 HRMS (ESI) spectrum of **ABTT-Glu** incubated with GGT for 1 h at 37 °C.

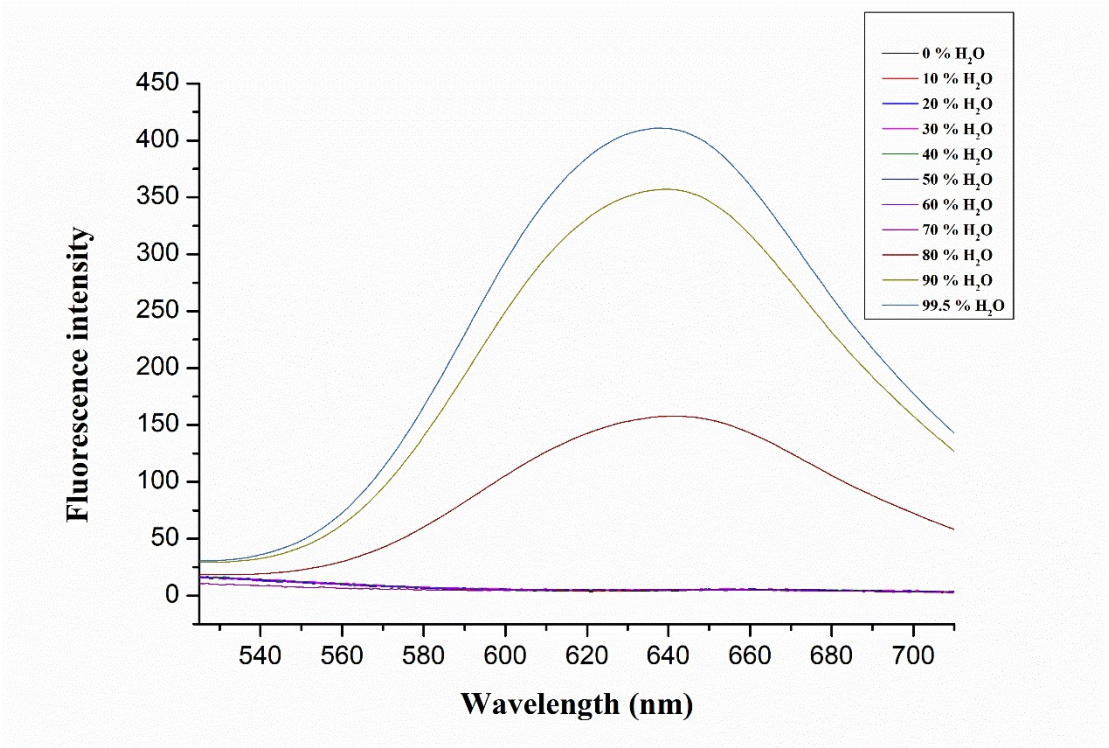

Fig. S9 The fluorescence spectra of ABTT-NH_2 in solution contained different ratios of water/methanol.

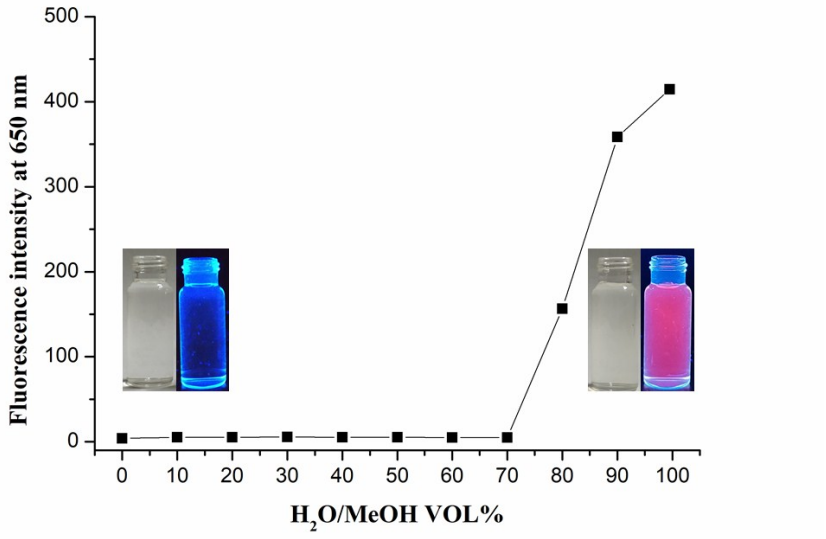

Fig. S10 The fluorescence emission intensity of ABTT-NH_2 at 650 nm in solution containing different ratios of water/methanol.

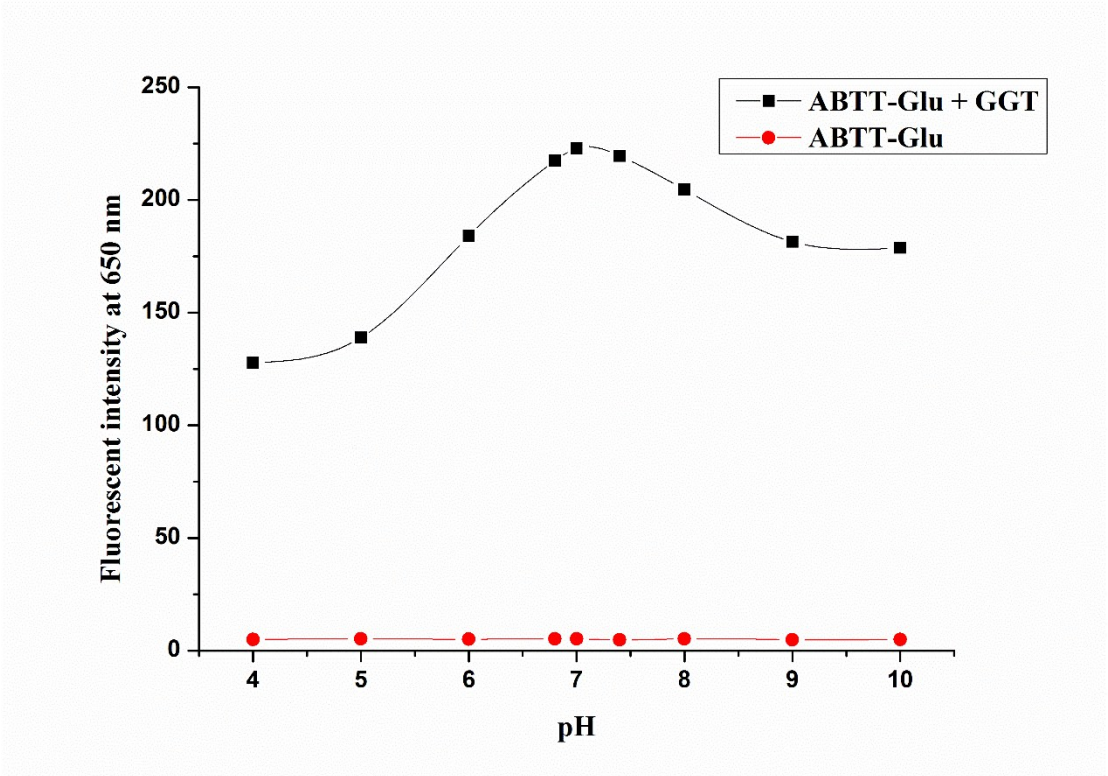

Fig. S11 Effects of pH on the fluorescence signal of **ABTT-Glu** (10 μM) without and with GGT (80 U/L). $\lambda_{ex/em}$= 365/650 nm.

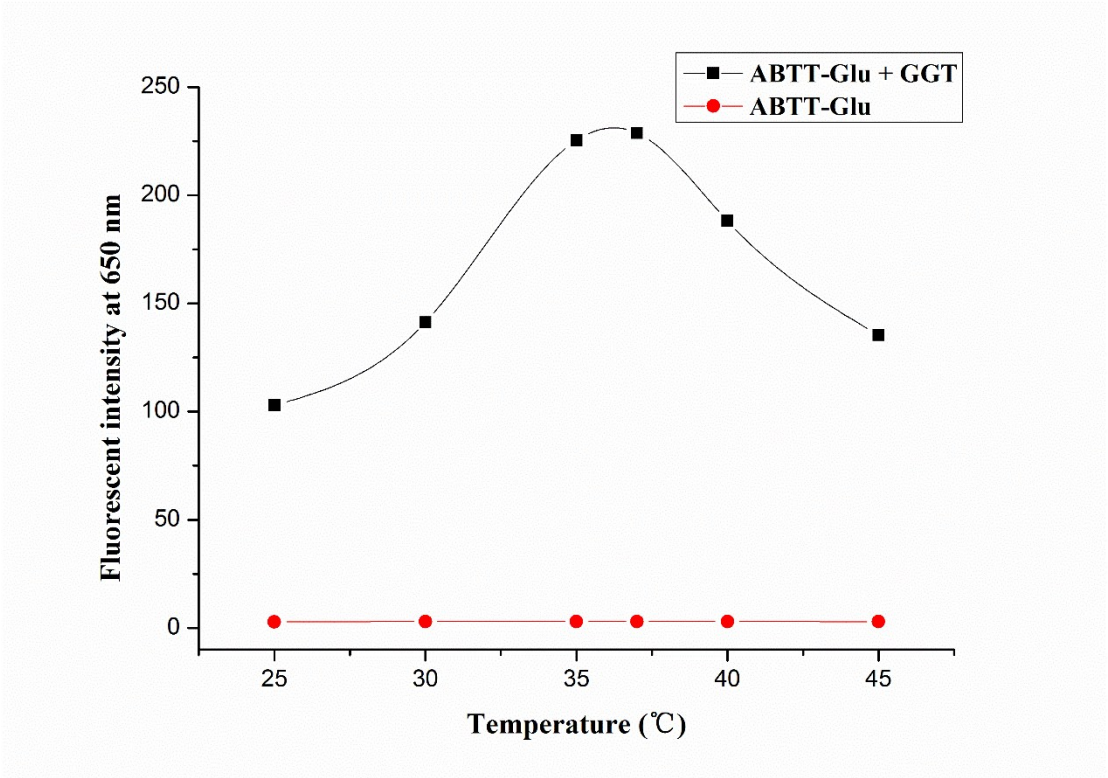

Fig. S12 Effects of temperature on the fluorescence signal of **ABTT-Glu** (10 μM) without and with GGT (80 U/L). $\lambda_{ex/em}$= 365/650 nm.

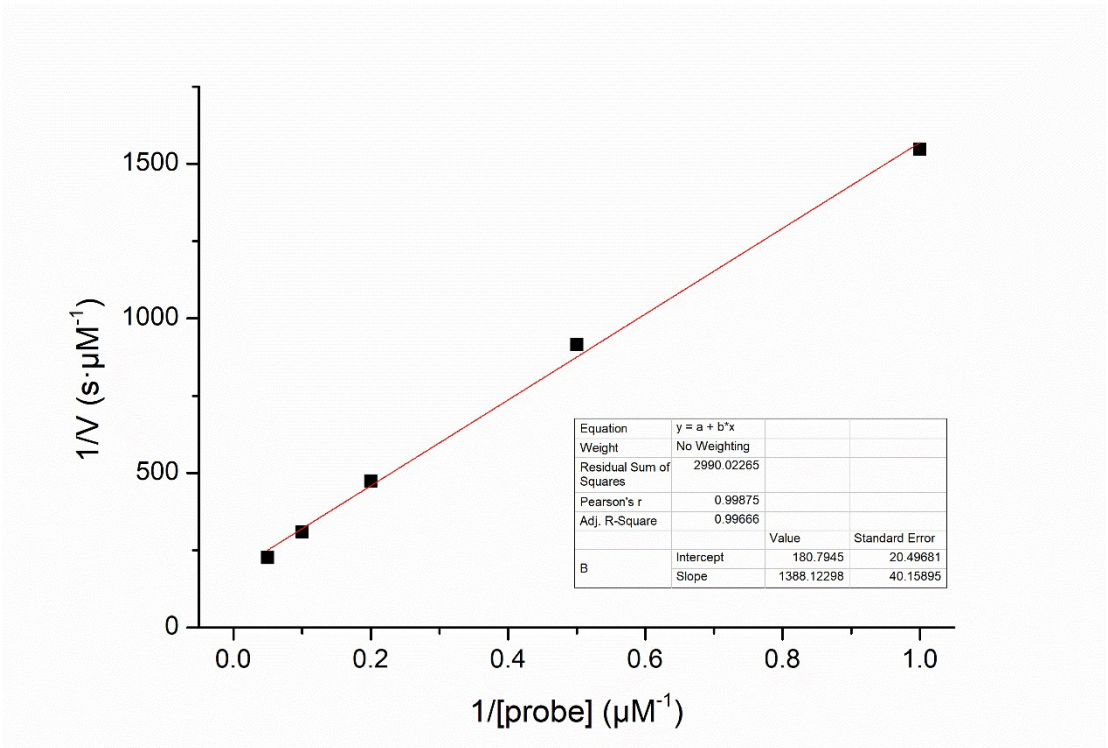

Fig. S13 Lineweaver-Burk plot for the GGT-catalyzed reaction. ([probe] is the **ABTT-**

Glu concentration, K_m is the Michaelis constant and was calculated to be 7.68 μM. Conditions: 80 U/L GGT, 1.0-20 μM of **ABTT-Glu**, $\lambda_{ex/em}$= 365/650 nm. Data points were fitted using a linear regression model with R^2= 0.996)

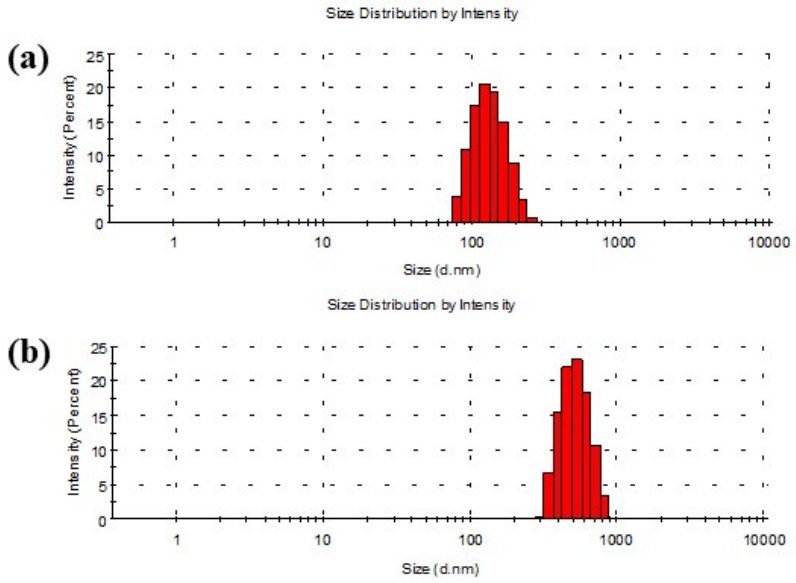

Fig. S14 The particle size of probe measured via DLS experiment. (a) the particle size of **ABTT-Glu**, (b) the particle size of **ABTT-Glu** incubated beforehand with GGT (80 U/L) at 37 °C for 1 h.

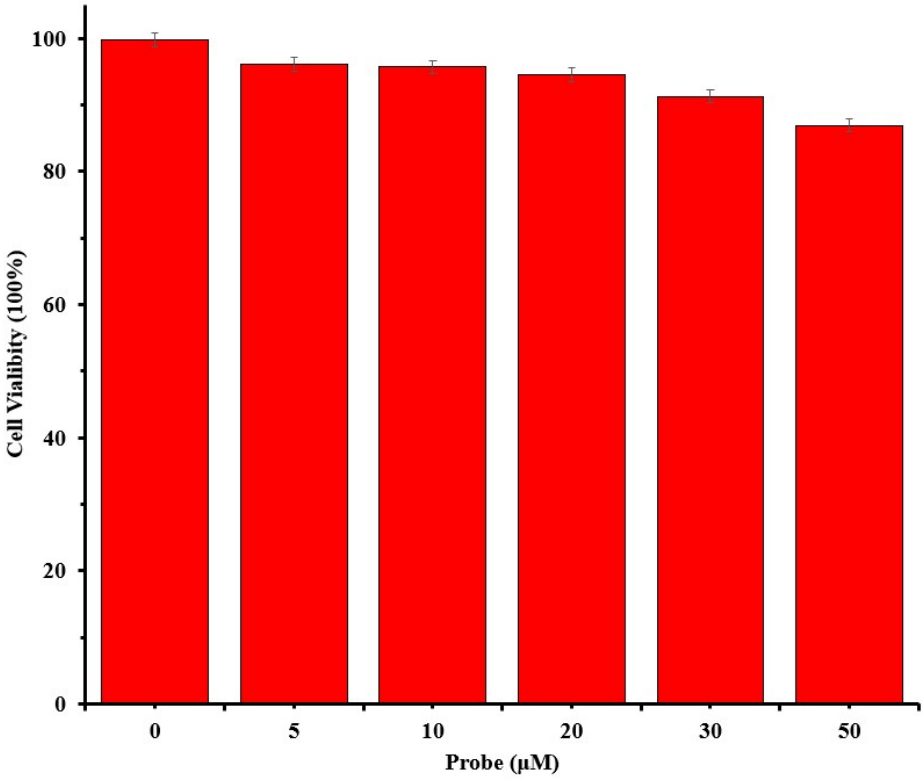

Fig. S15 Cell viability estimated by MTT assay. HepG2 cells were incubated with different concentrations of **ABTT-Glu** (0-50 μM) for 3 h. Error bars represent standard deviation of three repeated experiments.

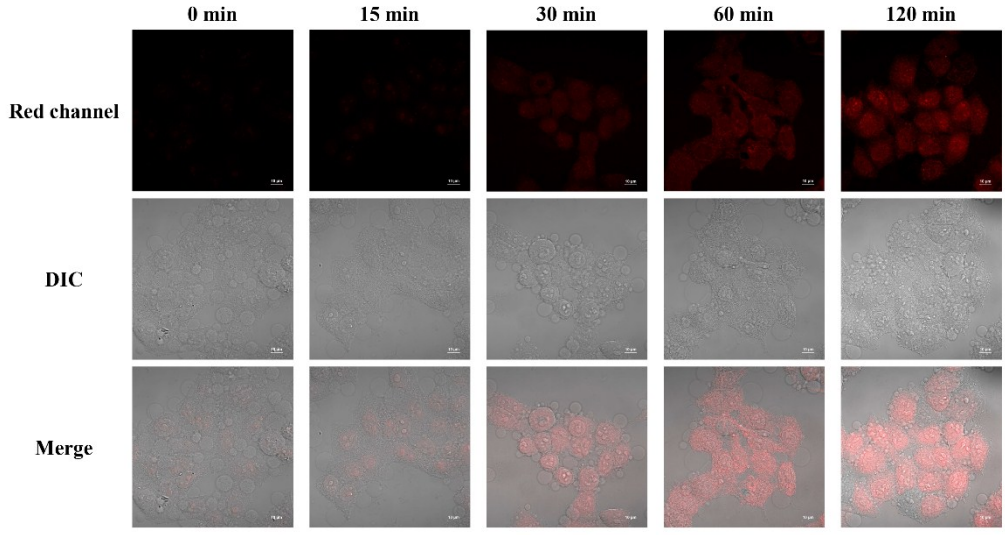

Fig. S16 Confocal fluorescence images of HepG2 cells incubated with **ABTT-Glu** (20 μM) obtained at different time intervals (0, 15, 30, 60 and 120 min).

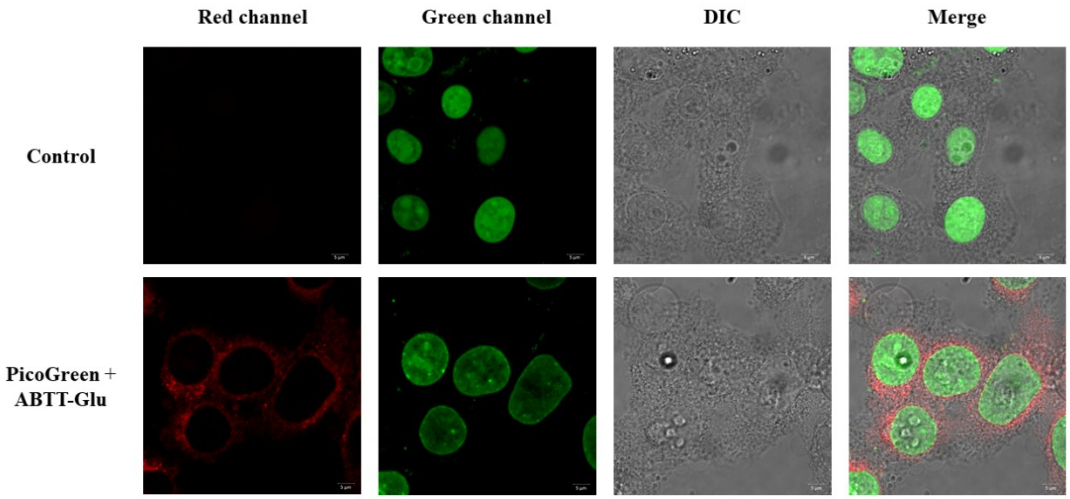

Fig. S17 Confocal fluorescence images of HepG2 cells incubated with **ABTT-Glu** (10 μM) at 37 °C for 1 h firstly, and then incubated with PicoGreen.

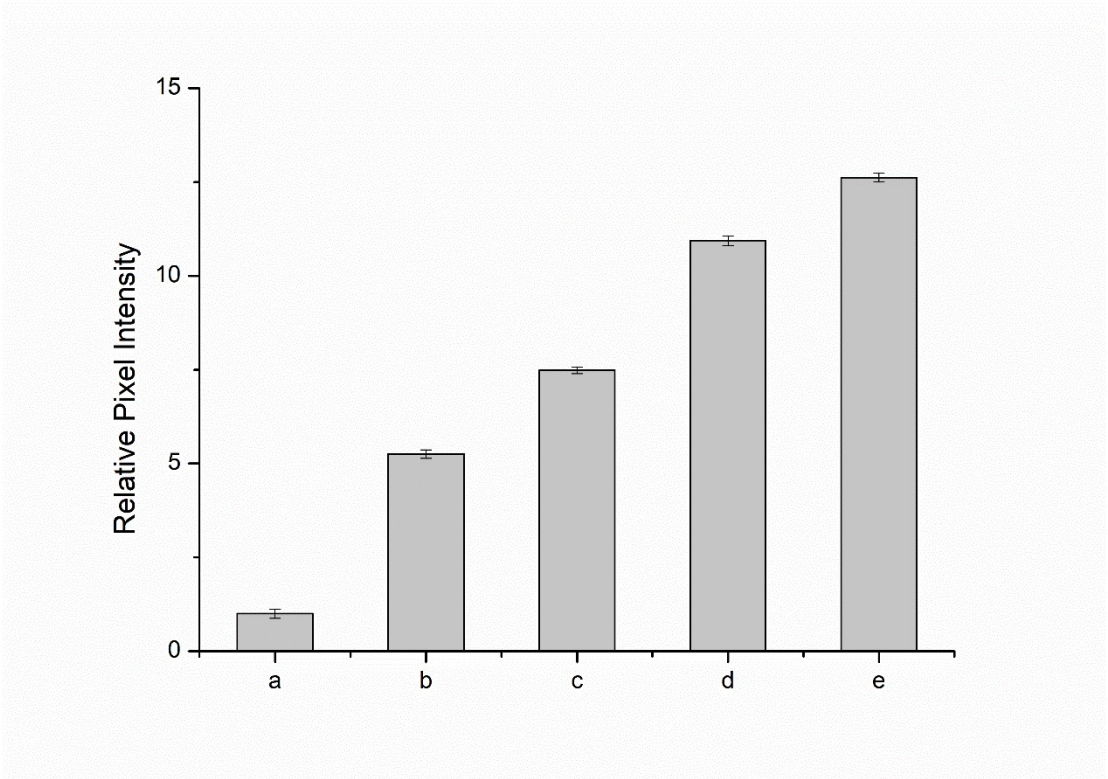

Fig. S18 The relative intensity levels of the cells shown in Fig. 5 was analyzed by ImageJ. (a) No **ABTT-Glu**. (b) 10 μM **ABTT-Glu** + 1 mM DON. (c) 5 μM **ABTT-Glu**. (d) 10 μM **ABTT-Glu**. (e) 20 μM **ABTT-Glu**. The intensity from images a were defined as 1.0, respectively.

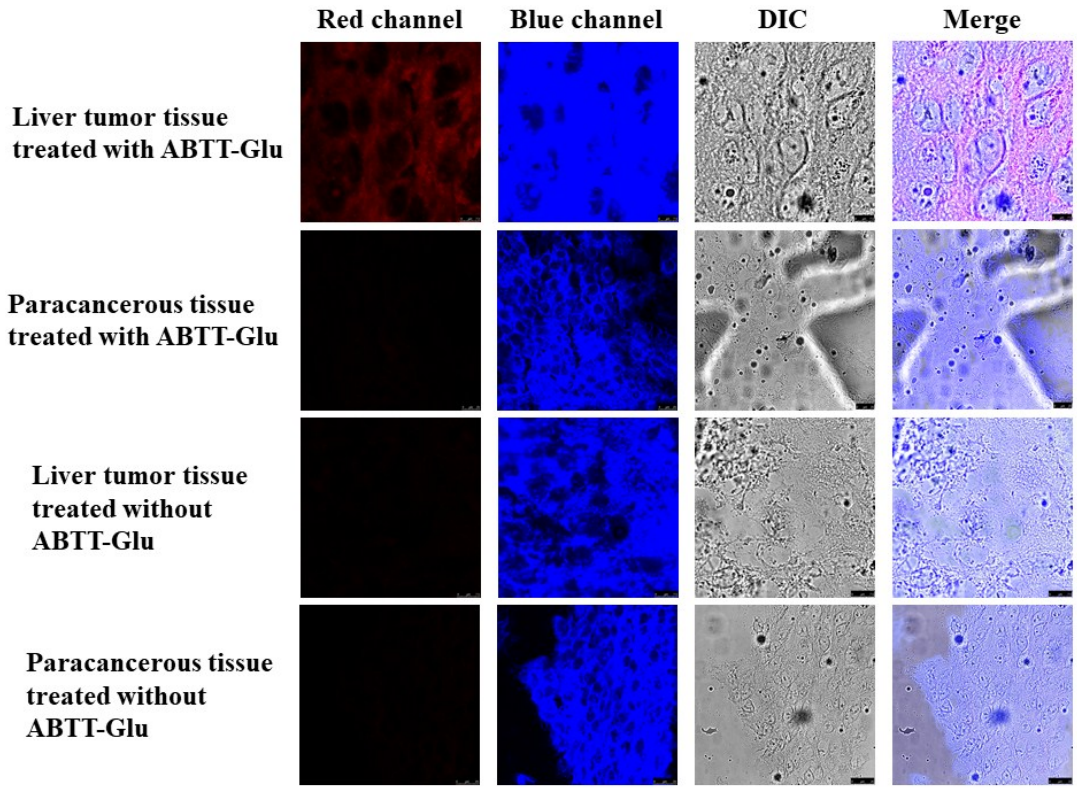

Fig. S19 Fluorescence images in human liver tumor tissues and normal human liver tissues. Two types tissues were cut to slices under frozen, and incubated with/without **ABTT-Glu** (10 μM) at 37 °C for 1 h firstly, and then incubated with DAPI.

9. Table S1 Spectroscopic properties of quinine sulphate (10 μM), **ABTT-Glu** (10 μM) and **ABTT- Glu** (10 μM) + GGT (80 U L^{-1}) in H_2O solution.

Compound	λ_{abs}/nm	A/Abs	λ_{em}/nm	Φ	Δν/cm^{-1}
Quinine sulphate	347	0.745	455	0.55	59013.4
ABTT-Glu	365	0.004	650	< 0.01	5.8
ABTT- Glu + GGT	365	0.191	650	0.13	3595.4