

## Supporting Information

### Construction and theoretical insights of the first ESIPT fluorescent probe for imaging FA in vitro and in vivo

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Electronic Supplementary

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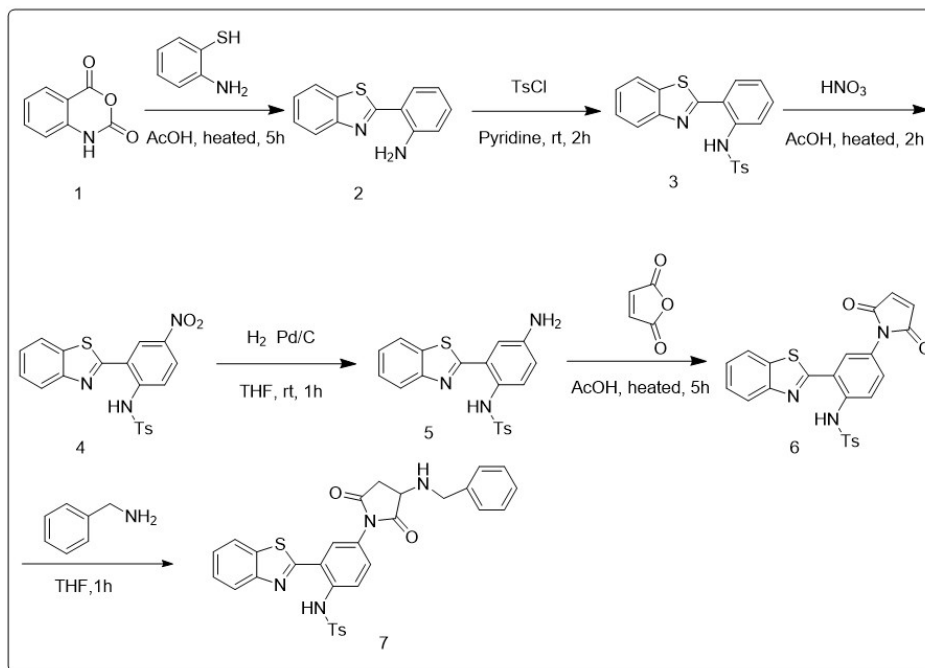
## **S1 Synthesis and characterization**

### **S1.1 Materials and methods**

All chemicals and reagents were used as received unless otherwise specified. Vitamin B1, benzaldehyde, and *p*-chloroaniline (99.5%) were purchased from Energy Chemical Co., Ltd (China). Pyridine and 4-Pyridine carboxaldehyde (98%) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). DAPI (4',6-diamidino-2-phenylindole) was purchased from Sigma-Aldrich. Roswell Park Memorial Institute (RPMI)-1640 medium, phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Invitrogen. The cell cytotoxicity assay kit (MTT) was a commercial product of Beyotime Biotechnology (China). The brain slice was obtained by Leica VT3000 vibrating-blade microtome.

<sup>1</sup>H and <sup>13</sup>C NMR spectra were measured on a Bruker ARX 500 MHz NMR spectrometer using CDCl<sub>3</sub> as the solvent and tetramethylsilane (TMS;  $\delta = 0$ ) as the internal reference. UV-vis Absorption spectra were performed on a UV-2450 scanning spectrophotometer (Shimadzu, Japan). ZORBAX Eclipse Plus C18 (Agilent) 1.8  $\mu$ m, 100 mm $\times$ 2.1 mm column. Fluorescent spectra were recorded on a Shimadzu RF-5301 equipped with a 1 cm quartz cell. Dynamic light scattering measurements were performed at 25 °C on Zestier Nano ZS (Malvern Instruments Ltd, UK). Fluorescent images were collected on Leica DMI 4000B fluorescence microscope.

**Cell line:** HeLa cell was continuously cultured in our laboratory.



Scheme S1 Synthesis of Probe **ABTB**

**Compound 2** (2-(benzo[d]thiazol-2-yl)aniline). Isatoic anhydride (1.0 g, 6.13 mmol) and 2-aminothiophenol (0.85 mL, 7.97 mmol) were dissolved in 15 mL of acetic acid, and then stirred at 45 °C for 2 h. Followed by neutralization using 5 mol/L NaOH solution, the precipitates were filtered and washed with sodium carbonate solution. The crude product was purified by the chromatographic column (silica gel, hexane: ethyl acetate 10: 1, v/v) to yield Compound **1** as a yellow solid (1.1 g, 80.1%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ ppm 8.01 (d, *J* = 8.1 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 6.42 (s, 2H).

**Compound 3** (N-(2-(benzo[d]thiazol-2-yl)phenyl)-4-methylbenzenesulfonamide). A mixture of Compound **2** (240 mg, 1 mmol) and p-toluenesulfonyl chloride (400 mg, 2 mmol) in 15 mL of pyridine was stirred at room temperature for 2 h, and then the reaction system was neutralized using aqueous HCl to give the white sediment. Followed by washing using saturated saline, the crude product was purified on chromatographic column (silica gel, hexane: dichloromethane 10: 1, v/v) to yield Compound **3** as a white solid (384 mg, 95.1%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ ppm 12.27 (s, 1H), 8.13 (d, *J* = 8.1 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.3 Hz,

1H), 7.71 (d,  $J = 7.8$  Hz, 1H), 7.67 (d,  $J = 7.7$  Hz, 2H), 7.56 (t,  $J = 7.6$  Hz, 1H), 7.45 (t,  $J = 7.5$  Hz, 1H), 7.38 (t,  $J = 7.8$  Hz, 1H), 7.12 (t,  $J = 7.5$  Hz, 1H), 7.06 (d,  $J = 7.8$  Hz, 2H), 2.28 (s, 3H).

**Compound 5** (N-(4-amino-2-(benzo[d]thiazol-2-yl) phenyl)-4-methylbenzene -sulfonamide). Compound **3** (190 mg, 0.50 mmol) was dissolved in 10 mL of acetic anhydride and stirred at 50 °C. 0.1 mL of nitric acid and 2.0 mL of glacial acetic acid were added into the above solution dropwise. After the dropping was completed, the reaction system was continued stirring at 50 °C for another 2 hours. Then, a 5 mol/L NaOH solution was employed to neutralize the excessive acid. The mixture was extracted with dichloromethane and the organic layer was dried with anhydrous NaSO<sub>4</sub>. The solvent was removed under reduced pressure to give 108 mg of a pale-yellow crude compound. Pd/C (10 %, 10 mg) was added to the crude product in tetrahydrofuran (20 mL). The resulting solution was stirred under hydrogen at room temperature for 5 h. The mixture was filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, hexane: dichloromethane 1: 3, v/v) to give a yellow solid (42 mg, 22.3%) <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ ppm 11.11 (s, 1H), 8.09 (d,  $J = 8.2$  Hz, 1H), 7.86 (d,  $J = 8.0$  Hz, 1H), 7.63 (d,  $J = 8.8$  Hz, 1H), 7.56 (t,  $J = 7.7$  Hz, 1H), 7.44 (t,  $J = 7.6$  Hz, 1H), 7.36 (d,  $J = 7.7$  Hz, 2H), 6.92 (s, 1H), 6.82 (d,  $J = 7.8$  Hz, 2H), 6.78 (d,  $J = 8.7$  Hz, 1H), 3.68 (s, 2H), 2.19 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 167.29, 152.77, 143.74, 143.03, 135.90, 133.68, 128.97, 127.71, 126.87, 126.71, 125.77, 125.44, 123.60, 123.06, 121.14, 118.67, 115.10, 21.33.

**Compound 6** Compound **5** (395 mg, 1 mmol) and maleic anhydride (147 mg, 1.5 mmol) were dissolved in acetic acid (20 mL) and refluxed at 120 °C for at least 6 h. After the fluorescent dots all turned into dark dots monitoring by TLC, the mixture was cooled to room temperature and diluted with deionized water (200 mL). Then 0.1 M NaHCO<sub>3</sub> was dropwise added into the solution to adjust the pH into 7~8. The sediment was filter under lower pressure and washed using saturated brine three times. After the sediments dried at 50 °C overnight, it was purified by column chromatography (silica gel, hexane: dichloromethane 1: 1, v/v) to afford the pure

product Compound **6** (210 mg, 44.2% yield) as a pale yellow solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 12.41 (s, 1H), 8.17 (d,  $J = 8.1$  Hz, 1H), 7.92 (d,  $J = 7.9$  Hz, 1H), 7.88 (d,  $J = 8.9$  Hz, 1H), 7.78 (d,  $J = 2.3$  Hz, 1H), 7.72 (d,  $J = 8.3$  Hz, 2H), 7.59 (t,  $J = 7.6$  Hz, 1H), 7.49 (t,  $J = 7.5$  Hz, 1H), 7.44- 7.39 (m, 1H), 7.13 (d,  $J = 8.1$  Hz, 2H), 6.91 (d,  $J = 10.3$  Hz, 2H), 2.32 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.18, 166.96, 152.46, 143.89, 136.48, 136.35, 134.36, 133.26, 129.66, 128.81, 127.14, 126.98, 126.93, 126.66, 126.25, 123.25, 121.39, 120.39, 120.36, 21.48. HR-MS ( $m/z$ ): calculated for  $\text{C}_{24}\text{H}_{17}\text{N}_3\text{O}_4\text{S}_2$   $[\text{M}+\text{H}]^+$ , 475.0733; found, 476.0744.

**Compound 7** Benzylamine (35  $\mu\text{L}$ , 0.32 mmol) was added to the solution of Compound **6** (475 mg, 1 mmol) in THF (2 mL), then the mixture was stirred for 1 h at room temperature. The solvent was removed by a rotavapor, and the product was purified by flash column chromatography (dichloromethane/methanol = 500:1 v/v) on silica gel to give Compound **7** (305 mg, 55%) as a pale yellow solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 12.52 (s, 1H), 8.17 (s,  $J = 8.1$  Hz, 1H), 8.14(d,  $J = 8.1$  Hz, 1H), 7.90 (d,  $J = 7.9$  Hz, 1H), 7.89 (d,  $J = 8.9$  Hz, 1H), 7.86 (d,  $J = 8.1$  Hz, 1H), 7.84(d,  $J = 8.1$  Hz, 1H), 7.74 (m,  $J = 8.3$  Hz, 2H), 7.73 (m,  $J = 8.3$  Hz, 2H), 7.59 (d,  $J = 7.5$  Hz, 1H), 7.56-7.54 (t,  $J = 7.5$  Hz, 1H), 7.47 (d,  $J = 8.1$  Hz, 1H), 7.45 (s,  $J = 8.1$  Hz, 2H), 7.35 (d,  $J = 7.9$  Hz, 1H), 7.33 (d,  $J = 7.9$  Hz, 1H), 7.28 (d,  $J = 8.1$  Hz, 1H), 7.15 (d,  $J = 7.5$  Hz, 1H), 7.13 (d,  $J = 7.5$  Hz, H), 3.98 (d,  $J = 10.3$  Hz, 2H), 3.81 (s, 1H), 3.07 (m,  $J = 7.6$  Hz, 1H), 2.75 (m,  $J = 7.6$  Hz, 1H), 2.32 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  176.51, 173.88, 167.76, 166.78, 165.28, 162.33, 152.38, 144.02, 136.94, 136.38, 133.20, 129.73, 129.23, 129.17, 128.77, 128.46 127.83, 127.38, 127.15, 126.98, 126.62, 126.21, 123.23, 121.41, 120.09, 119.88, 55.7, 53.47, 36.29, 21.49.

### **S3. Calculate the fluorescence quantum yield.**

Fluorescence quantum yield ( $\Phi_f$ ) was determined by using quinine sulfate ( $\Phi_f = 0.58$ , in 0.1 M  $\text{H}_2\text{SO}_4$  aqueous solution) as the fluorescence standard. The quantum yield was calculated using the following equation.  $\Phi_f(\text{X}) = \Phi_f(\text{S}) (\text{A}_{\text{SFX}}/\text{A}_{\text{XFS}}) (\text{n}_{\text{X}}/\text{n}_{\text{S}})^2$  Where  $\Phi_f$  is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvent used. Subscripts S and X refer to the standard and to the unknown,

respectively. The quantum yield of **ABTB** was calculated 0.37.

#### S4. Absorption and Fluorescence emission spectra of **ABTB**

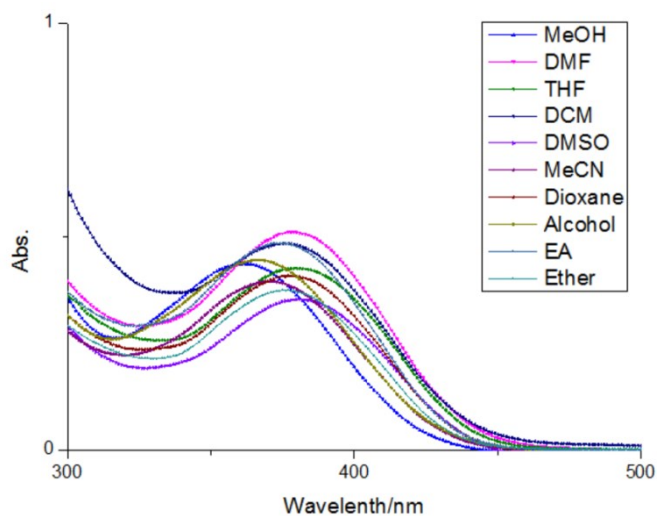


Figure S1. Absorption spectrum of **ABTB** in different solution.

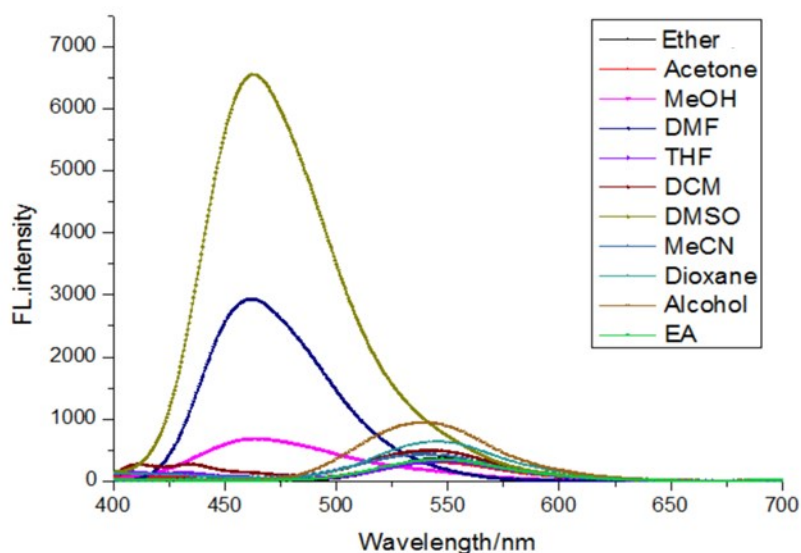


Figure S2. Fluorescent spectrum of **ABTB** in different solution.

#### S5. Determination of the detection limits

The detection limit was calculated according to the method used in the previous literature [1]. The fluorescence intensity was plotted versus the concentrations of FA. The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3 \sigma / k$$

Where  $\sigma$  is the standard deviation of blank measurement,  $k$  is the slope between the fluorescence intensity FA concentrations.

The detection limit was calculated 432 nM.

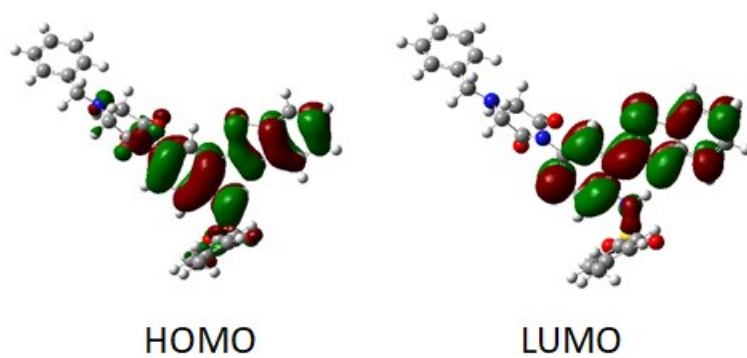
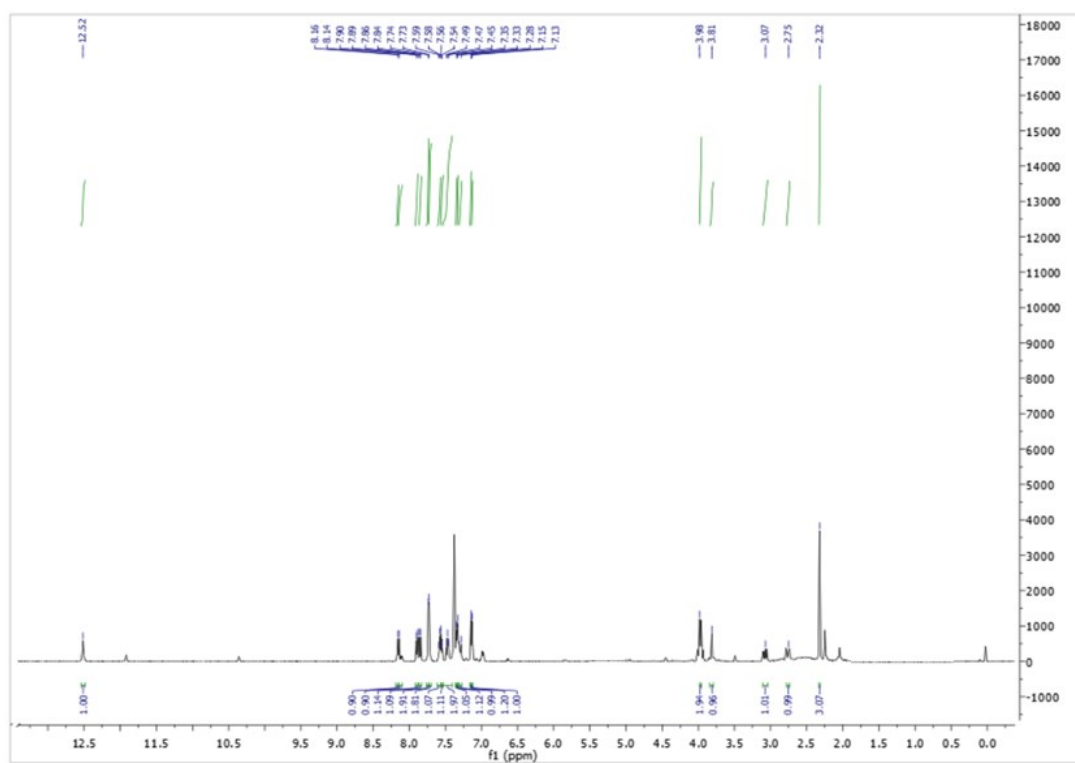
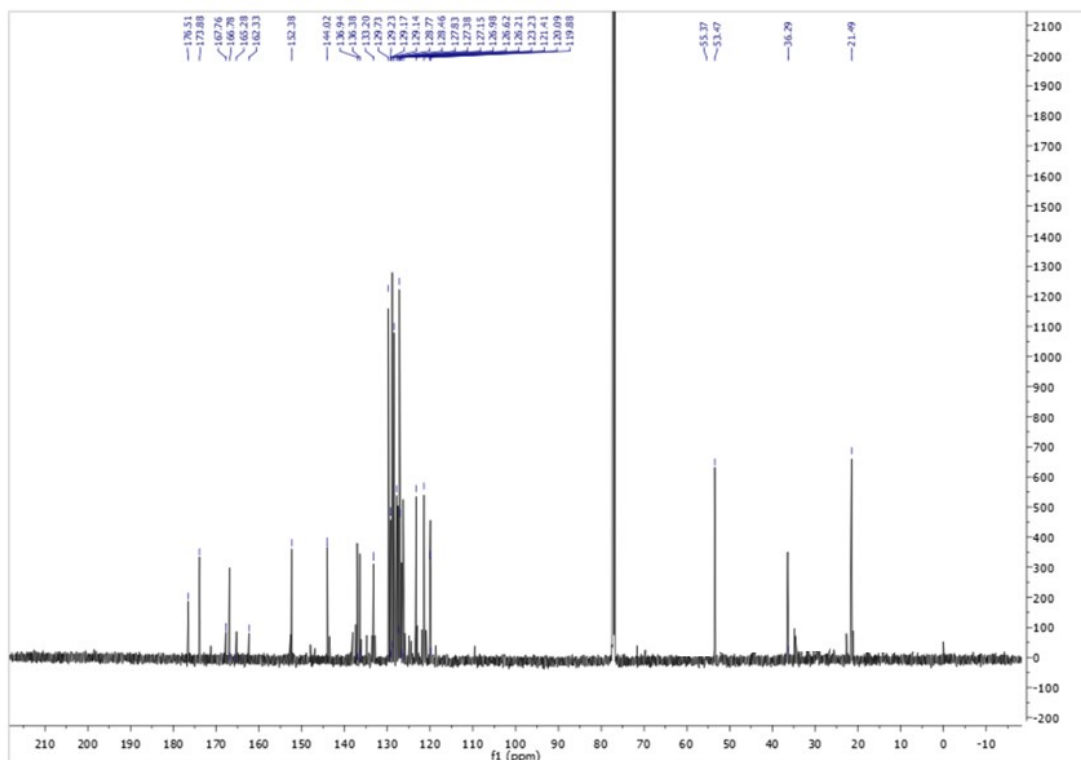


Figure S3. calculated HOMO and LUMO electron cloud distribution of **ABTB**.



<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) of **ABTB**



$^{13}\text{C}$ NMR (125 MHz,  $\text{CDCl}_3$ ) of **ABTB**

### UPLC-MS Studies of Hydrolysis Reaction of **ABTB**.

UPLC-MS analysis was performed in equipped with ZORBAX Eclipse Plus C18 (Agilent) 1.8  $\mu\text{m}$ , 100 mm $\times$ 2.1 mm column. All the samples were eluted using a gradient mixture from 30:70 to 95:5  $\text{CH}_3\text{CN}$ :  $\text{H}_2\text{O}$  containing 0.1 % formic acid at a flow rate of 0.2 mL/min in 10 min. LC peaks were monitored by UV absorption at 254 nm and MS was detected in ESI positive mode.

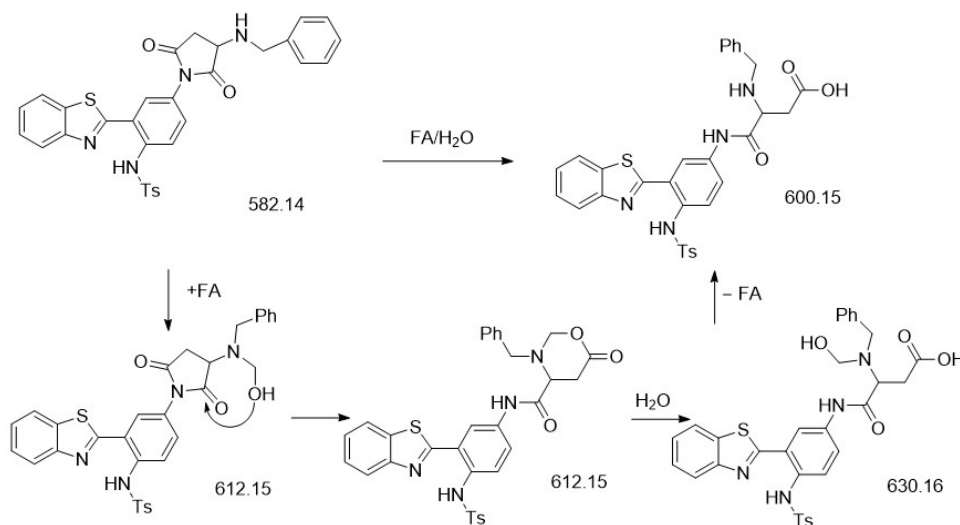


Fig. S4 The proposed mechanism of the reaction of **ABTB** and FA by MS.



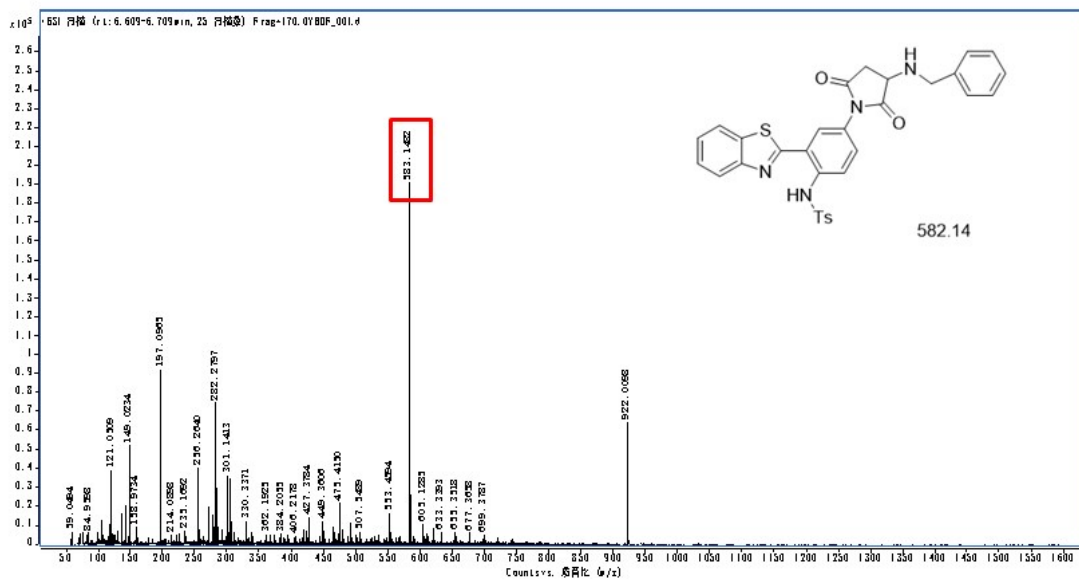
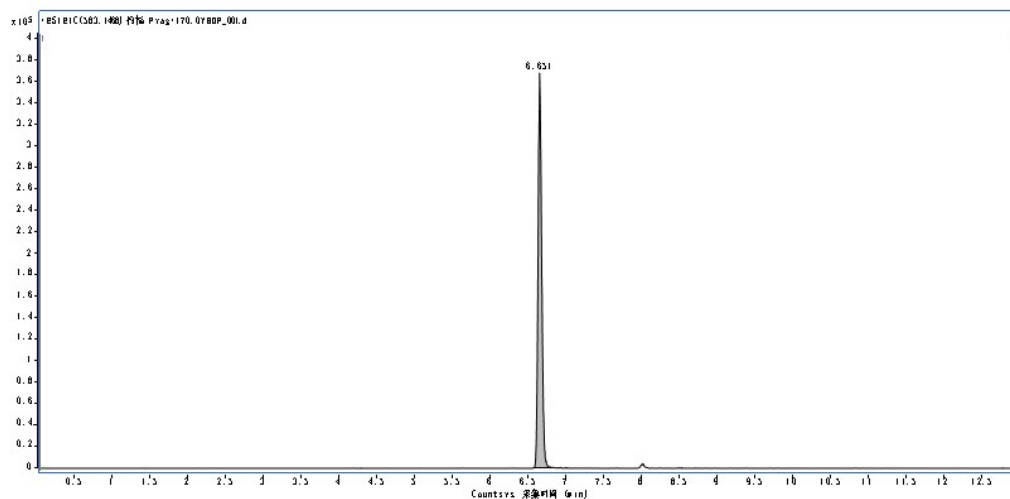


Fig. S5 HR-MS spectra of **ABTB**.

0 min FA Probe ABTB Cal: 583.1468, Found: 583.1482, Retention time: 6.5min



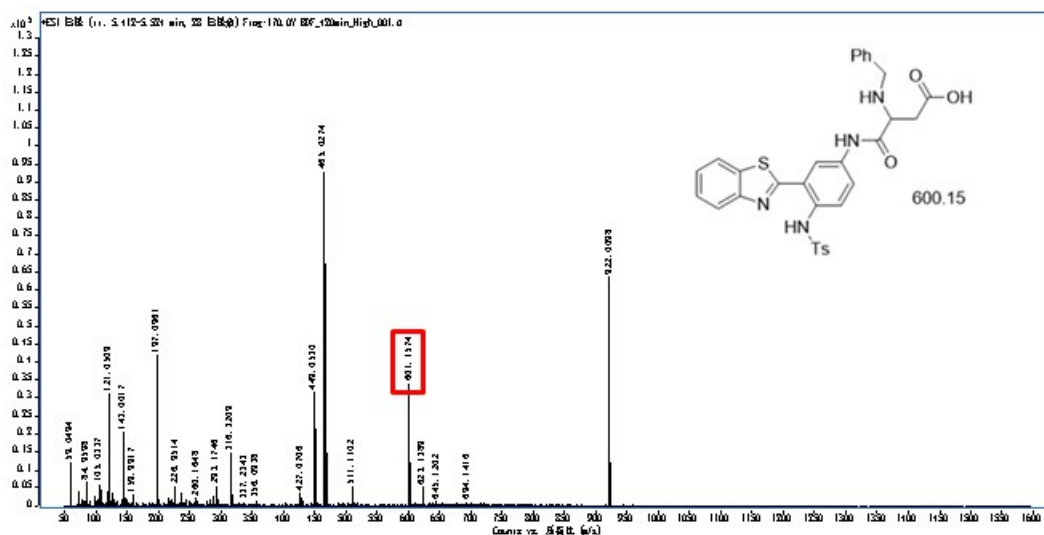
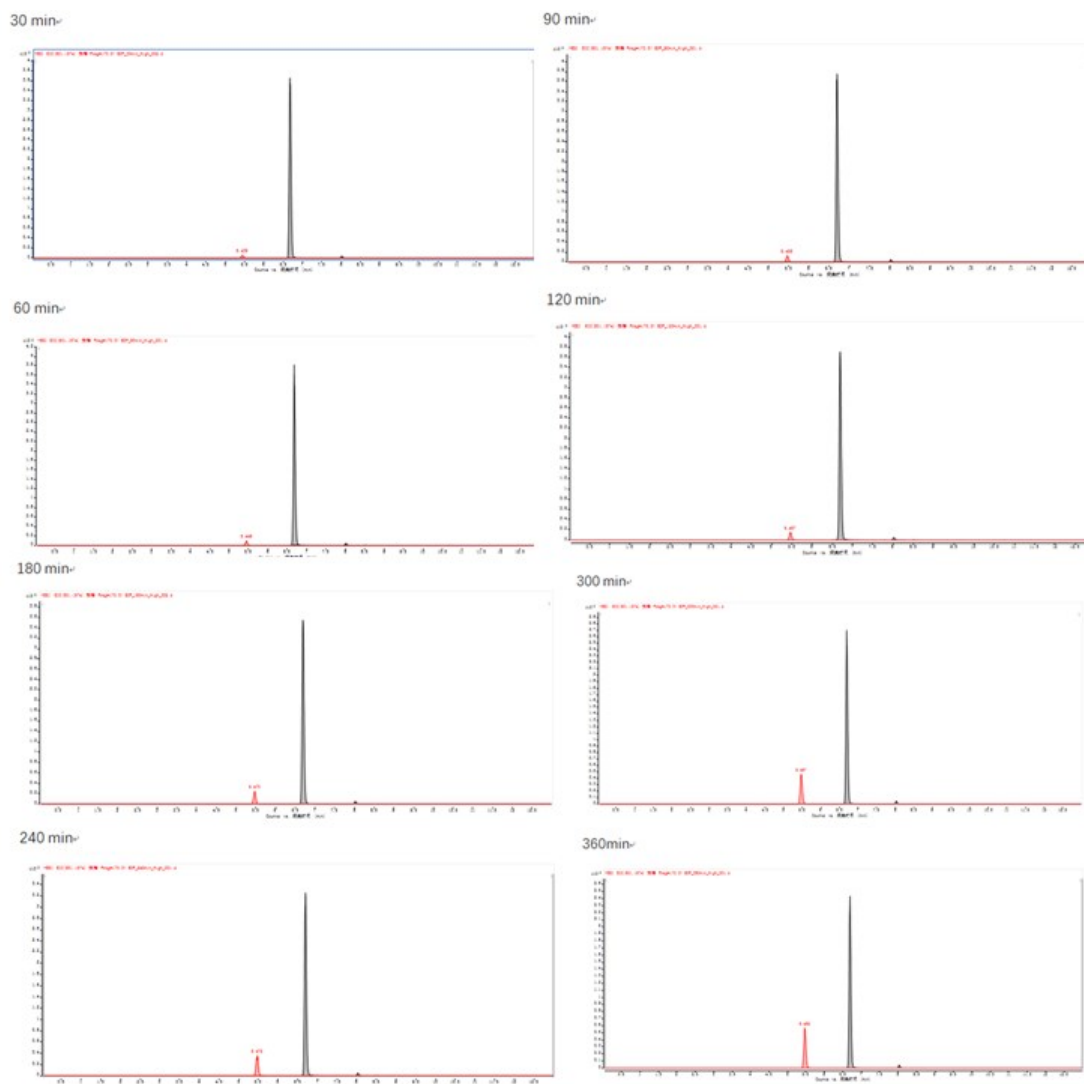


Fig. S6 UPLC-MS studies of mechanism of **ABTB** with reaction of FA, **ABTB** ( $m/z=582.14$ ) **ABTBF** ( $m/z=600.15$ ) at different time-points from 10 to

360 min.

### Cell viability assay

The cytotoxicity of **ABTB** was studied by MTT assay. Briefly, 100  $\mu\text{L}$  of cell suspension was placed in a 96-well plate. The plates were preincubated in an incubator for 24 h (37  $^{\circ}\text{C}$ , 5%  $\text{CO}_2$ ). A volume of 10  $\mu\text{L}$  of different concentrations of **ABTB** was added to the plates. The plates were incubated for 48 h in an incubator. A volume of 10  $\mu\text{L}$  of MTT solution was added to each well. The plates were incubated in an incubator for 1 h. The absorbance at 450 nm was measured with a microplate reader to reflect the cell viability of each well.

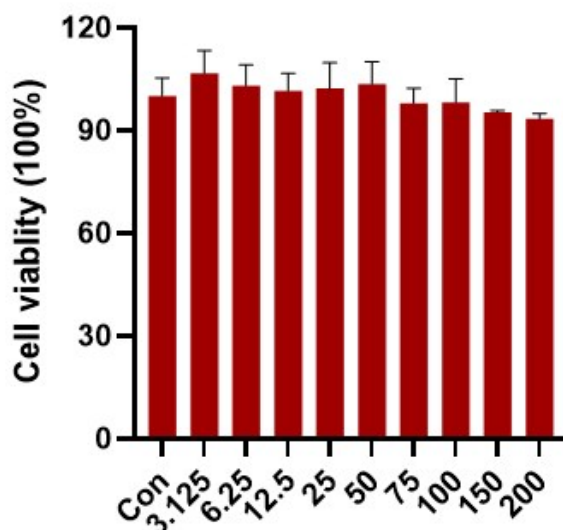


Figure S7. Cell viability of **ABTB**

**Cell line:** HeLa cells were maintained in DMEM (Thermo Fisher Scientific, USA), supplemented with 10% FBS (fetal bovine serum, Thermo Fisher Scientific, USA) and 1% penicillin–streptomycin (Thermo Fisher Scientific, USA). Cells were incubated at 37  $^{\circ}\text{C}$  with 5%  $\text{CO}_2$ .

### Animal Model

The animal experiment was approved by the Animal Ethics and welfare Committee, at the Second Xiangya Hospital, Central South University (No. 2020460). All animal studies were carried out using the Institutional Animal Care and Use Committee (IACUC) approved procedures.

Preparation of mouse brain tissue slices: The fresh mouse brain tissue slices were

prepared according to the reported procedure. Five-month-old AD mice were obtained from the Department of Pharmacy the Second Xiangya Hospital. The fresh mouse brain tissue slices were obtained using a Leica VT3000 vibrating-blade microtome (Germany) with a thickness of about 300  $\mu$ m. This step was fully operated in ice cold artificial cerebrospinal fluid (ACSF, NaCl 124.0 mM, KCl 3.0 mM, NaHCO<sub>3</sub> 26.0 mM, NaH<sub>2</sub>PO<sub>4</sub> 1.24 mM, MgSO<sub>4</sub> 8.0 mM, CaCl<sub>2</sub> 0.1 mM and D-glucose 10.0 mM) under a 95% O<sub>2</sub> and 5% CO<sub>2</sub> atmosphere.

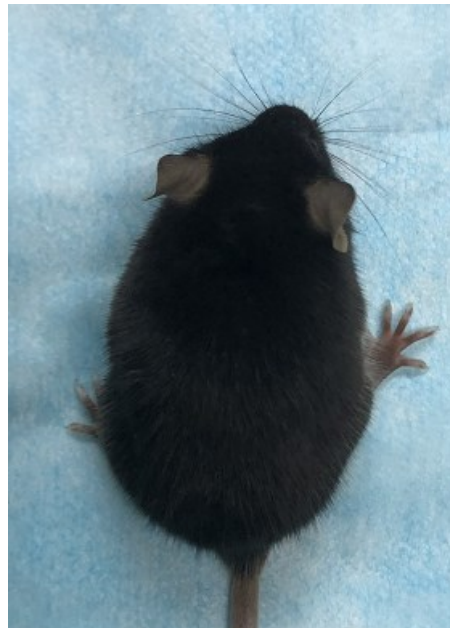


Figure S6. C57 AD mouse

#### References

- [1] A. Bi, T. Gao, X. Cao, J. Dong, M. Liu, N. Ding, W. Liao, W. Zeng, *Sens. Actuators B*, 2018, 255, 3292.