Supplementary information

NIR AIE luminogens for primary and metastasis tumor imaging and tracing applications

Yujiao Zhu, ‡[a] Yuhang Zeng, ‡ [b] Huimin Liu, [a] Yuting Yin, [b] Bin Chen *[a] and Rong Hu *[b]

[a.] *School of Material Science and Chemical Engineering, Ningbo University, Ningbo, Zhejiang, 315211, China.*
[b.] *School of Chemistry and Chemical Engineering, University of South China, Hengyang 421001, China.*

‡ Yujiao Zhu and Yuhang Zeng contributed equally to this work.
* Corresponding author. E-mail: chenbin2@nbu.edu.cn; hurong@usc.edu.cn

General information and methods

Materials

All chemical and reagents were commercially available and used as received without further purification. Murine 4T1 cancer cells were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). PBS buffer, DMEM (Dulbecco's modified Eagle medium), fetal bovine serum (FBS), Lyso-tracker Blue was purchased from Thermo Fisher Scientific (Shanghai, China). DSPE-PEG2000 was purchased from Ponsure (Shanghai, China). MTT was purchased from Tiangen Biotech (Beijing, China). Singlet Oxygen Sensor Green (SOSG) and Dihydrorhodamine 123 (DHR123) were purchased from Thermo Fisher Scientific (Shanghai, China). Reductant vitamin C (Vc) was purchased from TiTan (Shanghai, China). Chlorin e6 (Ce6) was purchased from Macklin (Shanghai,

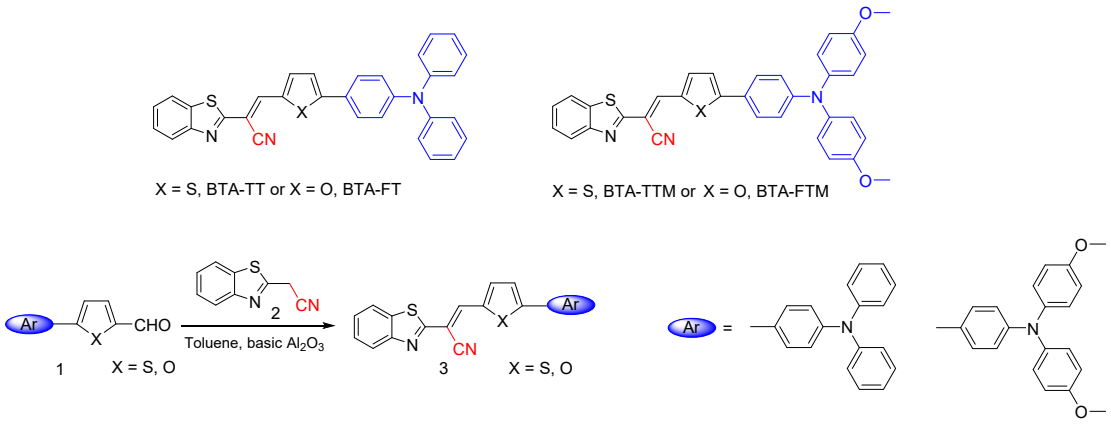
China). DCFH-DA was purchased from Bidepharm (Shanghai, China). The female BALB/c mice (6-week-old) were purchased from China Boryxin Biotechnology Co. (Hunan, China).

Measurements

^{1}H and ^{13}C NMR spectra were acquired using the Bruker ARX 500 NMR spectrometer utilizing dichloromethane-d_2 as solvents with tetramethylsilane (TMS; δ = 0 ppm) acting as the internal reference. High resolution mass spectra (HRMS) were measured on a TIMS-TOF of Bruker Daltonik to reported as m/z (relative intensity). UV-vis were obtained by PerkinElmer Lambda 365 Spectrophotometer. Fluorescence spectrum was acquired using FluoroMax-4 Spectrophotometer. Single crystal data were measured on Bruker D8 QUEST with Cu-Kα radiation (λ = 1.54178). Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (TI2-CTRE, Nikon, Japan). In vitro animals' fluorescence imaging was carried out by living imaging system (IVScope 8200, CliNX, China). The absorbance for MTT analysis was recorded on a microplate reader (Epoch, BioTek, Germany).

Experimental procedures.

Synthesis and characterization



Scheme S1. The synthetic path of BTA-TM, BTA-TTM, BTA-FT, BTA-FTM and their molecular structures.

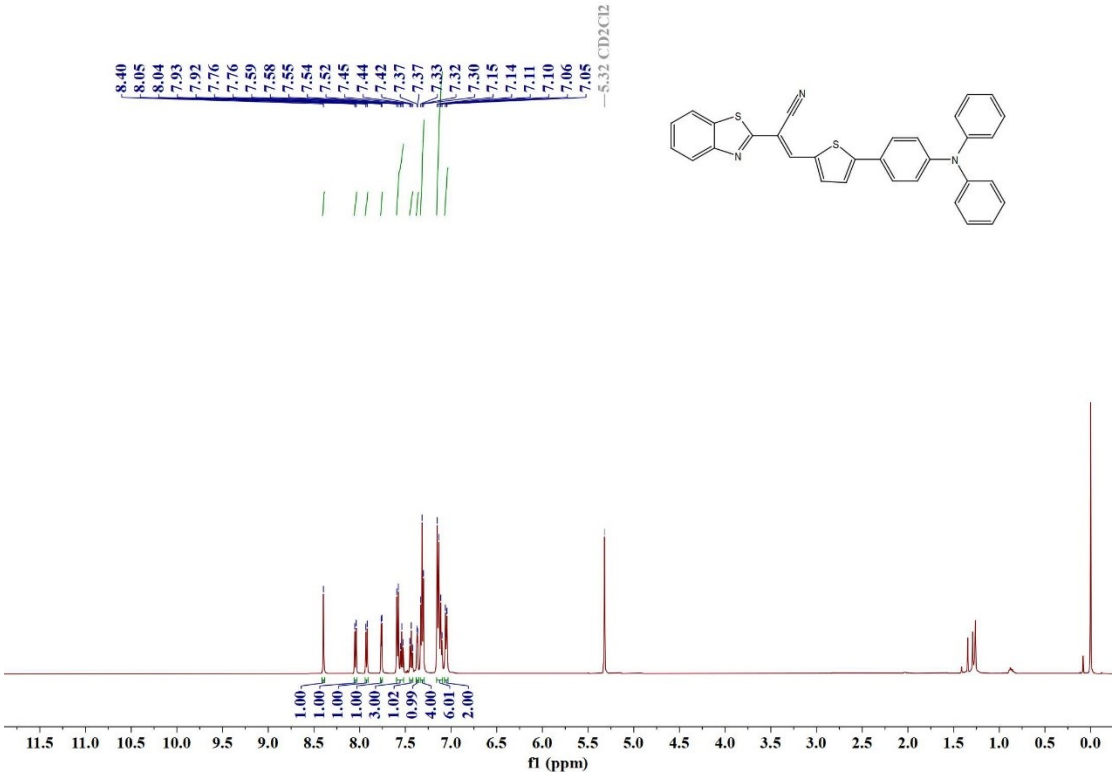


Figure S1. ^{1}H NMR spectrum of BTA-TT.

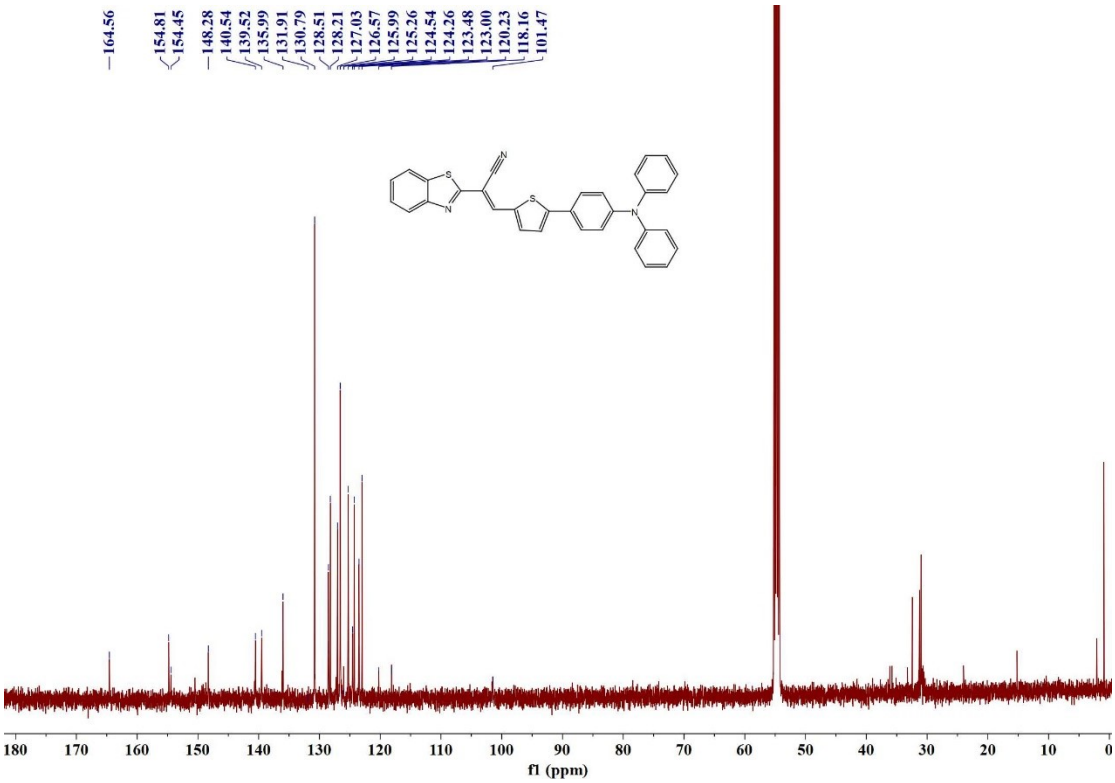


Figure S2. ^{13}C NMR spectrum of BTA-TT.

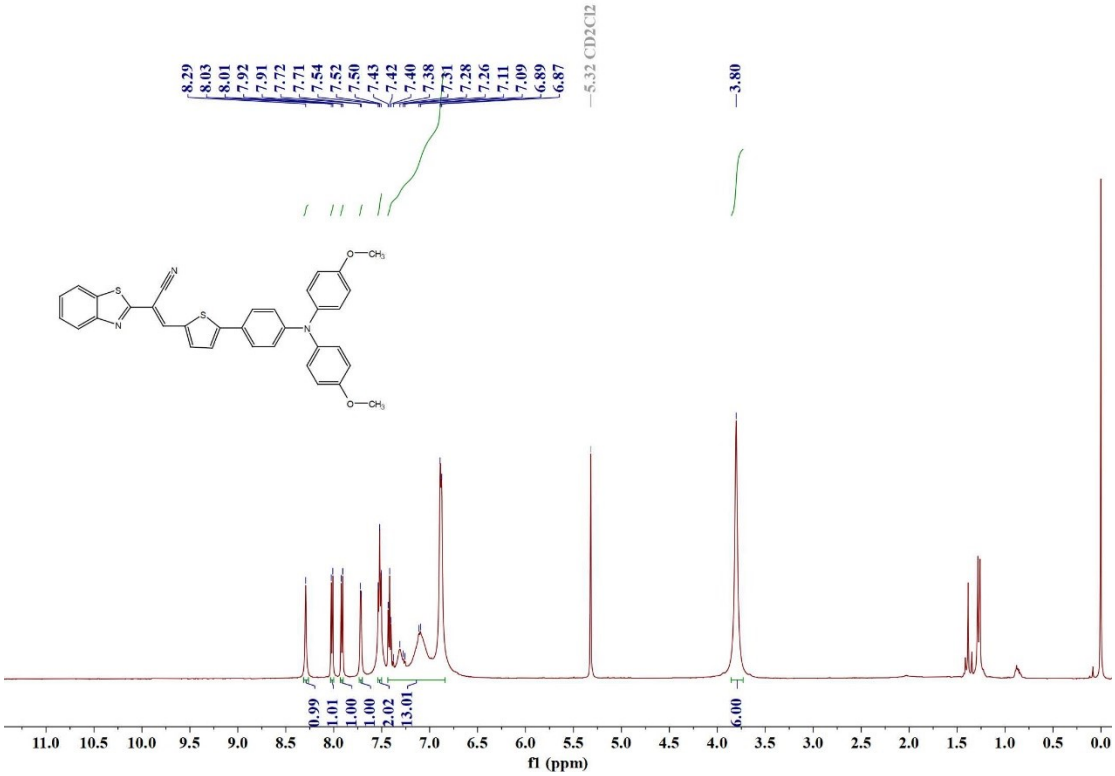


Figure S3. [1]H NMR spectrum of BTA-TTM.

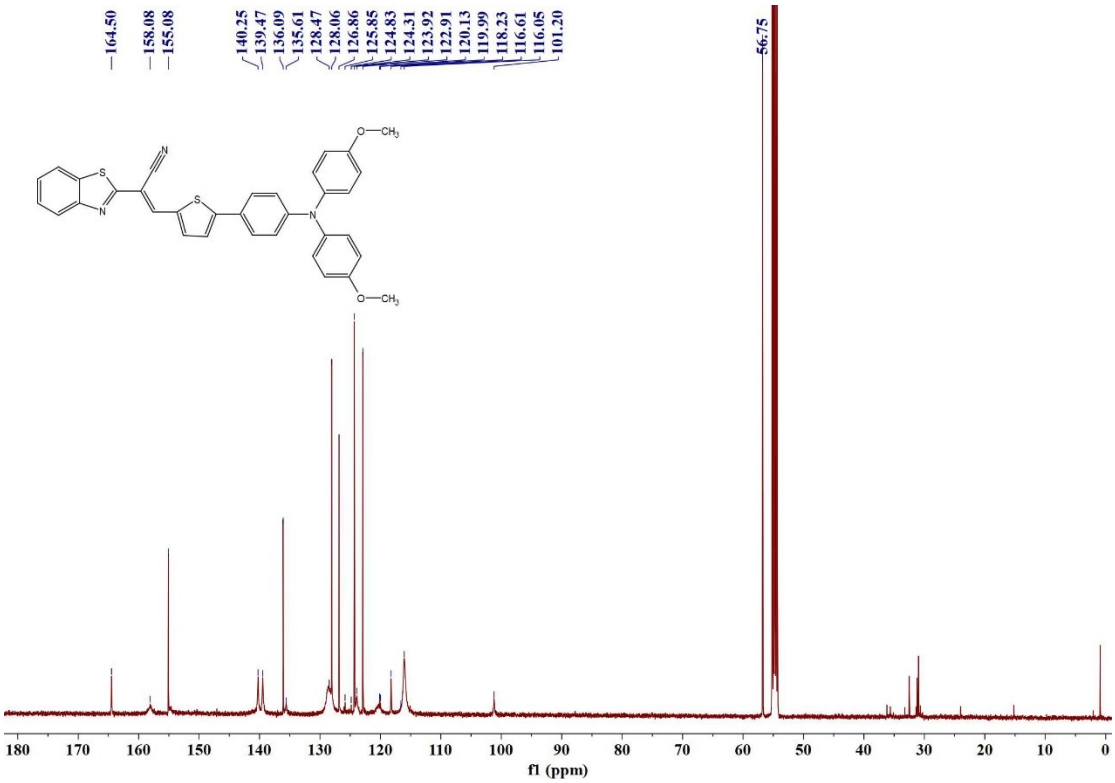


Figure S4. [13]CNMR spectrum of BTA-TTM.

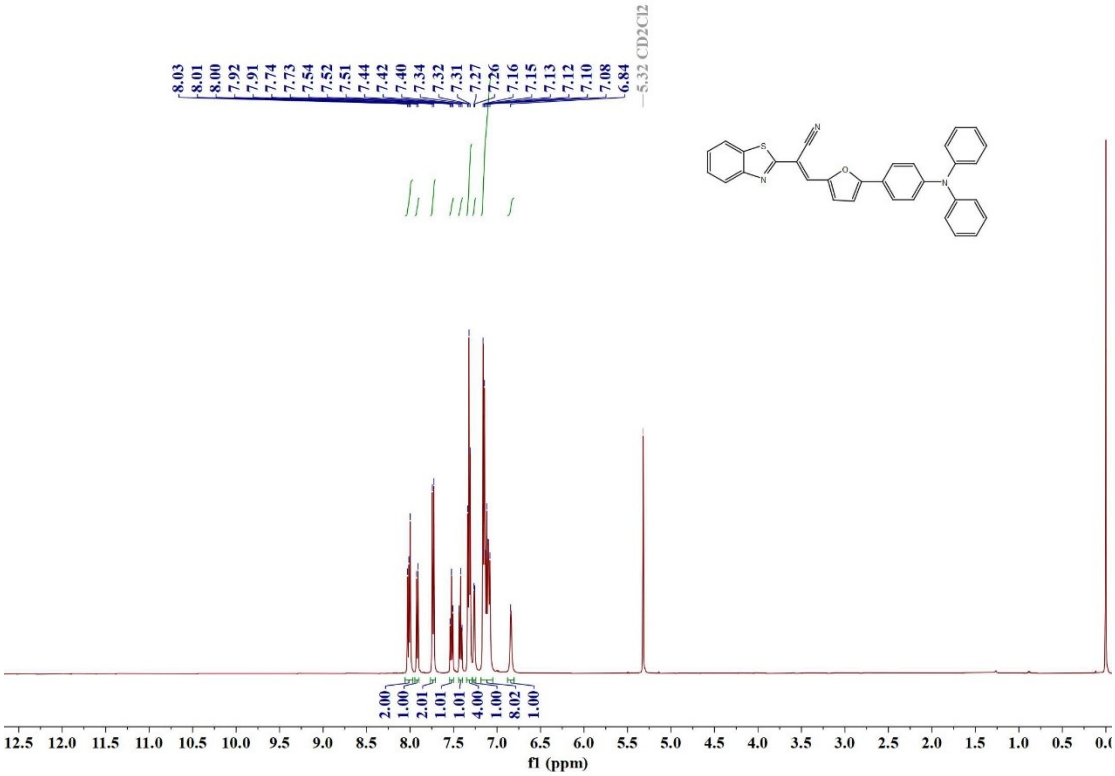


Figure S5. ^{1}H NMR spectrum of BTA-FT.

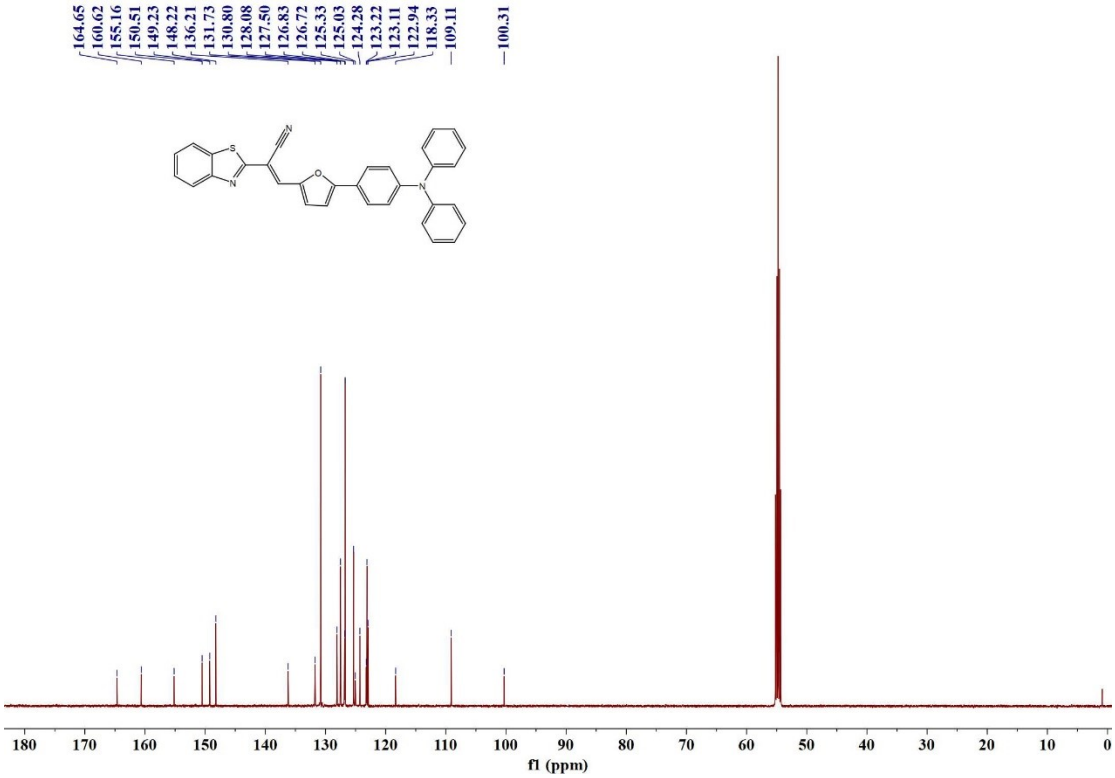


Figure S6. ^{13}C NMR spectrum of BTA-FT.

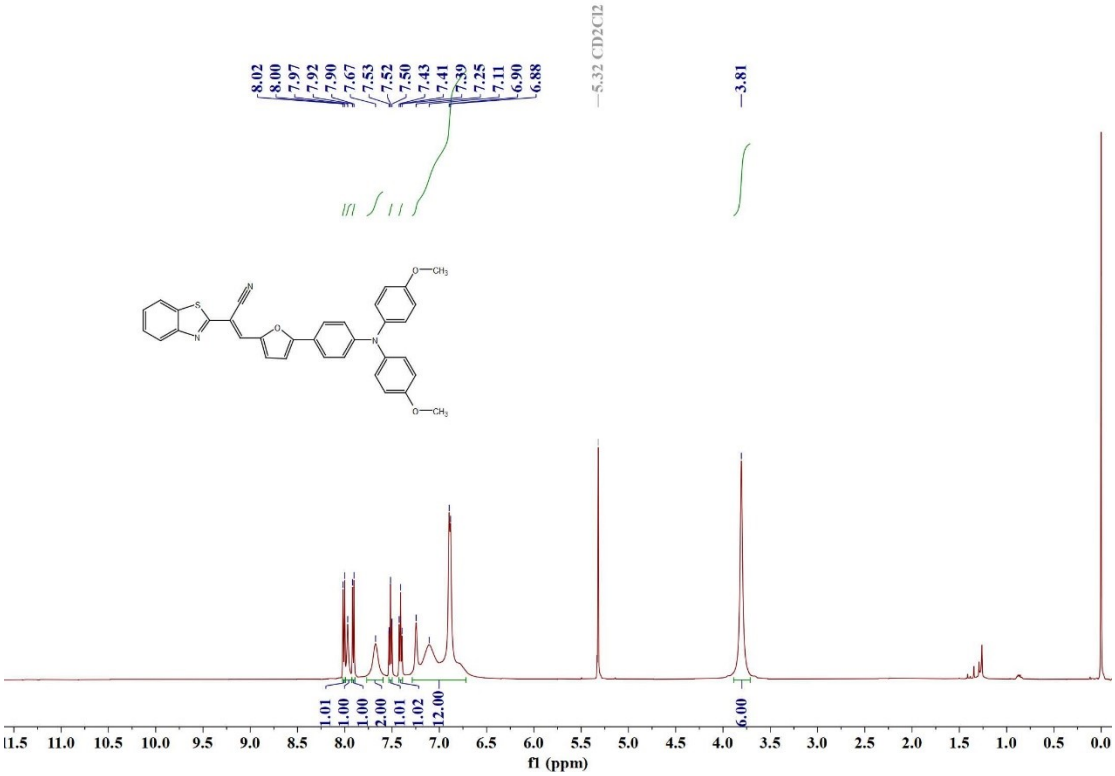


Figure S7. ^{1}H NMR spectrum of BTA-FTM.

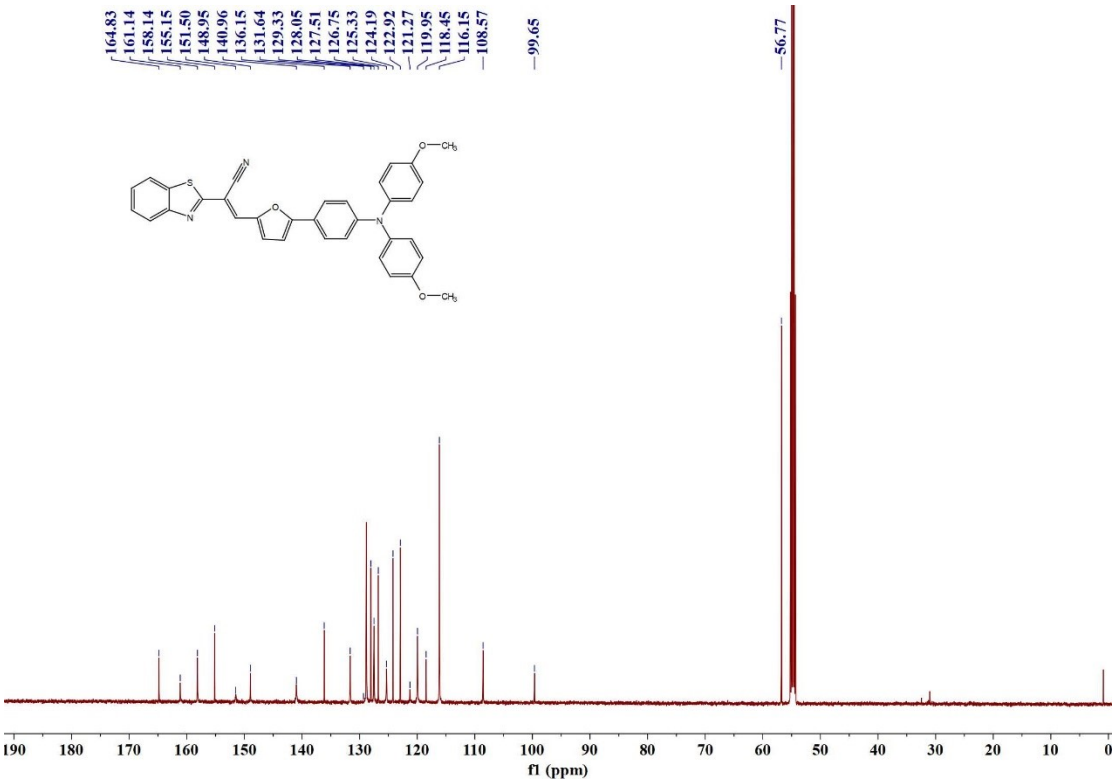


Figure S8. ^{13}C NMR spectrum of BTA-FTM.

Table S1 Crystallographic and structural refinement data of BTA-TTM and BTA-FT.

Compound	BTA-TTM	BTA-FT
CCDC	2378963	2378964
Empirical formula	$C_{34}H_{25}N_3O_2S_2$	$C_{32}H_{21}N_3OS$
Formula weight	571.69	496.617
Temperature/K	150.0	200.0
Crystal system	monoclinic	orthorhombic
Space group	C2/c	$Pna2_1$
a/Å	17.5570(5)	9.1476(3)
b/Å	17.4912(5)	19.4227(6)
c/Å	20.0444(5)	14.0083(4)
α/°	90	90
β/°	115.0550(10)	90
γ/°	90	90
Volume/Å^3	5576.3(3)	2488.87(13)
Z	8	4
ρ_{calc}g/cm^3	1.362	1.325
μ/mm^{-1}	2.029	1.396
F(000)	2384.0	1040.3
Crystal size/mm^3	0.105 × 0.091 × 0.068	0.141 × 0.121 × 0.099
Radiation	Cu-Kα (λ = 1.54178)	Cu-Kα (λ = 1.54178)
2θ range for data collection/°	7.512 to 144.214	7.78 to 133.26
Index ranges	-21 ≤ h ≤ 19, -21 ≤ k ≤ 21, -24 ≤ l ≤ 24	-10 ≤ h ≤ 10, -23 ≤ k ≤ 23, -16 ≤ l ≤ 14
Reflections collected	52751	29632
Independent reflections	5501 [R_{int} = 0.0540, R_{sigma} = 0.0235]	4175 [R_{int} = 0.0552, R_{sigma} = 0.0311]
Data/restraints/parameters	5501/0/372	4175/1/334
Goodness-of-fit on F^2	1.046	0.668
Final R indexes [I>=2σ (I)]	R_1= 0.0358, wR_2= 0.0991	R_1 = 0.0238, wR_2 = 0.0730
Final R indexes [all data]	R_1 = 0.0403, wR_2 = 0.1026	R_1 = 0.0255, wR_2 = 0.0759
Largest diff. peak/hole / e Å^{-3}	0.46/-0.51	0.09/-0.21

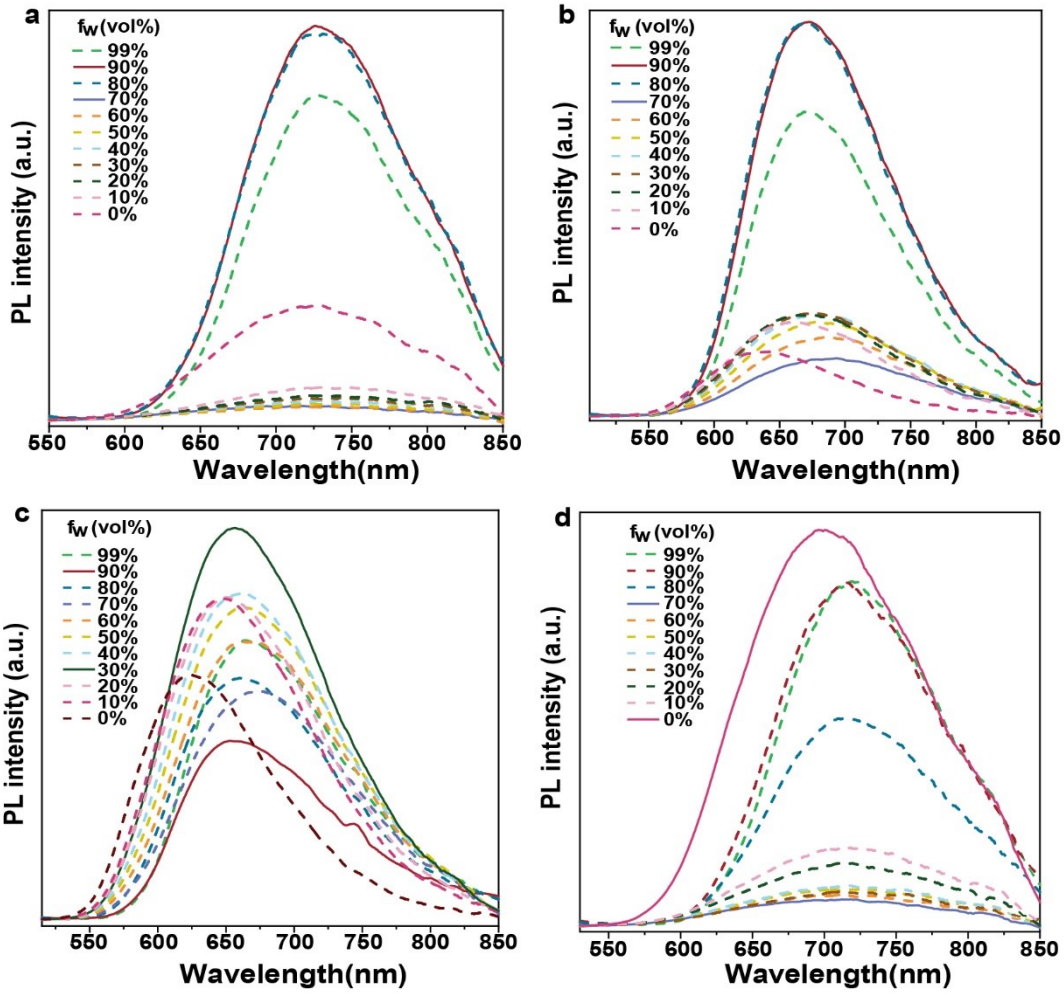


Figure S9. The fluorescence spectra of (a) BTA-TT, (b) BTA-TTM, (c) BTA-FT and (d) BTA-FTM were examined in THF/H_2O mixtures containing varying proportions of water.

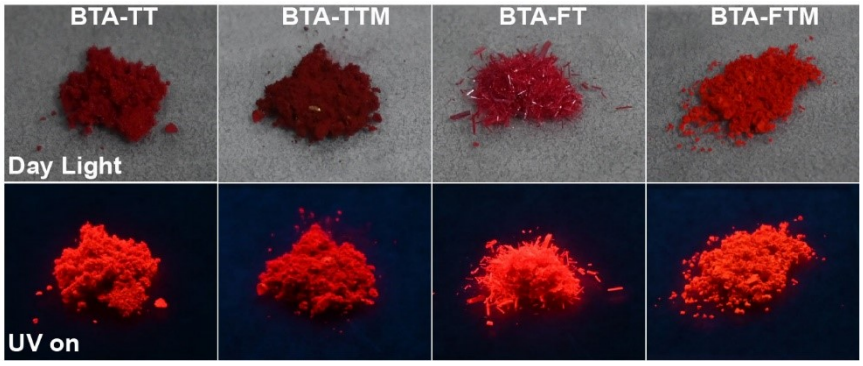


Figure S10. Pictures of BTA-TT, BTA-TTM, BTA-FT and BTA-FTM in solid under day light and UV lamp.

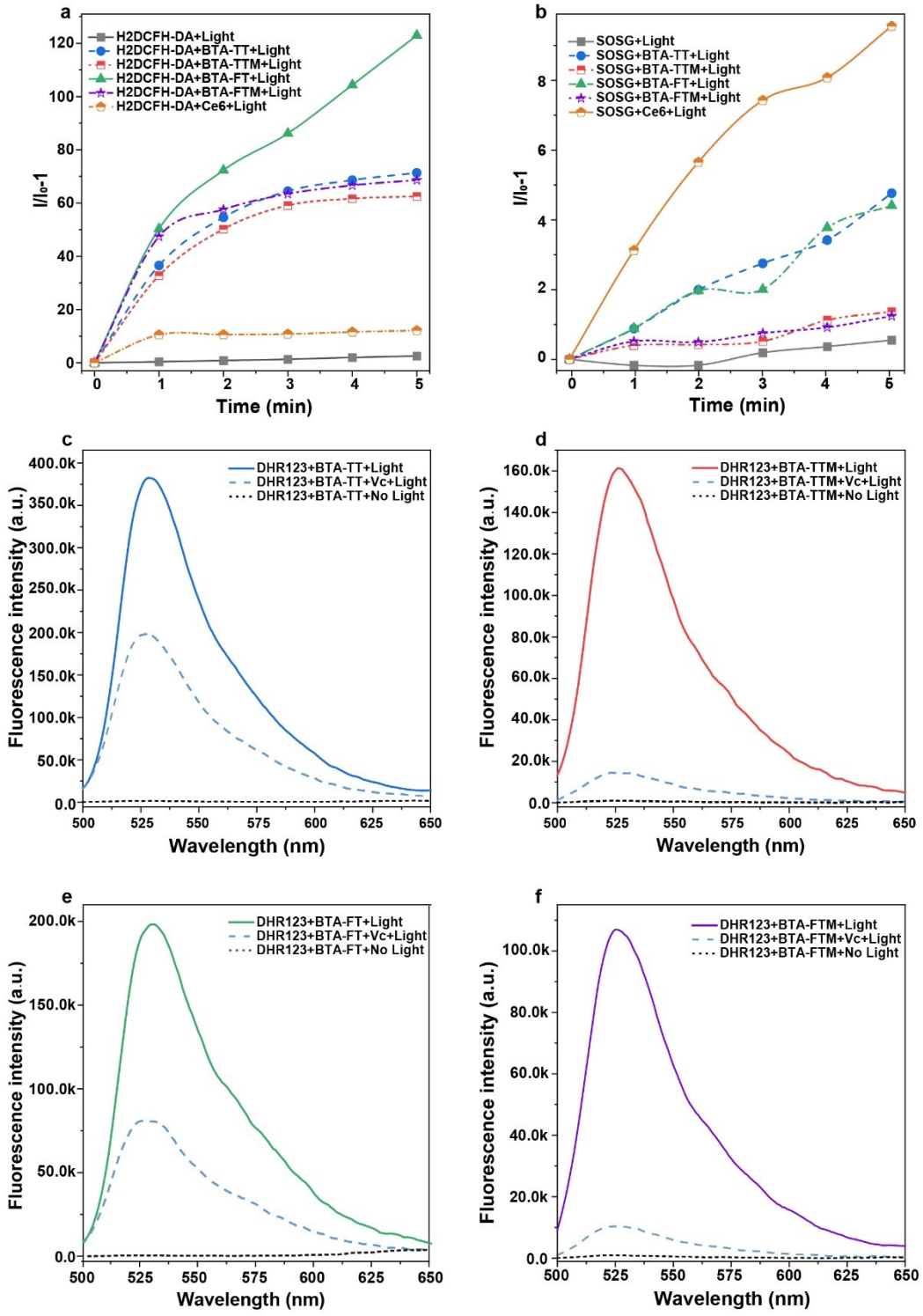


Figure S11. (a) Fluorescence spectra of four compounds (40 μM) in DMSO of H_2DCFH-DA at 525 nm under irradiation with 20 mw cm^{-2} of white light where I_0 was fluorescence intensity without white lamp. (b) SOSG test. (c-f) Fluorescence spectra of BTA-TTM-BTA-FTM in DMSO of DHR123, DHR123 and VC at 525 nm under irradiation with 20 mw cm^{-2} of white light in 2 minutes.

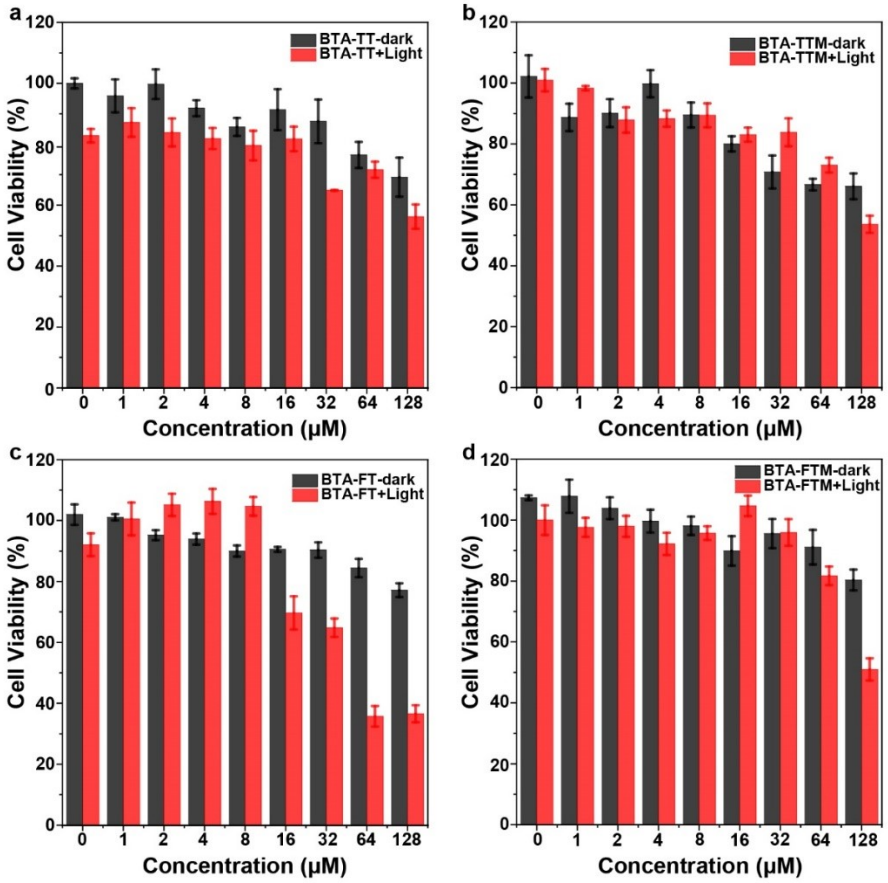


Figure S12. Cell phototoxicity of (a) BTA-TT, (b) BTA-TTM, (c) BTA-FT, (d) BTA-FTM in 4T1 cells.

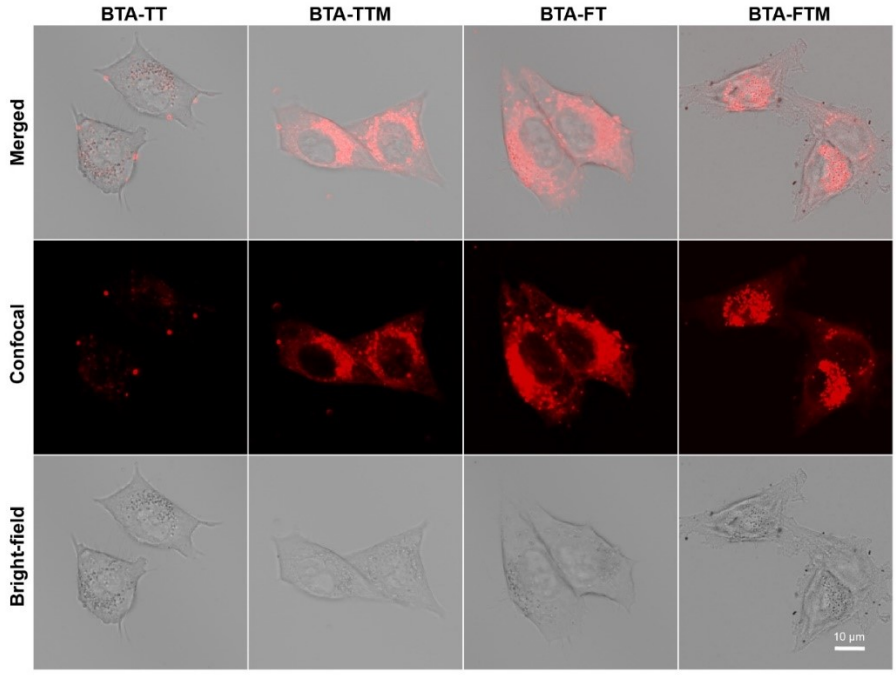


Figure S13. Confocal laser scanning microscopy images of 4T1 cells incubated with BTA-TT, BTA-TTM, BTA-FT, BTA-FTM. λ_{ex} = 488 nm, λ_{em} = 662 to 737 nm. Scale bar: 10 μm

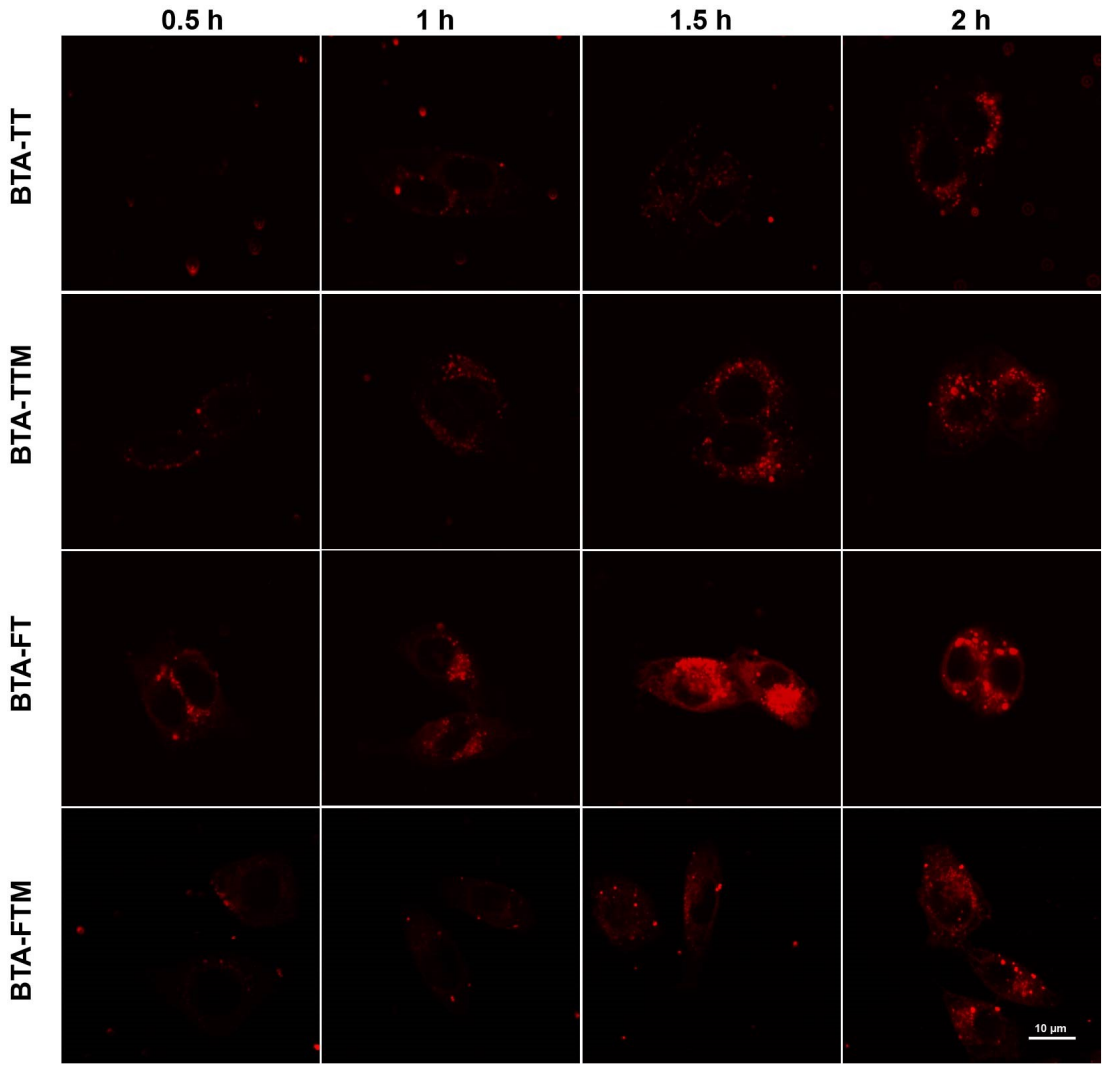


Figure S14. CLSM images of 4T1 cells were incubated with BTA-TT, BTA-TTM, BTA-FT, BTA-FTM, respectively, and then cells were treated with Lyso-tracker Blue for 30 min four molecules = 12.5 μM, Lyso-tracker Blue = 200 nM.

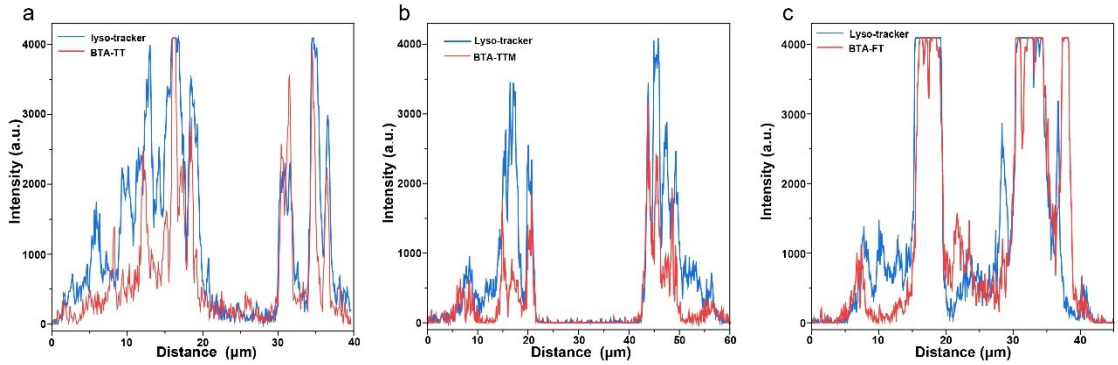


Figure S15. The line-plot graphs exhibit the fluorescence intensity profiles of the BTA-TT, BTA-TT, BTA-FT and Lyso-tracker (blue), respectively.

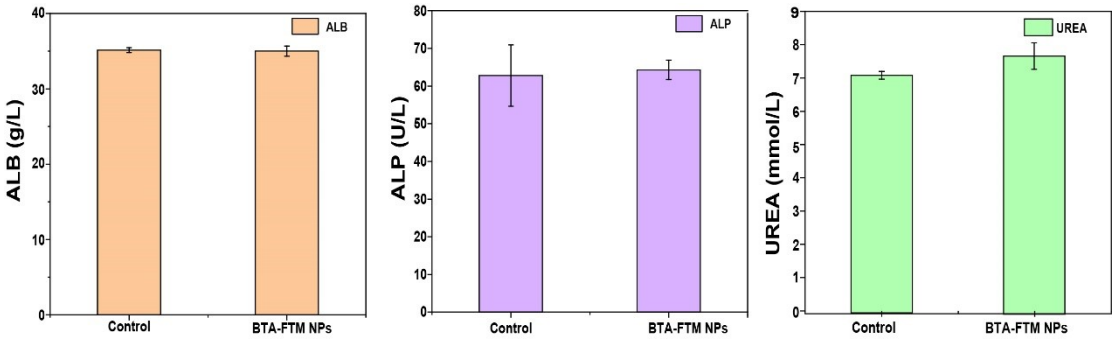


Figure S16. Levels of ALB, ALP and UREA in blood samples from mice

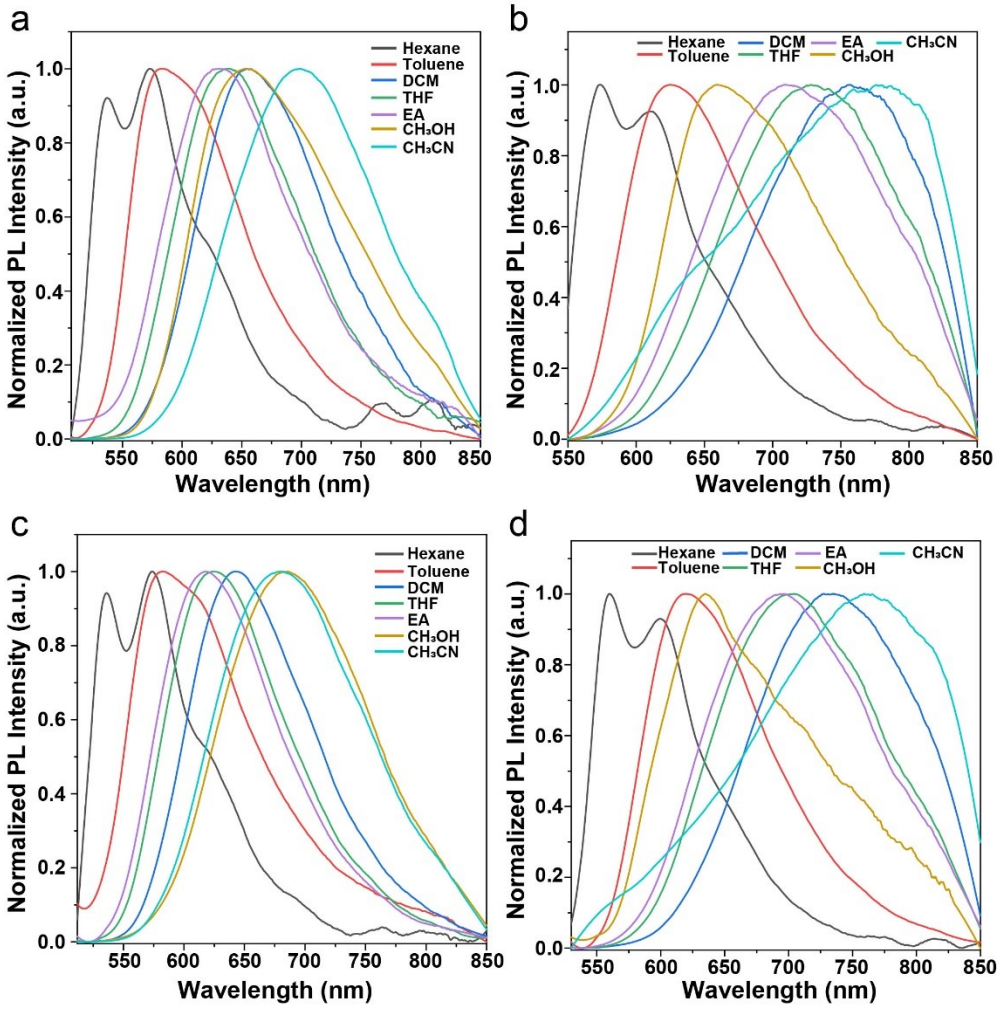


Figure S17. PL spectra of (a) BTA-TT, (b) BTA-TTM, (c) BTA-FT and (d) BTA-FTM in solvents with different polarities.

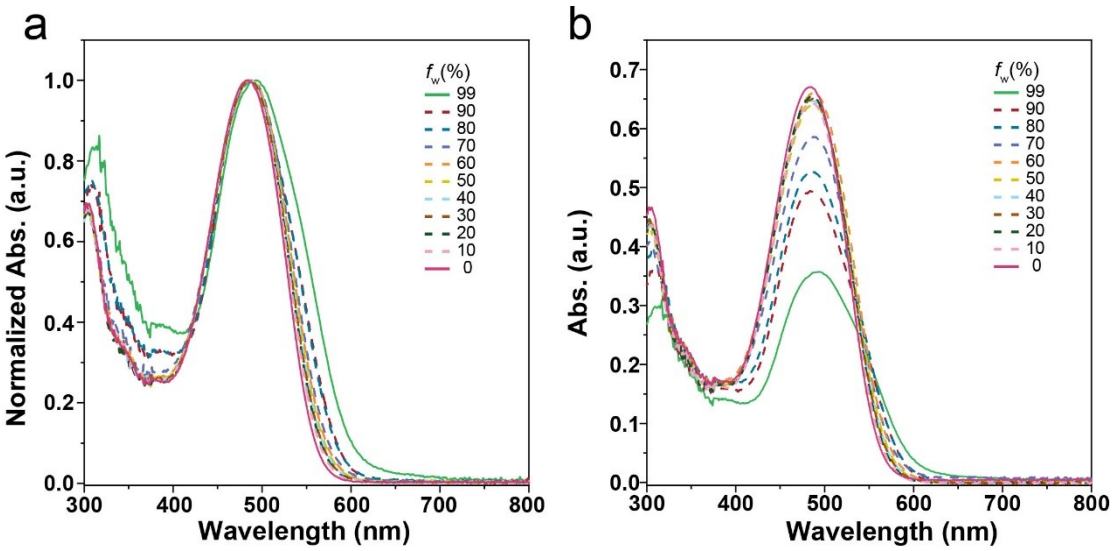


Figure S18. (a) Normalized absorption spectra and (b) absorption spectra of BTA-TT in different water fractions solvents.