Molecular Engineering to Design Bright Near-Infrared Red Photosensitizer: Cellular Bioimaging and Phototherapy

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Materials and instruments

Chemical agents were purchased from available commercial companies, such as Energy Chemical Co., Ltd., San-Bang Chemical Co., Ltd., Su Kailu Chemical Co., Ltd. Here, potassium carbonate, Tetrakis(triphenylphosphine)palladium, 4-Formylphenylboronic acid, Reactive oxygen probe (H2DCF-DA), Singlet oxygen probe (ABDA) were purchased from Energy Chemical Co., Ltd., 4-(Diphenylamino)phenylboronicacid was purchased from Su Kailu Chemical Co., Ltd., and 4, 9-dibromonaphtho-(2,3-c)(1,2,5)thiadiazole was provided from San-Bang Chemical Co., Ltd. 2,7-dichloro-

dihydrofluorescein diacetate (DCFH-DA), methyl thiazolyl tetrazolium (MTT) were purchased from Solarbio Biotechnology Co., Ltd. Hydroxyl radical probe (HPF) and superoxide anion radical (DHR123) were purchased from Shanghai Maokang Biotechnology Co., Ltd.

A Bruker VANCE III HD 500 MHz spectrometer (Germany) was used to record the ^{1}H and ^{13}C NMR spectra of molecules, Shimadzu RF-6000 and UV-1900i spectrophotometers were employed to measure the fluorescence emission and UV–Vis absorption spectra, respectively. The optimal ground structure and natural transition orbitals (NTOs) were calculated using the density function theory (DFT) and time-dependent density function theory (TD-DFT) method with M06-2X at the basis set level of 6-31G (d,p). All the calculations were performed using Gaussian software.

2.2 Materials synthesis

Synthesis of TNZ: TNZ was synthesized by adopting one-step Suzuki reaction. 4,9-dibromonaphtho-(2,3-c)(1,2,5)thiadiazole (1 mmol, 341.8 mg), 4-(Diphenylamino)phenylboronicacid (0.9 mmol, 260.2 mg), $Pd(PPh_3)_4$ (0.1 mmol, 115.5 mg) were put into two-neck flask. The flask maintains N_2 atmosphere by pumping and filling in with N_2 for three times. Toluene (20 mL) and K_2CO_3 aqueous solution (2 M, 12 mL) were injected into the flask. The reaction carried out at 110 ºC for 24 h. After the mixture was cooled down, the mixture was extracted with DCM for three times.

The pure product was obtained by chromatography using ethyl acetate/petroleum as the eluent to afford a light red powder. (206 mg), yield: 48%: ^{1}H NMR (500 MHz, CD_2Cl_2) δ = 8.60 (s, 1H), 8.10-8.07 (d, *J* = 15 Hz, 1H), 8.02-8.00 (d, *J* = 10 Hz, 11H), 7.53-7.50 (m, 2H), 7.46-7.42 (t, *J* = 20 Hz, 1H), 7.40-7.32 (m, 5H), 7.26-7.24 (m, 6H), 7.13-7.09 (t,2H). ^{13}C NMR (126 MHz, CD_2Cl_2), δ = 152.12, 152.04, 147.85, 147.58, 134.89, 132.25, 131.30, 130.73, 129.70, 129.40, 129.29, 126.86, 126.34, 126.31, 124.98, 1123.41, 122.23, 117.36.

Synthesis of TNZBr: 4,9-dibromonaphtho-(2,3-c)(1,2,5)thiadiazole (1 mmol, 341.8 mg), 4-(Diphenylamino)phenylboronicacid (1.2 mmol, 346.9 mg), $Pd(PPh_3)_4$ (0.02 mmol, 23.1 mg) were put into two-neck flask. The flask maintains N_2 atmosphere by pumping and filling in with N_2 for three times. Toluene (20 mL) and K_2CO_3 aqueous solution (2 M, 12 mL) were injected into the flask. The reaction carried out at 110 ºC for 24 h. After the mixture was cooled down, the mixture was extracted with DCM for three times. The pure product was obtained by chromatography using ethyl acetate/petroleum as the eluent to afford a black powder. (263.6 mg), yield: 52%: ^{1}H NMR (500 MHz, CD_2Cl_2) δ = 9.18-9.16 (d, *J* = 10 Hz, 1H), 8.85-8.83 (d, *J* = 10 Hz, 1H), 8.33-8.30 (t, *J* = 15 Hz, 1H), 8.2 (d, *J* = 5 Hz, 2H), 8.17-8.13 (t, *J* = 20 Hz, 1H), 8.08-8.05 (t, *J* = 15 Hz, 4H), 7.97-7.95 (t, *J* = 10 Hz, 6H), 7.84-7.81 (t, *J* = 15 Hz, 2H). ^{13}C NMR (126 MHz, CD_2Cl_2), δ = 157.99, 152.55, 150.67, 141.62, 134.29, 133.40, 129.52,

129.09, 128.82, 128.72, 128.49, 127.95, 127.63, 119.78, 116.15, 111.65.

Synthesis of TNZCHO: TNZBr (0.5 mmol, 253.7 mg), 4-Formylphenylboronic acid (1.8 mmol, 75 mg), $Pd(PPh_3)_4$ (0.01 mmol, 12 mg) were put into two-neck flask. The flask maintains N_2 atmosphere by pumping and filling in with N_2 for three times. Toluene (20 mL) and K_2CO_3 aqueous solution (2 M, 12 mL) were injected into the flask. The reaction carried out at 110 ºC for 24 h. After the mixture was cooled down, the mixture was extracted with DCM for three times. The pure product was obtained by chromatography using ethyl acetate/petroleum as the eluent to afford a deep red powder. (237.3 mg), yield: 89%: ^{1}H NMR (500 MHz, CD_2Cl_2) δ = 10.18 (s,1H), 8.19 (d, *J* = 10 Hz, 1H), 8.13 (d, *J* = 5 Hz, 2H), 7.94-7.92 (d, *J* = 10 Hz, 1H), 7.85 (d, *J* = 5 Hz, 2H), 7.56 (d, *J* = 10 Hz, 2H), 7.42-7.39 (m, 2H), 7.37-7.34 (t, *J* = 15 Hz, 4H), 7.28-7.25 (t, *J* = 15 Hz, 6H), 7.13-7.10 (t, *J* = 15 Hz, 2H). ^{13}C NMR (126 MHz, CD_2Cl_2), δ = 193.15, 152.76, 152.51, 149.31, 148.85, 144.47, 137.29, 133.63, 133.53, 133.19, 133.06, 132.45, 130.91, 130.82, 130.75, 129.34, 128.69, 128.24, 127.65, 127.52, 126.38, 124.82, 123.47.

2.3 ROS measurement

ROS detection: The ROS production from synthetic PSs could be detected by 2,7-dichlorodihydrofluorescein (DCFH) probe. Briefly, 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) dissolved in ethanol to prepare solution (1 mM), and was added to 2 mL NaOH (10 mM) and

allowed to stir at room temperature for 20 min. Then the solution was diluted with 10 mL of PBS to obtain the hydrolyzed DCFH with the concentration of 40 μM. Then, the PS solution (1 mM, 30 uL) was added into DCFH solution (20 uM, 2970 uL) for the PL spectra measurement under white light irradiation (50 mW/cm^2) at different intervals. The excitation wavelength was 488 nm and emissive range collected from 500 to 650 nm.

$^1O_2$ detection: ABDA was used to evaluate the $^1O_2$ generation ability of PSs. Three PSs (10 μL in DMSO) were mixed with ABDA (100 μL in ethanol) and ethanol (1890 μL) to make the final concentrations of ABDA and PSs were 100 μM and 10 μM, respectively. White light with a density of 50 mW/cm^2 was used to irradiate the PSs' solution for different time and the absorbance value at 400 nm was recorded.

$O_2^{\bullet-}$ detection: DHR123 probe was used to evaluate the $O_2^{\bullet-}$ generation of three PSs. DHR123 was resolved in DMSO. Then three PSs (10 μL in DMSO) were added to DHR123 solution (990 μL in PBS) to make the final concentrations of DHR123 and PSs were 10 μM and 1 μM, respectively. White light with a density of 50 mW/cm^2 was used to irradiate the solution for different time and the fluorescence intensity at 525 nm (excitation wavelength set as 490 nm) was recorded.

•OH detection: HPF was used to evaluate the •OH generation ability of three PSs. HPF in PBS (995 μL) was mixed with three PSs (5 μL

in DMSO) to make the final concentrations of HPF and PSs were 10 μM and 1 μM, respectively. White light with a density of 50 mW/cm^2 was used to irradiate the solution for 5 min and the fluorescence intensity at 512 nm (excitation wavelength set as 490 nm) was recorded.

2.4 Theoretical simulation

The energy levels and highest occupied molecule orbits (HOMOs) and lowest unoccupied molecule orbits (LUMOs) were operated based on Gaussian 09W. Density functional theory (DFT) and time-dependent density functional theory (TD-DFT) were used to simulate the geometries of PSs at the ground/excited states based on the M0-62X/6-31G (d,p) level.

2.5 Cellular fluorescent imaging

Human Umbilical Vein Endothelial (HUVEC) were incubated with medium containing 50 μg/mL PS-based nanoparticles for different time at 37 ^{o}C, the cells were imaged by CLSM in the channel mode as well as lambda mode at excitation of 488 nm without further washed.

2.6 Cytotoxicity studies

HepG2 cells were seeded in 96-well plates at a density of 1 × 10^5 cells/mL. After 24 h of culture, different concentrations of PSs-based nanoparticles were added and incubated at 37 ^{o}C for 12 h in dark. Without or with the exposure to white light irradiation of 50 mW cm^{-2} for 10 min, the cells were further incubated at 37 ^{o}C to 24 h. The sample and control wells were washed twice with PBS buffer and added with freshly prepared

100 μL MTT solution (0.5 mg/mL). After incubation at 37 °C for 4 h, the MTT solution was removed and washed twice with PBS buffer. 100 μL DMSO was then added into each well and the plate was gently shaken for 3 min at room temperature to dissolve all the precipitates formed. The absorbance of sample and control wells at 570 nm was then measured by a microplate Reader. Cell viability was then calculated by the ratio of the absorbance of sample wells to control cells.

2.7 Live/dead cell co-staining assay

HepG2 cells were grown in a confocal imaging dish at 37 °C in normal condition. After incubation with medium containing 50 μg/mL PSs-based NPs for 12 h in normal condition. With the exposure to white light irradiation of 50 mW cm^{-2} for 10 min, the cells were further incubated at 37 °C to 24 h in normal condition. Then after staining with medium containing 2 μM fluorescein diacetate (stock solution: 1 mM in DMSO) and 2 μg/mL PI (stock solution: 1 mg/mL in DMSO) for 10 min, the cells were imaged using CLSM. For fluorescein diacetate, the excitation was 488 nm, and the emission filter was 490–540 nm; For PI, the excitation was 543 nm, and the emission filter was 590–680 nm.

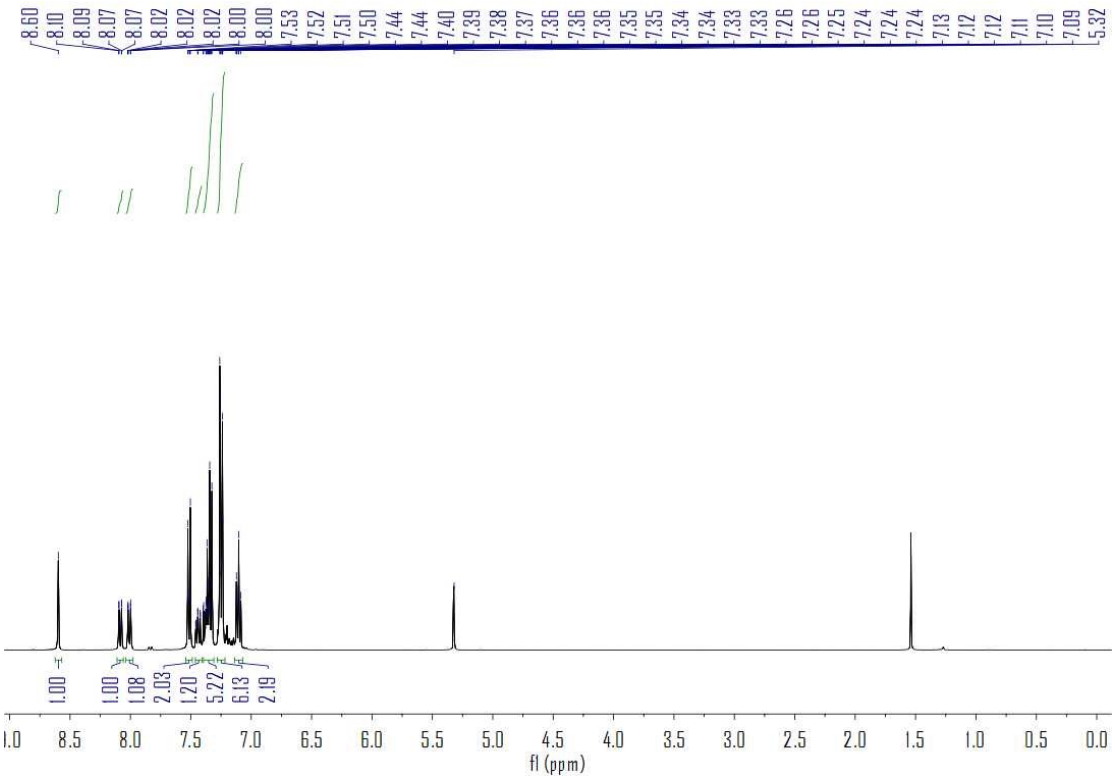

Figure S1 ^{1}H NMR spectrum of TNZ

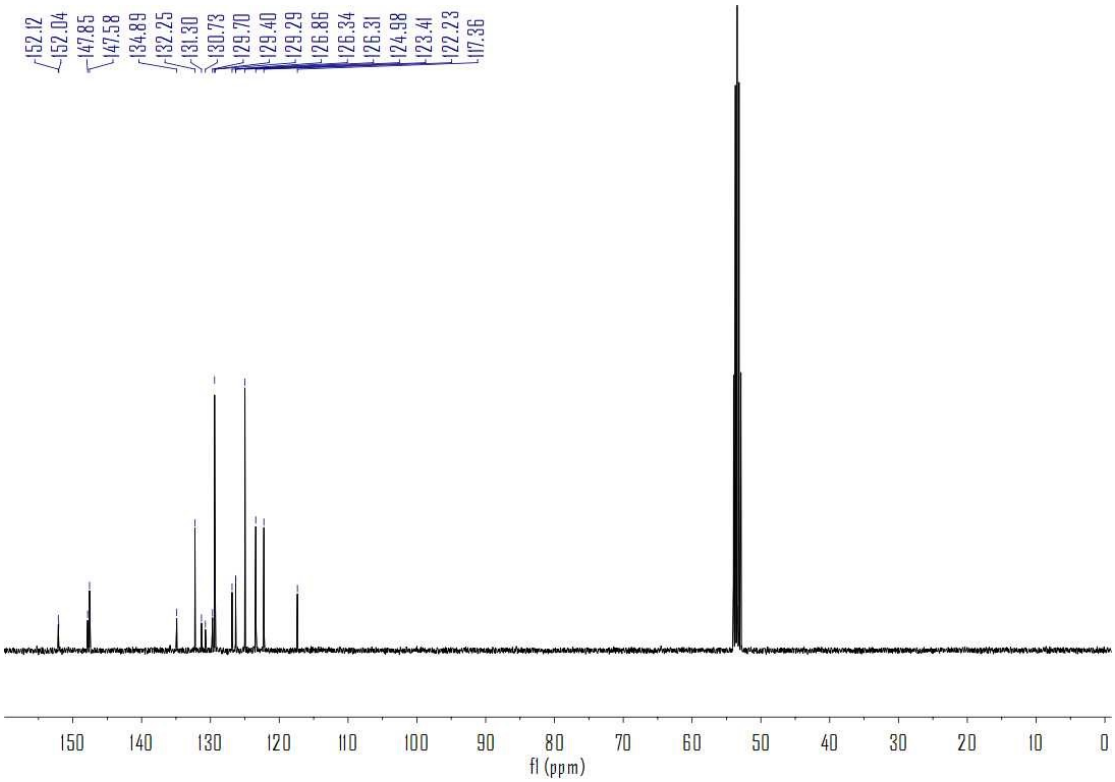

Figure S2 ^{13}C NMR spectrum of TNZ

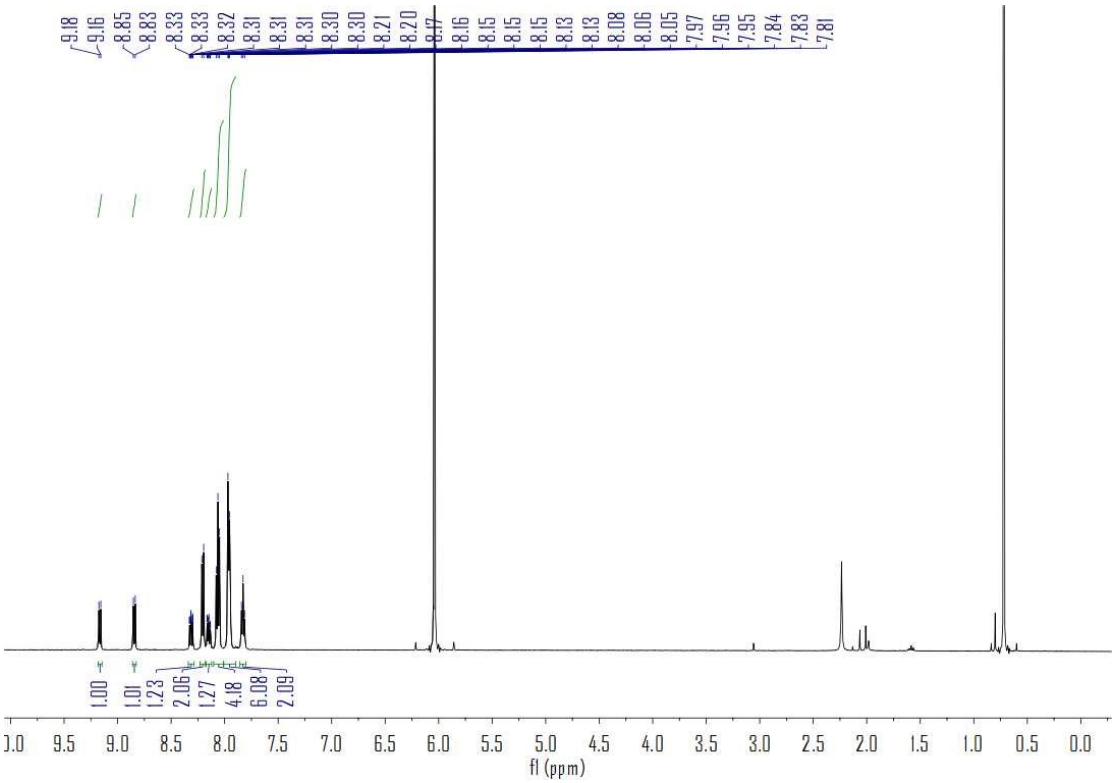

Figure S3 ^{1}H NMR spectrum of TNZBr

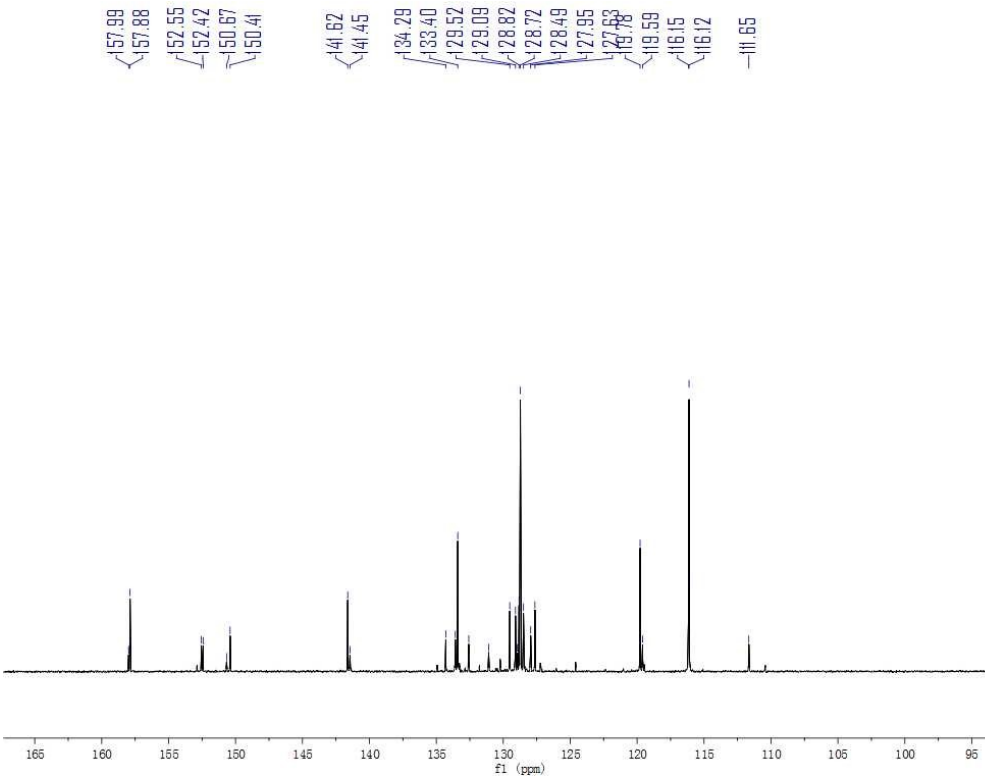

Figure S4 ^{13}C NMR spectrum of TNZBr

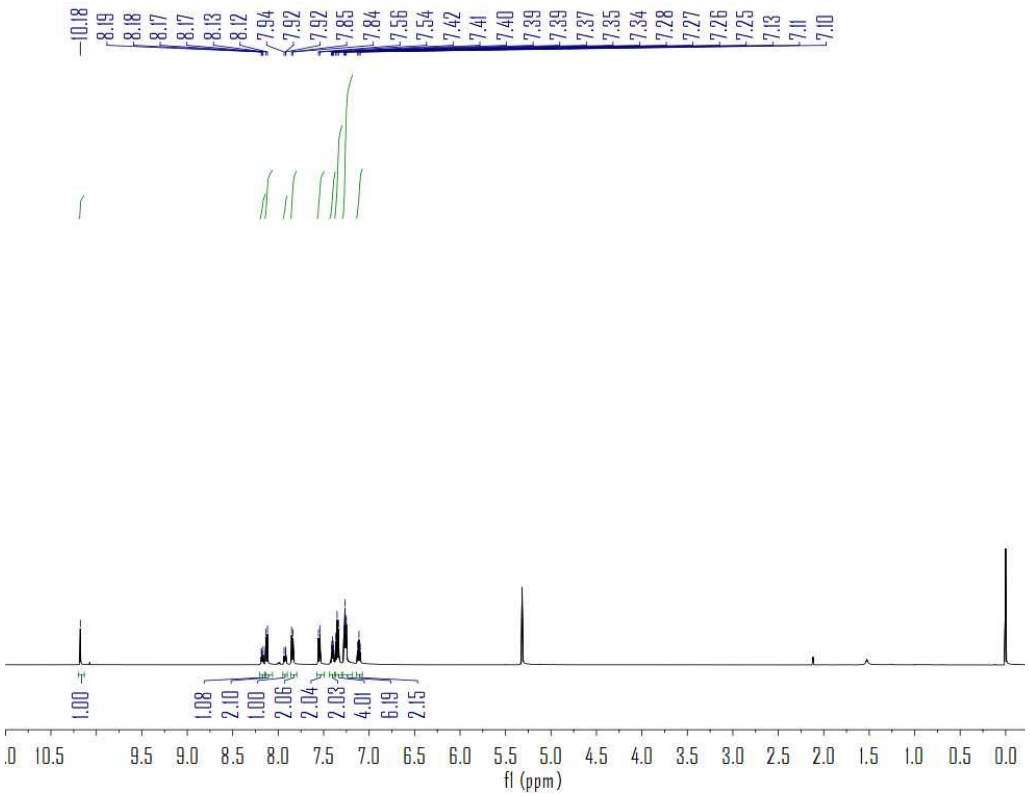

Figure S5 ^{1}H NMR spectrum of TNZCHO

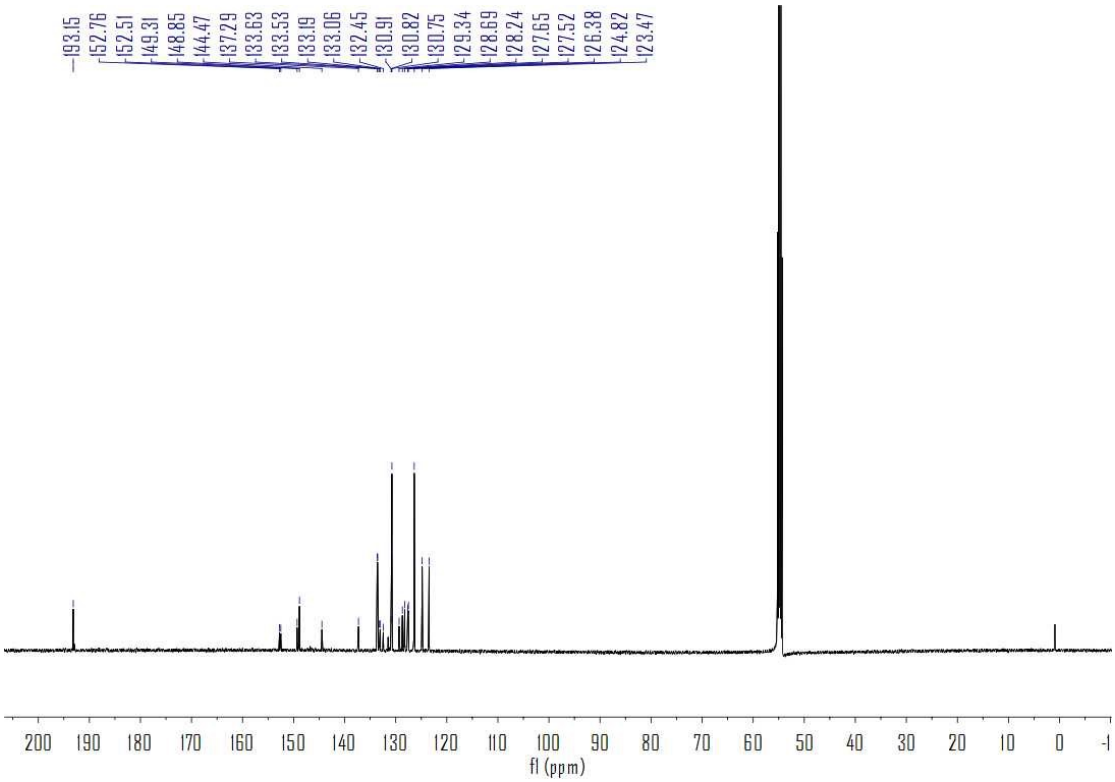

Figure S6 ^{13}C NMR spectrum of TNZCHO

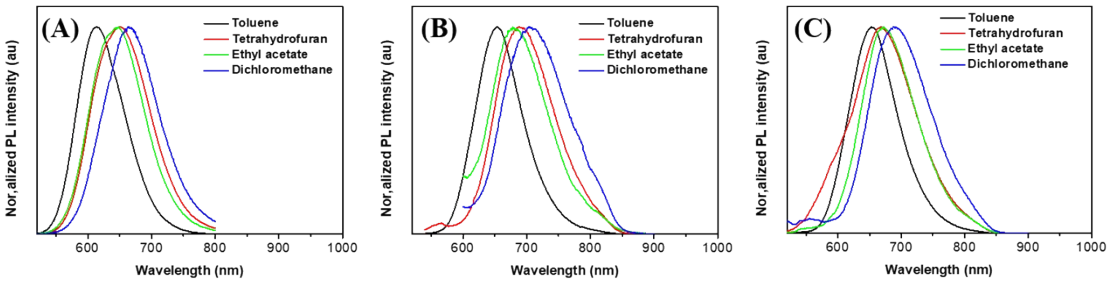

Figure S7 Fluorescent spectra of emitters in different solvents: (A) TNZ, (B) TNZBr, (C) TNZCHO. Emitter: 10 μM.

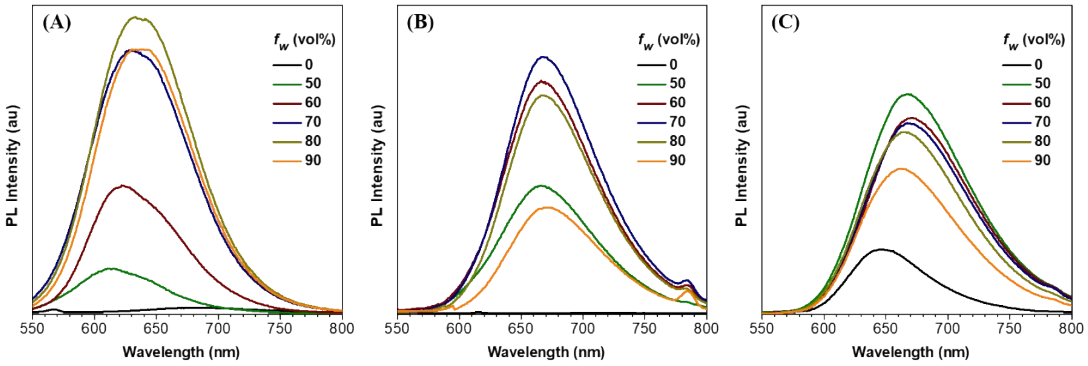

Figure S8 Fluorescent spectra of emitters in DMSO/H2O mixture with different water fraction: (A) TNZ, (B) TNZBr, (C) TNZCHO. Emitter: 10 μM for different time.

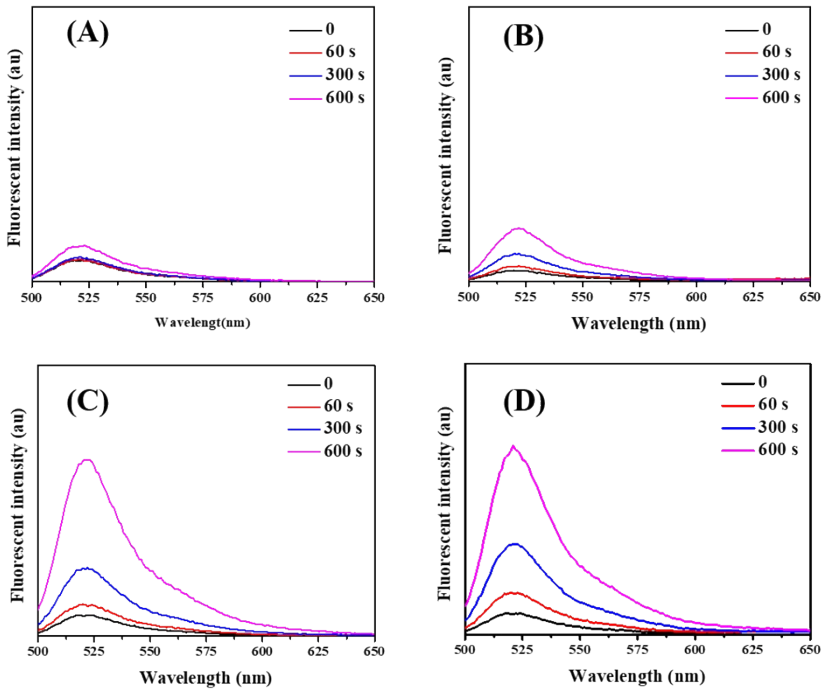

Figure S9 PL spectra of (A) pure DCFH (20 μM), (B) DCFH +TNZ (10 μM), (C) DCFH +TNZBr (10 μM), (D) DCFH + TNZBr (10 μM) upon white light (50 mW cm^{-2})

irradiation for different time.

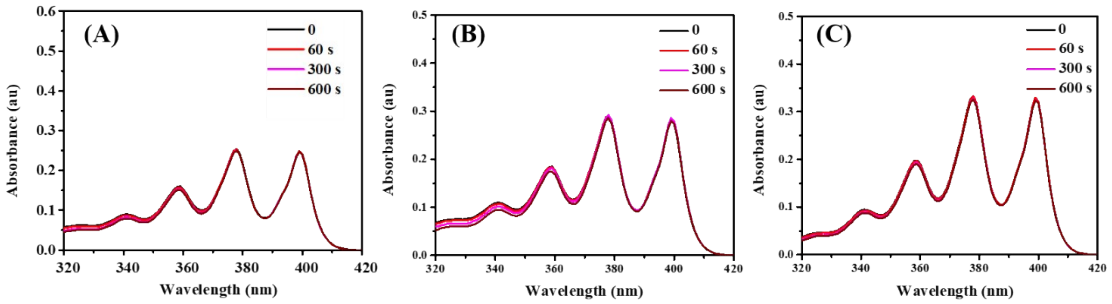

Figure S10 Absorption spectra of (A) pure ABDA (100 μM), (B) ABDA +TNZ (10 μM), (C) ABDA +TNZBr (10 μM), (D) ABDA +TNZCHO (10 μM) upon white light (50 mW cm^{-2}) irradiation for different time.

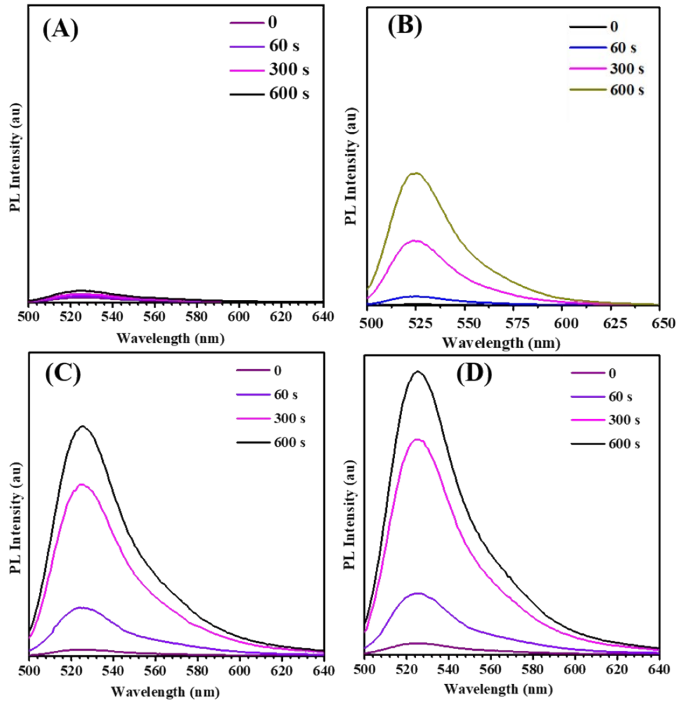

Figure S11 PL spectra of (A) pure DHR123 (10 μM), (B) DHR123+TNZ (1 μM), (C) DHR123+TNZBr (1 μM), (D) DHR123+TNZCHO (1 μM) upon white light (50 mW cm^{-2}) irradiation for different time.

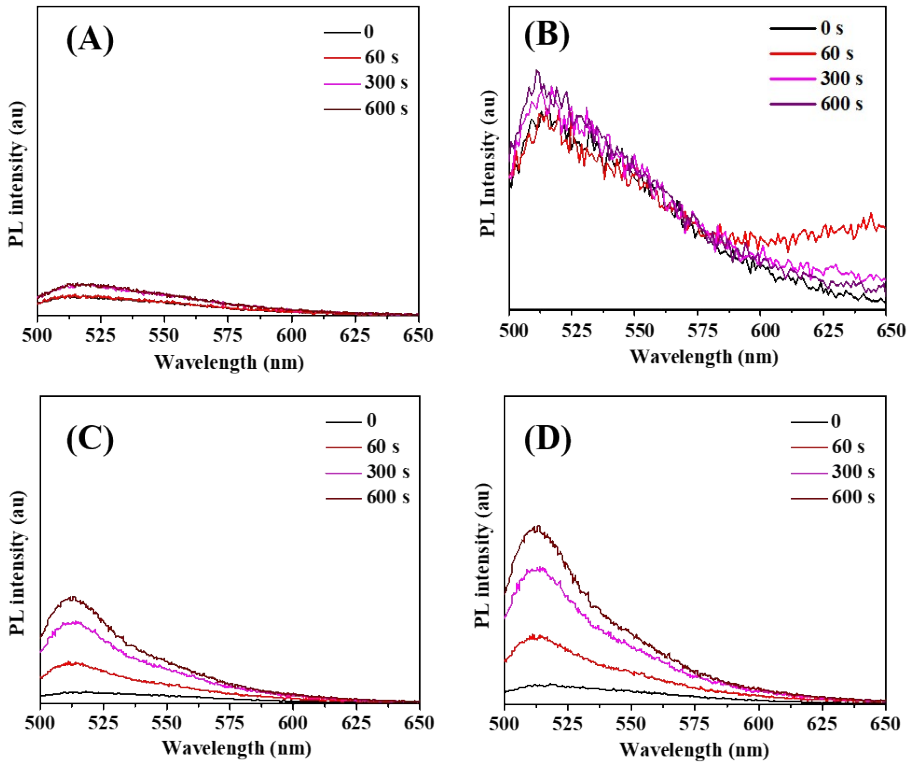

Figure S12 PL spectra of (A) pure HPF (10 μM), (B) HPF+TNZ (1 μM), (C) HPF+TNZBr (1 μM), (D) HPF+TNZCHO (11 μM) upon white light (50 mW cm^{-2}) irradiation for different time.

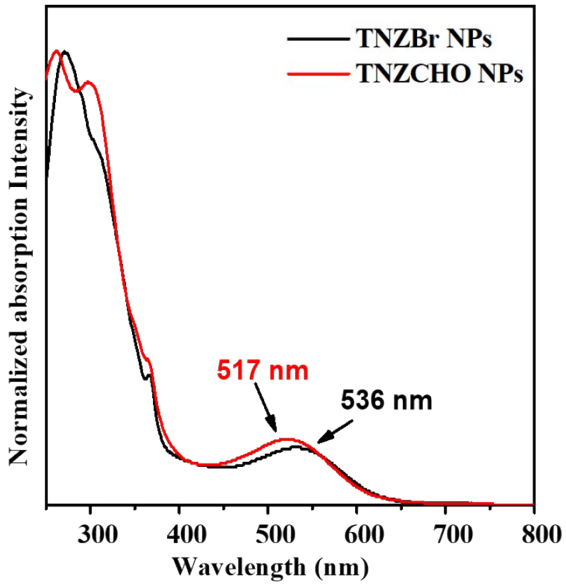

Figure S13 Absorption spectra of TNZBr NPs and TNZCHO NPs in aqueous solution (10 μg/mL).

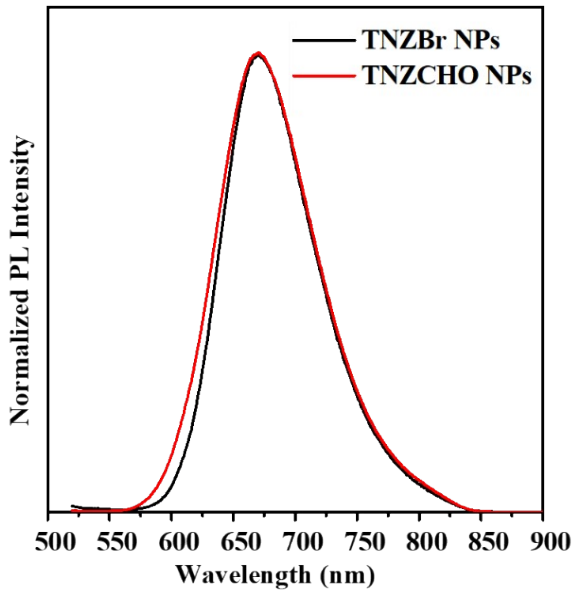

Figure S14 PL spectra of TNZBr NPs and TNZCHO NPs in aqueous solution (10 μg/mL).

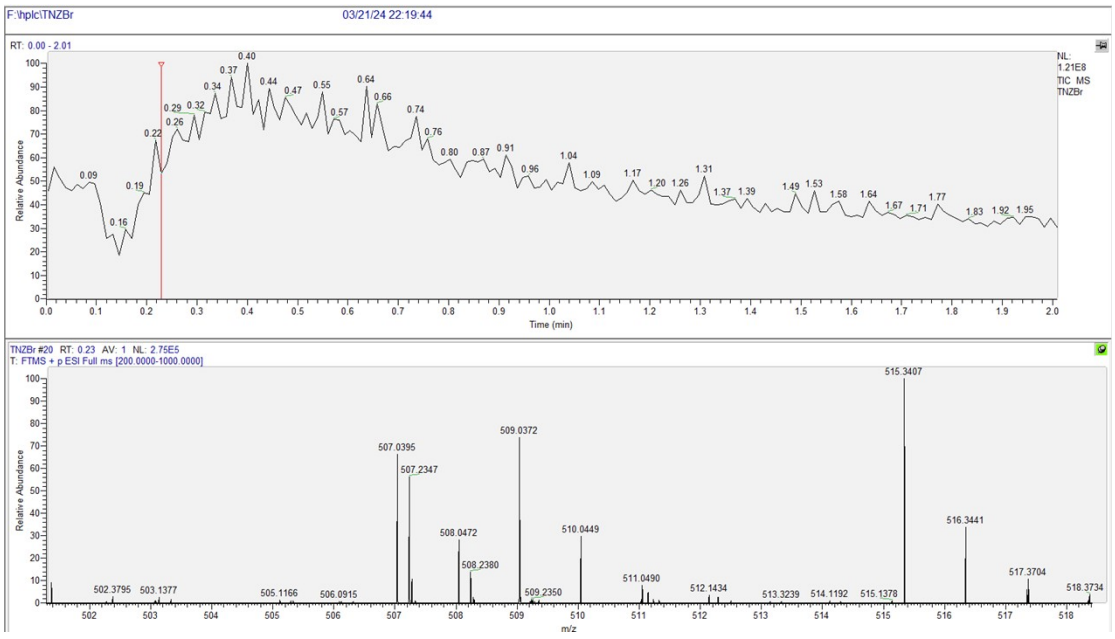

Figure S15 High-resolution mass spectrometry of TNZBr.

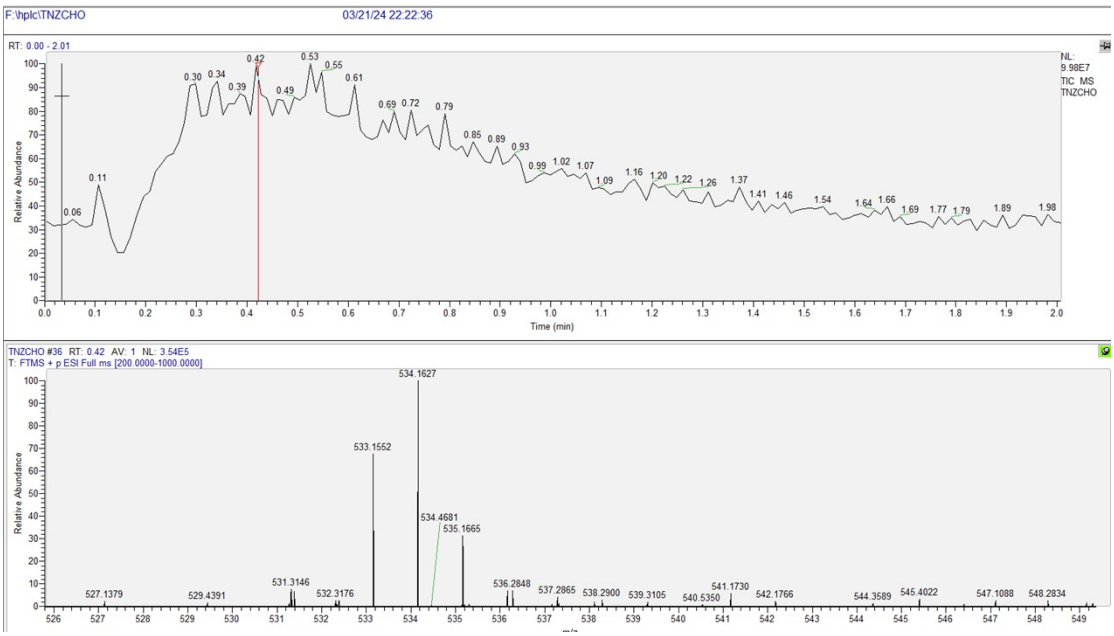

Figure S16 High-resolution mass spectrometry of TNZCHO.

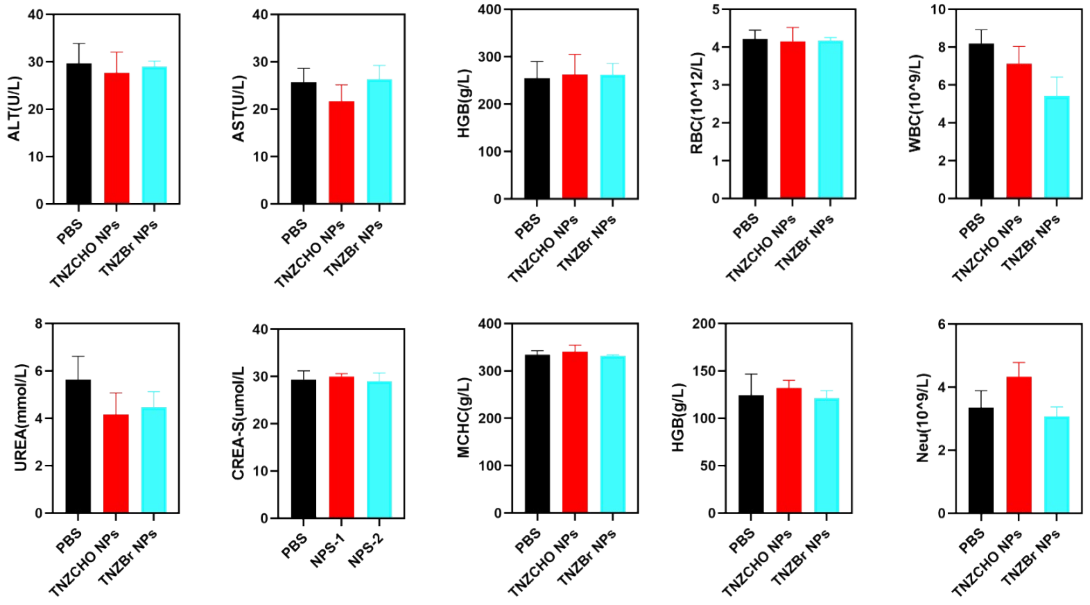

Figure S17 Blood routine assays and blood biochemistry test of the healthy mice with intra-tumoral injection of TNZBr NPs and TNZCHO NPs, and their hematopoietic system compared with mock treated group (PBS) at 10 days postinjection (n=5, *p < 0.05).

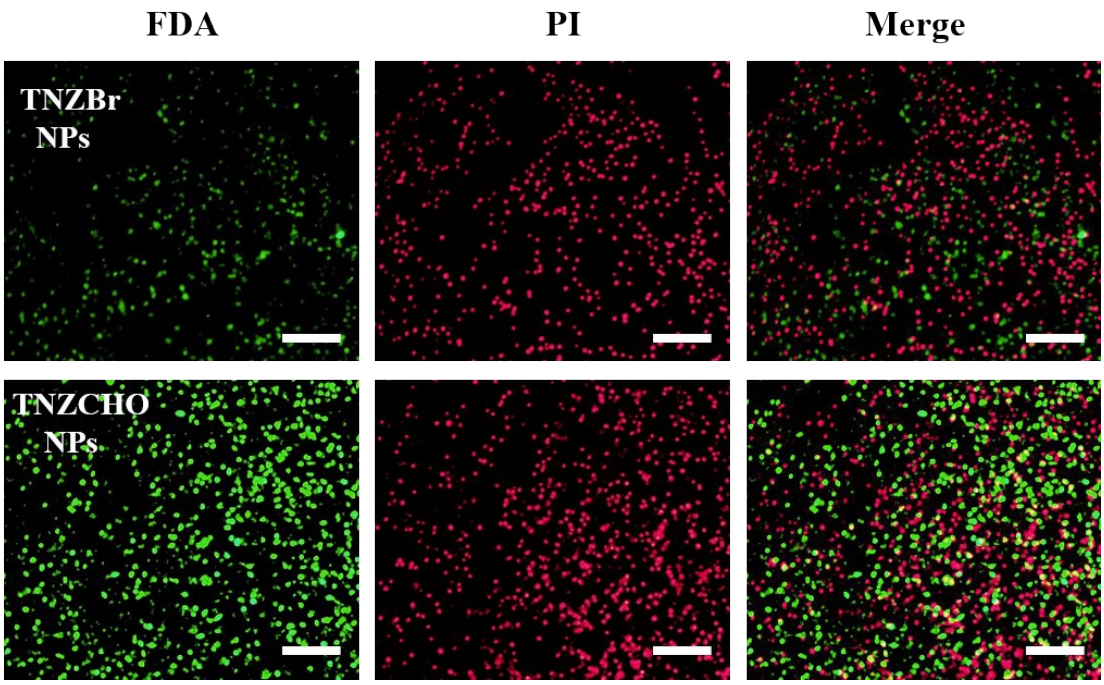

Figure S18 Confocal microscopic images of HepG2 cells treated TNZBr NPs and TNZCHO NPs under the hypoxia enviroment (concentration of 100 μg/mL, light power of 50 mW cm^{-2}).