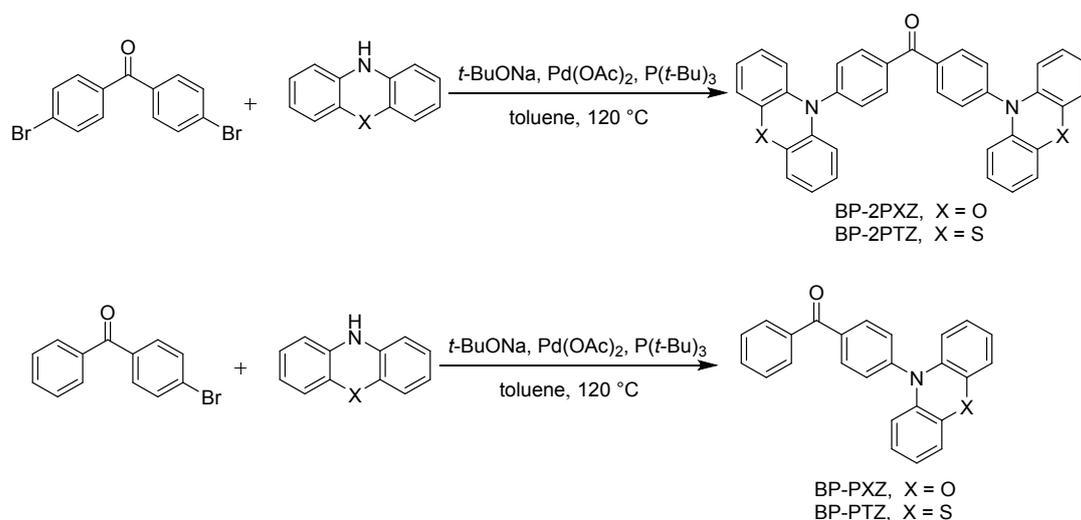


## *Electronic Supplementary Information (ESI)*

### **1. Materials and measurements**

THF was distilled from sodium benzophenone ketyl under dry nitrogen immediately prior to use. All chemicals were purchased from Energy Chemical and TCI Ltd. and used as received without further purification. Albumin from Bovine Serum (BSA) was purchased from Aldrich. Cell Counting Kit-8 (CCK8) was purchased from Dojindo, Japan. UV-vis absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. Fluorescence quantum yields were determined with a Hamamatsu C11347 Quantaury-QY absolute fluorescence quantum yield spectrometer. Fluorescence lifetimes were determined with a Hamamatsu C11367-11 Quantaury-Tau time-resolved spectrometer. The nanoparticles were formed by microtip probe sonicator with an 18 W output (XL2000, Misonix Incorp., USA). Fluorescence images were obtained by using confocal laser scanning microscope (Leica SP5, Germany). Time-resolved fluorescence images were performed on homemade system. The particle size was determined through dynamic light scattering (DLS) on a Malvern Nano-ZS90 particle size analyzer. NMR spectra were measured on a Bruker AV 500 spectrometer in deuterated chloroform using tetramethylsilane (TMS;  $\delta = 0$ ) as internal reference. High resolution mass spectra (HRMS) were recorded on a GCT premier CAB048 mass spectrometer operating in MALDI-TOF mode. All calculations were performed by using the D.01 version of the Gaussian09 package.

### **2. Synthesis**



**Scheme S1.** Synthesis of BP-2PXZ, BP-PXZ, BP-2PTZ and BP-PTZ.

**Bis(4-(10*H*-phenoxazin-10-yl)phenyl)methanone (BP-2PXZ):** In a three-necked flask, a mixture of bis(4-bromophenyl)methanone (0.68 g, 2mmol), phenoxazine (0.88 g, 4.8 mmol), *t*-BuONa (0.46 g, 6 mmol), *P*(*t*-Bu)<sub>3</sub> (0.4 ml, 0.08 mmol), Pd(OAc)<sub>2</sub> (10 mg, 0.04 mmol) and toluene (80 mL) was heated at 120 °C for 24 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with toluene for three times. The combined organic layers were washed with water and dried over anhydrous MgSO<sub>4</sub>. After filtration and solvent evaporation, the crude product was purified by silica-gel column chromatography using dichloromethane/petroleum ether as an eluent to afford a yellow solid in 68% yield (740 mg). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 8.11 (d, *J* = 8.2 Hz, 4H), 7.54 (d, *J* = 8.3 Hz, 4H), 6.77–6.61 (m, 12H), 6.02 (d, *J* = 7.8 Hz, 4H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 194.6, 144.1, 143.4, 136.9, 133.7, 132.8, 130.9, 123.3, 121.9, 115.8, 113.4. HRMS (C<sub>37</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>): *m/z* 544.1771 [M<sup>+</sup>, calcd 544.1787].

**(4-(10*H*-Phenoxazin-10-yl)phenyl)(phenyl)methanone (BP-PXZ):** In a three-necked flask, a mixture of (4-bromophenyl)(phenyl)methanone (0.52 g, 2 mmol), phenoxazine (0.44 g, 2.4 mmol), *t*-BuONa (0.23 g, 3 mmol), *P*(*t*-Bu)<sub>3</sub> (0.4 mL, 0.08 mmol), Pd(OAc)<sub>2</sub> (10 mg, 0.04 mmol) and toluene

(80 mL) was heated at 120 °C for 24 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with toluene for three times. The combined organic layers were washed with water and dried over anhydrous MgSO<sub>4</sub>. After filtration and solvent evaporation, the residue was purified by silica-gel column chromatography using dichloromethane/petroleum ether as an eluent. The product was obtained as a yellow powder in 55% yield (399 mg). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 8.04 (d, *J* = 8.4 Hz, 2H), 7.88 (dd, *J*<sub>1</sub> = 8.3 Hz, *J*<sub>2</sub> = 1.2 Hz, 2H), 7.66–7.62 (m, 1H), 7.54 (dd, *J*<sub>1</sub> = 10.7 Hz, *J*<sub>2</sub> = 4.8 Hz, 2H), 7.51–7.48 (m, 2H), 6.75–6.61 (m, 6H), 6.00 (d, *J* = 7.8 Hz, 2H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 195.7, 144.0, 143.0, 137.4, 137.2, 133.7, 132.8, 130.8, 130.0, 128.5, 123.3, 121.8, 115.7, 113.3. HRMS (C<sub>25</sub>H<sub>17</sub>NO<sub>2</sub>): *m/z* 363.1282 [M<sup>+</sup>, calcd 363.1259].

**Bis(4-(10*H*-phenothiazin-10-yl)phenyl)methanone (BP-2PTZ):** BP-2PTZ was synthesized similarly to that described for BP-2PXZ, using phenothiazine (0.95 g, 4.8 mmol) instead of phenoxazine. The product was obtained as a yellow powder in 72% yield (829 mg). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>), δ (ppm): 7.85–7.82 (m, 4H), 7.31–7.25 (m, 8H), 7.18–7.14 (m, 4H), 7.09–7.05 (m, 4H), 6.95 (dd, *J*<sub>1</sub> = 8.1 Hz, *J*<sub>2</sub> = 1.1 Hz, 4H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>), δ (ppm): 194.1, 147.3, 142.3, 133.3, 132.1, 128.4, 128.1, 127.1, 124.8, 122.3, 121.4. HRMS (C<sub>37</sub>H<sub>24</sub>N<sub>2</sub>OS<sub>2</sub>): *m/z* 576.1359 [M<sup>+</sup>, calcd 576.1330].

**(4-(10*H*-Phenothiazin-10-yl)phenyl)(phenyl)methanone (BP-PTZ):** BP-PTZ was synthesized similarly to that described for BP-PXZ, using phenothiazine (0.48 g, 2.4 mmol) instead of phenoxazine. The product was obtained as a yellow powder in 61% yield (462 mg). <sup>1</sup>H NMR (500 MHz, CD<sub>2</sub>Cl<sub>2</sub>), δ (ppm): 7.83–7.75 (m, 4H), 7.61–7.56 (m, 1H), 7.52–7.46 (m, 2H), 7.31 (dd, *J*<sub>1</sub> = 7.7 Hz, *J*<sub>2</sub> = 1.4 Hz, 2H), 7.26–7.16 (m, 4H), 7.10–7.07 (m, 2H), 7.01 (dd, *J*<sub>1</sub> = 8.0 Hz, *J*<sub>2</sub> = 1.2 Hz, 2H). <sup>13</sup>C NMR (125 MHz, CD<sub>2</sub>Cl<sub>2</sub>), δ (ppm): 196.3, 148.9, 143.5, 139.3, 133.9, 133.5, 133.3, 131.0,

130.26, 129.5, 129.3, 128.5, 126.3, 124.2, 121.8. HRMS (C<sub>25</sub>H<sub>17</sub>NOS): *m/z* 379.1004 [M<sup>+</sup>, calcd 379.1031].

### 3. Crystal data of BP-2PXZ and BP-PXZ

**Crystal data for BP-2PXZ (CCDC 1455109):** C<sub>37</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>, *MW* = 544.58, monoclinic, P2(1)/n, *a* = 24.2035(13), *b* = 9.4392(5), *c* = 24.3060(15) Å,  $\beta$  = 109.203(2)°, *V* = 5244.0(5) Å<sup>3</sup>, *Z* = 8, *D<sub>c</sub>* = 1.380 g cm<sup>-3</sup>,  $\mu$  = 0.088 mm<sup>-1</sup> (MoK $\alpha$ ,  $\lambda$  = 0.71073), *F*(000) = 2272, *T* = 173(2) K,  $2\theta_{\max}$  = 25.750 (98.0%)°, 30097 measured reflections, 9852 independent reflections (*R*<sub>int</sub> = 0.0943), GOF on *F*<sup>2</sup> = 1.032, *R*<sub>1</sub> = 0.1271, *wR*<sub>2</sub> = 0.1227 (all data),  $\Delta e$  0.545 and -0.232 eÅ<sup>-3</sup>.

**Crystal data for BP-PXZ (CCDC 1455110):** C<sub>25</sub>H<sub>17</sub>NO<sub>2</sub>, *MW* = 363.40, monoclinic, P(1)2(1)/c(1), *a* = 8.4792(8), *b* = 29.902(2), *c* = 7.9399(9) Å,  $\beta$  = 117.656(13)°, *V* = 1783.2(3) Å<sup>3</sup>, *Z* = 4, *D<sub>c</sub>* = 1.354 g cm<sup>-3</sup>,  $\mu$  = 0.086 mm<sup>-1</sup> (MoK $\alpha$ ,  $\lambda$  = 0.71073), *F*(000) = 760, *T* = 293(2) K,  $2\theta_{\max}$  = 25.350 (99.8%)°, 11082 measured reflections, 3266 independent reflections (*R*<sub>int</sub> = 0.0367), GOF on *F*<sup>2</sup> = 1.028, *R*<sub>1</sub> = 0.0641, *wR*<sub>2</sub> = 0.1005 (all data),  $\Delta e$  0.138 and -0.178 eÅ<sup>-3</sup>.

### 4. Fabrication of fluorogen-loaded BSA NPs

The BSA NPs loaded with the dyes were prepared by a modified desolvation method. The nanoparticles (NPs) were prepared with a feeding ratio of 2.5 wt%, defined as the ratio of the weight of the fluorogen (BP-2PXZ, BP-PXZ, BP-2PTZ or BP-PTZ) to that of the BSA in the mixture. In brief, 10 mg of BSA were dissolved in 5 mL of Milli-Q water. Subsequently, 0.5 mL of THF containing a predetermined amount of the dyes were added dropwise into the aqueous solution of BSA under sonication at room temperature, using a microtip probe sonicator with an 18 W output, leading to the formation of the fluorogen-loaded BSA NPs. A small amount (10  $\mu$ L) of glutaraldehyde solution (25%) was then added to cross-link the NPs at room temperature. The

mixture was then stirred overnight at room temperature. The THF was removed by rotary evaporation under vacuum. The cross-linked fluorogen-loaded BSA-NP suspension was filtered through a 0.45  $\mu\text{m}$  microfilter and was then washed with Milli-Q water.

## **5. Cell culture**

HeLa cells were purchased from ATCC and cultured in RPMI-1640 with 1% penicillin-streptomycin and 10% FBS at 37 °C in a humidified incubator with 5% CO<sub>2</sub>. The culture medium was changed every other day and the cells were collected by treating with 0.25% trypsin-EDTA solution after they reached confluence.

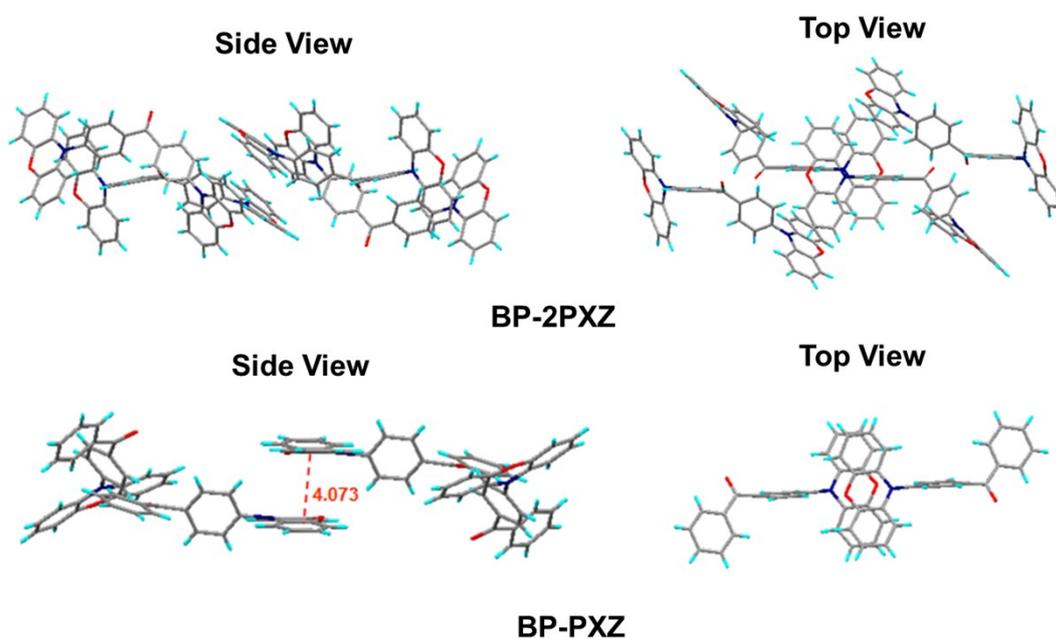
## **6. Cellular imaging**

HeLa cells were grown overnight on a cover slide in a 35-mm Petri dish. The living cells were stained with a solution of a BSA NPs (10  $\mu\text{M}$ ) and were further incubated for another 30 min. After careful washing, the cells were imaged by confocal laser scanning microscope (Leica SP5, Germany) using the LAS AF imaging software. The fluorescent signals from the BSA NPs were collected upon excitation with a long-pass barrier filter. Time-resolved fluorescence (or fluorescence lifetime) imaging was performed using the following system: the excitation source was the frequency doubled output (380 nm) of a cavity-dumped, mode-locked Titanium: sapphire laser (Coherent Mira 900f/APE PulseSwitch) operating at 5.4 MHz, which was delivered to a modified scanning confocal fluorescence microscope (Olympus IX71/FV300) by a single mode optical fibre (Thorlabs, SM300). Confocally detected emission was isolated with a highpass optical filter and images acquired with a time-correlated single photon counting-based FLIM system (Becker & Hickl, DCC-100 PMT, SPC-150 card), synchronized to the scanner and mode-locked laser. Images were analysed using the associated image software package (Becker & Hickl, SPCImage 5.5).

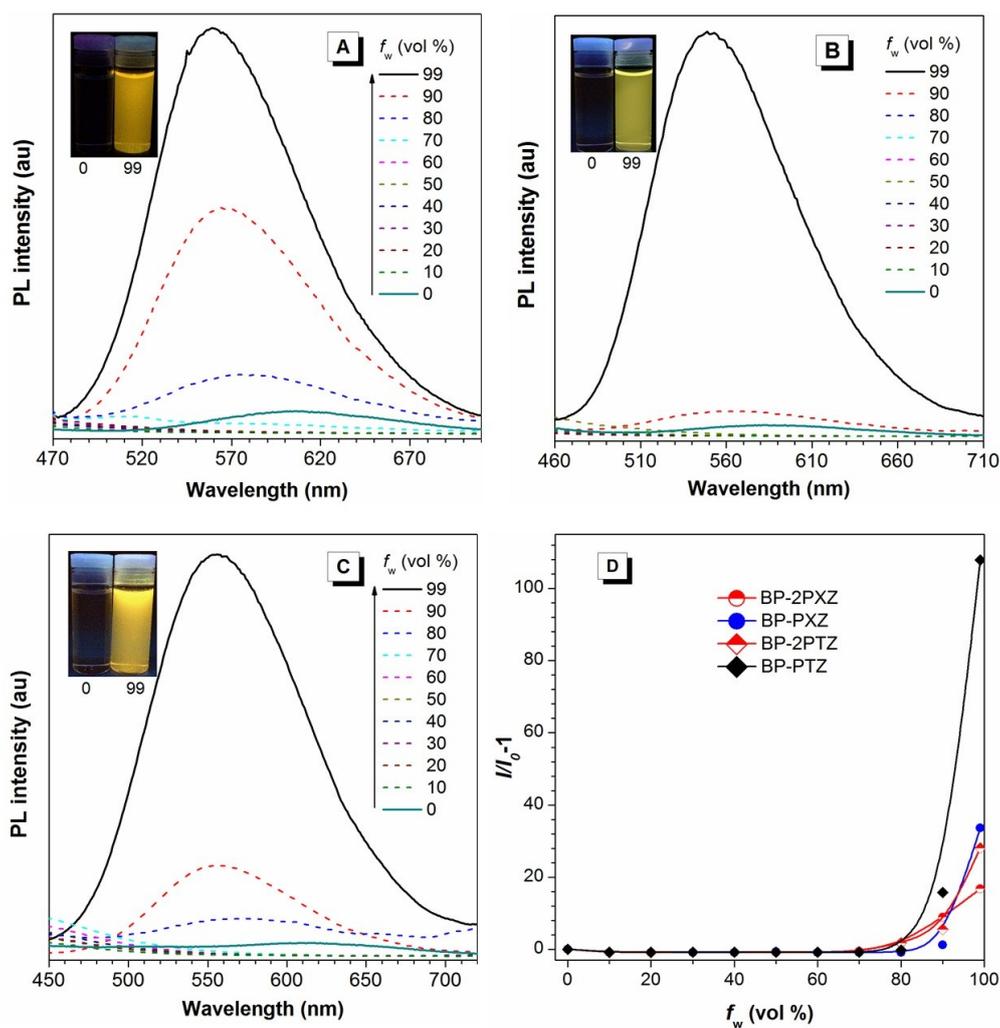
## 7. Cytotoxicity of fluorogen-loaded BSA NPs

Cytotoxicity of NPs was measured by the Cell Counting Kit-8 (CCK-8) assay ( $n = 5$ ). A suspension of HeLa (density of  $1 \times 10^4$  cells) was added into a 96-well plate. After 24 h of culture, different concentrations of BP-2PXZ BSA NPs (or BP-PXZ BSA NPs, BP-2PTZ BSA NPs and BP-PTZ BSA NPs) were added and further incubated for 24 h. The medium was then exchanged by 100  $\mu\text{L}$  of fresh medium containing 10  $\mu\text{L}$  of CCK-8 solution in each well and incubated at 37  $^\circ\text{C}$  for 2 h. The absorption was measured at 450 nm with a microplate reader (Perkin-Elmer Victor3<sup>TM</sup>).

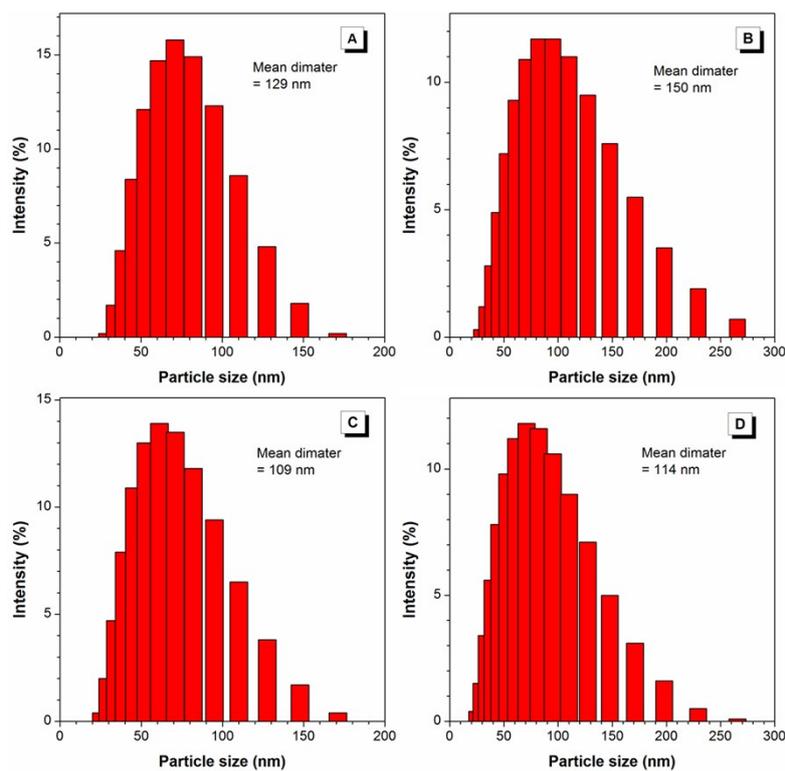
## 8. Additional spectra



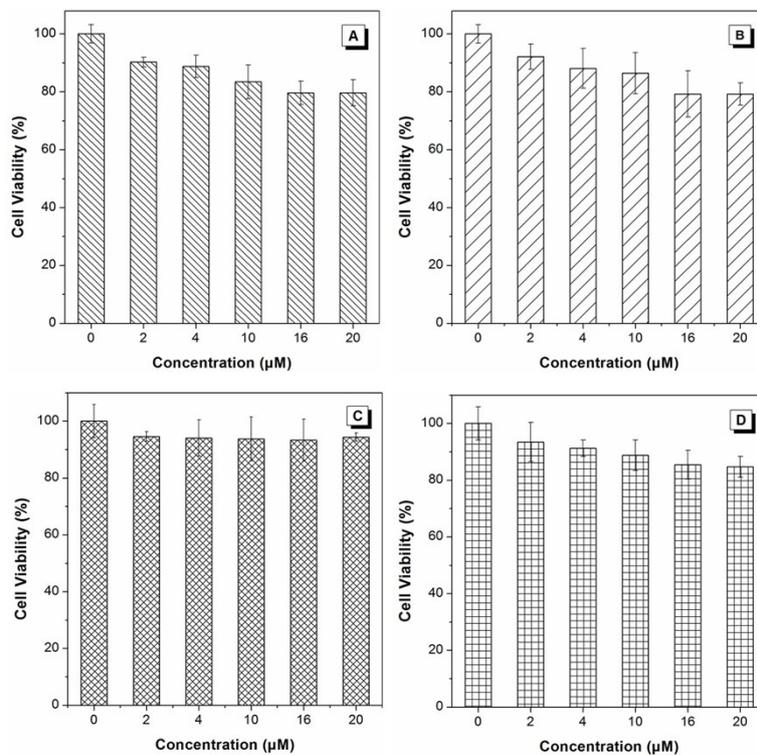
**Fig. S1** Packing pattern of BP-2PXZ and BP-PXZ in crystals.



**Fig. S2** PL spectra of (A) BP-2PXZ, (B) BP-PXZ, (C) BP-2PTZ in THF-water mixtures with different water fractions ( $f_w$ ) and (D) Plot of  $(I/I_0 - 1)$  values versus water fractions in THF-water mixtures of BP-2PXZ, BP-PXZ, BP-2PTZ and BP-PTZ.  $I_0$  is the PL intensity in pure THF solution.

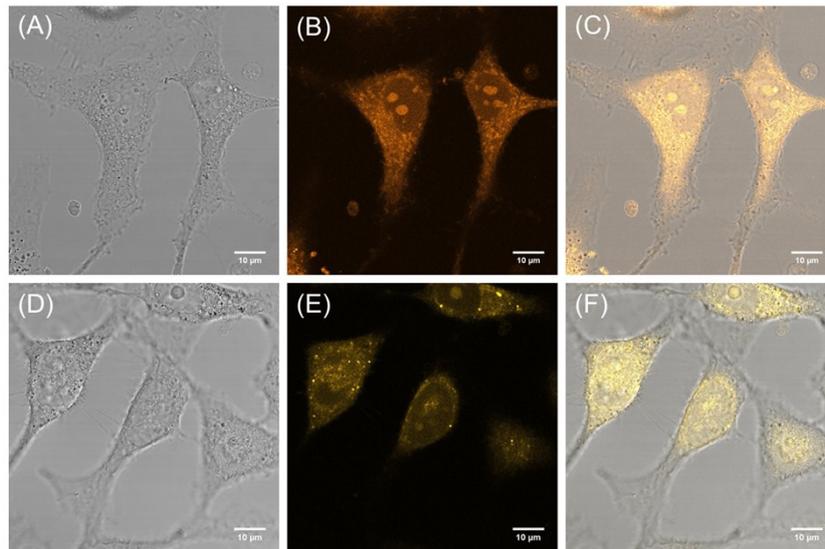


**Fig. S3** Particle size distribution of (A) BP-2PXZ BSA NPs, (B) BP-PXZ BSA NPs (C) BP-2PTZ BSA NPs and (D) BP-PTZ BSA NPs measured by dynamic light scattering.

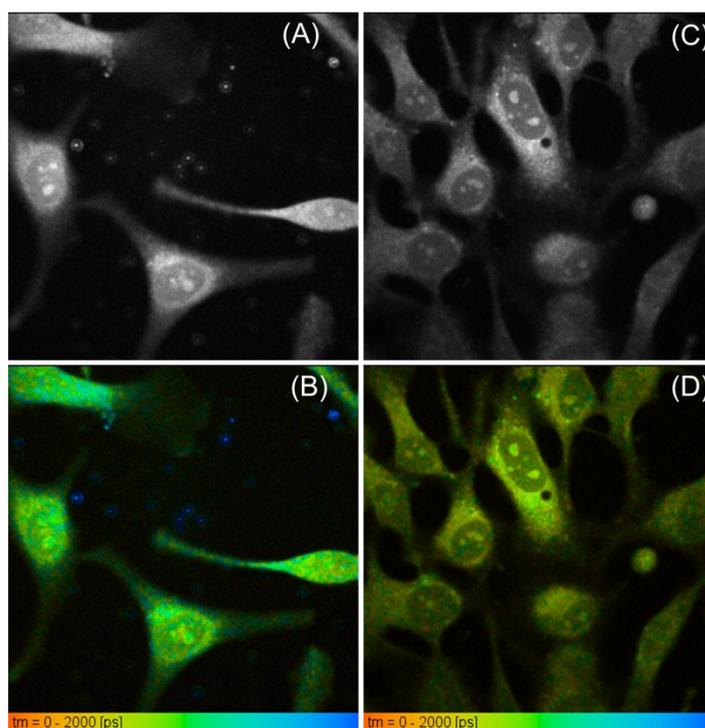


**Fig. S4** Cell viability of HeLa cells after incubation with different concentrations of (A) BP-2PXZ

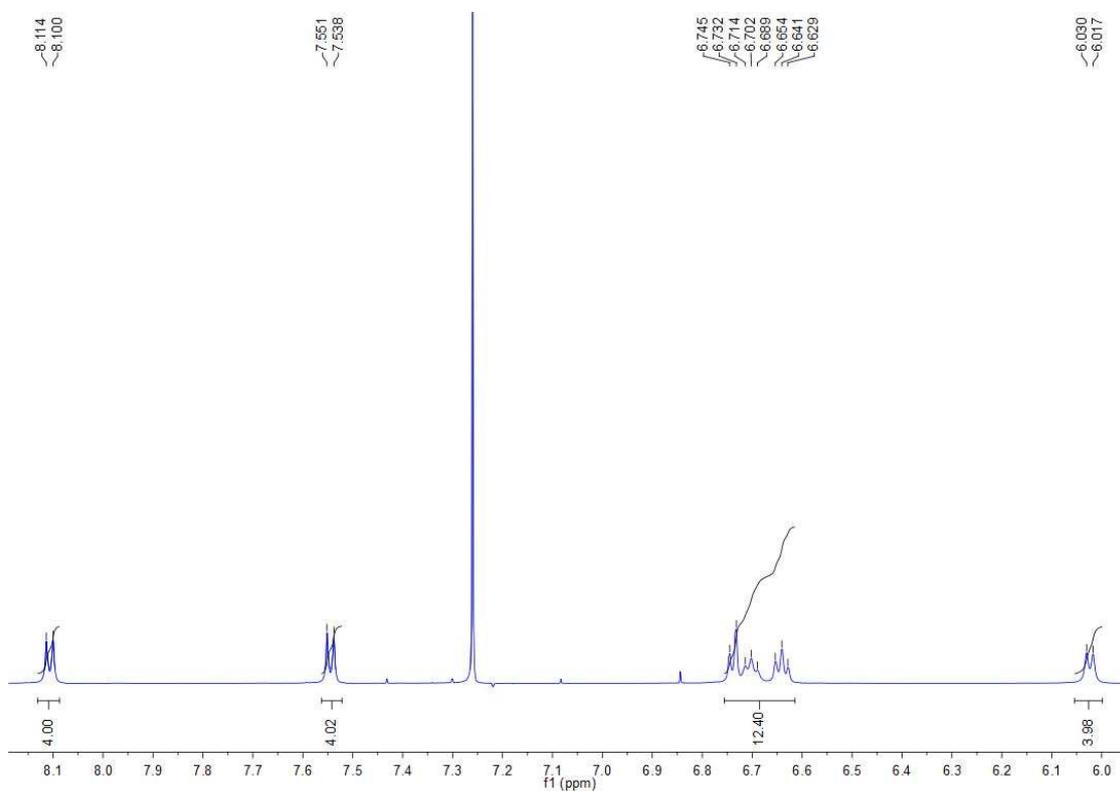
BSA NPs (0, 2, 4, 10, 16 and 20  $\mu\text{M}$  of BP-2PXZ), (B) BP-PXZ BSA NPs (0, 2, 4, 10, 16 and 20  $\mu\text{M}$  of BP-PXZ) for 24 h, (C) BP-2PTZ BSA NPs (0, 2, 4, 10, 16 and 20  $\mu\text{M}$  of BP-2PTZ), (D) BP-PTZ BSA NPs (0, 2, 4, 10, 16 and 20  $\mu\text{M}$  of BP-PTZ) for 24 h.



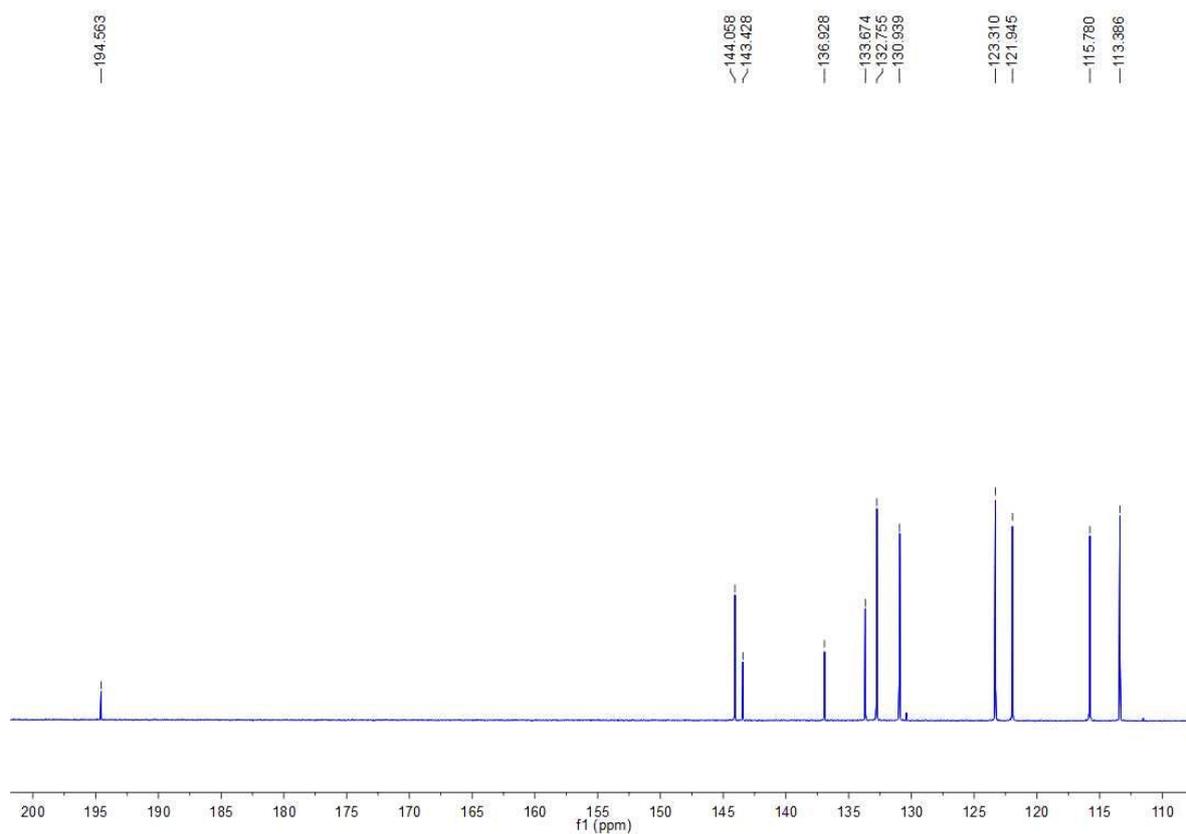
**Fig. S5** The confocal fluorescence images of HeLa cells after incubation with (A, B, C) BP-2PXZ BSA NPs (10  $\mu\text{M}$  of BP-2PXZ) and (D, E, F) BP-PTZ BSA NPs (10  $\mu\text{M}$  of BP-PTZ) at 37  $^{\circ}\text{C}$  for 0.5 h. (A, D) Bright-field images; (B, E) fluorescence images; (C) the merged image of (A) and (B); (F) the merged image of (D) and (E). Scale bar = 10  $\mu\text{M}$ .



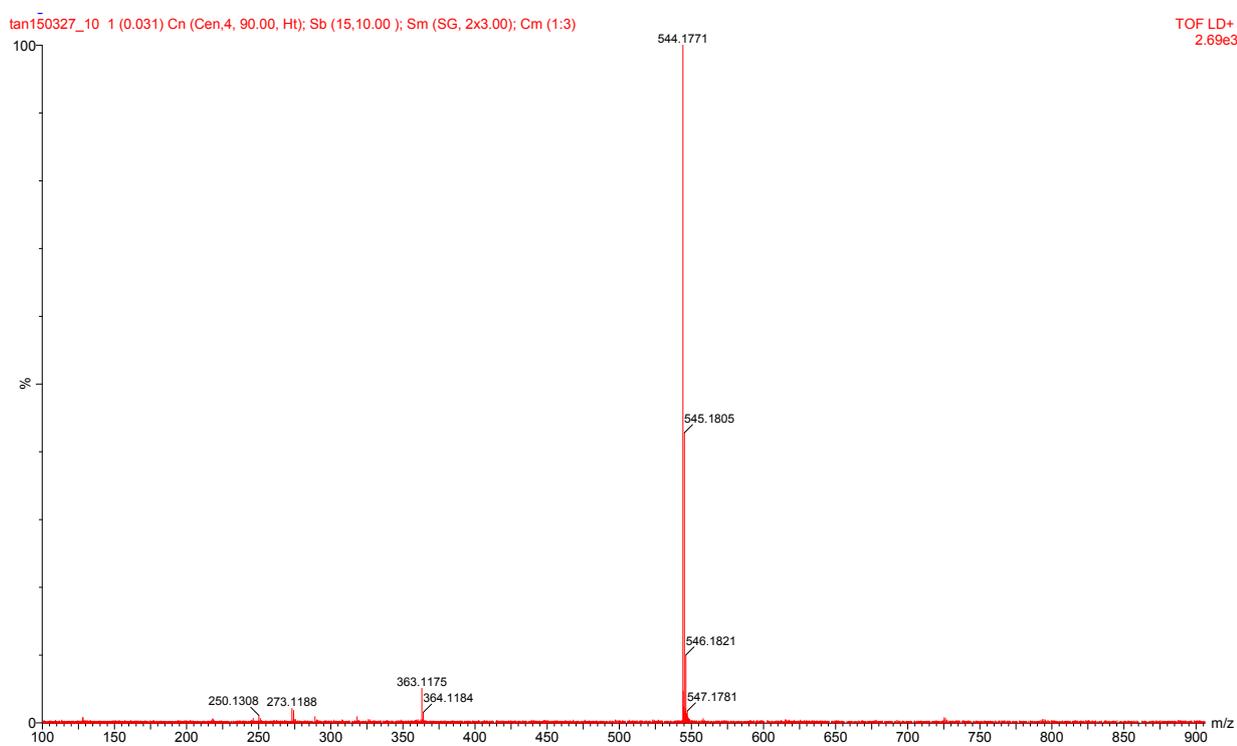
**Fig. S6** The fluorescence lifetime images of (A, B) HeLa cells stained with BP-2PXZ BSA NPs (10  $\mu$ M of BP-2PXZ) and (C, D) BP-PTZ BSA NPs (10  $\mu$ M of BP-PTZ) at 37  $^{\circ}$ C for 0.5 h.



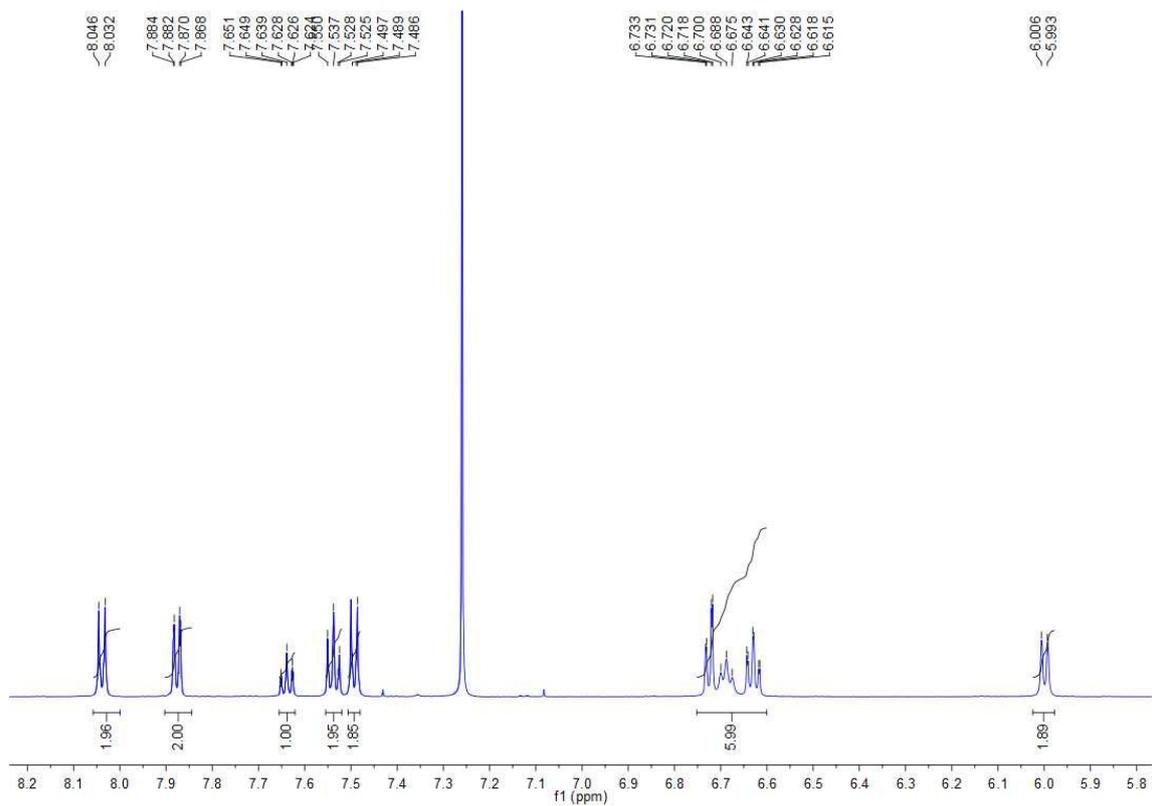
**Fig. S7**  $^1$ H NMR of BP-2PXZ in  $CDCl_3$ .



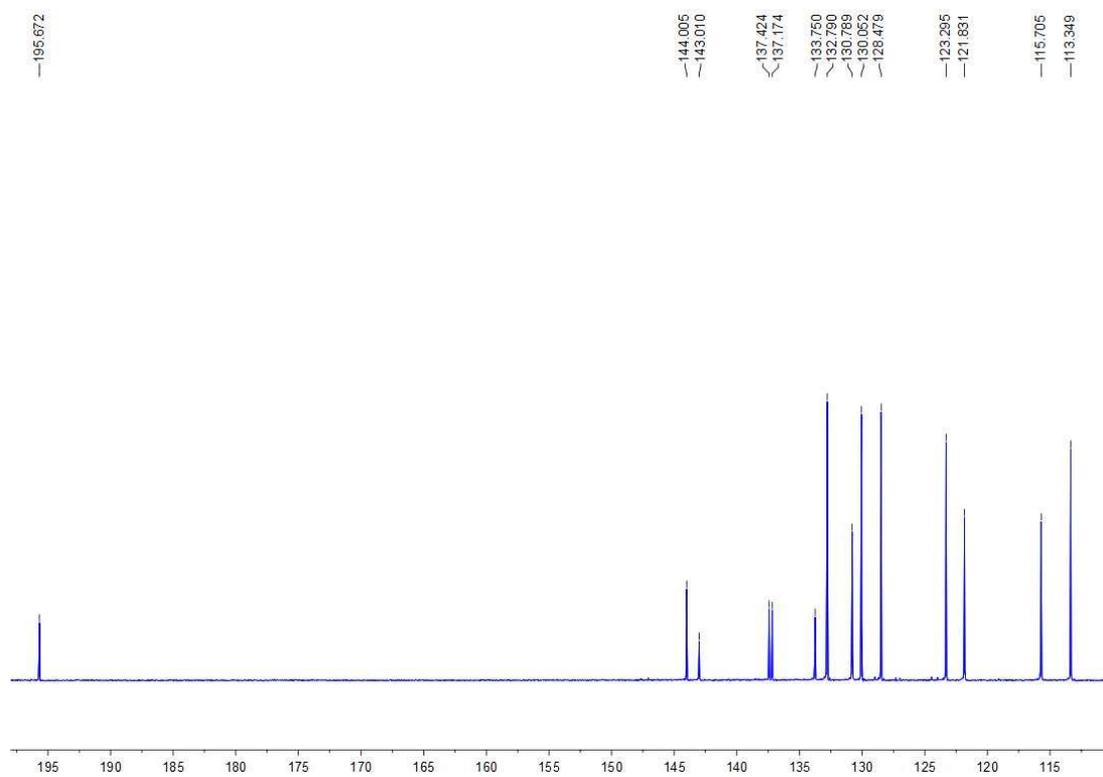
**Fig. S8**  $^{13}\text{C}$  NMR of BP-2PXZ in  $\text{CDCl}_3$ .



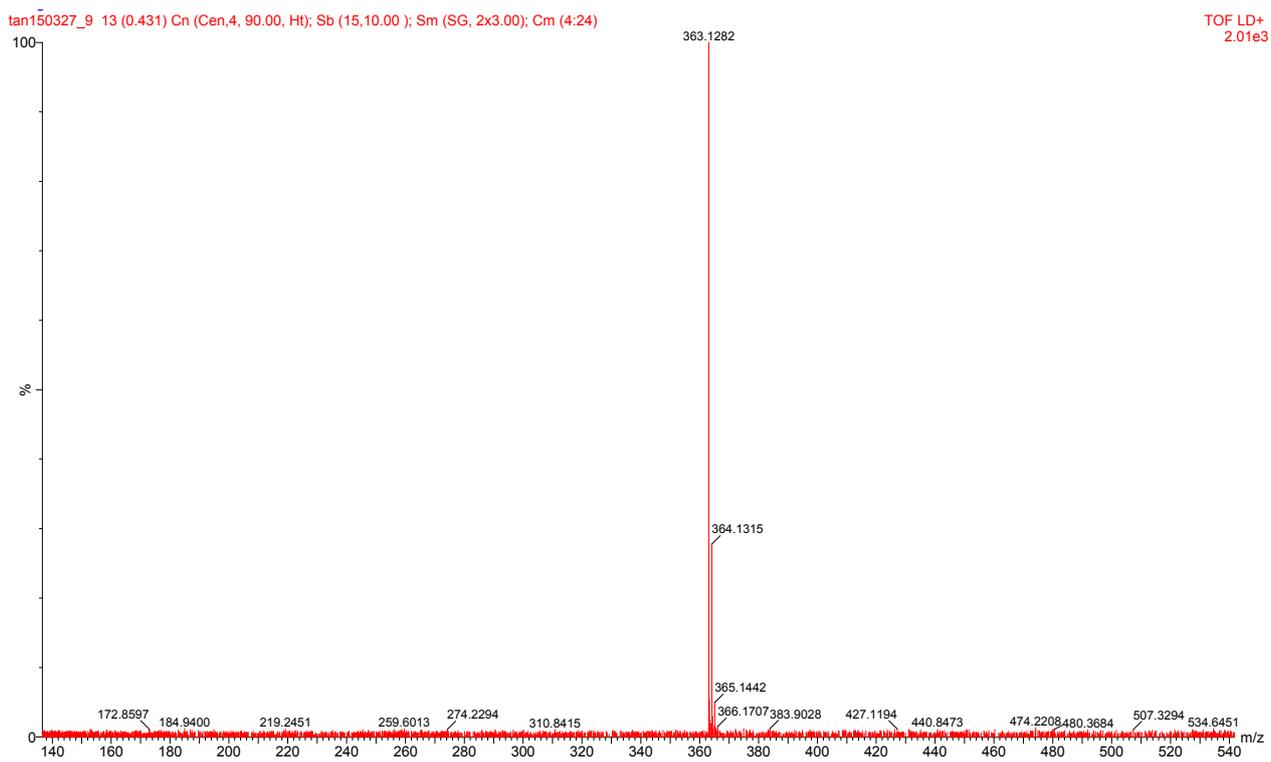
**Fig. S9** HRMS of BP-2PXZ.



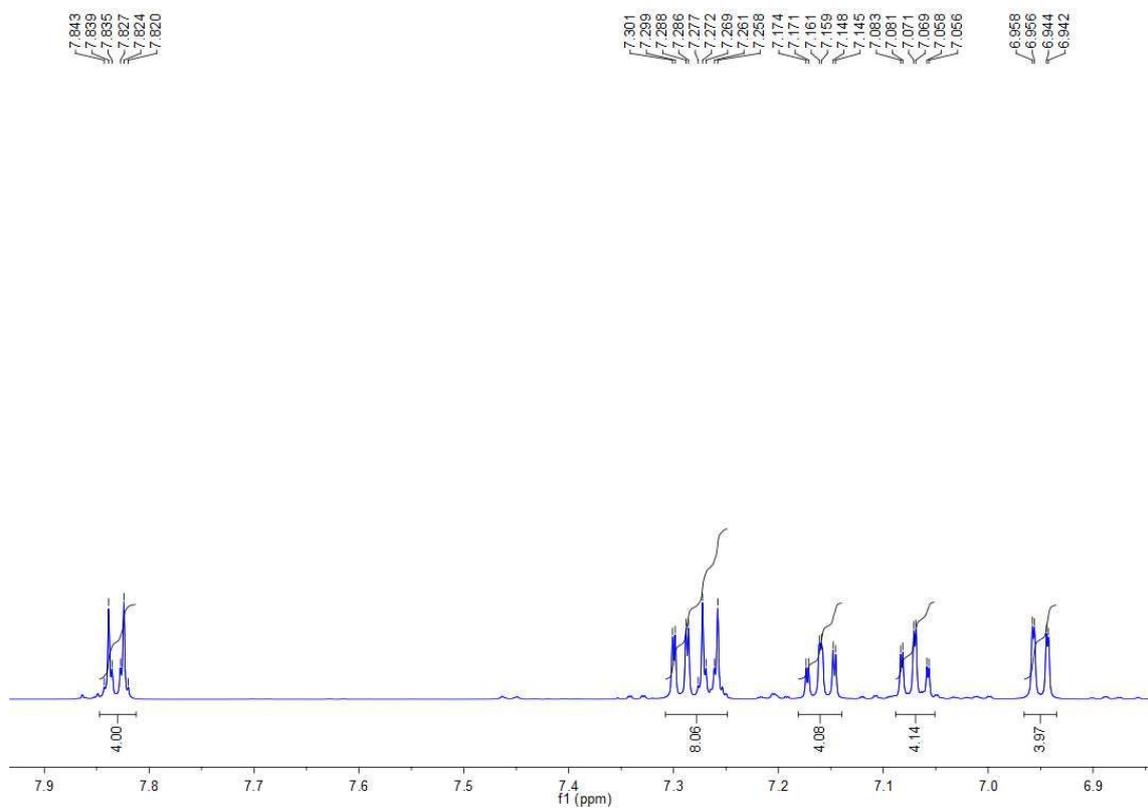
**Fig. S10**  $^1\text{H}$  NMR of BP-PXZ in  $\text{CDCl}_3$ .



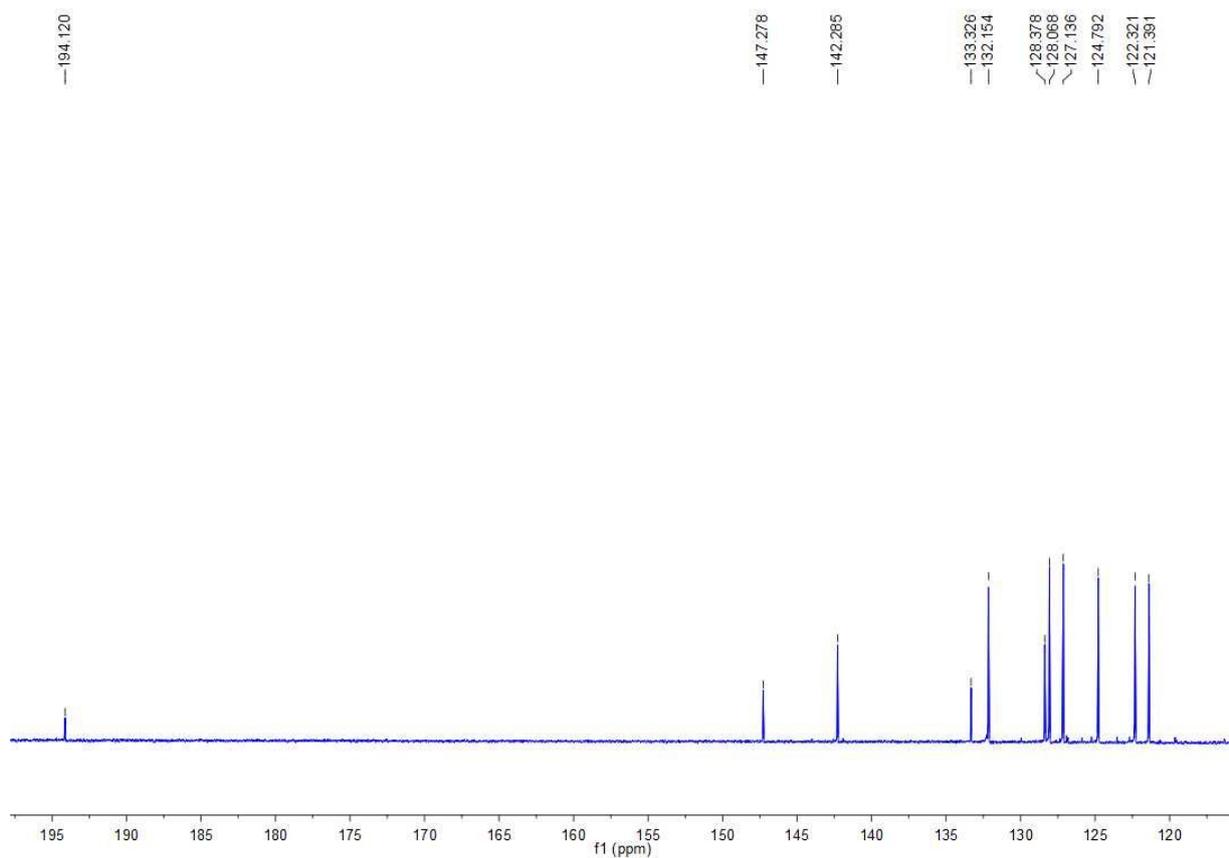
**Fig. S11**  $^{13}\text{C}$  NMR of BP-PXZ in  $\text{CDCl}_3$ .



**Fig. S12** HRMS of BP-PXZ.



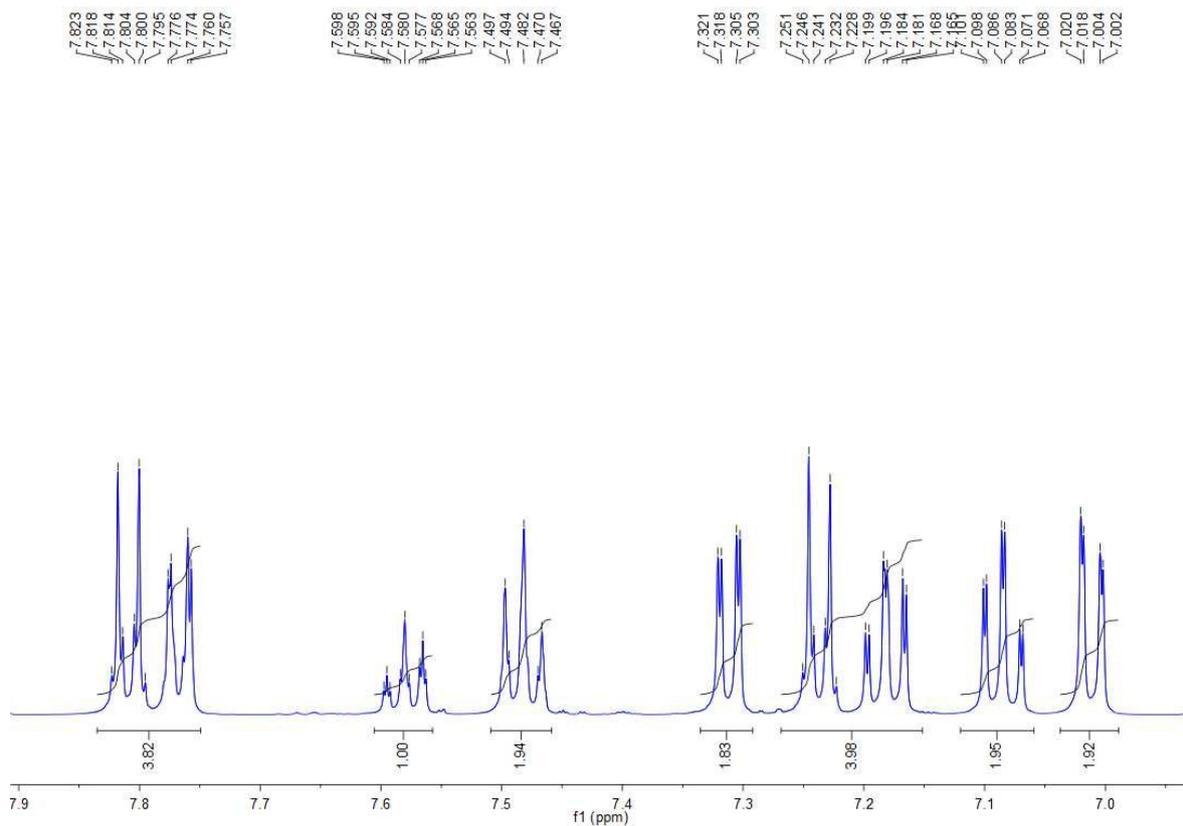
**Fig. S13**  $^1\text{H}$  NMR of BP-2PTZ in  $\text{CD}_2\text{Cl}_2$ .



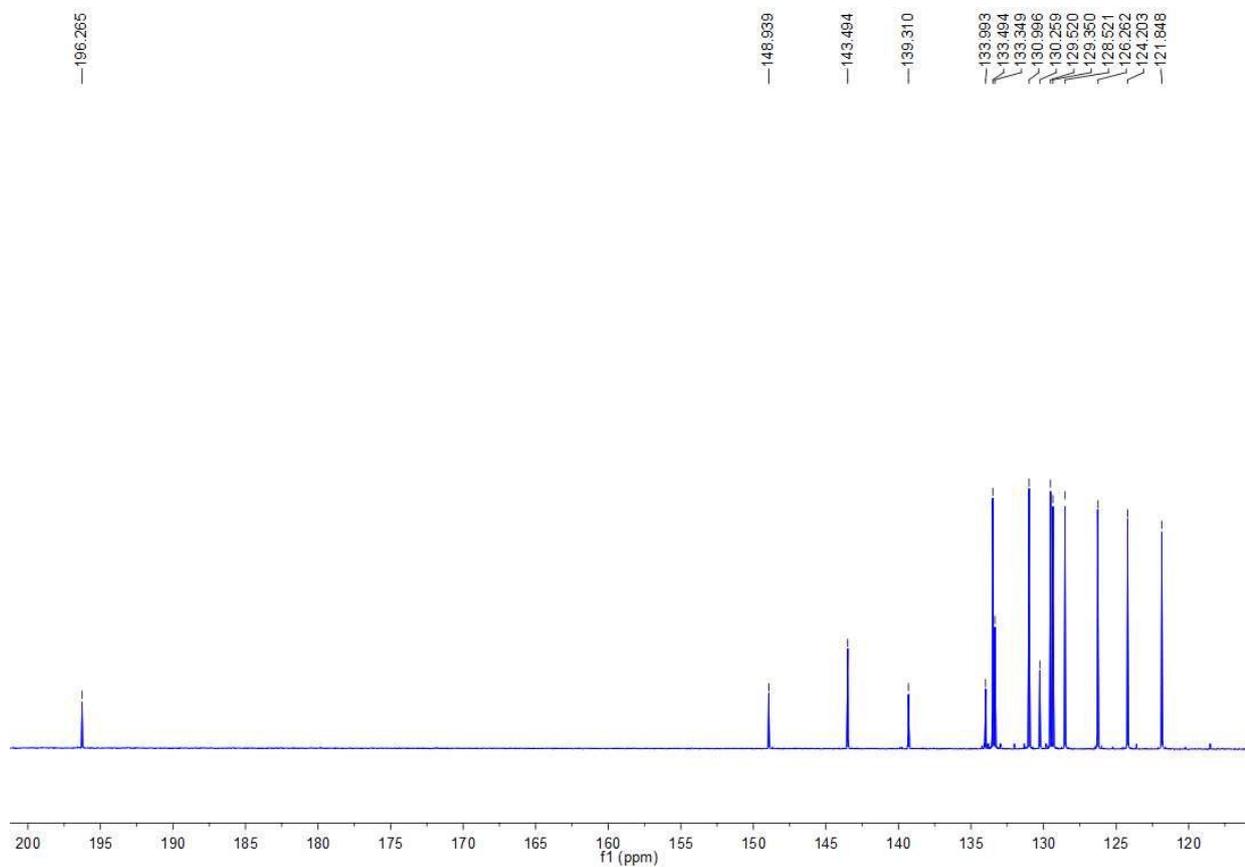
**Fig. S14**  $^{13}\text{C}$  NMR of BP-2PTZ in  $\text{CD}_2\text{Cl}_2$ .



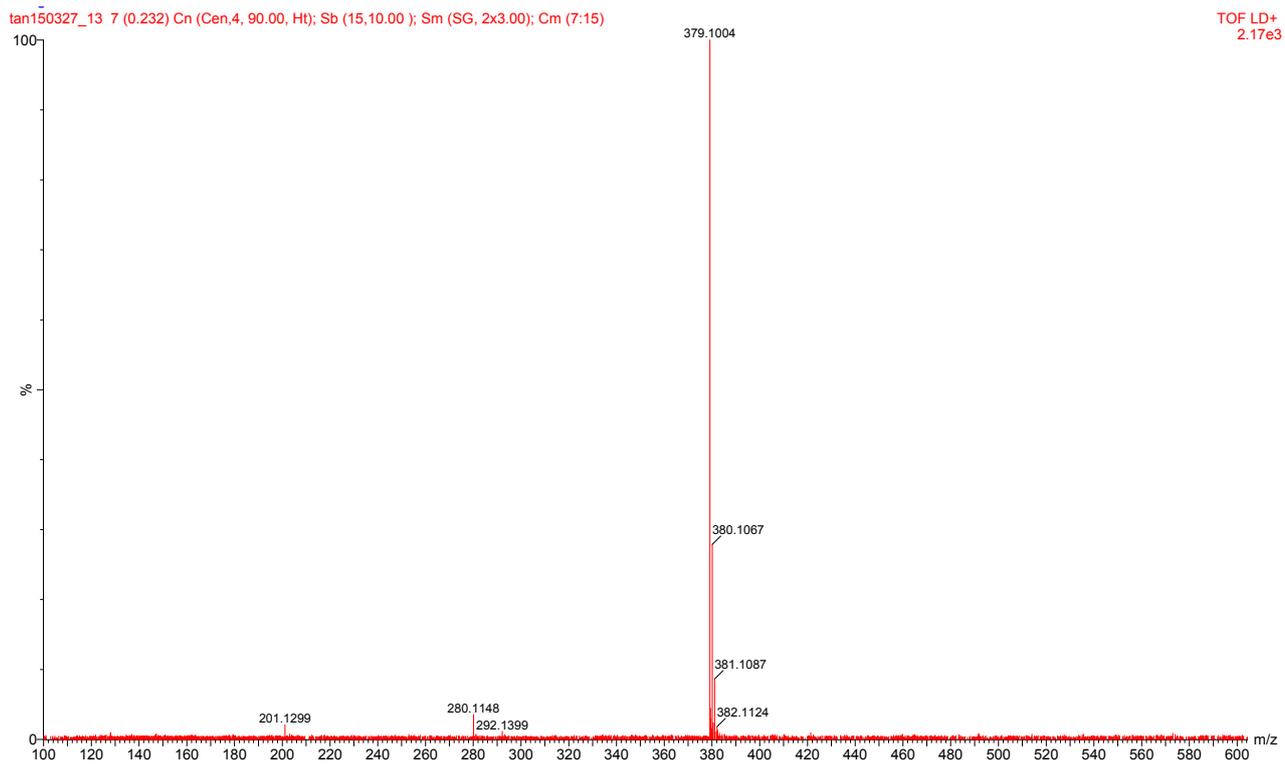
**Fig. S15** HRMS of BP-2PTZ.



**Fig. S16**  $^1\text{H}$  NMR of BP-PTZ in  $\text{CD}_2\text{Cl}_2$ .



**Fig. S17**  $^{13}\text{C}$  NMR of BP-PTZ in  $\text{CD}_2\text{Cl}_2$ .



**Fig. S18** HRMS of BP-PTZ.