Supporting Information

Stimuli-responsive nanotheranostics for real-time monitoring drug release by photoacoustic imaging

Zhen Yang,[#] Jibin Song,[#] Wei Tang,[#] Wenpei Fan, Yunlu Dai, Zheyu Shen, Lisen Lin, Siyuan Cheng, Yijing Liu, Gang Niu, Pengfei Rong, Wei Wang,* Xiaoyuan Chen*

Dr. Z. Yang, Dr. P. Rong, Prof. W. Wang
Cell Transplantation and Gene Therapy Institute, The Third Xiangya Hospital, Central South University, Changsha, Hunan, China.
Dr. Z. Yang, Dr. P. Rong, Prof. W. Wang
Engineering and Technology Research Center for Xenotransplantation of Human Province, Changsha, Hunan, China. E-mail: cjr.wangwei@vip.163.com
Pro. J. Song
MOE Key Laboratory for Analytical Science of Food Safety and Biology
College of Chemistry, Fuzhou University, Fuzhou 350108, China
Dr. Z. Yang, Dr. W. Tang, Dr. W. Fan, Dr. Y. Dai, Dr. Z. Shen, Dr. L. Lin, Dr. S. Cheng, Dr. Y. Liu, Dr. G. Niu, Prof. X. Chen
Laboratory of Molecular Imaging and Nanomedicine (LOMIN), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institutes of Health (NIH)
Bethesda, Maryland 20892, United States

[#] Z. Yang, J. Song and W. Tang contributed equally to this work

Corresponding Authors:

E-mail: cjr.wangwei@vip.163.com

E-mail: shawn.chen@nih.gov

Synthesis of product 2: A mixture of dibromo-perylene diimide (PDI) 1 (1 g, 1.7 mmol), Ethanolamine (0.23 g, 3.8 mmol), and propionic acid (5 g, 8.33 mmol) in Methyl-2-pyrrolidinone (NMP) (100 mL) was stirred at 100 °C under argon atmosphere for 8 h. After cooling down, the solution was dispensed into 2 L water, and the red precipitate was separated by suction filtration, with washing with deionized water. Next, the residue was dried under vacuum and obtained as a dark red powder. The two hydroxyl groups of the amide groups of PDI were protected using TBDMSCl to guarantee the solubility of perylene diimide in an organic solvent. The pure product 2 was obtained by column chromatography. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 9.47-9.31 (s, 2H), 8.85-8.81 (s, 2H), 8.68-8.55 (s, 2H), 4.47-4.33 (s, 4H), 4.12-3.98 (s, 4H), 0.95-0.91 (s, 18H), -0.12-0.21 (s, 12H). ESI-MS m/z: 867.73 $[M + H]^+$. The product structure was confirmed by ^{1}H NMR and ^{13}C NMR.

Synthesis of product 3: A solution of $mPEG_{2000}$-OH (1.00 g, 0.5 mmol) and NaH (0.05 g, 1 mmol) in tetrahydrofuran (THF) (100 mL) were stirred at room temperature. After stirring for 10 min, equivalent product 2 was added to the above solution. The reaction was stirred overnight at room temperature. The crude product was obtained by the rotary evaporator of the reaction solution. After precipitation in ether, excess product 2 was removed, then the excess $mPEG_{2000}$-OH was removed through dialysis (12 kDa) against water for 3 days and freeze drying for 2 days. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 9.31-9.22 (s, 2H), 8.51-8.23 (m, 4H), 4.58-4.45 (d, 4H), 4.35-4.13 (s, 4H), 4.02-3.78 (s, 170H), 3.59-3.53 (s, 3H), 3.53-3.26 (t, 2H), 0.95-0.91 (s, 18H), -0.12-0.21 (s, 12H). The pure structure was confirmed by ^{1}H NMR.

Synthesis of product 5 (HPDI): Solid product 3 was dissolved in anhydrous THF and dropped TFAB into the solution with trace acetic acid as catalyst, with vigorous stirring overnight. Subsequently, the solvent was removed by rotary evaporation. After precipitation by ether, precipitated product 4 was separated by suction filtration. To obtain product 5, product 4 was dissolved in NMP solution and reacted with excess 1-Methylpiperazine under 85°C for 2 h. Then the pure product 5 was obtained by precipitation in ether. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 9.96-9.22 (m, 2H), 8.51-8.12 (m, 4H), 4.88-4.75 (d, 2H), 4.60-4.55 (s, 2H), 4.25-4.03 (m, 8H), 4.02-3.78 (s, 170H), 3.53-3.26 (t, 2H), 2.85-2.71 (m, 3H), 2.52-2.21 (m, 4H). The synthesis of control molecule APDI was in the same procedure with the HPDI.

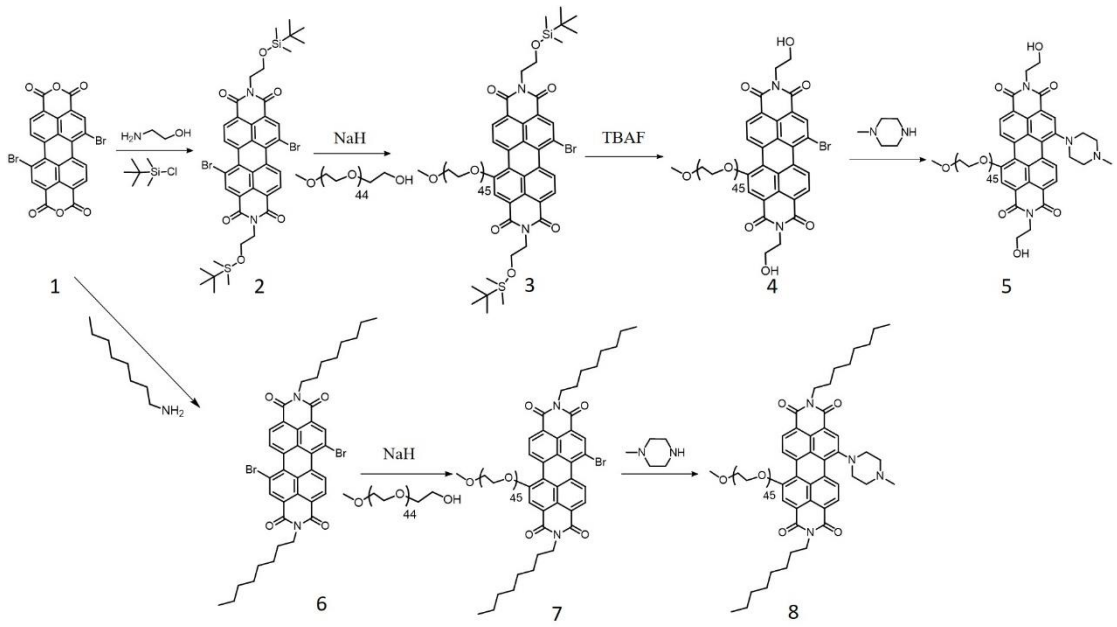

Figure S1. Synthesis routes of HPDI and APDI.

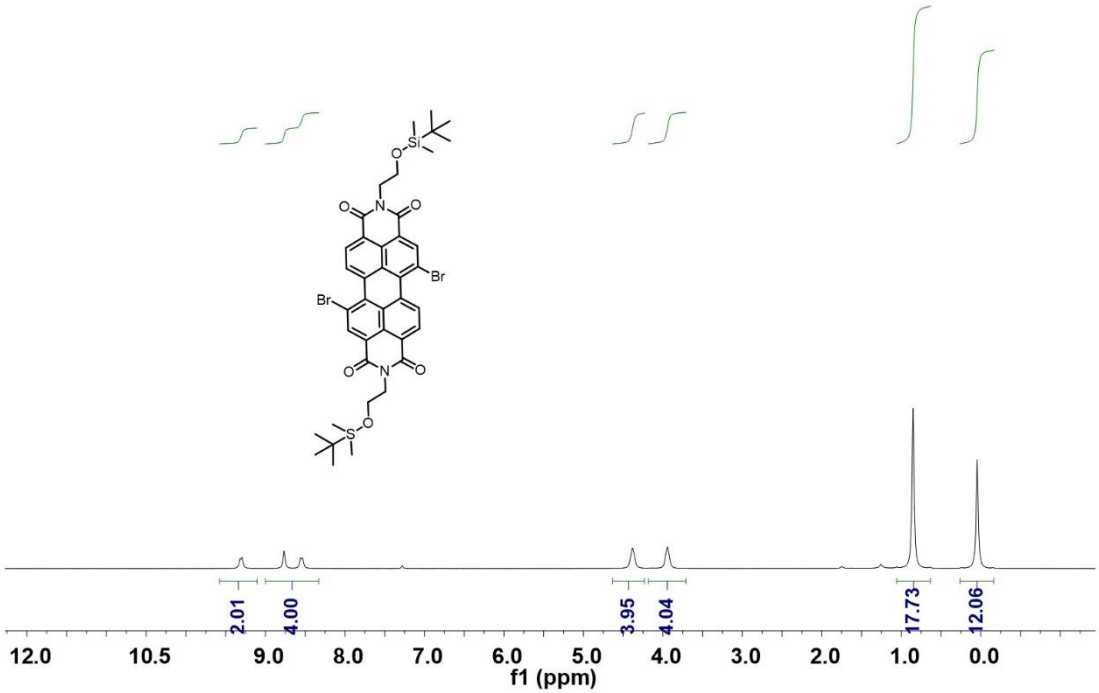

Figure S2. ^{1}H NMR spectrum of the compound 2. $CDCl_3$ was used as the solvent.

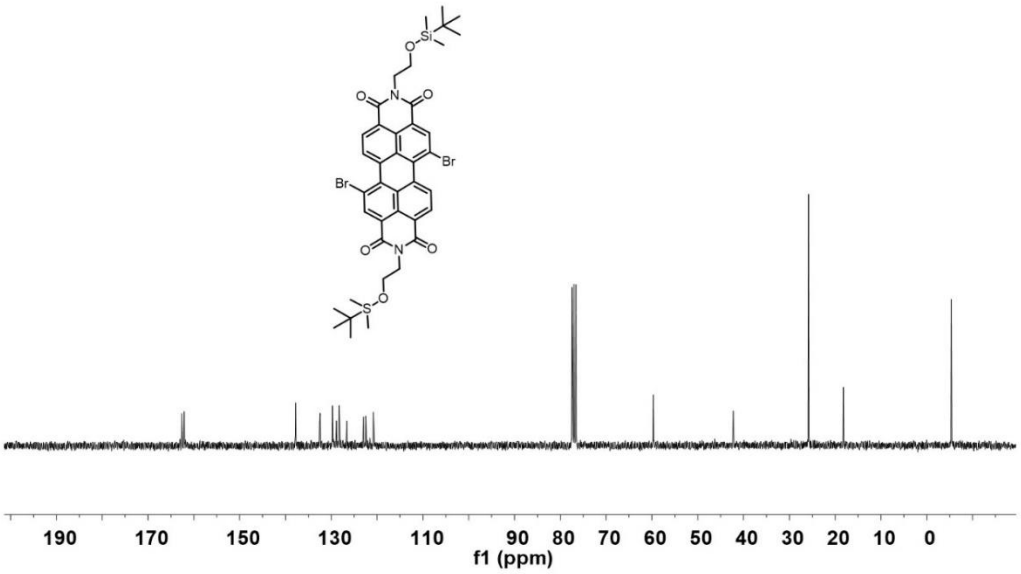

Figure S3. ^{13}C NMR spectrum of the compound 2. $CDCl_3$ was used as the solvent.

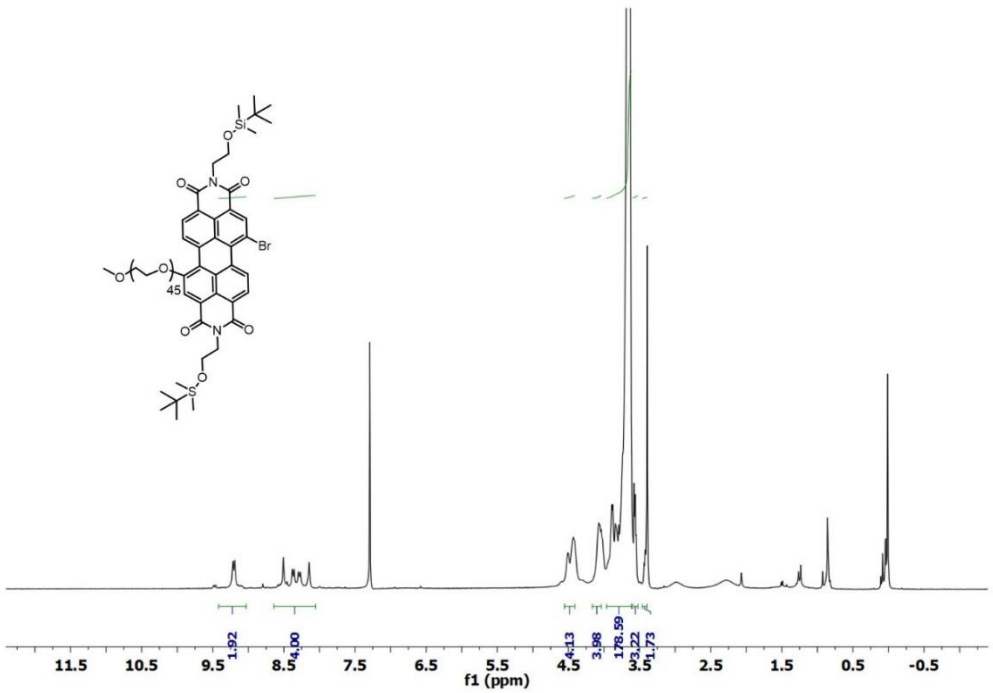

Figure S4. ^{1}H NMR spectrum of the compound 3. $CDCl_3$ was used as the solvent.

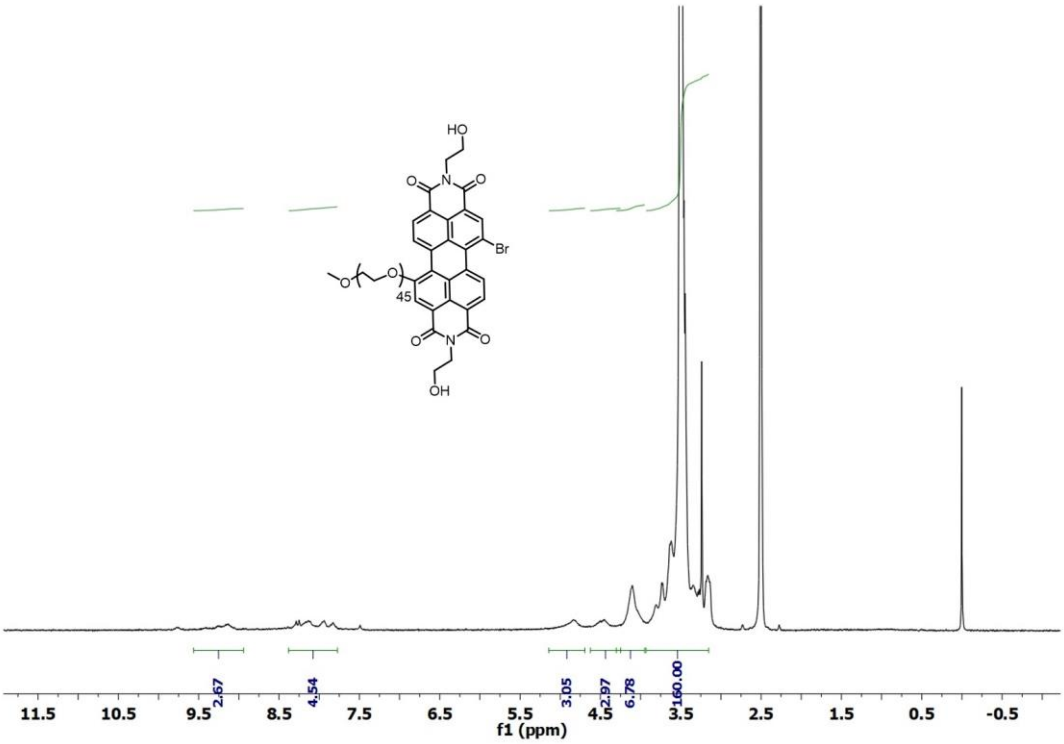

Figure S5. ^{1}H NMR spectrum of the compound 4. DMSO-6d was used as the solvent.

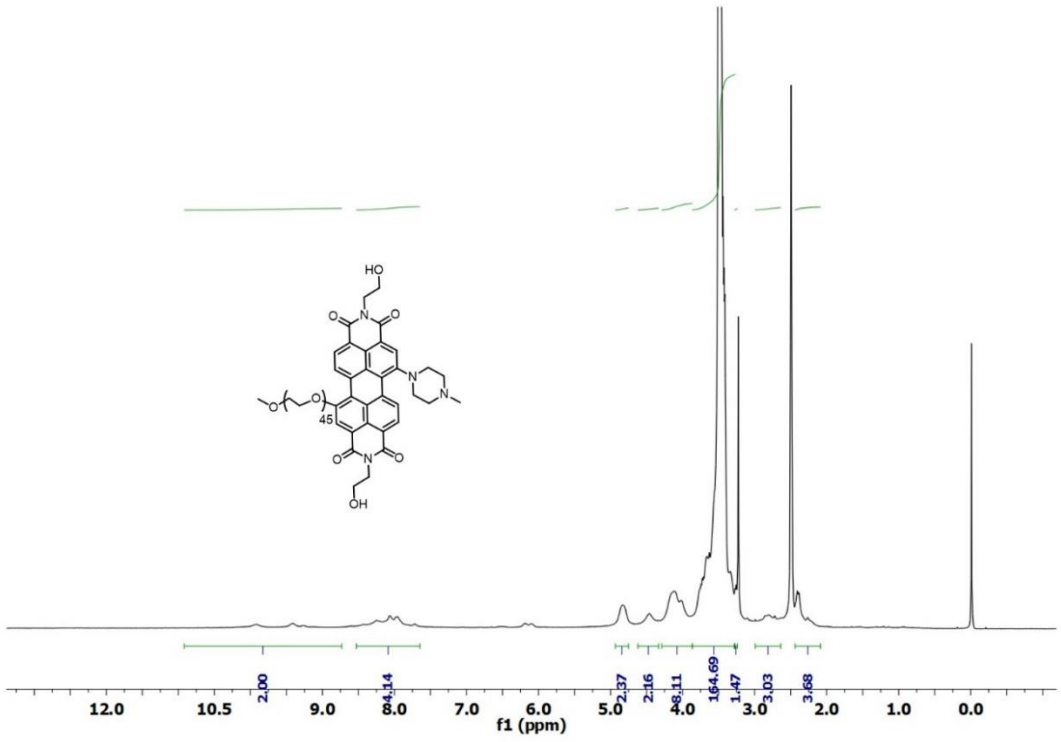

Figure S6. ^{1}H NMR spectrum of the compound 5. DMSO-6d was used as the solvent.

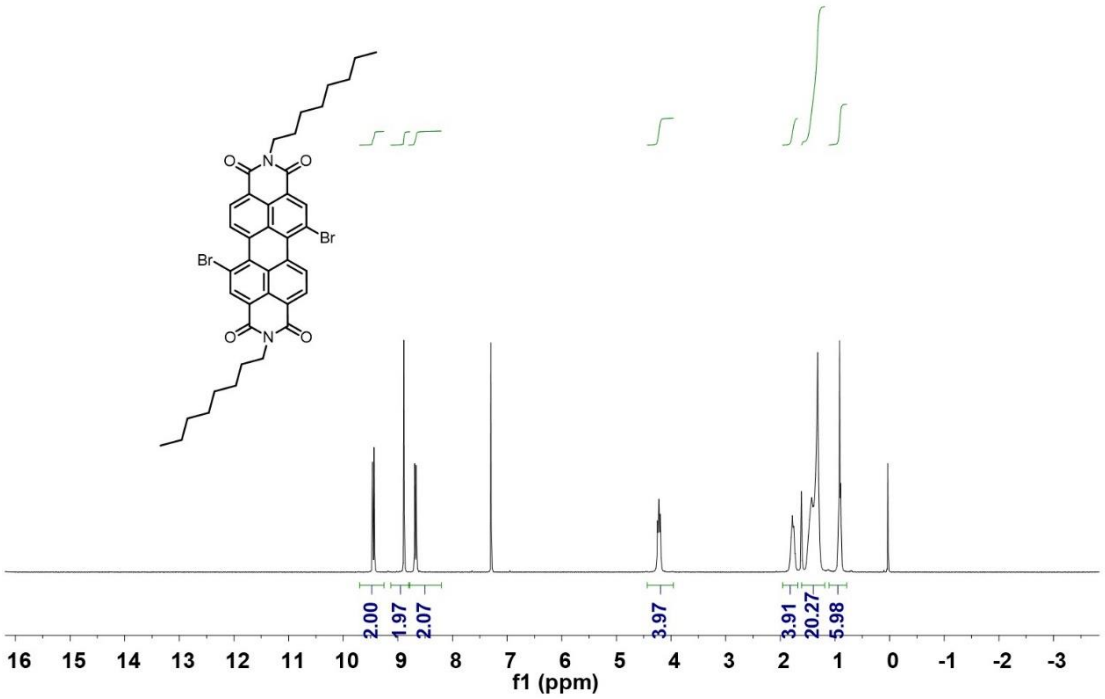

Figure S7. ^{1}H NMR spectrum of the compound 6. $CDCl_3$ was used as the solvent.

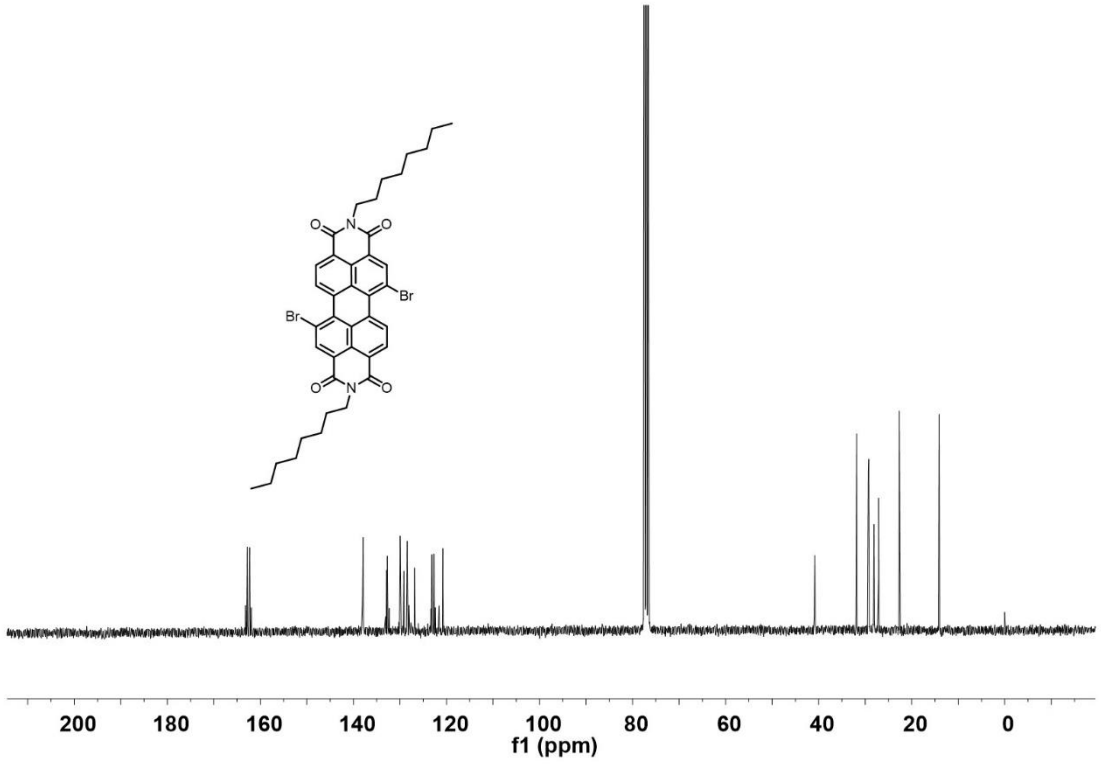

Figure S8. ^{13}C NMR spectrum of the compound 6. $CDCl_3$ was used as the solvent.

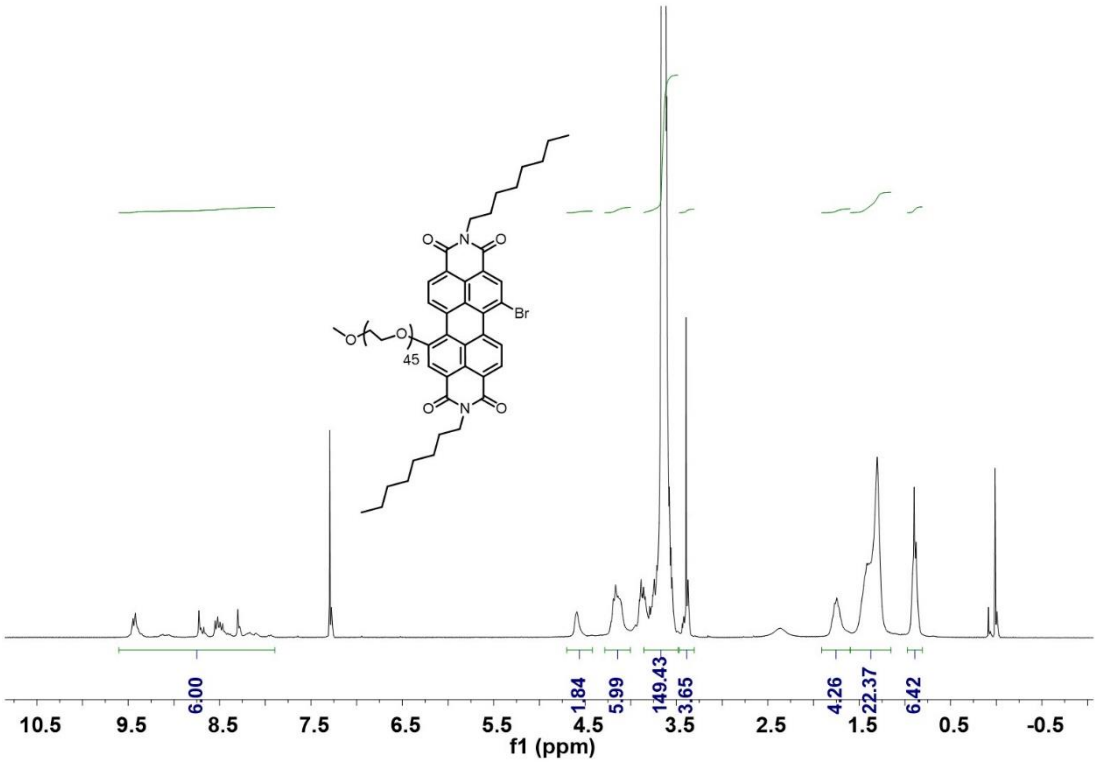

Figure S9. ^{1}H NMR spectrum of the compound 7. $CDCl_3$ was used as the solvent.

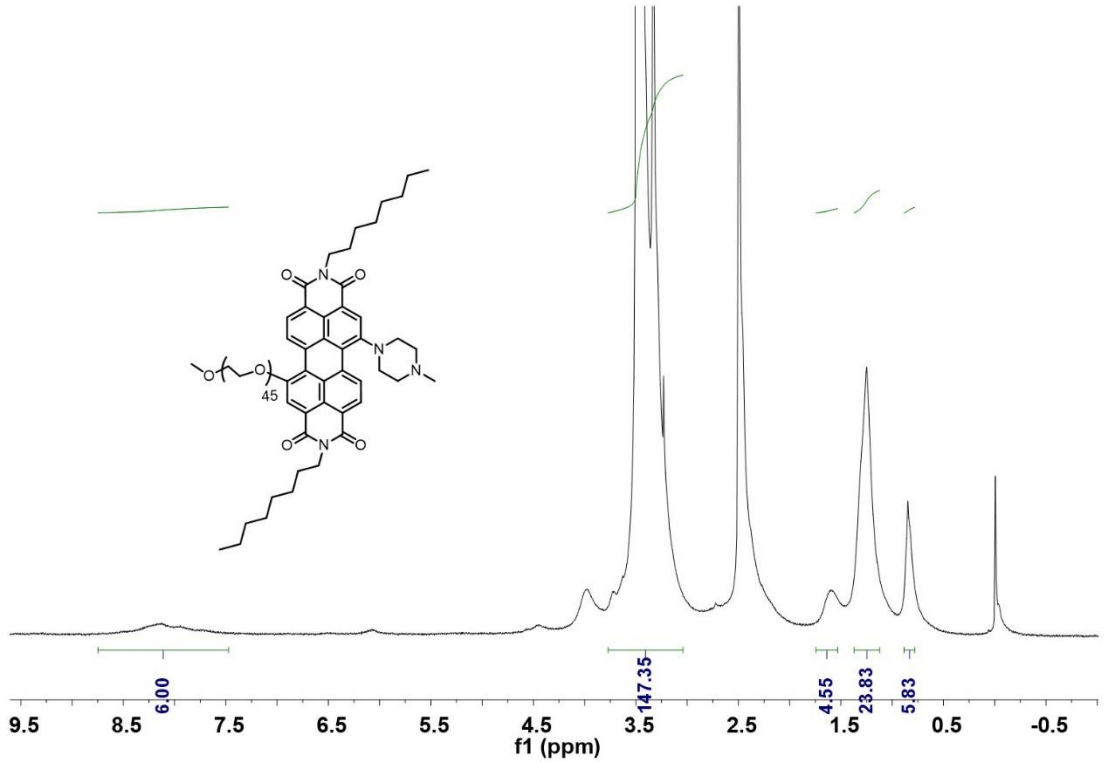

Figure S10. ^{1}H NMR spectrum of the compound 8. DMSO-6d was used as the solvent.

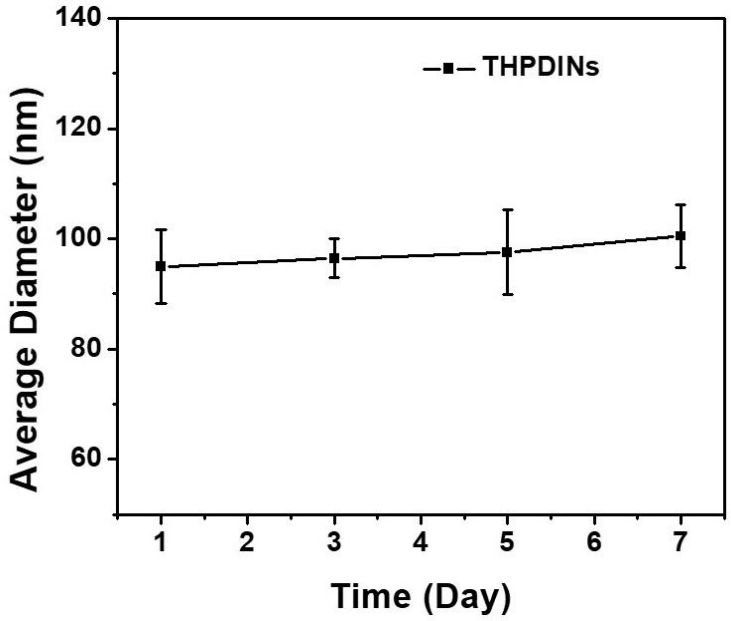

Figure S11. Average hydrodynamic diameters of the THPDINs stored in PBS (pH 7.4) for different time periods.

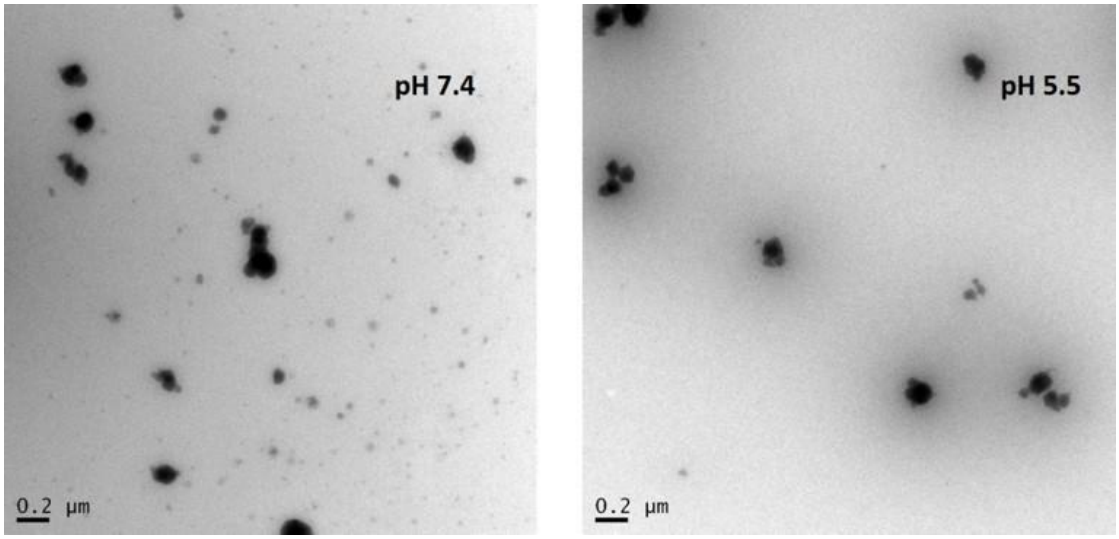

Figure S12. TEM results of the DOX loaded APDINs, which showed a relatively stable morphology in different pH solutions.

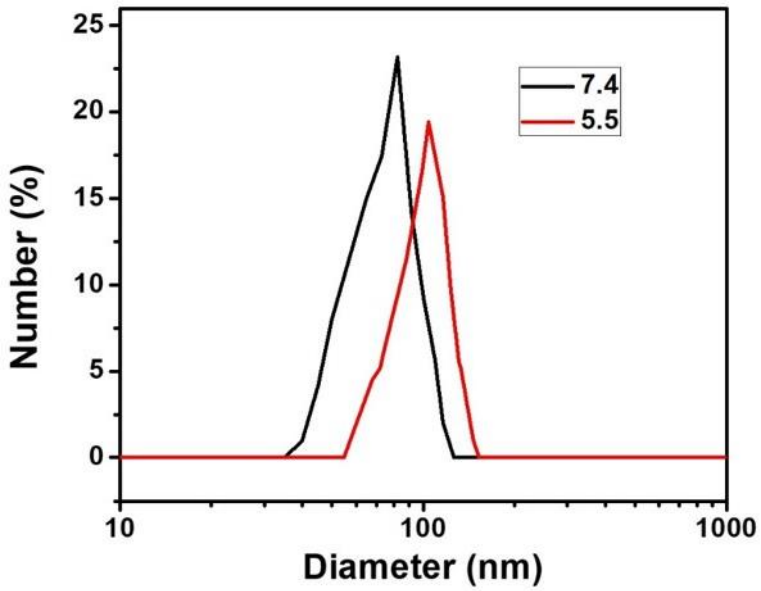

Figure S13. DLS results of the DOX loaded APDINs, which showed a relatively stable particle size in different pH solutions.

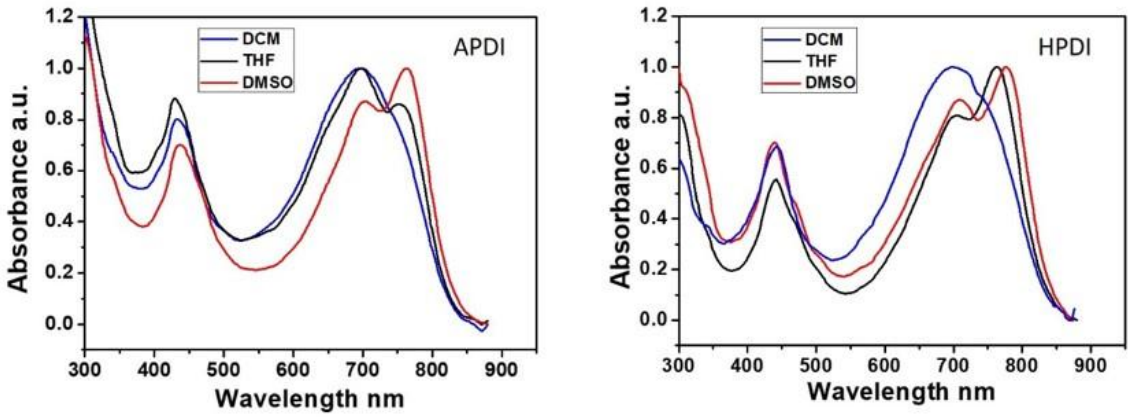

Figure S14. The UV-vis spectra of HPDI and APDI in different organic solvents.

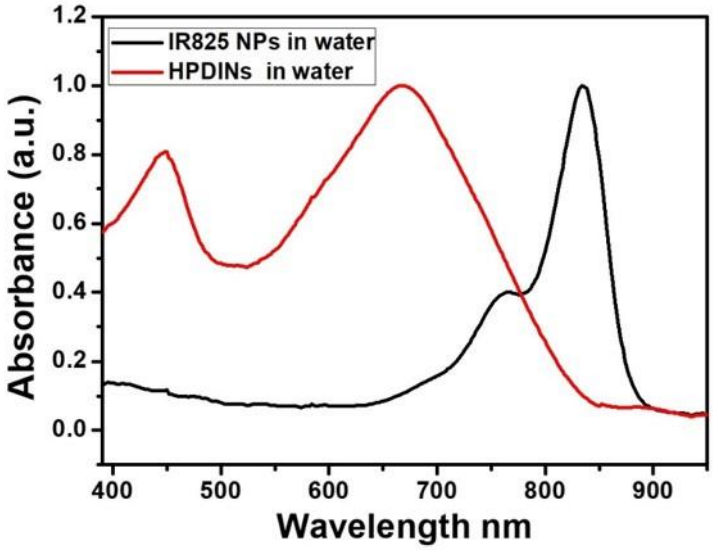

Figure S15. UV-vis spectra of HPDINs and IR825 NPs in neutral water (IR825 NPs was prepared by encapsulating of transparent PEG-PCL).

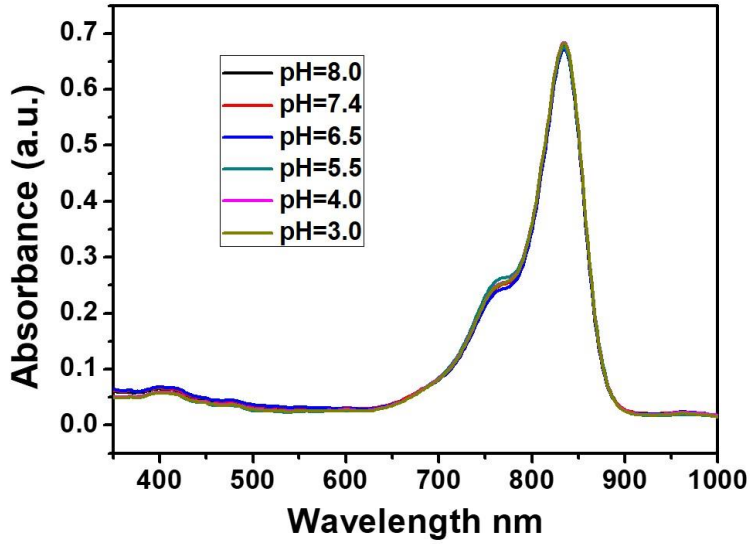

Figure S16. Independent absorption of IR825 under pH from 3.0 to 8.0.

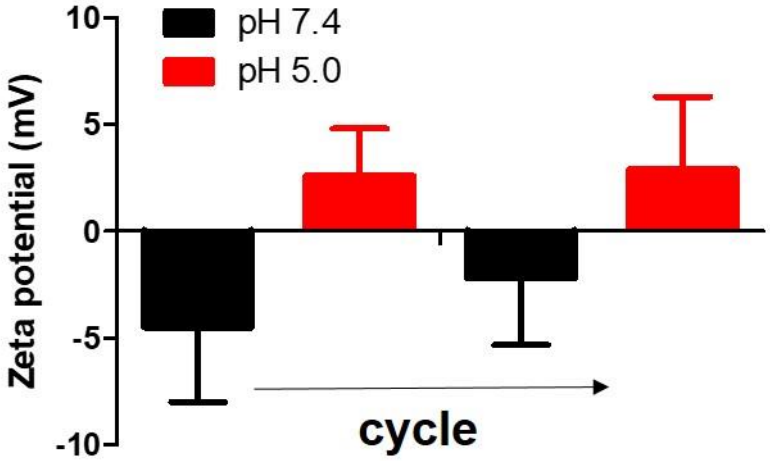

Figure S17. The zeta potential of the HPDI upon switching of pH from 7.4 to 5.0 repeatedly. Values are the mean ± s.d. (n = 3).

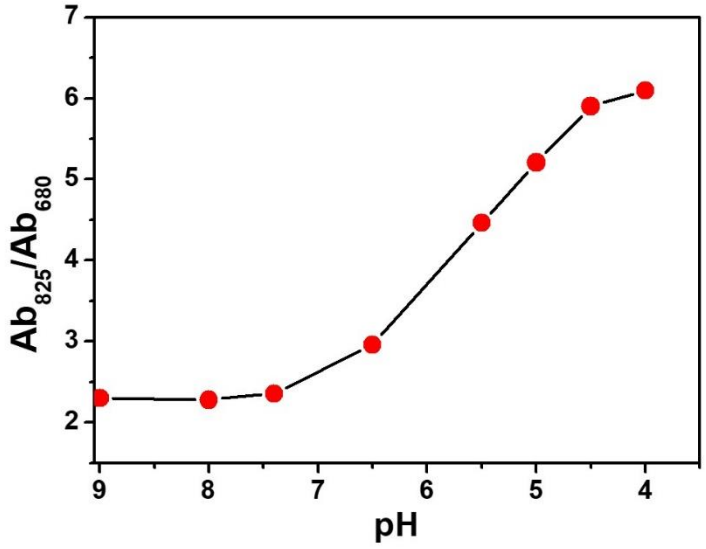

Figure S18. Absorption ratiometric signals of the THPDINs (Ab_{825}/Ab_{680}) as a function of various pH.

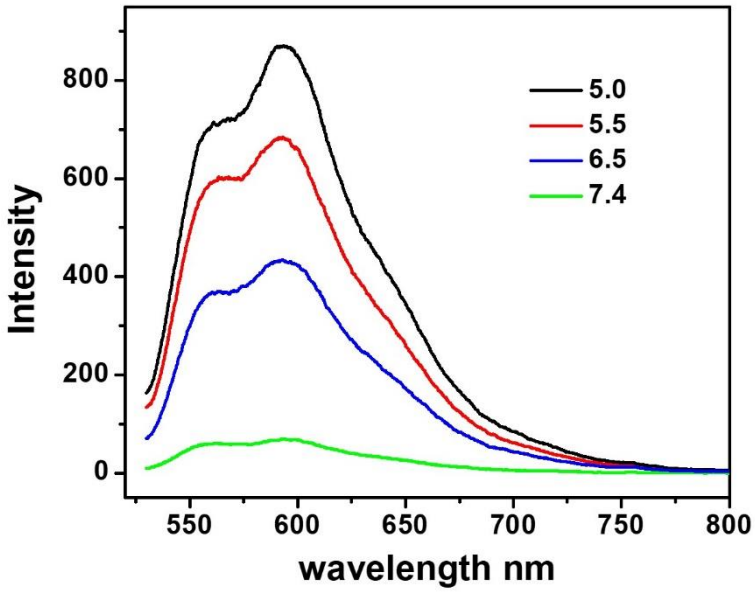

Figure S19. Fluorescence intensities of DOX in THPDINs as a function of various pH.

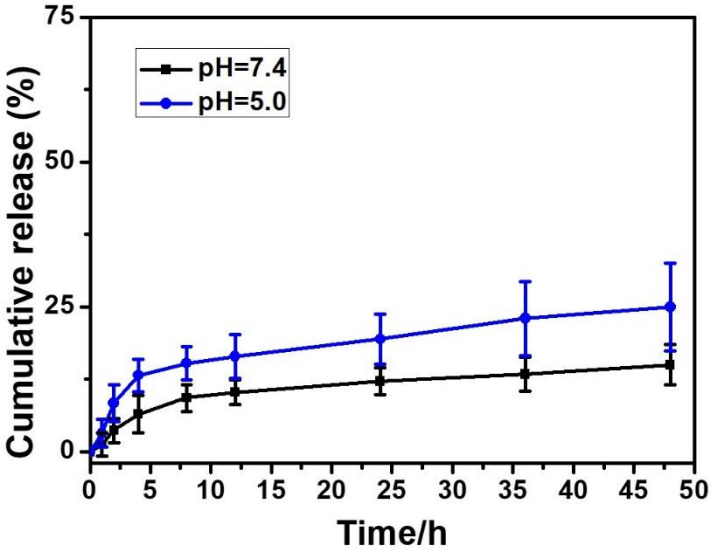

Figure S20. Release profiles of DOX-loaded AHPDINs under pH 5.0 and 7.4 PBS solution.

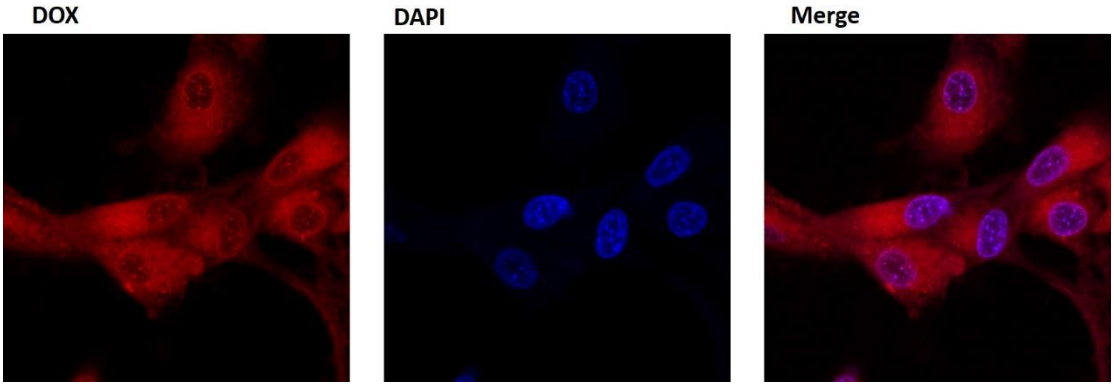

Figure S21. Zoomed in confocal images of U87MG cells after culture with THPDINPs at 6 h.

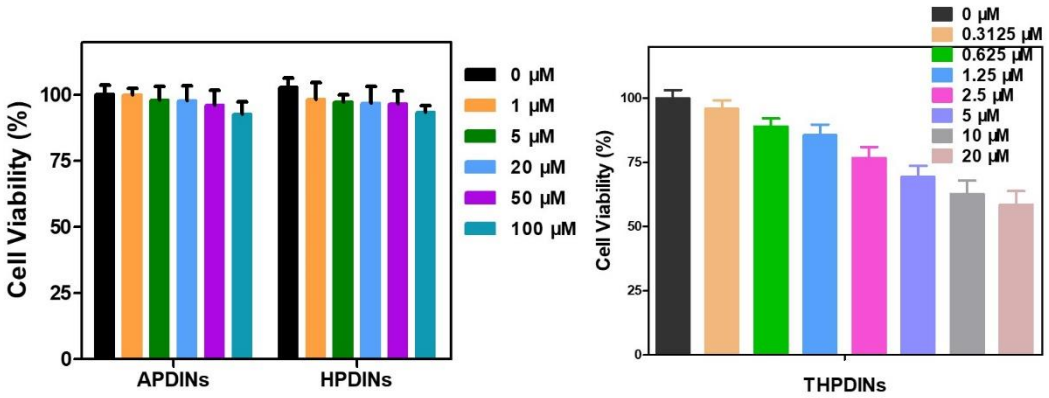

Figure S22. Cytotoxicity of U87MG cells with different treatments: APDINs and HPDINs (different concentration of PDI) and DOX loaded APDINs with various DOX concentration.

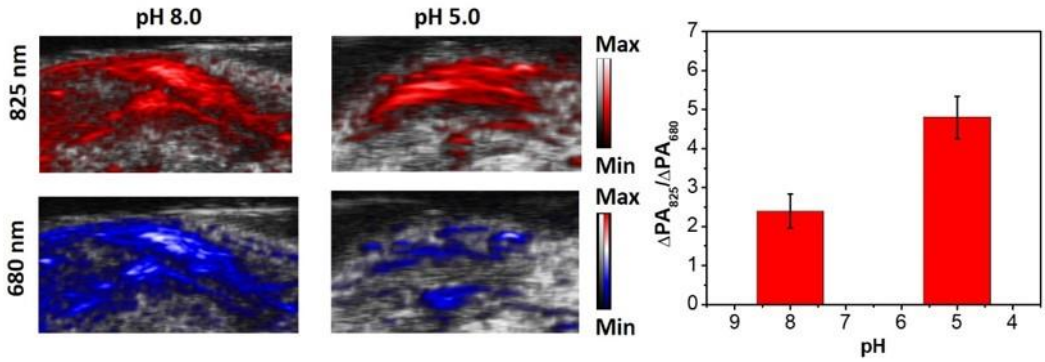

Figure S23. *In vivo* PA imaging of mice normal tissues by injection of THPDINs after changing the subcutaneous pH value by injection of 25 μL of citric buffer (pH 5.0) or $NaHCO_3$ solution (pH 8.0). PA images at 680 and 825 nm were performed and quantified at the two pH environments.

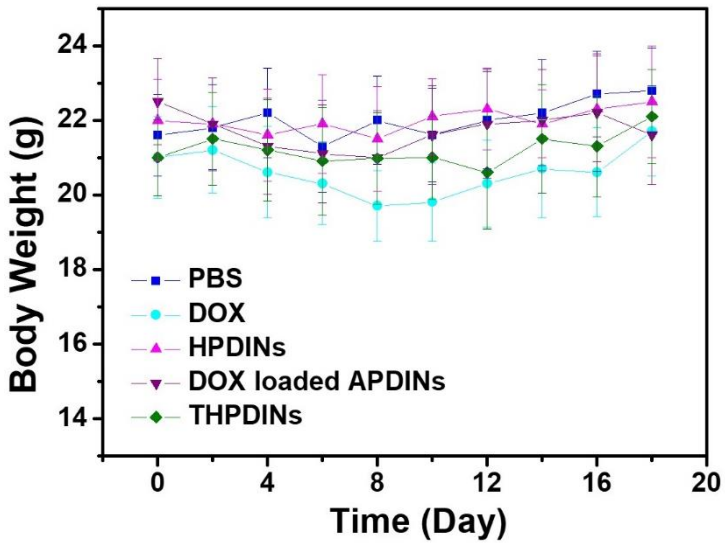

Figure S24. The body weight of Xenograft U87MG tumor bearing mice with different treatments.

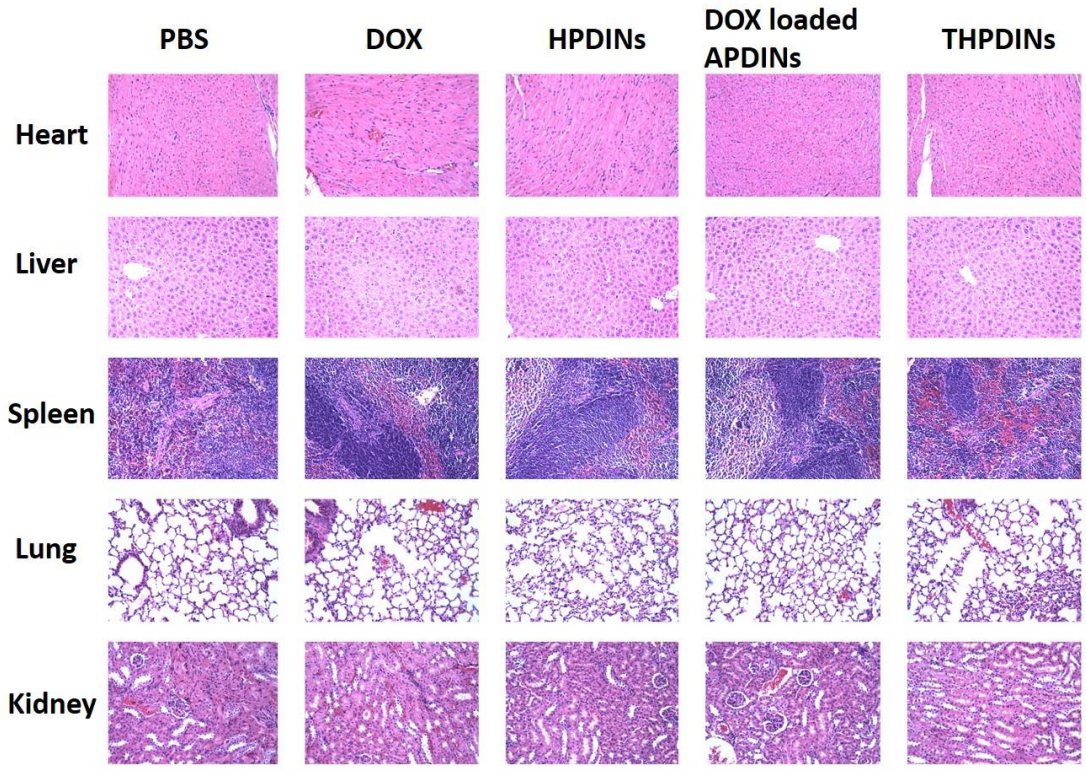

Figure S25. Hematoxylin and Eosin (H&E) staining of the major organs (heart, liver, spleen, lung and kidneys) from various groups at day 18 after treatment.