Electronic Supplementary Information

Encapsulating an acid-activatable phthalocyanine-doxorubicin conjugate and the hypoxia-sensitive tirapazamine in polymeric micelles for multimodal cancer therapy

Xuejiao Guo,[a,b] Honglin Jin[c] and Pui-Chi Lo*[a,b]

[a] *Department of Biomedical Sciences, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China. E-mail: gigi.lo@cityu.edu.hk*

[b] *Shenzhen Research Institute of City University of Hong Kong, Shenzhen 518057, China*

[c] *Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China*

Contents

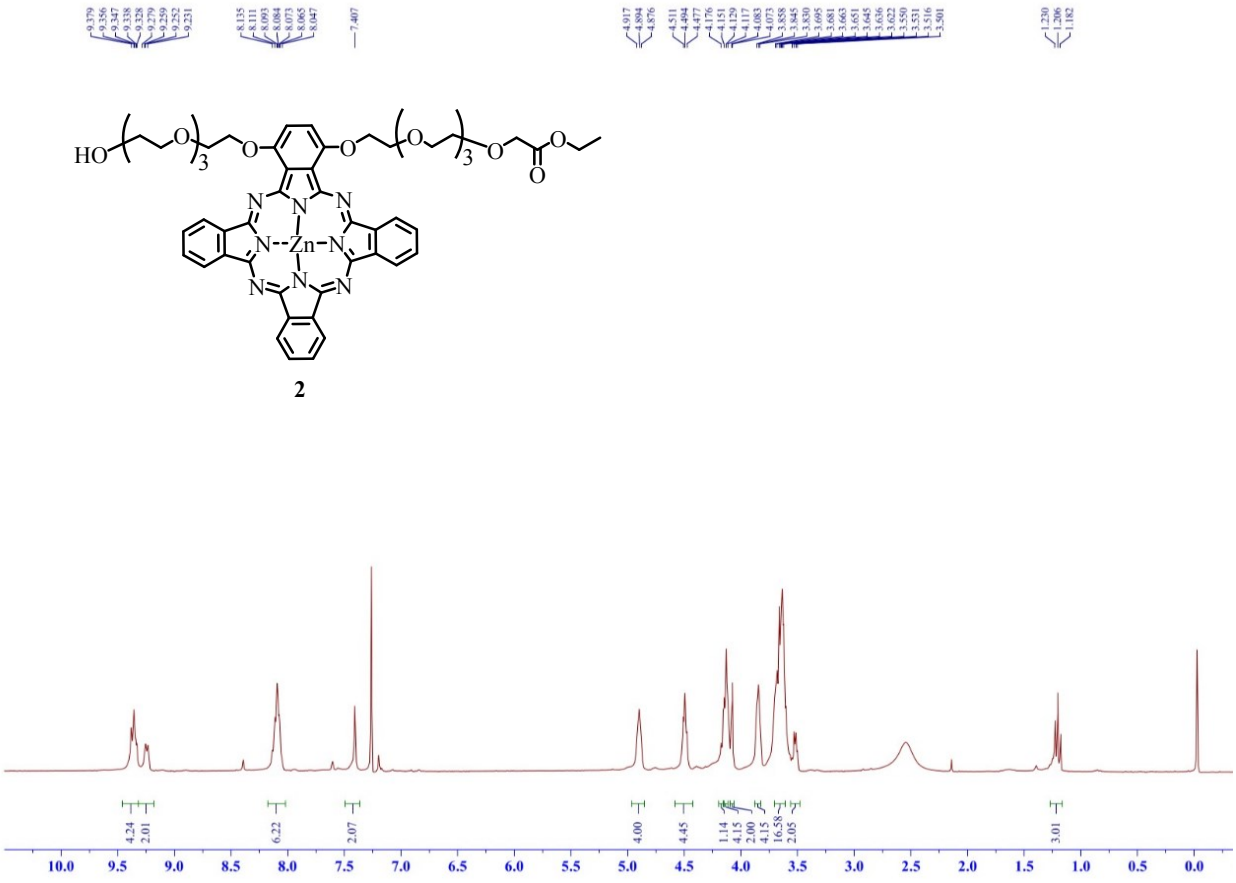


Fig. S1 ^{1}H NMR spectrum of **2** in $CDCl_3$ with a trace amount of pyridine-d_5.

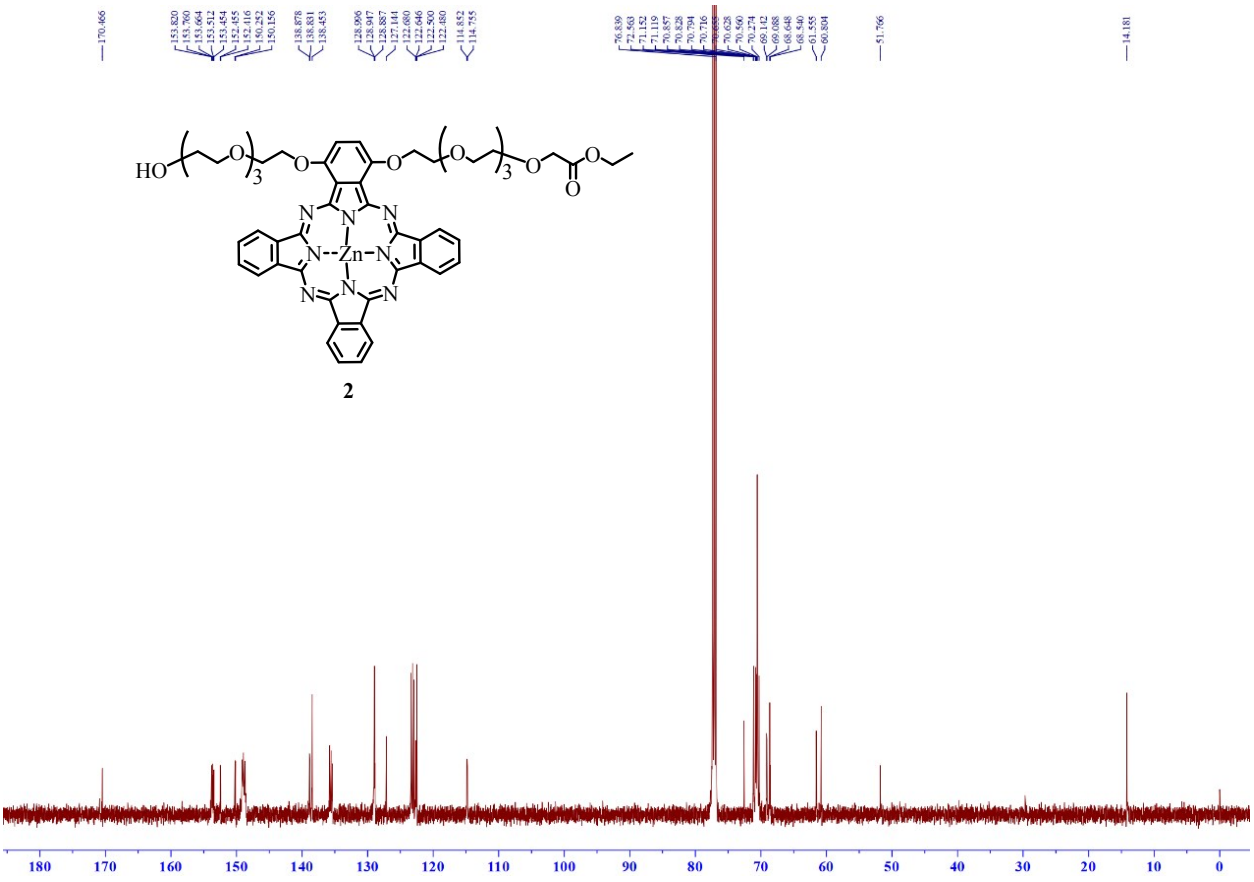


Fig. S2 $^{13}C\{^{1}H\}$ NMR spectrum of **2** in $CDCl_3$ with a trace amount of pyridine-d_5.

Molecular formula :	$C_{52}H_{54}N_8O_{12}Zn$
Experimental Mass $[M+Na]^+$:	1069.30494
Theoretical Mass $[M+Na]^+$:	1069.30449
Error (ppm) :	0.4

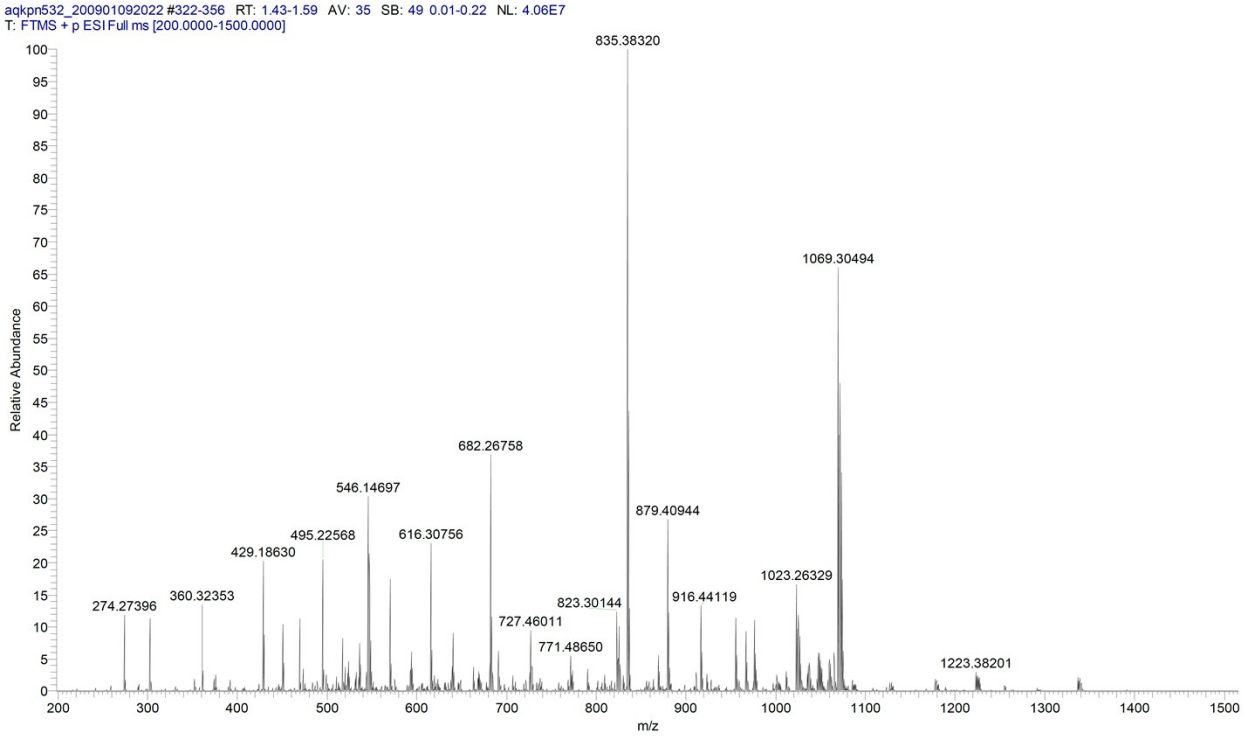


Fig. S3 ESI mass spectrum of **2**.

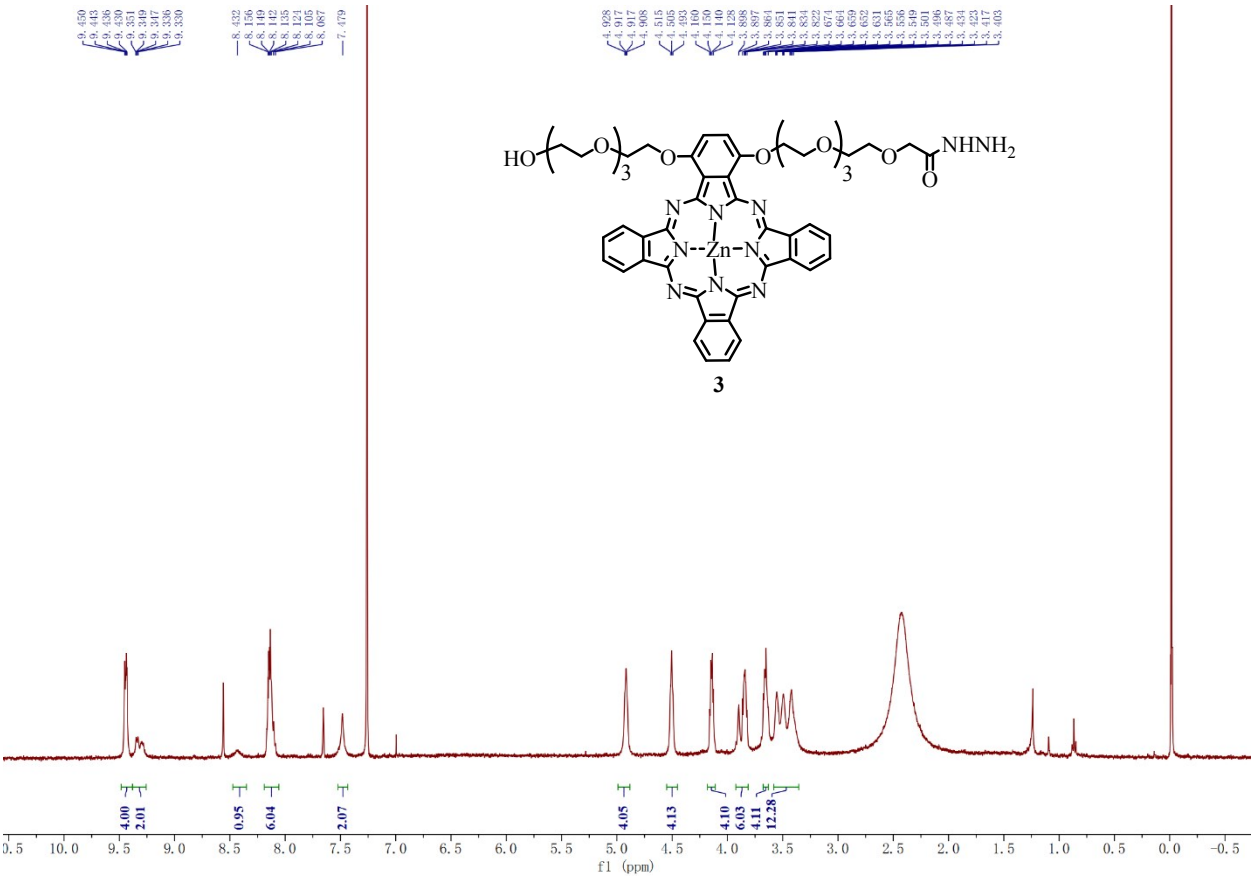


Fig. S4 ^{1}H NMR spectrum of **3** in $CDCl_3$ with a trace amount of pyridine-d_5.

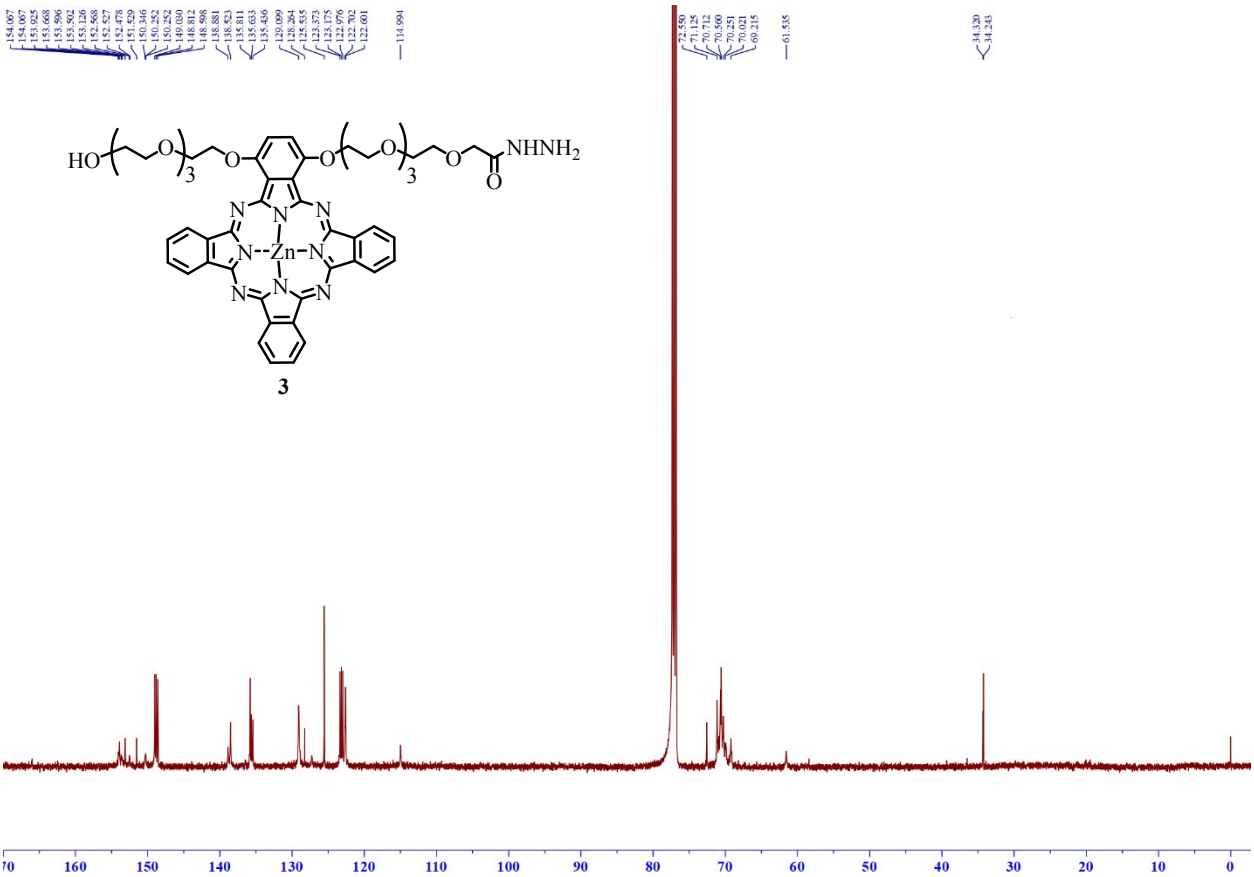


Fig. S5 $^{13}C\{^{1}H\}$ NMR spectrum of **3** in $CDCl_3$ with a trace amount of pyridine-d_5.

Molecular formula :	$C_{50}H_{52}N_{10}O_{11}Zn$
Experimental Mass $[M+H]^+$, $[M+2H]^{2+}$:	1033.31877, 517.16313
Theoretical Mass $[M+H]^+$, $[M+2H]^{2+}$:	1033.31813, 517.16270
Error (ppm) :	0.6, 0.8

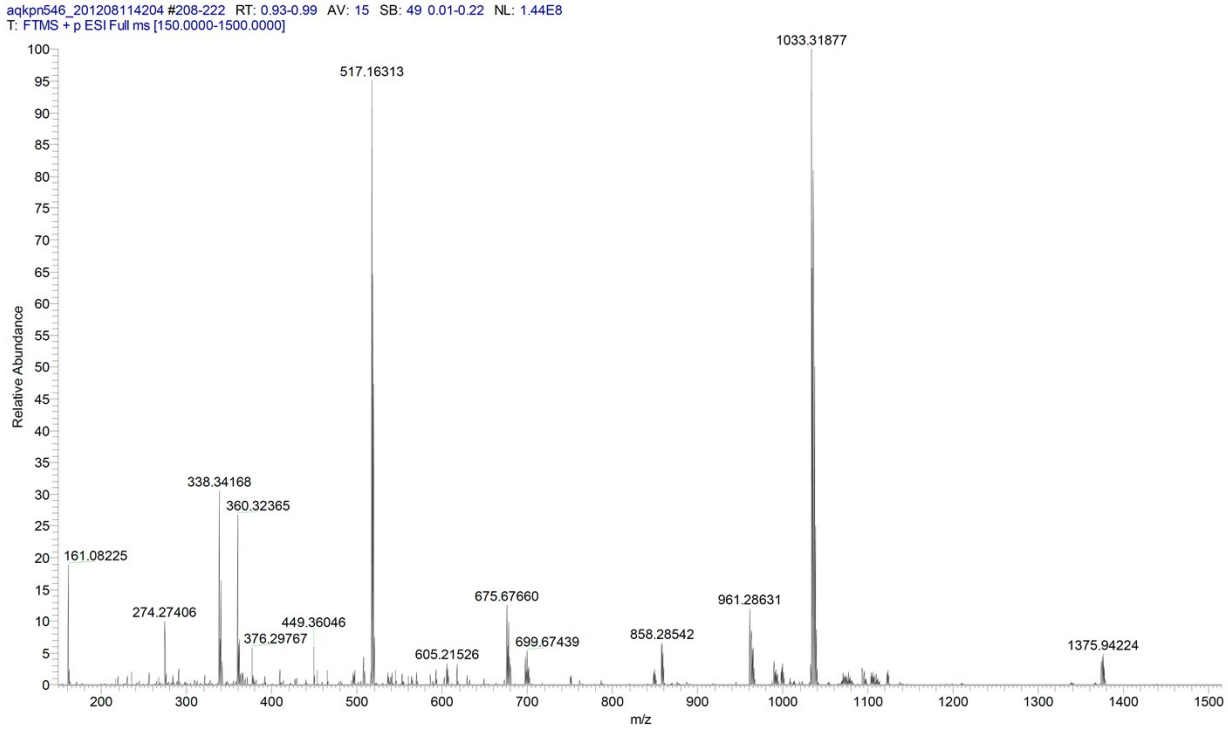
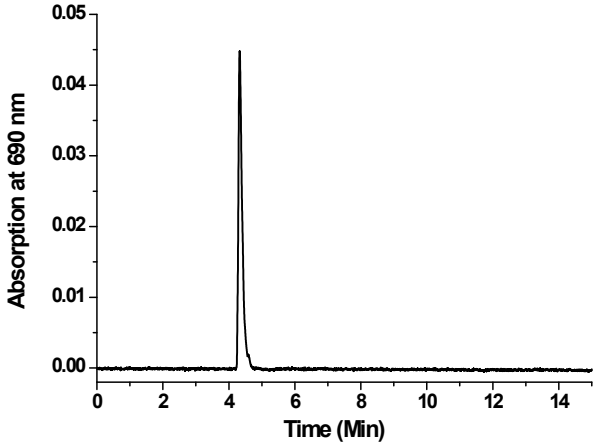


Fig. S6 ESI mass spectrum of **3**.

(a)



(b)

Molecular formula :	$C_{77}H_{79}N_{11}O_{21}Zn$
Monoisotopic Experimental Mass $[M+H]^+$, $[M+2H]^{2+}$:	1558.48194, 779.74458
Monoisotopic Theoretical Mass $[M+H]^+$, $[M+2H]^{2+}$:	1558.48162, 779.74445
Error (ppm) :	0.2, 0.2

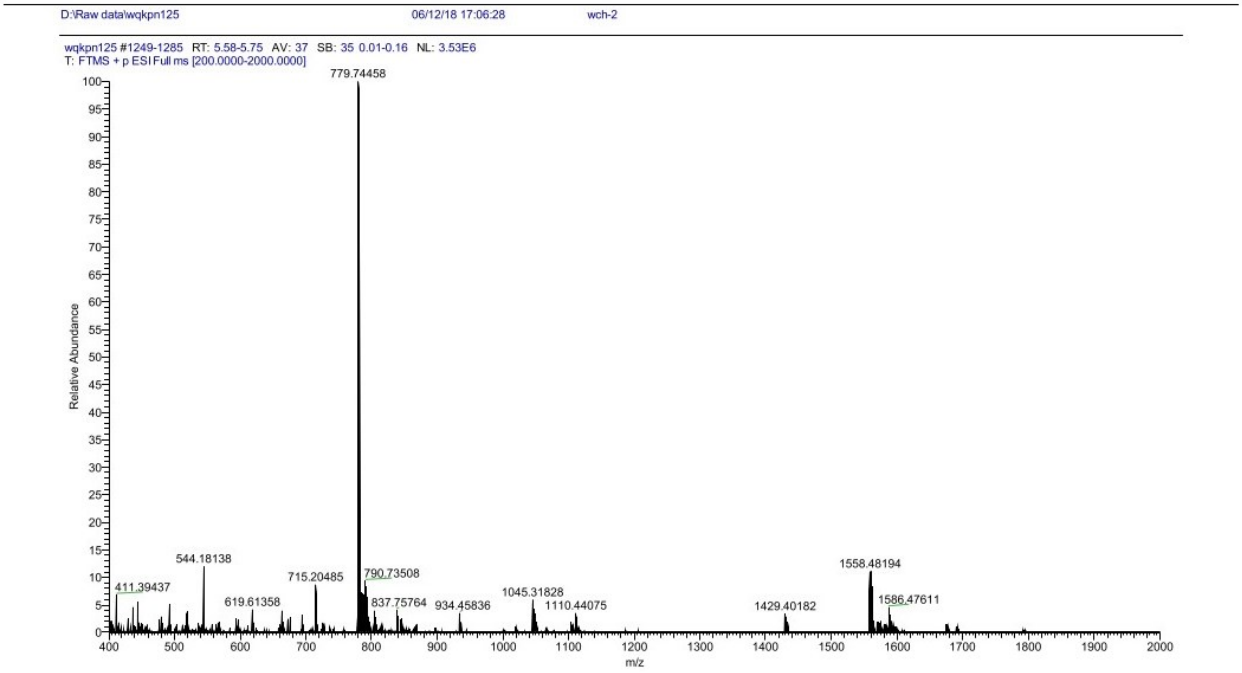


Fig. S7 (a) HPLC chromatograph and (b) ESI mass spectrum of ZnPc-Dox.

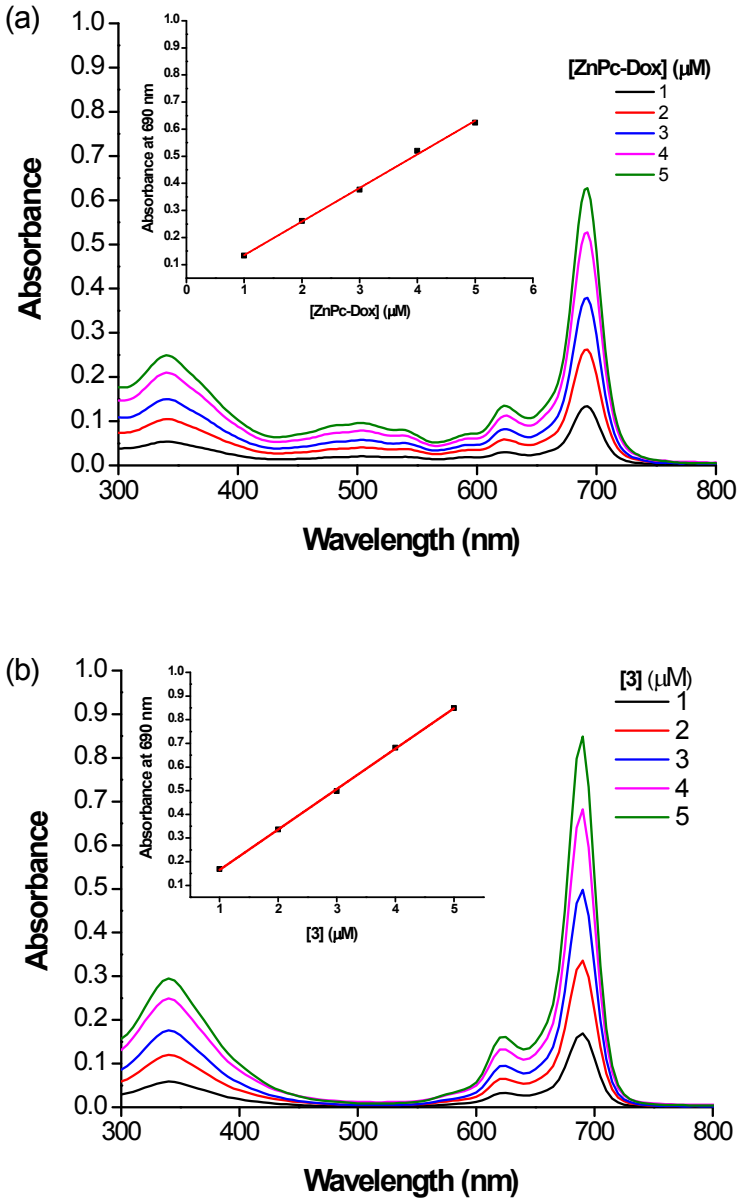


Fig. S8 Electronic absorption spectra of (a) ZnPc-Dox and (b) **3** at different concentrations in DMF. The inset of each figures plots the absorbance at 690 nm versus the concentration of ZnPc-Dox or **3**.

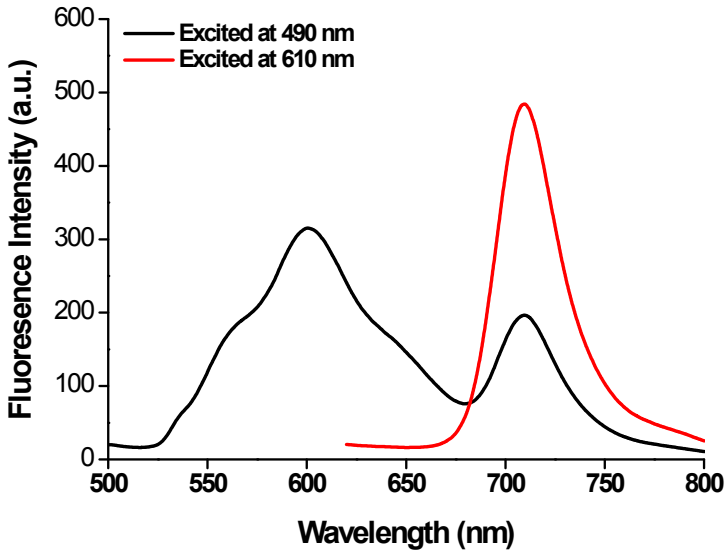


Fig. S9 Fluorescence spectra of ZnPc-Dox (1 μM) in DMF (λ_{ex} = 490 or 610 nm).

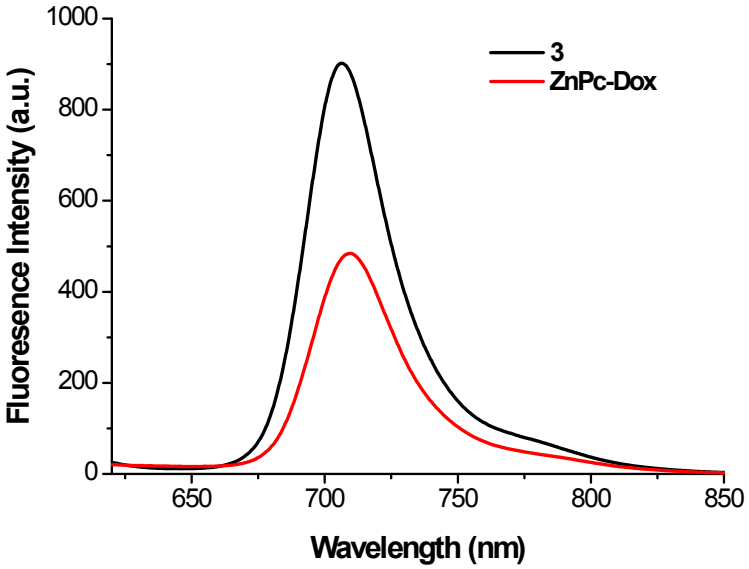


Fig. S10 Fluorescence spectra of **3** and ZnPc-Dox (both at 1 μM) in DMF (λ_{ex} = 610 nm).

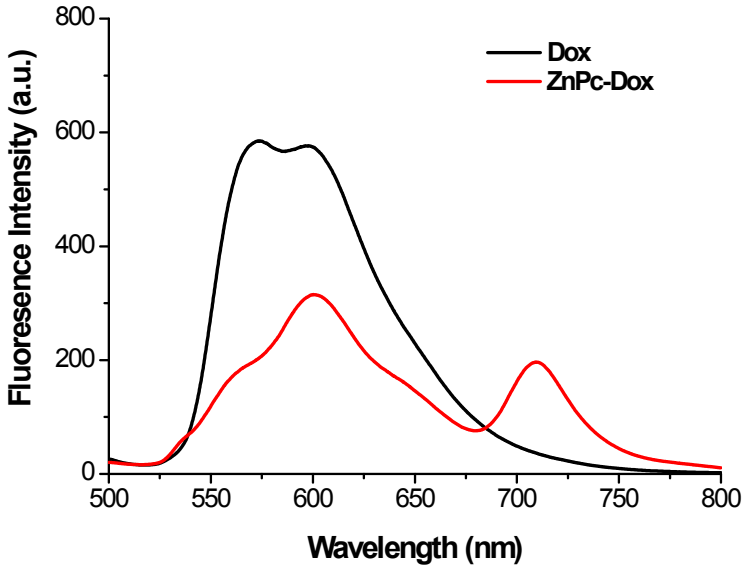


Fig. S11 Fluorescence spectra of ZnPc-Dox and Dox (both at 1 μM) in DMF (λ_{ex} = 490 nm).

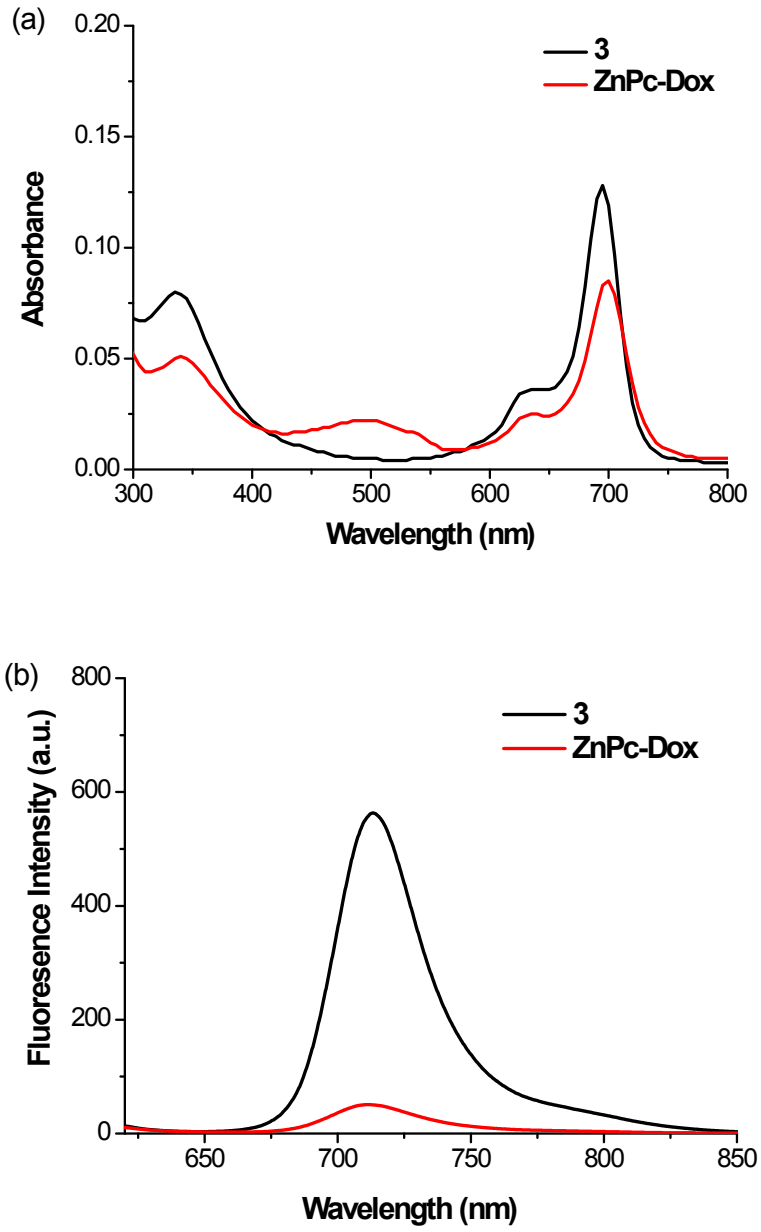


Fig. S12 (a) Electronic absorption and (b) fluorescence (λ_{ex} = 610 nm) spectra of **3** and ZnPc-Dox (both at 1 μM) in PBS with 0.05% Tween 80.

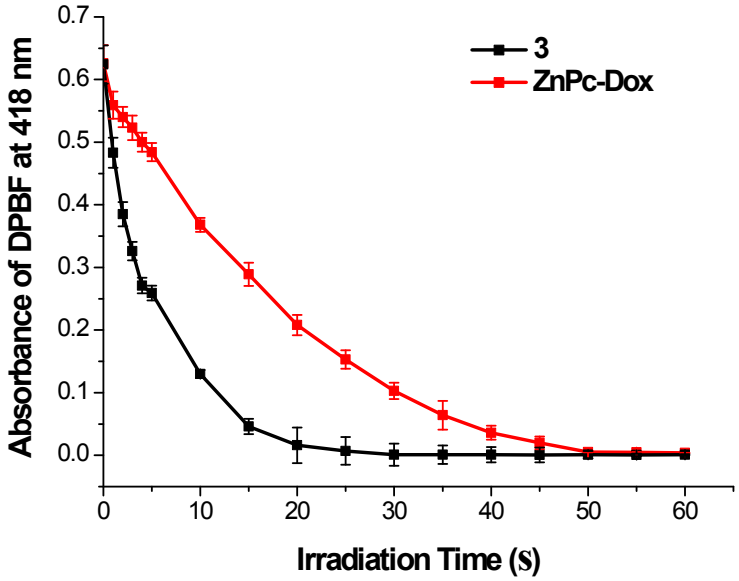


Fig. S13 Comparison of the rate of photoconversion of DPBF (initial concentration = 30 μM) sensitised by **3** or ZnPc-Dox in DMF. The concentration of **3** and ZnPc-Dox was fixed at 1 μM.

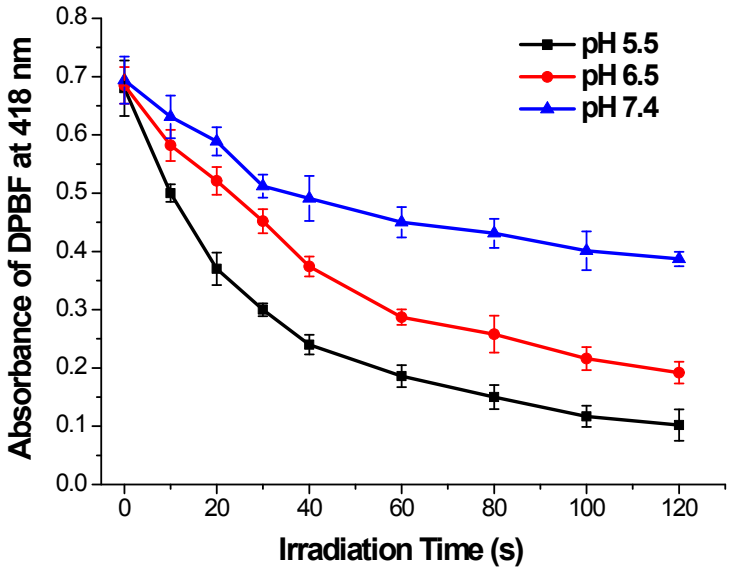


Fig. S14 Comparison of the rate of photoconversion of DPBF (initial concentration = 30 μM) sensitised by ZnPc-Dox (1 μM) upon incubation in a phosphate solution at different pH with 0.05% Tween 80 for 24 h. The concentration of ZnPc-Dox was fixed at 1 μM in all cases.

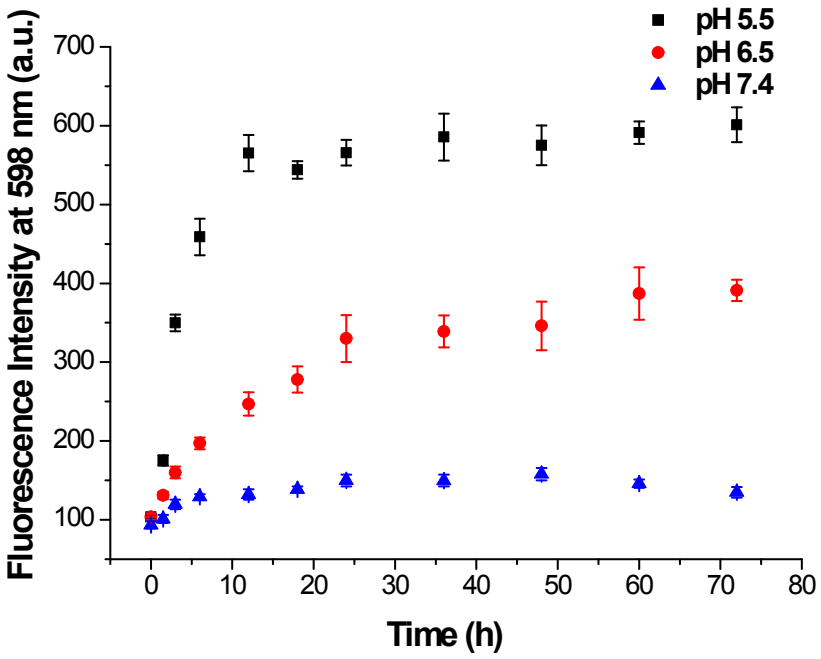


Fig. S15 Change in the fluorescence intensity of Dox during incubation of ZnPc-Dox (1 μM) in a phosphate solution at different pH with 0.05% Tween 80 at 37 °C over a period of 72 h (λ_{ex} = 490 nm).

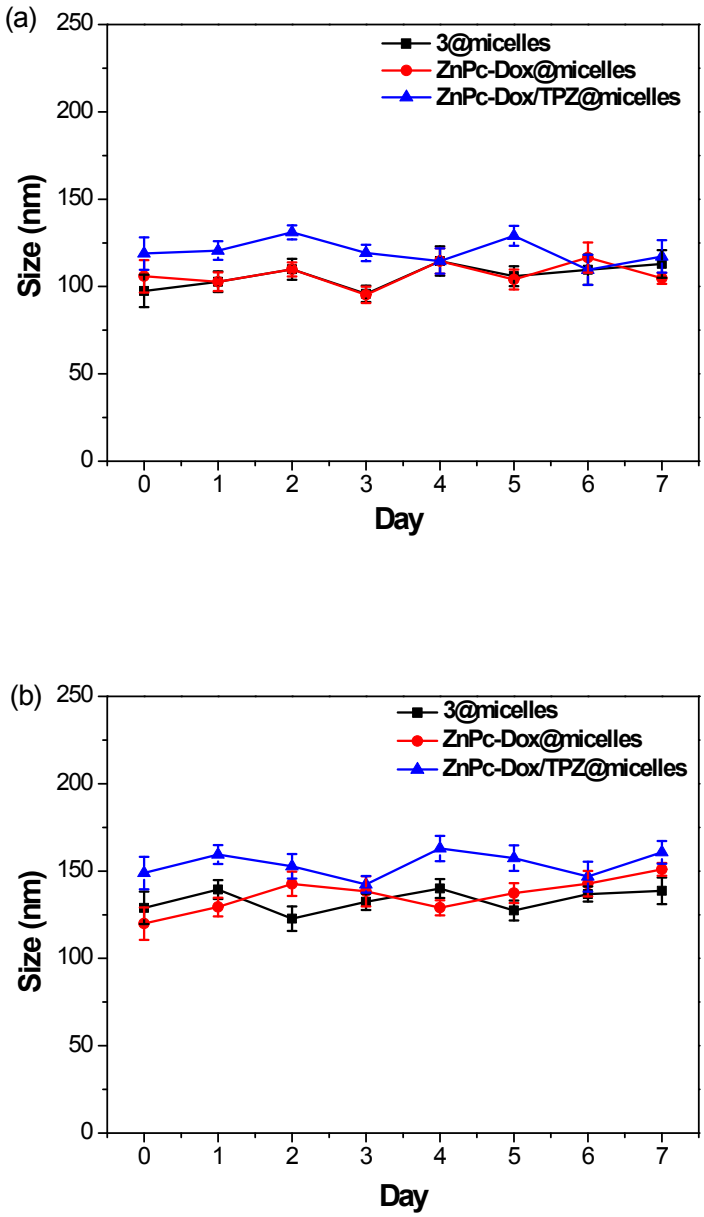


Fig. S16 Change in the size of different micelles (a) in PBS at pH 7.4 at 4 °C and (b) in PBS containing FBS (10%, v/v) at 37 °C over a period of 7 days. Data are shown as the mean ± SD (*n* = 3).

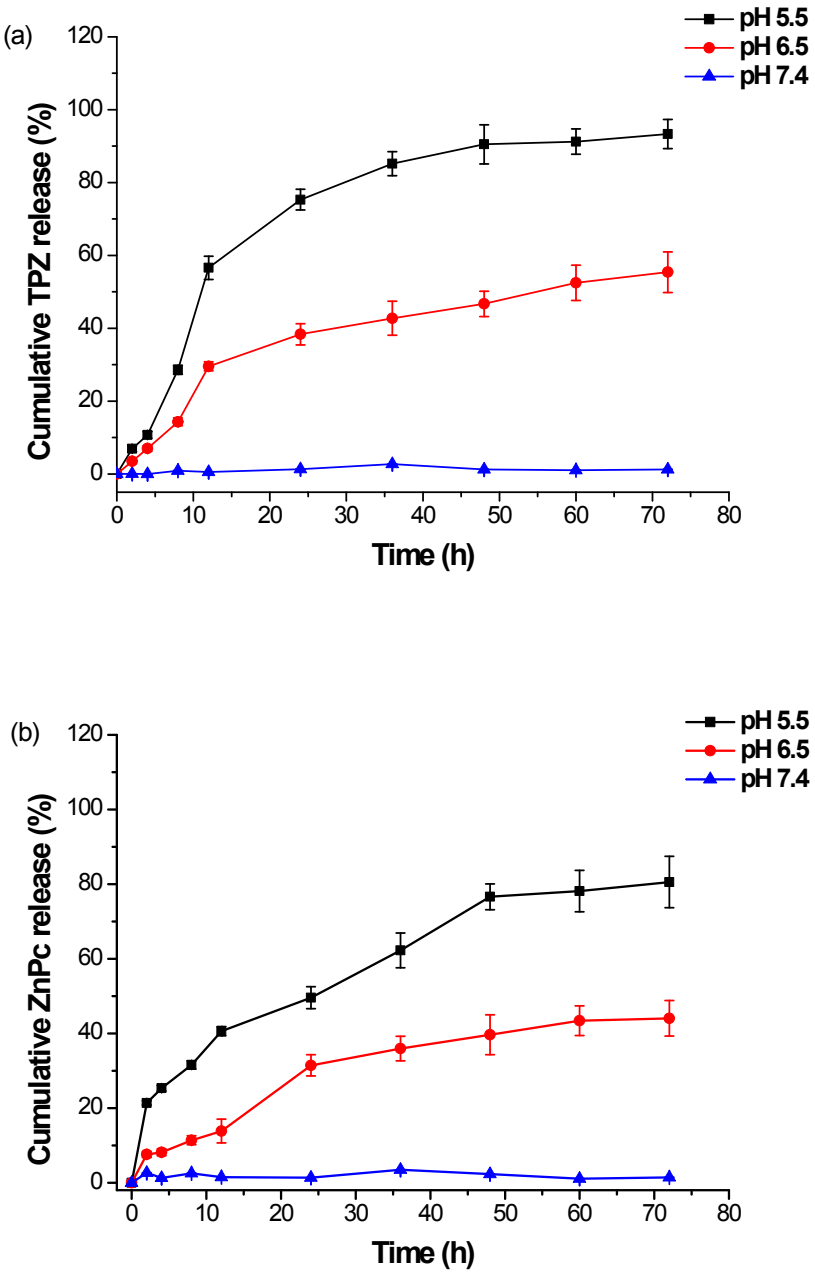


Fig. S17 Release profiles of (a) TPZ and (b) ZnPc-Dox from ZnPc-Dox/TPZ@micelles ([ZnPc-Dox] = 100 μM) in PBS at different pH at 37 °C over a period of 72 h

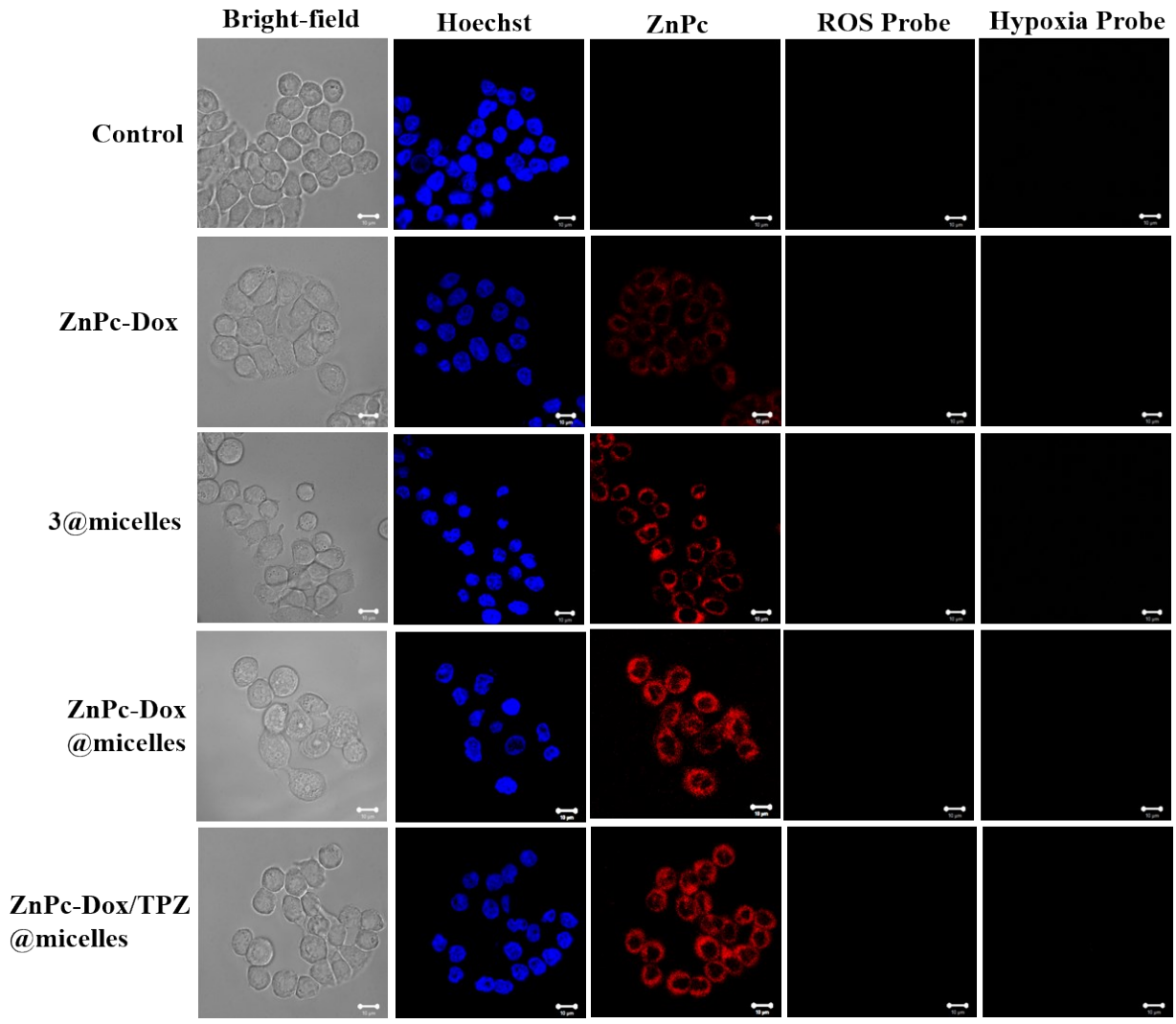


Fig. S18 Bright-field and fluorescence images of HT29 cells after incubation with ZnPc-Dox, 3@micelles, ZnPc-Dox@micelles and ZnPc-Dox/TPZ@micelles for 12 h. The cells were then stained with the hypoxia probe (0.5 μM) and the oxidative stress detection probe (1 μM) for 30 min and Hoechst 33342 (10 μg mL^{-1}) for 10 min in the absence of light irradiation. The concentration of ZnPc was fixed at 1 μM in all cases. Scale bar = 10 μm.

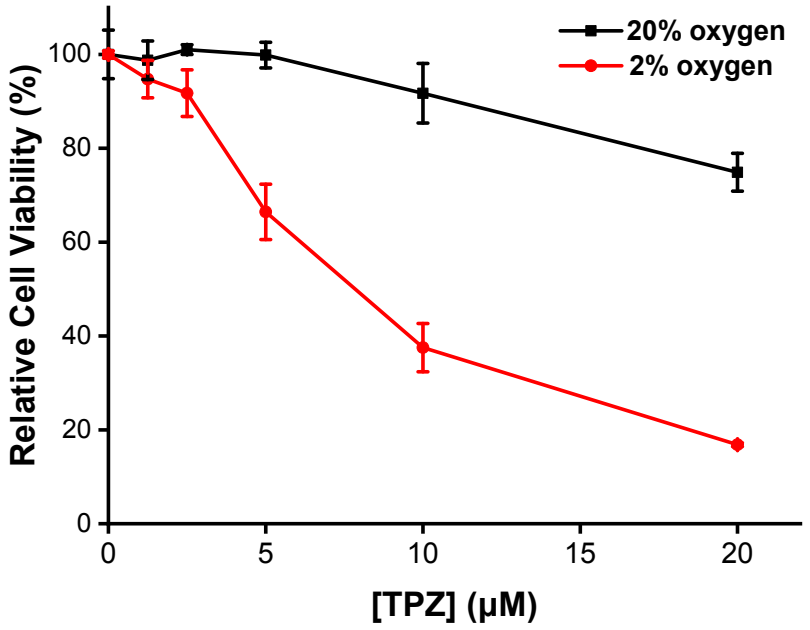


Fig. S19 Cytotoxicity of TPZ on HT29 cells after incubation for 12 h under a normoxic (20% oxygen) or hypoxic condition (2% oxygen). Data are expressed as the mean ± SEM of three independent experiments, each performed in quadruplicate.

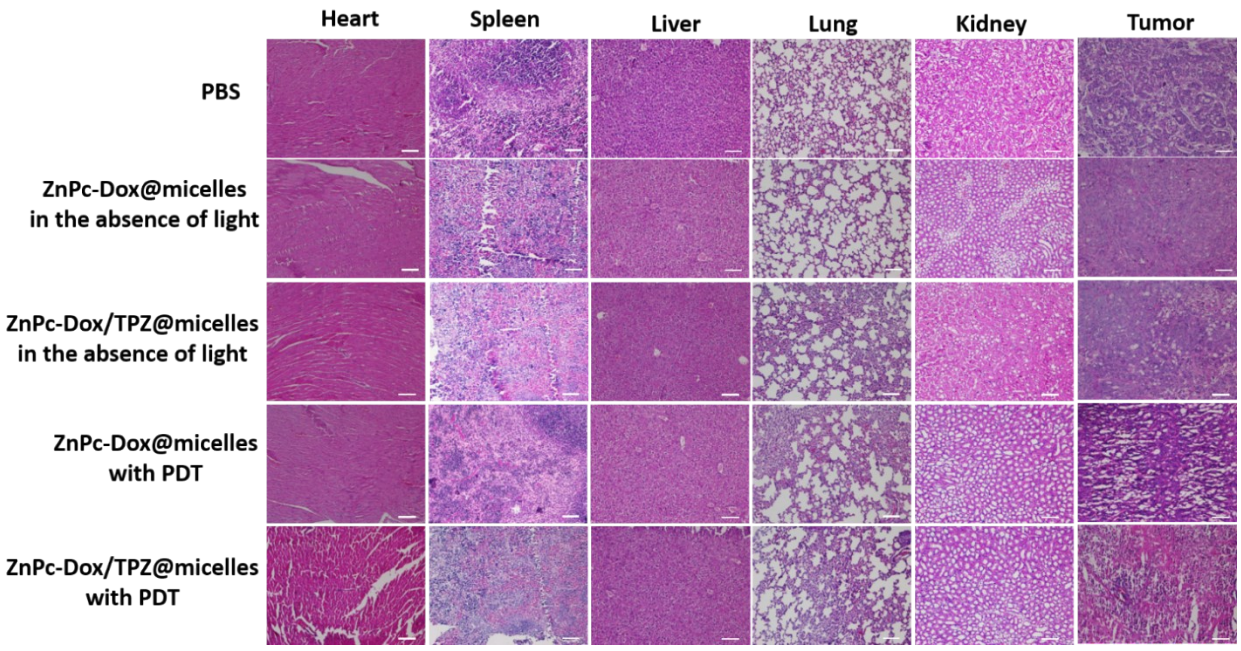


Fig. S20 H&E staining images of different organs and tumour slices in the mice after different treatments. Scale bar = 100 μm.

Table S1 Electronic absorption and photophysical data for **3** and ZnPc-Dox in DMF.

Compound	λ_{abs} (nm) (log ε)	λ_{em} (nm)[a]	Φ_F[b]	Φ_Δ[c]
3	340 (4.73), 625 (4.51), 690 (5.23)	710	0.15	0.43
ZnPc-Dox	340 (4.69), 500 (4.27), 625 (4.42), 690 (5.01)	710	0.12	0.35

[a] Excited at 610 nm. [b] Relative to unsubstituted ZnPc in DMF (Φ_F = 0.28). [c] Relative to unsubstituted ZnPc (Φ_Δ = 0.56).