

Supporting Information

Pronounced cellular uptake of pirarubicin versus that of other anthracyclines: Comparison of HPMA copolymer conjugates of pirarubicin and doxorubicin

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Supplemental figure 1

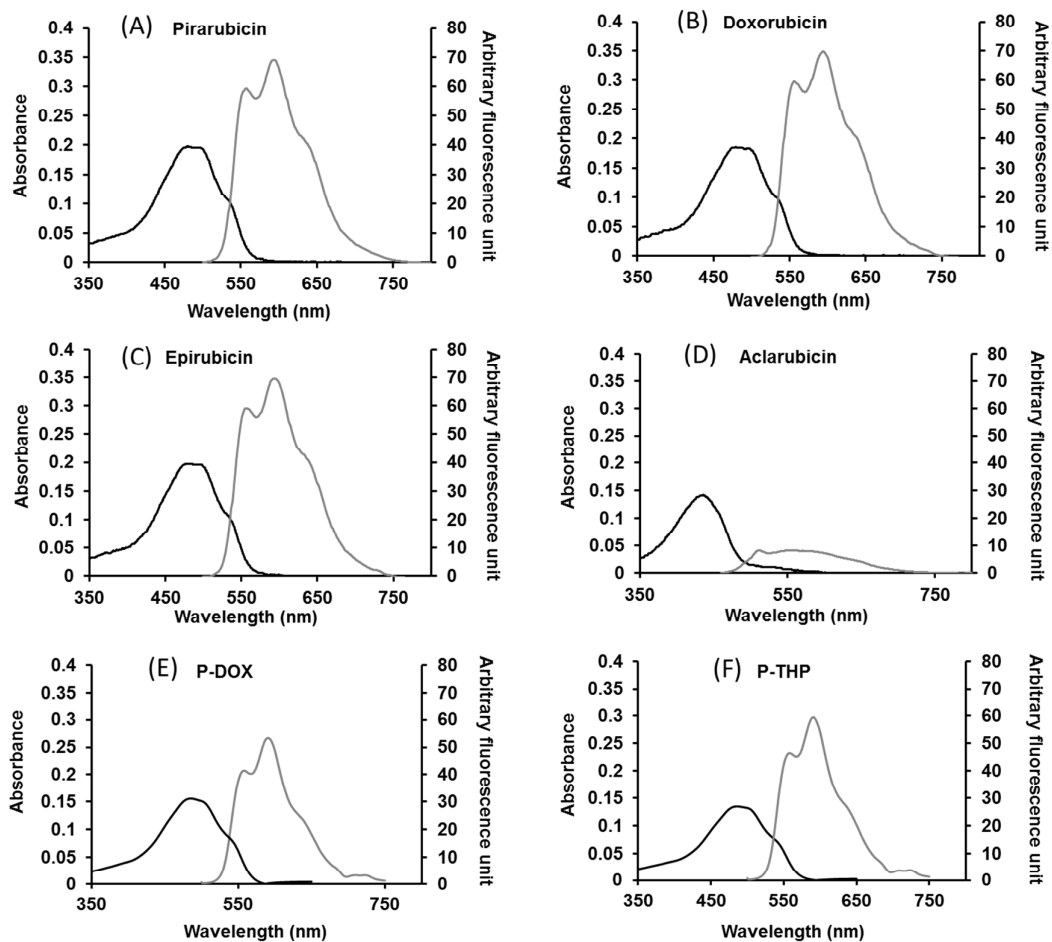


Fig. S1. Absorption and fluorescence spectra. Absorption spectra (black) and fluorescence spectra (gray), with excitation at 488 nm, of (A) THP, (B) DOX, (C) EPI, (D) aclarubicin, (E) P-DOX, and (F) P-THP in PBS.

Supplemental figure 2

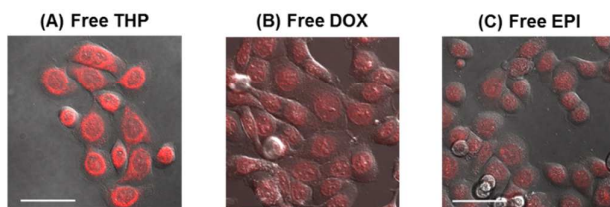


Fig. S2. Nuclear localization of anthracyclines. SUIT2 cells were treated with (A) free THP, (B) free DOX, and (C) free EPI, each at 5 $\mu\text{g}/\text{mL}$ after which cells were visualized by using confocal laser scanning microscopy under 5 % CO_2 at 37 $^\circ\text{C}$. The red indicates drug fluorescence. Display setting was each adjusted to see the nuclear localization of drugs clearly. Scale bars = 20 μm .

Supplemental figure 3

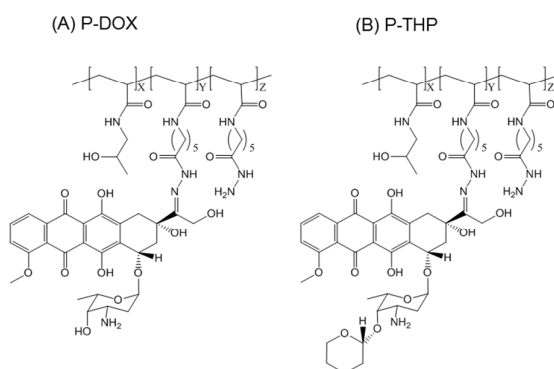


Fig. S3. Chemical structures of (A) P-DOX and (B) P-THP.

Supplemental figure 4

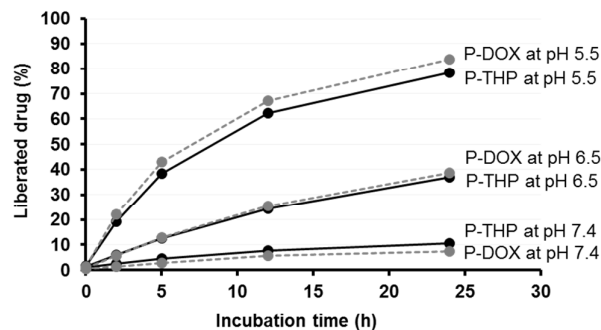


Fig. S4. Liberation of free drugs from polymer-drug conjugates. P-THP and P-DOX were incubated at pH 5.5, 6.5, and 7.4. Liberated free drug and polymer-bound drug were separated by using size exclusion chromatography and were detected by absorbance at 488 nm. The percentage of liberated drug was calculated by using the following equation: Liberated drug (%) = $\text{AUC of liberated drug} / (\text{AUC of liberated drug} + \text{AUC of polymer-bound drug}) \times 100$, where AUC = area under the curve.

Supplemental figure 5

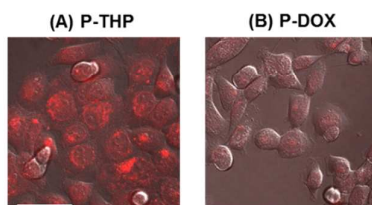


Fig. S5. Nuclear localization of P-THP and P-DOX. SUIT2 cells were treated with (A) P-THP, and (B) P-DOX, each at 5 $\mu\text{g}/\text{mL}$ after which cells were visualized by using confocal laser scanning microscopy under 5 % CO_2 at 37 $^\circ\text{C}$. The red indicates drug fluorescence. Display setting was each adjusted to see the nuclear localization of drugs clearly. Scale bars = 20 μm .

Supplemental figure 6

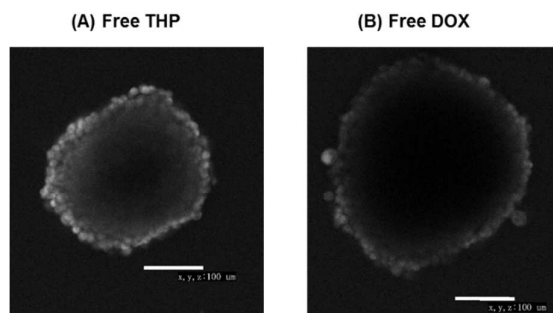


Fig. S6. Penetration and cellular uptake of free THP and free DOX in cultured tumor cell spheroid. HCT 116 cell spheroid having approx. 200 μm in diameter was treated with 30 $\mu\text{g}/\text{mL}$ of THP or DOX, and drugs were visualized by confocal laser microscopy at 60 min. Images are taken at the center core of spheroids. Scale bars = 100 μm . These images are representative image of triplicate experiments.

Supplemental figure 7

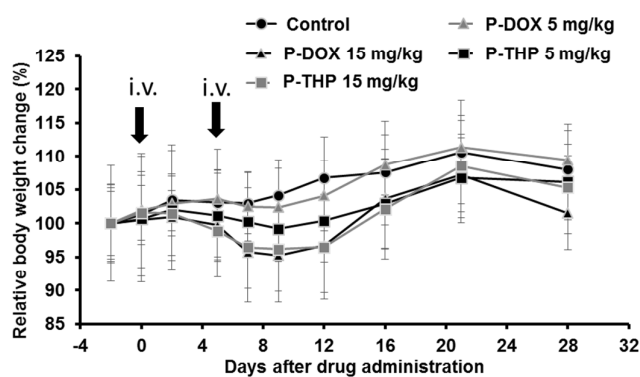


Fig. S7. Body weight change in SUIT2 tumor-bearing mice. Mice were given 5 or 15 mg/kg P-THP or P-DOX twice at the indicated times (arrows). Relative body weights were recorded. Values are means \pm SD; $n = 6$.

Table S1

Cytotoxicity of P-DOX and P-THP against various cell human lines.

Cell line (origin)	IC ₅₀ (µg/mL)	
	P-THP	P-DOX
SUIT2 (human pancreatic cancer)	0.062	0.44
MIAPaCa (human pancreatic cancer)	0.26	1.82
HeLa (human cervical cancer)	0.24	0.87
A549 (human non-small cell lung cancer)	0.092	1.07
HepG2 (human hepatocellular carcinoma)	0.18	1.65
SK-HEP (human hepatic adenocarcinoma)	0.14	0.94
DU145 (human prostate cancer)	0.045	0.30
HCT116 (human colon cancer)	0.10	0.56
HUVECs (human umbilical vein endothelial cells)	0.048	0.19
HEK293 (human embryonic kidney cells)	0.032	0.074
16HBE (human bronchial epithelial cells)	0.25	0.72
KYSE150 (human esophageal squamous cell carcinoma)	0.28	1.25

Table S2

Initial tumor volume of SUIT2 tumor bearing mice

Treatment	Control	LP-DOX 5 mg/kg	LP-DOX 15 mg/kg	LP-THP 5 mg/kg	LP-THP 15 mg/kg
Tumor volume (mm³)	158 ± 61	142 ± 53	199 ± 65	158 ± 50	188 ± 81