Supplementary figures







Fig.S2 ¹H-NMR spectrum of HA-VES in DMSO- d_6 (A), HA in D₂O (B), VES-NH₂ in CDCl₃ (C), and VES in CDCl₃ (D).



Fig.S3 The chemical structure of HA-VES characterized by FT-IR, and the FT-IR spectra of HA (a), VES (b), VES-NH₂ (c), HA-VES (d).



Fig.S4 The CMC determination of HA-VES copolymers with different DS.



Fig. S5 *In vitro* hemolysis assay of HA-VES4, HA-VES7 and HA-VES12 in comparison with Tween 80. (Mean \pm SD, n=3).



Fig.S6 *In vitro* cytotoxicity of HA-VES copolymers with different DS against MCF-7 (A-C) and MCF-7/Adr cells (D-F) at 48, 72 and 96 h post-treatment, respectively. (Mean \pm SD, n=3).



Fig.S7 *In vitro* cytotoxicity of DOX-Sol, the mixture of DOX-Sol and HA-VES4 copolymer, and HA-VES/DOX micelles against MCF-7 (A-C) and MCF-7/Adr cells (D-F) at 48, 72 and 96 h post-treatment, respectively. (Mean \pm SD, n=3).



Fig.S8 Western blot assay for caspase 3 and PARA.



Fig.S9 Endocytosis mechanism of HA-VES12/DOX after incubation with different endocytosis inhibitors in MCF7/Adr cells determined by FACS. (n=3, mean \pm SD, *p<0.05, **p<0.01 vs HA-VES12/DOX).



Fig. S10 (A and B) Confocal laser scanning microscopy images of DOX, VES4/DOX, HA-VES7/DOX and HA-VES12/DOX after incubation with 1 h (A) and 2 h (B) in

MCF7/Adr cells, respectively. Cells were counterstained with DAPI for nuclei and lysotracker green for lysosomes. Scale bar: 30 µm.



Fig.S11 Evaluation the levels of (A) CKMB, (B) CK, (C) LDH, and (D) AST after after intravenous administration with saline, DOX-Sol, HA-VES4/DOX, HA-VES7/DOX, and HA-VES12/DOX in 4T1-bearing mice. (n=10, mean \pm SD, *P<0.05, **P<0.01, ***P<0.001 compared with DOX-Sol group).

Supplementary tables

Table S1 The physicochemical characterizations of HA-VES/DOX. (Mean \pm SD,

		n=3)			
Sample	Size (nm)	PDI	Zeta (mV)	EE ^a (%)	DL ^b (%)
HA-VES4/DOX	253.2 <mark>0</mark> ±6.78	0.21 ± 0.03	-12.91±0.22	96.73±0.65	15.85 ± 0.15
HA-VES7/DOX	199.3 <mark>0</mark> ±7.36	$0.27{\pm}0.01$	-11.2 <mark>0</mark> ±0.55	97.22±0.93	16.14 ± 0.65
HA-VES12/DOX	196.6 <mark>0</mark> ±2.96	0.22 ± 0.04	-9.78±0.32	98.04 ± 3.15	16.33 ± 0.41

^a EE(%)=encapsulation efficiency.

^b DL(%)=drug loading content.

Table S2 The IC50 values, RI, and RF of DOX-Sol and HA-VES/DOX micelles in MCF-7 and MCF-7/Adr cells after 96 h incubation.

Time (h)	IC50 (µg/mL)	DI	DE	
Time (II)	MCF-7	MCF-7/Adr	KI	κι ^ν
DOX-Sol	0.43	38.04	88.57	_
HA-VES4/DOX	0.40	3.87	9.56	9.83
HA-VES7/DOX	0.37	3.10	8.47	12.27
HA-VES12/DOX	0.32	2.77	8.55	13.74

Table S3 The main pharmacokinetics parameters of DOX after intravenous administration of DOX-S0 and HA-VES/DOX. (Mean \pm SD, n=6)

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AUC _(0-t)	mg/L*h	659.07±134.62	1927.30±233.06	2179.07 ± 797.52	2668.84±1124.52
AUC _(0-∞)	mg/L*h	782.00 ± 258.86	2053.11±305.32	2286.35 ± 951.46	2901.29±1267.89
MRT _(0-t)	h	0.90±0.34	3.52±1.73	$3.20{\pm}1.56$	3.98 ± 0.64
MRT _(0-∞)	h	1.56±0.93	5.47±3.24	4.21±2.95	6.74±3.98
t _{1/2}	h	2.20±1.68	7.28±5.11	5.28 ± 3.02	8.46 ± 5.25
T_{max}	h	0.033	0.033	0.033	0.033
CLz	L/h/kg	0.007 ± 0.002	0.002 ± 0.001	0.003 ± 0.001	0.002
Vz	L/kg	0.025 ± 0.021	0.024 ± 0.016	$0.017 {\pm} 0.008$	0.022 ± 0.013
C_{max}	μg/L	3656.66±711.19	3973.33±711.72	4053.33±1320.74	4548.33±1823.81