Supporting Information for

Self-Complexation and Complexation Controlled Target Cancer Therapy

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General Methods: All coupling and deprotection were carried out under anhydrous conditions. *β*-Carboline-3-carboxylic acid (**1**),[1] and 6-(2-aminoethyleneamino)-6-deoxy-*β*-CD (**3**)[2] were synthesized according to a method as described in the literatures. ESI-MS experiment was performed on Quatteo micro ^{TM}API and Bruker micro OTOF-Q, respectively. NMR spectra were recorded on Bruker Avance-500 MHz. Elemental analysis was carried on a Perkin-Elmer-2400C instrument. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) at 25 °C on a RF-5301PC spectrometer equipped with a constant temperature water bath, the excitation wavelength was 495 nm. The statistical analysis of all the biological data was carried out using ANOVA with *p* value less than 0.001. Measurements at pH 5.5 were carried out in a citric buffer, 0.1 M, and those at pH 7.4 in a phosphate buffer, 0.1 M. pD 5.5 was obtained by adding a small amount of DCl mixed in D_2O to create a deuterated acidic solution.

Synthesis of 1-methyl-9*H*-*β*-carboline-3-carbonylamino-ethylamino)-6-deoxy-*β*-CD (**4**): *β*-Carboline-3-carboxylic acid (**1**) (0.137g, 0.61 mmol) was dissolved in dry DMF (8 mL). HOBT (0.099g, 0.73mmol) was added, and solution was cooled to 0 °C in an ice bath. DCC (0.150 g, 0.73 mmol) was then added, and temperature was maintained at 0 °C for a further 30 min. After that 0.706 g (0.60 mmol) of mono 6-ethylenediamino-6-deoxy-*β*-CD (**3**) was added to above solution. The reaction mixture was stirred at room temperature for 12h. After filtration, the filtrate was worked up with acetone (100 mL). The precipitates formed were collected on a glass filter and dried under reduced pressure. The crude product was purified by silica column chromatography, **4** was obtained as a pale yellow solid (0.125 g, 15 % yield). ESI-MS: *m/z* 1385 for $[M+H]^+$, calcd for $C_{57}H_{84}N_4O_{35}$ M 1384. ^{1}H NMR (500 MHz, D_2O): δ = 2.49 (s, 3H), 3.02 (t, 5.5Hz, 2H), 3.25 (t, 5.5Hz, 2H), 3.35-3.85 (m, 42H), 4.96 (s, 7H), 7.14 (t, 6.5Hz, 1H), 7.41 (t, 8Hz, 2H), 7.92 (d, 7.5Hz, 1H), 8.11 (s, 1H).^{13}C-NMR (125MHz D_2O), δ = 19.43, 48.18, 48.60, 60.19, 71.69, 71.97, 73.11, 81.07, 101.89, 112.05, 112.62, 120.31, 121.07, 121.34, 127.13, 128.56, 135.80, 137.01, 140.60, 141.89, 168.60. Anal. Calcd for $C_{57}H_{84}N_4O_{35}$ 9 H_2O.3NH_3: C 42.83, H 7.00, N 6.13%. Found: C 42.29, H 6.78, N 5.71%.

***In vitro* anti-proliferation assay:** The cytotoxicity of the DOX and self-complex/complex against colon carcinoma (HT-29) and leukemia (K562) cell lines was studied using the MTT

assay.[3] Cell suspension (100 µl of 5×10^4 cells/ml) was seeded in 96-well plates and incubated at 37ºC in a humidified atmosphere of 5% CO_2 for 4 h. The medium was replaced by medium containing different concentrations of test samples in PBS containing 0.1 % DMSO and tests were in triplicates. Incubation was carried out at 37 ºC in an incubator supplied with 5% CO_2 for 48 h. Following incubation, 25 µL of 5 mg/mL of MTT were added to each well and the plate was put back in the incubator for another 4 h at 37 ºC. Then culture medium was removed. The resulting MTT – formazan product was dissolved in 100µl DMSO. The amount of formazan was determined by measuring the optical density at 492 nm. The cytotoxic activities were observed to occur in a dose-dependent manner in the cells. The activity was expressed as an IC_{50} value, which is the concentration of test sample to give 50% inhibition of the growth of tumor cells.

***In vivo* survival study:** All studies described herein were performed under an approved review protocol by the ethics committee of Capital Medical University. The committee assured the welfare of the animals was maintained in accordance to the requirements of the animal welfare act and according to the guide for care and use of laboratory animals. The survival study was determined using ICR mice (Swiss, 10-12 weeks old) inoculated with S_{180} sarcoma. S_{180} ascites tumor cells were used to form solid tumors after subcutaneous injection. For initiation of subcutaneous tumors the cells were obtained as an ascitic form from the tumor-bearing mice, which were serially transplanted once per week. Subcutaneous tumors were implanted by injecting 0.2 ml of 0.9% saline containing 1×10^7 viable tumor cells under the skin on the right armpit. Twenty-four hours after implantation, the mice were randomly divided into different experimental groups with 13 in each group. The mice of the negative control group were given a daily injection of 0.2 ml of 0.9% saline for seven consecutive days. Furthermore, compound **4** with different doses was also used as a control. The mice of treatment groups were given a daily i.p injection for seven consecutive days, the doses ranged from 2 to 5 µM/kg (Table S1). The survival of the samples-treated mice was followed for 7 days. Twenty-four hours after the last administration, all mice were weighed, sacrificed by diethyl ether anesthesia and dissected to immediately obtain and weigh tumor samples.

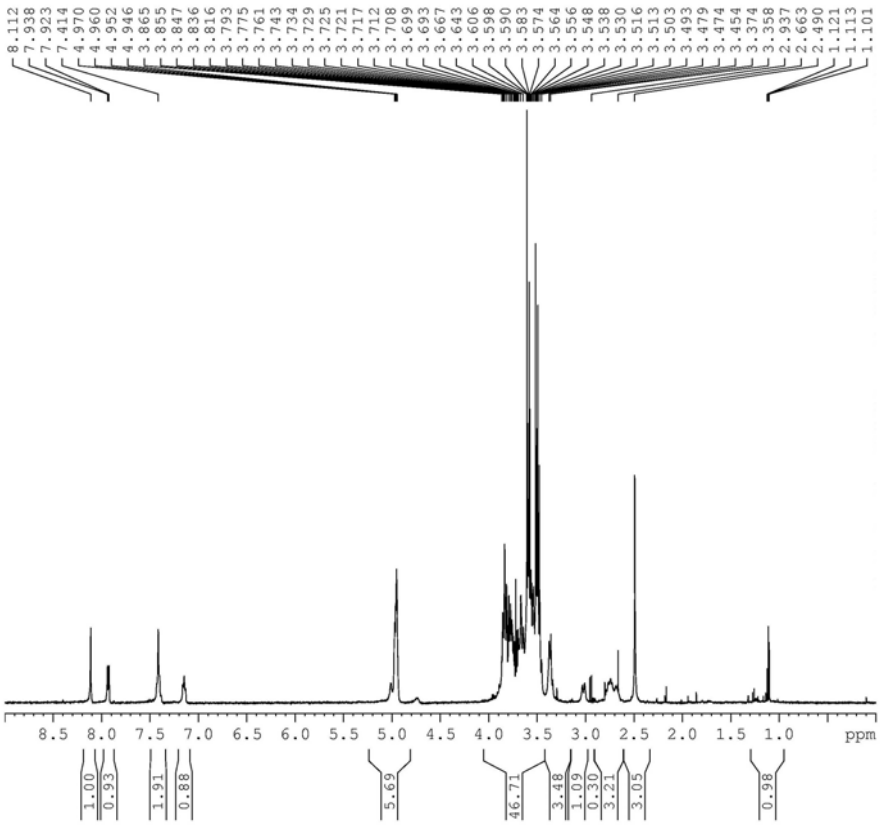

Figure S1. ^{1}H NMR spectra for **4** in D_2O

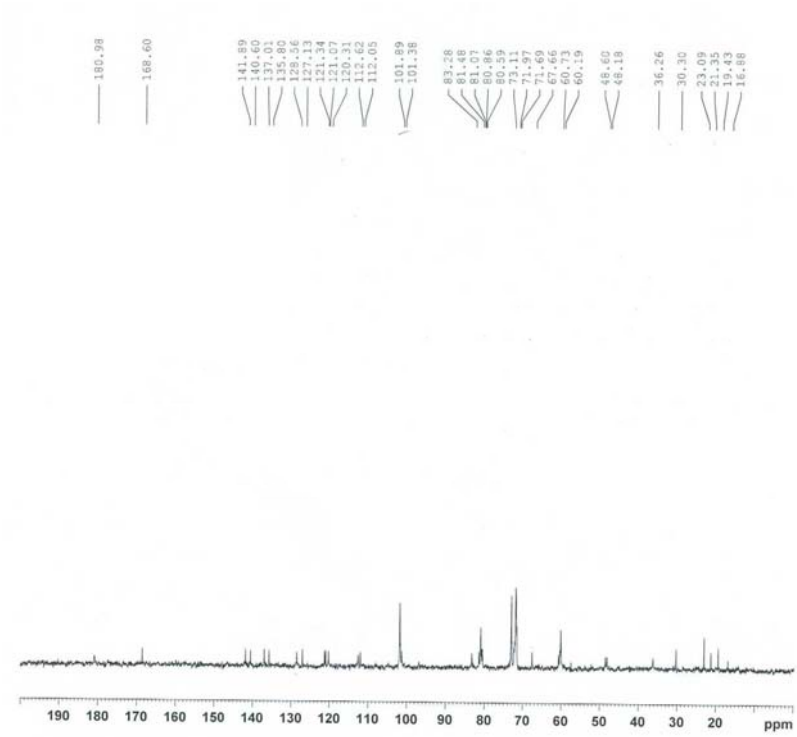

Figure S2. ^{13}C NMR spectra for **4** in D_2O

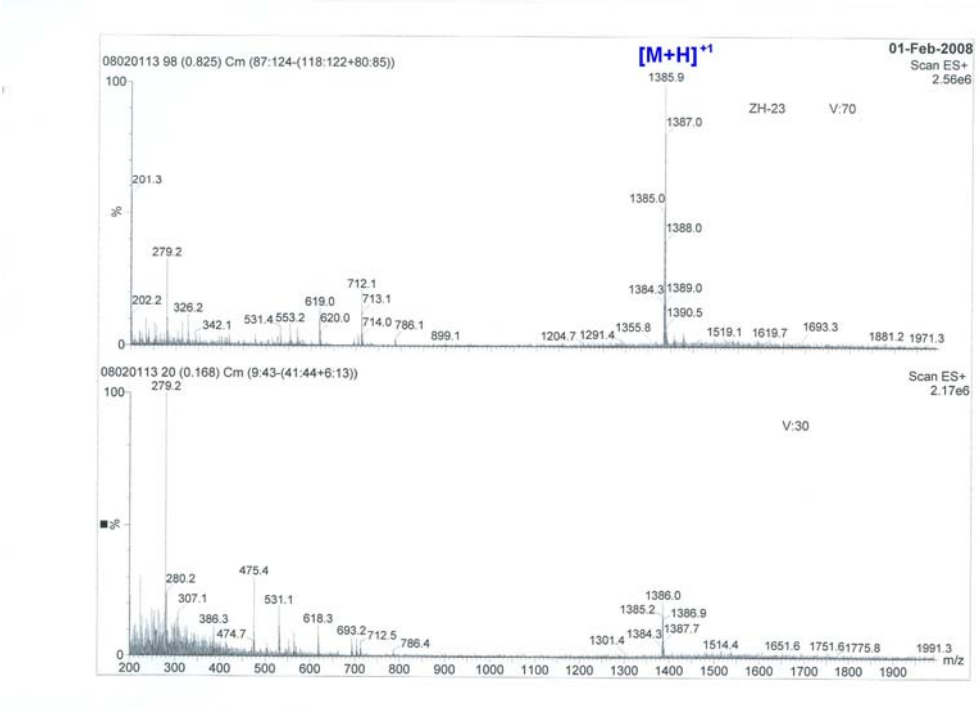

Figure S3. ESI-MS spectrum for **4**.

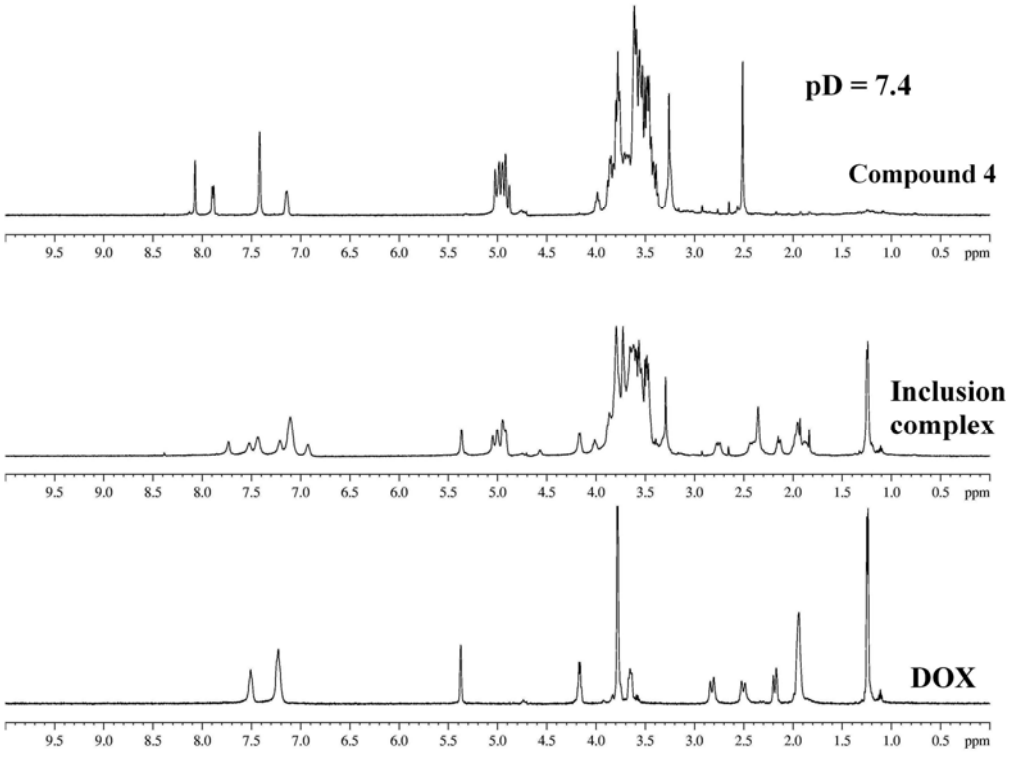

Figure S4. ^{1}H NMR spectra (500 MHz, D_2O, 298 K) for **4** (7.27 mM), **4** • (DOX) inclusion complex (7.27 mM), and DOX (7.27mM) at pD 7.4.

In control experiment, as shown in Fig S5, DOX binding with *β*-CD's cavity was accompanied by an increase in the fluorescence emission intensity. In Fig S6, a broad band occurred at 800-900 nm upon addition of *β*-carboline **1** to DOX solution. The red shift of broad band in Fig S6 indicated that the environment of π-π interaction between DOX and **1** was more hydrophilic.

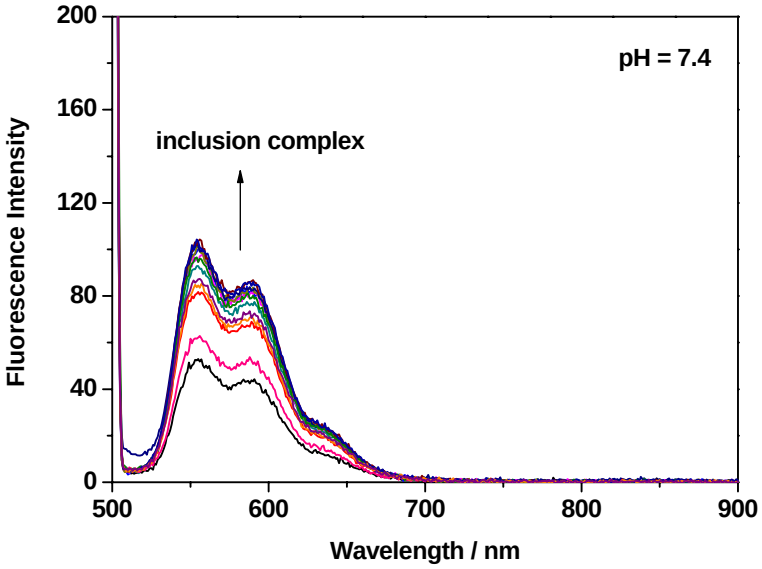

Figure S5. Fluorescence spectral changes for DOX (1.07 μM) upon addition of *β*-CD (from 0 to 106 μM) at pH 7.4.

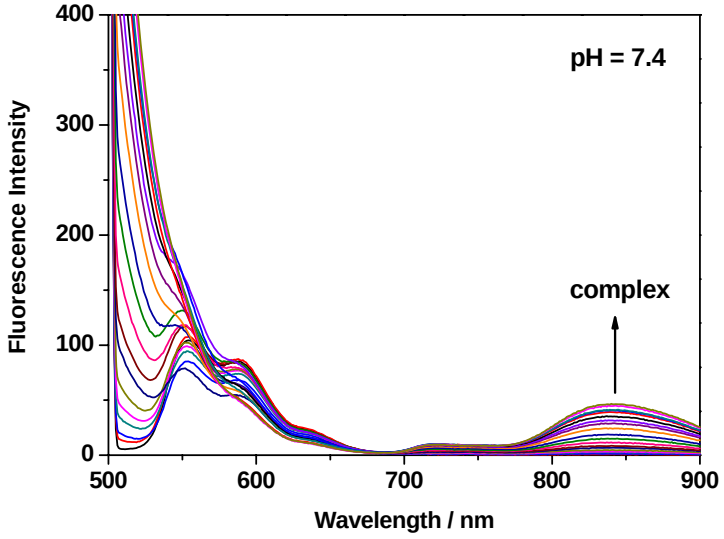

Figure S6. Fluorescence spectral changes for DOX (1.07 μM) upon addition of **1** (from 0 to 228 μM) at pH 7.4.

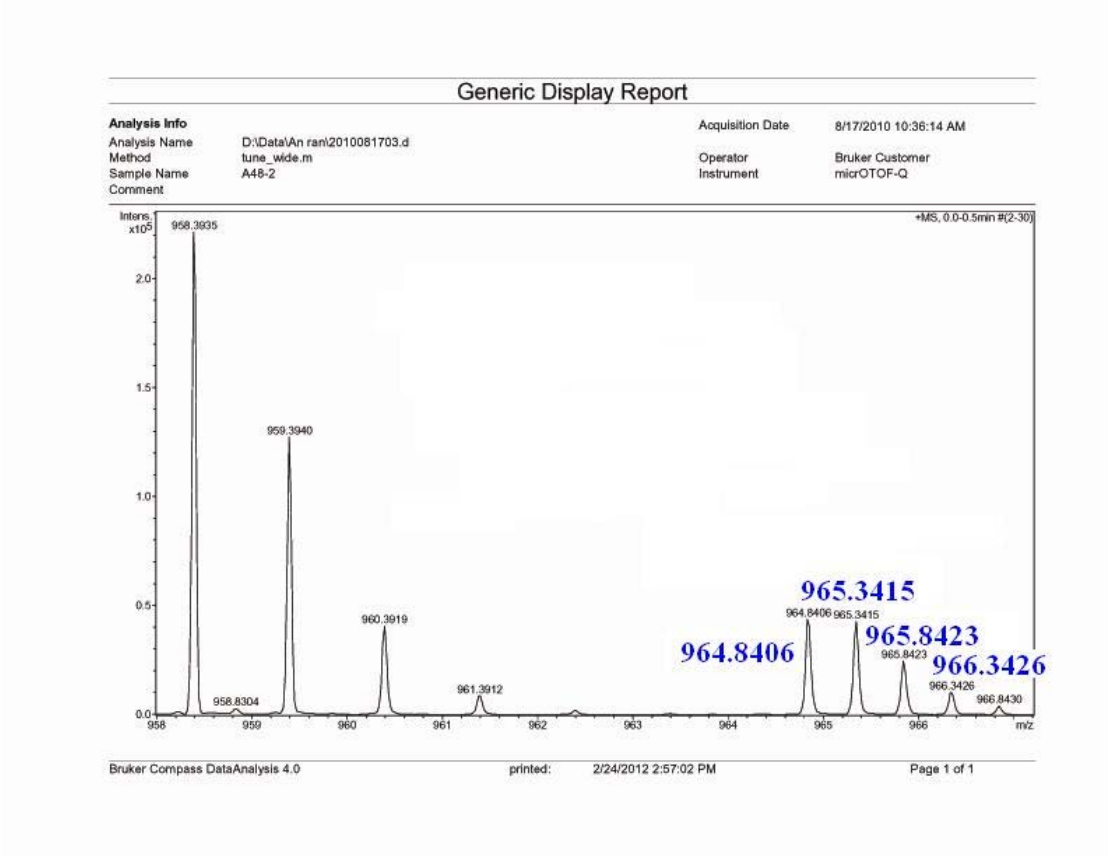

Figure S7. MS spectrum for inclusion complex

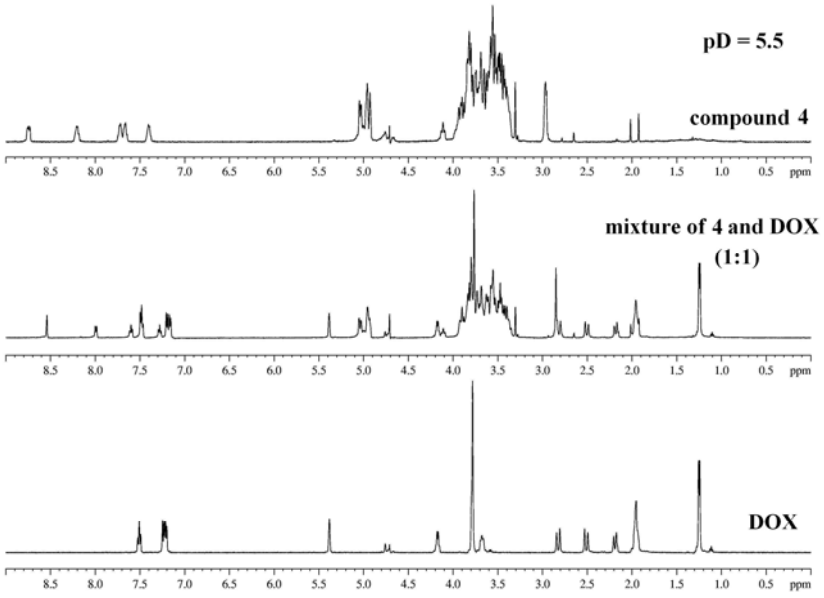

Figure S8. ^{1}H NMR spectra (500 MHz, D_2O, 298 K) for **4** (7.27 mM), 1:1 mixture of **4** (7.27 mM) and DOX (7.27mM), DOX (7.27mM) at pD 5.5.

In control experiment, as shown in Fig S9, a broad band appeared at 800-900 nm only upon addition of *β*-carboline **1** to DOX solution at pH 5.5.

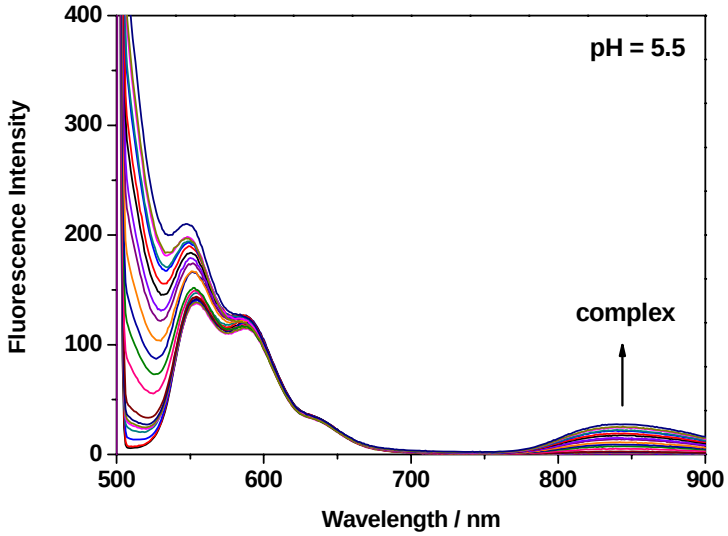

Figure S9. Fluorescence spectral changes for DOX (1.07 μM) upon addition of **1** (from 0 to 228 μM) at pH 5.5.

The control experiment of the fluorescence spectral changes of **4** at different pH proved that there was no interference for fluorescence spectral changes for DOX (Figure 2).

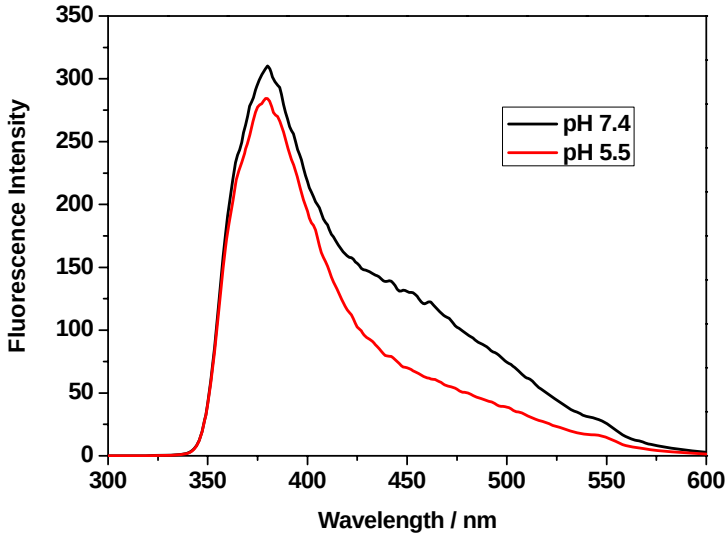

Figure S10. Fluorescence spectra for **4** (21.66μM) at pH 7.4 and pH 5.5, respectively.

Table S1. Dosing protocol for the survival study

Agent[a]	Dose (μ M/kg)	Tumor Weight (g)	% Survival
NS	0.2 ml/l	0.85 ± 0.07 [b]	100
Compound **4**	2	0.93 ± 0.14 [b]	100
	4	0.74 ± 0.14 [b]	100
	5	0.67 ± 0.25 [b]	100
	6	0.58 ± 0.09 [b]	92.31
DOX	2	0.46 ± 0.16 [b]	100
	4	0.26 ± 0.09 [b]	69.23
	5	0.15 ± 0.08 [b]	53.85
Inclusion Complex	2	0.45 ± 0.12 [b]	100
	4	0.32 ± 0.15 [b]	100
	5	0.19 ± 0.08 [b]	84.61

[a] Tumor weight is represented by X ± SD g; NS (normal saline) = vehicle; n = 13;

[b] Compared to NS $p < 0.01$.

References:

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2 Y. Liu, C.-C. You, B. Li, *Chem. Eur. J.* **2001**, *7*, 1281-1288.

3 Al-Allaf, T.A.K.; Rashan, L.J. *Eur. J. Med. Chem*. **1998**, 33, 817–820.