Electronic Supplementary Material (ESI) for ChemComm.

Supramolecular nanoprodrug based on boronate ester linked Curcumin complexing with water-soluble pillar[5]arene for synergistic chemotherapies

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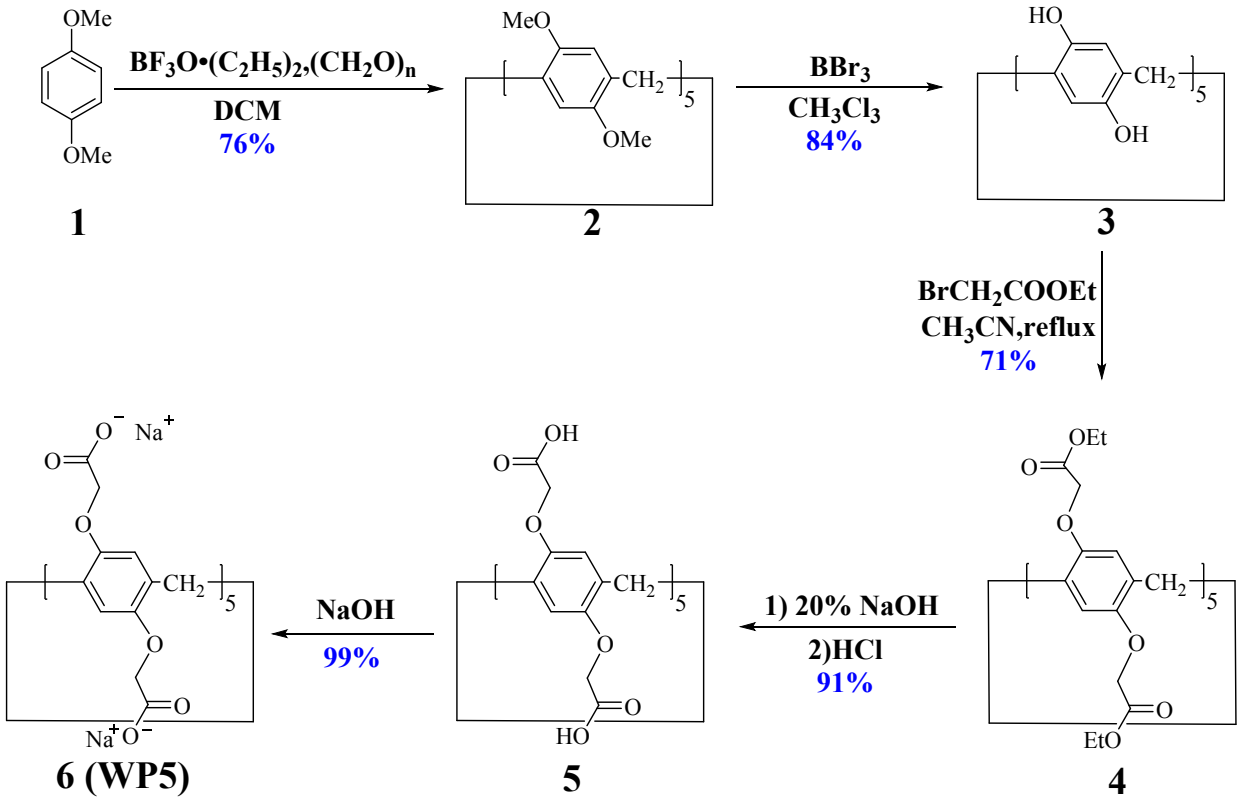
Supporting Information

1. General information

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled. Doxorubicin hydrochloride (Dox) was purchased from Sangon Biotech. ^{1}H NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei, respectively. SEM images were obtained using a S-4800 instrument (Hitachi Ltd.) with an accelerating voltage of 10.0 kV. DLS measurements were performed on a DelsaTM Nano system (Beckman Coulter, U.S.A.). UV-vis spectra were recorded with Shimadzu 1750 UV-visible spectrophotometer (Japan) at 298 K. Water surface tension was recorded with BZY-3B surface tension measurer (China). Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37 °C.

2. Synthesis and characterizations

Synthesis of compound WP5



Scheme S1: Synthesis of the host molecule WP5. [S1-S6]

As shown in Scheme S1, WP5 was synthesized and purified according to previously reported procedures (Scheme S1).[S1-S6] The ^{1}H NMR spectrum of 2 was shown in Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm):6.79 (s, 10H), 3.79 (s, 10H), 3.67 (s, 30H). The ^{1}H NMR spectrum of 4 was shown in Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) δ (ppm): 6.89 (s, 10H), 4.49 (s, 20H), 4.21 (q, J = 10.0 Hz, 20H), 3.89 (s, 10H), 1.24 (t, J = 10.0 Hz, 30H). The ^{1}H NMR spectrum of 5 was shown in Figure S3. ^{1}H NMR (500 MHz, DMSO-d_6, 298 K) δ (ppm): 12.97 (s, 10H), 7.10 (s, 10H), 4.71 (q, J = 15.0 Hz, 10H). 4.45 (q, J = 15.0 Hz, 10H), 3.76 (s, 10H). The ^{1}H NMR spectrum of 6 was shown in Figure S4. ^{1}H NMR (500 MHz, D_2O, 298 K) δ (ppm): 6.73 (s, 10H), 4.23 (s, 10H), 3.84 (s, 10H).

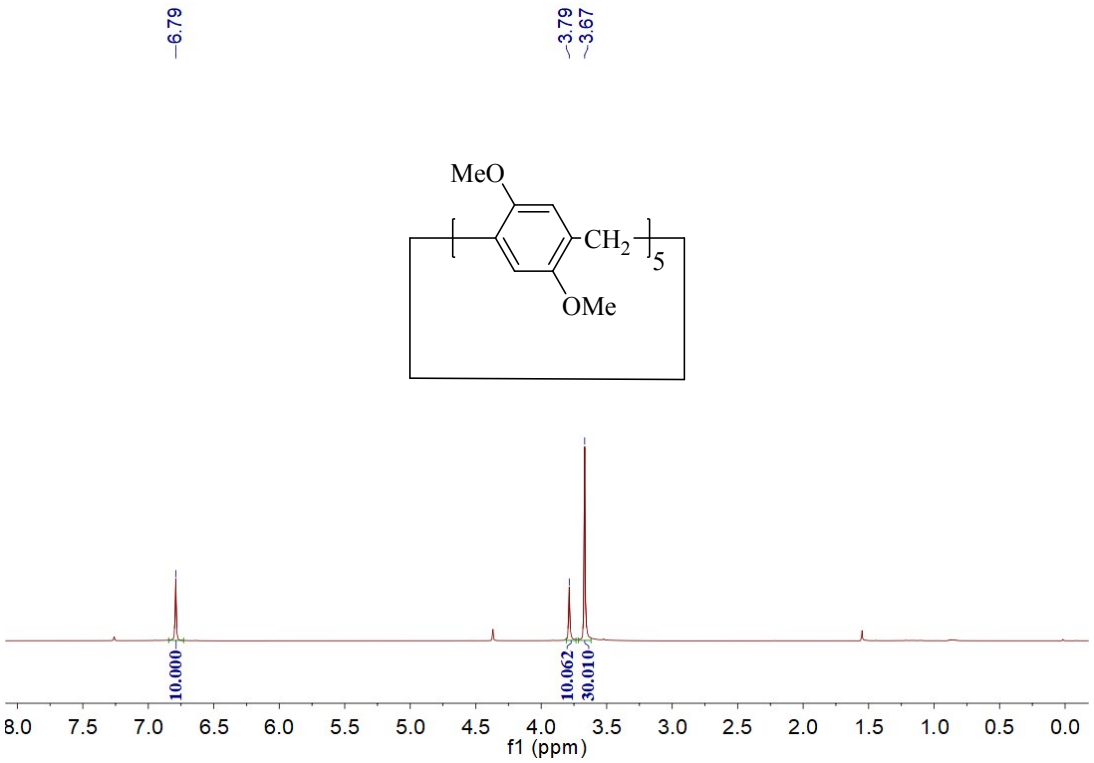


Figure S1: ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectrum of Compound 2.

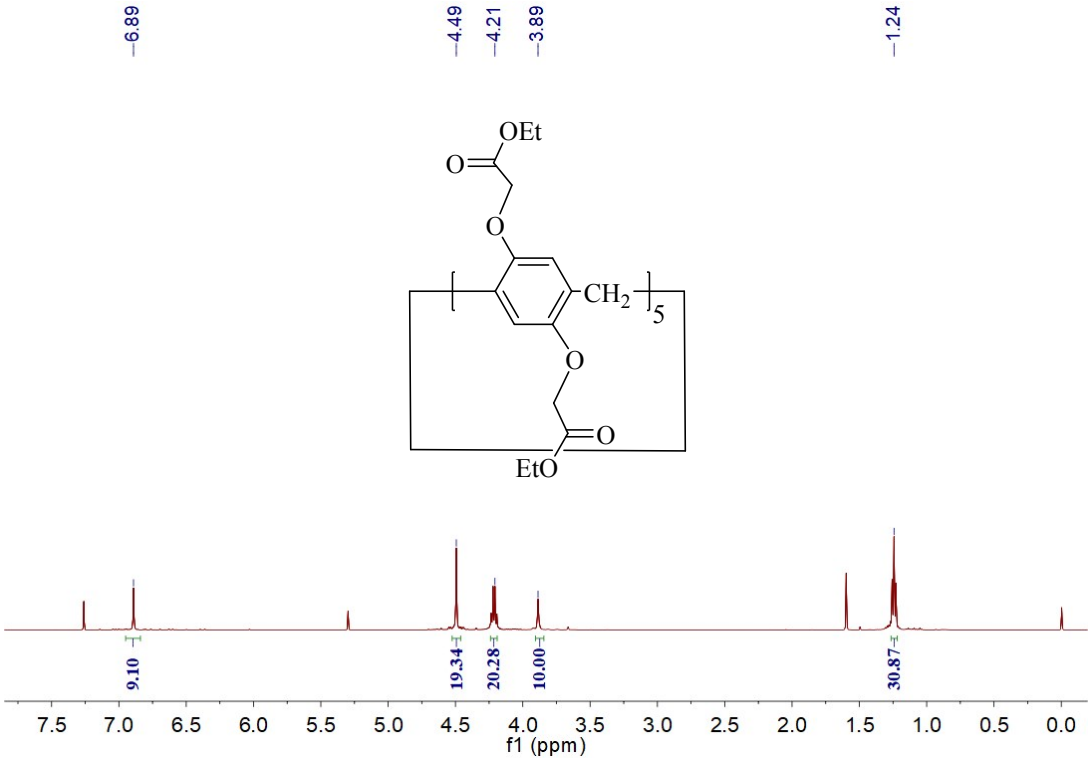


Figure S2: ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectrum of Compound 4.

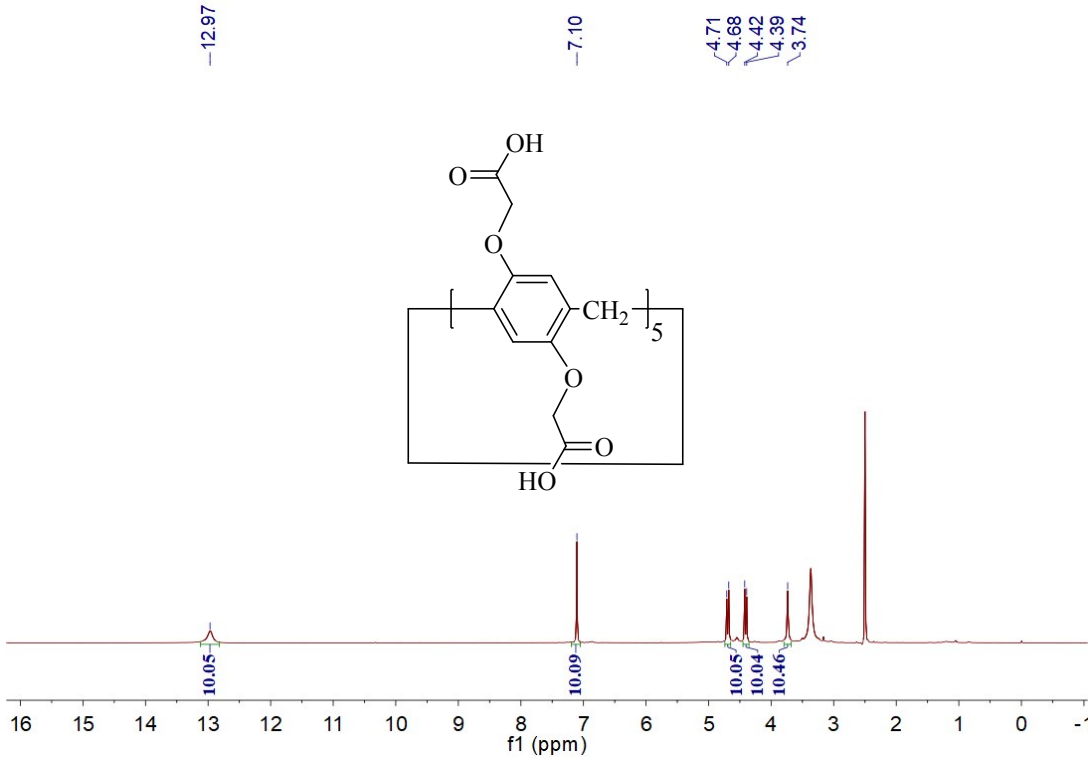


Figure S3: ^{1}H NMR (500 MHz, DMSO-d_6, 298 K) spectrum of Compound 5.

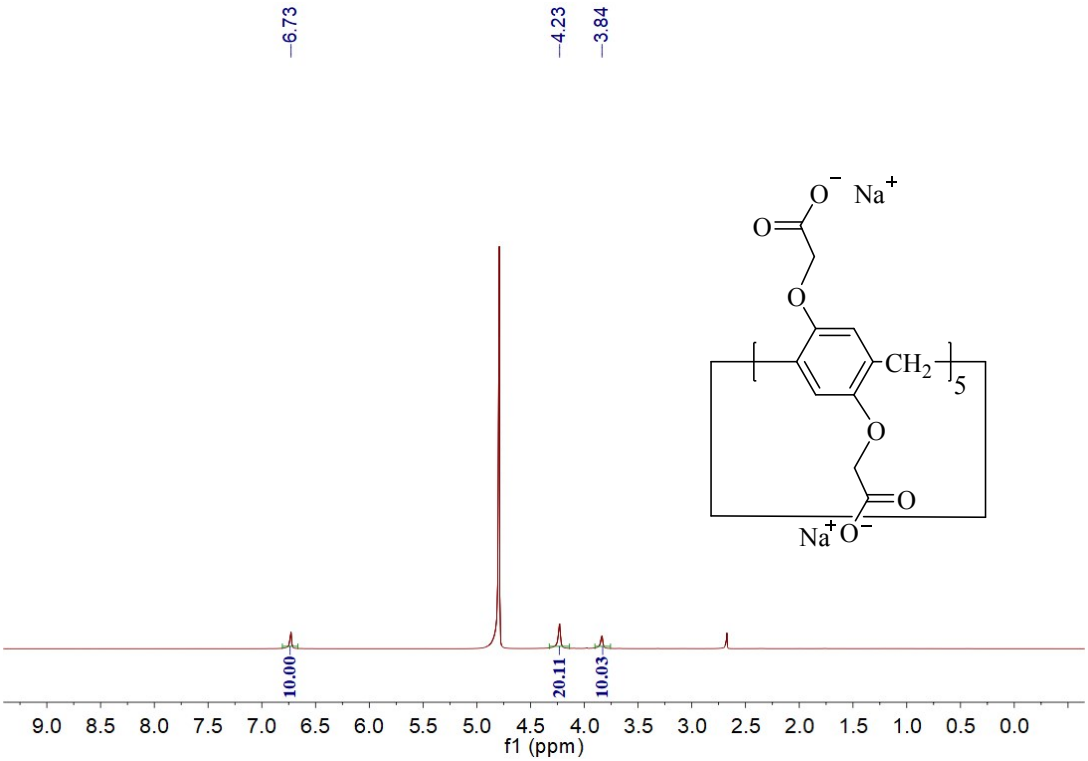
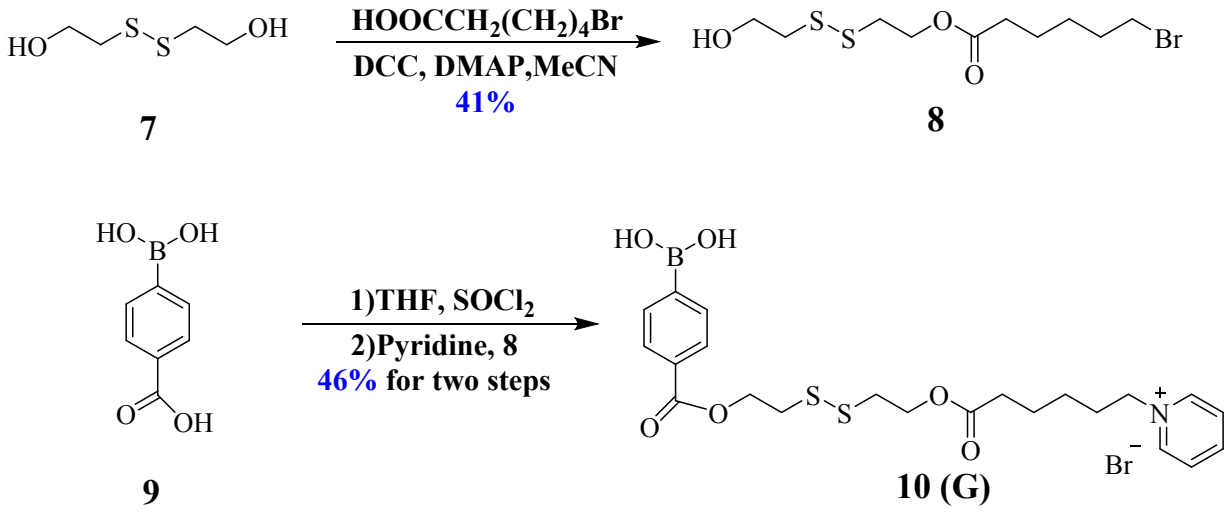


Figure S4: ^{1}H NMR (500 MHz, D_2O, 298 K) spectrum of Compound 6.

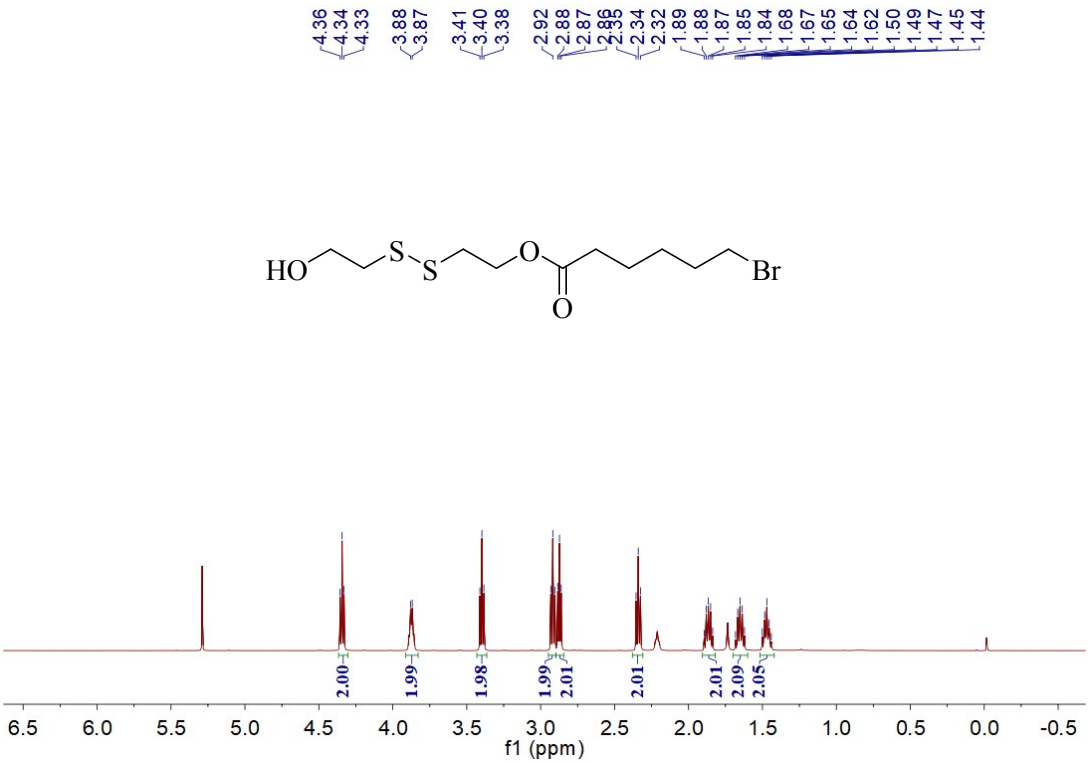
Synthesis of guest molecule G:



Scheme S2: Synthesis of the guest molecule G.

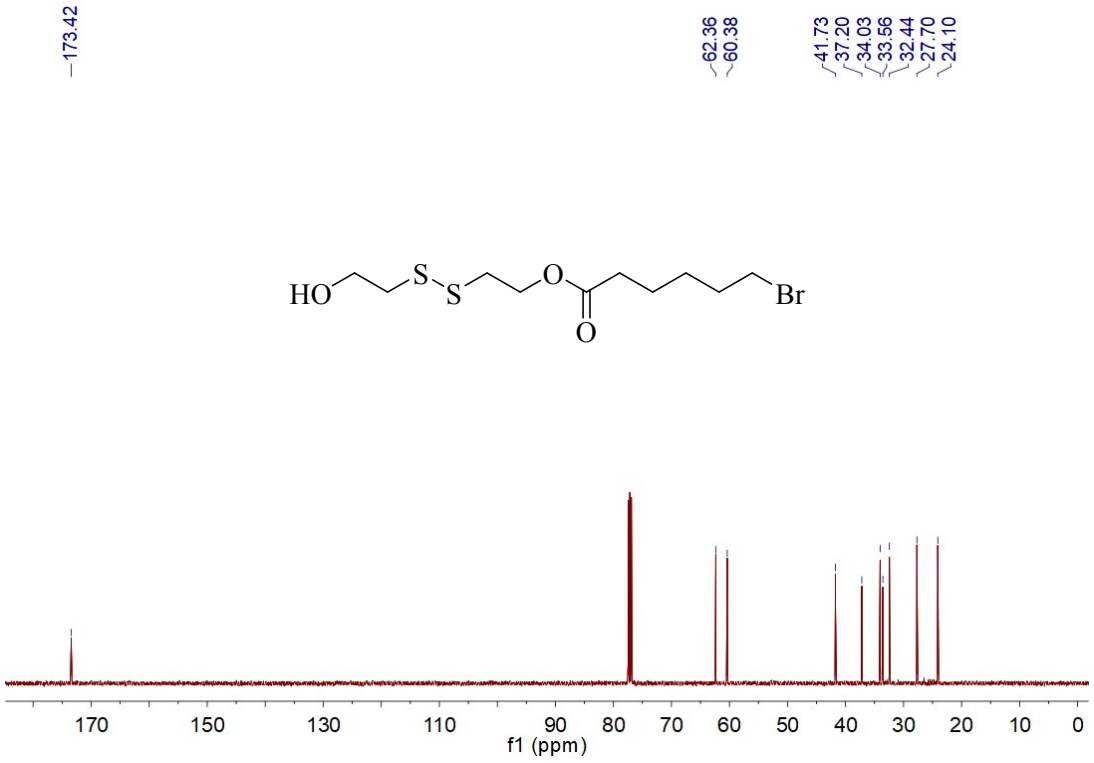
Synthesis of compound 8

A magnetically stirred solution of bis(2-hydroxyethyl) disulfide **7** (0.772 g, 5 mmol) and 6-bromohexanoic acid (0.975 g, 5 mmol) in MeCN (20 mL) was treated with DCC (1.236 g, 6 mmol) and DMAP (0.061 g, 0.5 mmol), and the ensuing solution was stirred for 72 hours at room temperature before being filtrated. The resulting filtrate was concentrated by rotary evaporator to obtain the crude product residue. This residue was dissolved in DCM and then washed with H_2O and sat. NaCl (3×10 mL), successively. The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography, using petroleum ether/ethyl acetate (PE/EA = 6:1, *v/v*) as eluent to afford a white solid (0.68 g, 2 mmol, 41 %). ^{1}H NMR(500 MHz, $CDCl_3$, 298 K) δ (ppm): 4.34 (t, J = 5.0 Hz, 2H), 3.88 (q, J = 5.0 Hz, 2H), 3.40 (t, J = 10.0 Hz, 2H), 2.92 (t, J = 10.0 Hz, 2H), 2.88 (t, J = 10.0 Hz, 2H), 2.34 (t, J = 10.0 Hz, 2H), 1.87 (m, 2H), 1.65 (m, 2H), 1.47 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ (ppm): 173.42, 62.36, 60.38, 41.73, 37.20, 34.03, 33.56, 32.44, 27.70, 24.10. MS



(ESI) $C_{10}H_{19}BrO_3S_2$ *calc.* for $[M+NH_4]^+$ 349.3367; found 350.0057.

Figure S5: ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectrum of Compound **8**.



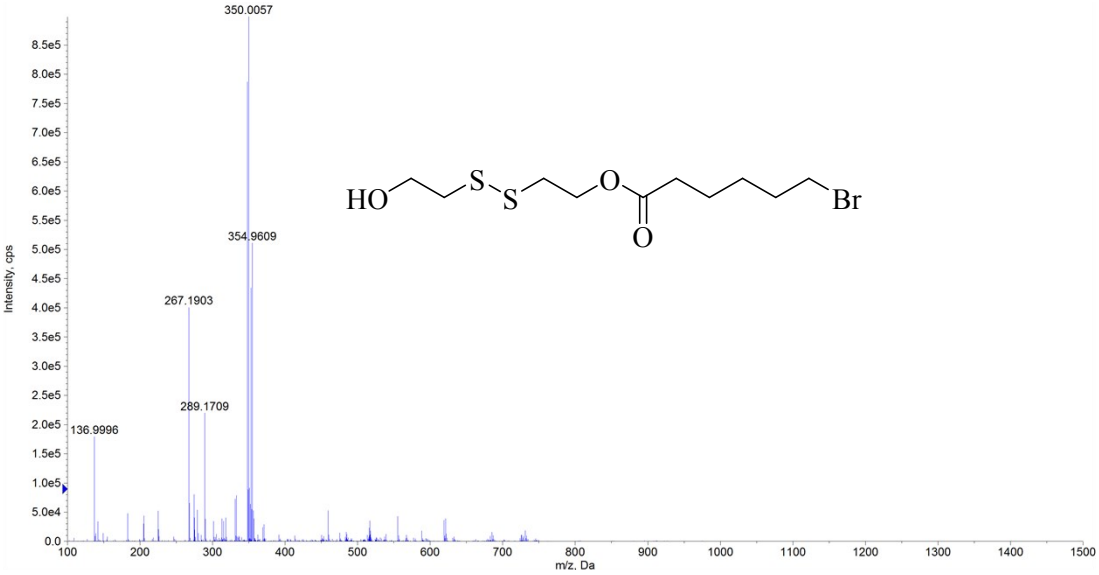
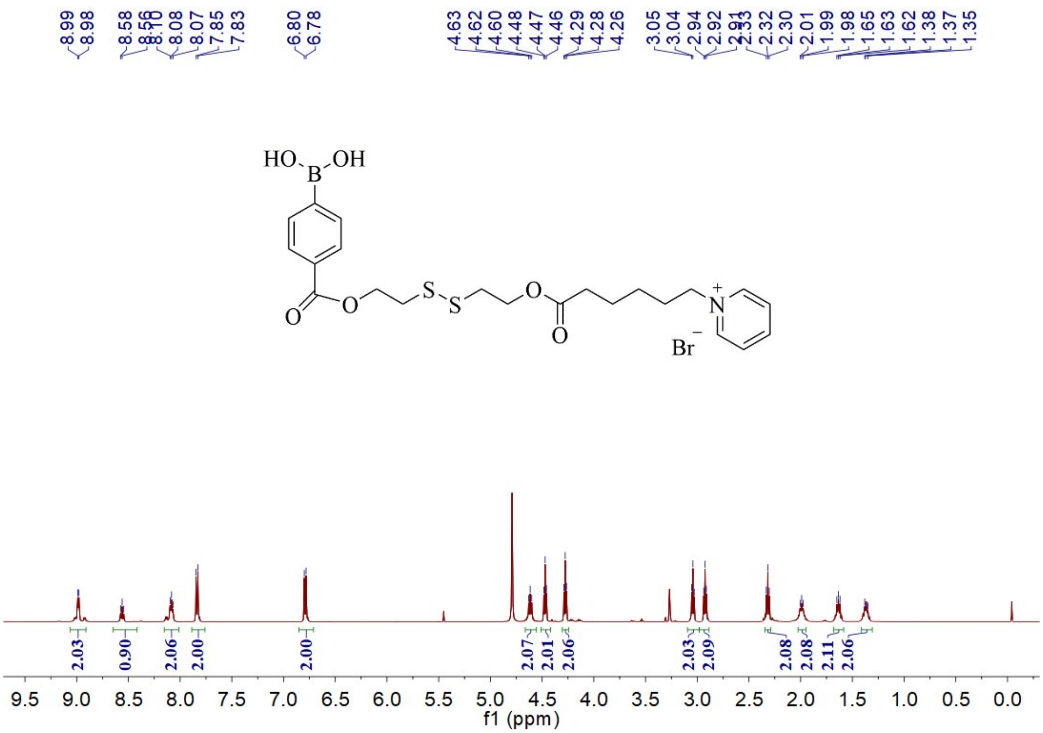


Figure S6: ^{13}C NMR (500 MHz, $CDCl_3$, 298 K) spectrum of Compound 8.

Figure S7: HR-MS spectrum of compound 8.

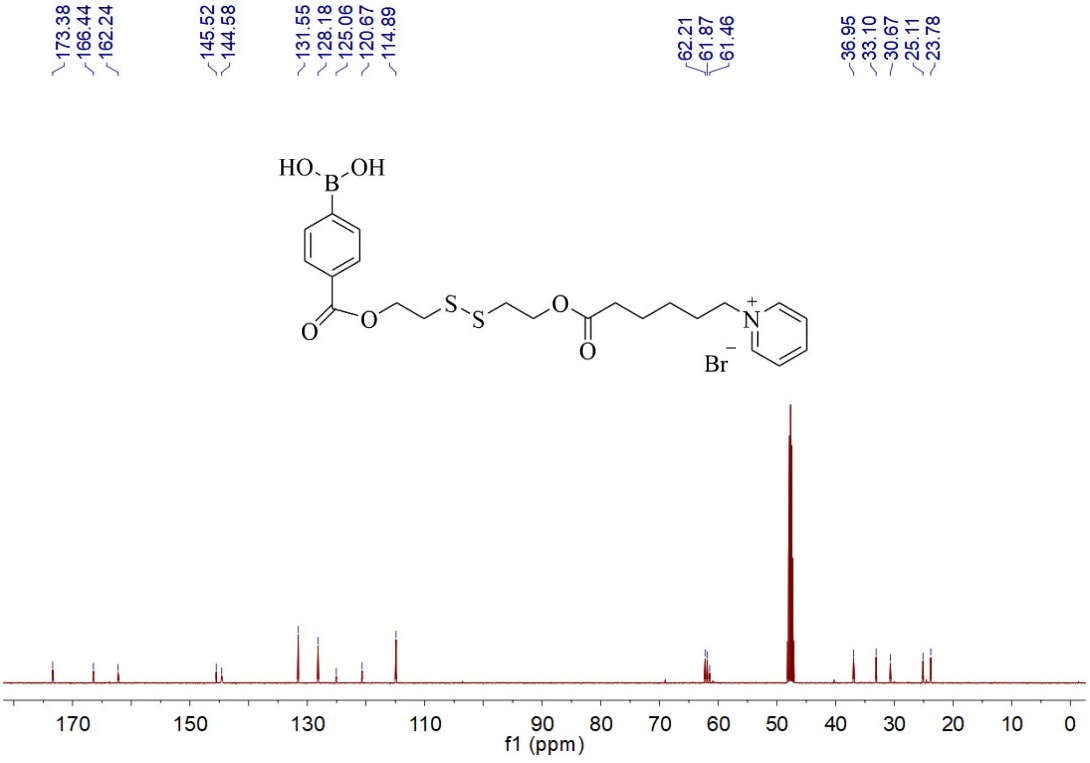
Synthesis of compound G

A magnetically stirred solution of 4-boronobenzoic acid 9 (0.830 g, 5 mmol) in anhydrous THF (15 mL) was treated with sulfurous dichloride (0.975 g, 5 mmol), and the ensuing solution was stirred for 12 hours at room temperature before being concentrated by rotary evaporator to remove the solvent. The resulting residue was dissolved in pyridine and treated with compound 8 (0.662 g, 2 mmol), the mixture was reflux under nitrogen atmosphere for 24 h before being cooled to room temperature. After removal of the solvent under reduced pressure, the crude product was purified by silica gel chromatography using DCM/MeOH (10:1, *v/v*) as the eluent to afford compound G as a pale solid (1.1 g, 2 mmol, 46 %). ^{1}H NMR(500 MHz, CD_3OD, 298 K) δ (ppm): 9.03 (d, *J* = 5.0 Hz, 2H), 8.60 (t, *J* = 5.0 Hz, 1H), 8.14 (t, *J* = 10.0 Hz, 2H), 7.88 (d, *J* = 10.0 Hz, 2H), 6.84 (d, *J* = 10.0 Hz, 2H), 4.66 (t, *J* = 10.0 Hz, 2H), 4.51 (t, *J* = 5.0 Hz, 2H), 4.32 (t, *J* = 5.0 Hz, 2H), 3.08 (q, *J* = 5.0 Hz, 2H), 2.97 (t, *J* = 5.0 Hz, 2H), 2.36 (t, *J* = 10.0 Hz, 2H), 2.03 (m, 2H), 1.68 (m, 2H), 1.41 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD, 298 K) δ (ppm): 173.38, 166.44, 162.24, 145.52, 144.58, 131.55, 128.18, 125.06, 120.67, 114.89, 62.21, 61.87, 61.46, 36.95, 33.10, 30.67, 25.11, 23.78.



MS (ESI) $C_{22}H_{29}BNO_3S_2$ calc. for $[M]^+$ 478.3774; found 478.1486.

Figure S8: ^{1}H NMR (500 MHz, CD_3OD, 298 K) spectrum of Compound G.



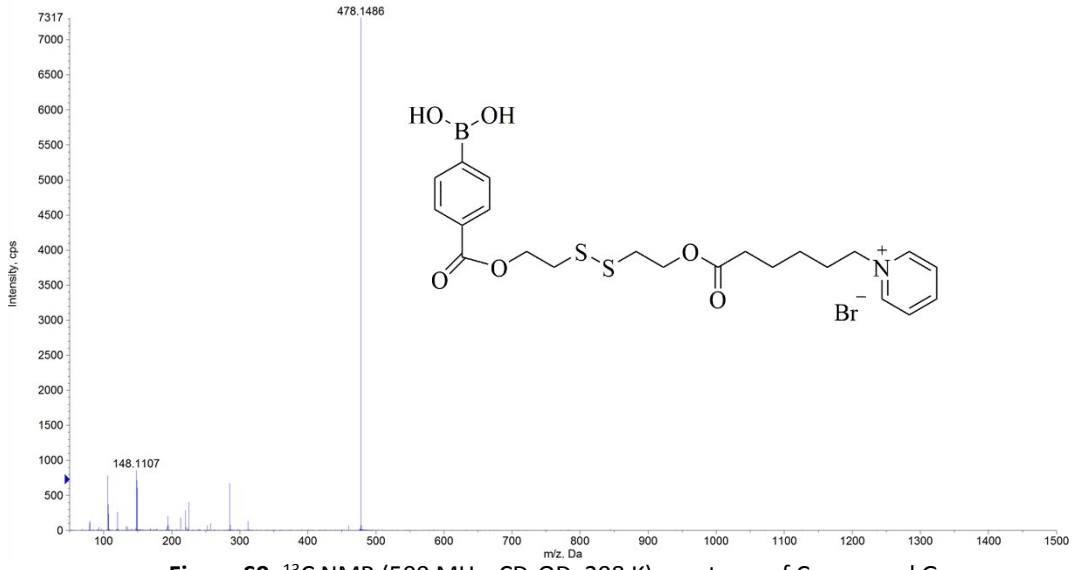
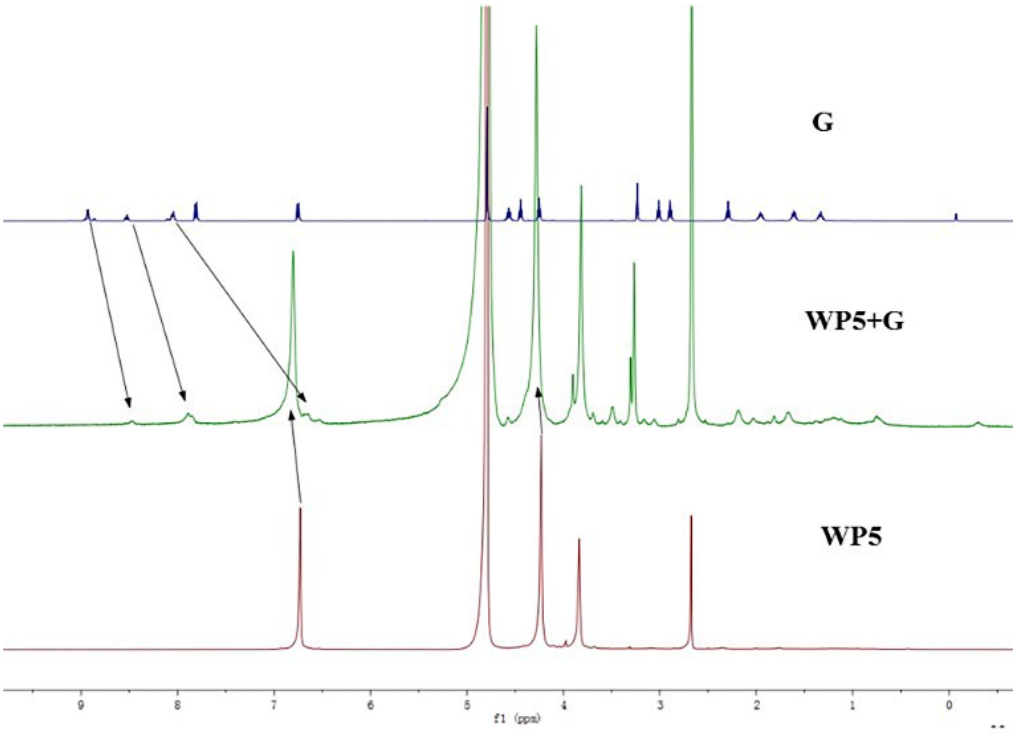


Figure S9: ^{13}C NMR (500 MHz, CD_3OD, 298 K) spectrum of Compound G.

Figure S10: HR-MS spectrum of compound G.



3. Host-guest complexation of WP5 and G

Fig. S11: ^{1}H NMR spectra (500 MHz, CD3OD:D2O = 1:1, 298 K) of G (10.00 mM), WP5+G (WP5:G = 1:1) and WP5 (10.00 mM).

4. Job's Plot for WP5G

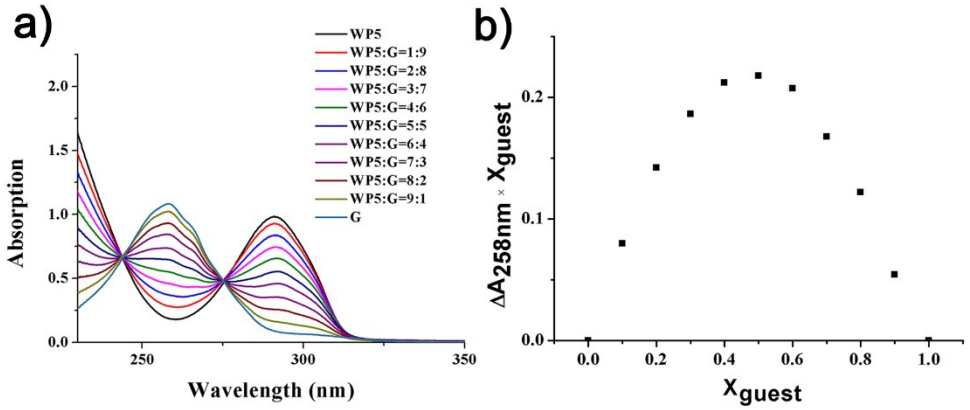


Figure S12: (a) Fluorescence intensity of the mixture of WP5 and G in water at different molar ratio while [WP5] + [G] = 5 ×10^{-5} M. (b) Job's Plot showing 1:1 stoichiometry of the complex between WP5 and G by plotting the difference fluorescence intensity at 258 nm.

5. Raw ITC data of WP5 with G in water

The binding constant between WP5 and G was performed using a thermostated and fully computer operated Nano-ITC SV calorimeter purchased from TA-Waters LLC. The microcalorimetric titrations were performed in D.I. water at atmospheric pressure. Each solution was degassed and thermostated before titration. [S7]

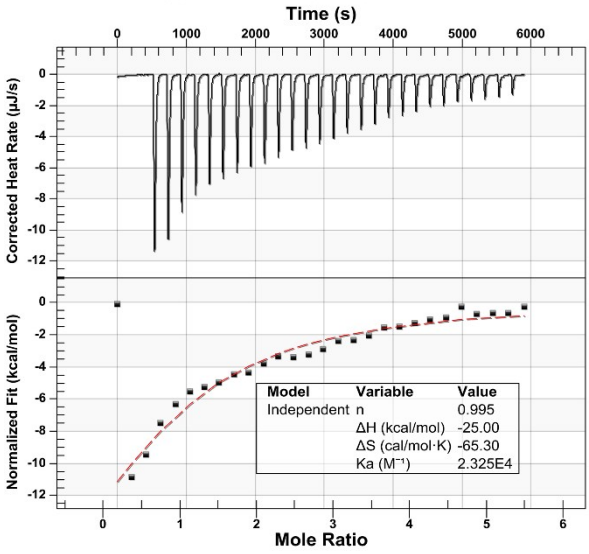


Figure S13: Microcalorimetric titration of WP5 and G in D.I. water at 298.15K. (TOP) Raw ITC data for 28 sequential injections (3.54 µL per injection) of a WP5 solution (2.00 mM) into a G solution (0.10 mM). (Bottom) Net reaction heat obtained from the integration of the calorimetric traces.

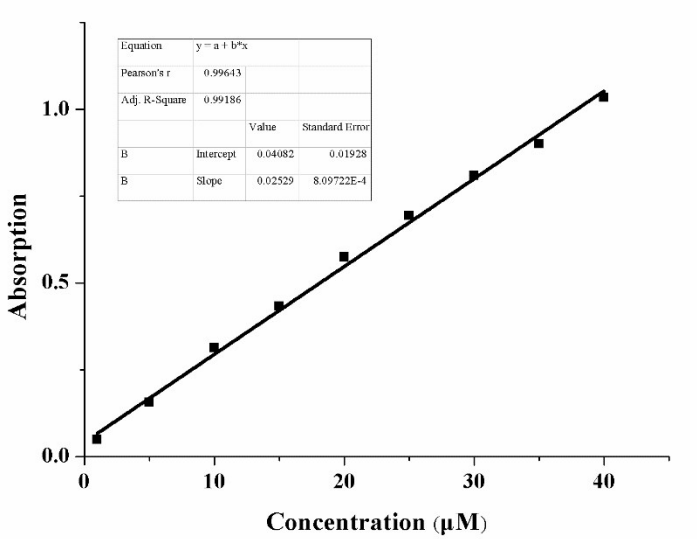
6. Preparation of WP5G-Cur

Firstly, WP5 (0.01 mmol) and G (0.01 mmol) were dissolved in 10 mL PBS (pH=8.5), and the resultant suspension was sonicated for 2 h at room temperature. Subsequently, a solution of Cur (0.01 mmol) in 5 mL of PBS (pH=8.5) and MeOH (*v:v* 9:1) was drop-wised into the above suspension under dark circumstance, and the mixture was reacted for 12 h at room temperature. Then, in order to remove the residual organic solvent in the reaction system, the mixed solution was dialyzed with distilled water for 24 h (molecular weight cut-off (MWCO) = 2,000 Da). After

centrifugation of the resulting mixture at 8,000 rpm for 5 min to remove any precipitates, the obtained solution was stocked at 4 °C for further use.

7. Dox Loading of WP5G-Cur

Firstly, a standard curve of Dox has been drawn. To prepare Dox loaded WP5G-Cur prodrug nanoparticles (Dox@WP5G-Cur), both Dox·HCl and WP5G-Cur were dissolved in PBS (pH=7.4) with the ratio of 1:1. The resultant mixture was sonicated for 2 h under dark circumstance, and then the mixture was left for 24h at room temperature to load Dox into the WP5G-Cur prodrug nanoparticles. After that, the unloaded Dox was removed by dialysis (molecular weight cut-off (MWCO) = 2,000 Da) against distilled water for 24 h until water outside the dialysis tube exhibited negligible Dox fluorescence. The Dox loading capability was calculated using the following equation:



Loading Capability(%) = ($m_{Dox\ loaded}/m_{Dox@WP5G\text{-}Cur}$) × 100%

$m_{Dox\ loaded}$ and $m_{Dox@WP5G\text{-}Cur}$ refer to the mass of Dox encapsulated in WP5G-Cur and the mass of the Dox@WP5G-Cur, respectively.

Figure S14: The standard curve of Dox (λ abs = 494 nm).

8. DLS and TEM of Dox@WP5G-Cur

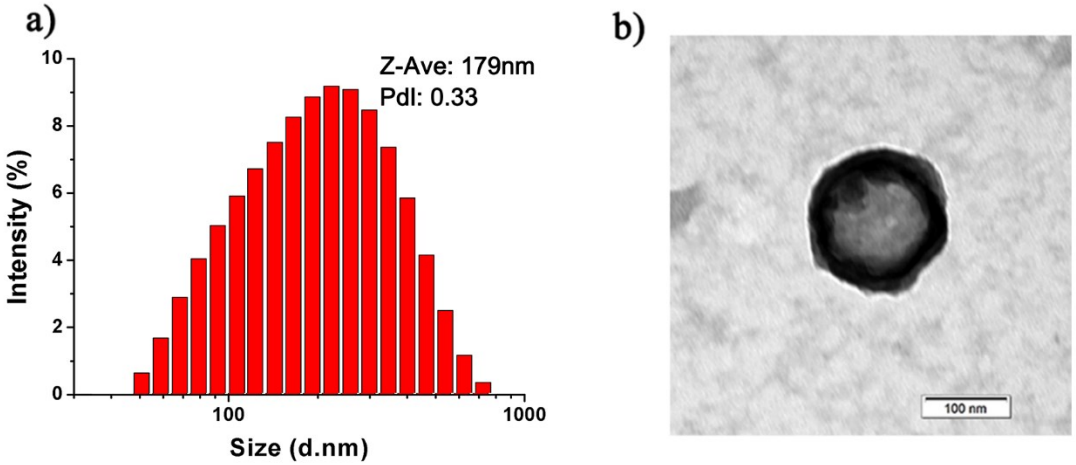


Figure S15: a) DLS of Dox@WP5G-Cur nanoparticles; b) TEM image of Dox@WP5G-Cur nanoparticles.

9. Confocal laser scanning microscopy (CLSM)

HepG2 cells and HepG2/ADR were seeded in 35 mm plastic bottomed μ-dishes for 24 h. The medium was replaced with a fresh one. Then the cells were incubated with free Dox, free Dox & Cur and Dox@WP5G-Cur for 4 h (The concentration of Dox is 5 μM). Next, the dishes were washed with PBS for three times before being added with 4% formaldehyde solution. Subsequently, the cells were stained with 5 M DAPI for 10 min. Finally, the cells were washed with PBS and then observed under a CLSM.

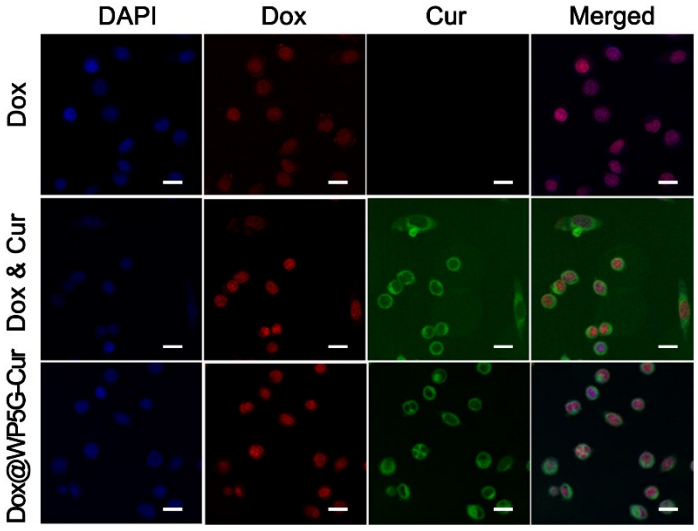


Figure S16: CLSM of HepG2 cells incubated with free Dox, free Dox & Cur and Dox@WP5G-Cur for 4 h. The scale bar is 20 μm.

10. Cytotoxicity evaluation

HepG2 cells and HepG2/ADR cells were cultured in RPMI 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete RPMI 1640 medium) in 5% CO_2 at 37 °C. 293T cells were cultured in DMEM medium containing 10% FBS, 1% penicillin/streptomycin (complete DMEM 1640 medium) in 5% CO_2 at 37 °C. The relative cytotoxicity of free Dox, free Dox & Cur and Dox@WP5G-Cur were evaluated *in vitro* by MTT assay, respectively. The cells were seeded in 96-well plates and incubated with the corresponding compounds at different concentrations for 24 h, 48 h. Subsequently, cells were washed and the fresh medium containing MTT (0.5 mg/mL) was added into each plate. The cells were incubated for another 4 h. After that, the medium containing MTT was removed and dimethyl sulfoxide (100 μL) was added to each well to dissolve the formazan crystals. Finally, the plate was gently shaken for 10 min and the absorbance at 490 nm was recorded with a microplate reader.

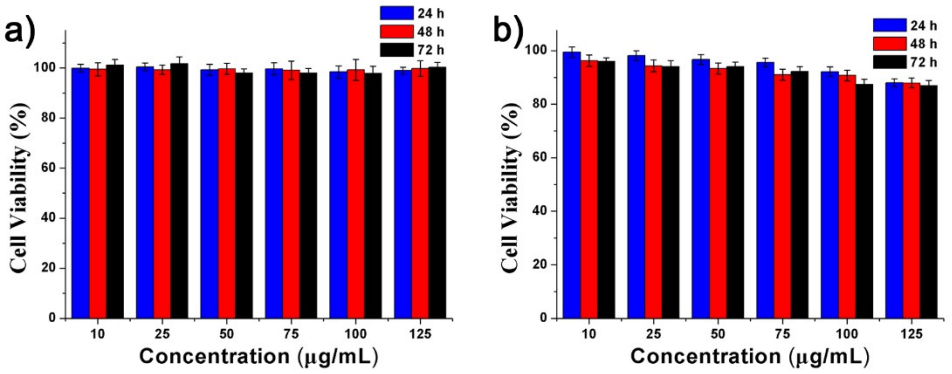


Figure S17: *In vitro* cytocompatibility of WP5G and WP5G-Cur nanorodrug against 293T normal cells after incubation for 24 h, 48 h and 72 h, respectively.

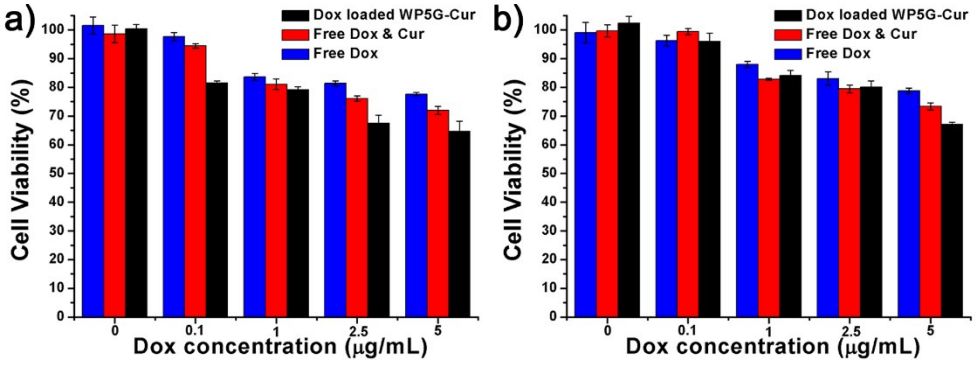


Figure S18: In vitro cytotoxicity results of free Dox, free Dox & Cur and Dox@WP5G-Cur on HepG2 cells (a) and HepG2/ADR cells (b) for 24 h.

11. References

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