Supplementary information

Nuclear delivery of dual anti-cancer drugs by molecular self-assembly

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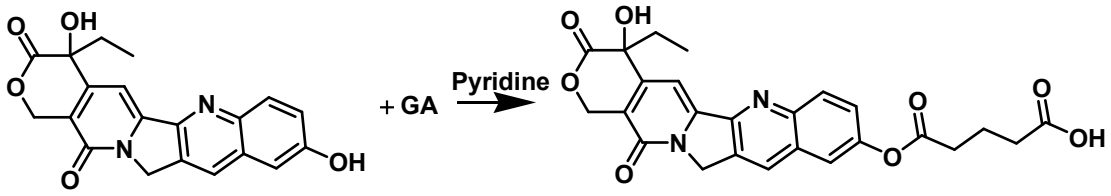
Synthesis of HCPT-GA:

0.3 g of 10-Hydroxycamptothecin and 0.282 g of Glutaric anhydride were dissolved in 60 mL of Pyridine and the resulting mixture was stirred at room temperature and in dark for 48 h. The Pyridine was then removed by a rotary evaporator. When adding 2 M HCl solution, we obtained yellowish solid (HCPT-GA) with the yield of 85 % and then it was directly used for solid phase peptide synthesis.



Scheme S1. The synthetic route of HCPT-GA

Synthesis of peptide derivatives

Peptide derivatives of CRB-K (HCPT) FFYG-PMI (***Comp. 1***), CRB-K (Nap) FFYG-PMI (***Comp. 2***), HCPT-K (Nap) FFYG-PMI (***Comp. 3***), CRB-K (HCPT) FFYG-scrambled PMI (***Comp. 4***), and CRB-K (HCPT) GGGG-PMI (***Comp. 5***) were prepared by standard Fmoc solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding amino acids with side chains protected by N-Fmoc. The C-terminal of the first aminoacid was excitated and conjugated to the resin. After washing by dichloromethane (DCM) and Anhydrous N, N'-dimethyl formamide (DMF), DMF containing 20 % piperidine was used to remove Fmoc protected group. To link the next amino acid to the free amino group, O-Benzotriazol-1-yl-N, N, N', N'-tetramethyluronium hexafluorophosphate (HBTU) was used as coupling reagent. After CRB or coupling to the last amino acid, excessive reagents were removed by 5 times DMF washing and following 5 times DCM washing. Lastly, 95 % trifluoroacetic acid (TFA) containing 2.5 % trimethylsilane (TIS) and 2.5 % H_2O was used to cleave peptide derivatives from resin. Nap or HCPT-GA were coupled with $-NH_2$ in Lysine by liquid phase reaction. And then the final products were purified by HPLC and lyophilization.

Characterization of HCPT-GA and peptide derivatives:

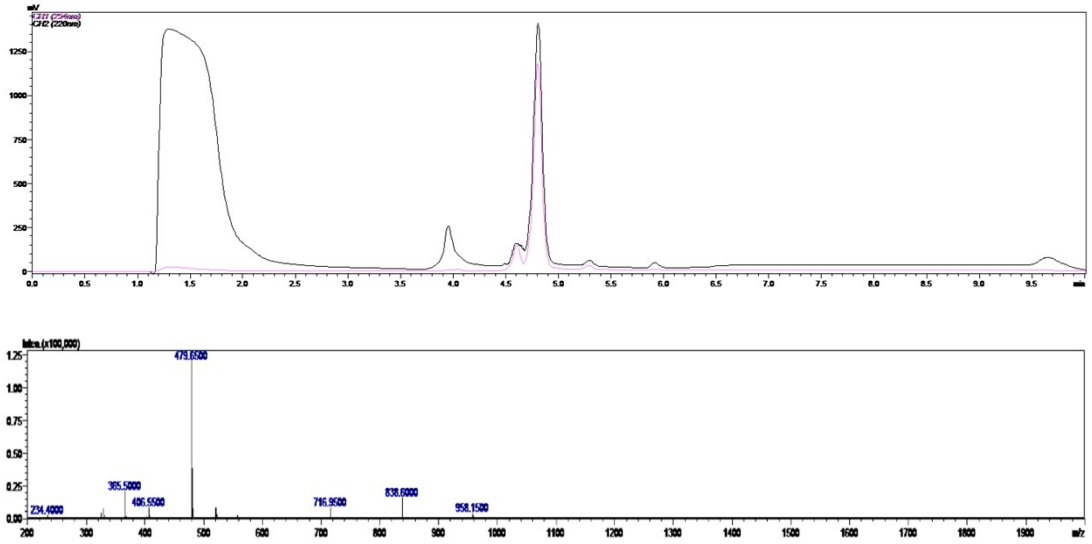


Figure S1. LC-MS spectrum of HCPT-GA

CRB-K (HCPT) FFYG-PMI (*Comp. 1*): ^{1}H NMR (400 MHz, DMSO) δ 8.64 (s, 1H), 8.28 (s, 1H), 8.07 (s, 1H), 7.84 (d, *J* = 35.6 Hz, 1H), 7.72 – 7.54 (m, 1H), 7.41 (s, 1H), 7.19 (d, *J* = 18.3 Hz, 1H), 6.99 (dd, *J* = 34.4, 12.8 Hz, 1H), 6.60 (dd, *J* = 27.5, 15.7 Hz, 1H), 5.41 (s, 1H), 5.30 (s, 1H), 4.95 (s, 1H), 4.45 (s, 1H), 4.41 – 4.22 (m, 1H), 3.17 (d, *J* = 8.0 Hz, 1H), 2.90 (s, 1H), 2.61 (s, 1H), 2.08 (s, 1H), 1.85 (d, *J* = 38.5 Hz, 1H), 1.75 – 1.56 (m, 1H), 1.39 (s, 1H), 1.12 (s, 1H), 0.97 (s, 1H), 0.84 (dd, *J* = 20.6, 5.6 Hz, 1H). MS: calc. M = 2816.93.

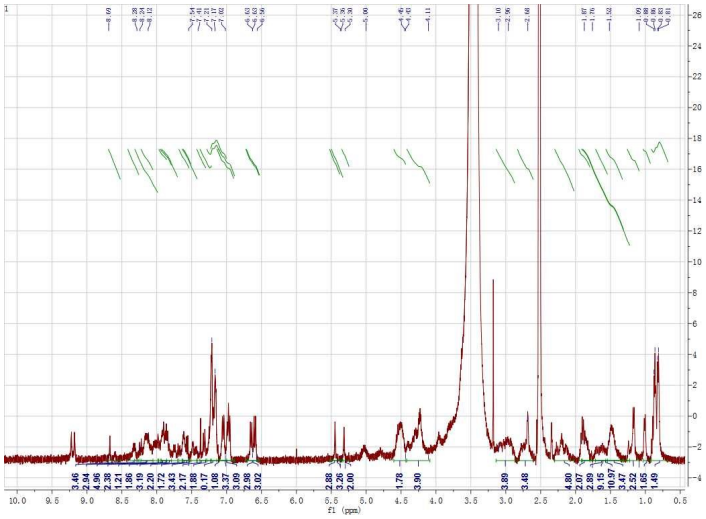


Figure S2. ^{1}H NMR spectrum of ***Comp. 1***

CRB-K (Nap) FFYG-PMI (*Comp. 2*): ^{1}H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 43.2 Hz, 3H), 7.88 (s, 3H), 7.52 (s, 1H), 7.20 (d, *J* = 29.7 Hz, 7H), 7.04 (s, 2H), 6.67 (s, 1H),

4.53 (s, 3H), 4.26 (s, 3H), 3.71 (s, 6H), 3.46 (s, 1H), 3.13 (s, 1H), 2.98 (s, 3H), 2.76 (s, 2H), 2.15 (d, *J* = 48.3 Hz, 3H), 1.92 (s, 1H), 1.72 (s, 2H), 1.62 (s, 1H), 1.49 (s, 2H), 1.29 (s, 2H), 1.19 (s, 2H), 1.03 (s, 1H), 0.86 (d, *J* = 17.5 Hz, 5H). MS: calc. M = 2524.69.

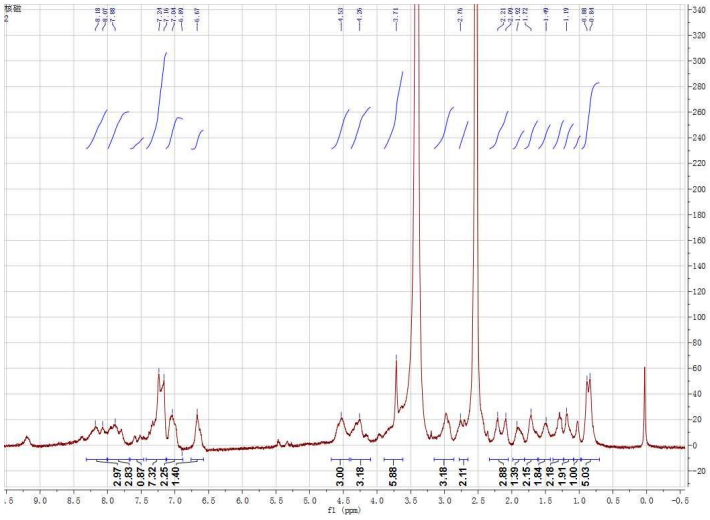


Figure S3. ^{1}H NMR spectrum of ***Comp. 2***

HCPT-K (Nap) FFYG-PMI (***Comp. 3***): ^{1}H NMR (400 MHz, DMSO) δ 8.12 (s, 5H), 7.98 (s, 5H), 7.19 (d, J = 23.8 Hz, 14H), 7.05 (s, 6H), 6.66 (s, 2H), 4.52 (s, 5H), 4.34 (s, 4H), 3.71 (s, 11H), 2.97 (s, 8H), 2.76 (s, 5H), 2.21 (s, 4H), 2.09 (s, 4H), 1.91 (s, 5H), 1.72 (s, 4H), 1.65 – 1.55 (m, 2H), 1.46 (s, 5H), 1.27 (s, 5H), 1.19 (s, 7H), 1.06 – 0.97 (m, 2H), 0.85 (s, 13H). MS: calc. M = 2698.93.

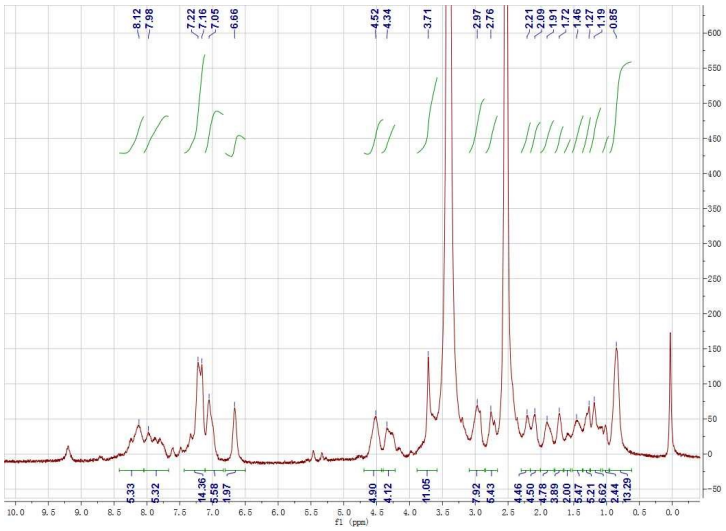


Figure S4. ^{1}H NMR spectrum of ***Comp. 3***

CRB-K (HCPT) FFYG-scrambled PMI (***Comp. 4***): ^{1}H NMR (400 MHz, DMSO) δ 8.19 (d, *J* = 23.7 Hz, 8H), 8.04 (s, 2H), 7.98 (s, 3H), 7.83 (s, 3H), 7.56 (d, *J* = 28.0 Hz, 2H), 7.29 (d, *J* = 32.8 Hz, 11H), 7.16 – 6.92 (m, 6H), 6.68 (d, *J* = 8.0 Hz, 3H), 5.46 (s, 1H), 5.30 (d, *J* = 27.3 Hz, 1H), 4.52 (s, 7H), 4.44 – 4.37 (m, 2H), 4.26 (s, 11H), 3.81 (dd, *J* = 61.0, 37.1 Hz, 30H), 3.19 (s, 1H), 3.02 (s, 5H), 2.92 (s, 2H), 2.73 (d, *J* = 23.1 Hz, 5H), 2.48 – 2.30 (m, 1H), 2.18 (d, *J* = 17.0 Hz, 5H), 2.06 (d, *J* = 25.4 Hz, 1H), 1.92 (s, 4H), 1.71 (s, 7H), 1.51 (s, 5H), 1.39 (s, 2H), 1.31 – 1.20 (m, 4H), 1.19 (s, 4H), 1.02 (s, 2H), 0.86 (d, *J* = 15.4 Hz, 12H). MS: calc. M = 2816.93.

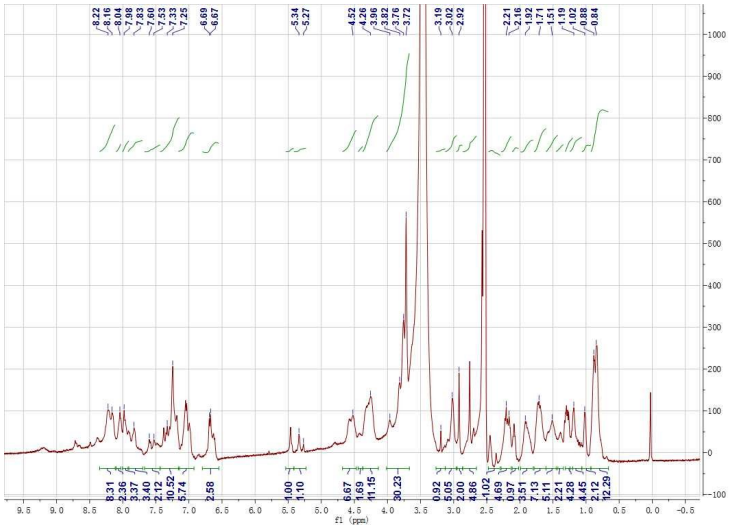


Figure S5. ^{1}H NMR spectrum of ***Comp. 4***

CRB-K (HCPT) GGGG-PMI (***Comp. 5***): ^{1}H NMR (400 MHz, DMSO) δ 8.06 (s, 1H), 7.82 (s, 1H), 7.54 (s, 1H), 7.41 – 7.31 (m, 1H), 7.18 (t, *J* = 19.1 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 6.94 (d, *J* = 17.1 Hz, 1H), 6.62 (dd, *J* = 21.5, 10.5 Hz, 1H), 5.42 (s, 1H), 5.28 (s, 1H), 4.46 (s, 1H), 4.17 (s, 1H), 3.30 – 3.10 (m, 1H), 2.63 (s, 1H), 2.33 (s, 1H), 2.15 (s, 1H), 1.85 (d, *J* = 41.0 Hz, 1H), 1.78 – 1.62 (m, 1H), 1.39 (s, 1H), 1.13 (s, 1H), 0.98 (s, 1H), 0.94 – 0.84 (m, 1H), 0.79 (d, *J* = 39.0 Hz, 1H). MS: calc. M = 2530.57.

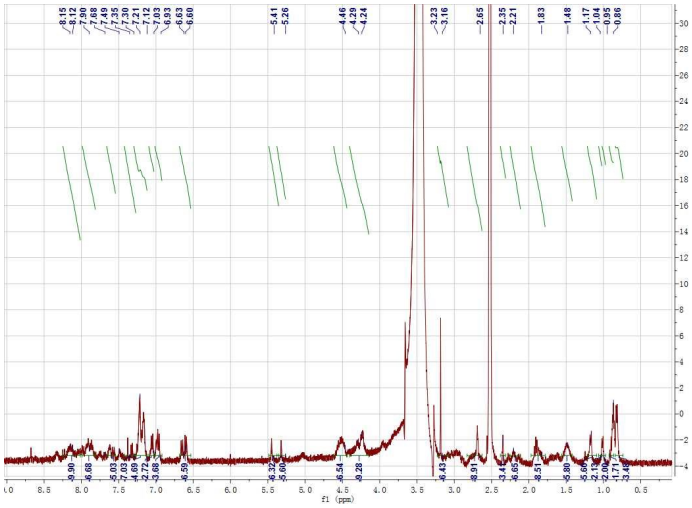


Figure S6. ^{1}H NMR spectrum of ***Comp. 5***

Optical images of solution

2 mg/mL of ***Comp. 1***, ***Comp. 2***, ***Comp. 4*** and ***Comp. 5*** were dissolved in PBS buffer with the supplement of 1 % of DMSO.

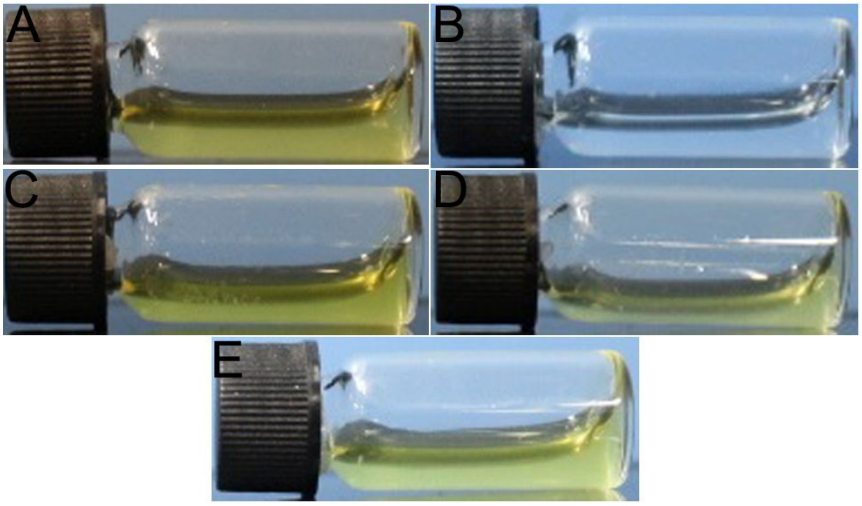


Figure S7. Optical image of A) ***Comp. 1***, B) ***Comp. 2***, C) ***Comp. 3***, D) ***Comp. 4*** and E) ***Comp. 5*** in PBS buffer with the supplement of 1 % of DMSO.

Critical aggregation concentration (CAC) and assembly capacity

The CAC values of ***Comp. 1-5*** were determined by dynamic light scattering (DLS). Solutions containing different concentrations of compound were tested and the light scattering intensity was recorded for each concentration analyzed. The lower CAC

values representative better assembly capacity.

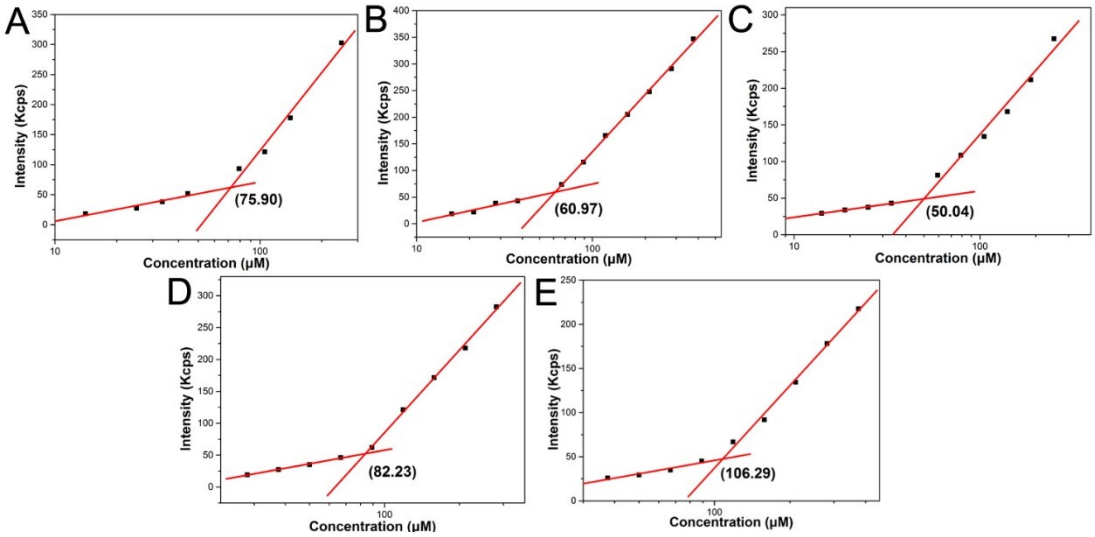


Figure S8. Critical aggregation concentration (CAC) value of A) ***Comp. 1***, B) ***Comp. 2***, C) ***Comp. 3***, D) ***Comp. 4*** and E) ***Comp. 5***

Cell viability analysis and IC_{50}

HepG2 cells (6×10^3 cells per well) were seeded into the 96-well plate for 24 h followed by culture medium removal and subsequently addition of culture medium containing the drugs with multiple dilution. After treatment for 48 hours, cell viability was performed by CCK8 kit (Dojindo, Japan) and OD value at 450 nm was measured by spectrophotometer. The experiment was repeated three times and the IC_{50} values of different drugs were calculated by GraphPad Prism.

***In vivo* evaluation of antitumor activity**

Female BALB/c nude mice (aged 6 weeks) were purchased from the Model Animal Research Center of Nanjing Medical University (Nanjing, China) and injected with approximately 1×10^6 HepG2 cells in 100 μL of PBS into the left inguinal region. Tumor growth was monitored every three days with a caliper. The tumor sizes were calculated as: volume = (tumor length) x (tumor width)^2/2.When tumors size reached about 75 mm^3 (12-14 days after tumor inoculation), forty mice were randomly divided into eight treatment groups as follows (five mice in each group): PBS (untreated group), HCPT, CRB, ***Comp. 1***, ***Comp. 2***, ***Comp. 3***, ***Comp. 4***, ***Comp. 5***. For the treatment, the drugs with dose as shown in Table 1 were injected into mice via the caudal vein, the day of

giving drugs was designated as day 0, all drugs were given with an interval of 3 days for a total of 4 times. Mice were sacrificed on the day 21 after drug withdrawal, and tumor tissues were taken.

Group	Drug	Dose (mg/kg bw)
1	PBS	100 μL/mouse
2	CRB	1.25 mg/kg, 100 μL/mouse
3	HCPT	1.5 mg/kg, 100 μL/mouse
4	***Comp. 1***	11.6 mg/kg, 100 μL/mouse
5	***Comp. 2***	10.4 mg/kg, 100 μL/mouse
6	***Comp. 3***	10.6 mg/kg, 100 μL/mouse
7	***Comp. 4***	11.6 mg/kg, 100 μL/mouse
8	***Comp. 5***	10.4 mg/kg, 100 μL/mouse

Table S1. Drug formulations and doses for evaluation of tumor inhibition.

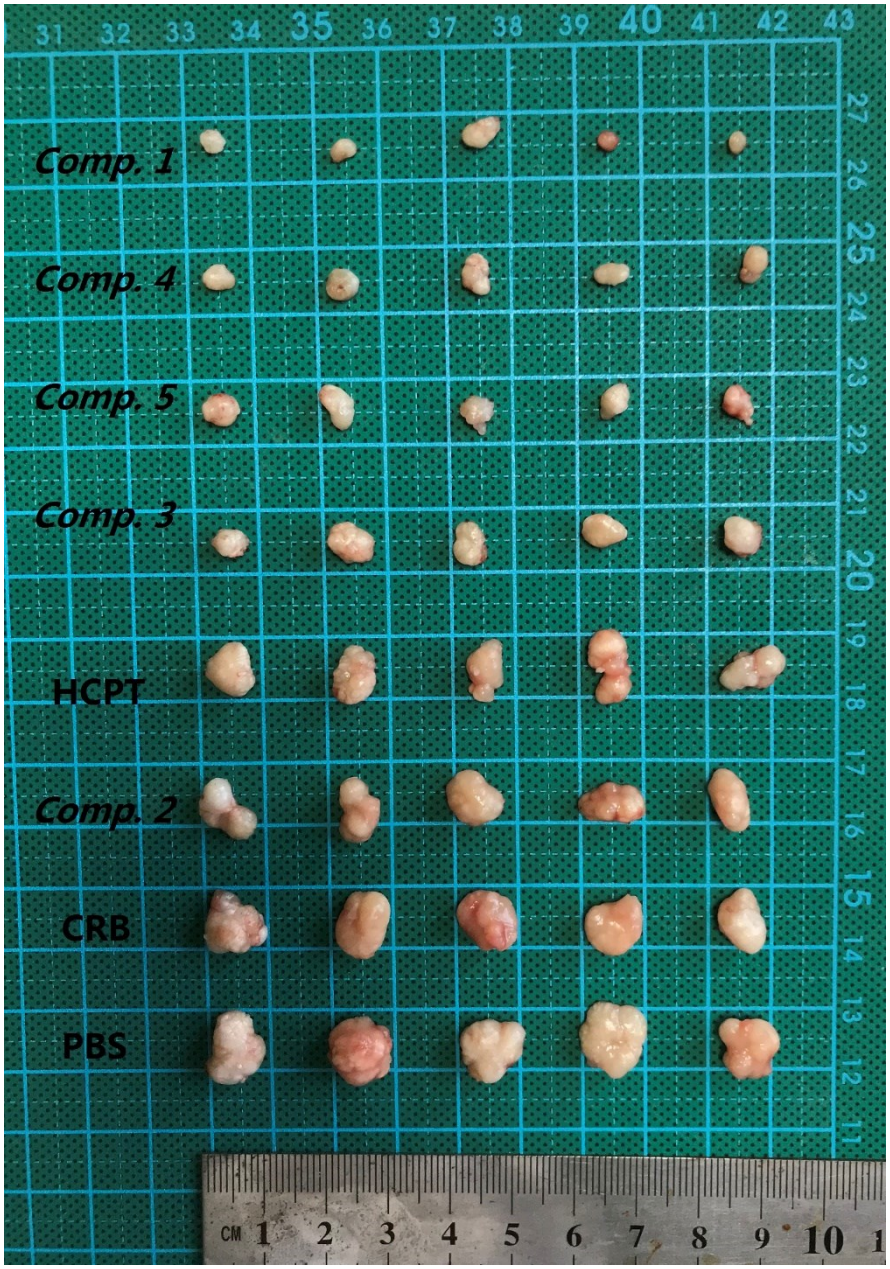


Figure S9. *Ex vivo* images of tumors extracted from HepG2 tumor-bearing Balb/c nude mice at day 21 after being *i.v.* injected with different drugs as the dosage of Table S1.

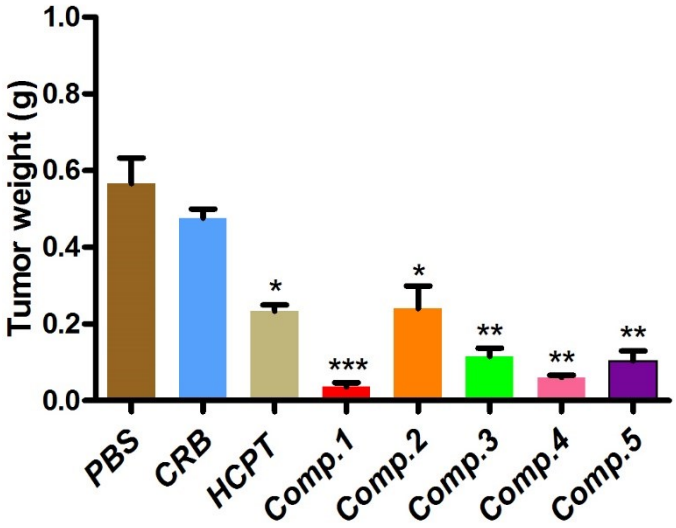


Figure S10. Weight of tumors extracted from HepG2 tumor-bearing Balb/c nude mice at day 21 after being *i.v.* injected with different drugs as dosage of Table S1. The data are shown as mean ± SEM (n = 5), * represents $P < 0.1$, ** represents $P < 0.05$, *** represents $P < 0.01$.