A supramolecular hybrid material constructed from pillar[6]arene-based host-guest complexation and ZIF-8 for targeted drug delivery

Kui Yang, Ke Yang, Shuang Chao, Jia Wen, Yuxin Pei and Zhichao Pei*

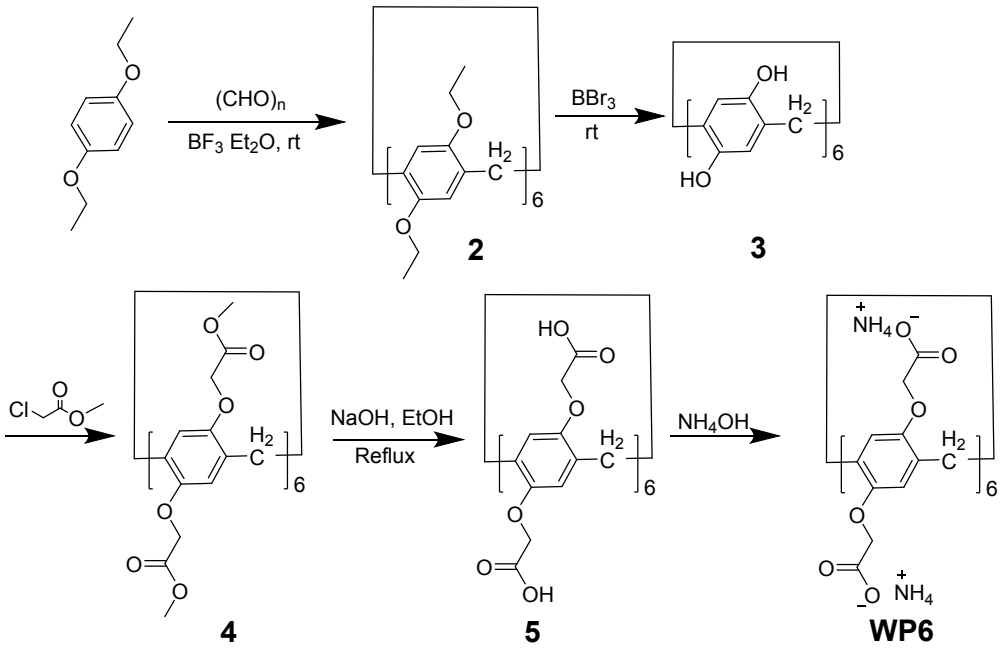
Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Yangling 712100, P. R. China.

Supporting Information

1. Materials and methods

All reagents were purchased from commercial suppliers and used without further purification unless specified. Water used in this work was triple distilled. NMR spectra were recorded on a Bruker 500 MHz Spectrometer, with working frequencies of 500 MHz for ^{1}H. Absorption spectra were collected by using a Shimadzu 1750 UV-visible spectrometer (Japan). Fluorescence spectra were measured with a Shimadzu RF-5301 fluorescence spectrometer (Japan). Cell culture was carried out in an incubator with a humidified atmosphere of 5% CO_2 at 37 ^{o}C. Cell toxicity was tested by microplate reader (KHB ST-360). The confocal laser microscope (CLSM) data were acquired using a CLSM (Andor REVOLUTION WD). SEM images were obtained from FEI Nova Nano SEM-450. TEM images were obtained from FEI TECNAI G2 SPIRIT BIO. Flow cytometry data were obtained from BD FACSAria™ III.

2. Synthesis and characterization of the compounds



Scheme S1. Synthetic route of **WP6**.[S1-S3]

As shown in Scheme S1, **WP6** was synthesized according to the literatures.[S1-S3] The ^{1}H NMR spectrum of **2** was shown in Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) δ

(ppm): 6.69 (s, 1H), 3.81 (m, 3H), 1.28 (t, J = 7.0 Hz, 3H). The ^{1}H NMR spectrum of **4** was shown in Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.86 (s, 12H), 4.50 (s, 24H), 3.89 (s, 12H), 3.74 (s, 36H). The ^{1}H NMR spectrum of **5** was shown in Figure S3. ^{1}H NMR (500MHz, DMSO) δ 12.87 (s, 12H), 6.83 (s, 12H), 4.50 (s, 24H), 3.71 (s, 12H). The ^{1}H NMR spectrum of **WP6** was shown in Figure S4. ^{1}H NMR (500 MHz, D_2O) δ 6.61 (s, 12H), 4.05 (s, 24H), 3.84 (s, 12H).

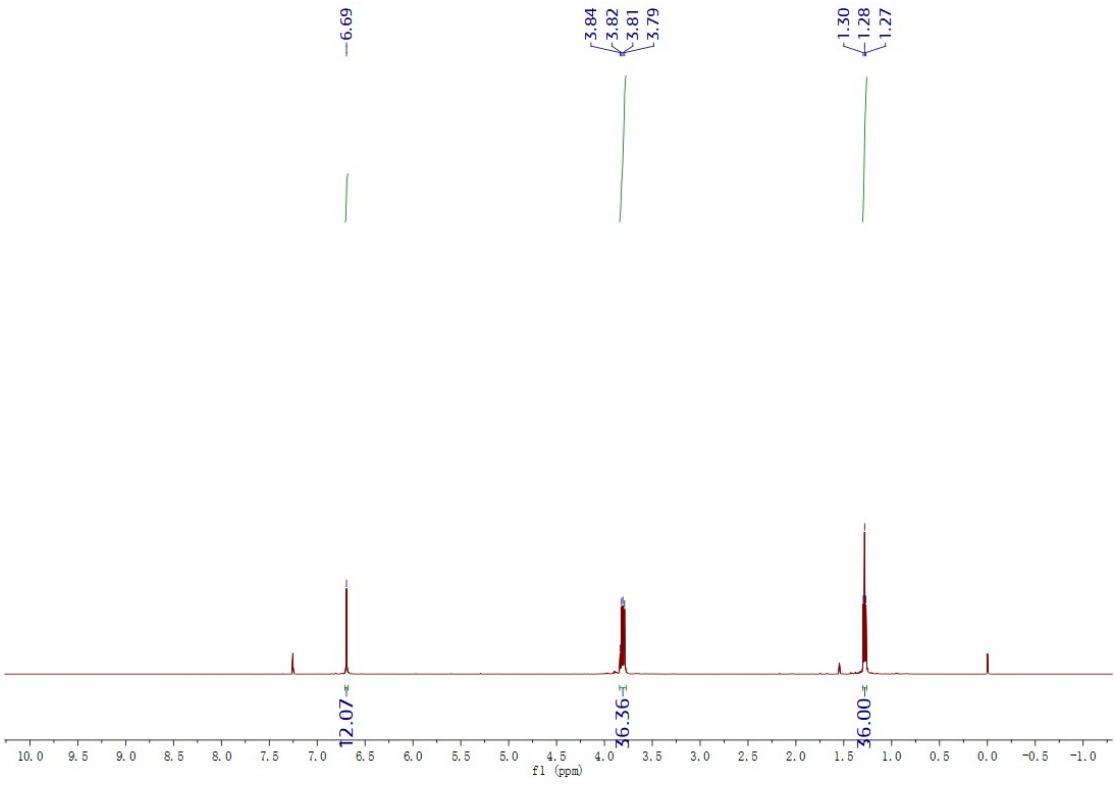


Figure S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **2**.

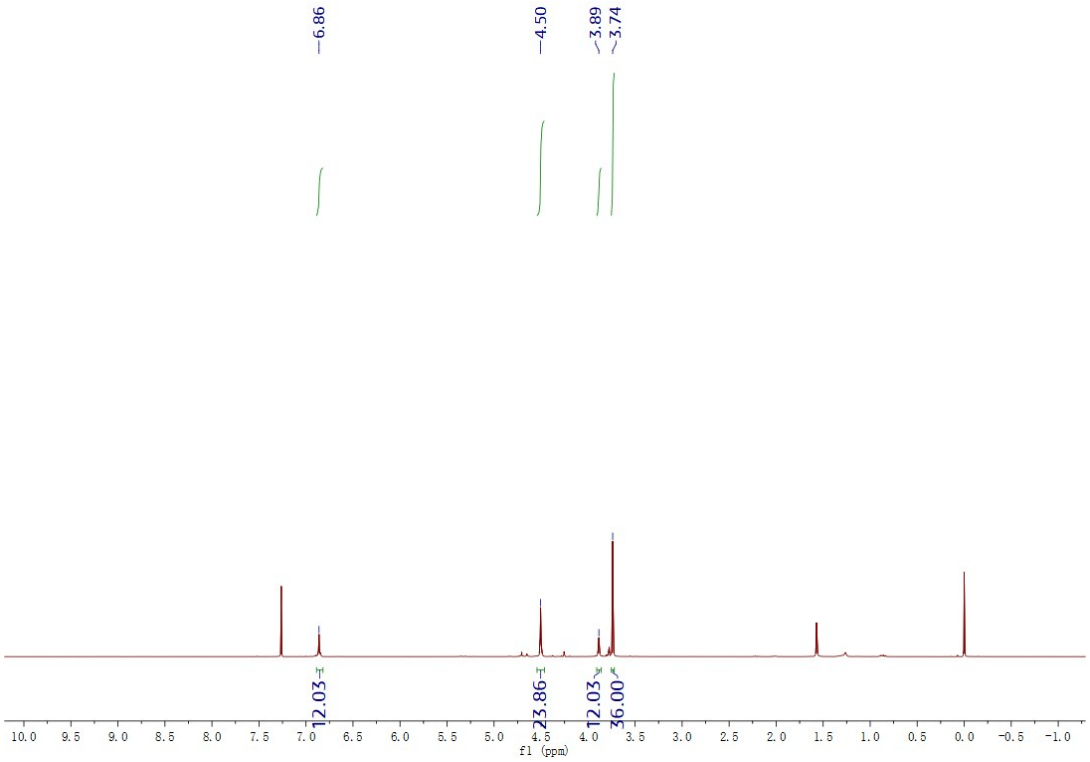


Figure S2. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **4**.

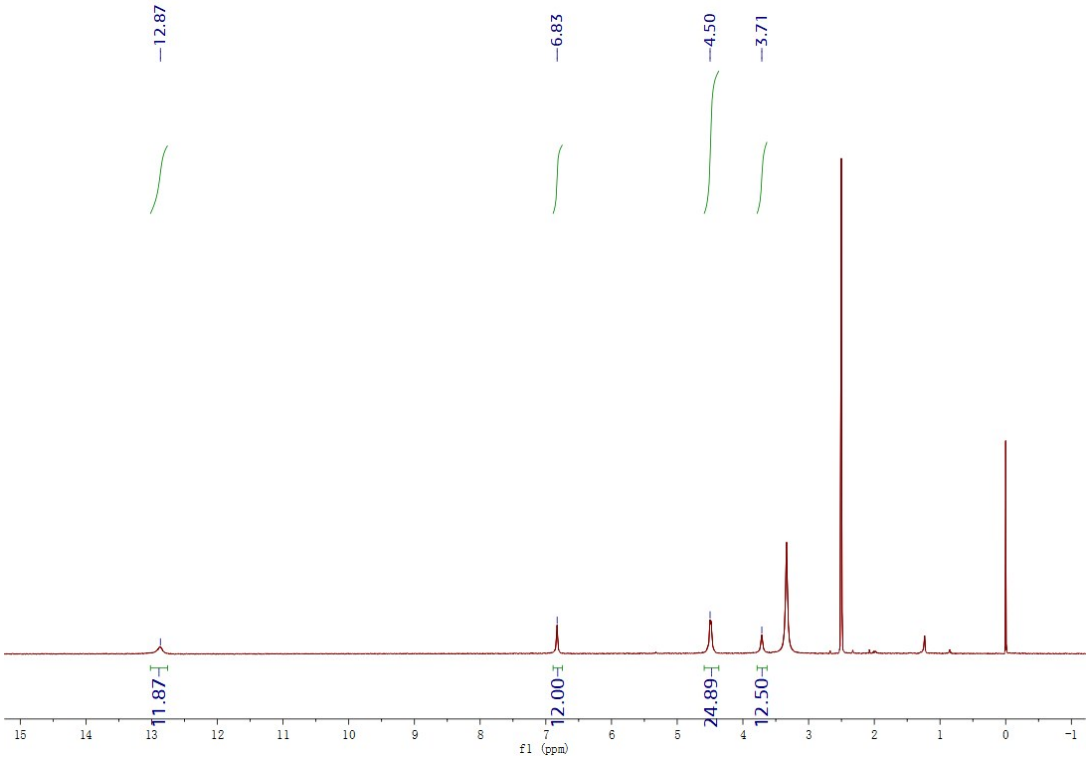


Figure S3. ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **5**.

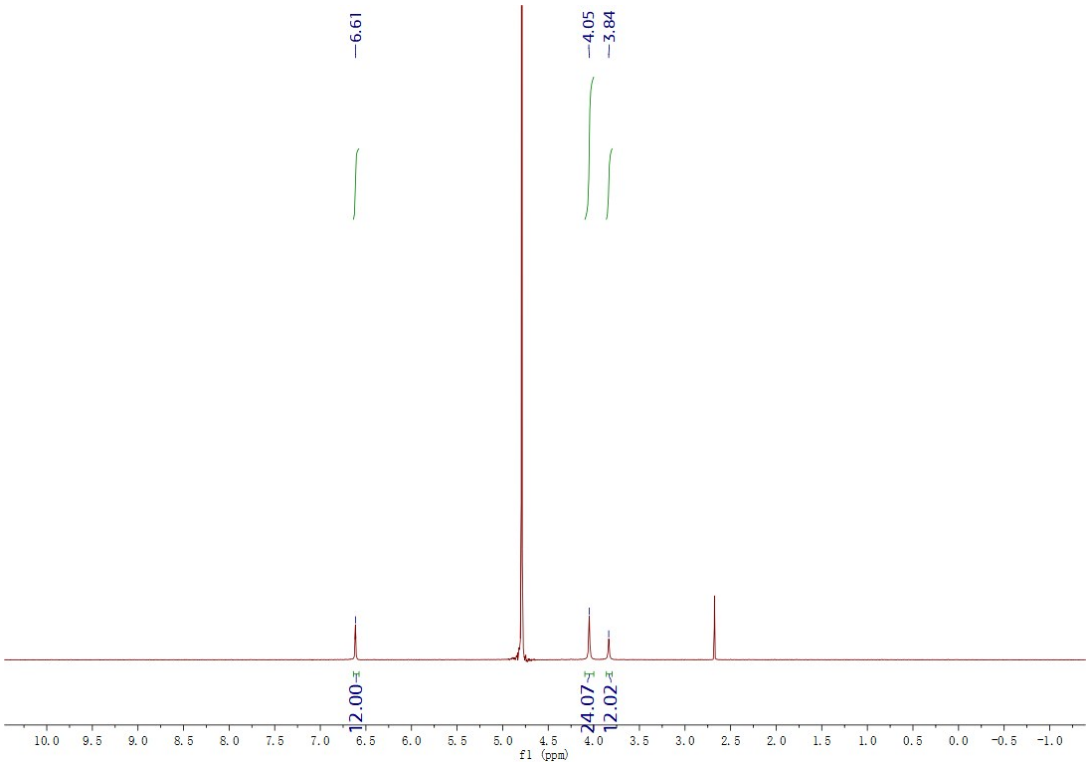
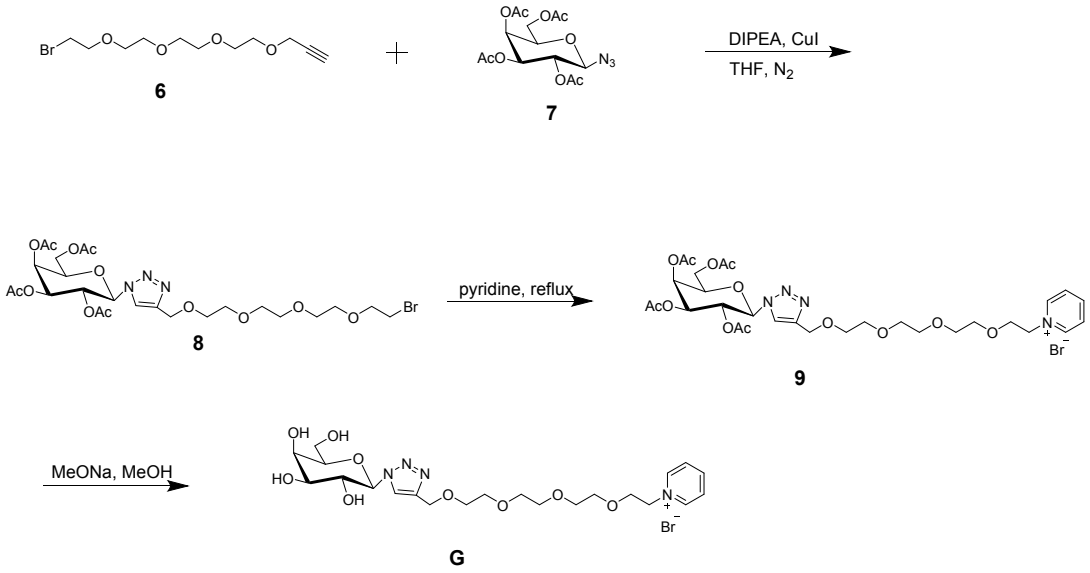


Figure S4. ^{1}H NMR spectrum (500 MHz, D_2O) of **WP6.**



Scheme S2. Synthetic route of **G.**[S4-S5]

Compound **8**: Compound **6** and **7** were synthesized as literatures reported before.[S4-S5] Compound **6** (58.8 mg, 0.2 mmol) and **7** (74.6 mg, 0.2 mmol) were dissolved in 5 mL THF. DIPEA (0.68 mL, 3.9 mmol) and CuI (18.6 mg, 0.1 mmol) were added in the solution. The mixture was stirred at room temperature under N_2 for 24 h. The mixture

was concentrated under reduced pressure. Then 20 mL DCM was added into the crude product. The mixture was washed with water (3 × 10 mL) and saturated NaCl 10 mL. The organic phase was dried with $MgSO_4$ and was concentrated under reduced pressure. The crude product was purified by flash column chromatography to give **8** (113 mg, 85%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 5.82 (d, J = 9.3 Hz, 1H), 5.54 (d, J = 11.5 Hz, 2H), 5.23 (d, J = 10.3 Hz, 1H), 4.68 (s, 2H), 4.26-4.04 (m, 3H), 3.79 (t, J = 6.2 Hz, 2H), 3.66 (m, 12H), 3.45 (t, J = 6.2 Hz, 2H), 2.21 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.36, 170.00, 169.83, 169.08, 145.87, 121.17, 86.28, 74.05, 71.22, 70.86, 70.66, 70.60, 70.53, 69.80, 68.26, 67.97, 66.93, 64.52, 61.24, 42.73, 30.33, 20.64, 20.63, 20.48, 20.26. HRMS: *m/z* calcd for $[M + H]^+$ $C_{25}H_{39}BrN_3O_{13}^+$, 668.1666, found 668.1672.

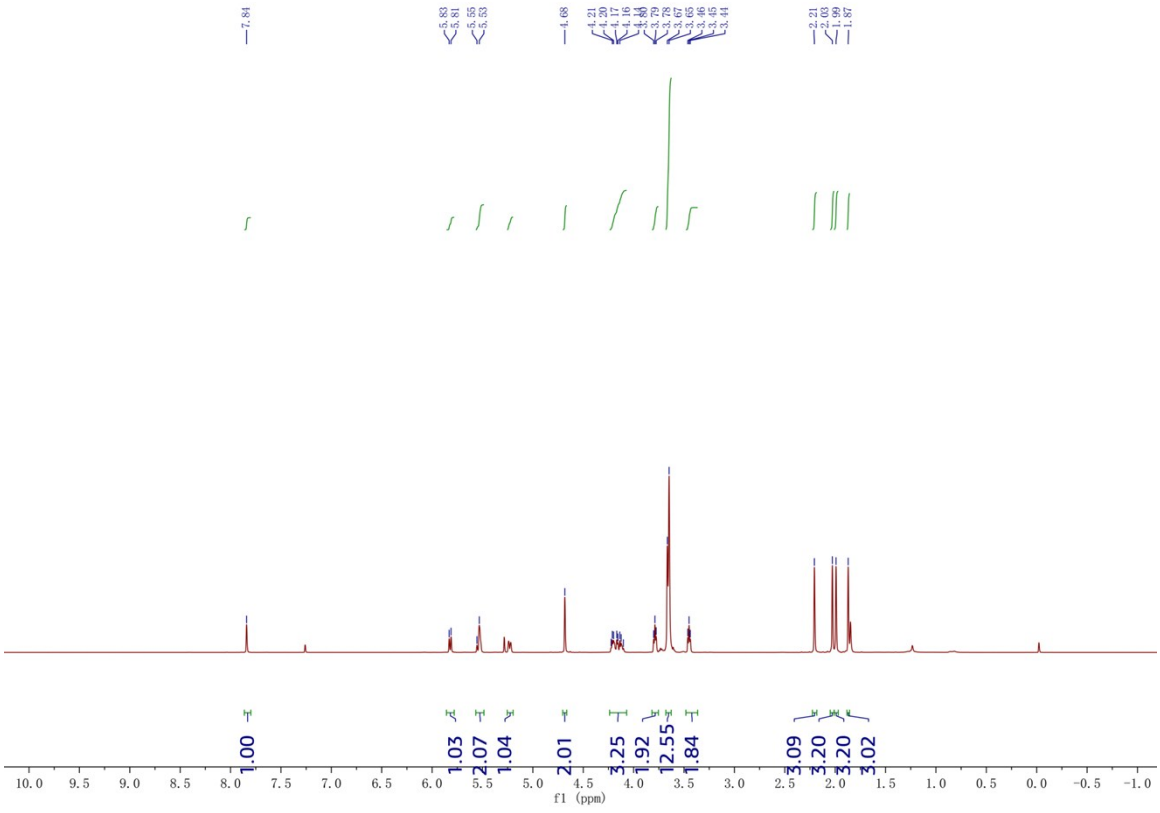


Figure S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **8**.

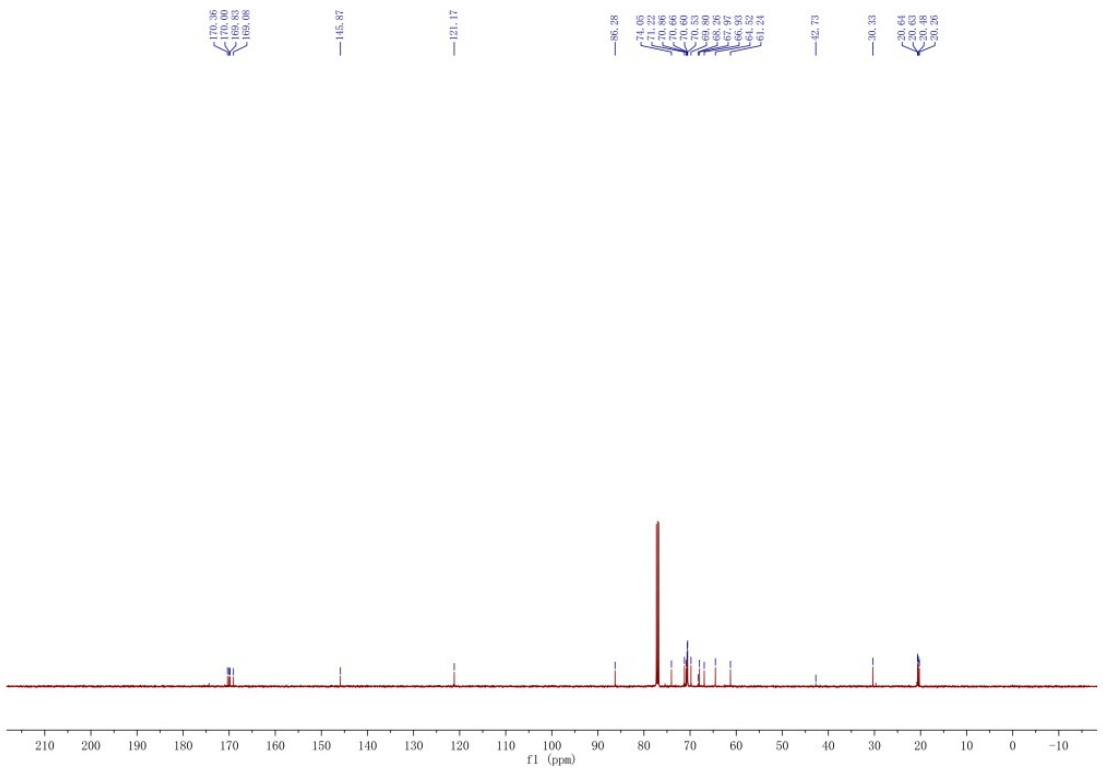


Figure S6. ^{13}C NMR spectrum (125 MHz, CD_3Cl) of **8**.

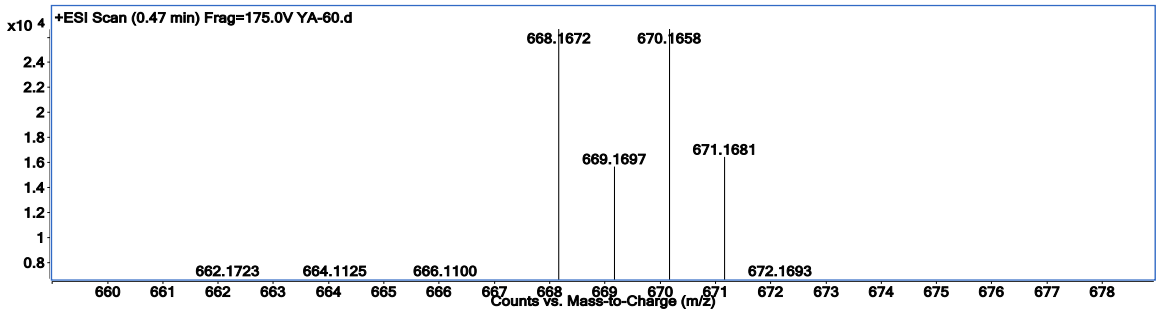


Figure S7. HRMS of **8**.

Compound **9**: The compound **8** (66.7 mg, 0.1 mmol) was dissolved in 3 mL pyridine. The mixture was reflux under nitrogen atmosphere for 12 h, then concentrated under reduced pressure. The crude product was purified by flash column chromatography (eluent: DCM/MeOH = 5 %) to give **9** as a yellow oil (48 mg, 65 %). ^1H NMR (500 MHz, $CDCl_3$) δ 9.56 (d, J = 5.8 Hz, 2H), 8.44 (t, J = 7.8 Hz, 1H), 8.05 (t, J = 7.0 Hz, 2H), 8.00 (s, 1H), 6.09 (d, J = 9.3 Hz, 1H), 5.67 – 5.56 (m, 2H), 5.32 (m, 1H), 5.28-5.15 (m, 2H), 4.62 (s, 2H), 4.46 (t, J = 6.4 Hz, 1H), 4.22 (m, 2H), 4.11 (t, J = 4.6 Hz, 1H), 3.72 (m, 4H), 3.70-3.65 (m, 4H), 3.64 (m, 2H), 3.58 (m, 2H), 2.26 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.89 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 170.38, 170.00, 169.74, 169.10, 145.98, 144.93, 144.86, 127.76, 122.05, 85.94, 73.95, 70.88,

70.50, 70.48, 70.44, 70.35, 70.22, 69.86, 69.59, 68.04, 67.03, 64.16, 61.27, 61.19, 20.71, 20.69, 20.49, 20.32. HRMS: *m/z* calcd for [M - Br]+ $C_{30}H_{43}N_4O_{13}^+$, 667.2821, found 667.2803.

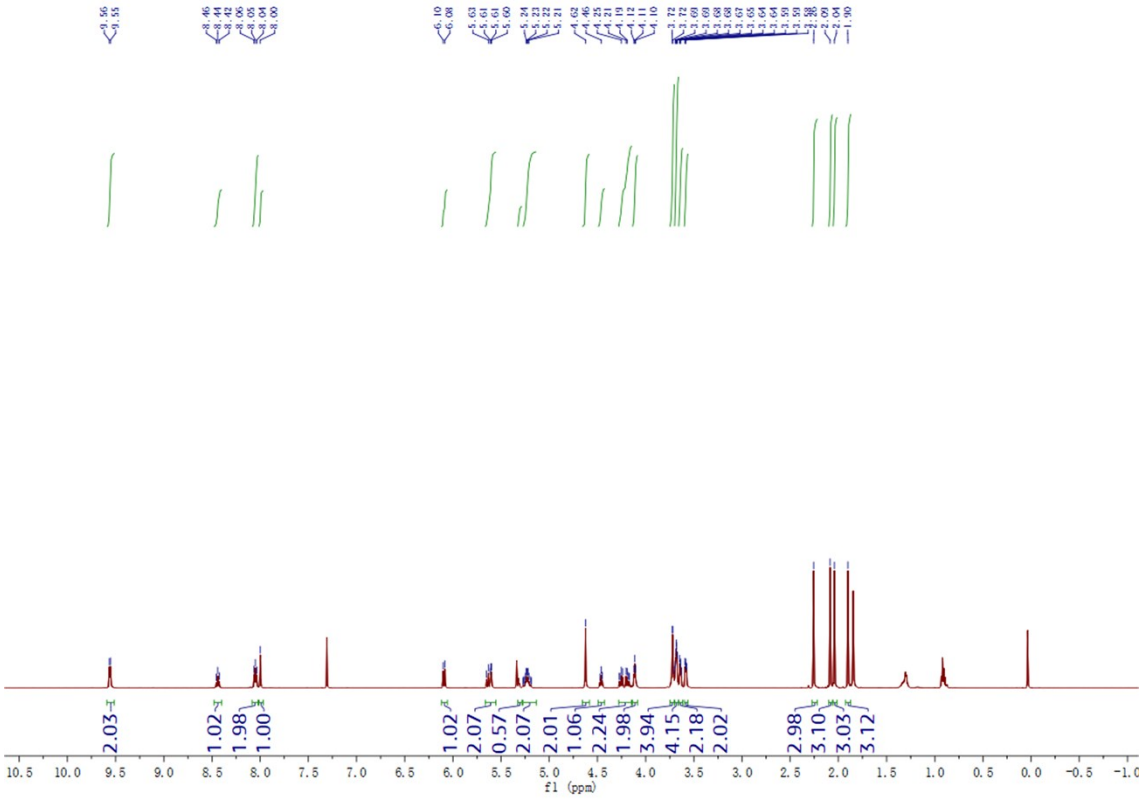


Figure S8. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **9**.

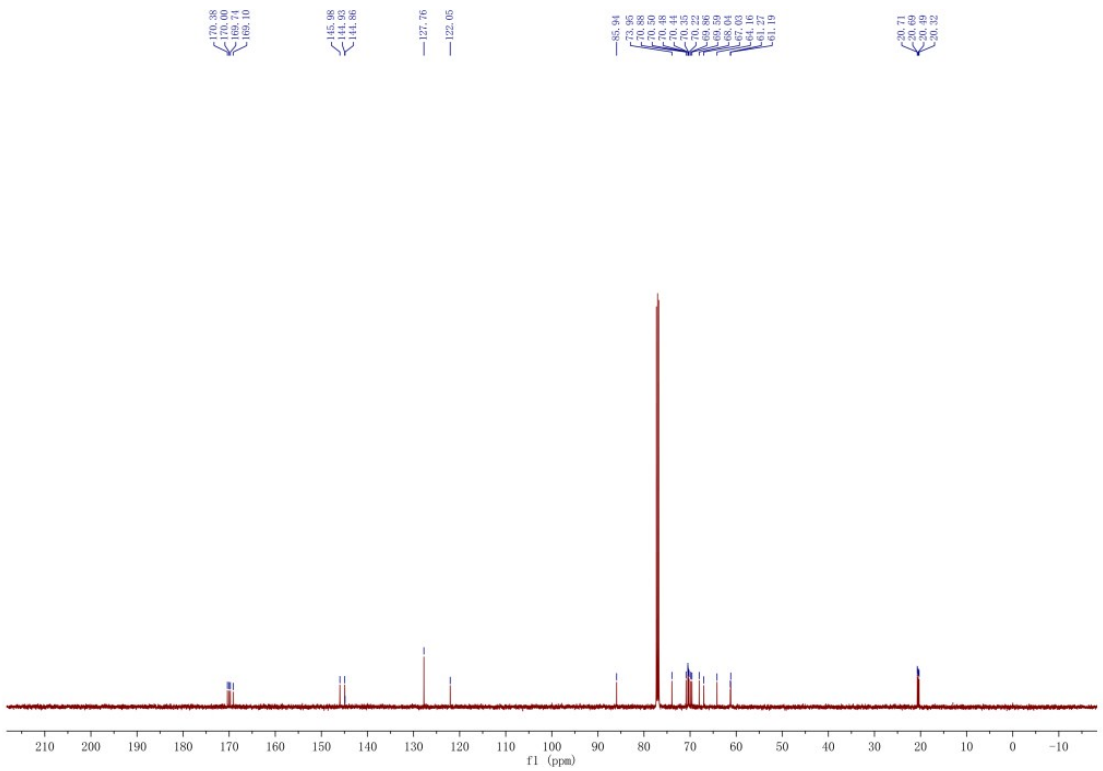


Figure S9. ^{13}C NMR spectrum (125 MHz, CD_3Cl) of **9**.

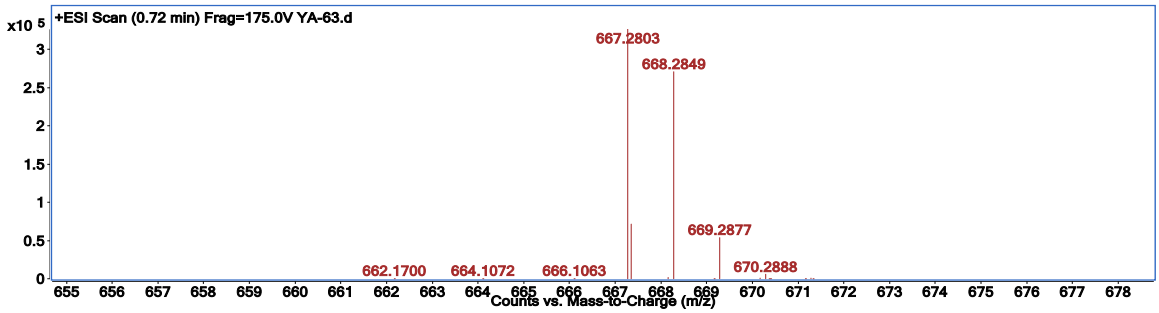


Figure S10. HRMS of **9**.

G: CH_3ONa (5.4 mg, 0.4 mmol) was added into a solution of compound **9** (74.6 mg, 0.1 mmol in 5 mL MeOH). The mixture was stirred at room temperature for 2 h, then neutralized by addition of ion-exchange resin (Amberlite IR 120 H+) until pH 7, filtered, and the solvent was removed under reduced pressure. The **G** was obtained as a yellow oil (52 mg, 90 %). ^1H NMR (500 MHz, D_2O) δ 8.90 (d, J = 5.9 Hz, 2H), 8.61 (t, J = 7.9 Hz, 1H), 8.35 (s, 1H), 8.12 (t, J = 7.0 Hz, 2H), 5.76 (d, J = 9.2 Hz, 1H), 4.85-4.81 (m, 2H), 4.77 (s, 2H), 4.28 (t, J = 9.5 Hz, 1H), 4.15 (d, J = 3.2 Hz, 1H), 4.07 (m, 3H), 3.94 (dd, J = 9.8, 3.3 Hz, 1H), 3.84 (m, 2H), 3.81 – 3.78 (m, 2H), 3.76 – 3.72 (m, 2H), 3.71 (m, 2H), 3.69 – 3.63 (m, 6H).

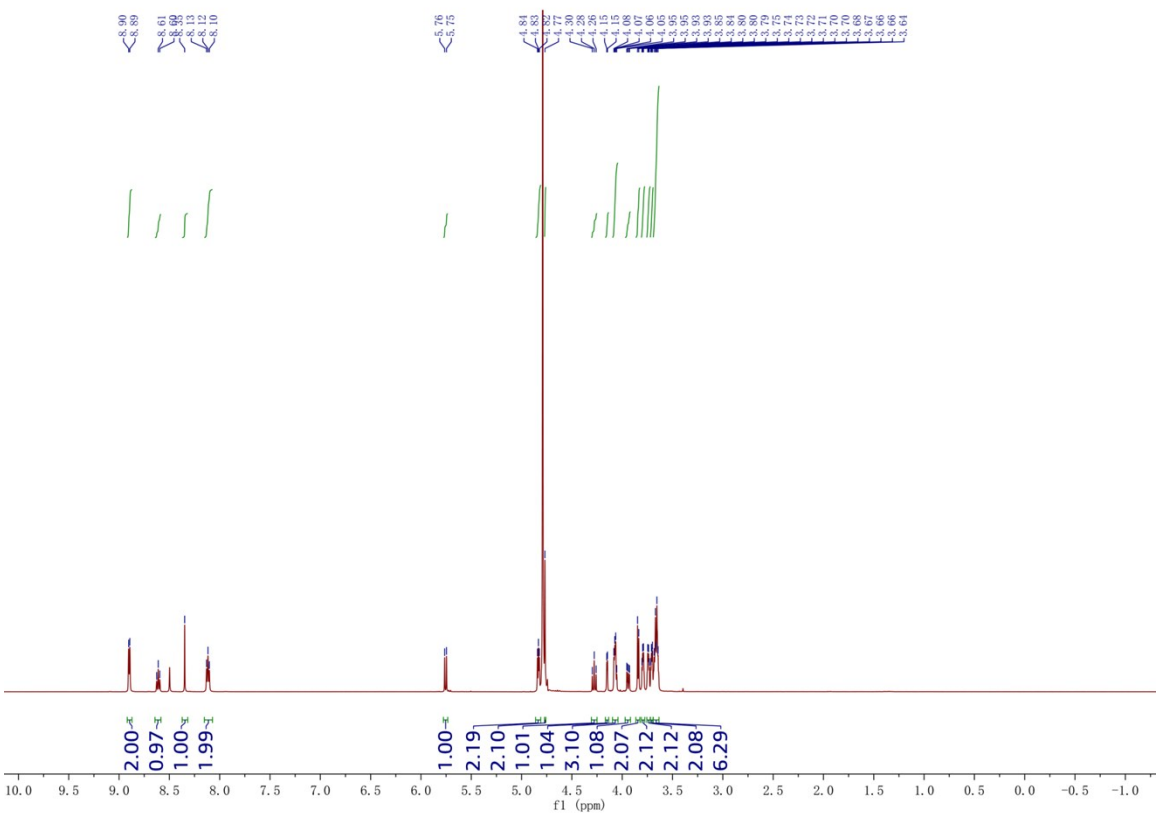


Figure S11. ^{1}H NMR spectrum (500 MHz, D_2O) of **G**.

3. Synthesis of nanoparticles

For typical preparation of ZIF-8@DOX, DOX (10 mg/mL), $Zn(NO_3)_26H_2O$ (0.25 g/mL) and 2-methylimidazole (2-mim, 0.25 g/mL) were prepared using deionized H_2O. 2 mL DOX solution was added to 0.4 mL $Zn(NO_3)_26H_2O$ solution. The mixture was stirred for 5 min. Then 0.4 mL 2-mim solution was added dropwise to the mixture. After being stirred for 10 min, the precipitation was obtained after centrifuge. The solid was washed with water more than 3 times. The ZIF-8@DOX was obtained after freeze drying.[S6]

3 mg ZIF-8@DOX, 3 mg WP6 was added into 1 mL H_2O. After sonication for 15 min, the ZIF-8@DOX@WP6 was obtained. Then 1 mL **G** (1 mg/mL) was added into the ZIF-8@DOX@WP6 solution. After stirred for 30 min, ZIF-8@DOX@WP6@G stock solution was obtained for further experiments.

4. Scanning electron microscope (SEM) and transmission electron microscope (TEM)

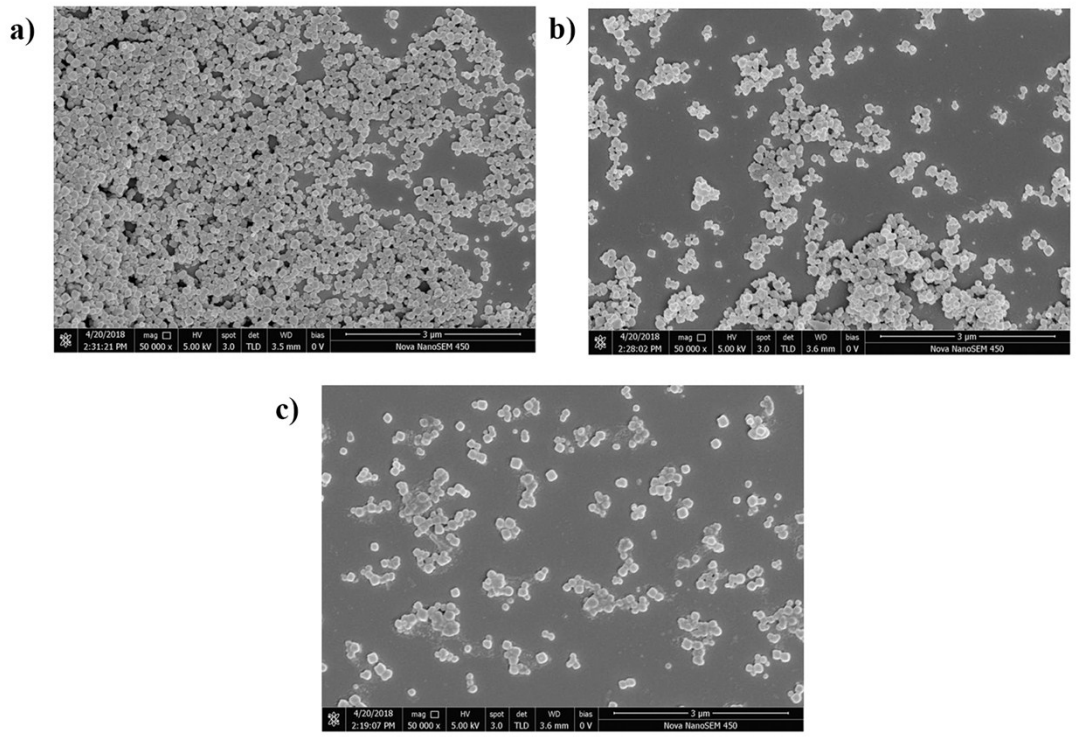


Figure S12. SEM images of ZIF-8@DOX (a), ZIF-8@DOX@WP6 (b) and ZIF-8@DOX@WP6@G (c). The scale bar is 3 μm.

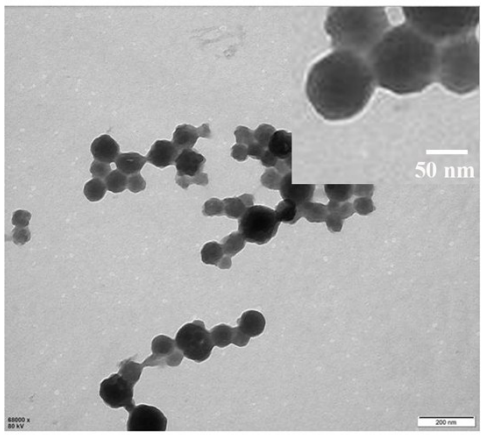


Figure S13. TEM images of ZIF-8@DOX@WP6. The scale bar is 200 nm. Inset: Partial enlarged images. The scale bar is 50 nm.

5. Fourier transform infrared (FTIR) spectroscopy

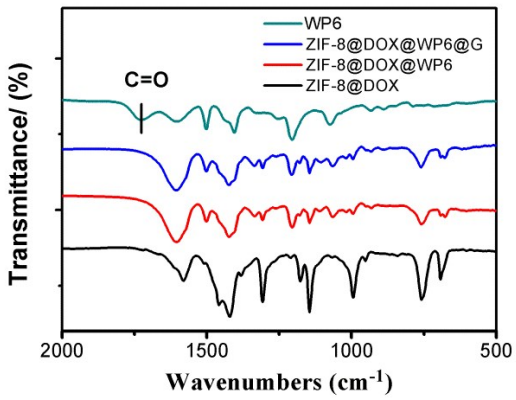


Figure S14. FTIR spectra of WP6, ZIF-8@DOX, ZIF-8@DOX-WP6 and ZIF-8@DOX@WP6@G. Compared with the sample of WP6, the peak around 1725 cm-1, which was assigned to the stretching vibration of C=O, was disappeared in the sample of ZIF-8@DOX@WP6 and ZIF-8@DOX@WP6@G owing to the coordination between the carboxyl group of WP6 and metal nodes of ZIF-8@DOX.

6. Dynamic light scattering

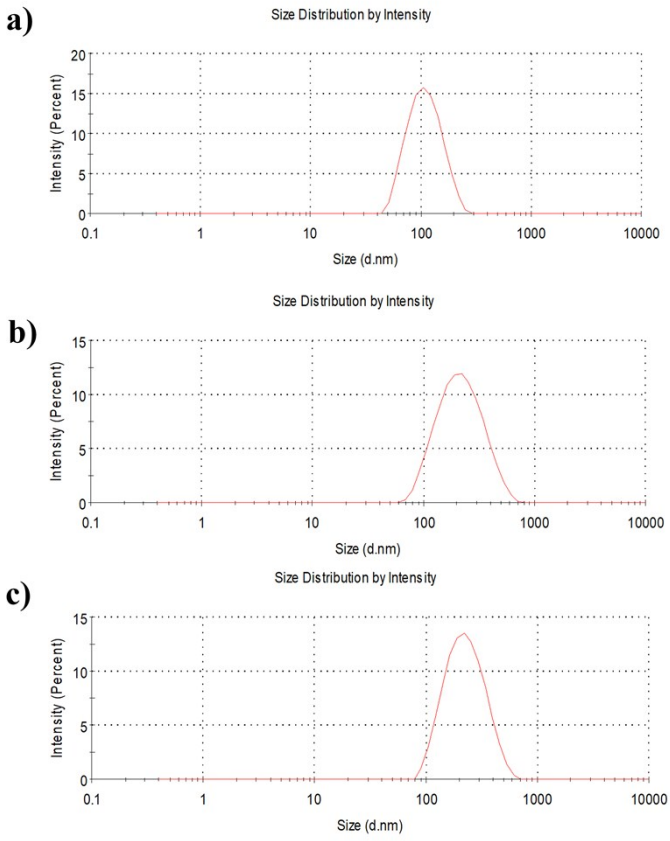


Figure S15. DLS data of ZIF-8@DOX (a), ZIF-8@DOX@WP6 (b) and ZIF-8@DOX@WP6@G (c). The diameters and PDIs are 104.2 nm, 0.162; 188.4 nm, 0.175; 203.5 nm, 0.148, respectively.

7. DOX release assay

The DOX release from ZIF-8@DOX@WP6@G was studied at different pH buffer solution. 1 mL of ZIF-8@DOX@WP6@G in a dialysis bag was added into corresponding release medium (30 mL). At specified time intervals, the concentration of DOX was determined by UV-Vis spectrophotometry.

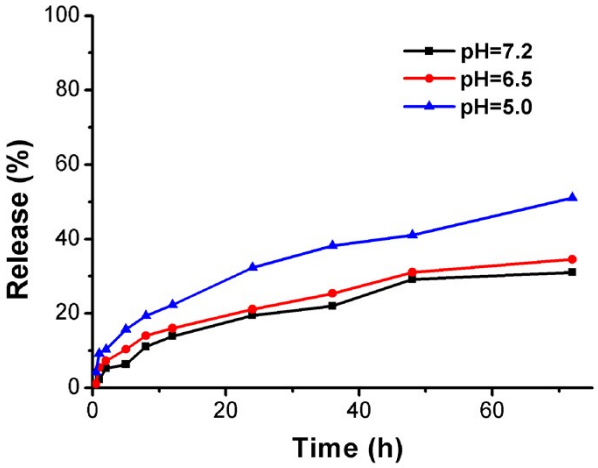


Figure S16. DOX release profiles from ZIF-8@DOX@WP6@G in PBS at different pH values.

8. The study of targeting effect by flow cytometry

HepG2 cells were cultured in 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete 1640) in 5% CO_2 at 37 °C. HepG2 cells were seeded in 6-well plates (1×10^5 cells/well) and cultured in complete medium for 24 h. The fresh medium containing 5 μg/mL free DOX, ZIF-8@DOX, or ZIF-8@DOX@WP6@G were added respectively. In contrast, one group was pretreated with lactobionic acid (2 mg/mL) for 4 h before the incubation with ZIF-8@DOX@WP6@G. After 2 h, the cells were harvested and washed for two times with cold PBS and resuspended in 50 μL PBS. Finally, cells were analyzed by a flow cytometer (BD FACSAria™ III).

9. Confocal laser scanning microscopy (CLSM)

HepG2 cells 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 ZIF-8@DOX@WP6@G for 30 min, 1 h and 6 h at the concentration of 5 μg/mL, respectively. Next, the dishes were washed with PBS for three times. After washing

with PBS, the cells were stained with Hoechst 33342 for 10 min. Finally, the cells were washed with PBS and then observed under a CLSM.

10. Cytotoxicity evaluation

HepG2 and HL7702 were cultured in RPMI 1640 medium containing 10% FBS, 1% penicillin/streptomycin (complete RPMI 1640 medium) in 5% CO_2 at 37 °C. The relative cytotoxicities of DOX, ZIF-8@DOX and ZIF-8@DOX@WP6@G were evaluated *in vitro* by MTT assay, respectively. The cells were seeded in 96-well plates at a density of 1×10^4 cells per well in 100 μL complete medium. Subsequently, cells were incubated with the corresponding compounds at different concentrations for 24 h, 48 h. The 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.

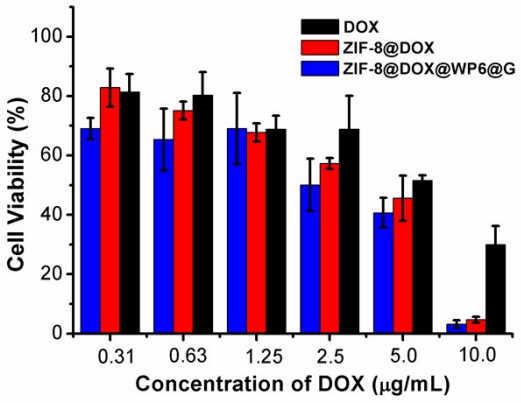


Figure S17. Relative cell viability of HepG2 cells after treatment with DOX, ZIF-8@DOX and ZIF-8@DOX@WP6@G at different concentrations for 48 h.

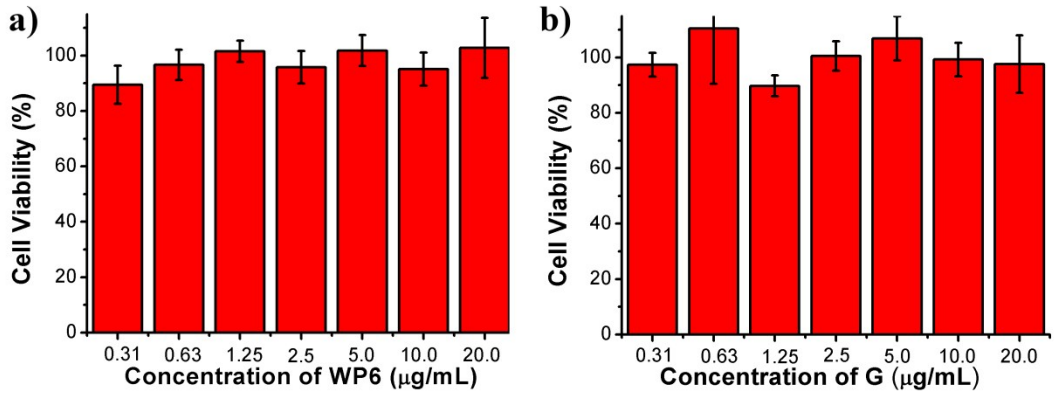


Figure S18. Relative cell viabilities of HepG2 cells after treatment with WP6 (a) and G (b) at different concentrations for 24 h.

Table S1. *In Vitro* IC_{50} of DOX, ZIF-8@DOX and ZIF-8@DOX@WP6@G against HepG2 cells after 24 h and 48 h Incubation.

Nanoparticles	24 h IC_{50} (μg/mL)	48 h IC_{50} (μg/mL)
DOX	8.410	4.604
ZIF-8@DOX	4.117	2.394
ZIF-8@DOX@WP6@G	3.476	1.724

11. References

S1. X. Hu, Z. Chen, L. Chen, L. Zhang, J. Hou and Z. Li, *Chem. Commun.*, 2012, **48**, 10999-11001.

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