Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Materials and general methods:

Chemicals materials: All Fmoc-amino acids and 2-chlorotrityl chloride resin were purchased from GL Biochem (Shanghai, China). 2-(1H-benzotriazol-1-yl)-1,1,3,3 –tetramethyluronium hexafluorophosph- ate (HBTU), 1-hydroxy-benzotriazole monohydrate (HOBT), trifluoroacetic acid, 4-dimethylamino-pyridine, diisopropylethylamine (DIPEA), tanshinone IIA and cryptotanshinone were obtained commercially from Aladdin Reagent Corporation (Shanghai, China). Danshen (Salvia miltiorrhiza Bunge) was purchased from Nanjing (Beijing tongrentang drugstore). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) was obtained from Biosharp Company (Hefei, China). The Human alveolar epithelial cell line (A549) was purchased from American Type Culture Collection (ATCC, China's district general agent, Beijing, China).

Peptide synthesis: The octa-peptides were synthesized via solid phase peptide synthesis method (SPPS). After cleavage from the resin and deprotection of the side groups and N-terminus using the TFA only, the peptides were collected in cold ether, centrifuged and freeze-dried. The obtained crude products were purified by reverse phase HPLC. MS, ^{1}H NMR, HPLC were used to verify the peptide structure and value their purity. TOF-MS of FHFDFHFD: m/z 1111.7 [M+H], 1149.7 [M+K]. 1H NMR

(300 MHz, DMSO) of FHFDFHFD δ 14.181 (wide peak, 2H), 8.95 (d, J = 10.2, 2H), 8.58-8.055 (m, 7H), 7.957 (d, J = 7.2, 2H), 7.428-7.303 (m, 8H), 7.279-7.210 (m, 8H), 7.181-7.135 (m, 4H), 7.119 (m, 2H), 4.649 (m, 5H), 4.608 (m, 1H), 4.589 (m, 1H), 4.561 (m, 1H), 3.400 (d, J = 6.9, 2H), 3.111 (d, J = 5.4, 2H), 2.935 (d, J = 4.2, 2H), 2.893 (d, J = 6, 2H), 2.847 (d, J = 7.2, 2H), 2.735 (d, J = 6.9, 2H), 2.676(d, J = 8.1, 2H), 2.617 (d, J = 9, 2H).

Chromatographic conditions：Chromatographic column: C18 ODS Hypersil column (250×4.6 mm, i.d., 5 μm).

Mobile phase: The mobile phase consisted of a mixture of two phases, A was water: TFA (0.1%), B was acetonitrile: TFA (0.1%).

Injection volume: 10 μL

Flow rate: 1.0 mL/min

T=25 °C

The DAD monitoring wavelength was 214 nm.

Time program for HPLC gradient elution

time (min)	A (%)	B (%)
0-15	90-40	10-60
15-50	40-20	60-80

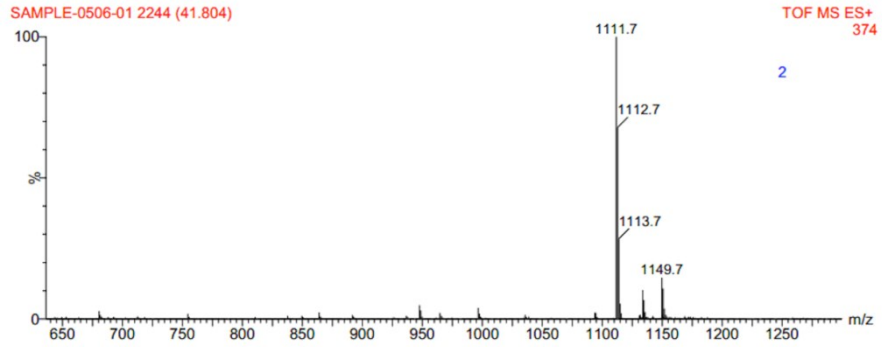

Fig. S-1. MS of FHFDFHFD peptide compound

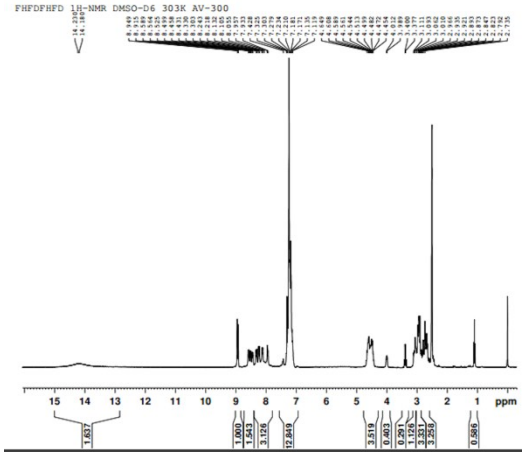

Fig. S-2. 1H-NMR spectrum of FHFDFHFD peptide compound

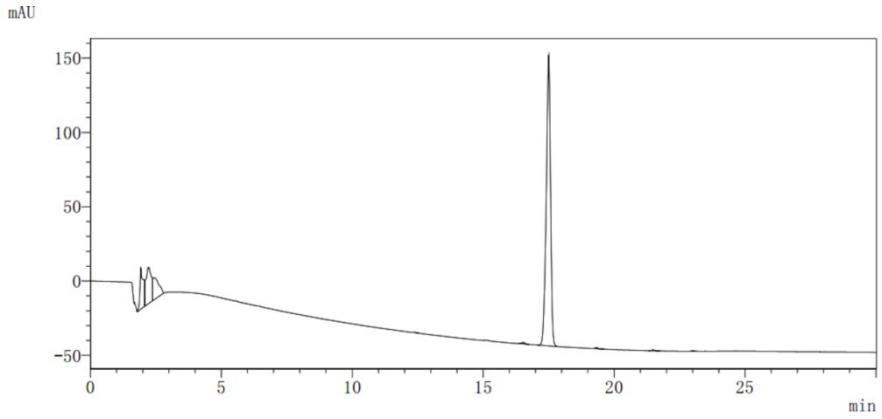

Fig. S-3. The chromatogram of pure FHFDFHFD peptide compound

Preparation of FHFDFHFD hydrogels: 10 mg of the peptide powders were first dissolved fully in 1 mL doubly distilled water (ddH_2O), then placing it at room temperature (RT) for hours (1.0 wt%).

Preparation of tanshinones: 30 g powders of the dried roots of Salvia

miltiorrhiza were extracted with 75% (V/V) ethanol (120 mL) by ultrasonic extraction at room temperature. The frequency of the ultrasonic was 40 KHz. After 60 minutes, the combined ethanol extracts were concentrated in vacuum. The residues were dispersed in warm water for three times and the temperature of the water was less than 60 °C. Finally, the concentrated residues were freeze-dried to the powders.

Determination the content of tanshinones: Tanshinone IIA and cryptotanshinone are chosen as a biomarker for quality control and the 2015 edition of Chinese Pharmacopoeia states that in qualified tanshinones the content of tanshinone IIA and cryptotanshinone should not be less than 9.8% and 2.1%, respectively. According to 2015 edition of Chinese Pharmacopoeia states we adopted HPLC (Shimadzu, Japan) by tanshinone IIA and cryptotanshinone standard curve to calculate their concentrations. The experiment was carried out three times at least.

Chromatographic conditions：Chromatographic column: C18 ODS Hypersil column (250×4.6 mm, i.d., 5 μm).

Mobile phase: The mobile phase consisted of two eluents, phase A was phosphoric acid solution (0.026%), phase B was acetonitrile.

Injection volume: 10 μL

Flow rate: 1.0 mL/min

T=25 °C

The DAD monitoring wavelength was 270 nm.

Time program for HPLC gradient elution

time (min)	A (%)	B (%)
0-20	80-40	20-60
20-50	40-20	60-80

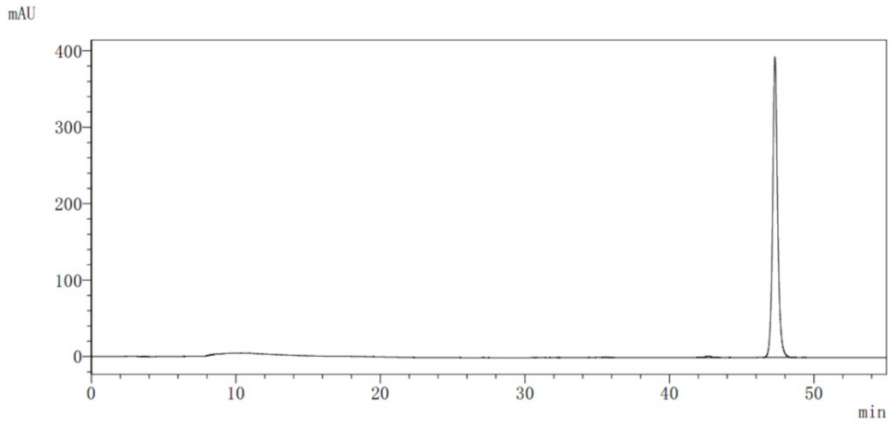

Fig. S-4. The chromatogram of pure tanshinone IIA

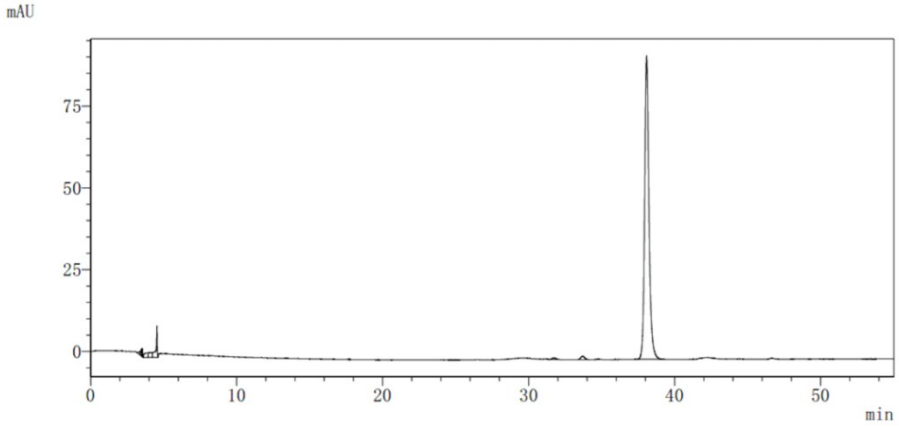

Fig. S-5. The chromatogram of pure cryptotanshinone

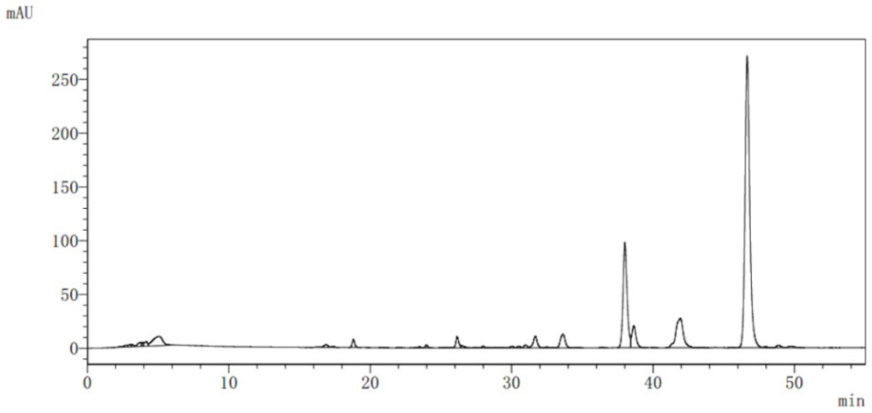

Fig. S-6. The chromatogram of tanshinones extrations

Preparation of drug-loaded hydrogels: The amount of tanshinone IIA stock solutions (DMSO)[1] in hydrogels was fixed to 2% (V/V) for all DMSO-containing hydrogels. For example, to form 1 mL of 1.0 wt% FHFDFHFD hydrogel loaded with 50 μg tanshinone IIA, 20 μL of tanshinone IIA stock solutions which were prepared in DMSO (2.5 mg/mL), was added to an appropriate peptide solution (10 mg FHFDFHFD in 980 μL doubly distilled water).

By determination the content of tanshinone IIA in tanshinones, nearly 10% (w/w) tanshinone IIA contained in extracted tanshinones. To obtain the similar concentration of tanshinone IIA in tanshinones, forming 1 mL of 1.0 wt% FHFDFHFD hydrogel loaded with 0.5 mg tanshinones was chose. The rest of the experimental process was the same as the above method.

Oscillatory rheology: Rheological tests including dynamic time sweep, dynamic frequency sweep, dynamic strain sweep, and gelation temperature were performed on a RheoStress 600 (Thermo) instrument

using 60 mm parallel plates at a gap of 0.5 mm. The tests were conducted to record the change between storage (G′) and loss (G″) modulus at 37 °C. Firstly, the gel was carried out dynamic time sweep with a constant frequency of 6.28 rad/s and constant strain value of 1% within 30 min. Then, the gel was characterized by the mode of dynamic frequency sweep within the region of 0.1-100 rad/s at the strain of 1%. Finally, the gel was conducted dynamic strain sweep test, in the strain region of 0.1-100% at the frequency of 6.28 rad/s. Gelation temperature test was conducted within 30 min, and the rise of temperature was at the rate of 2 °C per minute. The temperature at the cross point of G′ and G″ was defined as gelation temperature.

Transmission electron microscopy: 5 μL of diluted 1.0 wt% hydrogel, tanshinone IIA hydrogel and tanshinones hydrogel were applied to a carbon-coated grid and excess water was blotted away with filter paper after 1 min. Afterwards, the grids were left to dry in ambient conditions for 30 min at least. Transmission electron microscopy (TEM) samples were performed on a JEM-2100 electron microscope (Japan) operating at 220 KV.

Scan electron microscopy: the samples powders of 1.0 wt% hydrogel, tanshinone IIA hydrogel and tanshinones hydrogel freeze-dried previously were deposited on a copper stub and the images were obtained by S-4800 Scanning Electron Microscope (Japan).

Fluorescence spectroscopy: Fluorescence emission measurements were conducted in a RF-5301 spectrofluorimeter (Shimadzu, Japan). To investigate the aromatic stacking in the formed hydrogel, emission spectra were recorded from 300 nm to 600 nm with an excitation wavelength of 320 nm. In this experiment, we prepared octa-peptide saturated aqueous solution and diluted hydrogel solution (0.2 mg/mL).

The interaction of tanshinone IIA and tanshinones with peptides was testified also by fluorescence spectrophotometry. Emission spectra were recorded from 355 nm to 650 nm with an excitation wavelength of 320 nm. For this experiment, concentrations of tanshinone IIA were 0, 50 μg/mL in 1.0 wt% hydrogels. Tanshinone IIA solution without peptide was used to determine spectroscopic characteristic of tanshinone IIA. Concentrations of tanshinones were 0, 500 μg/mL in 1.0 wt% hydrogels. Tanshinones solution without peptide was used to determine spectroscopic characteristic of tanshinones.

In both experiments, the excitation and emission slits were set 5 nm with an assay volume of 1.0 mL.

Circular dichroism (CD)**:** The data were collected at 25 °C on a Jasco (Tokyo, Japan) J-810 CD spectropolarimeter. The concentration of peptide was 0.1 mg/mL in hydrogel as well as heat-treated peptide hydrogel. Additionally, for determination the drug-loaded hydrogels, 50, 500 μg/mL of tanshinone IIA, tanshinones in 1.0 wt% hydrogels were

diluted 100 times, respectively. Data was reported from 250 nm to 190 nm. Spectra were corrected by subtraction of the doubly distilled water which was the same as the solvent of the samples.

Release determination of drug-loaded hydrogels: In vitro release experiment was studied under physiological temperature (37 °C). For determination of tanshinone IIA release from hydrogels, 1.0 wt%, 1.5 wt%, 2.0 wt% peptide hydrogels were prepared in triplicate, and all fixed drug concentration was 50 μg/mL. Firstly, 0.2 mL of fresh phosphate buffer solution (PBS, pH 7.4) was added onto the top of each hydrogel. At a preset time point, the PBS supernatant was taken out for test of the drug release amount of tanshinone IIA from hydrogels. After that, an equal volume of fresh PBS buffer solution was added. Released tanshinone IIA concentrations were measured using HPLC (Shimadzu, Japan) at 270 nm by a tanshinone IIA standard curve. The experiment was carried out three times at least.

To study the similar release of tanshinone IIA in tanshinones hydrogel, prepared 1 mL of peptide hydrogel loaded with 0.5 mg tanshinones was chose. The rest of the experimental process was the same as the above method.

Chromatographic conditions：Chromatographic column: C18 ODS Hypersil column (250×4.6 mm, i.d., 5 μm).

Mobile phase: The mobile phase consisted of two eluents, phase A was

phosphoric acid solution (0.026%), phase B was acetonitrile.

Injection volume: 10 μL

Flow rate: 1.0 mL/min

T=25 °C

The DAD monitoring wavelength was 270 nm.

Time program for HPLC gradient elution

time (min)	A (%)	B (%)
0-25	40-10	60-90
25-35	10	90

To provide an explanation about the conceivable mechanism of the tanshinone IIA release from the hydrogels, the data were analyzed fitting to the following empirical equations:

Model 1: Higuch**i equ**ation[2]

$$\mathbf{M_t / M_\infty = k \cdot t^{0.5}} \quad (1)$$

Model 2: Ritger-Peppas equation[3]

$$\mathbf{M_t / M_\infty = k \cdot t^{n}} \quad (2)$$

Where M_t / M_∞ is fractional drug release, M_t is the amount of drug released at time t, M_∞ is the maximum amount of drug released at time ∞, t is the release time, k is a rate constant of kinetic release, and n is the diffusion exponent, characteristic of the drug release mechanism. For n< 0.5, it indicates that the drug release follows the Fickian diffusion, whereas the non Fickian drug release process has a value of n between

0.5 and 1.

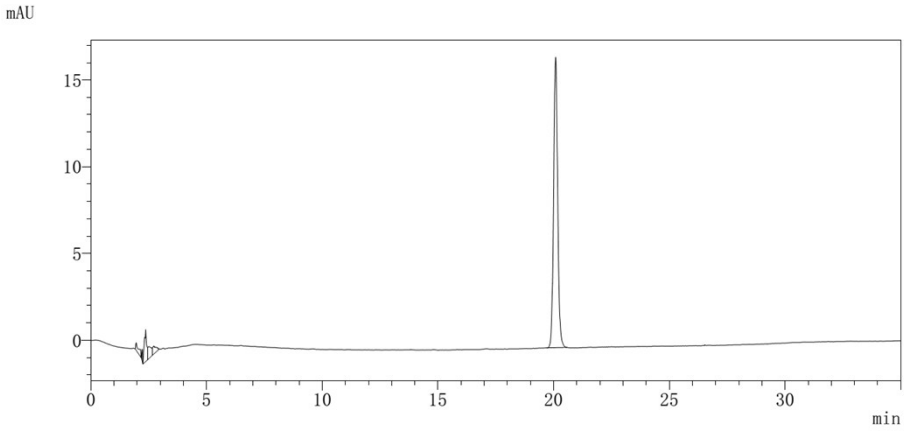

Fig. S-7. The chromatogram of pure tanshinone IIA

	Higuchi		Ritger-Peppas		
gel concentration	k	R^2	n	k	R^2
1.0 wt%	0.0506	0.9255	0.1339	0.1217	0.9826
1.5 wt%	0.0322	0.9892	0.1007	0.0708	0.9824
2.0 wt%	0.0158	0.9906	0.2342	0.0244	0.9729
	Stage1 (1-13h)		Stage2 (24-72h)		

Tab. S-1. The calculated data of tanshinone IIA hydrogel in drug release experiment.

	Higuchi		Ritger-Peppas		
gel concentration	k	R^2	n	k	R^2
1.0 wt%	0.0466	0.9349	0.1347	0.113	0.975
1.5 wt%	0.0262	0.9786	0.2633	0.0227	0.9374
2.0 wt%	0.0138	0.9910	0.3641	0.0246	0.9453
	Stage1 (1-13h)		Stage2 (24-72h)		

Tab. S-2. The calculated data of tanshinones hydrogel in drug release experiment.

Cell culture: The human non-small cell lung cancer cell line A549 were grown in RPMI-1640 supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS) at 37 °C in a 95% humidified air, 5% CO_2 atmosphere.

Drug-loaded hydrogels in contact with A549 cells: 500 μL of single cell suspension ($5x10^4$/mL) was shifted in 24-well plate and cultured in an

incubator for 24 hours. Thereafter, 1 mL of 1.0 wt% FHFDFHFD hydrogel loaded with 0, 50 μg tanshinone IIA so as to tanshinones were prepared as samples. The next day, 350 μL of each sample was added into each well and cultured for 48 hours with the phosphate buffered saline as control. After that, 0.5 mL of fresh PBS buffer was used to replace the medium for two times. Ultimately, the light microscope images were obtained by a confocal microscope (Olympus IX71, Japan).

In vitro anticancer efficacy evaluation: 100 μL of single cell suspension ($5x10^4$/mL) was injected into 96-well plate and cultured in an incubator for 24 hours. Subsequently, the same volume of the free drug and the drug-loaded hydrogel with six gradient concentrations were added to each well with the phosphate buffered saline as control (five parallel well for each concentration) for 48 hours. After that, the media was washed twice by 200 μL of fresh culture medium. 20 μL of MTT solution (5 mg/mL) was added to the plate before incubated for 4 hours at 37 °C. Then, the supernatant was pipetted out and 150 μL of DMSO was added into each well to dissolve the formazon crystals. Finally, the absorbance was obtained at 490 nm (Bio-RAD, America) after concussion for 10 min.

1. ER Smith, Z Hadidian and MM Mason, *Annals of the New York Academy of Sciences*, 1967, **141**, 101-113.
2. S Dash, P. N. Murthy, L Nath and P Chowdhury, *Acta Poloniae Pharmaceutica - Drug Research*, 2010, **67**, 217-223.
3. PL. Ritger and N. Peppas, *J. Controlled Release*, 1987, **5**, 23-26.