Electronic Supplementary Information

Sequential self-assembly and disassembly of curcumin hydrogel effectively alleviates inflammatory bowel disease

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Contents

1. General Methods

All the starting materials were obtained from Aladdin, Sigma or Sangon Biotech. Commercially available reagents were used without further purification, unless noted otherwise. All other chemicals were reagents grade or better. Calf intestinal alkaline phosphatase (ALP) was obtained from Takara Bio Inc. (Beijing, China). Esterase from porcine liver was obtained from Sigma. HPLC analyses were performed on an Agilent 1260 HPLC system equipped with a G7111A pump and an in-line diode array UV detector using an Agilent Zorbax 300SB-C18 RP column, with CH_3CN (0.1% of TFA) and ultrapure water (0.1% of TFA) as the eluent. Electrospray ionization (ESI) mass spectra were obtained on a Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFisher Corporation). H NMR spectrum was obtained on a JNM-ECZ400S. ^{13}C NMR spectrum was obtained on a JNM-ECZ600R. Rheology measurements were performed on a Haake RheoStress 6000 (Thermo Scientific), with cone-and-plate geometry (1 deg /20 mm) at the gap of 50 μm. Cryo transmission electron microscopy (cryo-TEM) images were obtained on a Tecnai F20 transmission electron microscope from FEI company, operating at 120 KV. Visible absorption spectra were obtained on a PerkinElmer Lambda 25 UV-vis spectrometer.

Cell culture

The cells required for this study were bone marrow-derived macrophages (BMDMs) extracted from an animal model. After the establishment of animal model, the bone marrow between the tibia and fibula of mice was taken and cultured with L929 mouse fibroblasts in DMEM of 10% FBS.

Cell inflammatory factor test

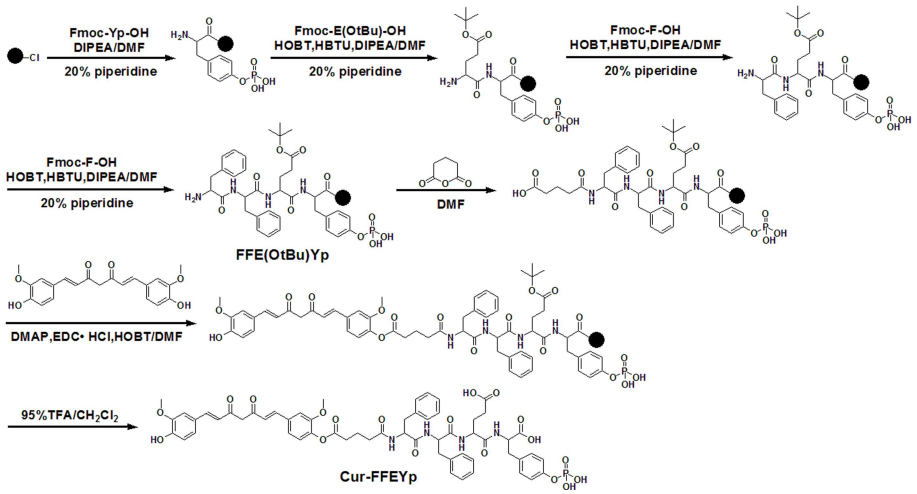
The differentiated and mature BMDMs cells were seeded in 24-well plates (5×10^5/well) and incubated overnight. Cells were pretreated with 10 μM Cur, **Cur-FFEYp** and DMEM for 2 h, and then continuously stimulated with 50 ng/mL LPS for 3 h. Total RNAs were isolated using Trizol reagent (Invitrogen). The cDNA was synthesized from 500 ng of total RNA using the HiScript III RT SuperMix kit (Vazyme, China) as manufacturer's instructions. After reverse transcription, quantitative real-time PCR (RT-qPCR) was performed on the Bio-Rad CFX384 real-time PCR system in a 10 μL reaction system using SYBR Green qPCR Master Mix (Vazyme, China). *Gapdh* was used as an endogenous control. The mRNA expression levels of the tested genes relative to *Gapdh* were determined using the $2^{-\Delta\Delta Ct}$ method and shown as fold induction.

Immunoblotting assay

After stimulation with LPS at the appropriate time (0, 15, 30 min), BMDMs were harvested using lysis buffer containing 50 mM Tris-HCl (pH 7.4), 2 mM EDTA, 150 mM NaCl, 0.5% NP-40, PMSF (50 μg/mL) and complete protease inhibitor mixtures (Sigma). Lysates were cleared by centrifugation and then the protein concentrations were measured, and equal amounts of lysates were used for immunoblotting and immunoprecipitation. Antibodies specific for p-p65 (3033, 1:1000), p65 (8242, 1:1000), p-IκBα (9246, 1:1000) and IκBα (4814, 1:1000) were from Cell Signaling Technology.

2. Syntheses and Characterizations of Cur-FFEYp and Cur-FFEY

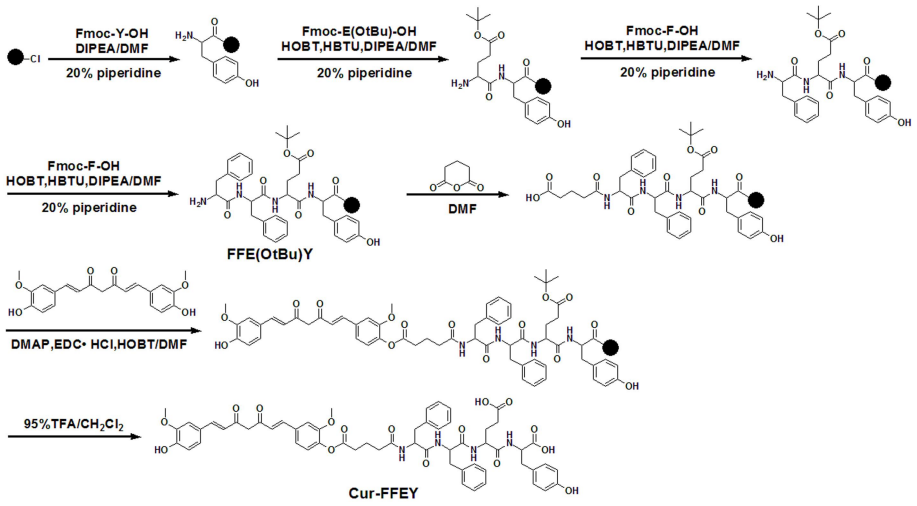
Scheme S1. The synthetic route for **Cur-FFEYp**.



Synthesis of ***Cur-FFEYp***: Peptide FFE(OtBu)Yp was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (800 mg, 0.88 mmol) and the corresponding Fmoc-protected amino acids with side chains properly protected. The solution of 20% piperidine in N, N -Dimethylformamide (DMF) was used to remove the Fmoc group. After removing the Fmoc group of the last amino acid, glutaric anhydride (GA, 225.9 mg, 1.98 mmol) in 4 mL DMF was added to react for 3 h. Then, curcumin (Cur, 486.3 mg,1.32 mmol), 4-dimethy- laminopyridine (DMAP, 161.26 mg, 1.32 mmol), 1-Hydroxybenzotriazole (HOBT, 178.36 mg, 1.32 mmol), and 1-ethyl-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC·HCl, 253.0 mg, 1.32 mmol) were added to react overnight. Finally, 95 % TFA/CH_2Cl_2 solution was used to deprotect the OtBu group and cut **Cur-FFEYp** (150.5 mg) from the resin. ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm): 8.23 (d, J = 6.9 Hz, 1 H), 8.18 – 7.99 (m, 3 H), 7.68 – 7.54 (m, 1 H), 7.52 – 7.42 (m, 1 H), 7.29 (d, J = 38.3 Hz, 4 H), 7.20 (d, J = 13.6 Hz, 5 H), 7.17 – 6.95 (m, 8 H), 6.89 – 6.75 (m, 2 H), 6.64 (dd, J = 23.0, 8.5 Hz, 1 H), 4.51 (s, 2 H), 4.40 (d, J = 5.5 Hz, 1 H), 4.32 (d, J = 6.3 Hz, 1 H), 3.85 – 3.77 (m, 6 H), 3.75 –

3.71 (m, 3 H), 2.98 (dd, *J* = 28.1, 13.2 Hz, 4 H), 2.36 (s, 2 H), 2.18 (s, 2 H), 2.12 (s, 2 H), 1.85 (s, 2 H), 1.73 (d, *J* = 24.1 Hz, 4 H), 1.35 – 1.12 (m, 2 H) (Figure S1).[13]C NMR (100 MHz, *d*$_6$-DMSO) δ (ppm): 176.42 (1 C), 174.52 (2 C), 173.16 (2 C), 171.88 (1 C), 171.72 (1 C), 171.53 (1 C), 171.26 (1 C), 151.61 (1 C), 150.70 (1 C),147.86 (1 C), 145.17 (1 C), 138.46 (2 C), 138.17 (2 C), 134.23 (1 C), 133.24 (1 C), 131.94 (1 C), 130.56 (4 C), 129.65 (4 C), 128.50 (4 C), 126.72 (1 C), 123.73 (1 C), 122.92 (1 C), 121.62 (1 C), 120.75 (1 C), 120.33 (3 C), 115.76 (1 C), 112.92 (1 C), 101.55 (1 C), 56.11 (3 C), 54.11 (2 C), 52.01 (1 C), 42.13 (1 C), 37.93 (1 C), 37.66 (1 C), 36.26 (1 C), 34.39 (1 C), 32.89 (1 C), 30.41 (1 C), 28.23 (1 C), 20.99 (1 C) (Figure S2). MS: calc $[M+H]^+$: 1149.37, obsvd. ESI-MS: m/z 1149.25 (Figure S3).

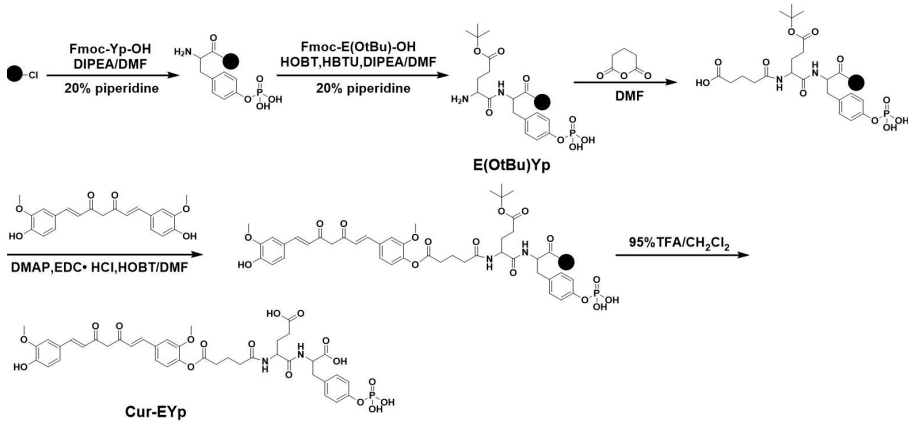
Scheme S2. The synthetic route for **Cur-FFEY**.



Synthesis of ***Cur-FFEY***: Peptide FFE(OtBu)Y was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (400 mg, 0.44 mmol) and the corresponding Fmoc-protected amino acids with side chains properly protected. The solution of 20% piperidine in N, N -Dimethylformamide (DMF) was used to remove the Fmoc group. After removing the Fmoc group of the last amino acid, glutaric anhydride (GA, 113 mg, 0.99 mmol) in 4 mL DMF was added to react for 3 h. Then, curcumin (Cur, 243.2 mg, 0.66 mmol), 4-dimethy- laminopyridine (DMAP, 80.7 mg, 0.66 mmol), 1-Hydroxybenzotriazole (HOBT, 89.2 mg, 0.66 mmol), and 1-ethyl-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC·HCl, 126.5 mg,0.66 mmol) were added to react overnight. Finally, 95 % TFA/CH_2Cl_2 solution was used to deprotect the OtBu group and cut **Cur-FFEY** (50.4 mg) from the resin. [1]H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm): 8.04 (d, *J* = 35.9 Hz, 6 H), 7.56 (d, *J* = 14.8 Hz, 2 H), 7.37 – 7.04 (m, 15 H), 6.78 (dd, *J* = 81.8, 63.9 Hz, 6 H), 6.09 (s, 1 H), 4.49 (s, 1 H), 4.30 (s, 2 H), 3.79 (d, *J* = 7.8 Hz, 2 H), 3.00 – 2.58 (m, 6 H), 2.20 (s, 2 H), 2.07 (s, 2 H), 1.86 (s, 2 H), 1.66 (s, 4 H), 1.19 (s, 2 H) (Figure S4). [13]C NMR (150 MHz, *d*$_6$-DMSO) δ (ppm): 185.40 (1 C), 181.98 (1 C), 174.55 (1 C), 173.34 (1 C), 171.87 (1 C), 171.79 (1 C), 171.42 (1 C),

171.25 (2 C), 156.50 (1 C), 151.70 (1 C), 150.09 (1 C), 148.55 (1 C), 142.08 (1 C), 141.34 (1 C), 139.57 (1 C), 138.51 (1 C), 138.21 (1 C), 134.30 (1 C), 130.53 (4 C), 129.69 (4 C), 128.54 (4 C), 127.85 (1 C), 126.75 (2 C), 125.08 (2 C), 123.88 (1 C), 121.79 (1 C), 116.25 (1 C), 115.54 (2 C), 112.47 (1 C), 111.98 (1 C), 56.51 (3 C),54.38 (2 C), 52.08 (1 C), 49.41 (1 C), 37.95 (1 C), 37.77 (1 C), 36.36 (1 C), 34.47 (1 C), 32.97 (1 C), 30.48 (1 C), 28.33 (1 C), 21.05 (1 C) (Figure S5） MS: calc $[M+H]^+$: 1069.40, obsvd. ESI-MS: m/z 1069.42 (Figure S6).

Scheme S3. The synthetic route for **Cur-EYp**.



Synthesis of ***Cur-EYp***: Peptide E(OtBu)Yp was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (800 mg, 0.88 mmol) and the corresponding Fmoc-protected amino acids with side chains properly protected. The solution of 20% piperidine in N, N -Dimethylformamide (DMF) was used to remove the Fmoc group. After removing the Fmoc group of the last amino acid, glutaric anhydride (GA, 225 mg, 1.98 mmol) in 4 mL DMF was added to react for 3 h. Then, curcumin (Cur, 486.3 mg, 1.32 mmol), 4-dimethy-laminopyridine (DMAP, 161.26 mg, 1.32 mmol), 1-Hydroxybenzotriazole (HOBT, 178.36 mg, 1.32 mmol), and 1-ethyl-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC·HCl, 253 mg, 1.32 mmol) were added to react overnight. Finally, 95 % TFA/CH_2Cl_2 solution was used to deprotect the OtBu group and cut **Cur-EYp** (70.6 mg) from the resin. ^{1}H NMR (600 MHz, d_6-DMSO) δ (ppm): 8.16 (d, J = 7.9 Hz, 1 H), 8.10 (d, J = 7.6 Hz, 1 H), 7.58 (d, J = 4.3 Hz, 1 H), 7.54 (d, J = 4.2 Hz, 1 H), 7.45 (s, 1 H), 7.31 – 7.25 (m, 2 H), 7.17 – 7.09 (m, 4 H), 7.02 (d, J = 8.1 Hz, 2 H), 6.95 – 6.88 (m, 1 H), 6.81 – 6.72 (m, 2 H), 6.10 (s, 1 H), 2.96 (dd, J = 14.0, 4.9 Hz, 1 H), 2.78 (dd, J = 13.9, 9.3 Hz, 1 H), 2.56 (t, J = 7.5 Hz, 2 H), 2.22 (t, J = 7.3 Hz, 2 H), 2.13 (dd, J = 10.1, 6.9 Hz, 2 H), 1.82 (t, J = 7.5 Hz, 2 H), 1.72 (s, 3 H), 1.27 – 1.11 (m, 8 H) (Figure S10). MS: calc $[M+H]^+$: 855.2475, obsvd. ESI-MS: m/z 855.2378 (Figure S11).

3. Supporting Figures and Tables

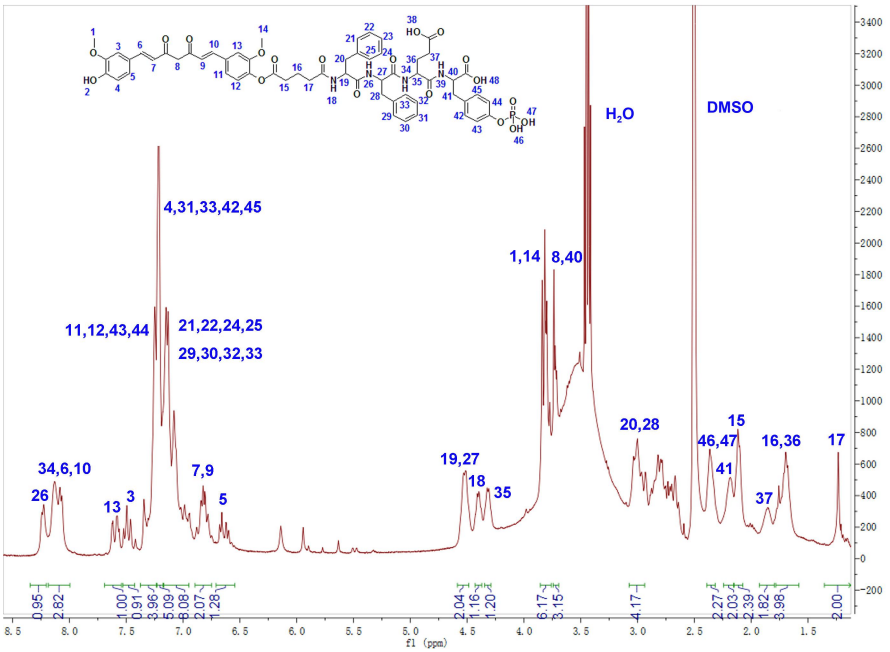


Figure S1. ^{1}H NMR spectrum of **Cur-FFEYp** in *d*$_6$-DMSO.

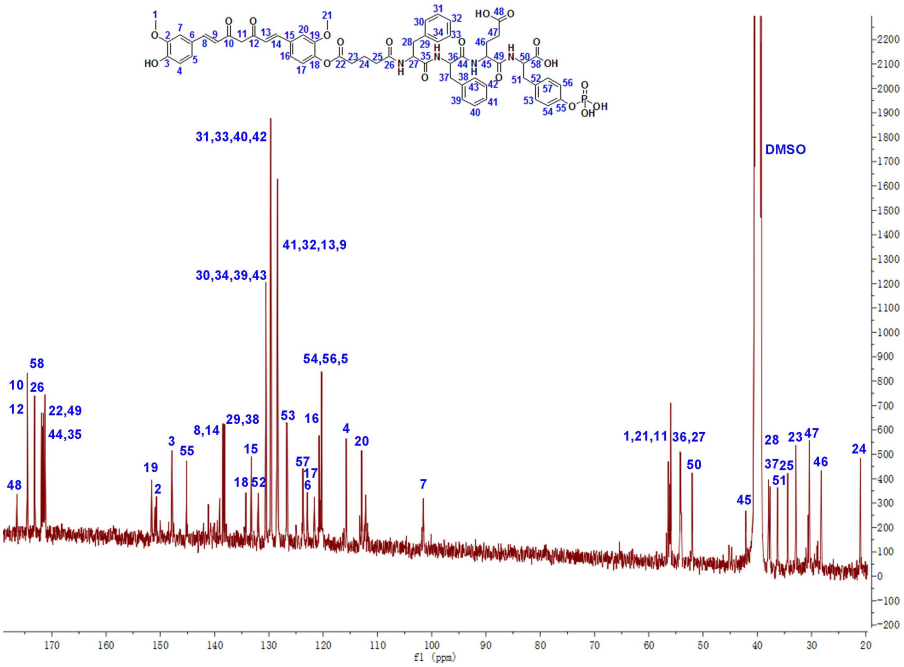


Figure S2. ^{13}C NMR spectrum of **Cur-FFEYp** in *d*$_6$-DMSO.

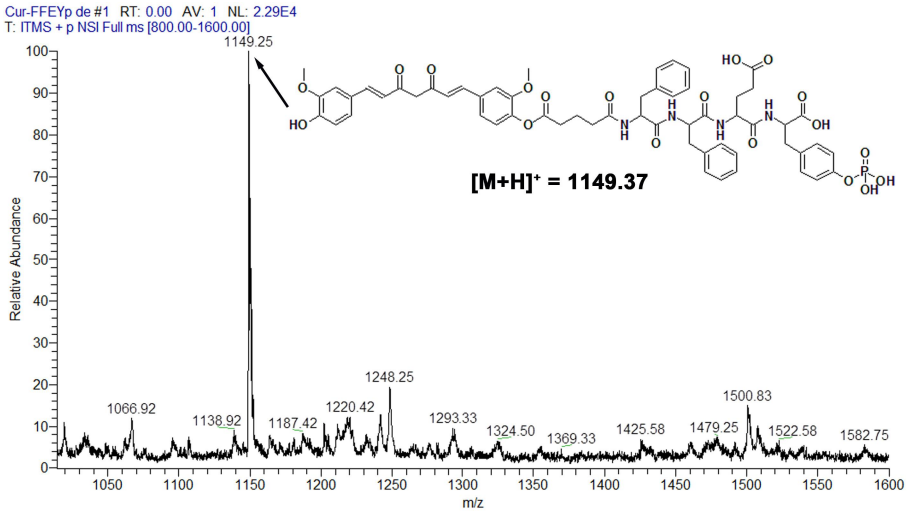


Figure S3. ESI-MS spectrum of **Cur-FFEYp**.

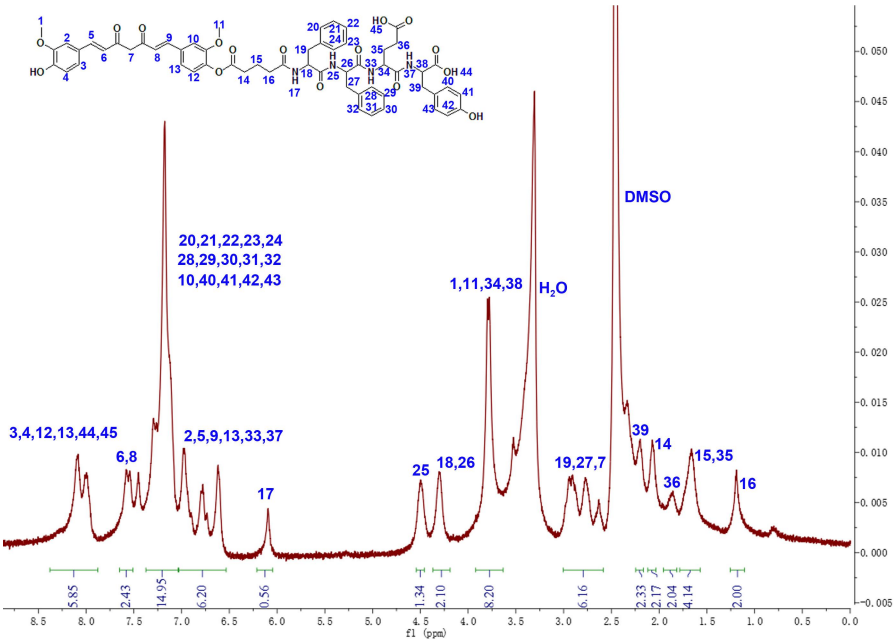


Figure S4. ^{1}H NMR spectrum of **Cur-FFEY** in *d*$_6$-DMSO.

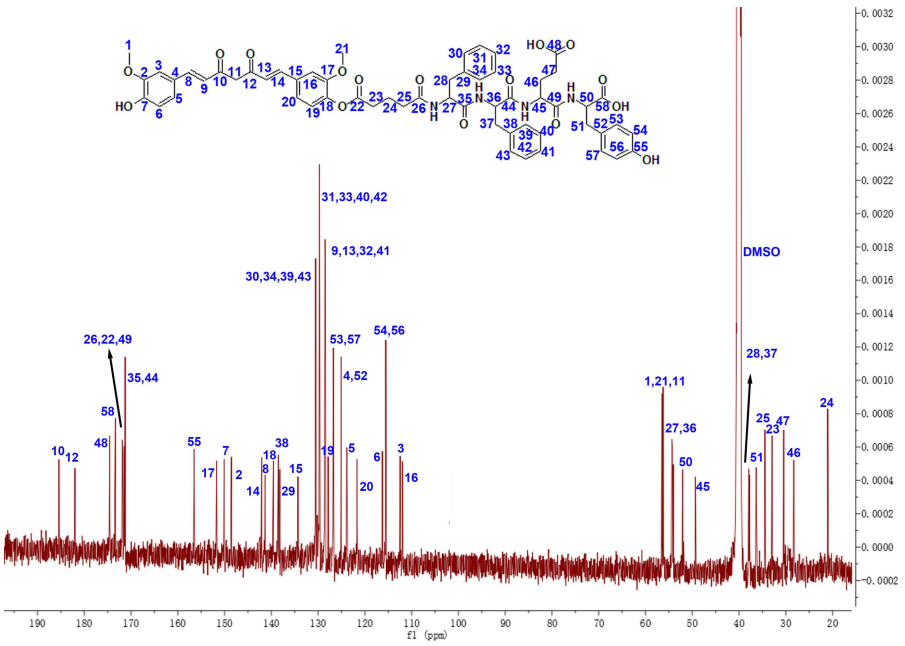


Figure S5. ^{13}C NMR spectrum of **Cur-FFEY** in *d*$_6$-DMSO.

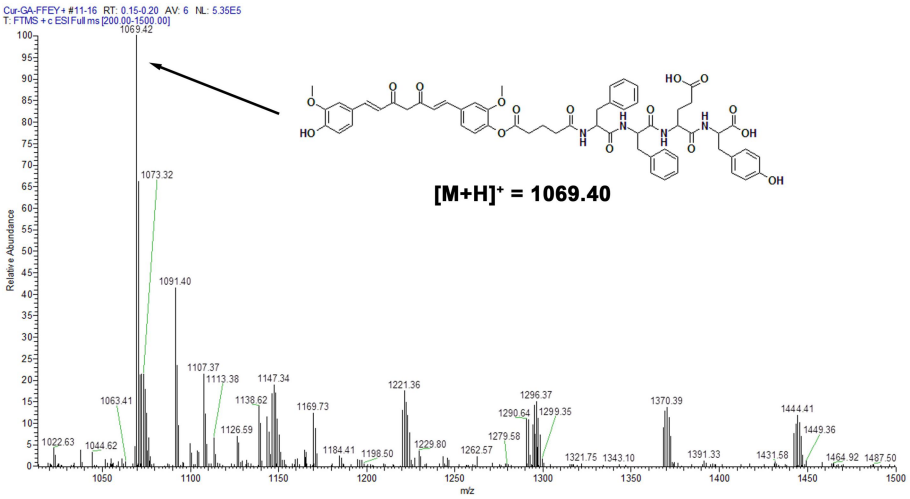


Figure S6. ESI-MS spectrum of **Cur-FFEY**.

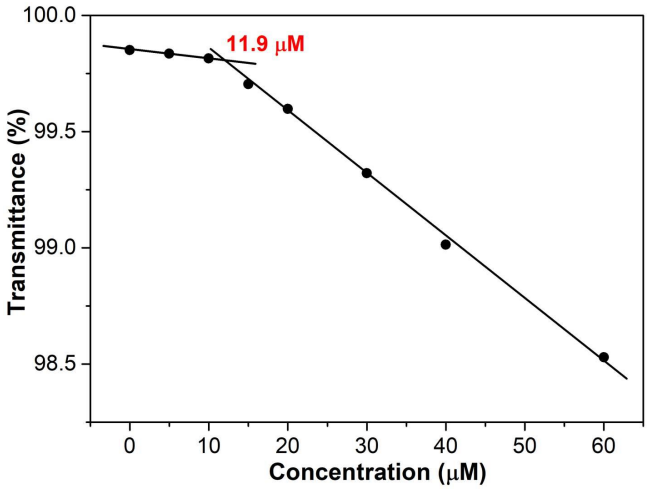


Figure S7. Concentration-dependent transmittance values at 600 nm of dilutions of Cur hydrogel.

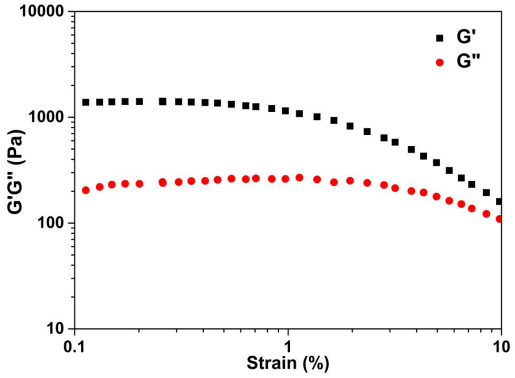


Figure S8. Dynamic strains of storage modulus (G') and the loss modulus (G") of Cur hydrogel at the frequency of 1 Hz, 25 °C.

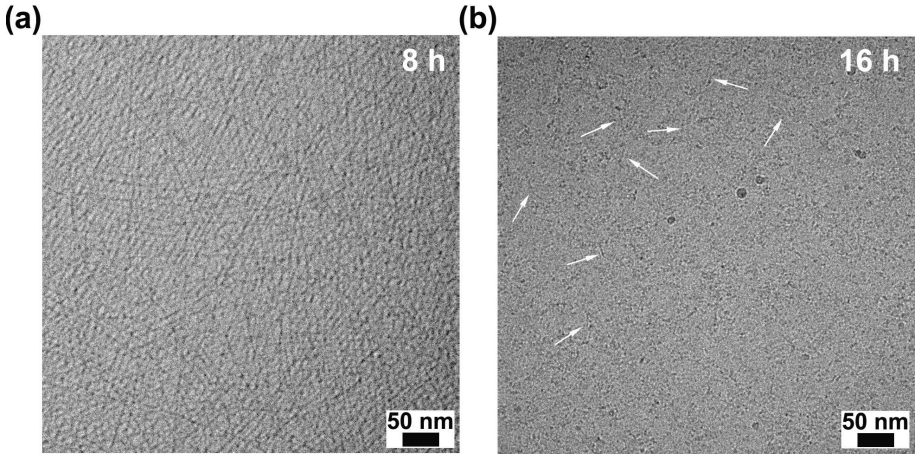


Figure S9. Cryo-TEM images of the **Cur-FFEYp** solution after incubation with ALP (0.04 U/μL) and esterase (0.18 U/μL) at 8 h (a) and 16 h (b).

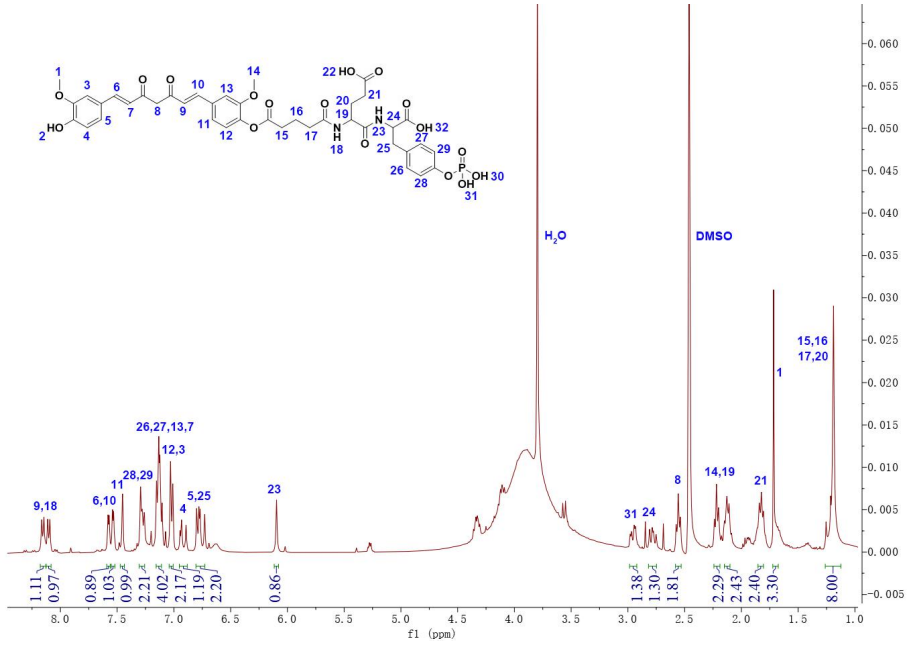


Figure S10. ^{1}H NMR spectrum of **Cur-EYp** in d_6-DMSO.

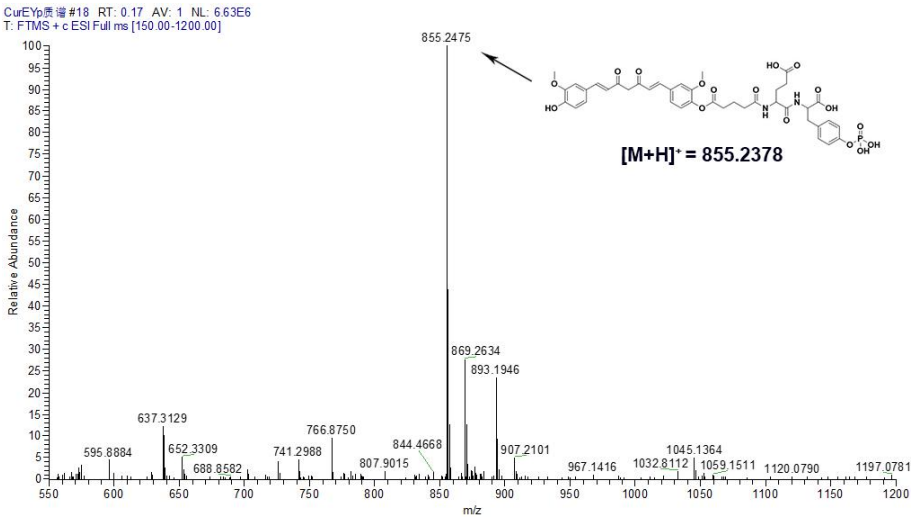


Figure S11. ESI-MS spectrum of **Cur-EYp**.

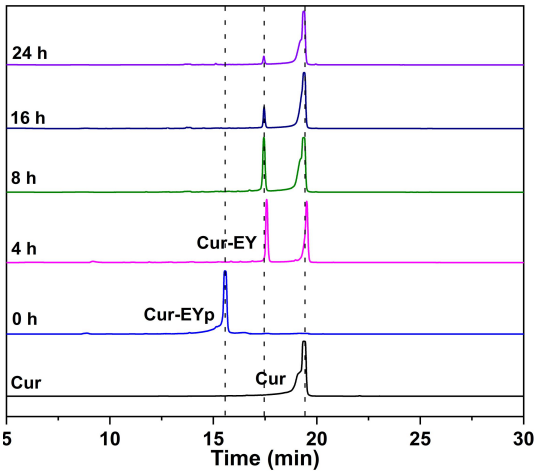


Figure S12. HPLC traces of Cur, **Cur-EYp** and a series of **Cur-EYp** solution incubated with 0.04 U/μL ALP and 0.18 U/μL esterase at different time points (0-24 h).

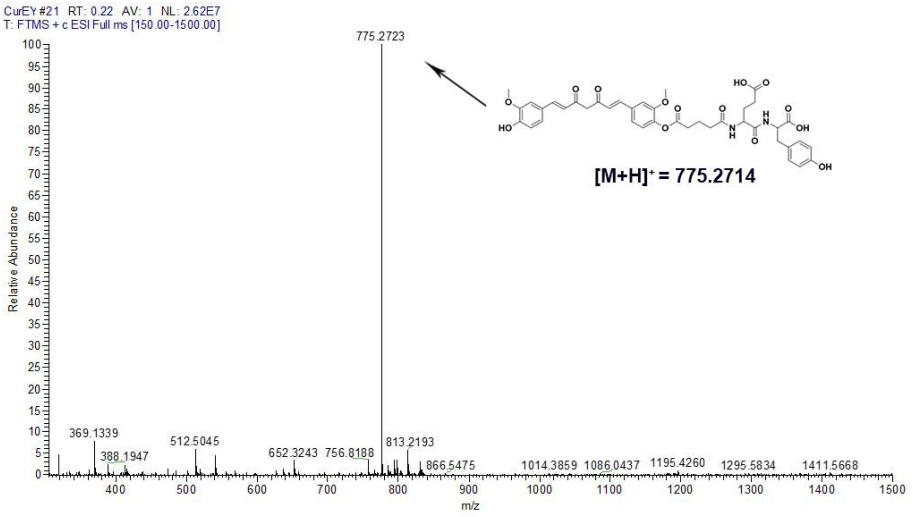


Figure S13. ESI-MS spectrum of **Cur-EY**.

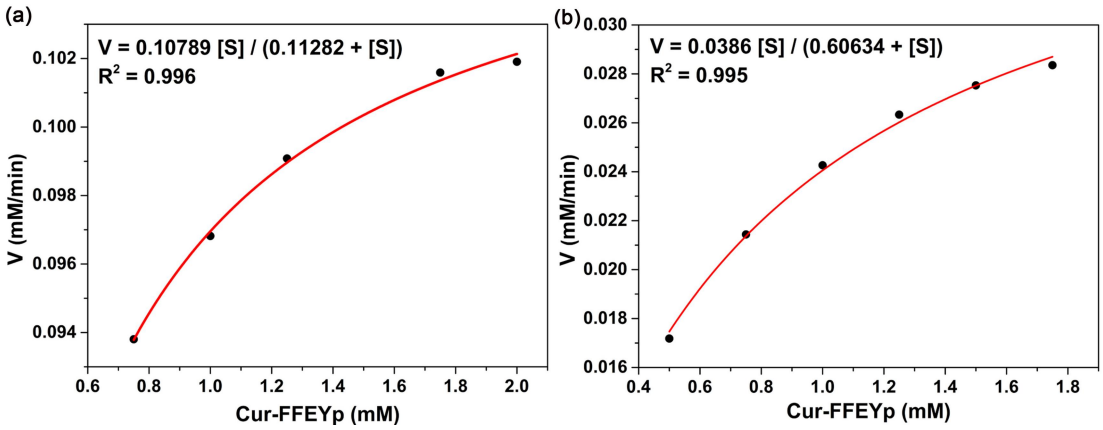


Figure S14. Initial velocities of ALP-catalyzed (a) or esterase-catalyzed (b) reaction plotted against the concentrations of **Cur-FFEYp** and fitted to the Michaelis-Menten model.

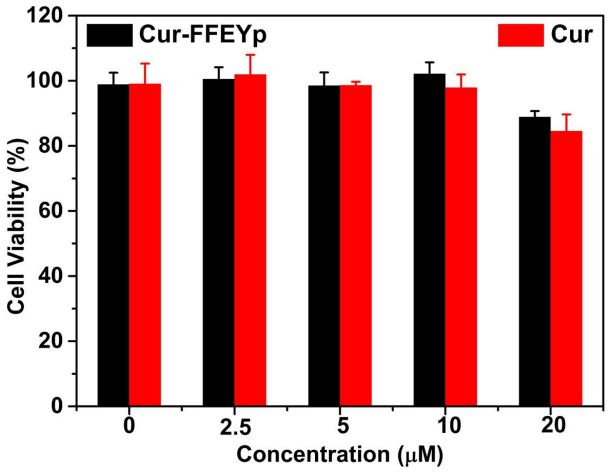


Figure S15. Cytotoxicity study of **Cur-FFEYp** and Cur on BMDMs. BMDMs was incubated with different concentrations of **Cur-FFEYp** and Cur for 12 h. Error bars represent the standard deviation of three independent experiments.

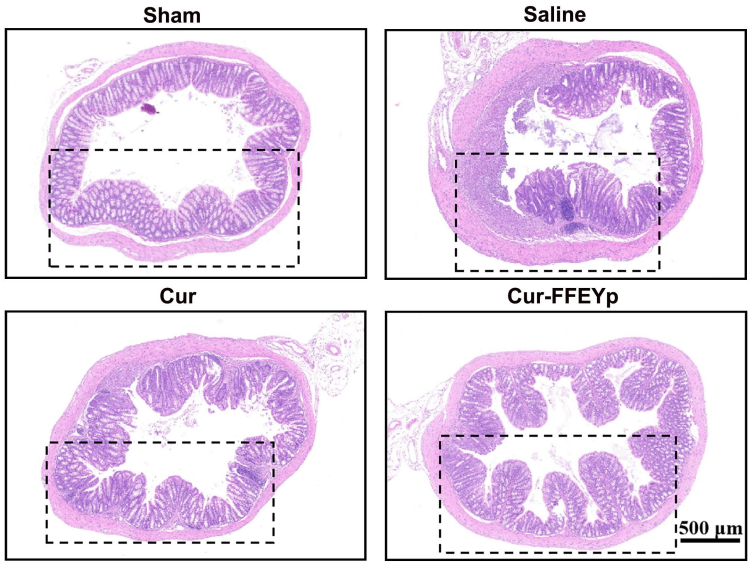


Figure S16. H&E staining of colon sections of mice in four groups after 8 days of administration for DSS-induced UC model.

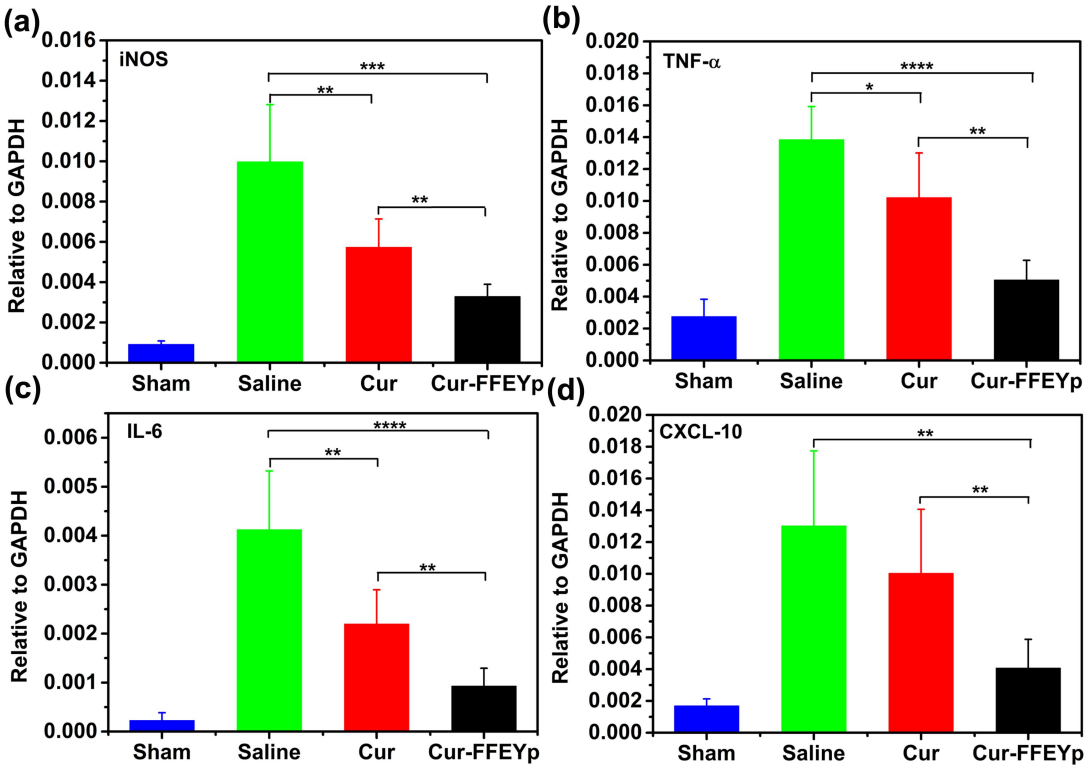


Figure S17. Inflammatory factors (iNOS (a), TNF-α (b), IL-6 (c) and CXCL-10 (d)) of colon of mice in four groups after 8 days of administration for DSS-induced UC model. Error bars represent the standard deviation of seven independent experiments. Data were shown as mean SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

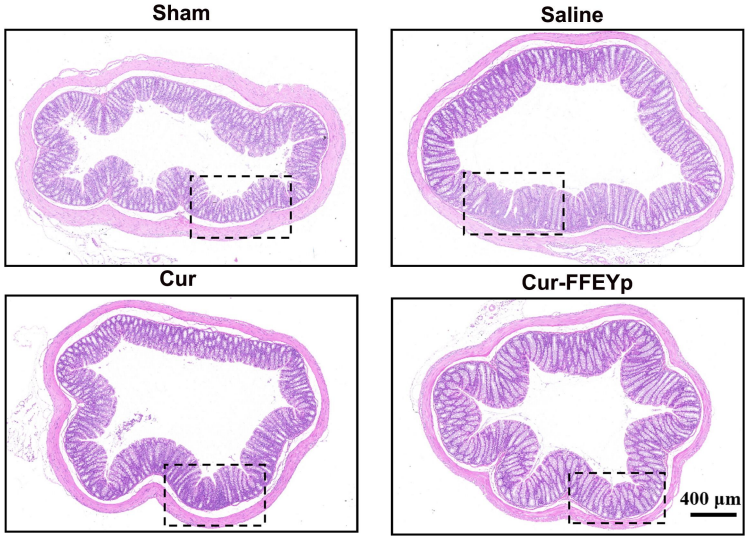


Figure S18. H&E staining of colon sections of mice in four groups after 12 days of administration for TNBS-induced CD model.

Table S1. Cumulative release percentage of Cur from **Cur-FFEYp** in the presence of ALP (0.04 U/μL) and esterase (0.18 U/μL) within 24 h.

Time (h)	4	8	16	24
Cumulative release percentage (%)	36.6	51.1	67.1	72.6

Table S2. Cumulative release percentage of Cur from **Cur-EYp** in the presence of ALP (0.04 U/μL) and esterase (0.18 U/μL) within 24 h.

Time (h)	4	8	16	24
Cumulative release percentage (%)	57.4	70.7	84.0	94.9

Table S3. Kinetic parameters for ALP-catalyzed or esterase-catalyzed reaction of **Cur-FFEYp**.

	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})
ALP	$1.13*10^{-4}$	1.64	$1.45*10^{4}$
Esterase	$6.06*10^{-4}$	0.14	$2.31*10^{2}$

Table S4. DAI comprehensive score table.

Weight loss (%)	Stool traits	Fecal occult blood	Score
0	normal	normal	0
1-5			1
5-10	soft stool but not sticking to the anus	occult blood positive	2
10-15			3
> 15	stool sticks to the anus and is mushy	gross bloody stool	4

Table S5. HPLC condition for Figure 2c, 2d and 2h.

Time (min)	Flow (mL/min)	H_2O % (0.1 % TFA)	CH_3CN % (0.1 % TFA)
0	1	60	40
3	1	60	40
35	1	20	80
37	1	20	80
39	1	60	40
40	1	60	40