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Electronic Supplementary Information

An esterase-activatable curcumin prodrug for tumor-targeting therapy

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Contents

1. Methods

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. 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). ^1H NMR spectrum was obtained on a JNM-ECZ400S at 400 MHz. ^{13}C NMR spectrum was obtained on a JNM-ECZ600R at 600 MHz. The MestReNova NMR software was used to perform the analysis. Fluorescence spectra were recorded on a spectrofluorometer FS 5 (Edinburgh Instruments). Confocal fluorescence images of cells were obtained on a Zeiss LSM 880 laser scanning confocal microscope.

Selectivity and anti-interference study *in vitro*

To study the selectivity of **Cur-RGD** toward biological substances, **Cur-RGD** was incubated with intracellular biothiols (100 μM Hcy, 100 μM Cys, or 1 mM GSH), reactive oxygen species (100 μM NaClO, 100 μM H_2O_2), tumor markers (100 U/L ALP, 30 U/L GGT), or 30 U/L CTB respectively. To study the anti-interference ability of **Cur-RGD** toward biological substances, **Cur-RGD** was co-incubated with above biological substances with 3.6 U/mL esterase.

Cell culture

The HepG2 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. The culture dishes were maintained at 37°C under a humid atmosphere with 5% CO_2.

FL imaging of living cells

HepG2 cells were seeded into glass bottom cell culture dishes (3.5 cm) at 1×10^5/well and cultured overnight. HepG2 cells in Group **Cur-RGD** were incubated with 50 μM

Cur-RGD in 1 mL of serum-free DMEM for 4 h. HepG2 cells in Group Cur were incubated with 50 μM Cur for 4 h. HepG2 cells in Group BNPP + **Cur-RGD** were pre-incubated with BNPP (200 μM) for 1 h and then incubated with 50 μM **Cur-RGD** for 4 h. HepG2 cells in Group RGD + **Cur-RGD** were pre-incubated with RGD (100 μM) for 1 h and then incubated with 50 μM **Cur-RGD** for 4 h. Before imaging, the cells were stained with Hoechst 33342.

Cell viability assay

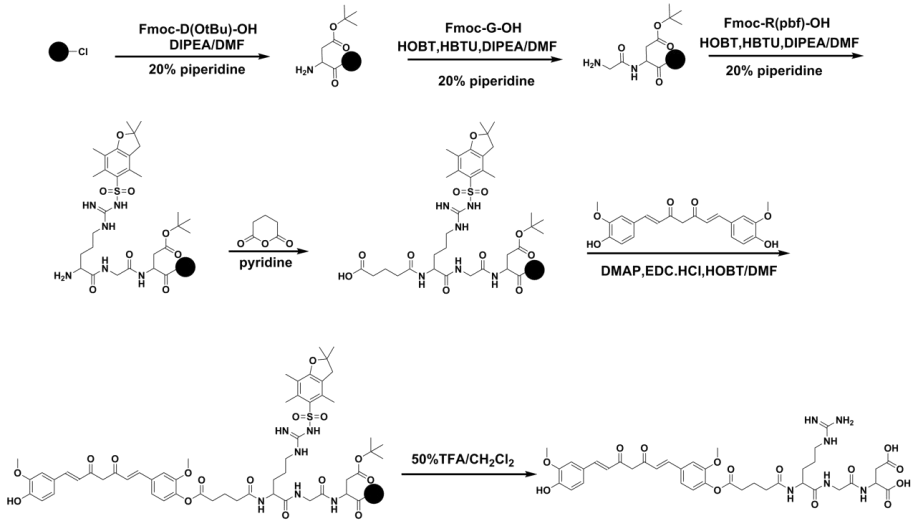
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with HepG2 cells was employed to evaluate the cytotoxicity. HepG2 cells were seeded into a 96-well plate at a density of 5×10^3 /well and cultured for 24 h at 37 °C under 5% CO_2. Cells in Group **Cur-RGD** were treated with 10, 20, 40, 80, 120 μM **Cur-RGD** for 24 or 48 h, respectively. Cells in Group Cur were treated with 10, 20, 40, 80, 120 μM Cur for 24 or 48 h, respectively. Cells in Group BNPP + **Cur-RGD** were co-incubated with 200 μM BNPP with 10, 20, 40, 80, 120 μM **Cur-RGD** for 24 or 48 h, respectively. Cells in Group RGD + **Cur-RGD** were co-incubated with 100 μM RGD with 10, 20, 40, 80, 120 μM **Cur-RGD** for 24 or 48 h, respectively. MTT (5 mg/mL, 10 μL) was added to each well and incubated with cells for 4 h. Next, 100 μL/well dimethylsulfoxide (DMSO) was added to dissolve the formazan. The absorption at 490 nm was detected using an ELISA reader (Varioskan Flash). The viability of cell growth was calculated: viability (%) = (mean of absorbance value of treatment group/mean of absorbance value of control) × 100.

Anti-tumor study *in vivo*. All animals received care according to the guidelines of the Care and Use of Laboratory Animals. The procedures were approved by the Anhui University Animal Care and Use Committee (2021-003). HepG2 cells (5×10^6) were subcutaneously inoculated into the left leg of mice. When the tumor volume was around 100 mm^3, mice were randomly divided into five groups (n = 3 for each group): (1) mice in Group saline were i.t. injected with saline every two days; (2) mice in Group Cur were i.t. injected with Cur (20 mg/kg) every two days; (3) mice in Group **Cur-RGD** group mice were i.t. injected with **Cur-RGD** (20 mg/kg Cur) every two

days; (4) mice in Group RGD + **Cur-RGD** were i.t. injected with the mixture of **Cur-RGD** (20 mg/kg Cur) and RGD (4.2 mg/kg) every two days; (5) mice in Group BNPP + **Cur-RGD** were i.t. injected with the mixture of **Cur-RGD** (20 mg/kg Cur) and BNPP (21 mg/kg) every two days. The tumor volumes and body weights of the mice were recorded every two days during treatment. At day14, the main organs and tumors of mice in five groups were dissected for H&E staining.

2. Syntheses and Characterizations of Cur-RGD

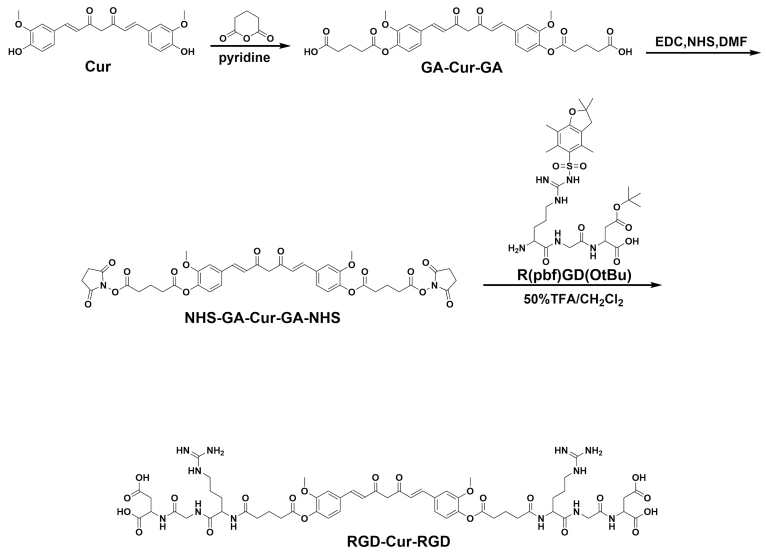
Scheme S1. The synthetic route for **Cur-RGD.**



Synthesis of ***Cur-RGD*****:** Peptide RGD 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 first amino acid Fmoc-D(OtBu)-OH (543 mg, 1.32 mmol) in 4 mL DMF was loaded onto the resin and DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. After loading the first amino acid to the resin, the capping reagent (DMF: MeOH: = 20:1, 4 mL) was used to ensure all the active sites of the resin were protected. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, Then the next Fmoc-G-OH (392 mg, 1.32 mmol) in 4 mL DMF was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, and the next Fmoc-R(pbf)-OH (856 mg, 1.32 mmol)

in 4 mL DMF was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The growth of the peptide chain followed the established Fmoc SPPS protocol. 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-dimethylaminopyridine (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, wash 12 times with 4 mL 50% TFA/CH_2Cl_2 solution to deprotect the OtBu and Pbf group and cut **Cur-RGD** from the resin. The pure **Cur-RGD** (543 mg, 0.67 mmol, yield: 76.1%) was obtained after HPLC purification. ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm): δ 8.16 (t, J = 5.9 Hz, 2H), 8.06 (d, J = 7.5 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.56 – 7.52 (m, 1H), 7.46 (s, 2H), 7.28 (d, J = 11.1 Hz, 3H), 7.17 – 7.07 (m, 3H), 6.92 (d, J = 15.9 Hz, 1H), 6.80 (d, J = 2.5 Hz, 1H), 6.79 – 6.76 (m, 1H), 6.73 (d, J = 2.2 Hz, 1H), 6.09 (s, 1H), 4.52 (q, J = 7.6, 7.2 Hz, 1H), 4.24 (d, J = 7.3 Hz, 1H), 3.80 (s, 6H), 3.70 (d, J = 5.6 Hz, 4H), 3.05 (t, J = 6.4 Hz, 2H), 2.63 (d, J = 5.4 Hz, 1H), 2.56 (q, J = 7.9, 6.9 Hz, 2H), 2.25 (t, J = 7.4 Hz, 2H), 1.83 (p, J = 7.6 Hz, 2H), 1.72 – 1.57 (m, 1H), 1.46 (h, J = 8.7, 6.9 Hz, 3H). (Figure S1).^{13}C NMR (150 MHz, *d*$_6$-DMSO) δ (ppm): 185.40 (1 C), 181.98 (1 C), 172.79 (2 C), 172.14 (2 C), 171.34 (1 C), 169.12 (1 C), 157.32 (1 C), 151.70 (1 C), 150.12 (1 C), 148.55 (1 C), 142.10 (1 C), 141.33 (1 C), 139.55 (1 C), 134.32 (1 C), 126.72 (1 C), 125.08 (1 C), 123.87 (1 C), 121.76 (1 C), 121.64 (1 C), 116.23 (1 C), 111.94 (2 C), 101.84 (1 C), 56.65 (1 C), 56.53 (1 C), 56.22 (1 C), 52.84 (1 C), 49.08 (1 C), 42.12 (1 C), 41.06 (1 C), 36.55 (1 C), 34.38 (1 C), 33.18 (1 C), 29.49 (1 C), 25.55 (1 C), 21.05 (1 C) (Figure S2) MS: calc $[M+H]^+$: 811.3150, obsvd. ESI-MS: m/z 811.3087 (Figure S3).

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



Synthesis of ***GA-Cur-GA*:** The Cur (368 mg. 1 mmol) was dissolved with GA (285.25 mg, 2.5 mmol) in pyridine and mixed overnight at room temperature. Removing the solvent using a rotary evaporator was purified using RP-HPLC to yield a yellow powder GA-Cur-GA (429.3 mg, yield: 71.9%). MS: calc $[M+H]^+$: 597.1972, obsvd. ESI-MS: m/z 597.1926.

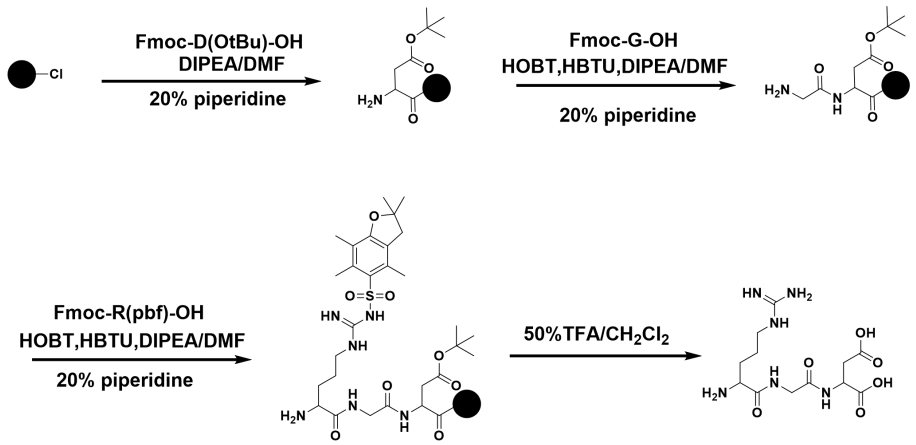
Synthesis of ***NHS-GA-Cur-GA-NHS*:** GA-Cur-GA (298.1 mg,0.5 mmol) was added to the DMF (2 mL) solution of NHS (172.6 mg,1.5 mmol) in an ice water bath for 30min, then added EDC HCl, (287.55 mg, 1.5 mmol) overnight. RP-HPLC purification was followed in NHS-GA-Cur-GA-NHS (355 mg, yield: 90%). MS: calc $[M+H]^+$: 791.2300, obsvd. ESI-MS: m/z 791.2300.

Synthesis of ***R(pbf)GD(OtBu)*:** Peptide R(pbf)GD(OtBu) 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 first amino acid Fmoc-D(OtBu)-OH (543 mg, 1.32 mmol) in 4 mL DMF was loaded onto the resin and DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. After loading the first amino acid to the resin, the capping reagent (DMF: MeOH: = 20:1, 4 mL) was used to ensure all the active sites of the resin were protected. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, then the next Fmoc-G-OH (392 mg, 1.32 mmol) in 4 mL

DMF was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, and the next Fmoc-R(pbf)-OH (856 mg, 1.32 mmol) in 4 mL DMF was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The growth of the peptide chain followed the established Fmoc SPPS protocol. Finally, wash 12 times with 4 mL 1% TFA/CH_2Cl_2 solution to cut R(pbf)GD(OtBu) from the resin. RP-HPLC purification was followed in R(pbf)GD(OtBu) (625 mg, yield: 89.8%). MS: calc $[M+H]^+$: 791.2300, obsvd. ESI-MS: m/z 791.2300.

Synthesis of ***RGD-Cur-RGD*****:** NHS-GA-Cur-GA-NHS (158 mg, 0.2 mmol) was added to the DMF mixing solution of RGD (261.7 mg, 0.4 mmol) then DIPEA (108.6 μL,0.624 mmol), the reaction mixture was stirred with a mixture at room temperature for 5 h, then 50% TFA / CH_2Cl_2 solution was stirred at room temperature for 2 h to remove OtBu protective base, remove the solvent and precipitated with a rotary evaporator. **RGD-Cur-RGD** (82.3 mg, yield: 52.0%) was obtained after RP-HPLC purification. ^{1}H NMR (400 MHz, d6-DMSO) δ (ppm): 8.16 (t, J = 7.8 Hz, 3H), 8.06 (d, J = 7.5 Hz, 2H), 7.63 (s, 1H), 7.59 (s, 1H), 7.56 - 7.51 (m, 1H), 7.47 (s, 2H), 7.29 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.1 Hz, 2H), 6.95 (d, J = 16.2 Hz, 2H), 4.51 (q, J = 6.8 Hz, 2H), 4.23 (s, 2H), 3.80 (s, 6H), 3.70 (d, J = 5.6 Hz, 8H), 3.06 (d, J = 6.6 Hz, 4H), 2.85 (s, 1H), 2.69 (s, 1H), 2.63 (d, J = 5.0 Hz, 1H), 2.55 (q, J = 6.3 Hz, 4H), 2.25 (t, J = 7.5 Hz, 4H), 1.82 (q, J = 7.8 Hz, 3H), 1.65 (s, 2H), 1.49 (d, J = 16.9 Hz, 5H), 1.19 (s, 2H).(Figure S5). ^{13}C NMR (150 MHz, d6-DMSO) δ (ppm): 183.74 (2 C), 173.70 - 170.85 (10 C), 169.14 (2 C), 157.32 (3 C), 151.72 (2 C), 141.48 (2 C), 140.38 (1 C), 134.20 (1 C), 125.12 (1 C), 123.88 (1 C), 121.97 (1 C), 116.43 (1 C).112.53 (2 C), 102.27 (1 C), 56.53 (3 C), 52.86 (2 C), 49.07 (2 C), 42.12 (2 C), 40.92 (2 C), 36.53 (2 C), 34.39 (2 C), 33.16 (2 C), 29.44 (2 C), 25.53 (2 C), 21.09 (2 C). (Figure S6) MS: calc $[M+H]^+$: 1253.4962, obsvd. ESI-MS: m/z 1253.5005. (Figure S7).

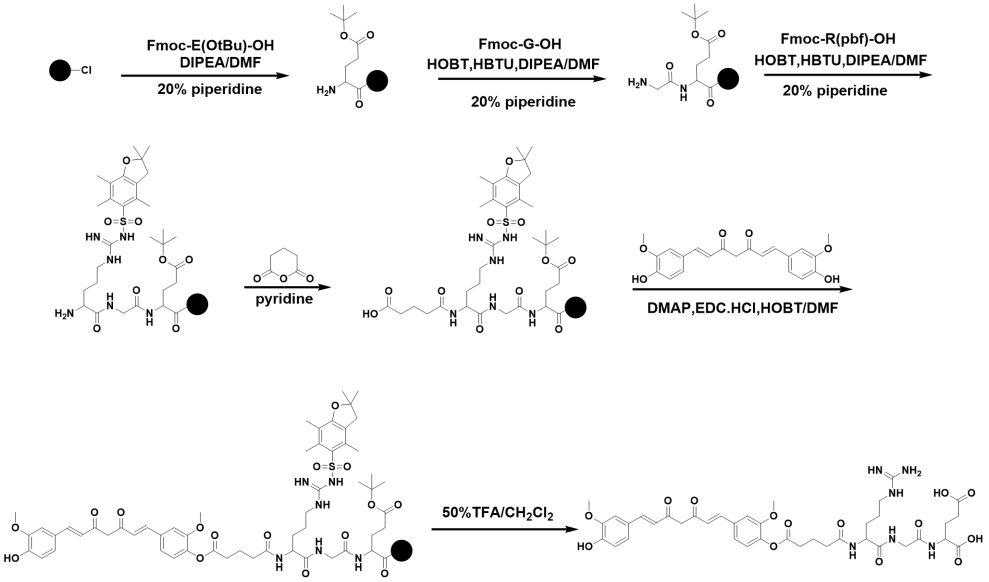
Scheme S3. The synthetic route for **RGD**.



*Synthesis of **RGD***: Peptide RGD 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 first amino acid Fmoc-D(OtBu)-OH (543 mg, 1.32 mmol) was loaded onto the resin. After loading the first amino acid to the resin, the capping reagent (DMF: MeOH: = 20:1, 4 mL) was used to ensure all the active sites of the resin were protected. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, the next Fmoc-G-OH (392 mg, 1.32 mmol) was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, and the next Fmoc-R(pbf)-OH (856 mg, 1.32 mmol) was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The growth of the peptide chain followed the established Fmoc SPPS protocol. Finally, wash 12 times with 4 mL 1% TFA/CH_2Cl_2 solution to deprotect the OtBu and Pbf group and cut RGD from the resin. The pure RGD (278 mg, yield: 91%) was obtained after HPLC purification. ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm): 8.75 (t, J = 5.8 Hz, 1H), 8.38 – 8.08 (m, 2H), 7.21 (s, 4H), 4.41 (q, J = 6.5 Hz, 1H), 3.98 – 3.49 (m, 4H), 3.09 (dd, J = 17.5, 10.9 Hz, 2H), 2.54 (d, J = 6.0 Hz, 2H), 1.72 (dd, J = 15.2, 7.9 Hz, 2H), 1.52 (q, J = 7.9, 7.4 Hz, 2H). (Figure S12). ^{13}C NMR (150 MHz, d6-DMSO) δ (ppm): 173.08 (1 C), 172.68 (1

C), 169 (1 C), 168.44 (1 C), 157.51 (1 C), 52.31 (1 C), 49.27 (2 C), 42.24 (1 C), 37.31 (1 C), 28.85 (1 C), 24.23 (1 C). (Figure S13) MS: calc $[M+H]^+$: 347.1679, obsvd. ESI-MS: m/z 347.1665 (Figure S14).

Scheme S4. The synthetic route for **Cur-RGE**.



Synthesis of ***Cur-RGE***: Peptide RGE 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 first amino acid Fmoc-E(OtBu)-OH (561 mg, 1.32 mmol) was loaded onto the resin and DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. After loading the first amino acid to the resin, the capping reagent (DMF: MeOH: = 20:1, 4 mL) was used to ensure all the active sites of the resin were protected. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. Then the next Fmoc-G-OH (392 mg, 1.32 mmol) was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. The solution of 20% piperidine in DMF (4 mL) was used to remove the Fmoc group, and the next Fmoc-R(pbf)-OH (856 mg, 1.32 mmol) was coupled to the free amino group using HBTU (496.6 mg, 1.32 mmol) and HOBT (178 mg, 1.32 mmol) as the coupling reagent. DIPEA (279 μL, 1.6 mmol) was used to provide alkaline environment. The growth of the peptide chain followed the established Fmoc SPPS protocol. 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 EDC·HCl (253.0 mg, 1.32 mmol) were added to react overnight. Finally, wash 12 times with 4 mL 50% TFA/CH_2Cl_2 solution to deprotect the OtBu and Pbf group and cut Cur-RGE from the resin. The pure **Cur-RGE** (565 mg, yield: 51.8 %) was obtained after HPLC purification. ^{1}H NMR (400 MHz, d6-DMSO) δ (ppm): 8.24 (t, J = 5.9 Hz, 1H), 8.10 (d, J = 7.2 Hz, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.65 – 7.38 (m, 4H), 7.28 (dd, J = 8.1, 4.8 Hz, 2H), 7.17 – 7.02 (m, 2H), 6.91 (d, J = 15.9 Hz, 1H), 6.83 – 6.69 (m, 2H), 6.09 (s, 1H), 4.19 (tq, J = 9.0, 4.4, 3.7 Hz, 2H), 3.80 (s, 6H), 3.70 (d, J = 5.9 Hz, 4H), 3.06 (q, J = 6.4 Hz, 2H), 2.55 (t, J = 7.4 Hz, 2H), 2.23 (q, J = 8.2 Hz, 4H), 1.99 – 1.71 (m, 4H), 1.68 – 1.35 (m, 4H). (Figure S15). ^{13}C NMR (150 MHz, d6-DMSO) δ (ppm): 185.43 (1 C), 181.96 (1 C), 175.19 – 170.80 (m) (5 C), 169.35 (1 C), 157.29 (1 C), 151.70 (1 C), 150.12 (1 C), 148.55 (1 C), 142.11 (1 C), 141.32 (1 C), 139.55 (1 C), 134.34 (1 C), 126.74 (1 C), 125.10 (1 C), 123.86 (1 C), 121.72 (d, J = 14.2 Hz) (2 C), 116.26 (1 C), 112.20 (d, J = 51.1 Hz) (2 C), 101.88 (1 C), 56.37 (d, J = 31.1 Hz) (3 C), 53.10 (1 C), 51.63 (1 C), 42.25 (1 C), 40.92 (1 C), 34.36 (1 C) ,33.16 (1 C), 30.44 (1 C), 29.25 (1 C), 26.84 (1 C), 25.56 (1 C), 21.07 (1 C). (Figure S16) MS: calc $[M+H]^+$: 825.3307, obsvd. ESI-MS: m/z 825.3299. (Figure S17).

3. Supporting Figures and Tables

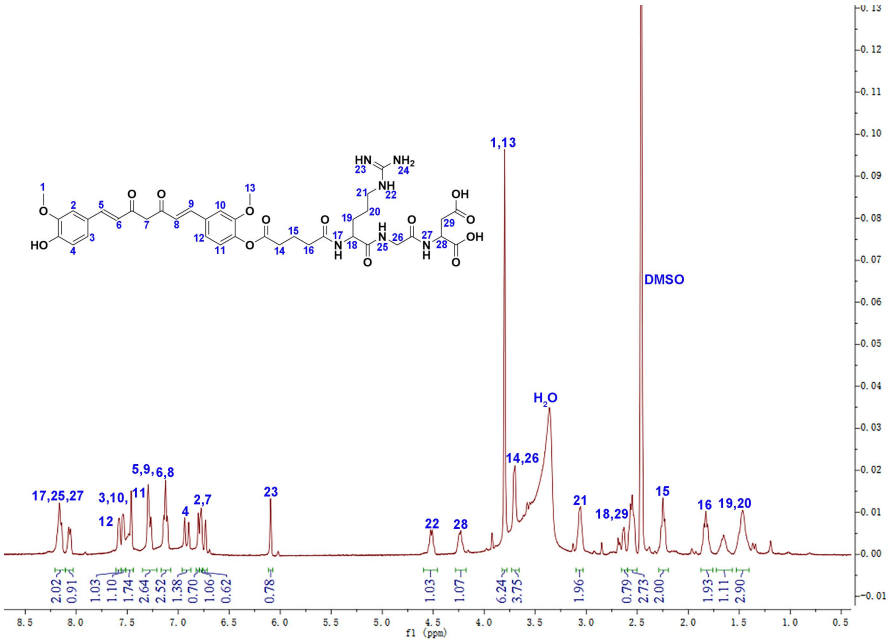


Figure S1. [1]H NMR spectrum of **Cur-RGD** in *d*$_6$-DMSO.

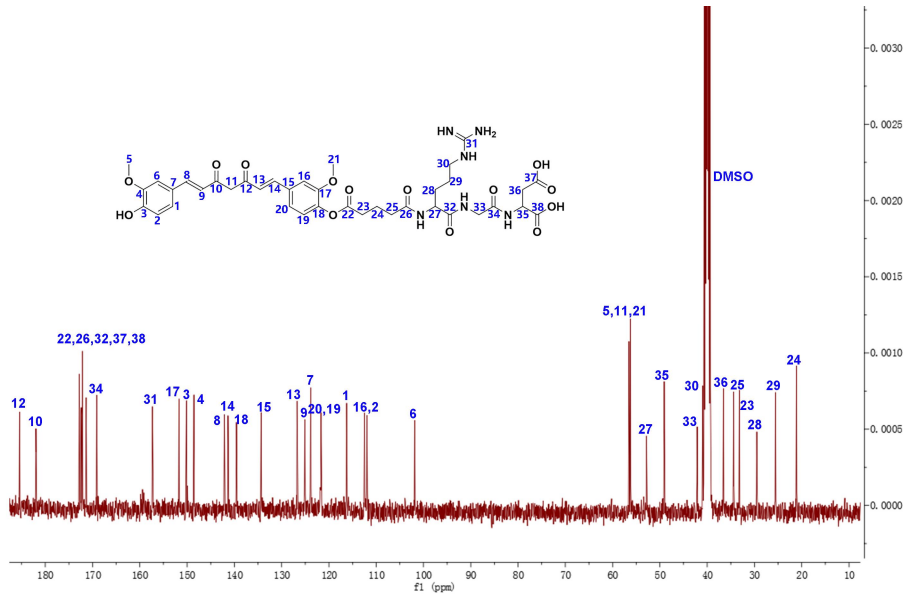


Figure S2. [13]C NMR spectrum of **Cur-RGD** in *d*$_6$-DMSO.

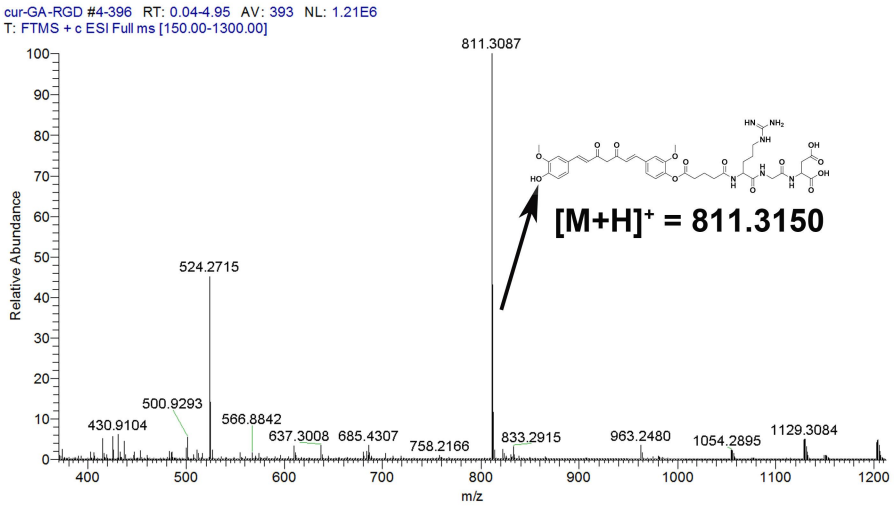


Figure S3. ESI-MS spectrum of **Cur-RGD**.

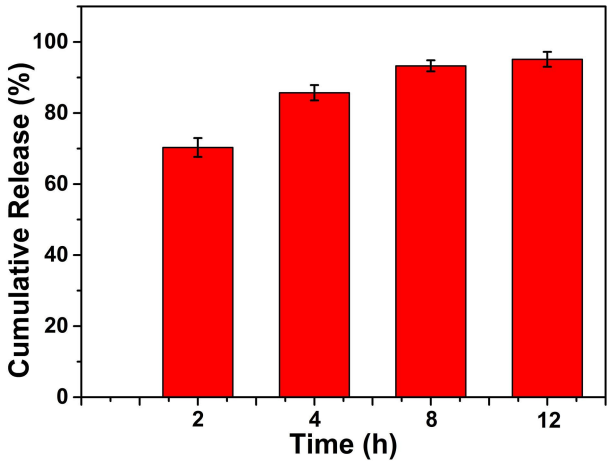


Figure S4. Cumulative drug release of **Cur-RGD** in the presence of esterase within 12 h.

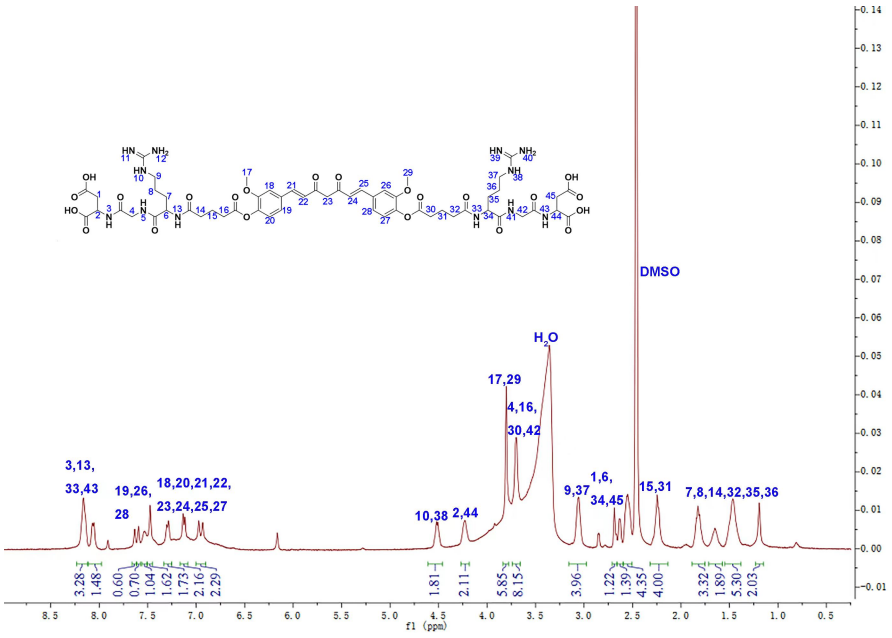


Figure S5. ^{1}H NMR spectrum of **RGD-Cur-RGD** in d_6-DMSO.

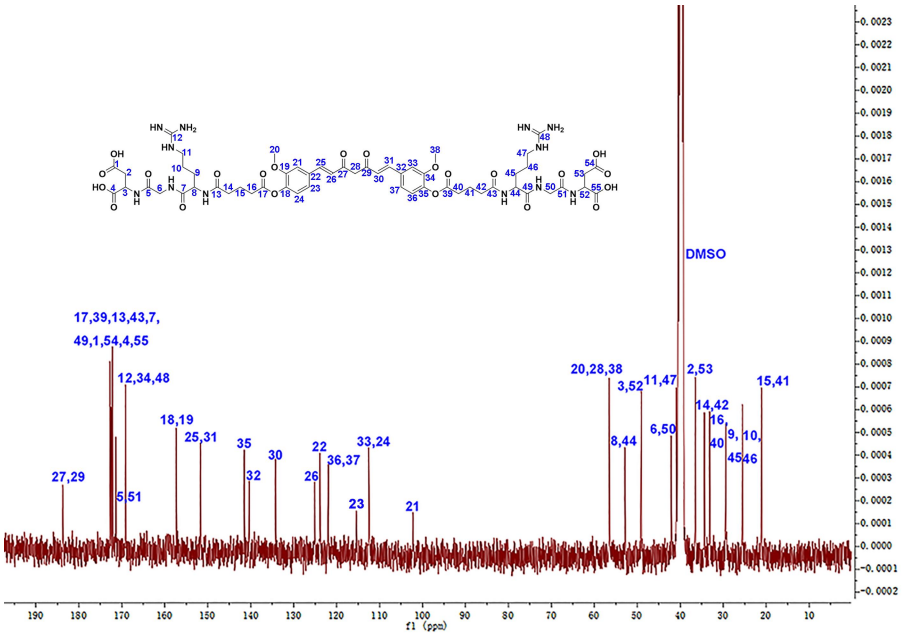


Figure S6. ^{13}C NMR spectrum of **RGD-Cur-RGD** in d_6-DMSO.

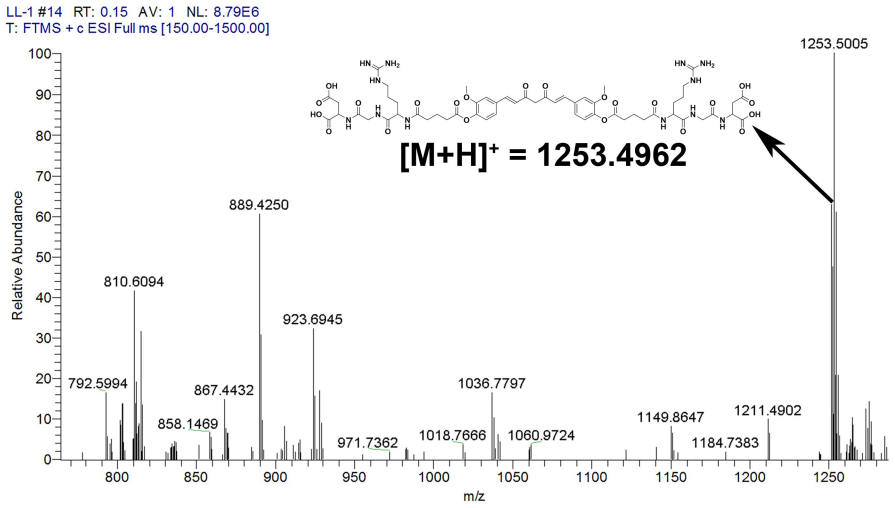


Figure S7. ESI-MS spectrum of **RGD-Cur-RGD**.

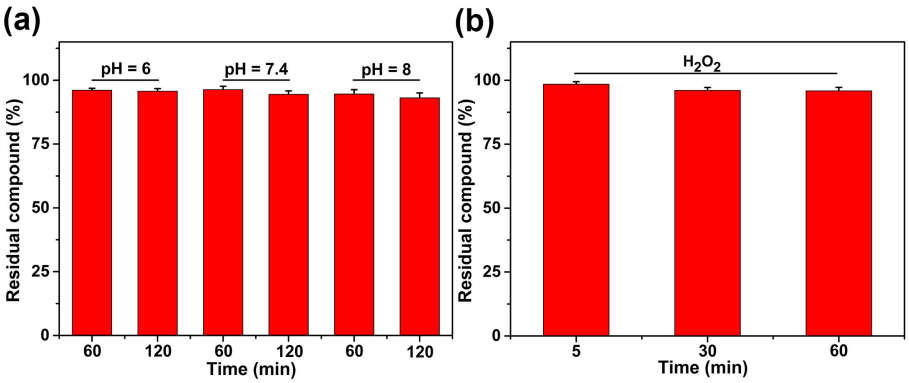


Figure S8. The stability of 250 μM **RGD-Cur-RGD** at different pH values in PB (a) and (b) in the presence of 100 μM H_2O_2 in PB.

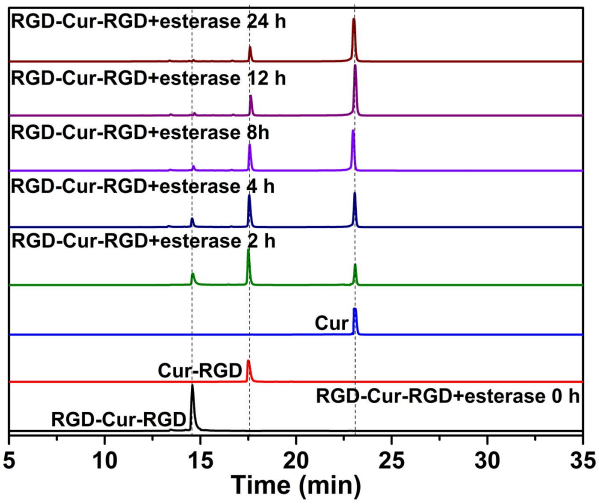


Figure S9. HPLC traces of **RGD-Cur-RGD** (black) and a series of **RGD-Cur-RGD** solution incubated with esterase at different time points (0-24 h).

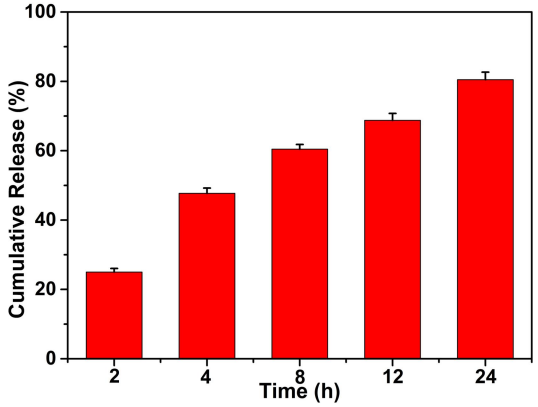


Figure S10. Cumulative drug release of **RGD-Cur-RGD** in the presence of esterase within 24 h.

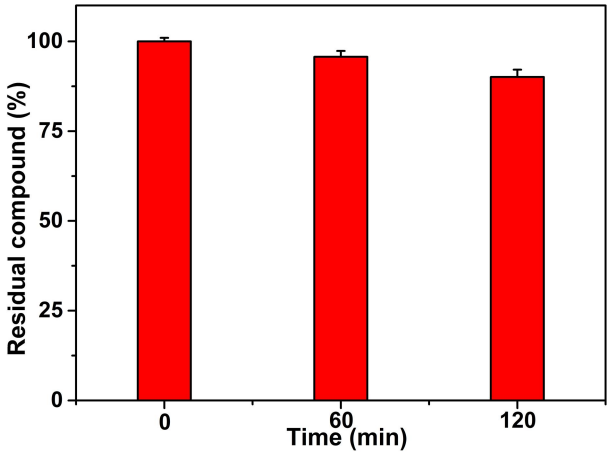


Figure S11. The stability of 250 μM **Cur-RGD** at complete medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum).

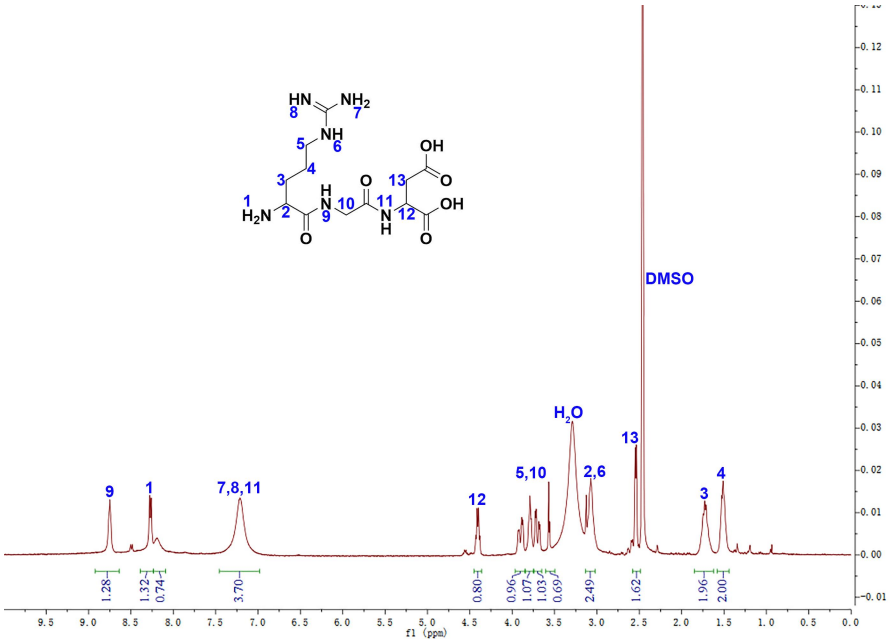


Figure S12. ^{1}H NMR spectrum of **RGD** in *d*$_6$-DMSO.

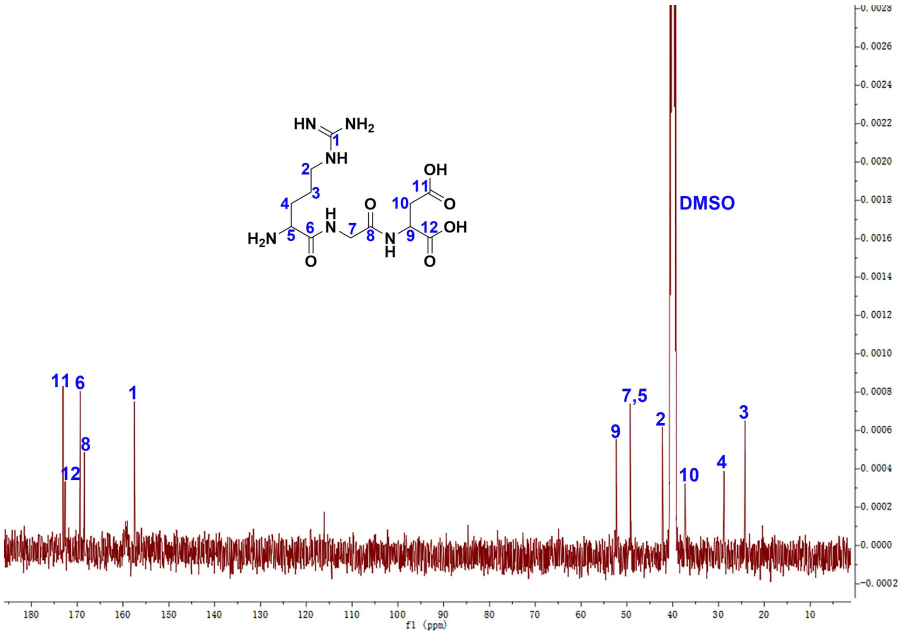


Figure S13. ^{13}C NMR spectrum of **RGD** in *d*$_6$-DMSO.

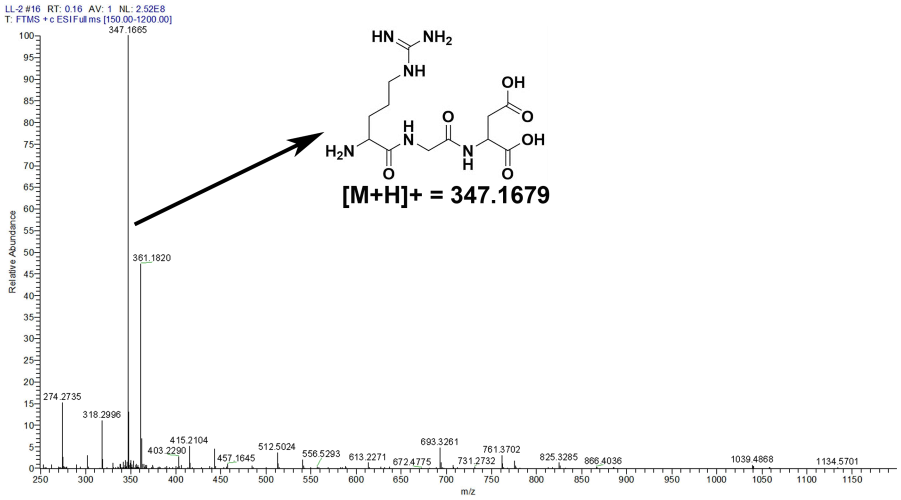


Figure S14. ESI-MS spectrum of **RGD**.

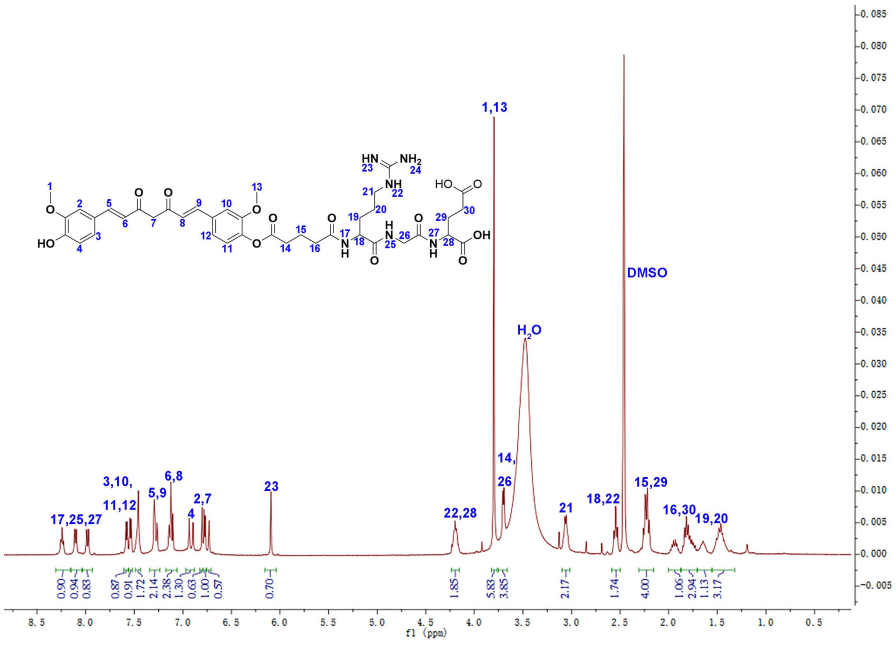


Figure S15. ^{1}H NMR spectrum of **Cur-RGE** in *d*$_6$-DMSO.

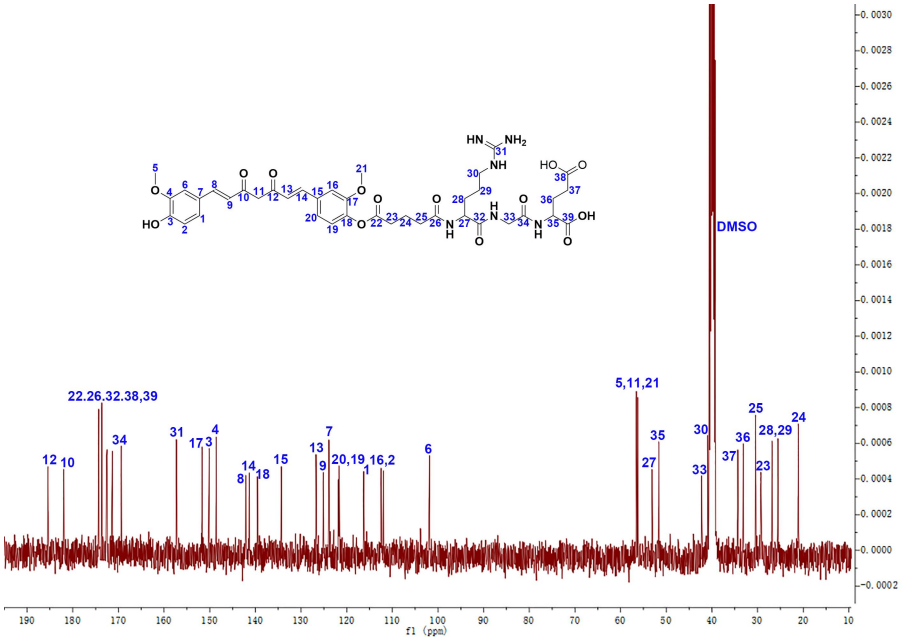


Figure S16. ^{1}C NMR spectrum of **Cur-RGE** in d_6-DMSO.

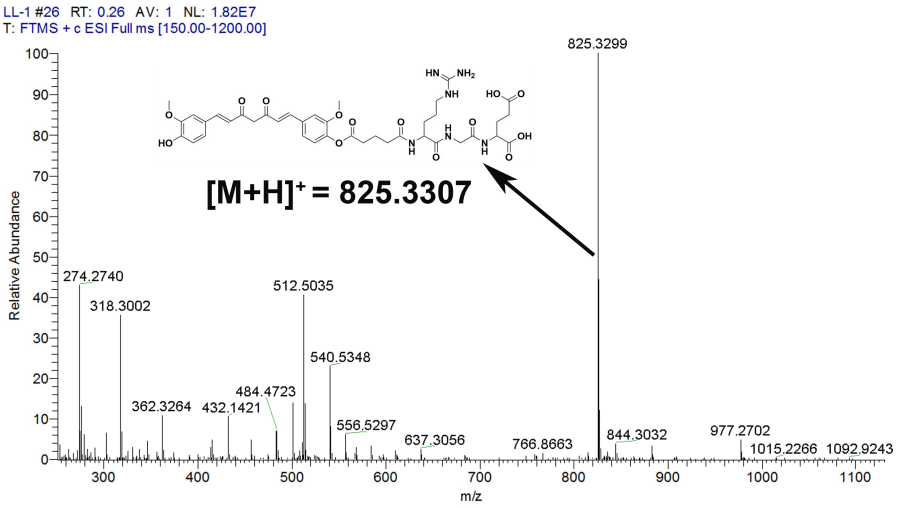


Figure S17. ESI-MS spectrum of **Cur-RGE**.

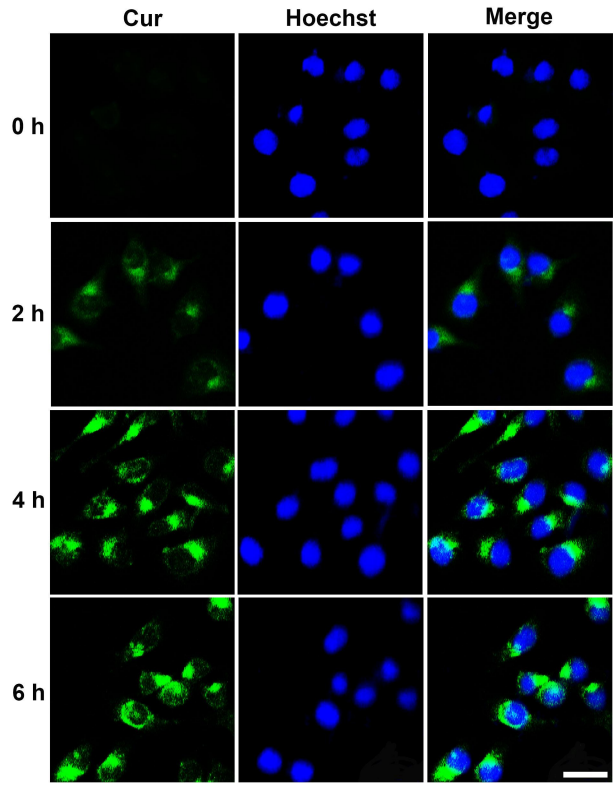


Figure S18. Time-course differential fluorescence, interference contrast (DIC), and overlay images of HepG2 cells incubated with 50 μM **Cur-RGD** in serum-free DMEM at 37 °C. Scale bar: 20 μm.

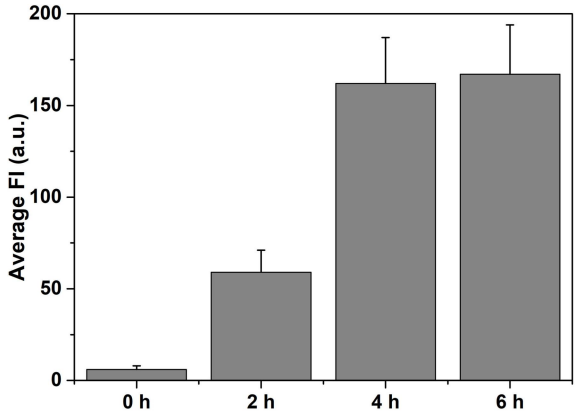


Figure S19. Average fluorescence intensities of HepG2 cell images from Cur channel in Figure S18.

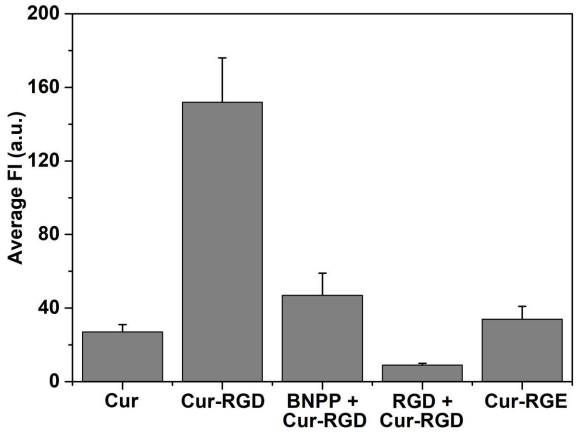


Figure S20. Average fluorescence intensities of HepG2 cell images from Cur channel within 4 h in Figure 3a.

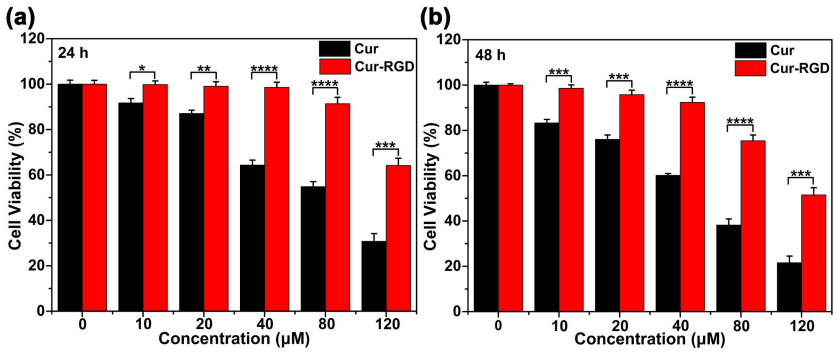


Figure S21. Relative viabilities of HUVEC cells after treatment with different concentrations of Cur and **Cur-RGD** at 24 h and 48 h. The error bars represent the standard deviation from three separate measurements. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

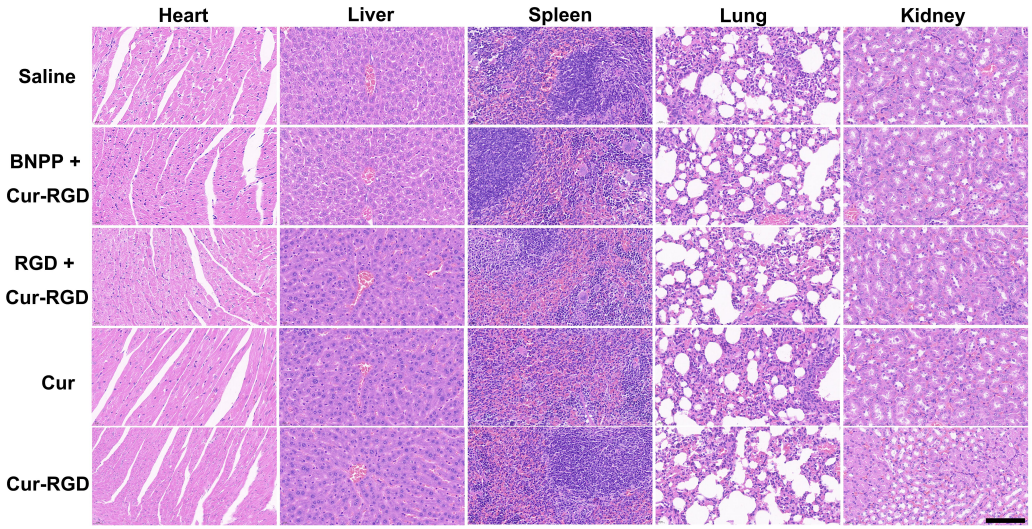


Figure S22. H&E staining of main organs from mice sacrificed at day 14.

Table S1. IC_{50} Values of prodrug **Cur-RGD** and Cur on HepG2 Cells.

IC_{50}	24 h	48 h
Cur-RGD	38.0 μM	21.9 μM
Cur	78.2 μM	62.5 μM

Table S2. IC_{50} Values of prodrug **Cur-RGD** and Cur on HUVEC Cells.

IC_{50}	24 h	48 h
Cur-RGD	233.7 μM	138.5 μM
Cur	86.4 μM	60.4 μM

Table S3. HPLC condition for Figure 2c, 2d and 2e.

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

Table S4. HPLC condition for purifying **Cur-RGD** and **Cur-RGE**.

Time (min)	Flow (mL/min)	H_2O % (0.1 % TFA)	CH_3CN % (0.1 % TFA)
0	12	55	45
5	12	55	45
35	12	20	80
37	12	20	80
39	12	55	45
40	12	55	45

Table S5. HPLC condition for purifying **RGD-Cur-RGD**.

Time (min)	Flow (mL/min)	H_2O % (0.1 % TFA)	CH_3CN % (0.1 % TFA)
0	12	50	50
5	12	50	50
35	12	0	100
37	12	0	100
39	12	50	50
40	12	50	50

Table S6. Column parameters for HPLC purification.

column length	column size	particle size	column temperature
12 cm	50 * 21.2 mm	5 μm	25 ℃