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# Supplementary Materials for
# Rational design of multifunctional small-molecule prodrugs for simultaneous suppression of cancer cell growth and metastasis in vitro and in vivo

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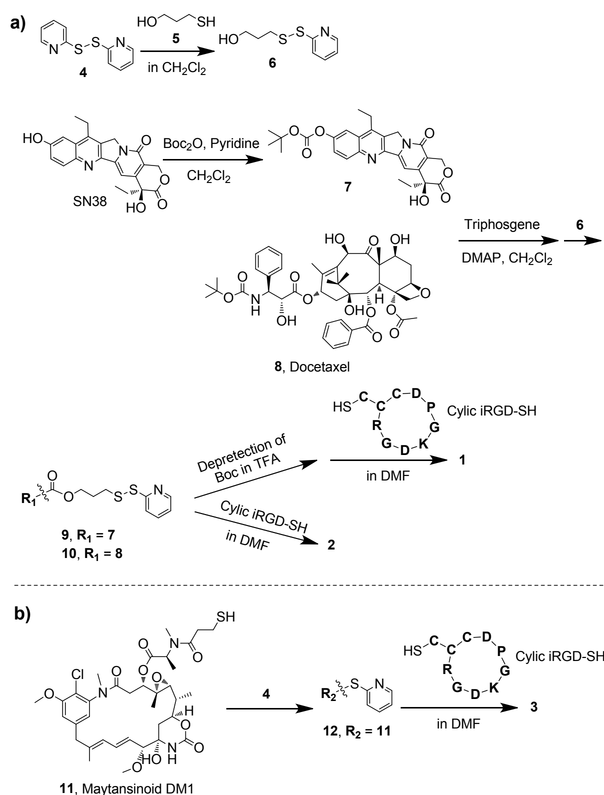



**Scheme S1** Synthesis of a) the iRGD-decorated prodrug conjugates iRGD-SN38, **1** and iRGD-docetaxel, **2** and b) iRGD-DM1, **3**.

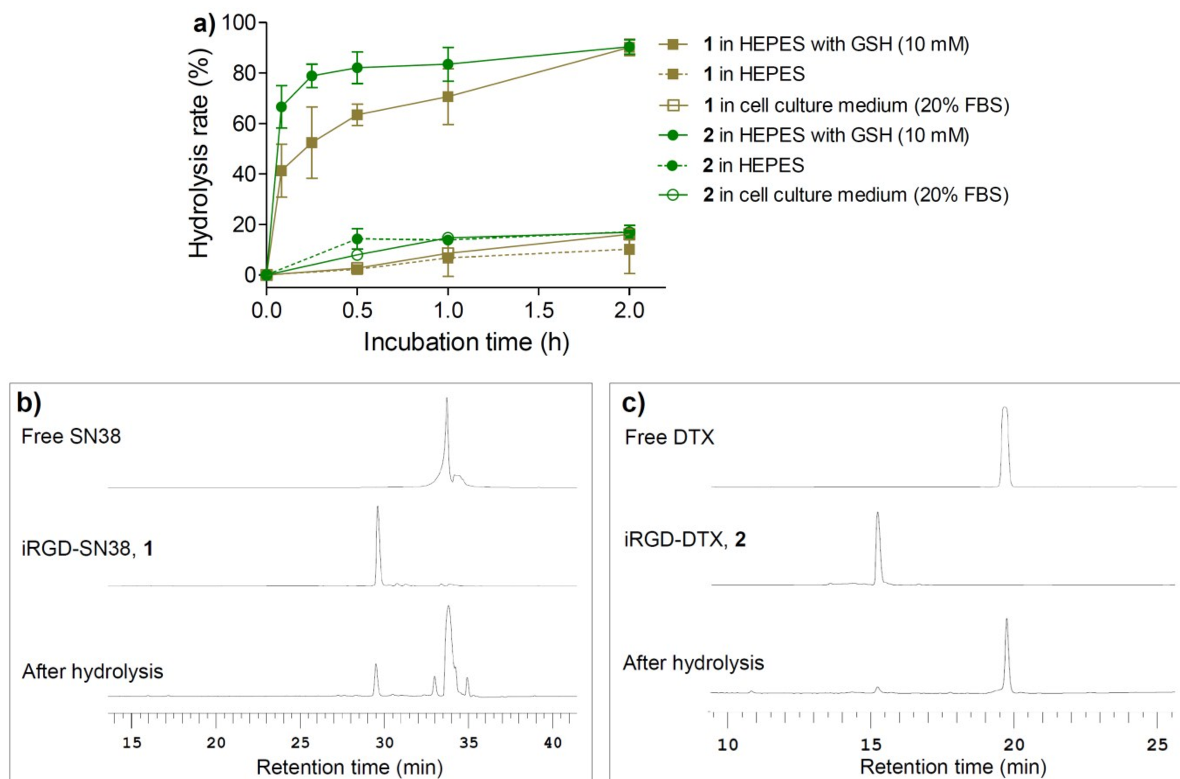



**Figure S1** (a) Hydrolysis profiles of iRGD-SN38 conjugate **1** and iRGD-DTX conjugate **2** in cell culture media containing 20% FBS or in HEPES buffer (pH 7.4) in the presence or absence of reduced glutathione (GSH, 10 mM) at 37 °C. HPLC curves of **1** (b) and **2** (c) before and after incubation with reduced glutathione (10 mM) for 2 h. Aliquots were obtained at indicated time points, and the hydrolytic yields were determined by HPLC.

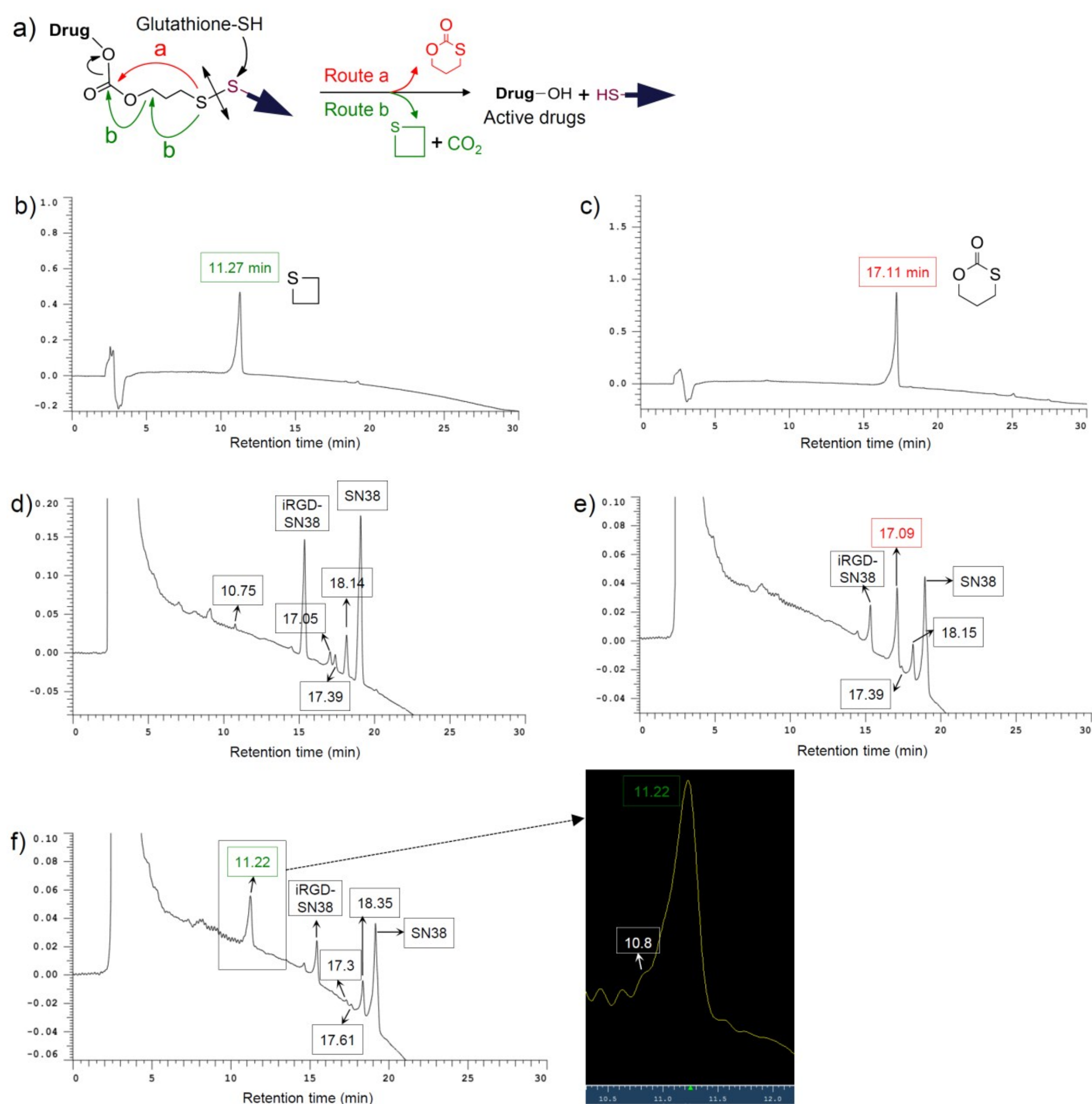



**Figure S2** (a) Proposed mechanism of prodrug hydrolysis in the presence of reduced glutathione. HPLC curves of the by-products, thietane (b) and 1,3-oxathian-2-one (c). (d) HPLC curve of the iRGD-SN38 conjugate **1** after incubation with reduced GSH (10 mM) for 1 h at 37 °C. (e) HPLC curve of the reaction mixture after addition of the standard sample of 1,3-oxathian-2-one. (f) HPLC curve of the reaction mixture after addition of the standard sample of thietane. The numbers in the square frame indicate the retention time. The results indicate that the new peak of 17.05 min in d) is the by-product 1,3-oxathian-2-one of prodrug **1**, but no peak corresponding to thietane was found. See the details in the experimental section.

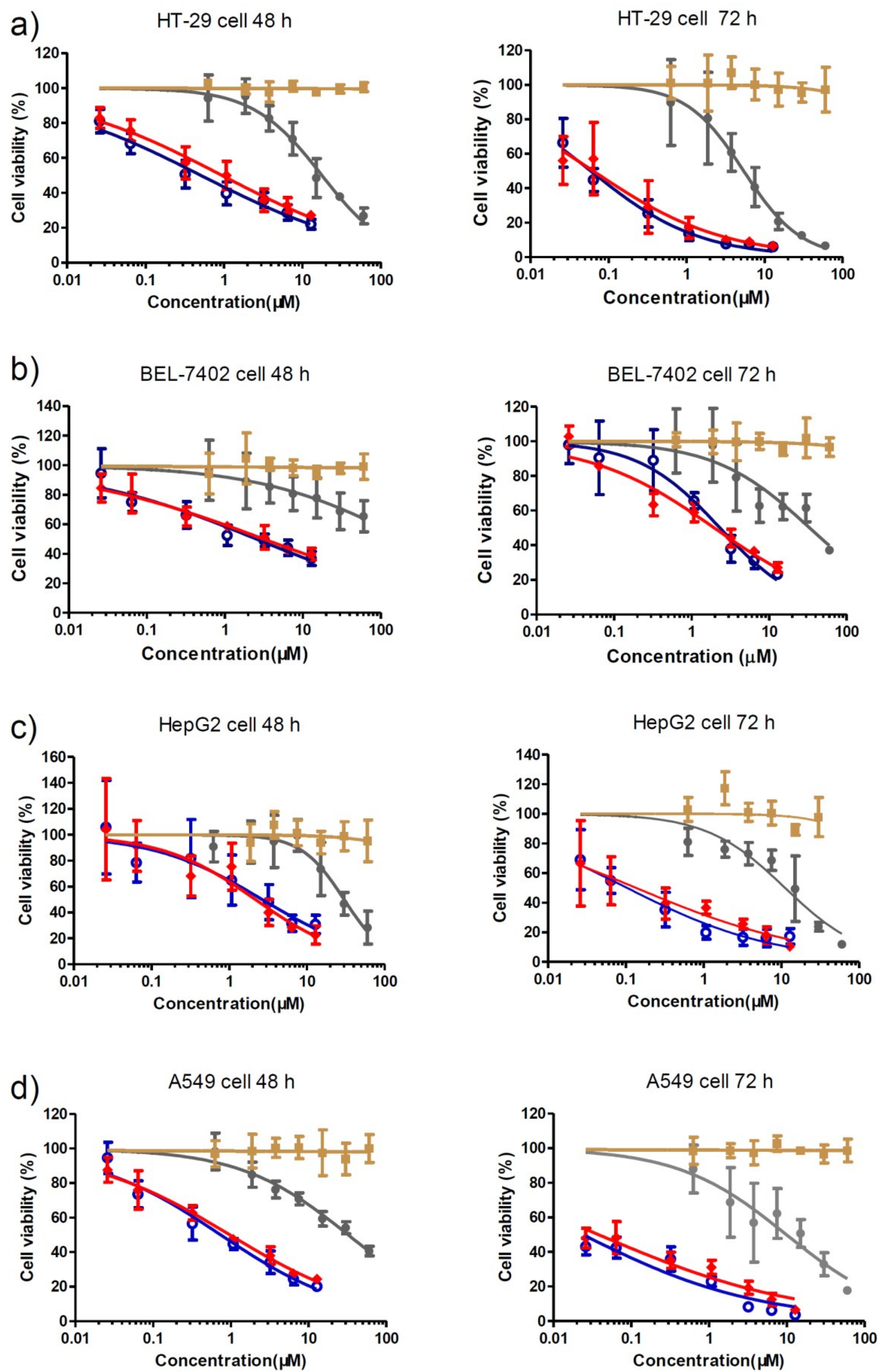



**Figure S3** Cell viability of cancer cells after 48 or 72 h incubation with CPT-11 (gray), free iRGD (brown), free SN38 (blue), and iRGD-SN38, **1** (red).

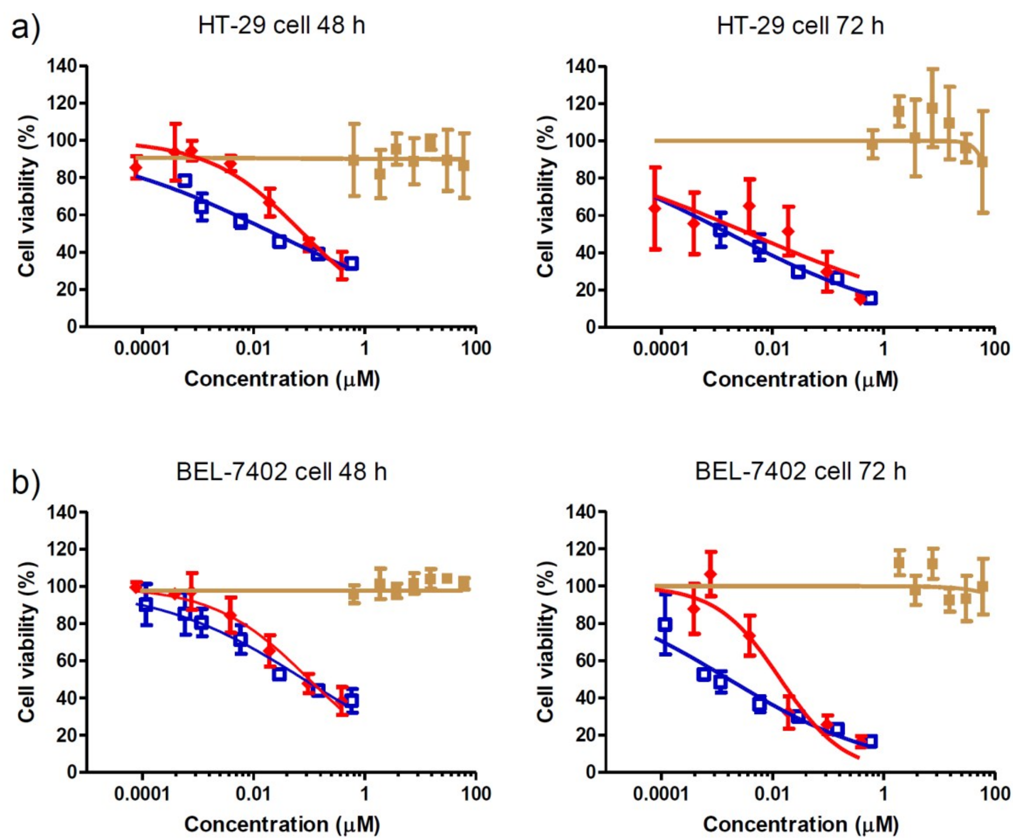



**Figure S4** Cell viability for free DTX, free iRGD peptide, and iRGD-DTX conjugate **2**, in human colon carcinoma HT-29 (a) and human HCC BEL-7402 (b) cells measured by the MTT assay (Mean ± SD). Brown line: free iRGD; Blue line: DTX; Red line: iRGD-DTX **2**.

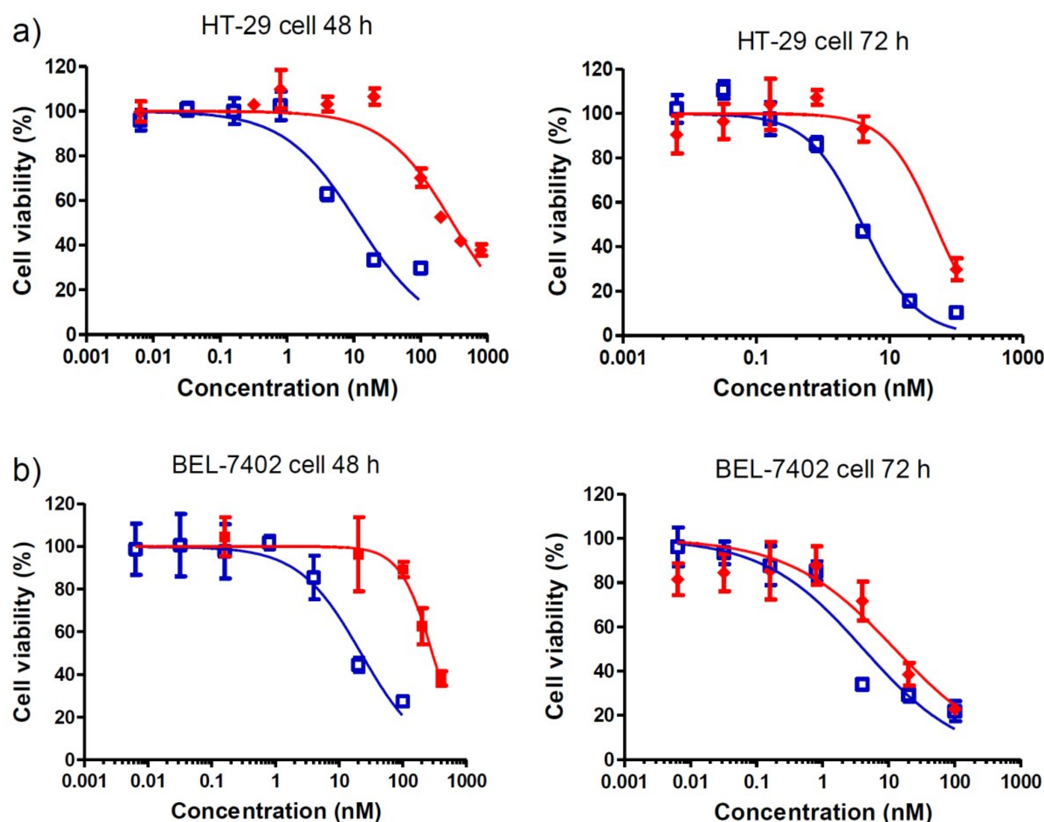



**Figure S5** Cell viability for free DM1 and iRGD-DM1 conjugate **3**, in human colon carcinoma HT-29 (a) and human HCC BEL-7402 (b) cells measured by the MTT assay (Mean ± SD). Blue line: DM1; Red line: iRGD-DM1, **3**.

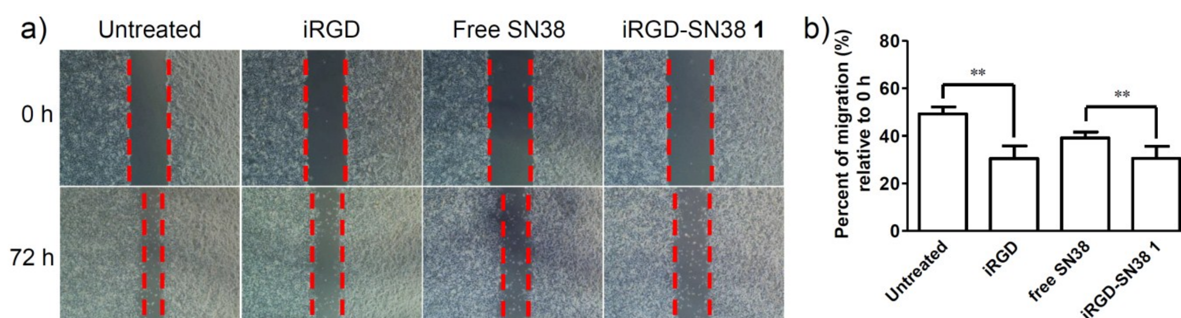



**Figure S6** (a) Photography of migration assay of HCC-LM3 cells treated with free iRGD peptide (5 μM), free SN38 (2 μM), and iRGD-SN38 **1** (2 μM), respectively. The untreated cell was used as control. (b) The wound healing rate in HCC-LM3 cells incubated with iRGD-SN38 (2 μM) was significantly decreased compared with SN38 (2 μM). ** $p<0.01$

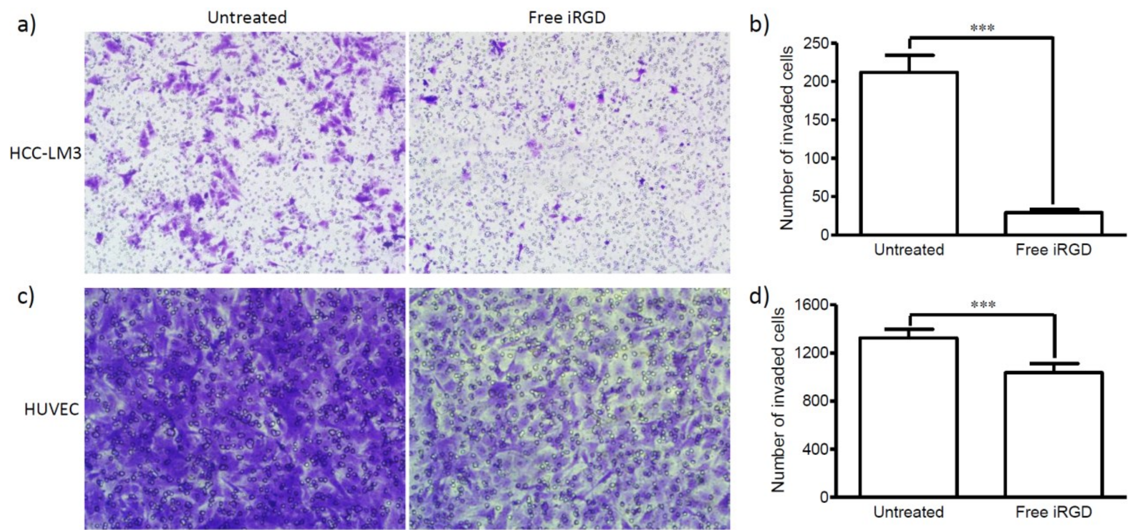



**Figure S7** iRGD peptide inhibited invasion of HCC-LM3 (a, b) and HUVEC (c, d) in Transwell assays. HCC-LM3 and HUVEC were seeded at $6\times10^4$ and $2\times10^5$ in the top chamber, respectively. The numbers of invaded HCC-LM3 and HUVEC in the iRGD-treated groups were significantly decreased compared with that of the untreated cells. *** $p<0.001$.

**Table S1** Antitumor activity of SN38-based drugs against a panle of cancer cell lines expressed as $IC_{50}$ (μM) [a]

| | CPT-11 | | iRGD | | Free SN38 | | iRGD-SN38, **1** | |
|---|---|---|---|---|---|---|---|---|
| | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h |
| HT-29 | 17.77±1.59 | 5.42±0.65 | N.E. | N.E. | 0.51±0.06 | 0.06±0.01 | 0.94±0.12 | 0.06±0.02 |
| BEL-7402 | 205.9±154.7 | 33.42±7.71 | N.E. | N.E. | 2.53±0.54 | 2.32±0.33 | 3.16±0.57 | 1.96±0.24 |
| HepG2 | 30.13±3.57 | 11.14±1.45 | N.E. | N.E. | 2.67±0.82 | 0.10±0.02 | 2.17±0.53 | 0.13±0.04 |
| HCC-LM3 | 92.92±28.28 | 31.48±4.12 | N.E. | N.E. | 3.92±0.64 | 0.71±0.10 | 18.32±4.63 | 3.74±0.39 |
| A549 | 32.38±3.55 | 9.89±2.19 | N.E. | N.E. | 0.74±0.10 | 0.02±0.01 | 0.97±0.10 | 0.04±0.01 |

[a] Determined by MTT assay.
N.E., not effective

**Table S2** Antitumor activity of DTX-based drugs against HT-29 and BEL-7402 cell lines expressed as $IC_{50}$ (nM) [a]

| | iRGD | | Free DTX | | iRGD-DTX, **2** | |
|---|---|---|---|---|---|---|
| | 48 h | 72 h | 48 h | 72 h | 48 h | 72 h |
| HT-29 | N.E. | N.E. | 23.67±4.20 | 1.74±0.91 | 77.90±15.3 | 4.04±2.83 |
| BEL-7402 | N.E. | N.E. | 81.08±18.47 | 1.64±0.40 | 99.94±16.81 | 13.85±3.27 |

[a] Determined by MTT assay.
N.E., not effective

**Table S3** Antitumor activity of DM1-based drugs against HT-29 and BEL-7402 cell lines expressed as $IC_{50}$ (nM) [a]

| | Free DM1 | | iRGD-DM1, **3** | |
|---|---|---|---|---|
| | 48 h | 72 h | 48 h | 72 h |
| HT-29 | 11.46±2.16 | 3.94±0.53 | 302.6±35.1 | 48.55±14.32 |
| BEL-7402 | 22.12±5.28 | 4.16±1.18 | 291.8±31.7 | 12.49±4.24 |

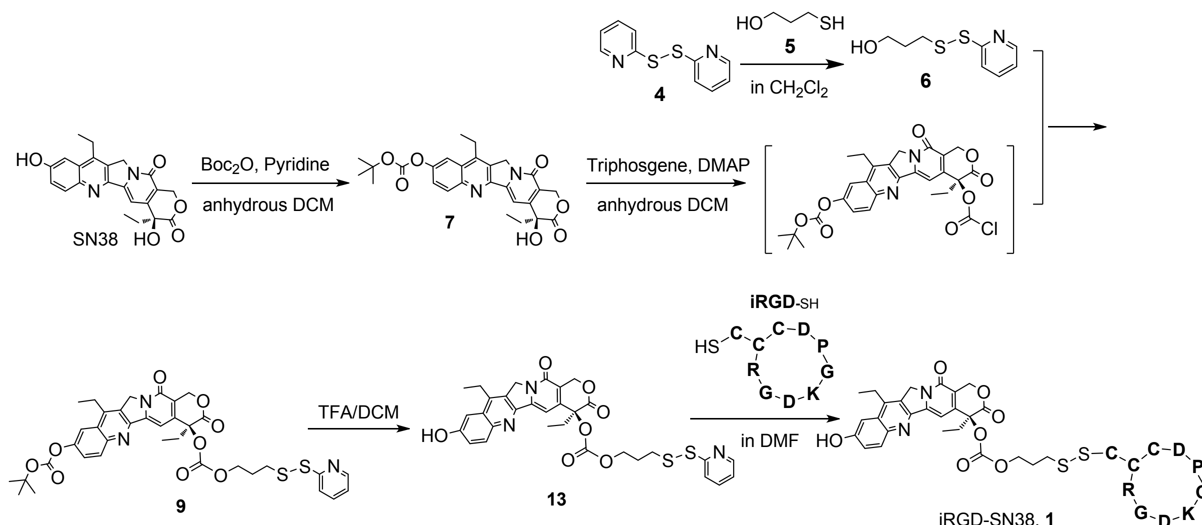
[a] Determined by MTT assay.

## General materials and methods for organic synthesis

7-Ethyl-10-hydroxycamptothecin (SN38) and docetaxel (DTX) were purchased from Knowshine Pharmachemicals Inc. (Shanghai, China). The Compound 2,2'-dithiodipyridine and 3-mercapto-1-propanol were purchased from J&K Chemical (Shanghai, China). The compound thietane was purchased from Tokyo Chemical Industry (Shanghai, China). The thiolated maytansine derivative DM1 was purchased from BrightGene (Suzhou, China) and confirmed by our lab using $^{1}$H NMR and mass spectra. Cyclic iRGD (CRGDKGPDC)C-SH with a free thiol was synthesized via a cysteine-cysteine disulfide bond formation between amino acid 1 and 9 by GL Biochem Ltd. (Shanghai, China). All other compounds and solvents were purchased from J&K Chemical (Shanghai, China).

All reactions were performed in a dry atmosphere. Thin layer chromatography (TLC) was performed on silica gel 60 $F_{254}$ pre-coated aluminium sheets (Merck) and visualized by fluorescence quenching. Chromatographic purification was accomplished Using flash column chromatography on silica gel (neutral, Qingdao Haiyang Chemical Co., Ltd). $^{1}$H NMR spectra were recorded in $CDCl_3$ or DMSO-d6 on a Bruker 400 (400 MHz) spectrometer and calibrated to the residual solvent peak or tetramethylsilane (= 0 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br = broad. High-resolution mass spectrometry (HRMS)-ESI was recorded on AB SCIEX 5600+Q-TOFMS instruments. Reverse phase HPLC (RP-HPLC) was carried out on a Hitachi Chromaster 5000 system. Compounds were purified using a YMC-Pack ODS-A column (5 μm, 250 × 2 mm) or their purities were analyzed by a YMC-Pack ODS-A column (5 μm, 250 × 4.6 mm). UV detection for SN38 and docetaxel were at 378 and 220 nm, respectively. All HPLC runs used linear gradients of acetonitrile (solvent A) and water (solvent B) containing 0.1% trifluoroacetic acid (TFA).

**Synthesis of iRGD-conjugated SN38 prodrug 1**




**Supplementary Scheme 1** Synthetic scheme of iRGD-SN38 conjugate, **1**

**Synthesis of compound 7**

To a solution of $Boc_2O$ (361.5 mg, 1.66 mmol) and SN38 (500 mg, 1.28 mmol) in 10 mL of anhydrous DCM were added pyridine (2 mL). The reaction mixture was stirred at 25 ºC overnight. After removing the solvent, DCM was added and washed with 0.3 N HCl, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to give compound **7** (510 mg, 81%).

$^1$H NMR (400 MHz, $CDCl_3$): δ 1.03-1.06 (q, 3H), 1.39-1.42 (q, 3H), 1.60-1.62 (q, 9H), 1.86-1.94 (m, 2H), 3.14-3.19 (q, 2H), 3.78-3.79 (d, 1H, $J$ = 2.0), 5.27-5.34 (m, 3H), 5.74-5.78 (q, 1H), 7.65-7.68 (t, 2H), 7.90 (s, 1H), 8.23-8.26 (q, 1H).

$^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): δ 7.84, 13.94, 23.17, 27.73, 31.65, 49.38, 66.28, 72.82, 84.34, 98.10, 114.04, 118.64, 125.13, 127.28, 127.38, 131.99, 145.34, 146.76, 147.23, 149.94, 150.21, 151.47, 151.82, 157.60, 173.79

HRMS: calcd for $[C_{27}H_{28}N_2O_7]^+$ $[M+H]^+$ = 493.1970; obsd 493.1890.

**Synthesis of compound 6**

To a solution of 3-mercapto-1-propanol, **5** (300 mg, 3.26 mmol) in 10 mL of ethanol were added 2,2'-dithiodipyridine, **4** (2.15 g, 9.78 mmol). The reaction mixture was stirred at 25 °C for 2 h. After removing the solvent, The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to give compound **6** (537 mg, 82%).

$^{1}H$ NMR (400 MHz, $CDCl_3$): δ 1.93-1.99 (m, 2H), 2.96-2.99 (t, 2H), 3.79-3.82 (t, 2H), 7.64 (s, 3H), 8.48 (s, 1H).

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 31.35, 35.76, 60.73, 120.22, 120.83, 137.06, 149.63, 159.92.

HRMS: calcd for $[C_8H_{11}NOS_2]^+$ $[M+H]^+$ = 202.0355; obsd 202.0356.

**Synthesis of compound 9**

To a solution of compound **7** (500 mg, 1.02 mmol) and Triphosgene (112 mg, 0.38 mmol) in 10 mL of anhydrous DCM were added DMAP (397 mg, 3.25 mmol). After stirring at 25 °C for 0.5 h, compound **6** (99 mg, 0.81 mmol) was added and further stirred for 1 h, then the reaction mixture was washed with water and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to give compound **9** (300 mg, 52%).

$^{1}H$ NMR (400 MHz, $CDCl_3$): δ 0.98-1.02 (t, 3H), 1.38-1.42 (t, 3H), 1.62 (s, 9H), 2.07-2.13 (m, 2H), 2.15-2.28 (m, 2H), 2.88-2.91 (t, 2H), 3.15-3.20 (q, 2H), 4.26-4.28 (t, 2H), 5.29-5.42 (t, 3H), 5.68-5.73 (d, 1H, *J* = 17.2), 7.34 (s, 1H), 7.68-7.71 (q, 1H), 7.80-7.86 (q, 3H), 7.92-7.95 (q, 1H), 8.25-8.27 (d, 1H, *J* = 9.6), 8.55 (s, 1H).

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 7.52, 13.90, 23.56, 27.70, 27.94, 31.65, 35.24, 49.82, 66.69, 66.78, 77.83, 84.87, 98.72, 114.44, 120.76, 122.54, 123.20, 126.81, 127.61, 127.87, 129.88, 142.55, 144.88, 144.93, 145.14, 146.95, 148.57, 149.93, 150.68, 151.23, 153.70, 157.39, 158.63, 159.90, 160.30, 167.25.

HRMS: calcd for $[C_{36}H_{37}N_3O_9S_2]^+$$[M+H]^+$ = 720.2044; obsd 720.2040.

**Synthesis of compound 1**

To a solution of the compound **9** (300 mg, 0.42 mmol) in 3 mL of anhydrous DCM were added TFA (3 mL). The reaction mixture was stirred at 25 ºC for 0.5 h, and then the solvent was removed under reduced pressure. After then, toluene was added, which forms azeotropes with TFA, to be coevaporated under reduced pressure. The compound **13** was used for next step without purification.

$^{1}H$ NMR (400 MHz, DMSO-$d_6$): δ 0.88-0.92 (t, 3H), 1.27-1.31 (t, 3H), 1.95-1.98 (t, 2H), 2.12-2.18 (m, 2H), 2.86-2.89 (t, 2H), 3.06-3.11 (q, 2H), 4.18-4.21 (t, 2H), 5.30 (s, 2H), 5.51(s, 2H), 6.94 (s, 1H), 7.19-7.22 (t, 1H), 7.40-7.41 (d, 2H, *J* = 5.6), 7.67-7.69 (d, 1H, *J* = 8.0), 7.72-7.76 (t, 1H), 8.01-8.03 (t, 1H ), 8.41-8.42 (d, 1H, *J* = 4.4), 10.35 (s, 1H).

$^{13}C\{^1H\}$ NMR (100 MHz, DMSO-$d_6$): δ 8.00, 13.81, 22.75, 28.03, 30.68, 34.63, 50.00, 66.90, 67.43, 78.30, 93.77, 105.23, 118.59, 119.72, 121.62, 122.90, 128.41, 128.74, 132.01, 138.10, 143.26, 144.08, 145.37, 147.68, 148.92, 150.00, 153.35, 156.99, 157.29, 159.39, 167.69.
HRMS: calcd for $[C_{31}H_{29}N_3O_7S_2]^+$ $[M+H]^+$ = 620.1520; obsd 620.1527.

The cyclic peptide, iRGD (84.8 mg, 0.08 mmol) and TEA (22.5 μL, 0.16 mmol) were added into a solution of compound **13** (50 mg, 0.08 mmol in DMF). The reaction mixture was stirred at 25 ºC for 7 h and then purified by HPLC using a C18 reverse-phase column (5 μm, 250 mm × 20 mm). A gradient elution of 10-50% acetonitrile/water was applied at a flow rate of 9.9 mL/min to give the compound iRGD-SN38, **1** (59 mg, 47%). The purity of obtained **1** was further determined to be >95% by analytic column (Figure S7).
$^1H$ NMR (400 MHz, DMSO-$d_6$): δ 0.89-0.92 (t, 3H), 1.22 (s, 2H), 1.28-1.31 (t, 3H),1.45-1.59 (m, 6H), 1.67-1.80 (m, 4H), 1.88-1.89 (d, 2H, *J* = 3.2), 1.95-1.98 (t, 2H), 2.00-2.07 (m, 2H), 2.13-2.19 (m, 2H), 2.57-2.68 (m, 2H), 2.72-2.83 (m, 6H), 2.93-3.00 (m, 2H), 3.07-3.12 (q, 6H), 3.16-3.25 (m, 4H), 3.78-3.82 (d, 2H, *J* = 16.8), 3.91-3.95 (t, 1H), 4.11-4.22 (m, 4H), 4.28-4.34 (m, 2H), 4.50-4.65 (m, 5H), 5.32 (s, 2H), 5.52 (s, 2H), 6.94 (s, 1H), 7.42-7.44 (q, 2H), 7.58 (s, 1H), 7.64-7.68 (t, 5H), 7.82-7.84 (t, 1H), 8.02-8.04 (t, 1H), 8.13-8.14 (d, 1H, *J* = 4.0), 8.24-8.26 (t, 2H), 8.33-8.41 (m, 4H), 8.45-8.47 (d, 1H, *J* = 7.6) , 8.55-8.57 (d, 1H, *J* = 7.2), 9.00-9.02 (d, 1H, *J* = 7.2), 10.40 (s, 1H).
$^{13}C\{^1H\}$ NMR (100 MHz, DMSO-$d_6$): δ 7.99, 13.82, 22.51, 22.77, 24.77, 25.56, 26.97, 28.07, 28.73, 29.43, 29.47, 30.48, 30.75, 31.57, 33.16, 33.57, 36.02, 46.74, 50.06, 50.22, 50.27, 51.69, 52.04, 52.62, 52.96, 53.23, 60.19, 61.07, 66.96, 67.60, 78.34, 93.79, 105.32, 123.07, 128.49, 128.82, 131.95, 143.43, 144.06, 145.32, 147.69, 148.91, 153.42, 157.02, 157.30, 157.43, 159.09, 167.52, 167.80, 168.28, 168.62, 169.63, 169.83, 170.75, 170.78, 171.87, 171.97, 172.33, 172.35, 172.51.
HRMS: calcd for $[C_{64}H_{86}N_{16}O_{22}S_4]^+$ $[M+H]^+$ = 1559.5063; obsd 1559.5080.

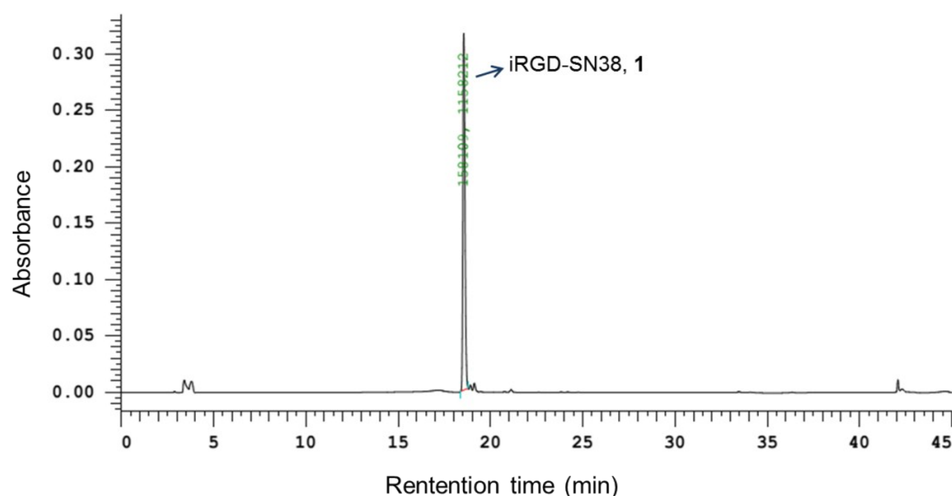
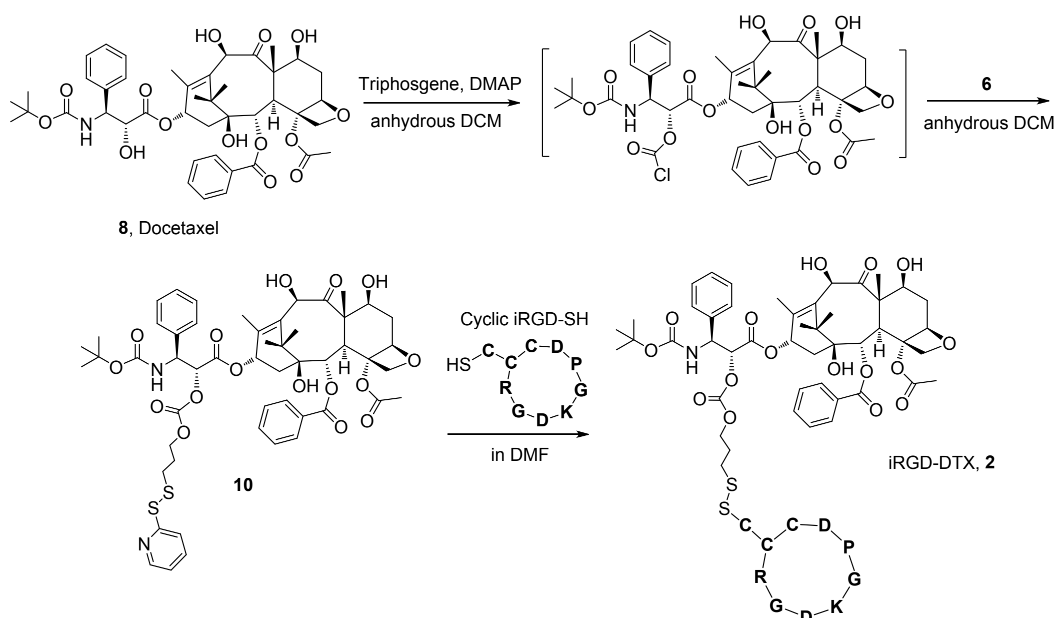



Figure S7 HPLC profile of iRDG-SN38, **1** observed at 220 nm.

**Synthesis of iRGD-conjugated DTX prodrug 2**




**Supplementary Scheme 2** Synthetic scheme of **2**

**Synthesis of compound 10**

To a solution of the compound docetaxel, **8** (500 mg, 0.62 mmol) and Triphosgene (68 mg, 0.23 mmol) in 10 mL of anhydrous DCM were added DMAP (397 mg, 3.25 mmol). The reaction mixture was stirred at 25 ºC over 0.5 h, and the compound **6** (60 mg, 0.49 mmol) was added. After stirring for 1 h, the mixture was washed with water and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated under vacuum. The residue was

purified by flash column chromatography on silica gel (DCM:MeOH = 80:1) to give compound **10** (249 mg, 39%).

$^1$H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H), 1.23 (s, 3H), 1.34 (s, 9H),1.64 (s, 9H), 1.77 (s, 3H), 1.91 (s, 3H), 2.04-2.12 (m, 2H), 2.56-2.63 (m, 1H), 2.82-2.86 (t, 2H), 3.91-3.93 (d, 1H, J = 7.2), 4.17-4.21 (m, 2H), 4.24 (s,1H), 4.96-4.98 (d, 2H, J = 6.8), 5.20 (s, 1H), 5.68-5.70 (d, 1H, J = 6.8), 6.21 (s, 1H), 7.09-7.13 (m, 1H), 7.30-7.32 (t, 3H), 7.38-7.41 (t, 2H), 7.48-7.52 (t, 2H), 7.59-7.63 (t, 1H), 7.66-7.72 (m, 2H), 8.10-8.12 (d, 2H, J = 6.8), 8.43-8.44 (d, 1H, J = 4.4).

$^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): δ 5.24, 9.49, 16.11, 17.88, 21.64, 23.12, 23.40, 24.95, 29.91, 30.88, 32.23, 38.38, 41.75, 48.69, 52.87, 62.45, 66.90, 67.59, 69.70, 70.23, 71.84, 72.50, 74.17, 75.68, 76.29, 79.55, 115.01, 116.14, 121.72, 123.52, 123.96, 124.18, 124.49, 125.43, 128.91, 130.95, 132.60, 134.06, 144.85, 149.25, 150.35, 155.14, 162.30, 163.16, 164.95, 206.69.

HRMS: calcd for $[C_{52}H_{62}N_2O_{16}S_2]^+$ $[M+H]^+$ = 1035.3619; obsd 1035.3615.

**Synthesis of compound 2**

iRGD (20.3 mg, 0.02 mmol) and TEA (5.5 μL, 0.04 mmol) were added into a solution of compound **10** (20 mg, 0.02 mmol) in DMF. The reaction mixture was stirred 16 h and purified by HPLC using a YMC C18 reverse-phase column (5 μm, 250 mm×20 mm). A gradient elution of 10-50% acetonitrile/water applied at a flow rate of 9.9 mL/min to give the compound iRGD-DTX, **2** (14 mg, 37%). The purity of obtained **2** was further determined to be >95% by analytic column (Figure S8).

$^1$H NMR (400 MHz, DMSO-*d₆*): δ 0.98 (s, 8H), 1.24 (s, 4H), 1.36 (s, 9H), 1.51 (s, 11H), 1.72 (s, 7H), 1.78-1.89 (m, 3H), 2.01-2.05 (t, 3H), 2.07 (s, 1H), 2.23 (s, 4H), 2.53-2.62 (m, 2H), 2.72-2.82 (m, 6H), 2.92-3.01 (m, 2H), 3.08-3.14 (m, 3H), 3.22-3.27 (m, 6H), 3.48-3.53 (m, 1H), 3.61-3.63 (d, 1H, *J* = 7.2), 3.78-3.82 (d, 1H, *J* = 14.8), 3.99-4.04 (t, 3H), 4.11(s, 1H), 4.18-4.32 (m, 5H), 4.43(s, 1H), 4.50-4.58 (m, 4H), 4.89-4.91 (d, 1H, *J* = 10), 4.99-5.05 (m, 2H), 5.07-5.09 (d, 2H, *J* = 7.2), 5.39-5.41 (d, 1H, *J* = 6.8), 5.77-5.79 (t, 1H), 7.10-7.18 (t, 1H), 7.35-7.37 (d, 1H, *J* = 8.0), 7.41-7.45 (t, 2H), 7.66-7.68 (d, 7H, *J* = 7.2), 7.72-7.75 (t, 2H), 7.86 (s, 1H), 7.91-7.94 (d, 1H, *J* = 9.2), 7.98-7.80 (d, 3H, *J* = 7.2), 8.12 (s, 1H), 8.25-8.27 (d, 2H, *J* = 6.0), 8.35-8.37 (m, 4H), 9.00-9.02 (d, 1H, *J* = 7.6).

$^{13}$C{$^1$H} NMR (100 MHz, DMSO-$d_6$): δ 10.23, 14.10, 21.21, 22.23, 22.49, 22.95, 24.78, 25.53, 25.59, 26.91, 26.98, 27.98, 28.59, 29.47, 31.61, 33.55, 33.58, 35.10, 35.96, 36.03, 36.91, 43.35, 46.42, 50.24, 50.29, 51.70, 51.73, 52.58, 52.98, 53.08, 55.63, 57.47, 67.31,

71.29, 71.99, 74.20, 75.24, 75.84, 77.25, 78.08, 79.06, 80.77, 84.20, 116.11, 119.07, 127.79, 129.13, 130.04, 130.48, 133.91, 136.25, 137.46, 137.62, 154.25, 155.58, 157.29, 158.67, 158.98, 165.77, 167.55, 168.59, 169.44, 169.61, 169.84, 170.05, 170.74, 170.78, 171.77, 171.86, 172.01, 172.30, 172.33, 172.52, 209.79.

HRMS: calcd for $[C_{85}H_{119}N_{15}O_{31}S_4]^+$ $[M+H]^+$ = 1974.7157; obsd 1974.7170.

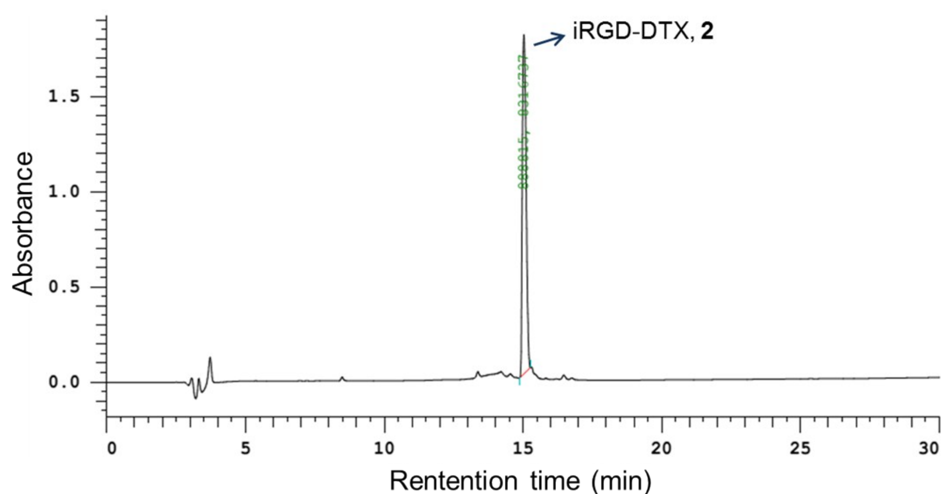
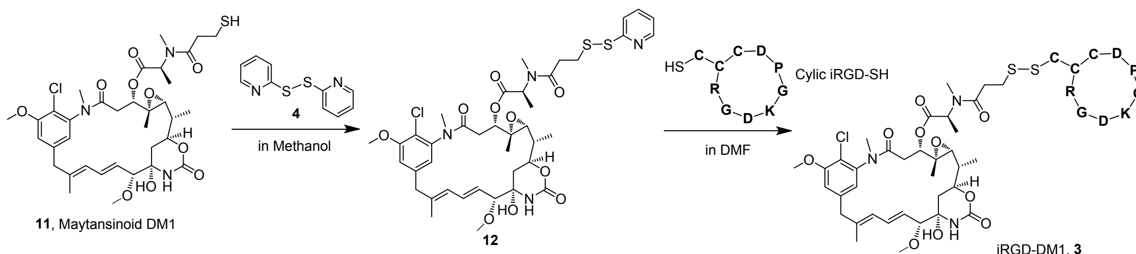



**Figure S8** HPLC profile of iRDG-DTX conjugate, **2** observed at 220 nm.

**Synthesis of iRGD-conjugated maytansinoid DM1 prodrug, 3**




**Supplementary Scheme 3** Synthetic scheme of **3**

**Synthesis of compound 12**

To a solution of 2,2'-Dithiodipyridine, **4** (22 mg, 0.10 mmol) in 5 mL of methanol were added thiolated maytansine derivative DM1, **11** (50 mg, 0.07 mmol). The reaction mixture was stirred at 25 °C for 2 h. After removing the solvent, The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 50:1) to give compound **12** (45 mg, 62%).

$^1$H NMR (400 MHz, DMSO-d6): δ 0.75 (s, 3H), 1.10-1.12 (d, 3H, $J$ = 6.4), 1.16-1.17 (d, 3H, $J$ = 6.8), 1.42-1.48 (m, 2H),1.55 (s, 3H), 1.99-2.03 (m, 2H), 2.43-2.47 (m, 2H), 2.65-2.67 (t,

4H), 2.77-2.80 (d, 1H, $J$ = 9.6), 2.88-2.96 (m, 2H), 3.05-3.08 (d, 5H, $J$ = 12.8), 3.25 (s, 3H), 3.92 (s, 3H), 4.02-4.08 (t, 1H), 4.49-4.52 (m, 1H),5.27-5.34 (m, 1H ), 5.53-5.59 (m, 1H), 6.41-6.42 (m, 1H), 6.53-6.60 (m, 2H),6.91(s, 1H), 7.02-7.03 (d, 1H, $J$ = 1.6), 7.16-7.19 ( m, 1H ), 7.58-7.60 (d, 1H, $J$ = 8.4), 7.69-7.73 (m, 1H), 8.32-8.34 (m, 1H).

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 12.11, 13.32, 14.55, 15.47, 30.53, 32.34, 33.35, 33.41, 35.48, 36.10, 38.82, 46.54, 52.32, 56.51, 56.61, 59.90, 67.18, 74.09, 78.18, 80.85, 88.43, 113.07, 118.71, 119.72, 120.80, 122.01, 125.32, 127.61, 133.30, 136.96, 139.27, 140.88, 142.06, 149.66, 152.24, 155.86, 159.45, 168.70, 170.54, 170.65.

HRMS: calcd for $[C_{40}H_{51}ClN_4O_{10}S_2]^+$ $[M+H]^+$ = 847.2815; obsd 847.2788.

**Synthesis of compound 3**

iRGD (24.8 mg, 0.02 mmol) were added into a solution of compound **12** (20 mg, 0.02 mmol) in DMF. The reaction mixture was stirred at 25 °C for 16 h and purified by HPLC using a YMC C18 reverse-phase column (5 μm, 250 mm × 20 mm). A gradient elution of 10-90% acetonitrile/water applied at a flow rate of 9.9 mL/min to give the compound iRGD-DM1, **3** (18 mg, 42%). The purity of obtained compound **3** was further determined to be >95% by analytic column (Figure S9).

$^{1}H$ NMR (400 MHz, DMSO-$d_6$): δ 0.78 (s, 3H), 1.12-1.13 (d, 3H, $J$ = 6.4), 1.18-1.20 (d, 3H, $J$ = 7.2), 1.24-1.27 (d, 4H, $J$ = 14), 1.44-1.50 (m, 8H), 1.58-1.59 (d, 4H, $J$ = 3.2), 1.71-1.79 (m, 3H), 1.85-1.94 (m, 2H), 1.97-2.03 (m, 1H), 2.05-2.08 (m, 2H), 2.54 (s, 6H), 2.72-2.74 (m, 6H), 2.79-2.81 (t, 2H,) 2.83-2.89 (m, 6H), 3.02-3.08 (m, 3H), 3.14 (s, 5H), 3.25 (s, 5H), 3.62-3.66 (m, 1H), 3.78-3.81 (d, 1H, $J$ = 15.2), 3.89 (s, 3H), 3.93-3.97 (m, 1H), 4.05-4.10 (m, 2H), 4.18-4.25 (m, 2H), 4.28-4.32 (t, 2H), 4.49-4.55 (m, 3H), 4.58-4.65 (m, 3H ), 5.30-5.33 (q, 1H), 5.52-5.58 (m, 1H), 5.92 (s, 1H), 6.52-6.61 (m, 3H), 6.91(s, 1H), 7.12 (s, 1H), 7.65-7.71 ( m, 6H ), 7.84 (s, 1H), 8.03-8.16 (m, 2H), 8.36-8.42 (m, 5H), 8.52-8.54 (d, 1H, $J$ = 7.2), 9.04-9.06 (d, 1H, $J$ = 7.6).

$^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-$d_6$): δ 11.86, 13.54, 14.89, 15.43, 22.23, 22.51, 24.75, 25.64, 26.97, 28.78, 29.49, 30.22, 32.43, 32.73, 33.38, 35.76. 36.00, 36.88, 38.18, 50.28, 51.14, 51.17, 52.19, 52.61, 53.04, 56.61, 56.93, 60.48, 61.04, 67.28, 73.60, 78.25, 80.47, 88.61, 114.29, 115.84, 117.58, 118.81, 122.09, 125.68, 129.06, 133.00, 138.88, 141.75, 141.82, 151.71, 155.70, 157.29, 158.41, 158.73, 159.04, 159.36, 167.60, 168.69, 169.60, 169.86, 170.74, 170.78, 170.90, 170.97, 171.75, 171.82, 171.92, 171.99, 172.28, 172.31, 172.51.

HRMS: calcd for $[C_{73}H_{108}N_{17}O_{25}S_4Cl]^+$ $[M+H]^+$ = 1787.6430; obsd 1787.6374.

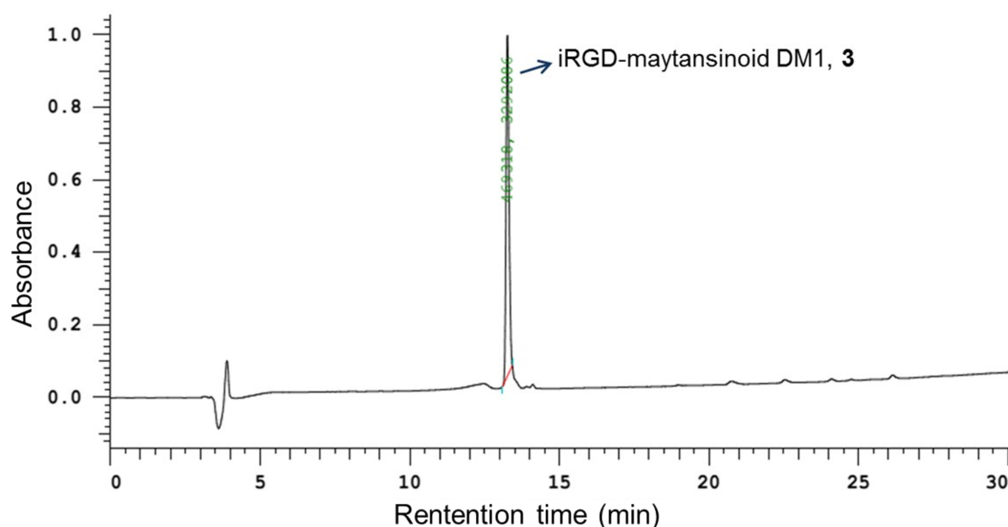
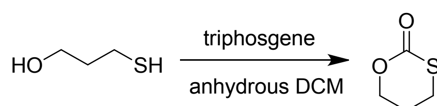



**Figure S9** HPLC profile of iRDG-maytansinoid DM1 conjugate, **3** observed at 220 nm.

**Synthesis of standard compound 1,3-oxathian-2-one**




To a solution of 3-mercapto-1-propanol (50 mg, 0.54 mmol) and 2,3-dimethyl-1-phenyl-5-pyrazolone-5-one (205 mg, 1.09 mmol) in 5 mL of anhydrous DCM were added triphosgene (54 mg, 0.18 mmol). The reaction mixture was stirred at room tembretrue for 16 h, and then washed with water and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated under vacuum. The crude residue was purified by flash column chromatography on silica gel (hexane:ethyl acetate = 15:1) to give the compound 1,3-oxathian-2-one (34 mg, 53.3%).

$^{1}H$ NMR (400 MHz, $CDCl_3$): δ 2.14-2.20 (m, 2H), 3.14-3.17 (t, 2H, *J* = 6.4), 4.46-4.48 (t, 2H, *J* = 6).

$^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 20.90, 32.78, 65.95, 155.10.

**Stability determination by high-performance liquid chromatography (HPLC)**

iRGD-decorated prodrugs **1** and **2** were dissolved in HEPES pH 7.4 with or without glutathione (GSH, 10 mM) and incubated in a 37 °C shaker. At specific time intervals, aliquots (100 µL) were withdrawn and analyzed by HPLC using a C18 reverse-phase column (5 µm, 250 mm × 4.6 mm). A gradient elution method of 10-50% acetonitrile/water was

applied at a flow rate of 1 mL/min. The releases of SN38 and DTX were detected by UV at 378 and 220 nm, respectively, and the hydrolysis was determined based on the presence of the free drugs. The experiments were performed in triplicate, and all data were expressed as the mean ± SD.

**Mechanistic studies on GSH-triggered hydrolysis of prodrug 1 by HPLC analysis**

To gain insight into the mechanism of prodrug hydrolysis upon the treatment of reduced GSH, we performed HPLC analysis to identify the by-products using the prodrug **1** as a model compound. 0.5 mL of HEPES buffer containing 20 mM of reduced GSH was added to 0.5 mL of compound **1** in HEPES buffer (pH 7.4, at a concentration of 1 mg/ml). The mixture was allowed to stir at 37 °C for 1 h and then subjected to HPLC to confirm the released metabolite(s).

The RP-HPLC analysis were performed on a Hitachi Chromaster 5000 system using a YMC-Pack ODS-A column (5 μm, 250 × 2 mm). The mobile phase was a gradient of acetonitrile (solvent A) and water (solvent B) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 1.0 mL/min. The linear gradient was 30% A:70% B to 100%A:0% B within 30 min. UV detection was monitored at 210 nm. The authentic compounds thietane and 1,3-oxathian-2-one were mixed with the GSH-treated hydrolytic samples, respectively, and co-injected into HPLC to verify the released metabolite(s). As a result, we confirmed the presence of 1,3-oxathian-2-one in the hydrolysis mixture of prodrug **1**.

**Cell culture**

Human colon carcinoma cells HCT-116 and HT-29, human umbilical vein endothelial cell (HUVEC), human hepatocellular carcinoma (HCC) BEL-7402 cell and human lung carcinoma A549 cell were maintained in RPMI-1640 media. HCC cells HCC-LM3 and HepG2 were maintained in DMEM medium. Unless otherwise specified, all media were supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells were incubated at 37 °C in a 5% $CO_2$ atmosphere.

***In vitro* MTT assay**

The *in vitro* cytotoxicity of drugs was determined by 3-(4, 5-dimethyl -2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay in a large panel of human tumor cell lines including A549, HepG2, HT-29, BEL-7402 and HCC-LM3. Briefly, tumor cells were evenly plated into 96-well plates at a density of 5000 cells per well and allowed to grow for 24 h. The

cells were then treated with iRGD-SN38 prodrug **1**, iRGD peptide, free SN38 or CPT-11, separately at varying concentrations, and further incubated for 48 and 72 h at 37 °C. Subsequently, 30 μL MTT (5 mg/mL) was added, the plates were incubated for additional 4 h, allowing viable cells to reduce the yellow tetrazolium salt into dark blue formazan crystals. Finally, the medium was removed, 100 μL of DMSO was added to dissolve the formazan crystals. The absorbance was determined at 490 nm on a SynergyHT plate reader (BioTek, Winooski, VT, USA). The *in vitro* cytotoxicity using iRGD-DTX **2** and related controls was also determined by MTT.

***In vitro* migration assay**

Wound healing assay was performed using HCC-LM3 and HUVEC cell lines. Briefly, cells were plated into 6-well plates at a density of $3 \times 10^5$ cells per well and allowed to grow for 24 h. Then the monolayer cells were wounded by scratching with pipette tips and washed with PBS. Fresh DMEM medium containing iRGD (5 μM), SN38 (2 μM) or iRGD-SN38, **1** (2 μM) was added. At specific time intervals, images were taken. The wound width was measured in order to evaluate the wound healing ability of tested cells.

**Transwell invasion assay**

Cell migration was analyzed using 24-well Transwell chambers coated with Matrigel. Chambers have upper and lower culture compartments that are separated by polycarbonate membranes with 8 μm pores. The bottom chamber was filled with 700 μL DMEM containing 20% FBS as a chemoattractant. HUVEC or HCC-LM3 cells in serum-free medium were seeded in the top chamber. The concentrations of drugs (free SN38 or iRGD-SN38, **1**) added to the upper chamber were 2 μM and 0.5 μM for HUVEC and HCC-LM3, respectively. After incubation in a humidified incubator containing 5% $CO_2$ at 37 °C for 72 h, non-migrated cells were scraped, and migrated cells were fixed with methanol and stained with crystal violet. The cells that migrated to the lower side of the membranes were imaged and counted with a microscope.

***In vivo* tumor growth and metastasis assays**

All studies involving animals were approved by The Animal Care and Use Committee of Zhejiang University, China. BALB/c nude mice (5 weeks old) were purchased from Shanghai Experimental Animal Center, Chinese Academy of Science. They are housed under aseptic conditions and given autoclaved rodent diet and sterile water.

Human HCC-LM3 cells ($5\times10^6$ cells) were subcutaneously injected into the flank of BALB/c nude mice. After the subcutaneous tumor reached to 500 $mm^3$ in volume, the tumor was recovered and dissected into 1 $mm^3$ sections, which were further implanted into the left hepatic lobe of BALB/c nude mice to establish orthotopic xenograft model. When the tumor volumes were estimated to be approximately 50 $mm^3$, the mice were randomized into four groups (n=5 for each group) for intravenous injection via the tail vein: (i) saline, (ii) CPT-11 (6 mg/kg), (iii) iRGD peptide (13 mg/kg in saline), (iv) iRGD-SN38 conjugate, **1** (5 mg/kg, SN38 equivalent in saline). The drugs were administered every other day for 5 times. Mice were sacrificed by $CO_2$ inhalation at day 25 after treatment. For the counting of lung metastatic lesions, lungs (n=3 for each group) were removed, fixed in formalin, and embedded in paraffin. The number of lung metastatic lesions was calculated in 10 randomly selected microscopy fields (magnification ×200).