Supporting Information

Activatable and tumor-targeting NIR fluorescent probe for imaging of histone deacetylase in cancer cells and in vivo

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Contents

Experimental Section

Materials

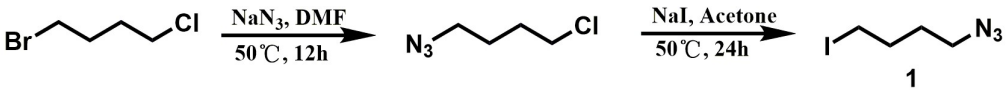
Cyclic pentapeptide c(Arg-Gly-Asp-D-Phe-Lys) (c-RGD) was purchased from GL Biochem Co, Ltd. (Shanghai, China). Histone deacetylase 6 (HDAC6), HDAC1 and HDAC5 were obtained from Cayman Chemical Company (USA). HDAC10 was obtained from OriGene Technologies, Inc (USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2h-tetrazoliubromide (MTT) and suberoylanilide hydroxamic acid (SAHA) were obtained from Macklin Biochemical Co., Ltd (Shanghai, China). 4',6-Diamidino-2-phenylindole (DAPI) were obtained from Thermo Fisher Scientific Co., Ltd (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. and Macklin Biochemical Co., Ltd (Shanghai, China). Organic solvents were purchased from Shanghai Titan Technology Co., Ltd (Shanghai, China). HeLa (human cervical cancer cells), HepG2 (human hepatocarcinoma cells) and LO2 (human hepatocytes) were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). The DMEM medium, 1640 medium and penicillin-streptomycin were obtained from Shanghai Yuanpei Biotechnology Co., Ltd (Shanghai, China). Fetal bovine serum was obtained from Thermo Fisher Technology Co., Ltd (Shanghai, China).

Instruments

Cary 60 spectrophotometer and Cary Eclipse spectrofluorophotometer were used to detect UV-vis absorption and fluorescence spectra, respectively (Agilent Technologies, Palo Alto, CA, USA). MTT assay was done by Cytation 3 Cell Imaging Multi-Mode

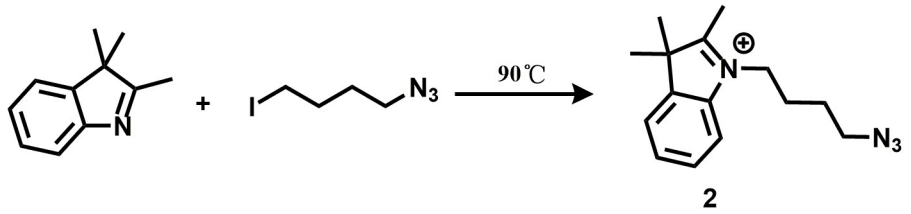
Reader (BioTek Instruments, Inc., Winooski, VT, USA). Confocal laser scanning microscopy (CLSM) images were acquired with a Leica TCS-SP8 confocal scanning microscope (Leica Microsystem Inc, Wetzlar, Germany). High Performance Liquid Chromatography (HPLC) data was acquired with an Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA). NMR spectra were collected on Bruker Advance spectrometer (500 MHz or 600 MHz, Germany). Mass spectra (MS) were measured on Thermo Fisher LTQ Orbitrap Elite (USA). High resolution mass spectrometer (HR-MS) was acquired with Agilent 1290-6545 UHPLC-QTOF mass spectrometer. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectra (MALDI-TOF-MS) was acquired with Bruker AUTOFLEX MALDI TOF/TOF™ mass spectrometer (Germany). In vivo fluorescence images were observed by Perkin Elmer Caliper IVIS Lumina II in vivo imaging system (USA).

Synthesis of compound CyAc-RGD



The 1-bromo-4-chlorobutane (2.55 g, 15 mmol) and sodium azide (1.02 g, 15.7 mmol) was dissolved in DMF at 50 °C for 12 h. After reaction, the residue was diluted with ethyl acetate and washed with DI water for three times. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to obtain compound without further purification. The compound with sodium iodide (0.6 g, 3.92 mmol) was dissolved and stirred in acetone at 50 °C for 24 h. The resulting residue was diluted

with ethyl acetate and washed with DI water for three times. Then the residue was dried over sodium sulfate and concentrated to obtain crude product 1.



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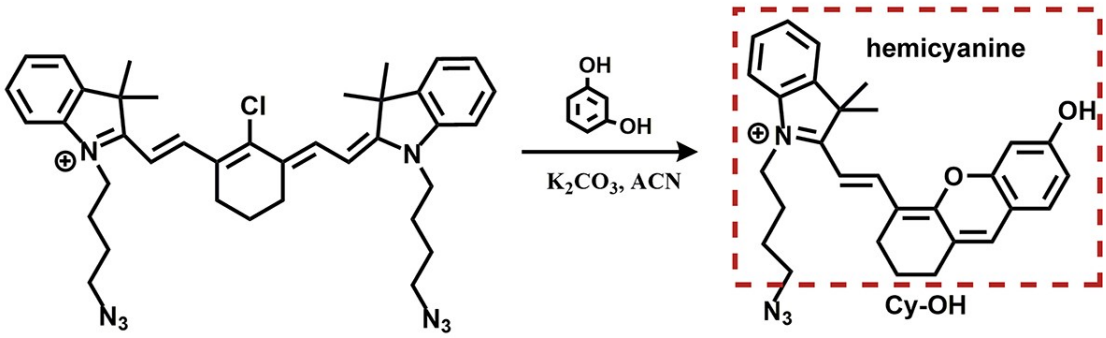
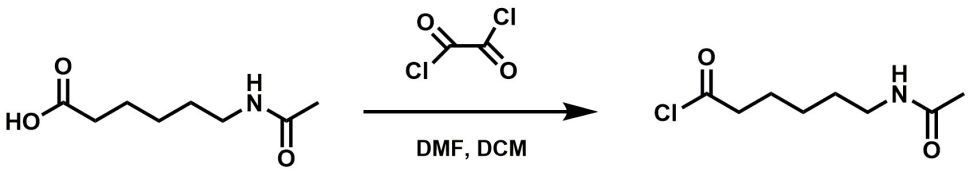
2,3,3-trimethylindolenine (477 mg, 3 mmol) and crude product 1 (675 mg, 3 mmol) were dissolved and stirred at 90 °C for 2 h. The residue was diluted with CH_2Cl_2, precipitated into excess diethyl ether, and washed three times with excess diethyl ether. The product 2 was obtained without further purification.

Product 2 (200 mg, 0.788 mmol), 2-chloro-3-(hydroxymethylidene)-cyclodex-1-ene-1-carbaldehyde (66 mg, 0.38 mmol) and sodium acetate (31mg, 0.38 mmol) were mixed in 5 mL acetic anhydride. The mixture was stirred at 90 °C for 4 h under N_2 atmosphere. Then the residue was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ for three times to remove residue acid. The product was purified by silica gel chromatography (CH_2Cl_2/CH_3OH = 40:1) to obtain pure CyCl (150 mg, 70%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.36 (d, *J* = 14.0 Hz, 2H), 7.45 – 7.36 (m, 4H), 7.28 – 7.19 (m, 4H), 6.27 (d, *J* = 14.1 Hz, 2H), 4.28 (t, *J* = 7.6 Hz, 4H), 3.46 (t, *J* = 6.4 Hz, 4H), 2.76 (t, *J* = 6.3 Hz, 4H), 2.01 – 1.92 (m, 6H), 1.83 (dd, *J* = 8.8, 6.1 Hz, 4H), 1.73 (s, 12H). ^{13}C

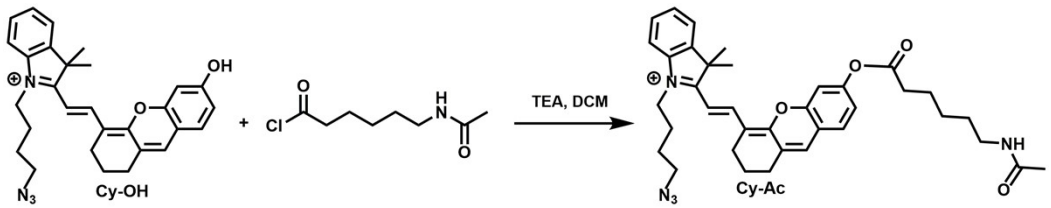
NMR (126 MHz, Chloroform-*d*) δ 172.27, 150.67, 144.45, 142.06, 140.98, 128.94, 127.79, 125.40, 122.29, 111.02, 101.47, 50.99, 49.36, 44.46, 28.19, 26.83, 26.24, 24.70, 20.71. MS-ESI: m/z 649.4.



Potassium carbonate (100 mg, 0.68 mmol) and resorcinol (77 mg, 0.68 mmol) were dissolved in 3 mL CH_3CN at 55 °C for 15 minutes, then CyCl (150 mg, 0.23 mmol) dissolved in 7 mL CH_3CN was added dropwise into the mixture. The reaction mixture was stirred for 2 h. The residue was diluted with CH_2Cl_2 and washed with DI water for three times. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH = 25:1) to obtain pure CyOH (75 mg, 60%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.72 (t, 1H), 7.64 (dd, J = 7.5, 2.7 Hz, 1H), 7.50-7.42 (m, 5H), 6.86 (m, J = 10.8, 6.3, 2.3 Hz, 2H), 6.43 (dd, J = 14.6, 8.7 Hz, 1H), 4.33 (m, J = 7.8, 3.3 Hz, 2H), 3.47 (t, J = 6.5 Hz, 2H), 2.77 (dd, J = 26.0, 6.2 Hz, 4H), 2.07 – 1.92 (m, 4H), 1.84 – 1.82 (m, 6H), 1.81 – 1.77 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 175.93, 162.52, 155.30, 144.24, 141.7, 136.02, 129.21, 128.72, 126.21, 122.33, 114.46, 111.71, 101.67, 50.42, 43.76, 28.46, 27.16, 25.63, 24.33, 23.74, 20.41. MS-ESI: m/z 467.3.

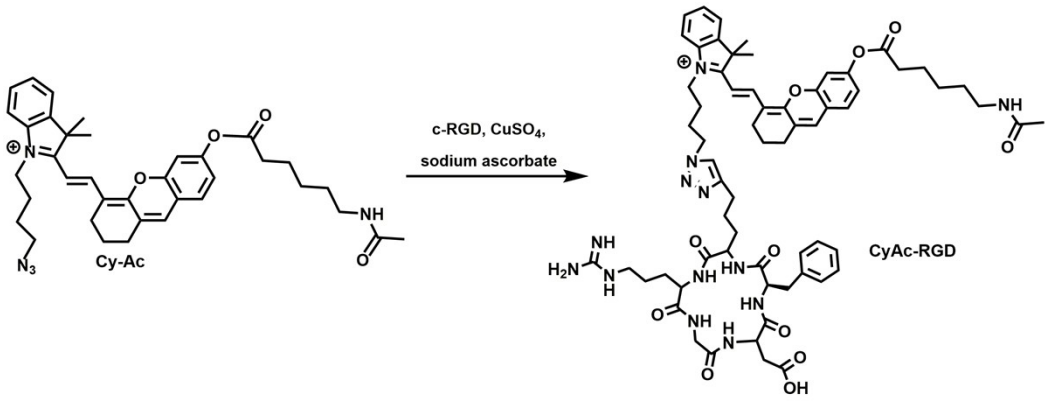


6-Acetamidohexanoic acid (30 mg, 0.17 mmol), oxalyl chloride (40 μL, 0.50 mmol) were dissolved in 2 mL DCM with a drop of DMF under N_2 atmosphere. The mixture was vigorously stirred at 0 °C for 30 min. Then the solvent was evaporated under reduced pressure for next step synthesis without further purification.



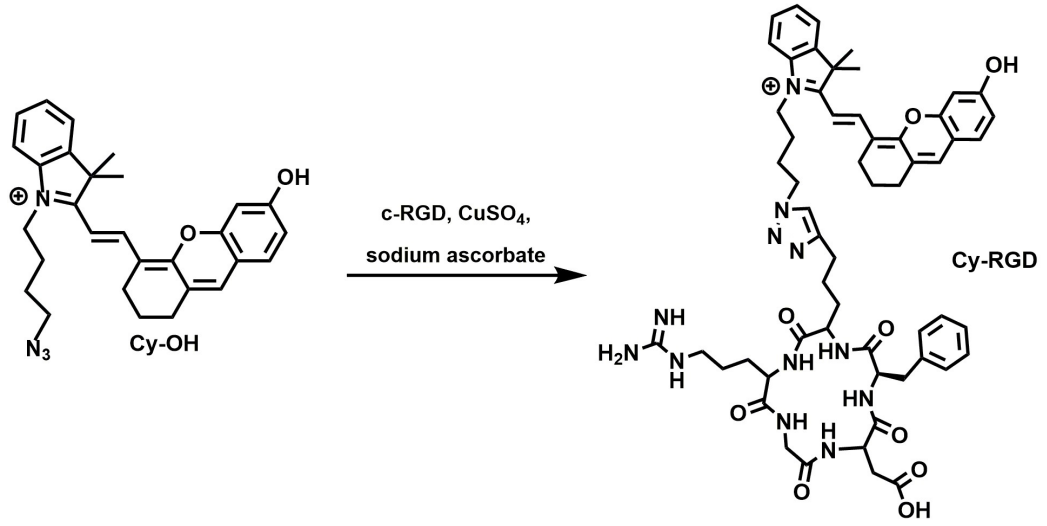
Cy-OH (30 mg, 0.064 mmol) was dissolved in anhydrous DCM in ice bath under N_2 atmosphere, then the above product dissolved in 1 mL anhydrous DCM was added dropwise into the mixture. The reaction mixture was then stirred for 2 h. The residue was diluted with CH_2Cl_2 and washed with DI water for three times. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was concentrated and purified after silica gel chromatography (DCM/CH_3OH=20:1) to obtain pure Cy-Ac (35 mg, 90%). ^1H NMR (500 MHz, Chloroform-*d*) δ 8.6 (d, *J* = 14.9 Hz, 1H), 7.6 – 7.4 (m, 3H), 7.4 (dd, *J* = 17.8, 8.0 Hz, 2H), 7.2 – 7.1 (m, 2H), 7.0 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.7 (d, *J* = 14.9 Hz, 1H), 4.6 (s, 2H), 3.5 (t, *J* = 6.0 Hz, 2H), 3.2 (m, *J* = 6.7 Hz, 2H), 2.8 (s, 2H), 2.7 (m, 2H), 2.6 (t, *J* = 7.4 Hz, 2H), 2.3 (t, *J* = 7.3 Hz, 1H), 2.0 (s, 3H), 2.0 – 1.9 (m, 2H), 1.9 (m, 2H), 1.9 –

1.8 (m, 2H), 1.8 (s, 6H), 1.6 (m, *J* = 19.5, 12.2, 9.6 Hz, 2H), 1.5 (m, *J* = 14.6, 6.8 Hz, 2H) 1.32 (d, *J* = 15.3 Hz, 2H). ^{13}C NMR (151 MHz, Chloroform-d) δ 178.54, 176.34, 171.68, 170.65, 170.40, 160.15, 153.11, 152.87, 146.67, 142.13, 141.18, 131.23, 130.20, 129.49, 128.08, 128.03, 122.67, 119.59, 119.23, 113.28, 109.63, 50.89, 45.83, 39.30, 34.18, 33.80, 31.51, 30.14, 29.71, 29.21, 29.02, 28.19, 26.21, 25.95, 25.33, 24.31, 23.31. HRMS-ESI: m/z =622.3388.



Compound Cy-Ac (30 mg, 0.048 mM), c-RGD (15.0 mg, 0.024 mM), $CuSO_4$•$5H_2O$ (8 mg, 0.030 mmol) and sodium ascorbate (3.0 mg, 0.014 mmol) were dissolved and stirred in THF/H_2O (2:1 v/v) for 1.5 h with a microwave reaction device at 40 °C. The reaction mixture was concentrated and then purified with preparative HPLC to obtain pure CyAC-RGD (10 mg, 34%). ^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 8.70 (d, *J* = 15.0 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 7.59 (s, 1H), 7.52 (dd, *J* = 15.9, 7.6 Hz, 3H), 7.48 (m, 1H), 7.28 (s, 2H), 7.23 (t, *J* = 7.5 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 2H), 7.16 (t, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.46 (d, *J* = 15.0 Hz, 1H), 4.46 (t, *J* = 7.9 Hz, 1H), 4.42 – 4.36 (m, 3H), 4.28 (m, *J* = 8.1 Hz, 3H), 4.17 (s, *J* = 5.5 Hz, 1H), 3.88 (d, *J* = 14.8 Hz, 1H), 3.71 (d, *J* = 14.8 Hz, 1H), 3.00 (m, *J* = 9.6, 6.4 Hz, 4H), 2.88 (m, *J* = 13.5, 7.2 Hz,

1H), 2.75 (s, 3H), 2.64 (m, *J* = 14.8, 6.8 Hz, 6H), 2.07 – 1.99 (m, 6H), 1.85 (m, *J* = 7.4 Hz, 3H), 1.76 (s, 6H), 1.60 – 1.39 (m, 11H). MALDI-MS found: m/z 1220.4344.



Compound Cy-OH (20 mg, 0.042 mM), c-RGD (15.0 mg, 0.024 mM), $CuSO_4$•$5H_2O$ (8 mg, 0.030 mmol) and sodium ascorbate (3.0 mg, 0.014 mmol) were dissolved and stirred in THF/H_2O (2:1 v/v) for 1.5 h with a microwave reaction device at 40 °C. The reaction mixture was concentrated and then purified with preparative HPLC to obtain pure Cy-RGD (8 mg, 33%). ^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 8.68 (d, *J* = 14.7 Hz, 1H), 7.63 – 7.58 (m, 2H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.42 (m, *J* = 7.9, 4.1 Hz, 3H), 7.40 (s, *J* = 5.6 Hz, 1H), 7.22 (m, *J* = 18.3, 7.3 Hz, 4H), 7.16 (t, 1H), 6.95 (s, *J* = 2.2 Hz, 1H), 6.89 (dd, *J* = 8.5, 2.3 Hz, 1H), 6.32 (d, *J* = 14.7 Hz, 1H), 4.45 (t, *J* = 7.9 Hz, 1H), 4.40 (t, *J* = 6.9 Hz, 2H), 4.37 (t, *J* = 6.6 Hz, 1H), 4.27 (t, *J* = 6.9 Hz, 1H), 4.22 (t, *J* = 7.5 Hz, 2H), 4.16 (dd, *J* = 10.4, 5.0 Hz, 1H), 3.89 (dd, *J* = 14.6, 4.6 Hz, 1H), 3.71 (dd, *J* = 14.9, 4.3 Hz, 1H), 3.00 (m, *J* = 16.1, 7.8 Hz, 4H), 2.89 (dd, *J* = 13.5, 7.4 Hz, 1H), 2.77 – 2.73 (m, 4H), 2.65 (t, *J* = 6.2 Hz, 2H), 2.62 (s, *J* = 9.8 Hz, 3H), 2.03 (t, *J* =

7.9 Hz, 2H), 1.88 (d, *J* = 5.9 Hz, 2H), 1.83 (t, *J* = 7.9 Hz, 2H), 1.75 (s, 6H), 1.54 (m, 3H), 1.50 – 1.43 (m, 3H). MALDI-MS found: m/z 1065.4625.

The optical properties of CyAc-RGD and Cy-RGD

CyAc-RGD (25 μM) and Cy-RGD (25 μM) were dissolved in PBS buffer solution (10 mM, pH 7.4). Then the absorption and fluorescence spectra were measured.

Fluorescence response of CyAc-RGD towards HDAC6. To measure the response of CyAc-RGD towards HDAC6, CyAc-RGD (25 μM) was dissolved in PBS buffer solution (10 mM, pH 7.4), then HDAC6 (100 nM) was added into the solution. The mixture was incubated at 37 °C for different time (0-24 min). Next, CyAc-RGD (25 μM) was added into PBS buffer solution, and various concentration of HDAC6 (0-100 nM) were added to the solution. The mixture was incubated at 37 °C for 24 min. The absorption and fluorescence spectra were detected. The excitation wavelength of fluorescence spectra is 680 nm, and the emission collection range is 700-900 nm.

The selectivity of CyAc-RGD towards HDAC6. HDAC6 (50 nM) was mixed with different concentrations of suberoylanilide hydroxamic acid (SAHA, HDAC6 inhibitor) (0-300 nM) in PBS buffer solution (10 mM, pH 7.4). CyAc-RGD (25 μM) was added into the system and shaked for 24 min at 37 °C. Then the fluorescence spectra were measured. CyAc-RGD (25 μM) was dissolved in PBS buffer solution, and 100 μM of Na^+, K^+, Ca^{2+}, Mn^{2+}, Zn^{2+}, vitamin C, glucose, L-cysteine, L-tryptophan, L-glutamate, glutathione (GSH), hyaluronidase, lysozyme, collagenase, and HDAC1, HDAC5, HDAC10 or HDAC6 (100 nM) was added to the solution. Then the mixture was incubated at 37 °C for 24 min and the fluorescence spectra were monitored.

Cell Culture. Hela cells were cultured in DMEM medium at 37 °C in 5% CO_2 atmosphere. The DMEM medium contained 10% fetal bovine serum and 1% penicillin-streptomycin solution. HepG2 and LO2 cells were grown in DMEM and 1640 medium, respectively.

Cytotoxicity assay (MTT). Hela cells (5000/well) were incubated in a 96-well plate and cultured at 37 °C with 5% CO_2 for 24 h. Change the DMEM medium and add various concentration of CyAc-RGD (0-30 μM) in DMEM medium for another 24 h. The medium in each well was removed carefully, and cells was gently washed with PBS for 3 times. Add 0.5 mg/mL of the prepared MTT solution to each well and culture in the incubator for 4 h. 100 μL DMSO was added to each well to dissolve formazan after removing MTT solution. After shaking for 15 min, the optical density values were detected at 479 nm by Cytation 3 Cell Imaging Multi-Mode Reader.

Fluorescence cell imaging. Then CyAc-RGD (0, 5, 10, 20 μM) in DMEM medium was added into each chamber and incubated for 2 h. The medium was removed and fixed with 4% paraformaldehyde for 10 min. Then the cells were incubated with DAPI (10 μg/mL) for 10 min. After incubation, the medium was removed and washed lightly with PBS buffer for 3 times. Then 200 uL PBS was added to dishes and the cells were used to confocal imaging with excitation wavelength of 638 nm and emission wavelength of 700 ~ 900 nm on a Leica TCS-SP8 confocal scanning microscope.

Hela cells ($1x10^4$/well) were seeded in a 4-chamber glass bottom dish and incubated overnight at 37 °C with 5% CO_2. The DMEM medium was removed, and the CyAc-RGD (10 μM) in DMEM medium was added into each chamber and incubated for 0, 1,

2, 4, or 6 h, respectively. 4% paraformaldehyde was added and fixed for 10 min. The medium was removed, and DAPI (10 μg/mL) was added and incubated for 10 min. Finally, the culture medium was removed and the cells were gently washed with PBS for 3 times, then CLSM imaging experiment was followed.

To inhibit HDAC6 activity and blocked $\alpha_v\beta_3$ integrin, Hela cells were pre-incubated with SAHA (500 nM) and c-RGD (50 μM) for 1 h. Then the culture medium was removed and incubated with CyAc-RGD (10 μM) for 2 h. The medium was removed, then fixed with 4% paraformaldehyde for 10 min and incubated with DAPI (10 μg/mL) for another 10 min. After incubation, the medium was removed. Wash with PBS buffer three times, then 200 uL of PBS was added. The cells were used for confocal imaging.

In order to prove the high expression of HDAC6 in tumor cells, we used tumor cells (HepG2) and normal cells (LO2) for comparison. HepG2 cells and LO2 cells were incubated in 4-chamber dish. After incubating for 12 h, CyAc-RGD (10 μM) in DMEM medium or MEM medium were added into the two chambers and incubated for 2 h, respectively. Then fix with 4% paraformaldehyde for 10 min, and gently wash with PBS buffer for 3 times. The cells were then added with 200 uL of PBS and used for confocal imaging.

Imaging of HDAC6 in three-dimensional multicellular spheroids (MCSs). 150 μL of hot agarose solution (10 mg/ml) was added to 96-well plates. After cooling, agarose solution formed a layer of agaropectin. Hela cells were seeded into well at 2×10^3 cells/well and incubated for a week to grow into spheroids. Then MCSs were incubated with CyAc-RGD (10 μM) in DMEM medium for 2 h. After the medium was removed,

the spheroids were washed gently with PBS buffer for 3 times and transferred to 4-chamber glass. Then the MCSs were used for confocal imaging.

Animals and tumor models. Female nude mice aged 5-6 weeks were purchased from Shanghai Experimental Animal Center (Shanghai, China). All animal experiments were conducted in accordance with the guidelines approved by East China Normal University Institutional Animal Care and Use Committee (Approval number: m20200406).

2×10^6 HeLa cells suspended in 100 μL PBS were injected subcutaneously into the right hind limb and abdomen of nude mice to establish tumor models. The tumor was grown for about 3-4 weeks to a size of about 150 mm^3 for the following experiment.

Firstly, nude mice were i.v. injected with Cy-RGD (50 μM, 100 μL). The fluorescent images at 0.3, 0.6, 1, 1.5, 2, 19, 24 and 48 h (λ_{ex} = 675 nm and λ_{em} = 710 nm) were obtained using Perkin Elmer Caliper IVIS Lumina II in vivo imaging system.

Then nude mice were divided into three groups. The mice in first group were i.v. injected PBS (100 μL), and the mice in second group were i.v. injected CyAc-RGD (50 μM, 100 μL). To inhibit HDAC6 activity, the third group of mice were firstly intratumor injected with SAHA (1 mM, 25μL) for 1 h, then i.v. injected with CyAc-RGD (50 μM, 100 μL). The fluorescent images at 0, 1, 1.5 and 2 h (λ_{ex} = 675 nm and λ_{em} = 710 nm) were obtained by IVIS.

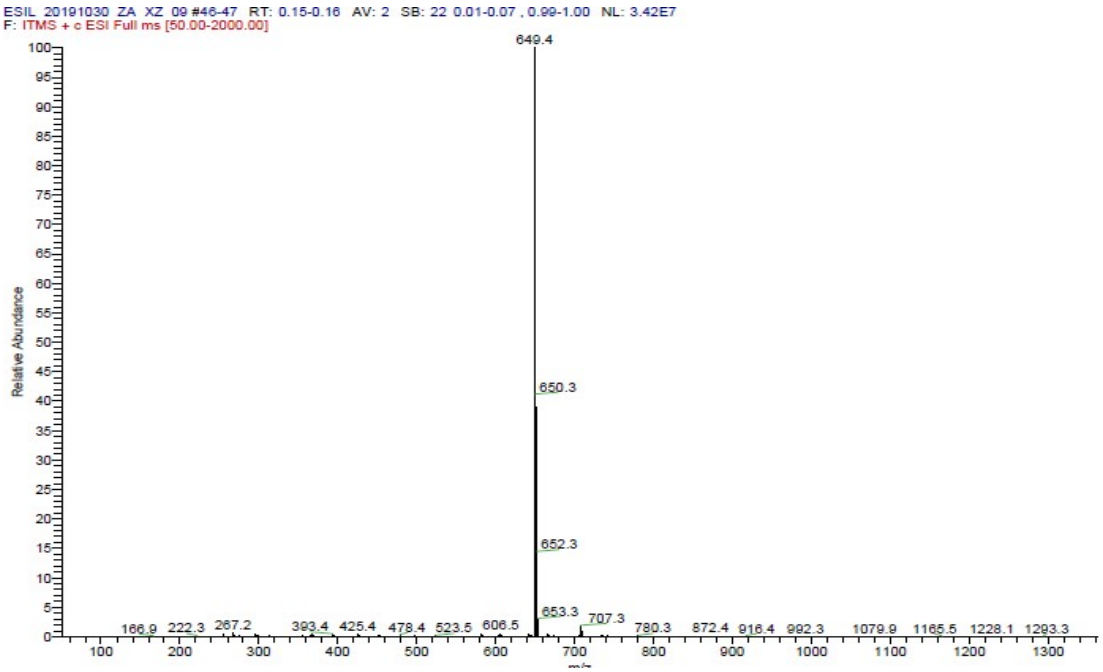


Figure S1. MS spectrum of CyCl.

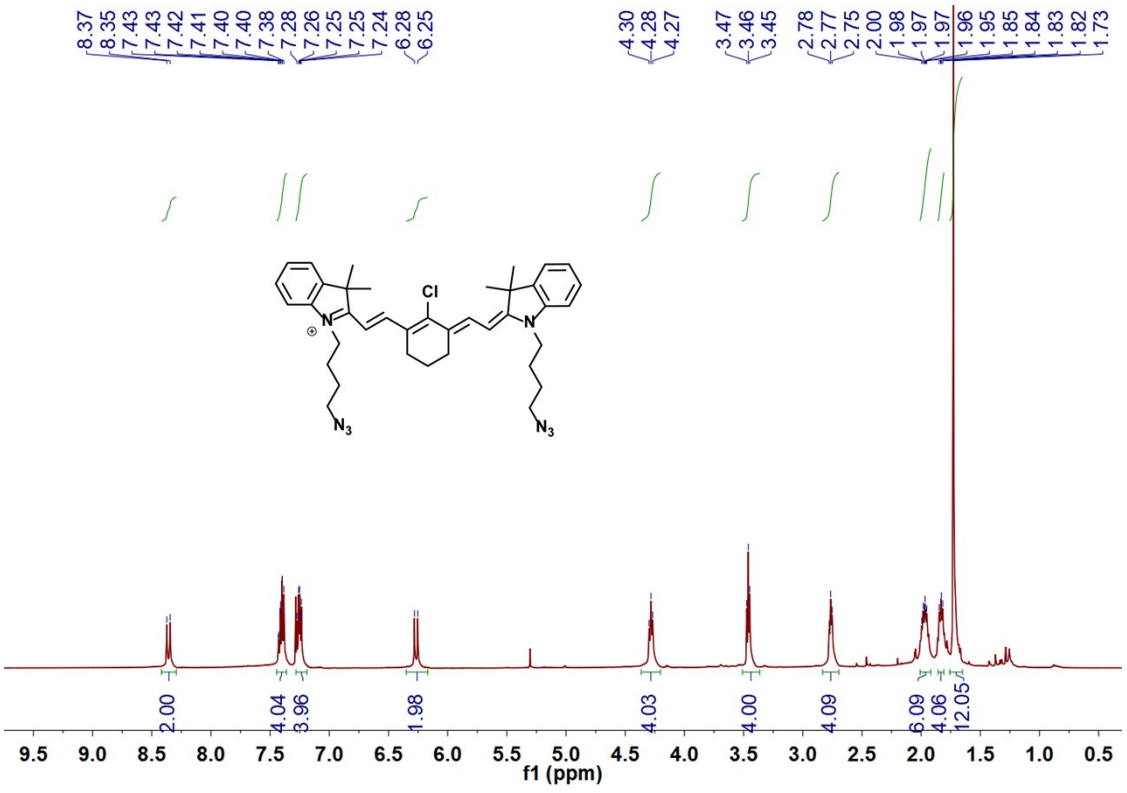


Figure S2. ^{1}H NMR spectrum of CyCl in $CDCl_3$.

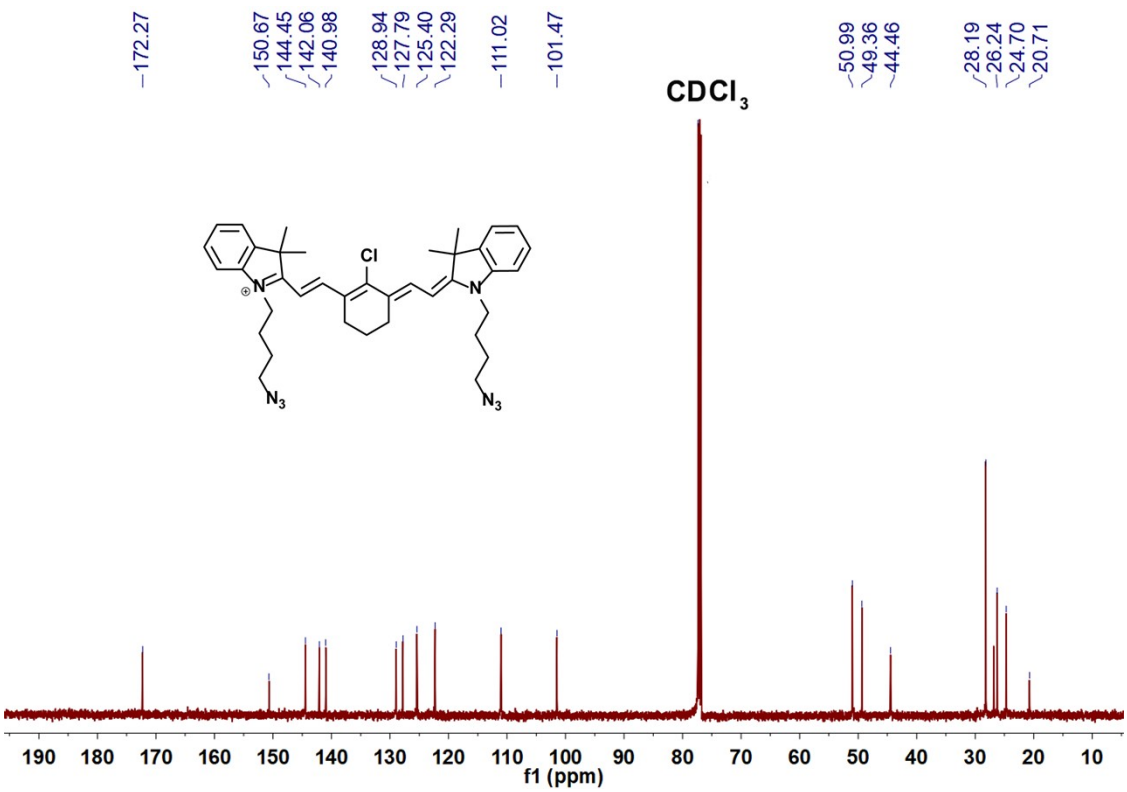


Fig. S3. ^{13}C NMR of CyCl in $CDCl_3$.

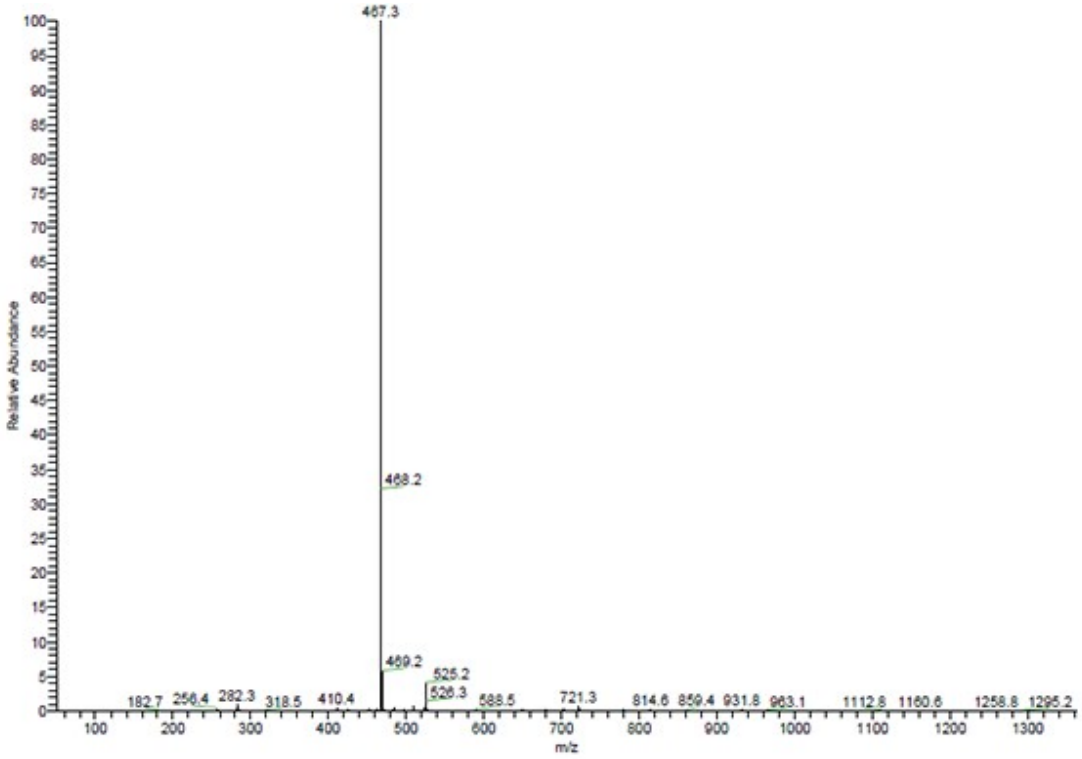


Figure S4. MS spectrum of Cy-OH.

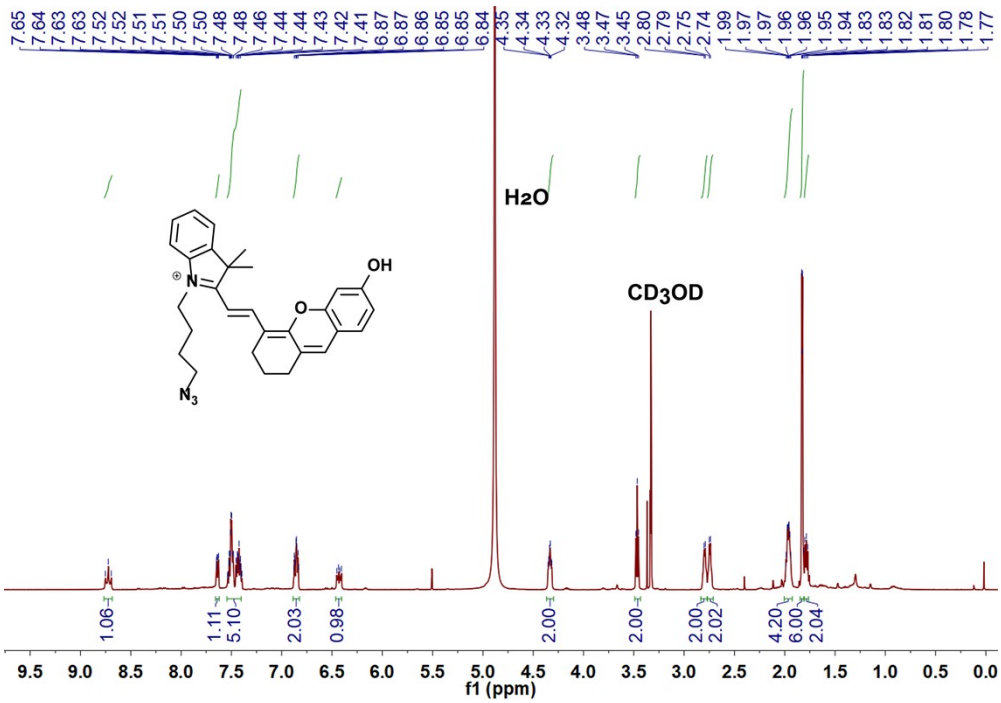


Fig. S5. ^{1}H NMR of Cy-OH in CD_3OD.

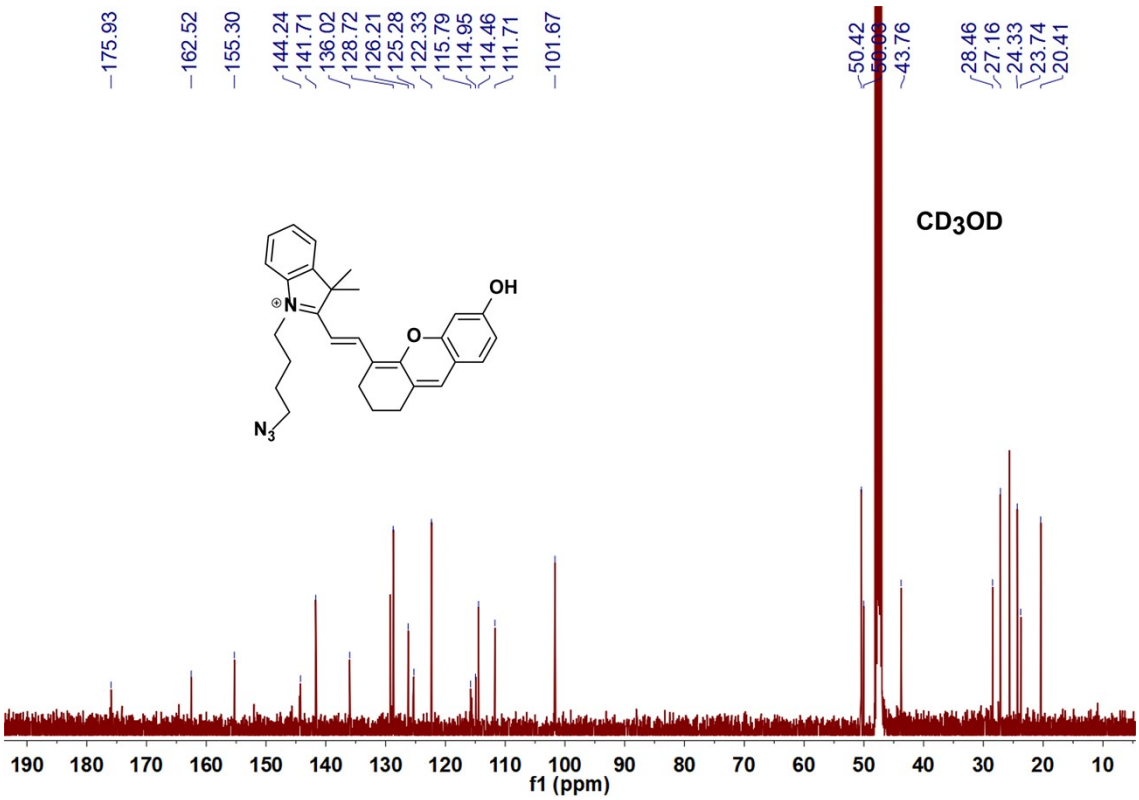
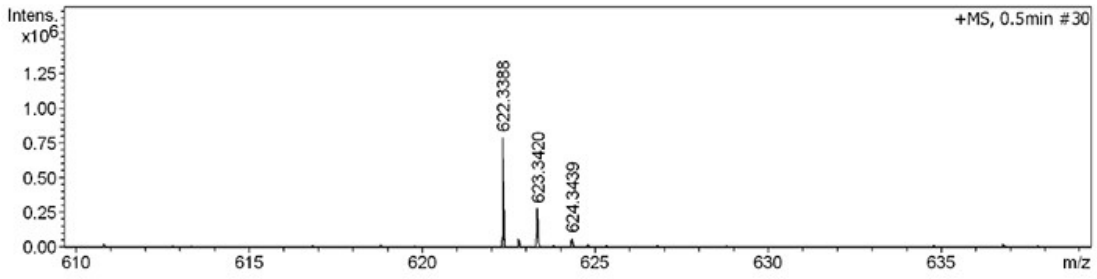


Fig. S6. ^{13}C NMR of Cy-OH in CD_3OD.



#	m/z	Res.	S/N	I	I %	FWHM
1	622.3388	29805	1147.9	792752	100.0	0.0209
2	623.3420	21880	406.9	280992	35.4	0.0285
3	624.3439	18629	90.6	62540	7.9	0.0335

Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma		Score	rdb	e¯ Conf	N-Rule
622.3388	1	C37H44N5O4	622.3388	-0.1	36.4	3	100.00	18.5	even	ok

Fig. S7. HR-MS spectrum of Cy-Ac.

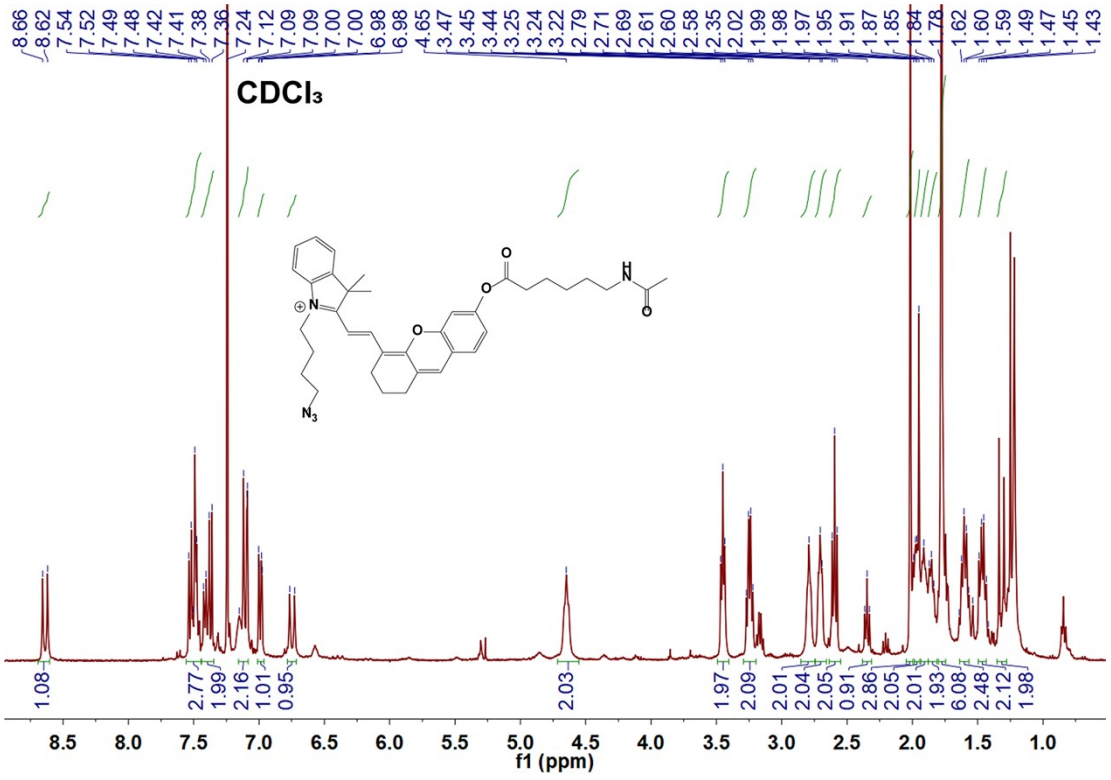


Fig. S8. ^{1}H NMR of Cy-Ac in $CDCl_3$.

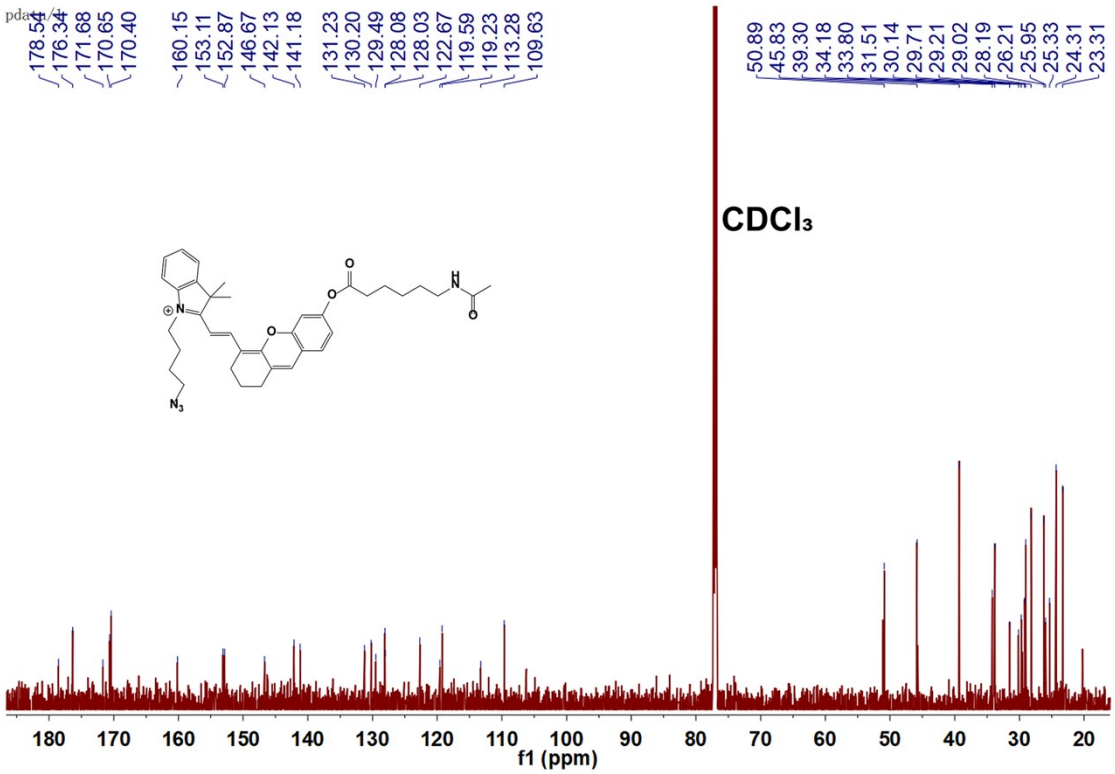


Fig. S9. ^{13}C NMR of Cy-Ac in $CDCl_3$.

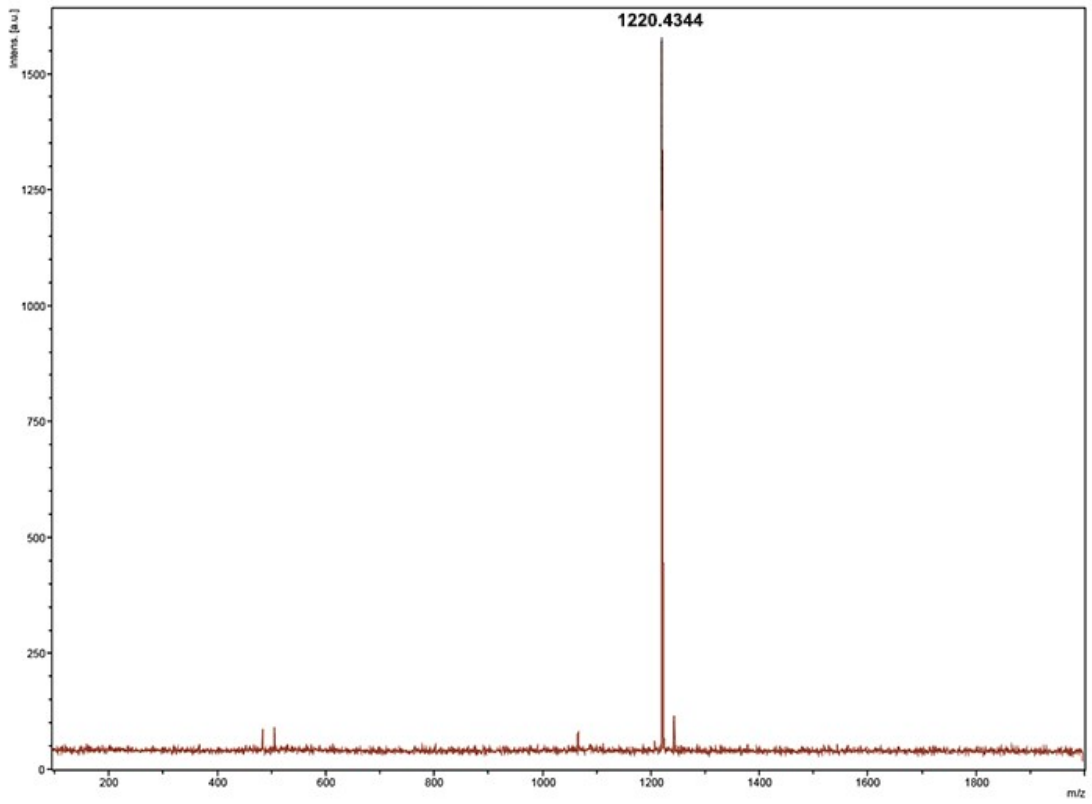


Fig. S10. MALDI-MS of CyAc-RGD.

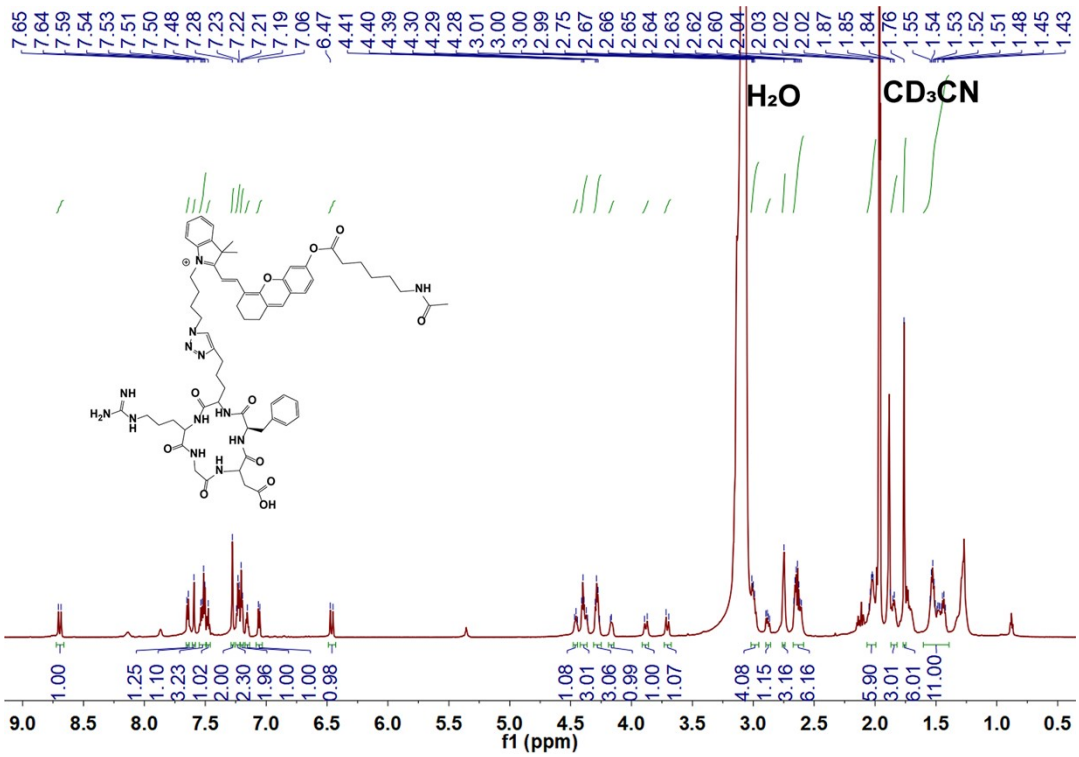


Fig. S11. ^{1}H NMR of CyAc-RGD in CD_3CN.

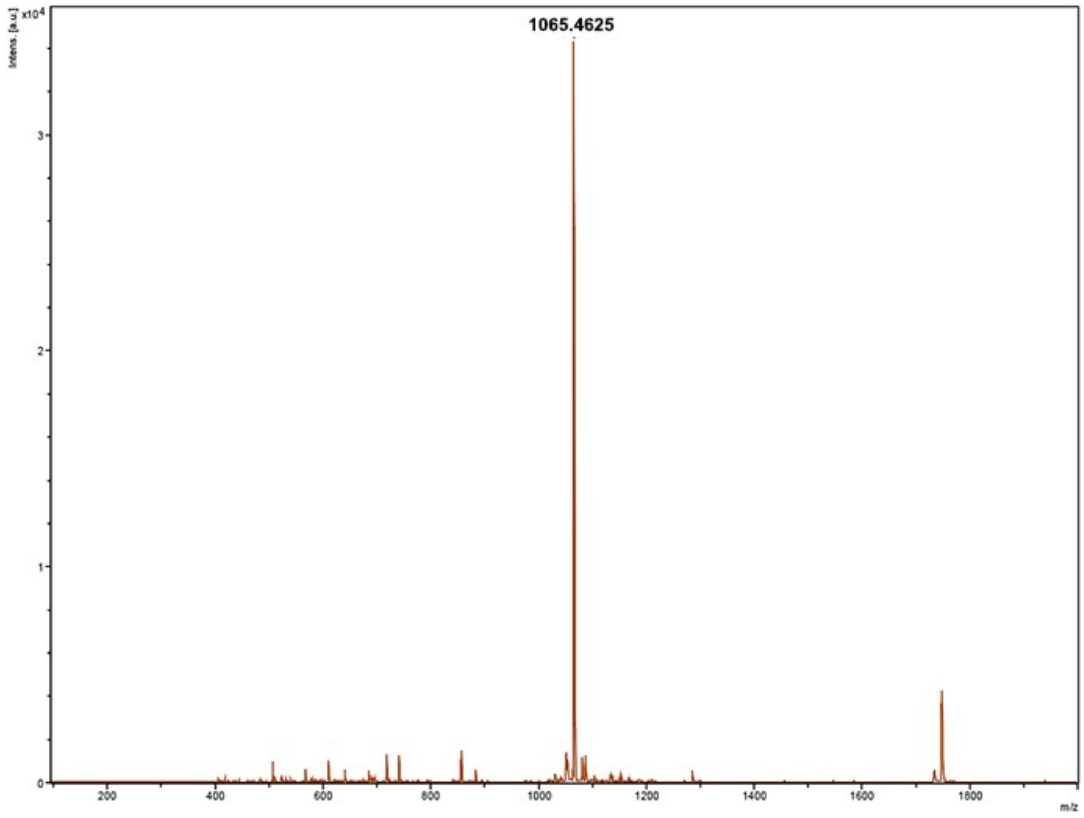


Fig. S12. MALDI-MS of Cy-RGD.

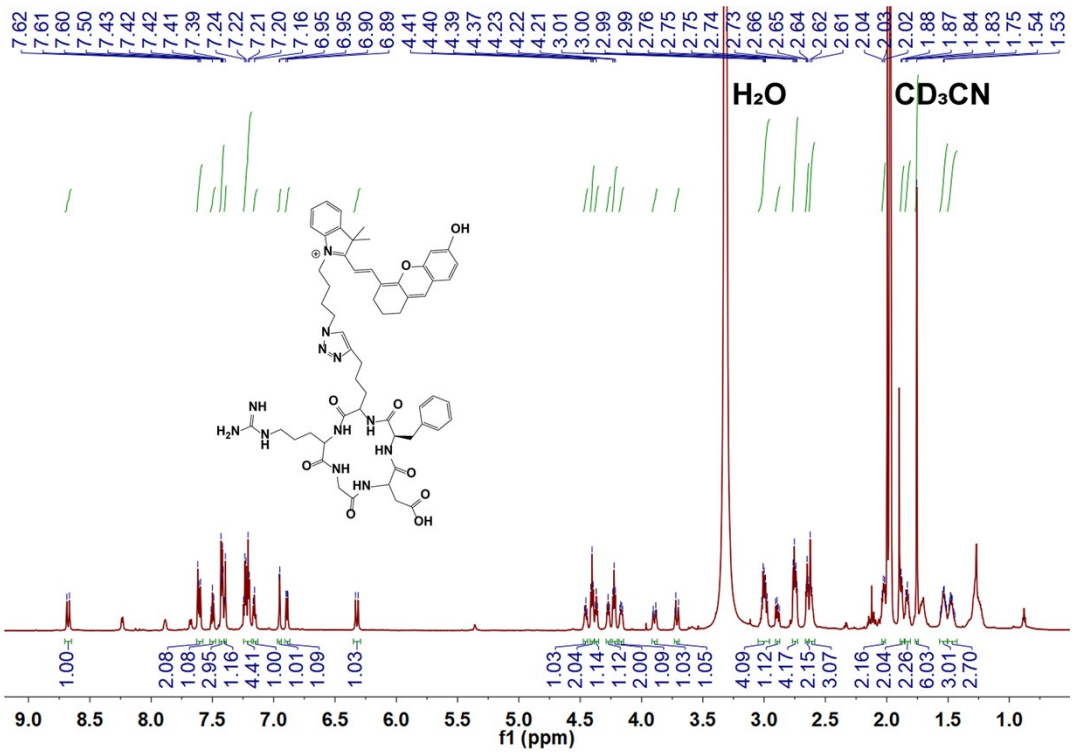


Fig. S13. ^{1}H NMR of Cy-RGD in CD_3CN.

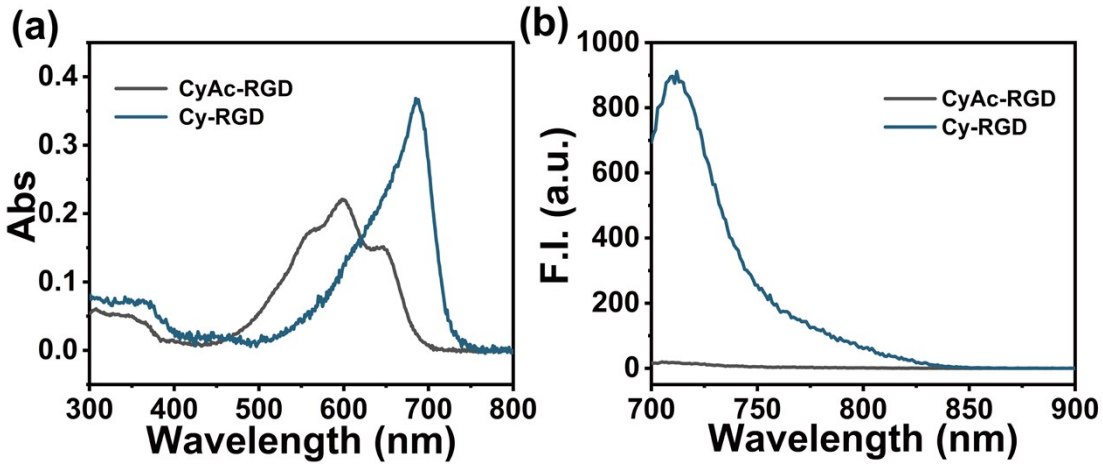


Fig. S14. (a) UV-vis absorption and (b) fluorescence spectra of CyAc-RGD (25 μM) and Cy-RGD (25 μM) in PBS buffer (10 mM, pH 7.4, 37 °C).

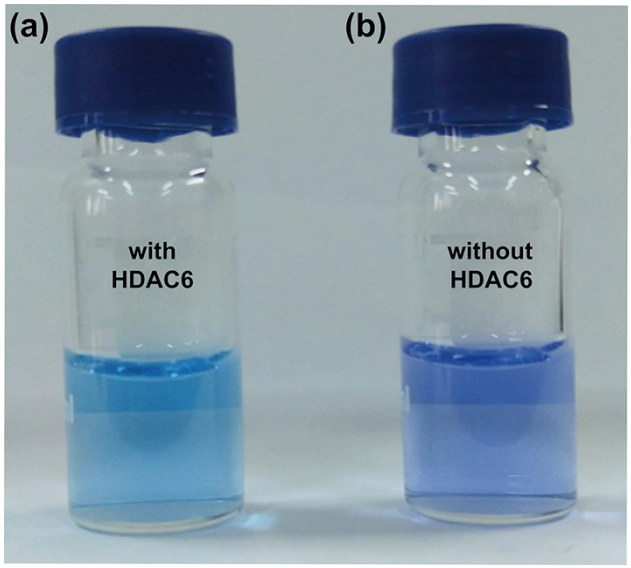


Fig. S15. Color change of CyAc-RGD (25 μM) with and without HDAC6 (100 nM)

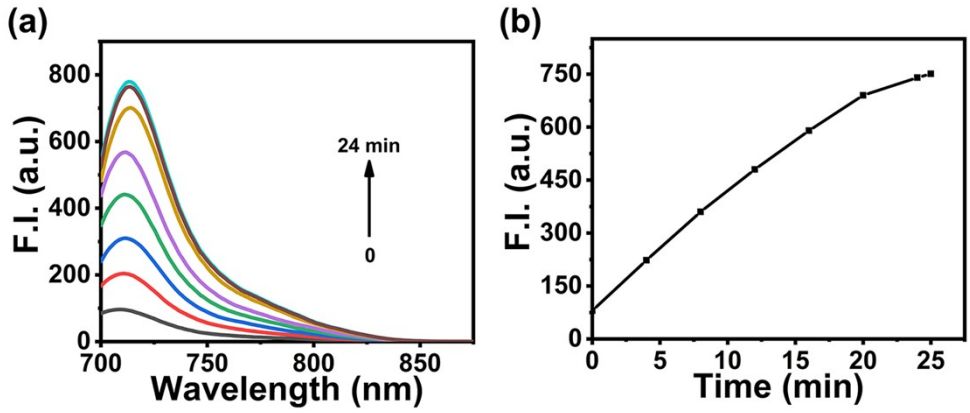


Fig. S16. (a) Fluorescence spectra of CyAc-RGD (25 μM) incubated with HDAC6 (100 nM) in PBS buffer (10 mM, pH 7.4, 37 °C) for different time (0-24 min). (b) The fluorescence intensity of CyAc-RGD (25 μM) at 710 nm as a function of incubation time.

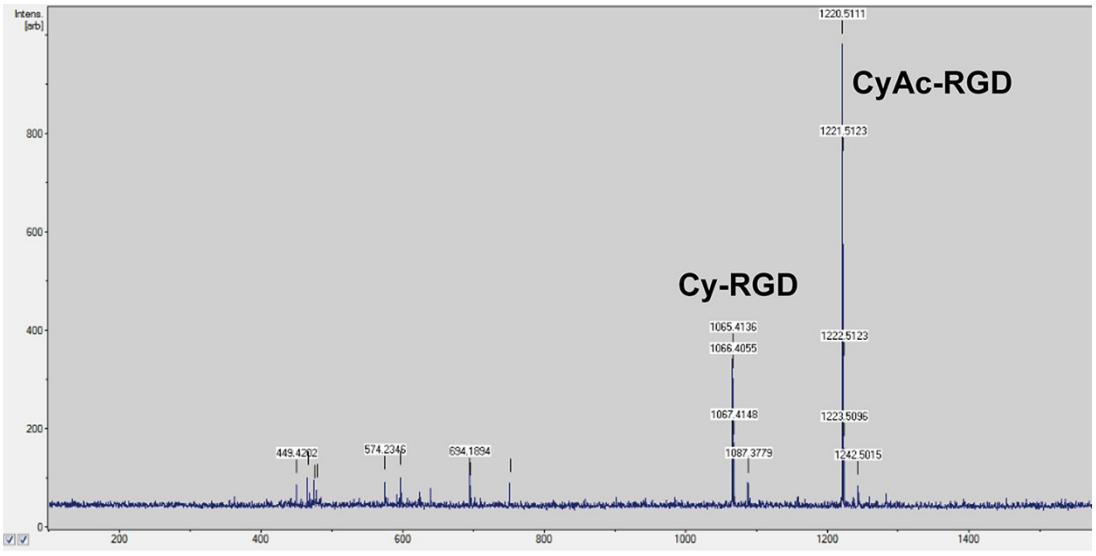


Fig. S17. MALDI-MS analysis of CyAc-RGD after the incubation with HDAC6.

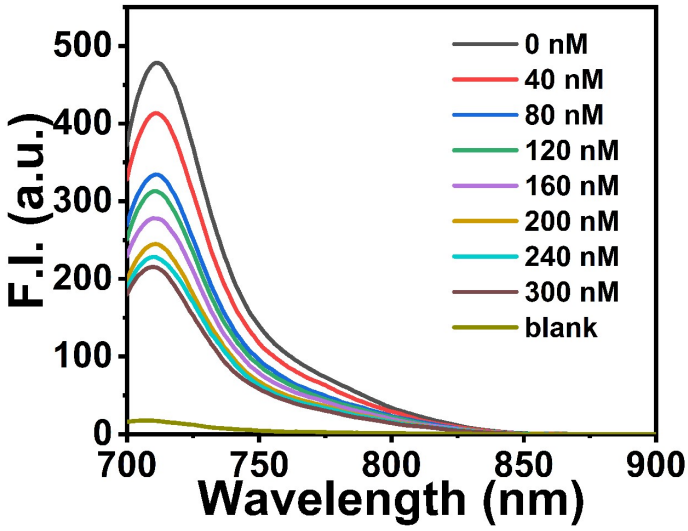


Fig. S18. Fluorescence spectra of CyAc-RGD (25 μM) and HDAC6 (50 nM) with different concentrations of SAHA (0, 40, 60, 80, 120, 160, 200, 240, 300 nM) in PBS buffer (10 mM, pH 7.4) at 37 °C

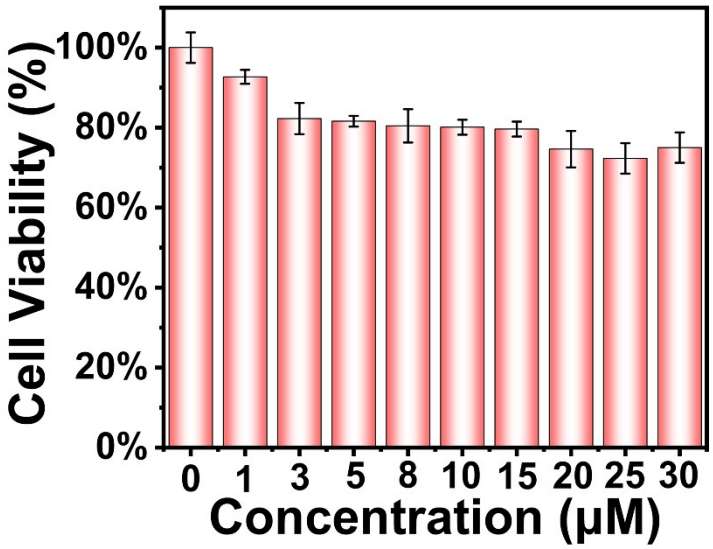


Fig. S19. The cell viability of Hela cells incubated with different concentrations of CyAc-RGD by MTT assay.

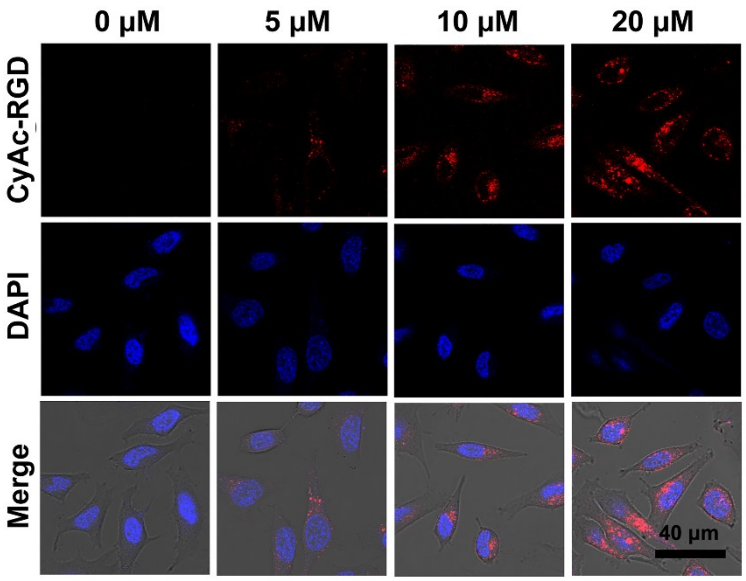


Fig. S20. Fluorescence images of Hela cells incubated with CyAc-RGD (0, 5, 10 or 20 μM) for 2 h.

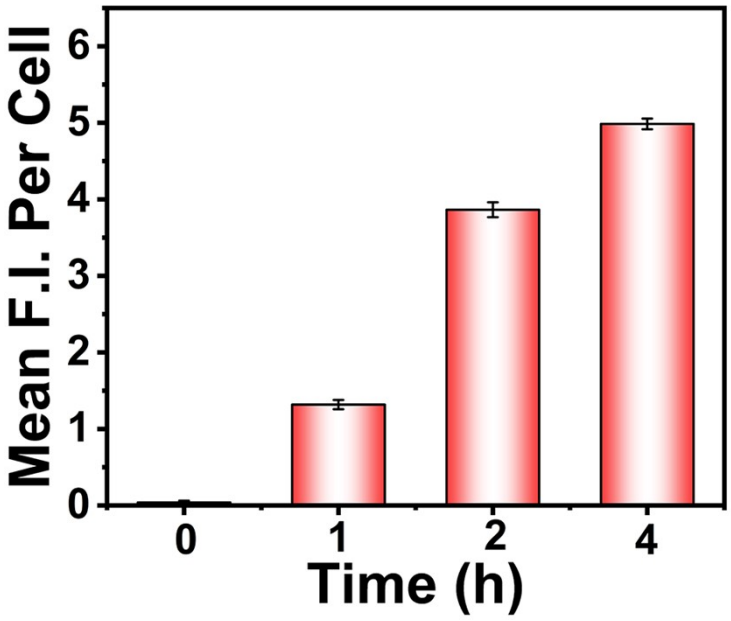


Fig. S21. Mean fluorescence intensities of Hela cells incubated for different time (0, 1, 2 or 4 h) and measured by CLSM.

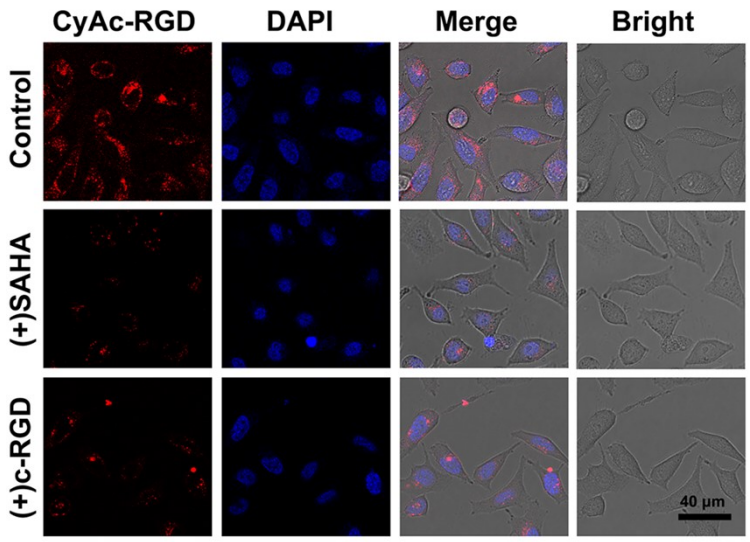


Fig. S22. Fluorescence images of Hela cells incubated with CyAc-RGD (10 μM), CyAc-RGD (10 μM) and SAHA (500 nM), CyAc-RGD (10 μM) and c-RGD (50 μM).

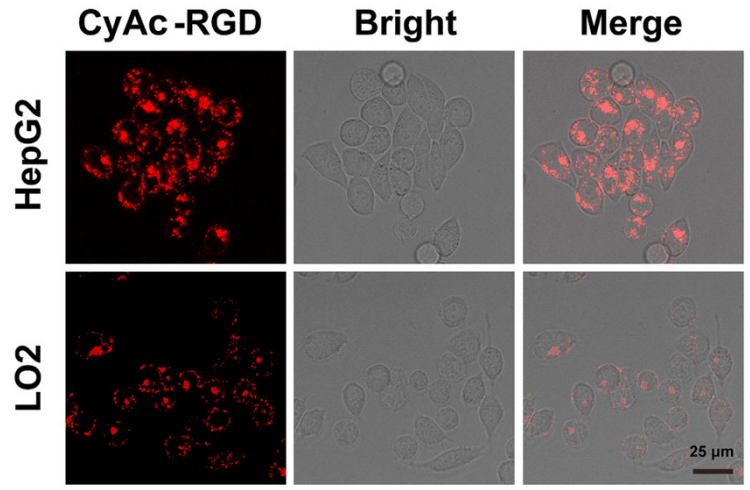


Fig. S23. Fluorescence images of HepG2 and LO2 cells incubation with CyAc-RGD (10 μM) for 2 h.

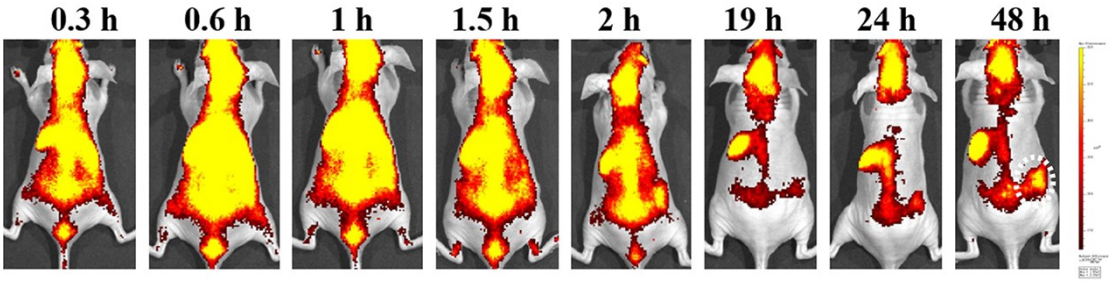


Fig. S24. Real-time imaging of Cy-RGD in living mice.