Electronic Supplementary Material (ESI) for ChemComm.


Supplementary data for

# Selective degradation of histone deacetylase 8 mediated by a proteolysis targeting chimera (PROTAC)

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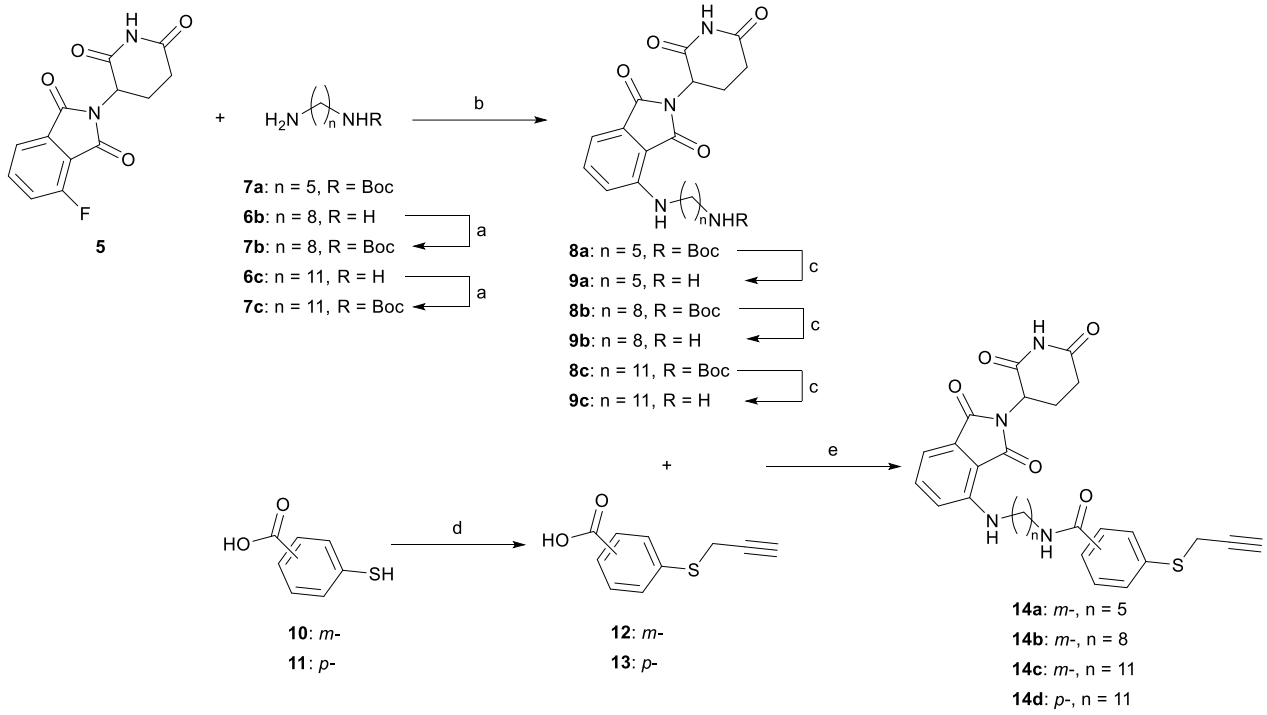
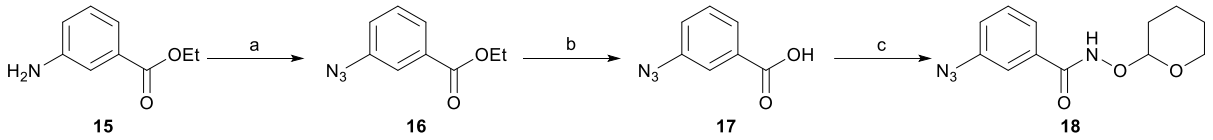
**Contents**

**Scheme S1.** Synthesis of compounds **14**.[a]



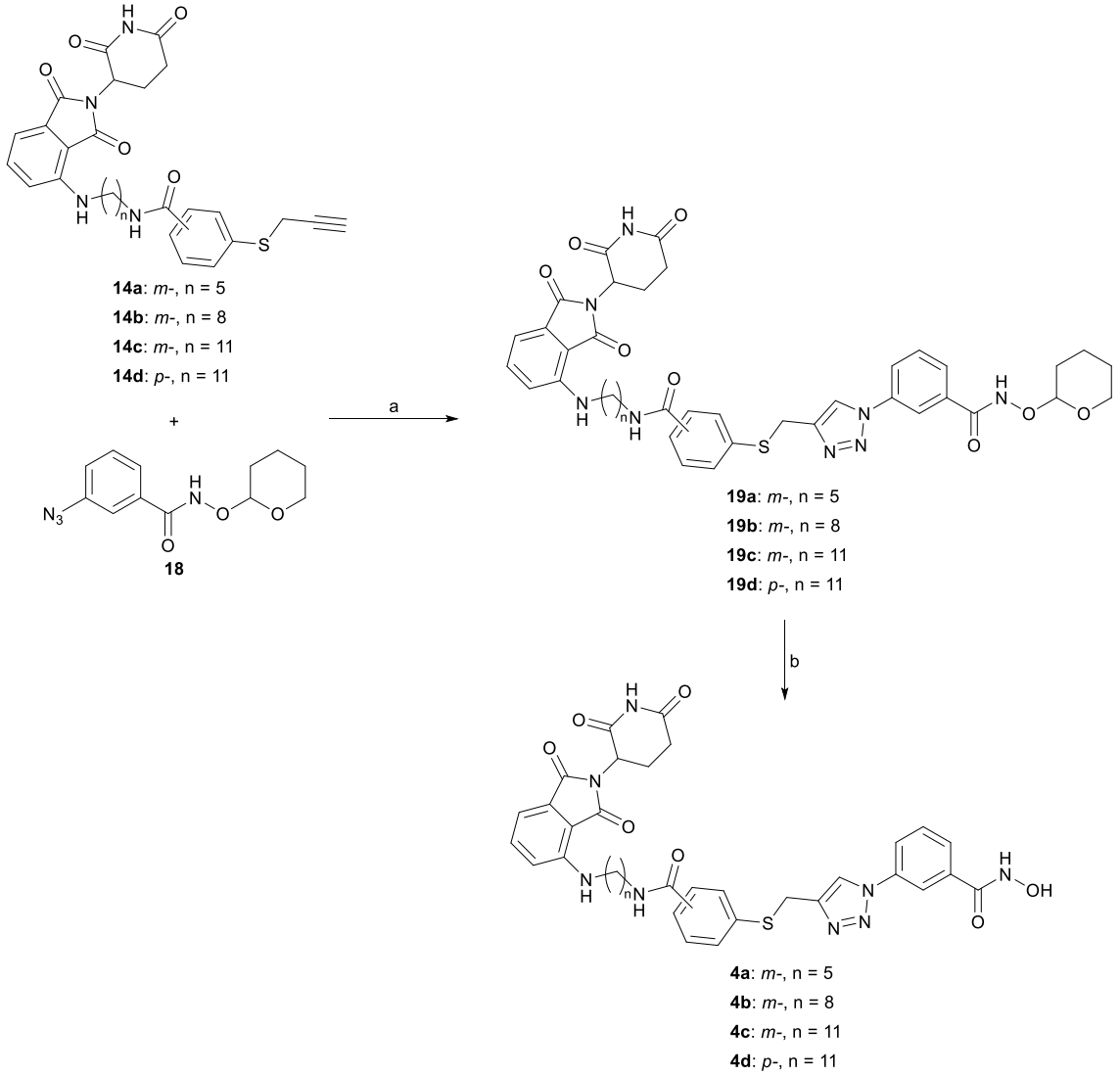

[a] Reagents and conditions. (a) $Boc_2O$, $Et_3N$, $CHCl_3$, room temperature, 54% for **7b** or 29% for **7c**; (b) *i*-$Pr_2NEt$, DMF, 90°C, 25% for **8a**, 33% for **8b**, or 27% for **8c**; (c) trifluoroacetic acid, $CH_2Cl_2$, 0°C; (d) propargyl bromide, $Et_3N$, THF, room temperature, 85% for **12** or 89% for **13**; (e) 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b] pyridinium 3-oxide hexafluorophosphate (HATU), *i*-$Pr_2NEt$, DMF, room temperature, 44% for **14a** (2 steps from **8a**), 51% for **14b** (2 steps from **8b**), 32% for **14c** (2 steps from **8c**), or 45% for **14d** (2 steps from **8c**).

**Scheme S2.** Synthesis of compound **18**.[a]




[a] Reagents and conditions. (a) *t*-BuONO, $TMSN_3$, MeCN, room temperature, 1 h, 78%; (b) NaOH, THF, MeOH, $H_2O$, room temperature, 94%; (c) *O*-(Tetrahydro-2*H*-pyran-2-yl)hydroxylamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole monohydrate ($HOBt \cdot H_2O$), DMF, room temperature, 87%.

**Scheme S3.** Synthesis of compounds **4**.[a]




[a] Reagents and conditions. (a) $CuSO_4$, sodium ascorbate, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA), THF, $H_2O$, room temperature, 77% for **19a**, 55% for **19b**, 100% for **19c**, or 81% for **19d**; (b) $TsOH \cdot H_2O$, $CH_2Cl_2$, MeOH, room temperature, 82% for **4a**, 87% for **4b**, 66% for **4c**, or 60% for **4d**.

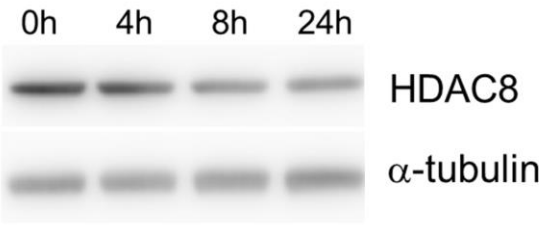



**Fig. S1.** Western blot detection of HDAC8 in Jurkat cells after 4, 8, and 24 h treatment with **4c**.

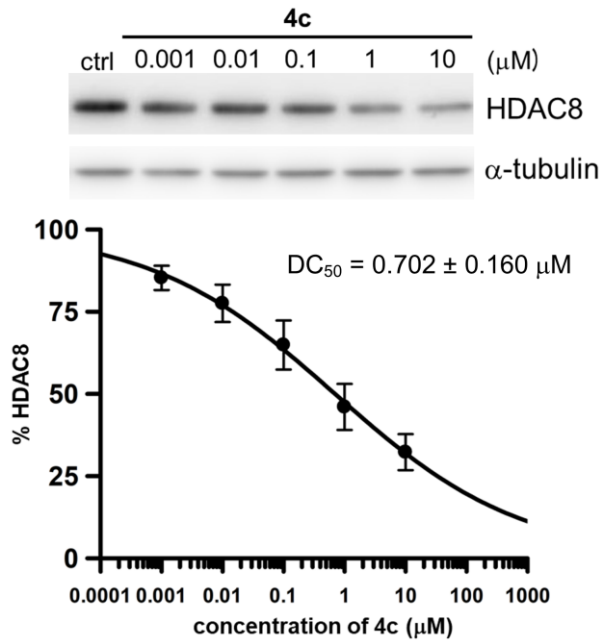



**Fig. S2.** Representative western blot detection of HDAC8 levels in Jurkat cells treated with compound **4c** at several concentrations for 24 hours and a $DC_{50}$ value determination. The dose-response curve was drawn and the $DC_{50}$ value was calculated based on values of HDAC8 ratio determined by optical density measurement of the blots. The $DC_{50}$ value is the mean± SD of three experiments.

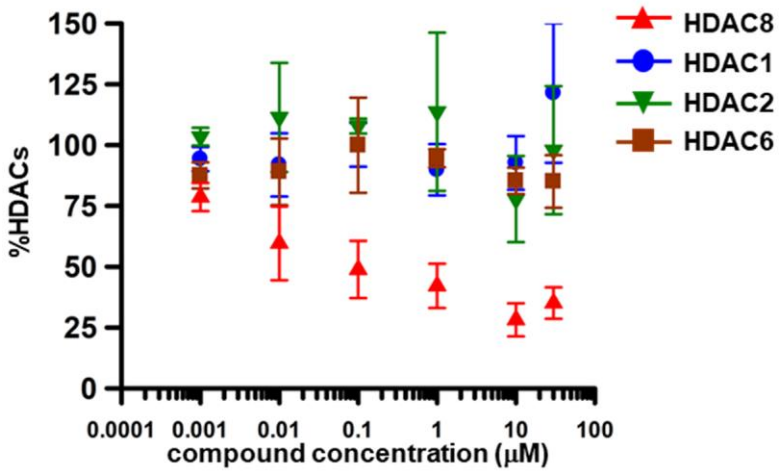



**Fig. S3.** Optical density measurement of western blotting analysis of HDAC1, 2, 6, and 8 levels. The values were calculated based on values of HDAC1, 2, 6, and 8 ratio determined by optical density measurement of the blots including the one shown in Figure 3B. The value is the mean± SD of three experiments.

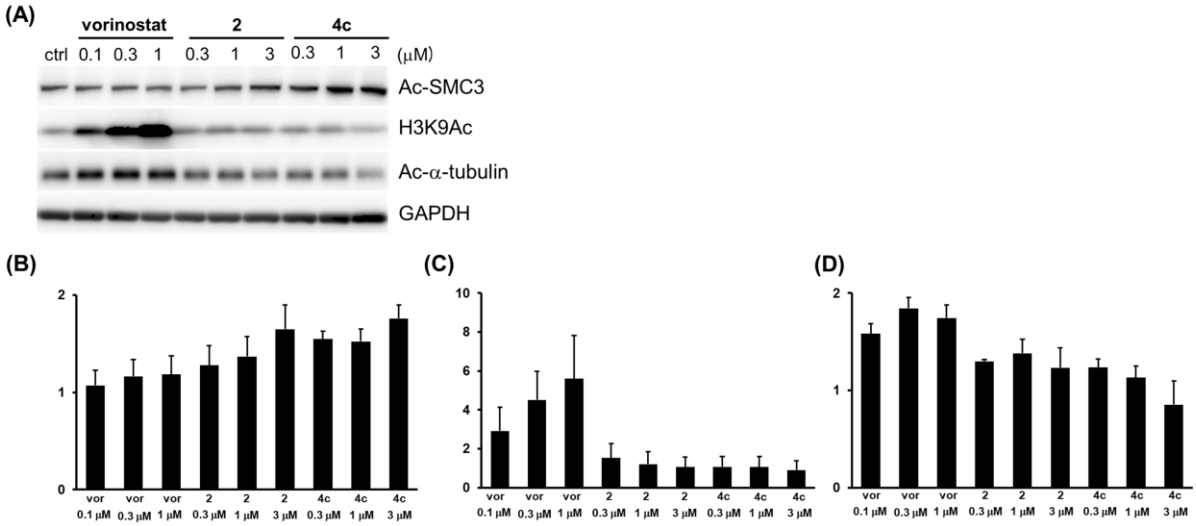



**Fig. S4.** Effect of compound **4c** on acetylated SMC3 (Ac-SMC3), acetylated lysine 9 of histone H3 (H3K9Ac), or acetylated α-tubulin (Ac-α-tubulin) in Jurkat cells. (A) Representative western blot detection of acetylated Ac-SMC3, H3K9Ac, or Ac-α-tubulin in Jurkat cells after 24 h treatment with **4c, 2**, or vorinostat. Compound **2** or vorinostat was used as a positive control. (B, C, and D) Optical density measurement. The bars show (B) Ac-SMC3/GAPDH, (C) H3K9Ac3/H3, and Ac-α-tubulin/GAPDH ratios determined by optical density measurement of the blots. The values are the mean± SD of three experiments.

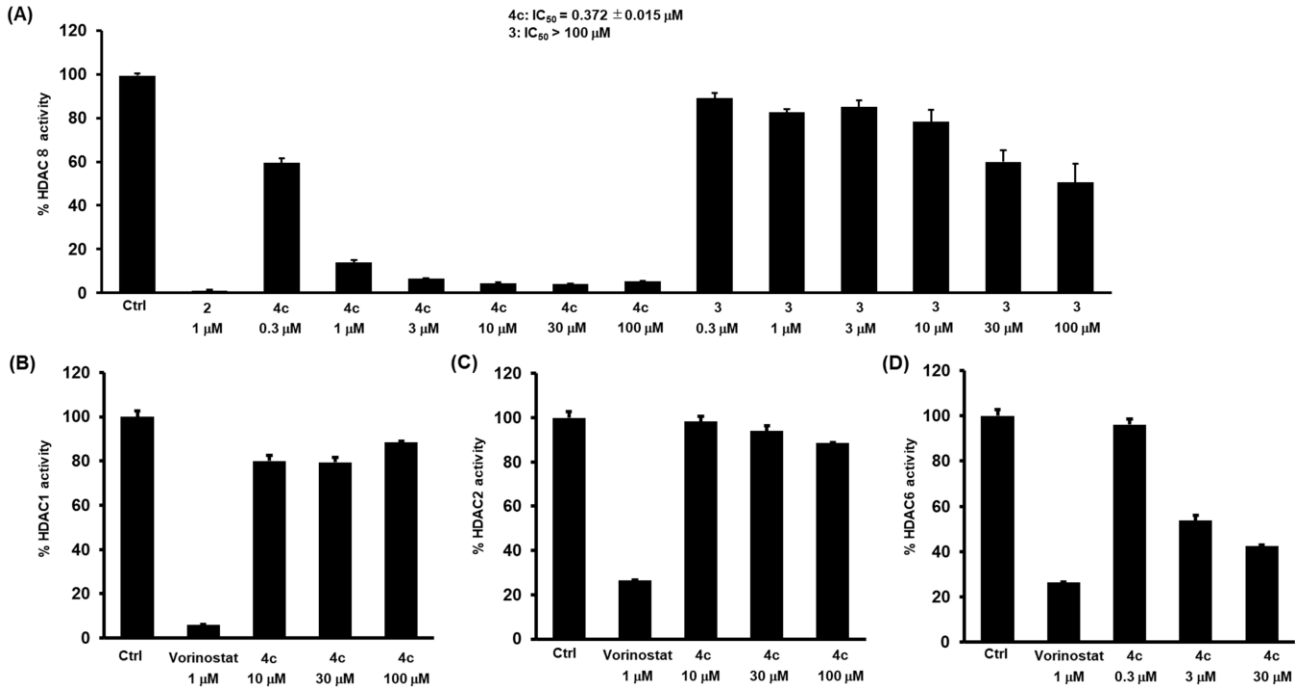



**Fig. S5.** Inhibitory activity of compound **4c** towards HDAC1, 2, 6, and 8**.** (A) HDAC8, (B) HDAC1, (C) HDAC2, and (D) HDAC6. Compound **2** or vorinostat was used as a positive control. The $IC_{50}$ values of compound **4c**, **2**, and **3** against HDAC8 are 0.372, 0.053,[S1] and > 100 μM, respectively.

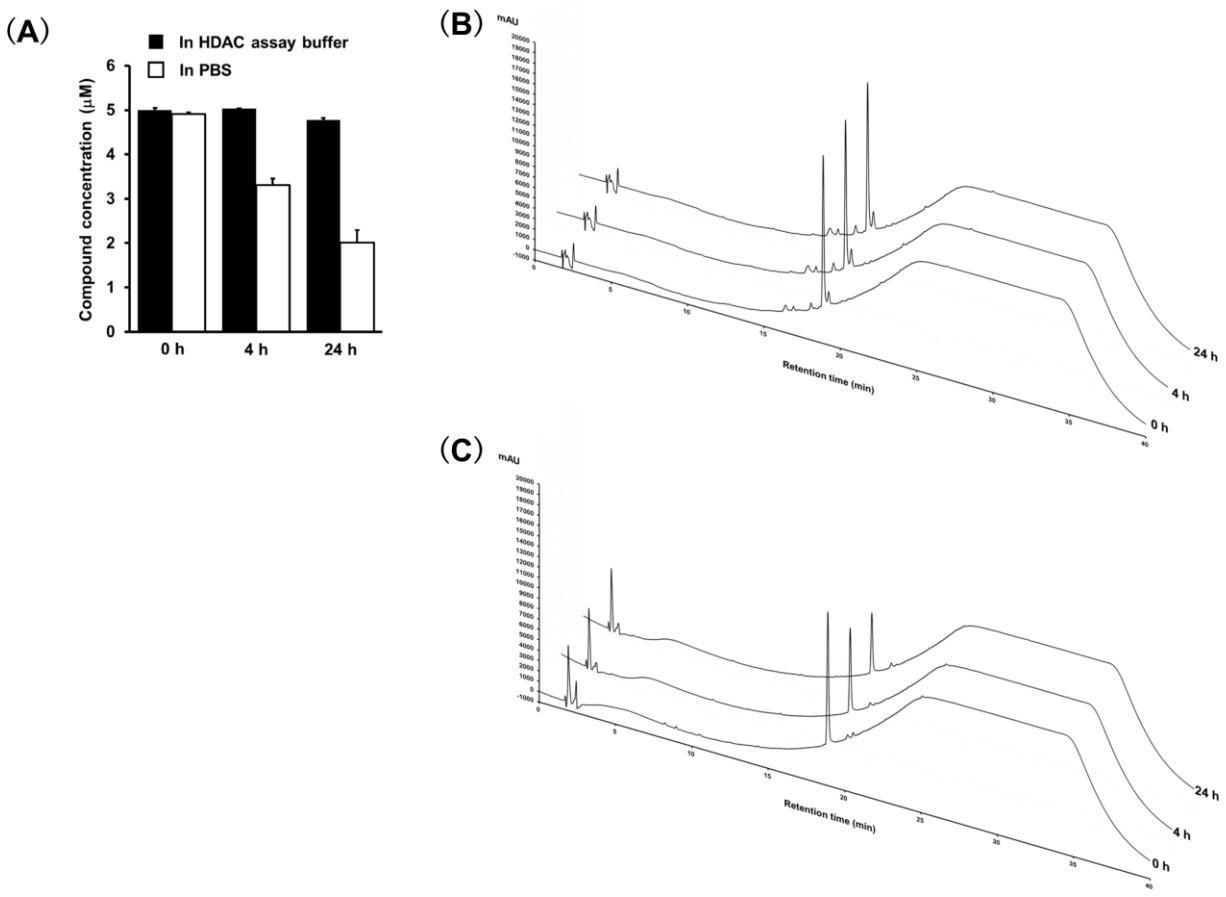



**Fig. S6.** Stability tests of compound **4c** by means of HPLC analysis. The initial concentration of **4c** was 5 μM and the graph bars indicate that concentrations of compound **4c** in HDAC assay buffer at 25 ℃ or phosphate buffered saline (PBS, pH 7.4) at 37 ℃ after 4h- or 24h-incubation. (A) Quantitative analysis. Values are the mean ± SD of at least three experiments. (BC) Original HPLC charts for analysis of compound **4c** in (B) HDAC assay buffer or (C) PBS. The retention time of compound **4c** is around 19 min. These data indicate that at least 95% and 40% of compound **4c** remain after 24 h-incubation in HDAC assay buffer and in PBS, respectively. The stability of **4c** is higher than that of reported pomalidomide-based PROTACs.[S2]

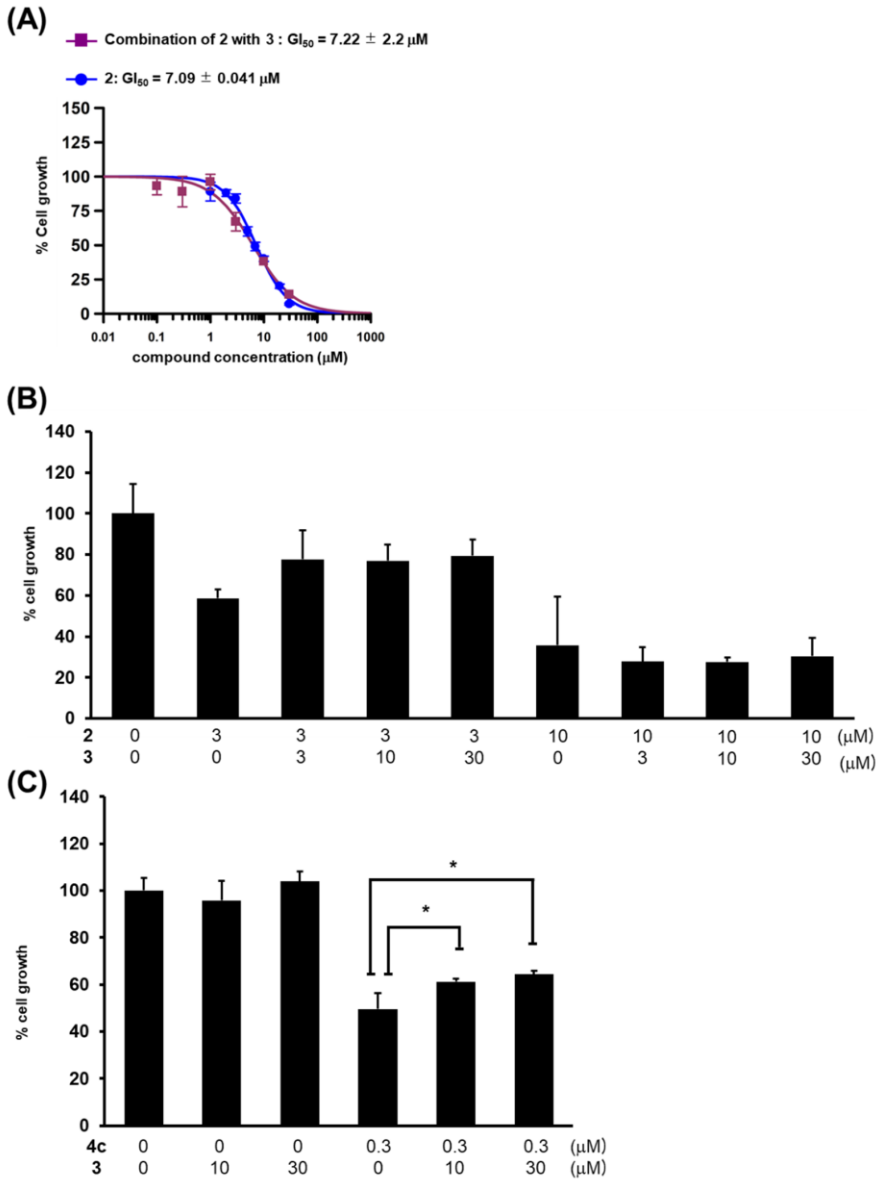



**Fig. S7.** Cell growth-inhibition of Jurkat cells co-treated with compounds **2** and **3** after 72-h treatment. Values are the mean ± SD of at least three experiments. (A) The Jurkat cells were treated with at the same concentrations of each compound. The same data of compound **2** as Fig. 5 is used. (B) Cell growth-inhibitory activity of 3 or 10 μM of compound **2** in the presence of 3, 0, 3, 10, or 30 μM of compound **3**. (C) Cell growth-inhibitory activity of 0.3 μM of compound **4c** in the presence of 30 μM of compound **3**. The *p* values were determined using Turkey's multiple comparisons test. **p* < 0.05.

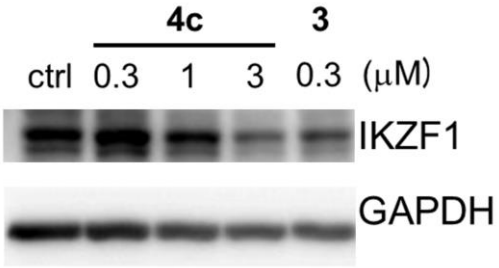



**Fig. S8.** Western blot detection of IKZF1 levels in Jurkat cells treated with compounds **4c** and **3** for 24 hours. The data indicate that the treatment with compound **4c** induced weak IKZF1 degradation relative to that compound **3**.

**Experimental Procedures.**

**Chemistry.** The chemical reagents and solvents used in this study were commercial products of the highest available purity. Reagents and solvents were purchased from Sigma Aldrich, FUJIFILM Wako Chemical Co., and TCI Tokyo Chemical Industry Co., Ltd. All air- and moisture-sensitive reactions were performed under an argon (Ar) atmosphere in dried glassware. Analytical thin layer chromatography was performed using 0.25 mm silica gel plate (Merck TLC Silica gel 60 F254). Organic solvents were dried over anhydrous sodium sulfate. NMR spectra were recorded on a JEOL ESC-400 spectrometer operating at 400 MHz ($^{1}$H) or 100 MHz ($^{13}$C), JEOL ECA-600 spectrometer operating at 150 MHz ($^{13}$C) or Bruker JMS 700 (Bruker Biospin, Switzerland) spectrometer operating at 700 MHz ($^{1}$H) or 175 MHz ($^{13}$C). $^{1}$H NMR and $^{13}$C NMR chemical shifts values are reported as δ (ppm) relative to the solvent peak or trimethylsilane (TMS) ($CDCl_3$: $^{1}$H NMR TMS 0.00, $^{13}$C NMR 77.16; DMSO-$d_6$: $^{1}$H NMR 2.50, $^{13}$C NMR 39.52; $CD_3OD$: $^{1}$H NMR 3.31, $^{13}$C NMR 49.00) and coupling constants are given in Hz. The purity of all tested compounds was > 95% as determined by HPLC using a Shimadzu UFLC (SPD-M20A UV detector, DGU-20A3R degassing unit, LC-20AD

solvent delivery unit and CBM-20A system) and a C18 column (Inert Sustain, 4.6*150, 5 μM), at a flow rate of 1 mL/min, with UV detection (λ = 220 or 254 nm). HPLC conditions: eluent A: $H_2O$ containing 0.1% TFA; eluent B: acetonitrile containing 0.1% TFA. Gradient: B: 0 to 20 min, 10–90%; 20 to 30 min, 90%; 30 to 40 min, 90–10%. Positive/negative LRMS ion mass spectra were recorded on a Bruker HCT-Plus. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-SX102A mass spectrometer or a Shimadzu LCMS-IT-TOF mass spectrometer.

***tert*-Butyl (8-aminooctyl)carbamate (7b)**. To a solution of 1, 8-diaminooctane (2.64 g, 18.3 mmol) in chloroform (180 mL) was added triethylamine (7.42 g, 73.2 mmol), and then a solution of $Boc_2O$ (2.00 g, 9.16 mmol) in chloroform (46 mL) was added dropwise. The reaction mixture was stirred at room temperature for 25 h, then concentrated in vacuo, and the residue was purified by flash column chromatography ($CHCl_3$/MeOH/triethylamine = 89 /10 /1) to obtain 1.22 g (54%) of **7b** as a colorless oil: $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 4.58 (1H, br), 3.10 (2H, brq, *J* = 6.5 Hz), 2.68 (2H, t, *J* = 7.0 Hz), 1.48–1.42 (12H, m), 1.30 (9H,

s); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 156.10, 79.09, 42.33, 40.69, 33.89, 30.16, 29.51, 29.36, 28.53, 26.90, 26.86; MS (ESI) *m/z* 245 ($MH^+$).

***tert*-Butyl (11-aminoundecyl)carbamate (7c)**. Compound **7c** was prepared from compound **6c** using the same procedure described for **7b**: colorless solid (29% yield); $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 4.53 (1H, br), 3.10 (2H, brq, *J* = 6.5 Hz), 2.68 (2H, t, *J* = 7.0 Hz), 1.48–1.42 (12H, m), 1.32–1.24 (15H, m; $^{13}$C NMR ($CDCl_3$, 175 MHz, δ; ppm) 156.13, 79.15, 50.84, 42.37, 40.76, 33.94, 30.18, 29.72, 29.67, 29.64, 29.61, 29.42, 28.56, 27.01, 26.93; MS (ESI) *m/z* 287 ($MH^+$).

***tert*-Butyl (5-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}pentyl)carbamate (8a).** To a mixture of *N*-(*tert*-butoxycarbonyl)-1, 5-diaminopentane (**7a**) (500 mg, 2.47 mmol) and DIPEA (1.17 g, 9.05 mmol) in DMF (5 mL) was added thalidomide 4-fluoride (**5**) (456 mg, 1.65 mmol). The reaction mixture was stirred under reflux for 14 h, then cooled to room temperature, diluted with water, and extracted with AcOEt. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration, concentration, and purification by flash column chromatography (*n*-hexane/AcOEt = 4/1 to 7/3) afforded 187 mg (25%) of **8a** as a green solid:

$^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.05 (1H, br), 7.50 (1H, dd, *J* = 8.4, 7.2 Hz), 7.09 (1H, d, *J* = 6.8 Hz), 6.88 (1H, d, *J* = 8.4 Hz), 6.23 (1H, brt, *J* = 5.4 Hz), 4.92 (1H, dd, *J* = 12.2, 5.4 Hz), 4.56 (1H, br), 3.27 (2H, q, *J* = 6.7 Hz), 3.14 (2H, q, *J* = 6.7 Hz), 2.92–2.73 (3H, m), 2.16–2.12 (1H, m), 1.69 (2H, quin, *J* = 7.3 Hz), 1.54 (2H, quin, *J* = 7.1 Hz), 1.47–1.40 (11H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.55, 169.59, 168.73, 167.73, 156.08, 146.95, 136.20, 132.53, 116.70, 111.48, 109.90, 79.24, 48.92, 42.59, 40.42, 31.48, 29.89, 28.95, 28.49, 24.21, 22.84; MS (ESI) *m/z* 459 ($MH^+$).

***tert*-Butyl (8-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}octyl)carbamate (8b)**.

Compound **8b** was prepared from compounds **5** and **7b** using the same procedure described for **8a**: green oil (33% yield); $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.05 (1H, br), 7.50 (1H, dd, *J* = 8.2, 7.4 Hz), 7.09 (1H, d, *J* = 7.6 Hz), 6.88 (1H, d, *J* = 8.0 Hz), 6.23 (1H, brt, *J* = 5.0 Hz), 4.92 (1H, dd, *J* = 12.2, 5.4 Hz), 4.53 (1H, br), 3.26 (2H, q, *J* = 6.4 Hz), 3.11 (2H, q, *J* = 6.7 Hz), 2.92–2.72 (3H, m), 2.16–2.12 (1H, m), 1.66 (2H, quin, *J* = 7.0 Hz), 1.40 (12H, s), 1.41–1.28 (9H, m); $^{13}$C NMR ($CDCl_3$, 175 MHz, δ; ppm) 171.05, 169.63, 168.43,

167.78, 147.13, 136.27, 132.59, 116.80, 111.52, 109.91, 79.24, 48.97, 42.73, 40.72, 31.57, 31.12, 30.15, 29.31, 29.28, 28.57, 26.95, 26.82, 22.96; MS (ESI) *m/z* 523 ($MNa^+$).

***tert*-Butyl (11-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecy)carbamate (8c).** Compound **8c** was prepared from compounds **5** and **7c** using the same procedure described for **8a**: green oil (27%); $^1H$ NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.23 (1H, br), 7.49 (1H, dd, *J* = 8.4, 7.2 Hz), 7.09 (1H, d, *J* = 6.8 Hz), 6.88 (1H, d, *J* = 8.0 Hz), 6.23 (1H, brt, *J* = 5.6 Hz), 4.92 (1H, dd, *J* = 12.0, 5.2 Hz), 4.52 (1H, br), 3.26 (2H, q, *J* = 6.8 Hz), 3.10 (2H, q, *J* = 6.3 Hz), 2.96–2.69 (3H, m), 2.16–2.10 (1H, m), 1.66 (2H, quin, *J* = 7.2 Hz), 1.44 (12H, s), 1.41–1.27 (15H, m); $^{13}C$ NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.17, 169.64, 168.50, 167.79, 156.12, 147.16, 136.25, 132.61, 116.79, 111.48, 109.911, 79.17, 53.57, 48.98, 42.80, 40.76, 31.56, 30.19, 29.62, 29.58, 29.39, 28.56, 27.06, 26.91, 22.95; MS (ESI) *m/z* 565 ($MNa^+$).

**4-[(5-Aminopentyl)amino]-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (9a).** To a solution of **8a** (187 mg, 0.407 mmol) in $CH_2Cl_2$ (3.5 mL) in an ice bath under an Ar atmosphere was added trifluoroacetic acid (3.5 mL) in a dropwise fashion. The reaction mixture was stirred in an ice bath under Ar for 1 h, then diluted

with MeOH and concentrated in vacuo to obtain crude **9a**, which was used for the next step without further purification.

**3-(Prop-2-yn-1-ylthio)benzoic acid (12).** To a solution of 3-mercaptobenzoic acid (**10**) (1.04 g, 6.75 mmol) and triethylamine (1.81 g, 17.9 mmol) in THF (20 mL) in an ice bath under an Ar atmosphere was added propargyl bromide (1.55 g, 13.1 mmol) in a dropwise fashion. The reaction mixture was stirred at room temperature for 5 h, then poured into saturated aqueous $NaHCO_3$ solution and washed with AcOEt. The aqueous phase was acidified by adding 1 N aqueous HCl and extracted with AcOEt. The organic layer was dried over $Na_2SO_4$ and concentrated in vacuo to give 1.07 g (85%) of **12** as a yellow solid: $^1$H NMR ($CD_3OD$, 400 MHz, δ; ppm) 8.07 (1H, t, *J* = 1.6 Hz), 7.88 (1H, dt, *J* = 8.0, 1.4 Hz), 7.65 (1H, dq, *J* = 7.6, 0.9 Hz), 7.44 (1H, t, *J* = 7.8 Hz), 3.74 (2H, d, *J* = 2.8 Hz), 2.59 (1H, t, *J* = 2.6 Hz); $^{13}$C NMR ($CD_3OD$, 100 MHz, δ; ppm) 169.13, 137.51, 134.86, 132.74, 131.531, 130.082, 128.84, 80.493, 72.84, 22.43; MS (ESI) m/z 193 ($MH^+$).

**4-(Prop-2-yn-1-ylthio)benzoic acid (13).** Compound **13** was prepared from compound **11** using the same procedure described for **12**: yellow solid (89% yield); $^1$H NMR ($CD_3OD$, 400 MHz, δ; ppm) 7.95 (2H, dt, *J* =

8.8, 2.0 Hz), 7.45 (2H, dt, *J* = 8.8, 1.8 Hz), 3.81 (2H, d, *J* = 2.8 Hz), 2.61 (1H, t, *J* = 2.8 Hz); $^{13}$C NMR ($CD_3OD$, 100 MHz, δ; ppm) 169.47, 143.93, 131.14, 129.12, 127.99, 80.20, 72.82, 21.01; MS (ESI) *m/z* 193 ($MH^+$).

***N*-(5-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}pentyl)-3-(prop-2-yn-1-ylthio) benzamide (14a).** To a solution of crude **9a** and **12** (163 mg, 0.848 mmol) in DMF (6 mL) was added diisopropylethylamine (574 µL, 3.29 mmol) followed by HATU (636 mg, 1.67 mmol). The reaction mixture was stirred at room temperature for 16 h, then diluted with water and extracted with AcOEt. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration, concentration, and purification by flash column chromatography (*n*-hexane/AcOEt = 4/1 to 1/4) gave 205 mg (44%, 2 steps from **8a**) of **14a** as a yellow oil: $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.65 (1H, s), 7.83 (1H, brt, *J* = 1.6 Hz), 7.61 (1H, d, *J* = 7.6 Hz), 7.54 (1H, dt, *J* = 8.0, 1.4 Hz), 7.47 (1H, dd, *J* = 8.2, 3.4 Hz), 7.36 (1H, t, *J* = 7.8 Hz), 7.05 (1H, d, *J* = 7.2 Hz), 6.86 (1H, d, *J* = 8.8 Hz), 6.52 (1H, brt, *J* = 5.4 Hz), 6.23 (1H, brt, *J* = 5.6 Hz), 4.91 (1H, dd, *J* = 12.2, 5.4 Hz ), 3.64 (2H, d, *J* = 2.4 Hz), 3.43 (2H, q, *J* = 6.7 Hz), 3.26 (2H, q, *J* = 6.4 Hz), 2.87–2.68 (3H, m), 2.26 (1H, t, *J* = 2.6 Hz),

2.15–2.08 (1H, m), 1.67 (4H, heptet, $J$ = 7.6 Hz), 1.51–1.44 (2H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.56, 169.63, 168.84, 167.74, 167.05, 146.96, 136.27, 135.88, 135.53, 132.46, 129.23, 128.29, 125.42. 116.79, 111.54, 109.88, 79.53, 72.06, 50.87, 48.95, 42.51, 39.99, 31.49, 29.39, 28.87, 24.35, 22.84, 22.35; MS (ESI) *m/z* 555 ($MNa^+$).

***N*-(8-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}octyl)-3-(prop-2-yn-1-ylthio) benzamide (14b).** Compound **14b** was prepared from compounds **8b** and **12** using the same procedure described for **9a** and **14a**: yellow solid (51% yield); $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.50 (1H, s), 7.83 (1H, s), 7.62 (1H, d, $J$ = 8.0 Hz), 7.55 (1H, d, $J$ = 8.0 Hz), 7.48 (1H, t, $J$ = 7.8 Hz), 7.37 (1H, t, $J$ = 7.8 Hz), 7.07 (1H, d, $J$ = 6.8 Hz), 6.87 (1H, d, $J$ = 8.0 Hz), 6.32 (1H, brt, $J$ = 5.4 Hz), 6.23 (1H, t, $J$ = 5.4 Hz), 4.91 (1H, dd, $J$ = 12.0, 5.6 Hz), 3.64 (2H, d, $J$ = 2.8 Hz), 3.43 (2H, q, $J$ = 6.5 Hz), 3.25 (2H, q, $J$ = 6.5 Hz), 2.89–2.68 (3H, m), 2.25 (1H, t, $J$ = 2.4 Hz), 2.14–2.10 (1H, m), 1.65 (2H, quin, $J$ = 7.2 Hz), 1.60 (2H, quin, $J$ = 7.3 Hz), 1.40–1.25 (8H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.38, 169.62, 168.65, 167.76, 166.95, 147.08, 136.22,

135.88, 135.70, 132.51, 129.24, 128.30, 125.41, 116.78, 111.45, 109.85, 79.55, 72.03, 50.90, 48.93, 42.64, 40.23, 40.10, 31.49, 29.64, 29.17, 26.88, 26.84, 22.89, 22.39; HRMS (ESI) *m/z* 597 ($MNa^+$).

***N*-(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)-3-(prop-2-yn-1-ylthio)benz amide (14c).** Compound **14c** was prepared from compounds **8c** and **12** using the same procedure described for **9a** and **14a**: yellow oil (32%, 2 steps from **8c**); $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.48 (1H, s), 7.83 (1H, t, *J* = 1.6 Hz), 7.62 (1H, dt, *J* = 7.6, 1.4 Hz), 7.54 (1H, dt, *J* = 8.4, 1.5 Hz), 7.48 (1H, dd, *J* = 8.4, 7.2 Hz), 7.37 (1H, t, *J* = 7.8 Hz), 7.07 (1H, d, *J* = 6.8 Hz), 6.87 (1H, d, *J* = 8.0 Hz), 6.30 (1H, brt, *J* = 5.4 Hz), 6.23 (1H, t, *J* = 5.6 Hz), 4.92 (1H, dd, *J* = 11.8, 5.4 Hz), 3.64 (2H, d, *J* = 2.4 Hz), 3.43 (2H, q, *J* = 6.8 Hz), 3.25 (2H, q, *J* = 6.5 Hz), 2.89–2.68 (3H, m), 2.25 (1H, t, *J* = 2.6 Hz), 2.13–2.09 (1H, m), 1.65 (2H, quin, *J* = 7.1 Hz), 1.60 2H, quin, *J* = 7.5 Hz), 1.42–1.28 (14H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.36, 169.59, 168.62, 167.76, 166.91, 147.10, 136.19, 135.87, 135.75, 132.51, 129.22, 128.32, 125.42, 116.76, 111.40, 109.84, 79.55, 72.02, 48.93, 42.73, 40.27, 31.50, 29.70, 29.54, 29.50, 29.34, 29.28, 27.04, 26.98, 22.88, 22.41; HRMS (ESI) *m/z* 639 ($MNa^+$).

***N*-(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)-4-(prop-2-yn-1-ylthio) benzamide (14d).** Compound **14d** was prepared from compounds **9c** and **13** using the same procedure described for **14a**: yellow solid (45%, 2 steps from **8c**); $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.43 (1H, s), 7.71 (2H, dt, *J* = 8.4, 2.1 Hz), , 7.48 (1H, dd, *J* = 8.2, 7.4 Hz), 7.41 (2H, dt, *J* = 8.4, 2.1 Hz), 7.07 (1H, d, *J* = 6.8 Hz), 6.88 (1H, d, *J* = 8.8 Hz), 6.26–6.21 (2H, m), 4.91 (1H, dd, *J* = 12.2, 5.4 Hz ), 3.66 (2H, d, *J* = 2.8 Hz), 3.43 (2H, q, *J* = 6.7 Hz), 3.25 (2H, q, *J* = 6.5 Hz), 2.90–2.68 (3H, m), 2.26 (1H, t, *J* = 2.4 Hz), 2.14–2.10 (1H, m), 1.65 (2H, q, *J* = 7.3 Hz), 1.60 (2H, q, *J* = 7.6 Hz), 1.40–1.28 (14H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.33, 169.60, 168.60, 167.77, 166.94, 147.10, 139.64, 136.20, 132.61, 128.20, 127.52, 116.76, 111.41, 109.82, 79.28, 71.95, 48.93, 42.73, 40.22, 31.50, 29.75, 29.55, 29.51, 29.35, 29.28, 27.06, 26.98, 22.88, 21.45; HRMS (ESI) *m/z* 639 ($MNa^+$).

**Ethyl 3-azidobenzoate (16).** To a solution of 3-aminobenzoic ethyl ester (1.06 g, 6.42 mmol) in MeCN (12 mL) in an ice bath were added *t*-BuONO (0.919 g, 8.91 mmol) and trimethylsilyl azide (1.66 g, 14.4 mmol) in a dropwise fashion. The reaction mixture was stirred at room temperature for 1 h, then concentrated in vacuo.

The residue was purified by flash column chromatography (*n*-hexane/AcOEt = 9/1) to obtain 961 mg (78%) of **16** as a yellow oil: $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.81 (1H, dt, *J* = 8.0, 1.4 Hz), 7.70 (1H, t, *J* = 2.0 Hz), 7.42 (1H, t, *J* = 8.0 Hz), 7.19 (1H, ddd, *J* = 8.2, 2.4, 1.0 Hz), 4.39 (2H, q, *J* = 7.1 Hz), 1.40 (3H, t, *J* = 7.0 Hz); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 165.78, 140.61, 132.38, 129.86, 126.06, 123.37, 120.03, 61.44, 14.38; MS (ESI) *m/z* 192 ($MH^+$).

**3-Azidobenzoic acid (17).** To a solution of **16** (1.07 g, 5.60 mmol) in THF (5 mL) and MeOH (5 mL) at room temperature was added 4 N aqueous NaOH (5.25 mL, 21.0 mmol). The reaction mixture was stirred at room temperature for 2.5 h, then cooled in an ice bath, acidified by adding 1 N aqueous HCl, and extracted with AcOEt. The organic layer was dried over $Na_2SO_4$. Filtration and concentration in vacuo afforded 858 mg (94%) of **17** as a yellow solid: $^{1}$H NMR ($CD_3OD$, 400 MHz, δ; ppm) 7.81 (1H, dt, *J* = 7.6, 1.3 Hz), 7.67 (1H, t, *J* = 1.8 Hz), 7.49 (1H, t, *J* = 8.0 Hz), 7.29 (1H, dd, *J* = 7.8, 2.2 Hz); $^{13}$C NMR ($CD_3OD$, 100 MHz, δ; ppm) 168.73, 142.00, 133.89, 131.41, 127.20, 124.41, 120.87; MS (ESI) *m/z* 164 ($MH^+$).

**3-Azido-*N*-[(tetrahydro-2*H*-pyran-2-yl)oxy]benzamide (18).** To a mixture of **17** (784 mg, 4.81 mmol), EDCI (2.79 g, 14.6 mmol) and HOBt·$H_2O$ (1.97 g, 14.6 mmol) in DMF (15 mL) at room temperature was added *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.71 g, 14.6 mmol). The reaction mixture was stirred at 25°C for 23 h, then poured into saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, and dried over $Na_2SO_4$. Filtration, concentration, and purification by flash column chromatography (*n*-hexane/AcOEt = 4/1) gave 1.19 g (87%) of **18** as a yellow oil: $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 9.03 (1H, s), 7.49 (1H, dt, *J* = 7.6, 1.4 Hz), 7.44 (1H, t, *J* = 1.8 Hz), 7.41(1H, t, *J* = 8.0 Hz), 7.17 (1H, ddd, *J* = 8.0, 1.2, 0.9 Hz), 4.01 (1H td, *J* = 10.4, 2.8 Hz), 3.68–3.64 (1H, m), 1.91–1.84 (3H, m), 1.71–1.58 (3H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 141.04, 133.88, 130.25, 123.45, 122.58, 118.19, 102.93, 62.88, 28.15, 25.11, 18.72; MS (ESI) *m/z* 263 ($MH^+$).

***N*-(5-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}pentyl)-3-({[1-(3-{[(tetrahydro-2*H*-pyran-2-yl)oxy]carbamoyl}phenyl)-1*H*-1,2,3-triazol-4-yl]methyl}thio)benzamide (19a).** To a mixture of **14a** (205 mg, 0.385 mmol), **18** (134 mg, 0.512 mmol), TBTA (22.2 mg, 0.0418 mmol) in THF (5 mL) were added

$H_2O$ (2 mL), $Cu_2SO_4 \cdot 5H_2O$ (11.1 mg, 0.0444 mmol) and sodium ascorbate (41.8 mg, 0.211 mmol). The reaction mixture was stirred at room temperature for 19.5 h, then diluted with water and extracted with AcOEt. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration, concentration, and purification by flash column chromatography ($CH_2Cl_2$/MeOH = 99/1 to 19/1) gave 239 mg (77%) of **19a** as a yellow solid: $^1$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.87 (1H, br), 9.42 (1H, s), 7.94 (1H, s), 7.86 (1H, s), 7.78 (2H, t, *J* = 9.4 Hz), 7.71 (1H, s), 7.57 (1H, d, *J* = 7.6 Hz), 7.40–7.36 (3H, m), 7.23 (2H, t, *J* = 7.8 Hz), 6.95 (1H, d, *J* = 6.8 Hz), 6.76 (1H, d, *J* = 8.4 Hz), 6.11 (1H, br), 5.10 (1H, s,), 4.94–4.90 (1H, m), 4.18 (2H, s), 4.05 (1H, brt, *J* = 8.0 Hz), 3.563 (1H, d, *J* = 10.4 Hz), 3.34 (2H, d, *J* = 5.2 Hz); 3.14 (2H, d, *J* = 5.2 Hz), 2.79–2.71 (3H, m), 2.13–2.00 (1H, m), 1.88–1.71 (3H, m), 1.69–1.45 (7H, m), 1.40–1.30 (2H, m); $^{13}$C NMR ($CDCl_3$, 400 MHz, δ; ppm) 172.17, 169.56, 169.46, 167.72, 167.36, 164.38, 146.78, 145.38, 136.63, 135.15, 135.83, 135.37, 133.66, 132.29, 130.02, 129.19, 128.23, 127.89, 125.44, 123.40, 120.84, 118.81, 116.76, 111.30, 109.58, 102.71, 62.71, 53.55, 48.88, 42.39, 39.94, 31.46, 29.06, 28.74, 28.33, 28.21, 25.02, 24.23, 22.74, 18.77; MS (ESI) *m/z* 817 ($MNa^+$).

***N*-(8-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}octyl)-3-{[(1-(3-{[(tetrahydro-2*H*-pyr an-2-yl)oxy]carbamoyl}phenyl)-1*H*-1,2,3-triazol-4-yl)methyl]thio)benzamide (19b).** Compound **19b** was prepared from compounds **14b** and **18** using the same procedure described for **19a**: yellow solid (55% yield); $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.65 (1H, brd, *J* = 13.2 Hz), 9.00 (1H, d, *J* = 9.2 Hz), 7.92 (1H, s), 7.85 (2H, d, *J* = 7.6 Hz), 7.80 (1H, d, *J* = 3.6 Hz), 7.69 (1H, s), 7.59 (1H, d, *J* = 7.2 Hz), 7.49–7.43 (3H, m), 7.29 (1H, t, *J* = 7.6 Hz), 7.04 (1H, d, *J* = 6.8 Hz), 6.85 (1H, d, *J* = 8.4 Hz), 6.74 (1H, d, *J* = 2.8 Hz ), 6.19 (1H, t, *J* = 5.2 Hz), 5.12 (1H, s), 4.94 (1H, br), 4.23 (2H, s), 4.13–4.07 (1H, m), 3.60 (1H, d, *J* = 10.4 Hz), 3.35 (2H, q, *J* = 6.3 Hz), 3.22 (2H, q, *J* = 6.1 Hz), 2.87–2.73 (3H, m), 2.11 (1H, brt, *J* = 6 Hz), 1.91–1.80 (3H, m), 1.67–1.48 (7H, m), 1.37–1.21 (8H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.76, 169.63, 169.25, 169.19, 167.77, 167.33, 164.35, 147.04, 145.51, 136.81, 136.24, 135.73, 135.56, 133.80, 132.95, 132.49, 130.17, 129.37, 128.80, 128.70, 128.04, 125.53, 123.71, 120.84, 118.91, 116.82, 111.43, 109.77, 102.95, 102.88, 62.96, 62.89, 48.96, 42.60, 40.30, 31.53, 29.41, 29.07, 28.64, 28.31, 26.81, 26.76, 25.10, 22.88, 18.97; MS (ESI) *m/z* 859 ($MNa^+$).

***N*-(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)-3-({[1-(3-{[(tetrahydro-2*H*-pyran-2-yl)oxy]carbamoyl}phenyl)-1*H*-1,2,3-triazol-4-yl]methyl}thio)benzamide (19c).** Compound **19c** was prepared from compounds **14c** and **18** using the same procedure described for **19a**: yellow solid (100% yield); $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.74 (1H, br), 9.02 (1H, s), 7.94 (1H, s), 7.86 (2H, d, *J* = 6.8 Hz), 7.83 (1H, d, *J* = 4.4 Hz), 7.68 (1H, s), 7.57 (1H, d, *J* = 8.0 Hz), 7.46 (3H, quin, *J* = 8.0 Hz), 7.28 (1H, t, *J* = 7.6 Hz), 7.03 (1H, d, *J* = 7.6 Hz), 6.85 (1H, d, *J* = 8.8 Hz), 6.77 (1H, brt, *J* = 5.4 Hz), 6.20 (1H, t, *J* = 5.6 Hz), 5.12 (1H, s), 4.96–4.91 (1H, m), 4.22 (2H,s), 4.08 (1H, brt, *J* = 10.6 Hz), 3.60 (1H, d, *J* = 10.8 Hz), 3.35 (2H, q, *J* = 6.5 Hz), 3.23 (2H, q, *J* = 6.4 Hz), 2.86–2.70 (3H, m), 2.12–2.09 (1H, m), 1.90–1.79 (3H, m), 1.66–1.49 (7H, m), 1.38–1.19 (14H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.85, 169.55, 169.07, 167.77, 167.31, 164.42, 147.03, 145.39, 136.74, 136.16, 135.79, 135.56, 133.79, 132.84, 132.46, 130.13, 129.30, 128.76, 128.06, 125.41, 123.62, 120.81, 118.86, 116.76, 111.32, 109.72, 102.77, 62.77, 48.91, 42.65, 40.33, 31.49, 29.49, 29.46, 29.42, 29.29, 29.26, 29.18, 28.64, 28.25, 26.98, 26.89, 25.07, 22.83, 18.82; MS (ESI) *m/z* 901 ($MNa^+$).

**3-{4-[({4-[(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)carbamoyl]phenyl}thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-[(tetrahydro-2*H*-pyran-2-yl)oxy]benzamide (19d)** Compound **19d** was prepared from compounds **14d** and **18** using the same procedure described for **19a**: yellow solid (81% yield); $^{1}$H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.16 (1H, br), 8.77 (1H, s), 8.02 (1H, s), 7.91 (1H, s), 7.83 (2H, dd, *J* = 12.8, 8.0 Hz), 7.61 (2H, d, *J* = 8.8 Hz), 7.49 (1H, t, *J* = 8.0 Hz), 7.47 (1H, t, *J* = 8.0 Hz), 7.28 (2H, d, *J* = 7.2 Hz), 7.04 (1H, d, *J* = 7.6 Hz), 6.86 (1H, d, *J* = 8.8 Hz), 6.51 (1H, brt, *J* = 5.6 Hz), 6.20 (1H, brt, *J* = 5.4 Hz), 5.09 (1H, s), 4.92 (1H, dd, 7.49, *J* = 11.8, 5.4 Hz), 4.28 (2H,s), 4.04 (1H, t, *J* = 9.8 Hz), 3.60 (1H, d, *J* = 11.2 Hz), 3.38 (2H, q, *J* = 6.7 Hz), 3.23 (2H, q, *J* = 6.4 Hz), 2.88–2.72 (3H, m), 2.12–2.09 (1H, m), 1.98–1.77 (3H, m), 1.67–1.53 (7H, m), 1.39–1.24 (14H, m); $^{13}$C NMR ($CDCl_3$, 100 MHz, δ; ppm) 171.74, 169.61, 168.99, 167.81, 167.19, 147.11, 145.68, 139.86, 136.94, 136.23, 133.86, 132.53, 132.48, 130.22, 127.95, 127.66, 123.57, 120.63, 119.08, 116.81, 111.40, 109.81, 102.98, 62.95, 48.97, 42.72, 40.26, 31.54, 29.66, 29.49, 29.44, 29.29, 29.25, 28.26, 27.70, 27.03, 26.94, 25.08, 22.90, 18.89; MS (ESI) *m/z* 901 ($MNa^+$).

***N*-(5-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}pentyl)-3-[({1-[3-(hydroxycarbamoyl) phenyl]-1*H*-1,2,3-triazol-4-yl}methyl)thio]benzamide (4a).** To a solution of **19a** (239 mg, 0.301 mmol) in MeOH (16 mL) and $CH_2Cl_2$ (6 mL) was added *p*-toluenesulfonic acid monohydrate (6.70 mg, 0.0352 mmol). The reaction mixture was stirred at room temperature for 17.5 h, then concentrated in vacuo. The residue was purified by flash column chromatography ($CH_2Cl_2$/MeOH = 19/1) to obtain 167 mg (82%) of **4a** as a yellow solid; $^1$H NMR (DMSO-$d_6$, 700 MHz, δ; ppm) 11.43 (1H, br), 11.12 (1H, s), 9.26 (1H, br), 8.76 (1H, s), 8.52 (1H, t, *J* = 5.6 Hz), 8.22 (1H, s), 8.01 (1H, dd, *J* = 8.4, 1.4 Hz), 7.84 (1H, s), 7.83 (1H, s), 7.67 (1H, t, *J* = 8.1 Hz ), 7.65 (1H, d, *J* = 7.7 Hz), 7.58–7.56 (2H, m), 7.42 (1H, t, *J* = 7.7 Hz), 7.10 (1H, d, *J* = 8.4 Hz), 7.01 (1H, d, *J* = 7.0 Hz), 6.55 (1H, brt, *J* = 6.0 Hz), 5.04 (1H, dd, *J* = 11.6, 5.6 Hz), 4.46 (2H, s), 3.30 (2H, q, *J* = 6.8 Hz), 3.26 (2H, q, *J* = 6.5 Hz), 2.88 (1H, ddd, *J* = 18.2, 13.0, 5.3 Hz ), 2.59 (1H, dt, *J* = 18.2, 3.3 Hz), 2.53–2.47 (1H, m), 2.03–2.01 (1H, m), 1.60 (2H, quin, *J* = 7.4 Hz), 1.56 (2H, quin, *J* = 7.4 Hz), 1.38 (2H, quin, *J* = 7.5 Hz); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, δ; ppm) 172.81, 170.10, 168.94, 167.31, 165.47, 162.87, 146.41, 144.76, 136.52, 136.27, 135.90, 135.41, 134.40, 132.19, 130.62, 130.16, 129.02, 126.95, 124.96, 122.48, 121.66,

118.43, 117.20, 110.38, 109.01, 48.54, 41.80, 40.06, 30.99, 28.78, 28.43, 27.19, 23.80, 22.15; HRMS calcd for $C_{35}H_{34}N_8NaO_7S^+$ 733.2169, found 733.2164; HPLC $R_t$: 14.14 min, 99% purity.

***N*-(8-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}octyl)-3-[({1-[3-(hydroxycarbamoyl)phenyl]-1*H*-1,2,3-triazol-4-yl}methyl)thio]benzamide (4b).** Compound **4b** was prepared from compound **19b** using the same procedure described for **4a**: yellow solid (87%); $^1$H NMR (DMSO-$d_6$, 700 MHz, δ; ppm) 11.12 (1H, br), 8.75 (1H, s), 8.50 (1H, brt, *J* = 5.6 Hz), , 8.22 (1H, s), 8.00 (1H, d, *J* = 7.7 Hz), 7.84 (1H, s), 7.83 (1H, s), 7.66 (2H, q, *J* = 7.2 Hz), 7.57 (2H, d, *J* = 7.7 Hz), 7.42 (1H, t, *J* = 7.7 Hz), 7.08 (1H, d, *J* = 9.1 Hz), 7.02 (1H, d, *J* = 7.0 Hz), 6.54 (1H, brt, *J* = 6.0 Hz), 5.05 (1H, dd, *J* = 13.0, 5.3 Hz), 4.45 (2H, s), 3.28 (2H, q, *J* = 6.8 Hz), 3.23 (2H, q, *J* = 6.5 Hz), 2.88 (1H, ddd, *J* = 18.2, 12.6, 5.3 Hz), 2.60–2.57 (1H, m), 2.55–2.48 (1H, m), 2.04–2.01 (1H, m), 1.56 (2H, quin, *J* = 7.2 Hz), 1.50 (2H, quin, *J* = 6.3 Hz), 1.33–1.24 (8H, m); $^{13}$C NMR (DMSO-$d_6$, 150 MHz, δ; ppm) 172.80, 170.09, 168.95, 167.30, 165.40, 162.79, 146.42, 144.75, 136.50, 136.26, 135.88, 135.42, 134.42, 132.18, 130.62, 130.14, 129.01,126.95, 126.87, 124.95, 122.42, 121.64,

118.38, 117.16, 110.35, 108.99, 48.53, 41.83, 40.05, 30.97, 29.00, 28.69, 27.19, 26.41, 26.28, 22.14; HRMS calcd for $C_{38}H_{40}N_8NaO_7S^+$ 775.2638, found 775.2634; HPLC $R_t$, 16.42 min, 99% purity.

***N*-(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)-3-[({1-[3-(hydroxycarbamoyl)phenyl]-1*H*-1,2,3-triazol-4-yl}methyl)thio]benzamide (4c).** Compound **4c** was prepared from compound **19c** using the same procedure described for **4a**: yellow solid (66% yield).; $^1$H NMR (DMSO-$d_6$, 700 MHz, δ; ppm) 11.42 (1H, s), 11.12 (1H, s), 9.24 (1H, s), 8.76 (1H, s), 8.49 (1H, brt, *J* = 5.6 Hz), 8.23 (1H, s), 8.01 (1H, dd, *J* = 8.4, 1.4 Hz), 7.85 (1H, s), 7.83 (1H, d, *J* = 1.4 Hz), 7.67 (1H, t, *J* = 8.1 Hz), 7.65 (1H, dt, *J* = 7.7, 1.2 Hz), 7.59–7.56 (2H, m), 7.42 (1H, t, *J* = 7.7 Hz), 7.09 (1H, d, *J* = 8.4 Hz), 7.02 (1H, d, *J* = 7.0 Hz), 6.53 (1H, brt, *J* = 6.0 Hz), 5.06 (1H, dd, *J* = 13.3, 5.6 Hz), 4.46 (2H, s), 3.28 (2H, q, *J* = 6.8 Hz), 3.23 (2H, q, *J* = 6.5 Hz), 2.88 (1H, ddd, *J* = 17.5, 12.6, 5.3 Hz), 2.59 (1H, d, *J* = 17.5 Hz), 2.54–2.48 (1H, m), 2.04–2.01 (1H, m), 1.55 (2H, quin, *J* = 7.2 Hz), 1.49 (2H, quin, *J* = 6.7 Hz), 1.32–1.25 (14H, m); $^{13}$C NMR (DMSO-$d_6$, 175 MHz, δ; ppm) 172.90, 170.18, 168.98, 167.35, 165.41, 162.89, 146.44, 144.79, 136.55, 136.33, 135.93, 135.43, 134.40, 132.22, 130.59, 130.22, 129.07, 126.93, 126.89, 124.96, 122.51, 121.71, 118.44, 117.23, 110.41,

108.99, 48.55, 41.84, 40.02, 31.02, 29.07, 29.05, 29.00, 28.81, 28.71, 27.14, 26.51, 26.36, 22.18; HRMS calcd for $C_{41}H_{46}N_8NaO_7S^+$ 817.3108, found 817.3101; HPLC $R_t$ 18.84 min, 96% purity.

**3-{4-[({4-[(11-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}undecyl)carbamoyl]phenyl}thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-hydroxybenzamide (4d).** Compound **4d** was prepared from compound **19d** using the same procedure described for **4a**: yellow solid (60% yield); $^1$H NMR (DMSO-$d_6$, 700 MHz, δ; ppm) 11.42 (1H, br), 11.12 (1H, s), 9.25 (1H, s), 8.82 (1H, s), 8.41 (1H, brt, *J* = 5.6 Hz), 8.23 (1H, s), 8.02 (1H, d, *J* = 8.4 Hz), 7.84 (1H, d, *J* = 8.4 Hz), 7.78 (2H, dt, *J* = 8.4, 1.6 Hz), 7.67 (1H, t, *J* = 7.7 Hz), 7.58 (1H, dd, *J* = 8.4, 7.7 Hz), 7.47 (2H, dt, *J* = 9.1, 2.1 Hz), 7.09 (1H, d, *J* = 8.4 Hz), 7.02 (1H, d, *J* = 7.0 Hz), 6.54 (1H, brt, *J* = 5.6 Hz), 5.06 (1H, dd, *J* = 12.6, 5.6 Hz), 4.48 (2H, s), 3.28 (2H, q, *J* = 6.8 Hz), 3.21 (2H, q, *J* = 6.5 Hz), 2.88 (1H, ddd, *J* = 18.2, 13.0, 5.3 Hz), 2.59 (1H, d, *J* = 18.2 Hz), 2.54–2.47 (1H, m), 2.04–2.00 (1H, m), 1.56 (2H, quin, *J* = 7.2 Hz), 1.49 (2H, quin, *J* = 6.7 Hz), 1.33–1.25 (14H, m); $^{13}$C NMR (DMSO-$d_6$, 175 MHz, δ; ppm) 172.91, 170.19, 168.98, 167.36, 165.43, 162.90, 146.45, 144.78, 139.68, 136.53, 136.33, 134.42, 132.23, 131.69, 130.24, 127.78, 126.94, 126.60, 122.51, 121.75, 118.41, 117.24, 110.40, 108.99, 48.55, 41.84,

40.01, 31.01, 29.13, 29.04, 29.02, 29.00, 28.81, 28.70, 26.52, 26.35, 26.19, 22.18; HRMS calcd for $C_{41}H_{46}N_8NaO_7S^+$ 817.3108, found 817.3099; HPLC $R_t$ 18.67 min, 95% purity.

**Docking study.** The X-ray structure of humanized mutant HDAC8 (PDB code 6HSG) was used as a model for docking. Protein preparation, receptor grid generation and ligand docking were performed using the docking software Glide. Water molecules were removed before the docking calculation. Compound **2** was docked into the active site of the prepared protein as described above. The standard precision mode of Glide was used to determine favorable binding poses, which allowed the ligand conformation to be flexibly explored while holding the protein as a rigid structure during docking.

**Western blotting.** T-cell leukemia Jurkat cells were provided by RIKEN BRC through the National Bio-Resource Project of MEXT, Japan and were cultured in RPMI1640 containing 10% heat-inactivated fetal bovine serum (FBS), penicillin, and streptomycin mixture at 37 °C in a humidified atmosphere of 5% $CO_2$ in air. The cells (350,000–6,000,000 cells/well) were treated with the test compounds at the indicated concentrations in the culture medium. The cells were collected and extracted with SDS buffer, and the protein

concentrations of the lysates were determined using a BCA protein assay. Equivalent amounts of protein from each lysate were resolved in 8% SDS-polyacrylamide gels for HDAC6 or 12.5% or 5–20% SDS-polyacrylamide gels for the other proteins, and transferred onto PVDF membranes. After blocking, the transblotted membranes were probed with primary antibodies. The probed membranes were washed three or four times with TBS-T, incubated with HRP-linked secondary antibody, and again washed three or four times with TBS-T. The immunoblots were visualized by enhanced chemiluminescence with Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, P90718). The conditions of the blocking and antibody reactions are included in the table listing the reagents and antibodies (see page S39). The concentration of the test compound that results in 50% degradation ($DC_{50}$) was determined by plotting log[Inh] versus the logit function of % degradation.

**HDAC8 assay.** The HDAC8 activity assay was performed using an HDAC8 deacetylase fluorometric assay kit (Cyclex, CY-1158V2), according to the supplier's instructions. 5 μL of recombinant HDAC8 solution was added to a mixture of 5 μL of substrate solution (0.2 mM), 5 μL of assay buffer, 5 μL of developer, 5 μL of

test compound solution (10%DMSO in assay buffer), and 25 μL of distilled water in a 96-well plate. After 30 min incubation, the fluorescence of the wells was measured on an Ensight™ microplate reader (PerkinElmer, Inc.) with excitation set at 360 nm and emission detection set at 460 nm. The % inhibition was calculated from the corrected fluorescence intensities relative to those of control wells. The concentration of the test compound that results in 50% inhibition ($IC_{50}$) was determined by plotting log[Inh] versus the logit function of % inhibition. $IC_{50}$ values were determined by regression analysis of the concentration/inhibition data.

**HDAC1, HDAC2, and HDAC6 assays.** The HDAC1, HDAC2, and HDAC6 activity assays were performed using HDAC1 (Signalchem, H83-30G), HDAC2 (Signalchem, H84-30G), HDAC6 (BPS, 50006), HDAC assay buffer (BPS, 50031), FLUOR DE LYS® deacetylase substrate based on residues 379–382 of p53 (Arg-His-Lys-Lys(Ac), Enzo, BML-KI177), and HDAC developer (BPS, 50030). For HDAC1 and HDAC2 assays, 20 μL of substrate solution (12.5 μM) was added to a mixture of 20 μL of HDAC1 or HDAC2 solution (1.0 ng/μL), and the mixtures were diluted with the HDAC assay buffer and 10 μL of test compound solution (5% DMSO in assay buffer) in a 96-well plate. The resulting mixture was incubated at

30 °C for 30 min. For HDAC6 assay 2.5 μL of HDAC6 solution (21.0 ng/μL) diluted with the HDAC assay buffer was added to a mixture of 2.5 μL of substrate solution (200 μM), 2.5 μL of test compound solution (10%DMSO in assay buffer), and 2.5 μL of 0.1% BSA aqueous solution in a 384-well plate, and the resulting mixture was incubated at 30 °C for 15 min. Then, the fluorescence of the wells was measured on an Ensight™ microplate reader (PerkinElmer, Inc.) with excitation set at 360 nm and emission detection set at 460 nm. The % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells.

**Stability test.** Compound **4c** (2 mM in DMSO) was diluted 1:400 in HDAC assay buffer or compound **4c** (125 μM in DMSO) was diluted 1:24 in phosphate buffered saline (PBS, pH 7.4) to afford solutions of 5 μM compound **4c** in 300 μL of HDAC assay buffer (0.25% DMSO) or PBS (4% DMSO), respectively. After incubated at 25 °C (in HDAC assay buffer) or 37 °C (in PBS) for 4 or 24 hours, the solutions were analyzed by the above-mentioned HPLC systems. The residual concentrations of compound **4c** were calculated using

linear calibration curves created by analysis of authentic samples of **4c** (0.0625, 0.625, 1.25, 2.5, 5, and 7.5 μM for HDAC assay buffer or 0.625, 1.25, 2.5, 5, and 7.5 μM for PBS).

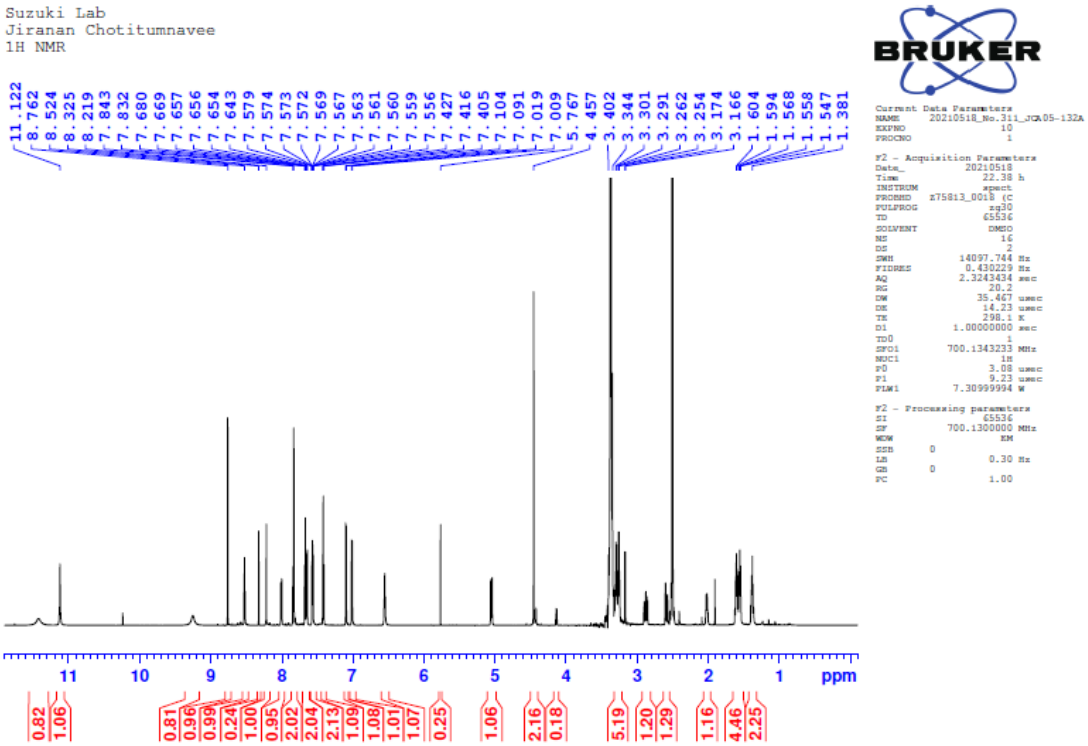
**Cell Growth Assay.** Jurkat cells were placed in 96-well plates at the initial density of 1,500 cells/well (50 μL/well) and incubated (37 °C). Test compound solutions of varying concentrations in RPMI1640 were added (50 μL/well) and incubation was continued (37 °C; 3 days; humidified atmosphere of 5% $CO_2$ in air), then 10 μL of alamar Blue™ was added to each well. The cells were further incubated (37 °C, 3 hr), and the fluorescence in each well was measured on a Ensight™ multilabel reader (PerkinElmer, Inc.; excitation: 540 nm; detection: 590 nm). The fluorescence of control (*C*) and test wells (*T*) was measured, as well as the fluorescence of the test wells at t = 0 s ($T_0$; addition of the compounds). From these data, the inhibition of cell growth (% growth) and the inhibition of cell viability (% viability) at each concentration of a test inhibitor were calculated according to the following equations: % growth = $100\times[T-T_0]/[C-T_0]$ and % viability = $100\times T/C$.

**List of regents and antibodies for western blotting analysis.**

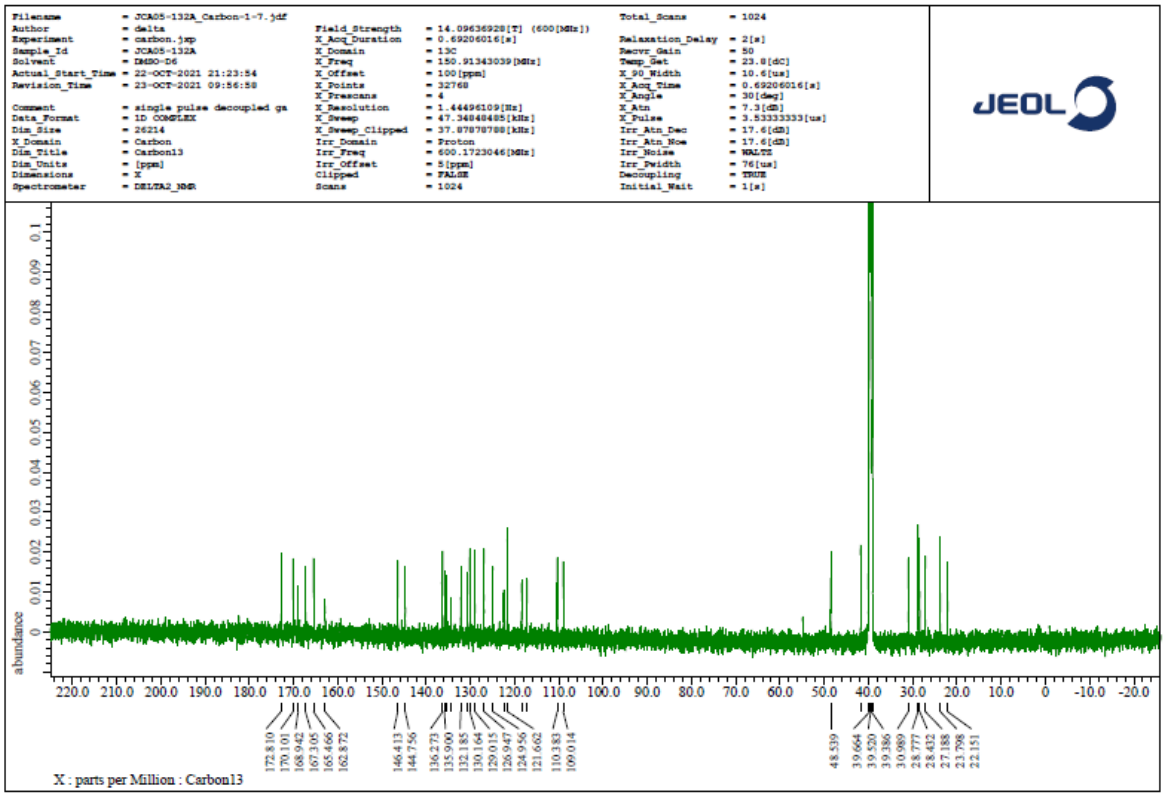
| Reagents | Supplier | Code | Blocking and probing conditions [a] | Dilution for probing |
|---|---|---|---|---|
| Solutions for blocking or antibody reaction | | | | |
| Blocking One | Nacalai | 03953-66 | | |
| Can get signal solutions | TOYOBO | NKB-101T | | |
| Primary Antibodies | | | | |
| Mouse monoclonal anti-HDAC8 antibody | SANTA CRUZ | sc-374180 | Method A, B, or C [b] | 1:500 dilution |
| Rabbit monoclonal anti-HDAC1 antibody | Abcam | Ab109411 | Method A | 1:2000 dilution |
| Rabbit polyclonal anti-HDAC2 antibody | Abcam | Ab7029 | Method D | 1:2000 dilution |
| Rabbit monoclonal anti-HDAC6 antibody | CST | D21B10 | Method A | 1:1000 dilution |
| Mouse monoclonal anti-α-tubulin antibody | Sigma Aldrich | T8203 | Method D | 1:1000 dilution |
| Rabbit monoclonal anti-GAPDH antibody | Abcam | ab9485 | Method E | 1:10000 dilution |
| Mouse monoclonal anti-acetylated SMC3 antibody | Merck | MABE1073 | Method C | 1:2000 dilution |
| Mouse monoclonal anti-acetylated α-tubulin antibody | Sigma Aldrich | T6793 | Method E | 1:4000 dilution |
| Rabbit monoclonal acetyl-histone H3 (Lys9) antibody | CST | C5B11 | Method E | 1:2000 dilution |
| Rabbit polyclonal anti-IKZF1 antibody | CST | 5443 | Method F | 1:2000 dilution |
| Secondary Antibody | | | | |
| ECL rabbit IgG, HRP-linked whole Anti-body | GE Healthcare Life Science | NA934 | | 1:2500 dilution [c] |
| ECL mouse IgG, HRP-linked whole Anti-body | GE Healthcare Life Science | NA931 | | 1:2500 dilution [c] |

[a] Methods for blocking and probing conditions: Method A,TBS containing 1% skimmed milk for blocking buffer and can get signal solution for antibody dilution; Method B, TBS containing 5% skimmed milk for blocking buffer and can get signal solution for antibody dilution; Method C, Blocking one for blocking buffer and TBS-T containing 5% blocking one for antibody dilution; Method D, TBS containing 5% skimmed milk for blocking buffer and TBS containing 5% skimmed milk for antibody dilution; Method E, TBS-T containing 5% skimmed milk for blocking buffer and TBS-T containing 5% skimmed milk for antibody dilution; Method F, TBS-T containing 5% BSA for blocking buffer and TBS-T containing 5% BSA for antibody dilution. [b] Detection of HDAC8, Method A for Figs. 3B and 4EF, and Supplementary Figs. S1 and S2.; Method B for Fig. 3A; Method C for Fig. 4A-D. [c] 1:5000 Dilution with TBS-T containing 5% skimmed milk for GADPH and acetylated α-tubulin probing.

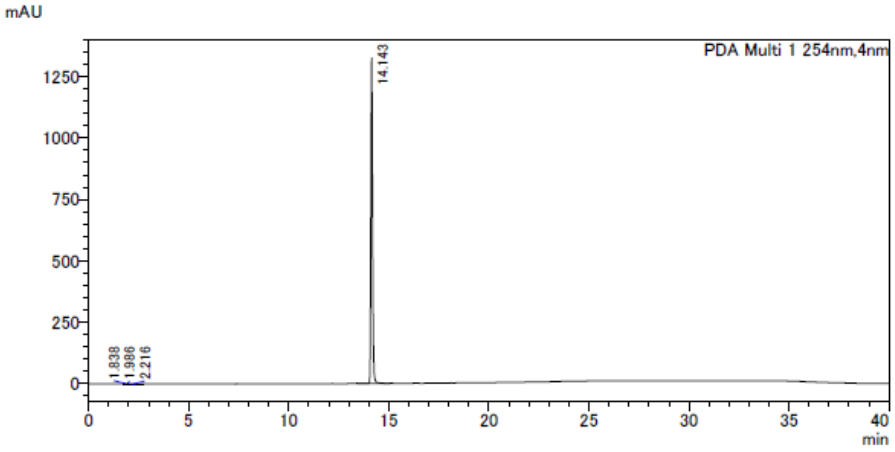
$^{1}$H NMR spectrum of compound **4a**.



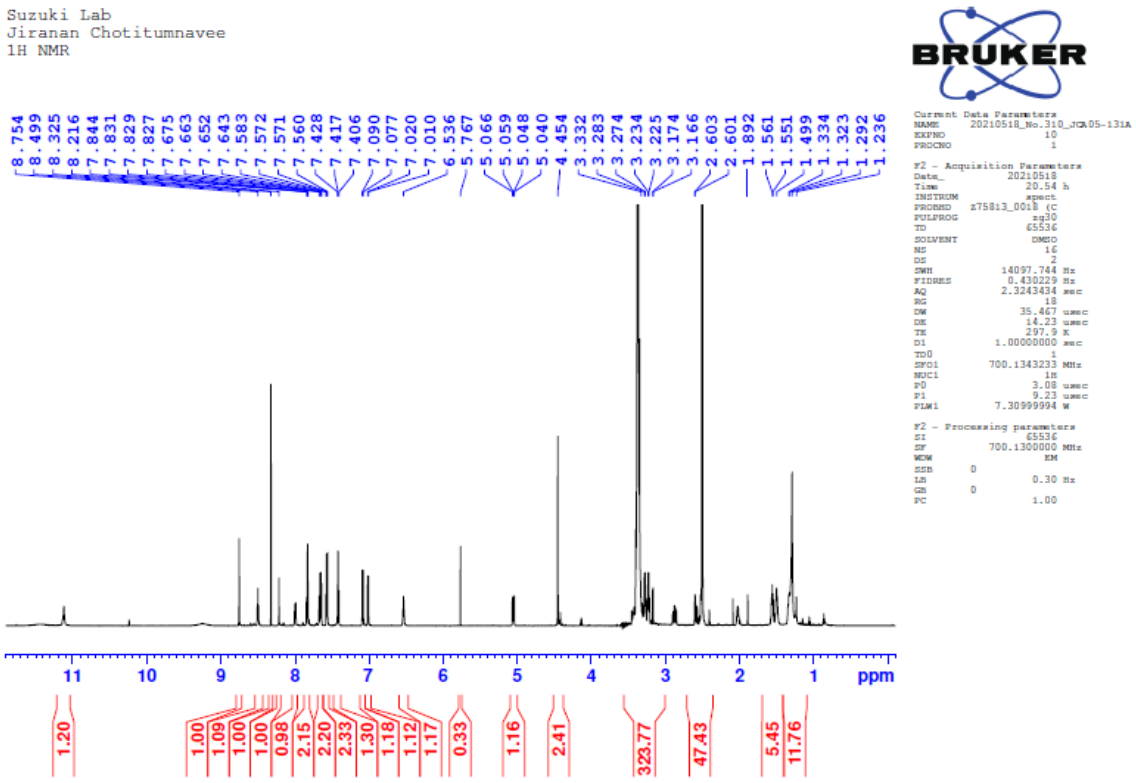

$^{13}$C NMR spectrum of compound **4a**.



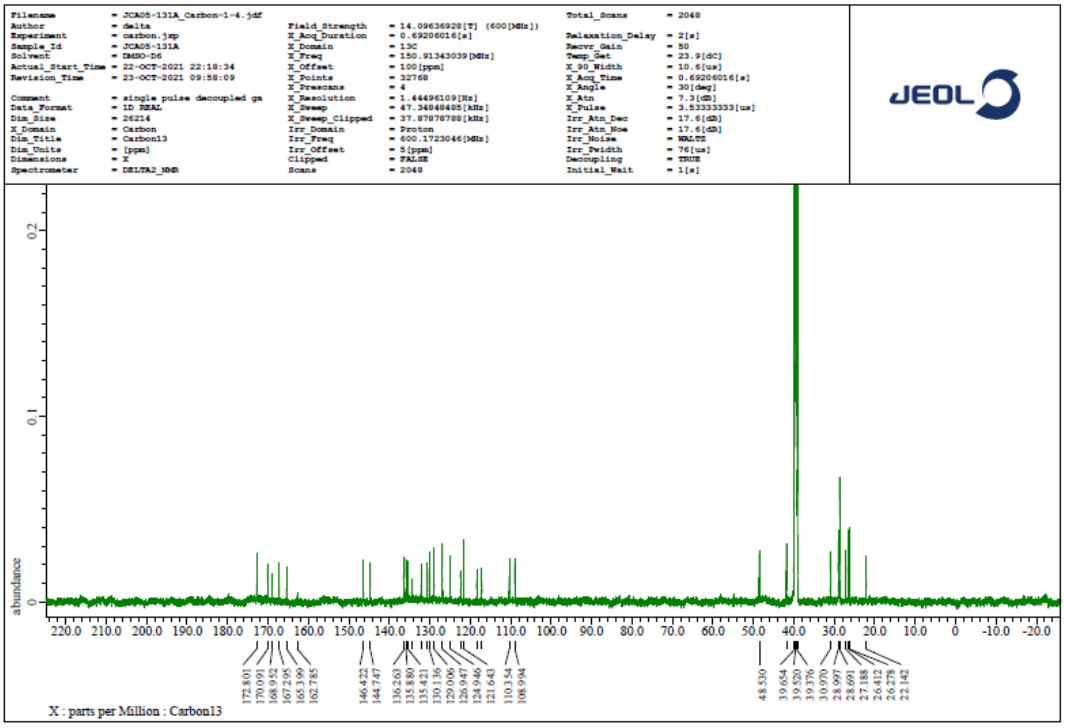
HPLC chart of compound **4a.**



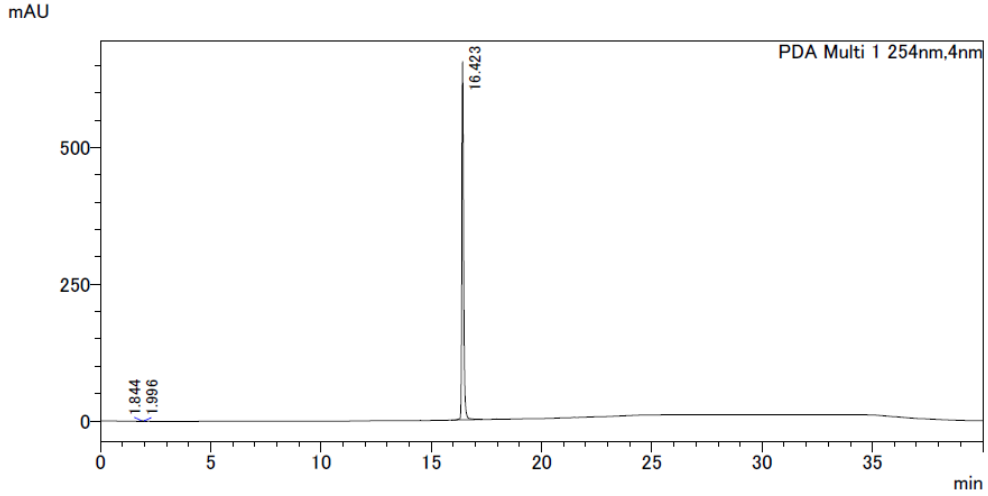
$^{1}$H NMR spectrum of compound **4b**.



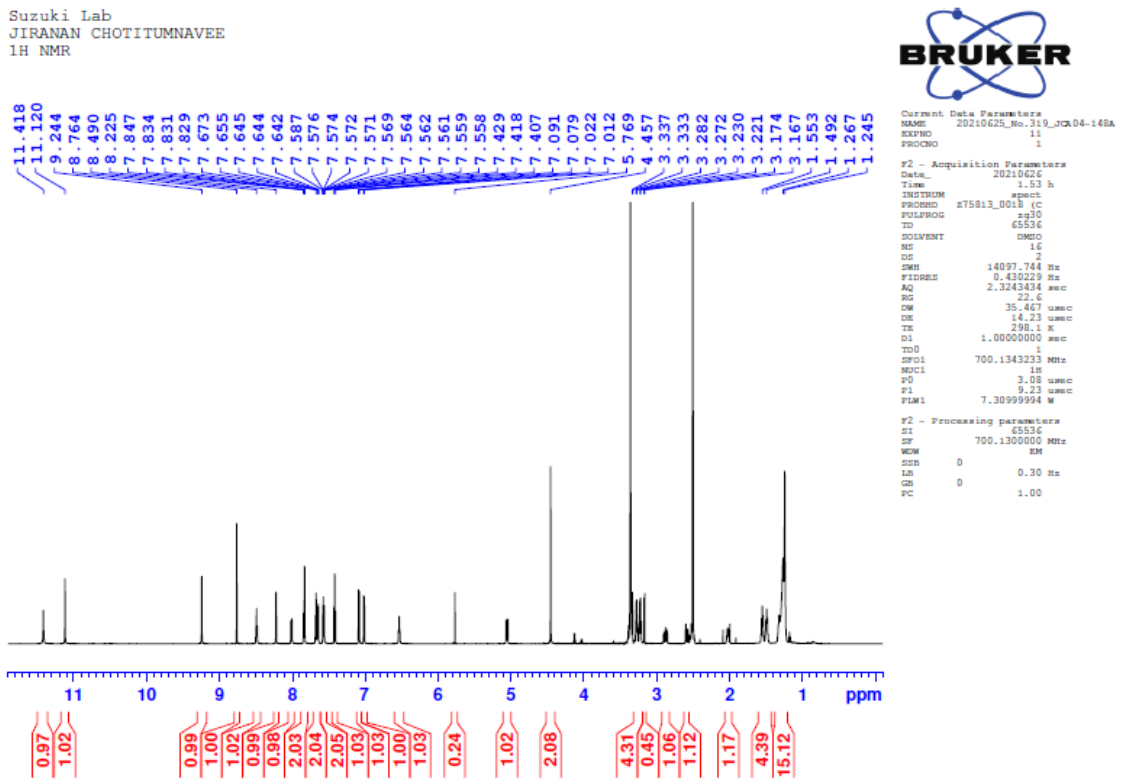

$^{13}$C NMR spectrum of compound **4b**.



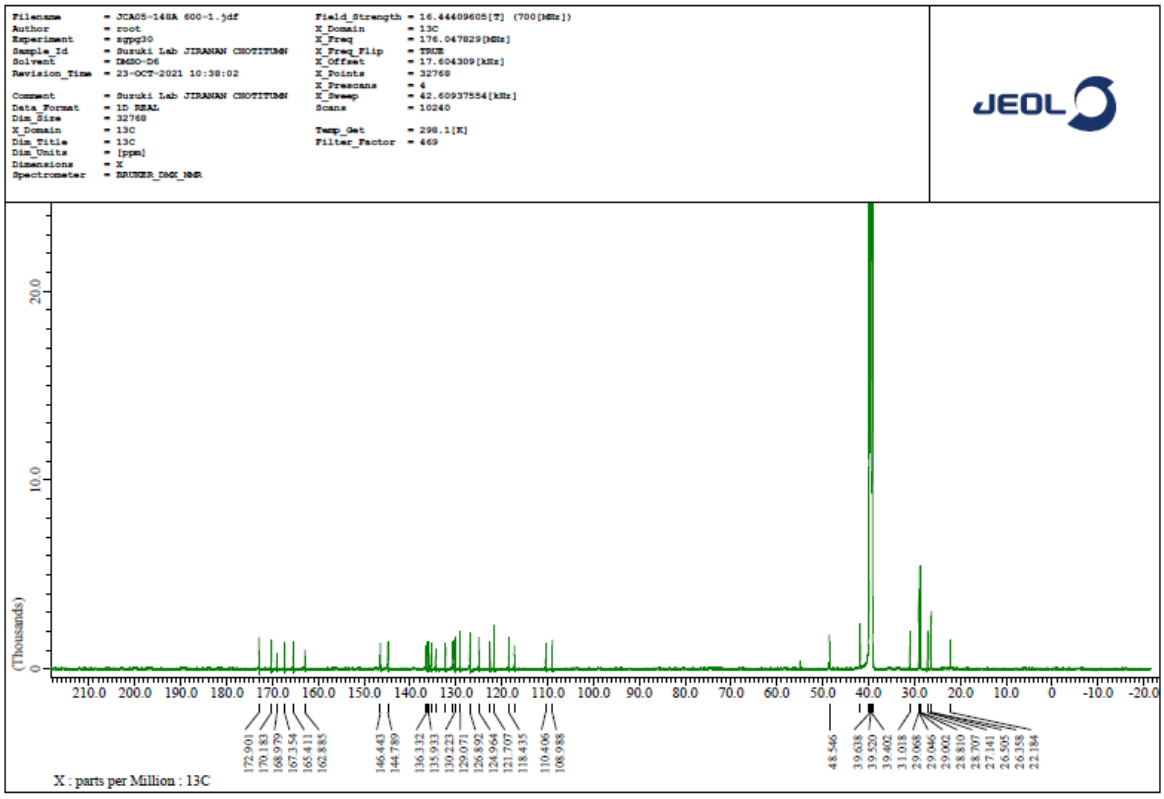
HPLC chart of compound **4b**.



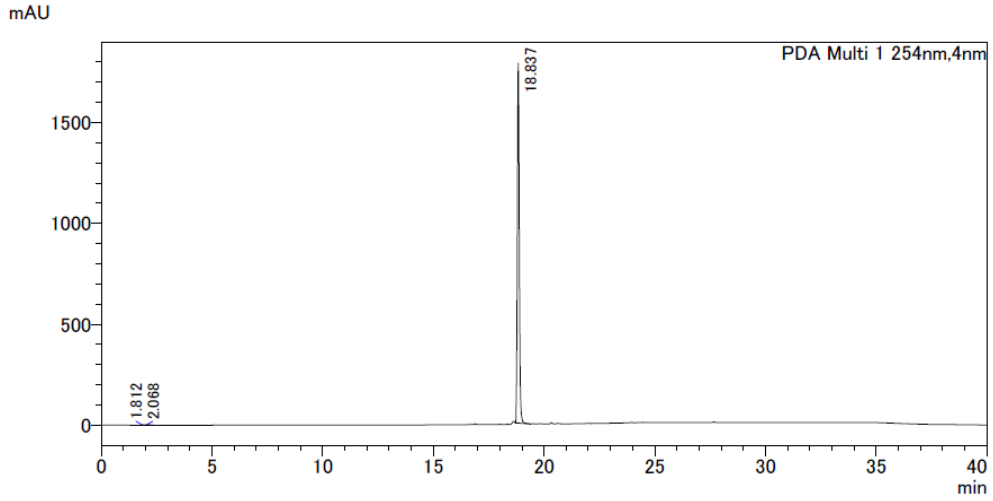
$^{1}$H NMR spectrum of compound **4c**.



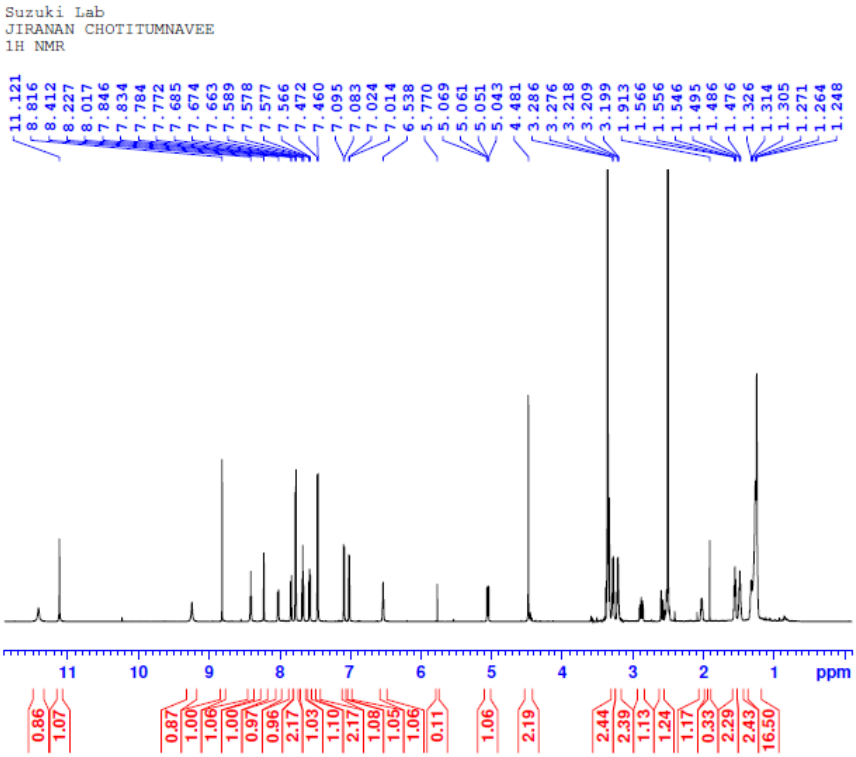

$^{13}$C NMR spectrum of compound **4c**.

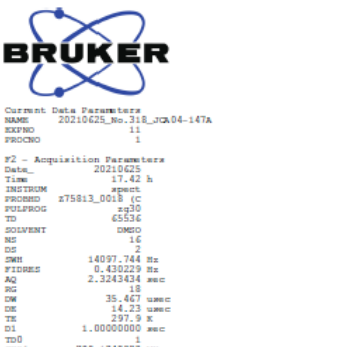


HPLC chart of compound **4c**.

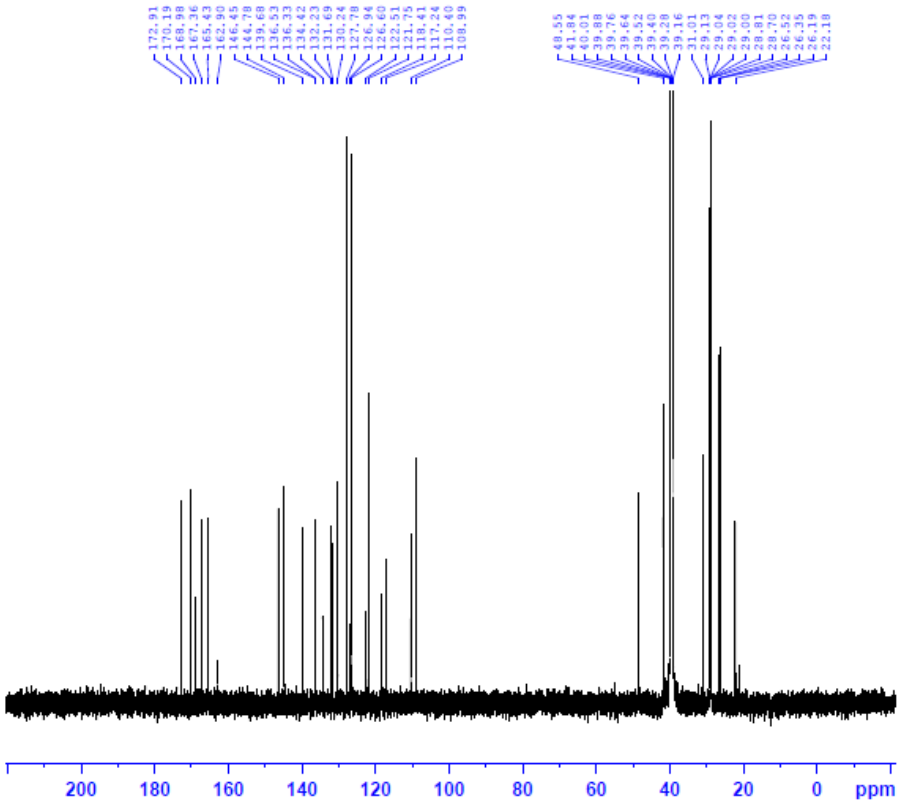


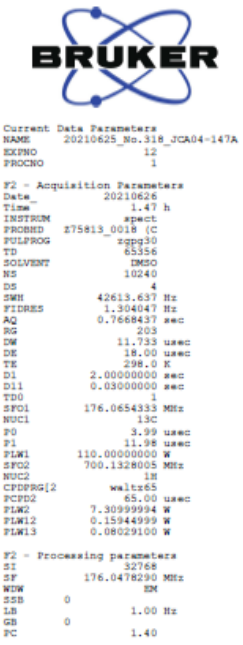
$^{1}$H NMR spectrum of compound **4d**.



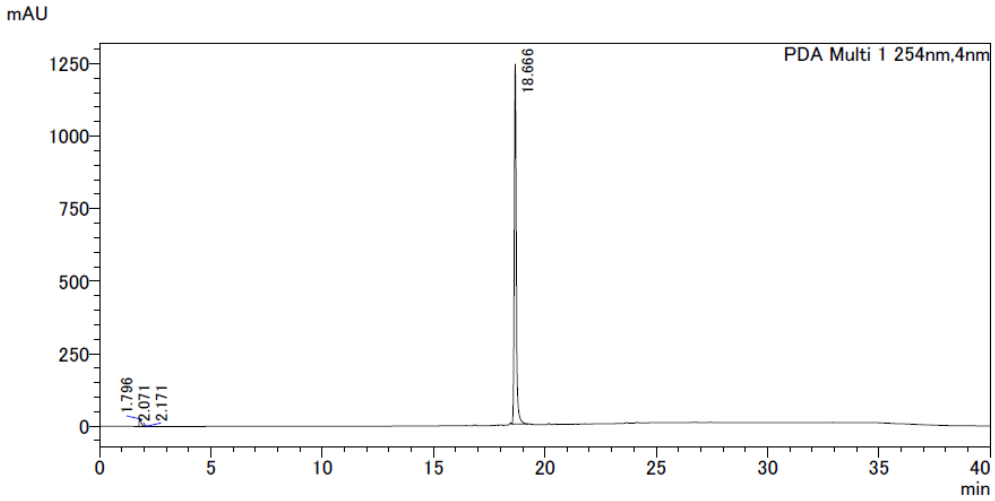




$^{13}$C NMR spectrum of compound **4d**.

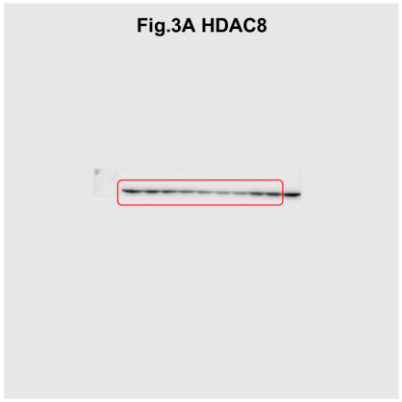


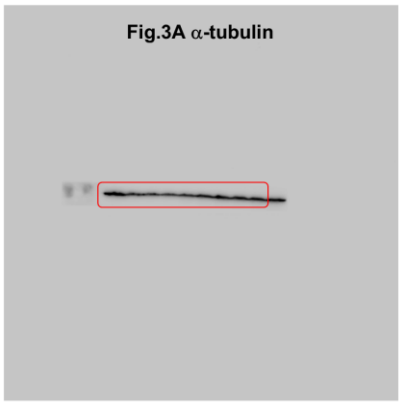



HPLC chart of compound **4d**.

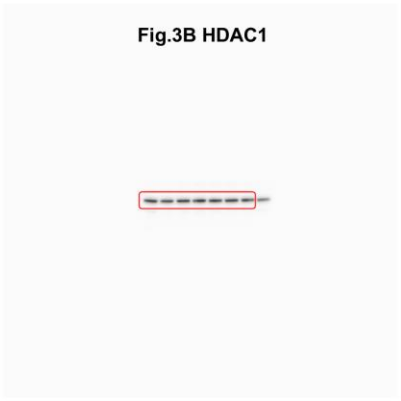


Full western blot images

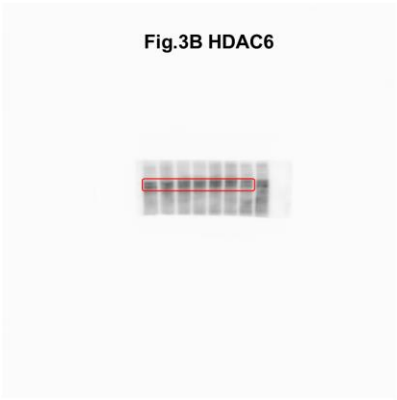


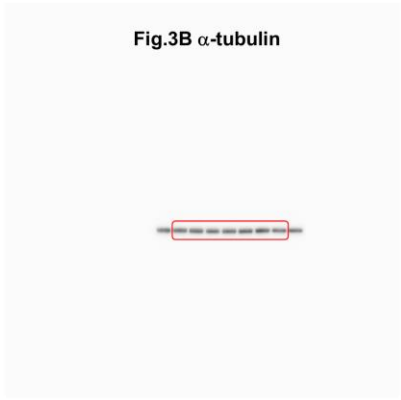





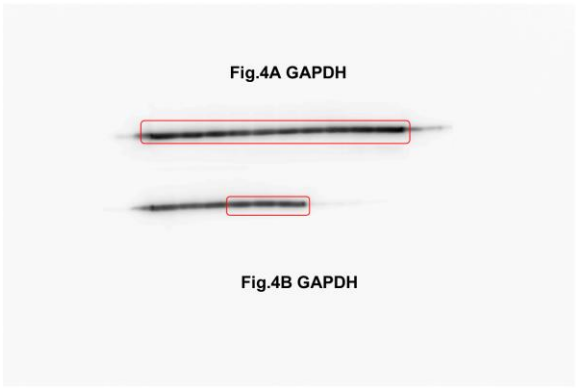










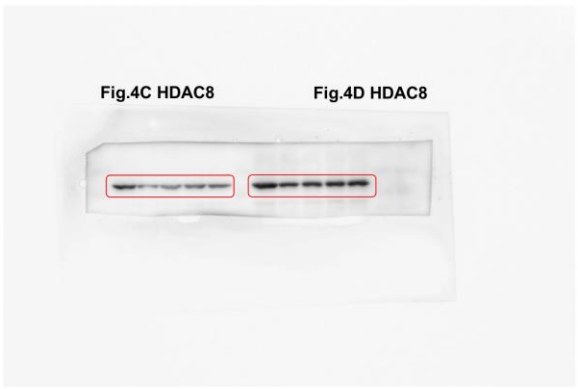


Fig.4C HDAC8
Fig.4D HDAC8

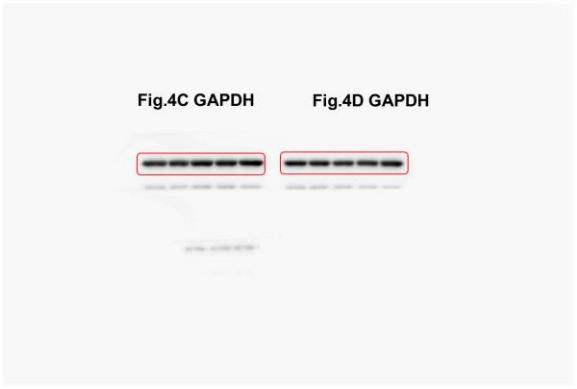


Fig.4C GAPDH
Fig.4D GAPDH

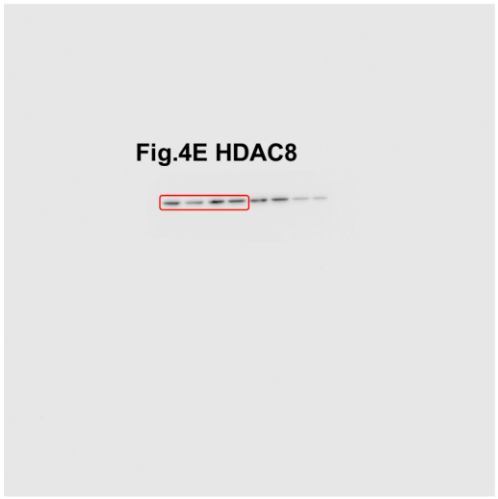


Fig.4E HDAC8

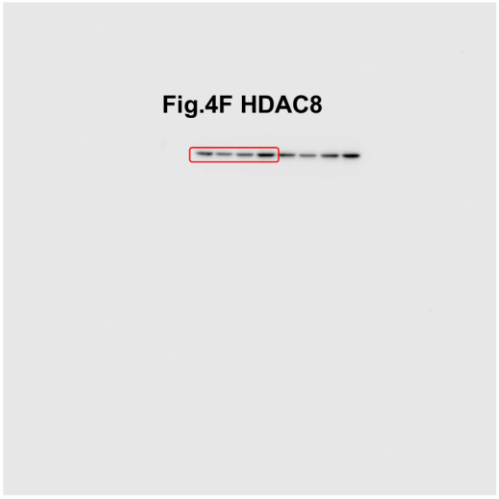


Fig.4F HDAC8

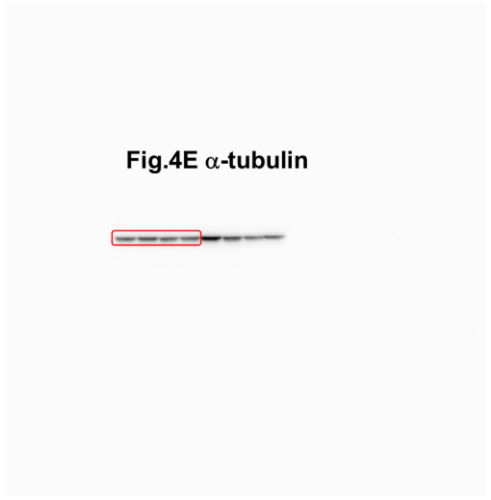


Fig.4E α-tubulin


Fig.4F α-tubulin

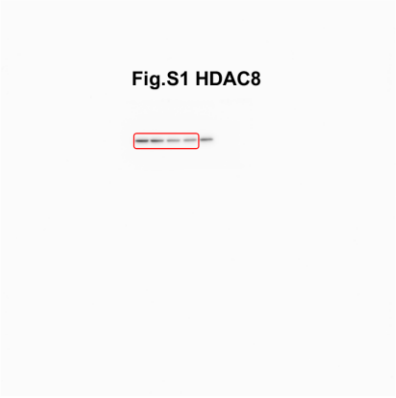


Fig.S1 HDAC8

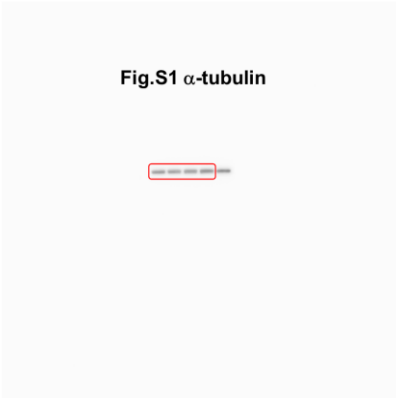


Fig.S1 α-tubulin


Fig.S2 HDAC8

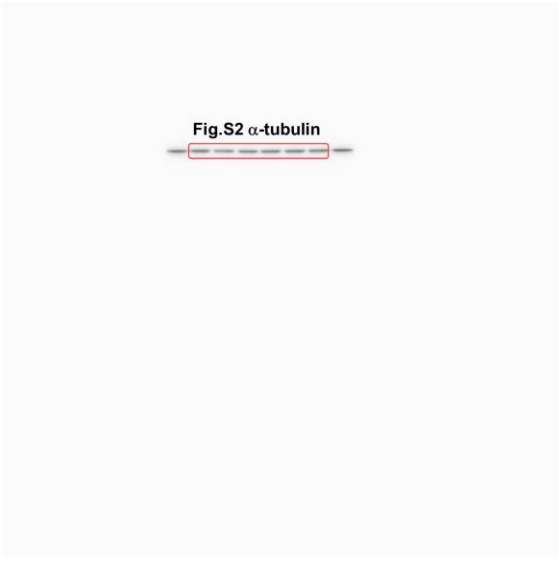


Fig.S2 α-tubulin

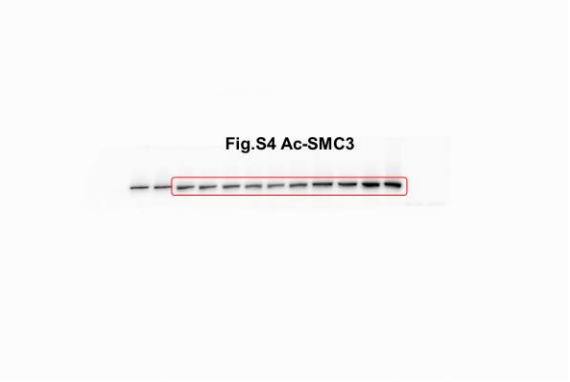


Fig.S4 Ac-SMC3

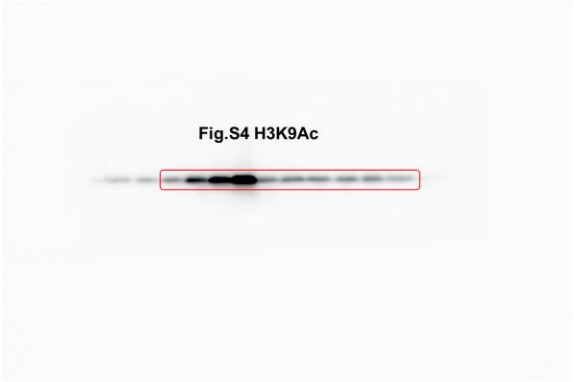


Fig.S4 H3K9Ac

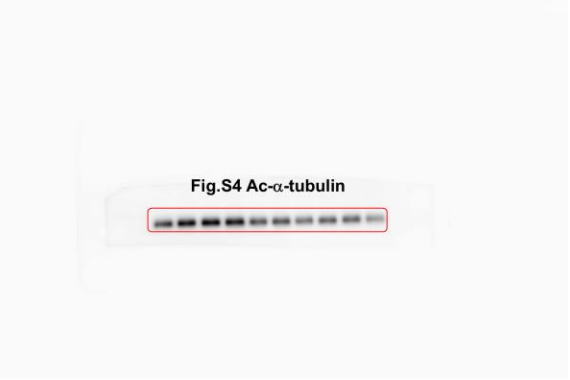


Fig.S4 Ac-α-tubulin


Fig.S4 GAPDH

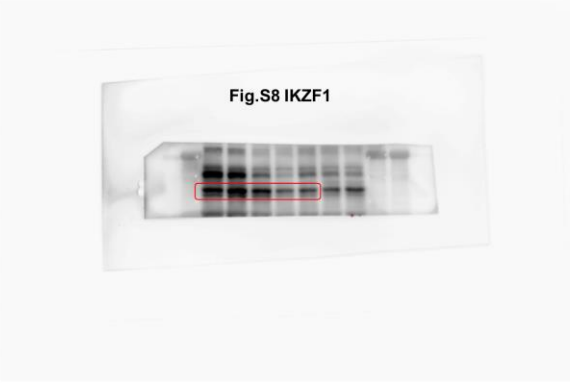


Fig.S8 IKZF1


Fig.S8 GAPDH

**Supplementary References**

S1. T. Suzuki, N. Muto, M. Bando, Y. Itoh, A. Masaki, M. Ri, Y. Ota, H. Nakagawa, S. Iida, K. Shirahige, N. Miyata N, *ChemMedChem*, 2014, **9**, 657–664.

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