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# Design, Synthesis, and Biological Activity of NCC149 Derivatives as Histone Deacetylase 8-Selective Inhibitors

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We recently discovered *N*-hydroxy-3-[1-(phenylthio)methyl-1*H*-1,2,3-triazol-4-yl]benzamide (NCC149) as a potent and selective histone deacetylase 8 (HDAC8) inhibitor from a 151-member triazole compound library using a click chemistry approach. In this work, we present a series of NCC149 derivatives bearing various aromatic linkers that were designed and synthesized as HDAC8-selective inhibitors. A series of in vitro assays were used to evaluate the newly synthesized compounds, four of which showed HDAC8 inhibitory activity similar to that of NCC149, and one of which displayed HDAC8 selectivity superior to that of NCC149. In addition, these top four compounds induced the increase of acetylated cohesin (an HDAC8 substrate) in HeLa cells in a dose-dependent manner, indicating inhibition of HDAC8 in the cells. While none of these compounds enhanced the acetylation of H3K9 (a substrate of HDAC1 and 2), only one compound refrained from increasing α-tubulin acetylation, a substrate of HDAC6, indicating that this compound is more selective for HDAC8 than the other derivatives. Furthermore, this HDAC8-selective inhibitor suppressed the growth of T-cell lymphoma cells more potently than did NCC149. These findings are useful for the further development of HDAC8-selective inhibitors.

## Introduction

Post-translational ε-acetylation and methylation of lysine residues on histones and non-histone proteins is important for the regulation of fundamental life processes, including gene expression and cell cycle progression.[1] The lysine acetylation state of cellular proteins is determined by the activities of histone acetyltransferases and histone deacetylases (HDACs).[1a,b] Eighteen HDAC subtypes have been identified so far, and they are subdivided into two families: zinc-dependent HDACs (HDAC1–11) and $NAD^+$-dependent HDACs (SIRT1–7).[2] Among these, HDAC8 is unique in that it deacetylates non-histone proteins, such as cohesin,[3] estrogen-related receptor α,[4] and HSP20,[5] and is involved in genetic repression[6] and the actin cytoskeleton.[7] Recent studies have indicated that HDAC8 is associated with several disease states, particularly T-cell lymphoma[8] and neuroblastoma.[9] Therefore, HDAC8-selective inhibitors are of great interest, not only as tools for probing the biological functions of HDAC8, but also as candidate anticancer agents with potentially few side effects.

Several classes of HDAC8-selective inhibitors have been identified so far (Figure 1). These include SB-379278A (**1**),[10] "linkerless" hydroxamic acid **2**,[11] PCI-34051 (**3**),[8] azetidinone **4**,[12] A8B4 (**5**),[13] and *ortho*-aryl *N*-hydroxycinnamide **6**.[14] We recently discovered NCC149 (**7**) (Figure 1) as a potent and selective HDAC8 inhibitor from a 151-member triazole compound library using a click chemistry approach.[15] Here we report the design, synthesis, in vitro HDAC inhibitory activity, cohesin acetylation activity, and T-cell lymphoma cell growth inhibitory activity of NCC149 derivatives.

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## Results and Discussion

### Design

The molecular model of NCC149 (**7**) docked into the HDAC8 catalytic core suggested that the triazole ring interacts with the methylene group of HDAC8 Phe152 through CH-π or hydrophobic interactions[15b] (Figure 2). The triazole ring is also considered to be important in fixing the orientation of the zinc-binding hydroxamate and the hydrophobic pocket-binding phenylthiomethyl group appropriately, thereby contributing to the potent HDAC8 inhibitory activity and HDAC8 selectivity of NCC149. On the basis of the simulated HDAC8/NCC149 (**7**) complex, we designed compounds **8**–**12** (Figure 3),

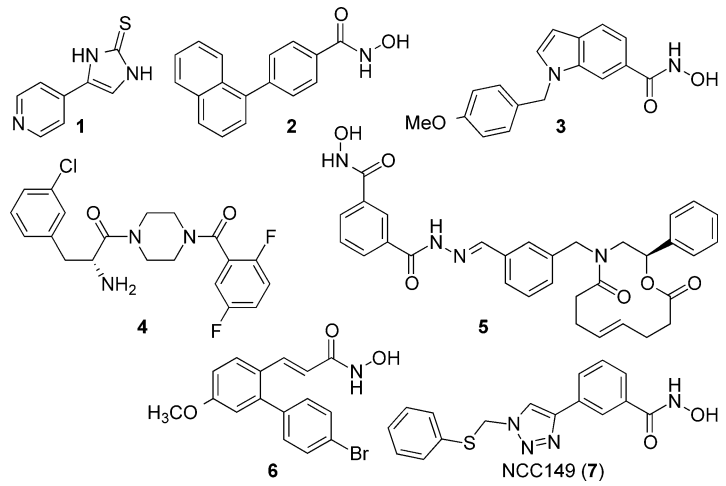



**Figure 1.** Reported HDAC8-selective inhibitors.

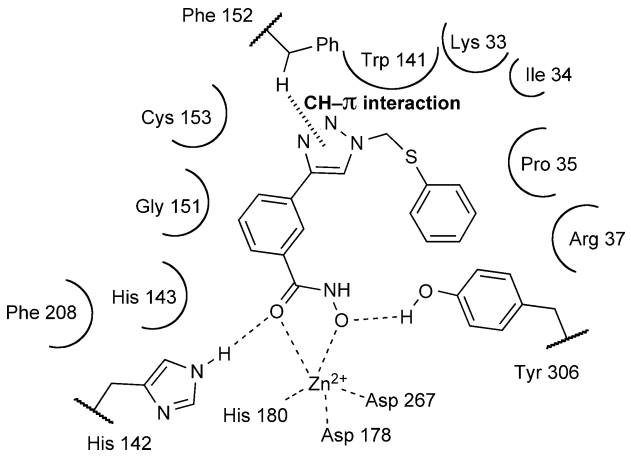



**Figure 2.** Schematic view of the conformation of NCC149 (**7**) docked into the HDAC8 catalytic core.

in which the triazole ring of NCC149 (**7**) was converted into various aromatic rings. We anticipated that these aromatic rings would interact with Phe152 of HDAC8 more efficiently, thereby leading to more potent and selective inhibition of HDAC8.

## Synthesis

A series of compounds (**8**–**12**) modeled after NCC149 were synthesized, as shown in Schemes 1–5 below. The synthetic route for compound **8**, in which the triazole ring of NCC149 (**7**) is replaced with a benzene ring, is described in Scheme 1. Suzuki coupling[16] of 3-iodobenzyl alcohol **13** with 3-(methoxycarbonyl)phenylboronic acid **14** provided biphenyl derivative **15**. Bromination of the hydroxy group in **15** and coupling with thiophenol gave sulfide compound **17**. Hydrolysis of the methyl ester of **17** afforded carboxylic acid **18**, which was then treated with *O*-THP hydroxylamine in the presence of EDCI and HOBt to give *O*-THP hydroxyamide **19**, after which removal of the THP group under acidic conditions yielded desired compound **8**.

Thiazole **9** was synthesized from 3-acetylbenzoic acid **20**, as shown in Scheme 2. Methyl esterification of **20** and subsequent bromination gave α-bromo-3-acetylbenzoic acid methyl ester **22**. Alternatively, phenylthioacetic acid **23** was converted into amide **24**, and subsequent treatment with Lawesson's reagent afforded thioacetamide **25**. α-Keto compound **22** was then reacted with thioacetamide **25** in EtOH at 70 °C to give thiazole **26**. Hydrolysis of the methyl ester of **26**, *O*-THP hydroxyamide formation, and deprotection of the THP group gave thiazole **9**.

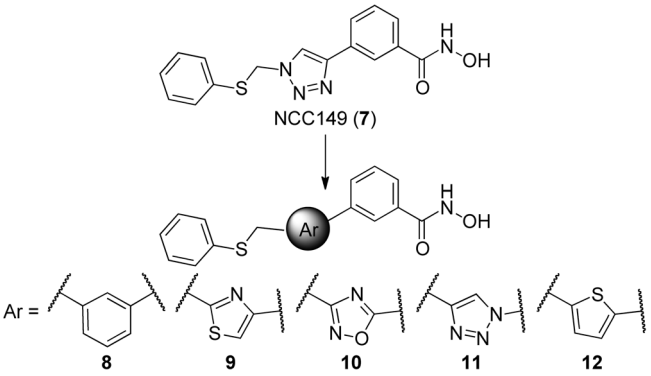
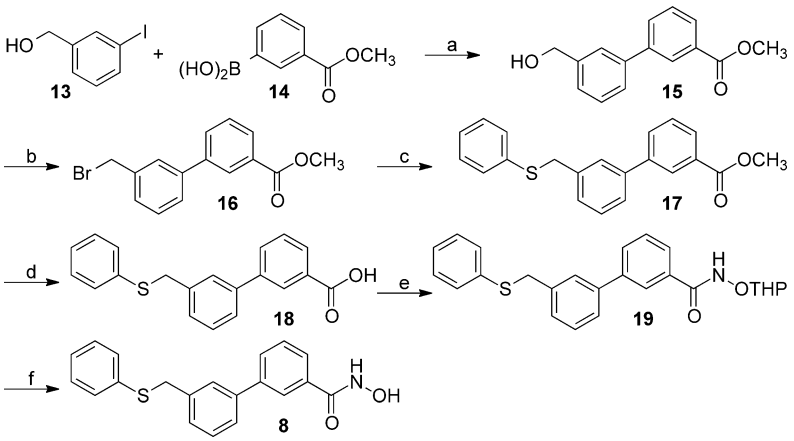
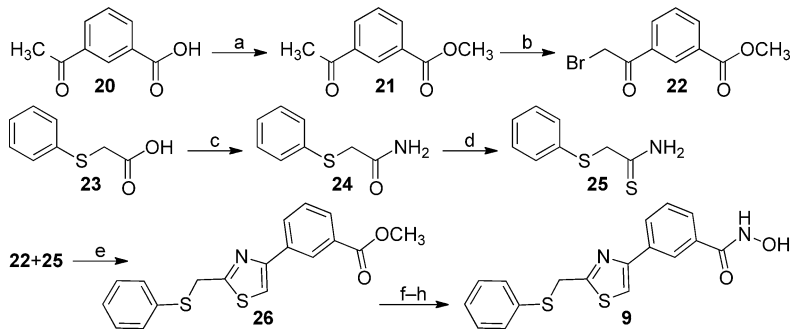


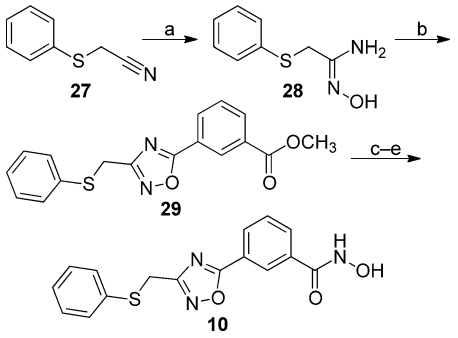

**Figure 3.** Conversion of the triazole ring of NCC149.




**Scheme 1.** Synthesis of **8**. *Reagents and conditions*: a) $Pd(OAc)_2$, $Na_2CO_3$, DMF, $H_2O$, 55 °C, 1.5 h, (quant.); b) $PBr_3$, 1,4-dioxane, 0 °C, 1.5 h, 83%; c) PhSNa, $Et_3N$, acetone, $H_2O$, RT, 19.5 h, 50%; d) NaOH, MeOH, $H_2O$, THF, RT, 5 h, 50%; e) $NH_2OTHP$, EDCI, HOBt, DMF, RT, 30 h, 84%; f) TsOH, MeOH, RT, 5.5 h, 40%.



**Scheme 2.** Synthesis of **9**. *Reagents and conditions*: a) $H_2SO_4$, MeOH, reflux, 26.5 h, 97%; b) $Br_2$, HBr, AcOH, $CHCl_3$, RT, 2 h, (quant.); c) 1. $(COCl)_2$, DMF, THF, 0 °C 0.5 h, 2. $NH_3$, $H_2O$, THF, 0 °C, 1.5 h, 83%; d) Lawesson's reagent, toluene, 80 °C, 3.5 h, 21%; e) MS (4 Å), EtOH, 70 °C, 3 h, 78%; f) NaOH, MeOH, $H_2O$, THF, RT, 5 h, 68%; g) $NH_2OTHP$, EDCI, HOBt, DMF, RT, 30 h, 96%; h) TsOH, MeOH, RT, 5.5 h, 73%.



**Scheme 3.** Synthesis of **10**. *Reagents and conditions*: a) $NH_2OH$, KOH, MeOH, RT, 20 h, 43%; b) 1. monomethyl isophthalate, CDI, DMF, RT, overnight; 2. microwave, 140 °C, 2 min, 33%; c) NaOH, MeOH, $H_2O$, THF, RT, 5 h, 86%; d) $NH_2OTHP$, EDCI, HOBt, DMF, RT, 30 h, 99%; e) TsOH, MeOH, RT, 5.5 h, 20%.

The preparation of oxadiazole **10** was achieved via key intermediate **28** (Scheme 3). (Phenylthio)acetonitrile **27** was converted into hydroxyamidine **28** by reaction with hydroxylamine in the presence of KOH. Compound **28** was allowed to react with monomethyl isophthalate in the presence of CDI to give an *O*-acyl hydroxyamidine intermediate. Ring closure of the intermediate to oxadiazole **29** was effected under microwave irradiation at 140 °C. Compound **29** was converted into hydroxamate **10** in three steps using the same procedure as described for compound **8**.

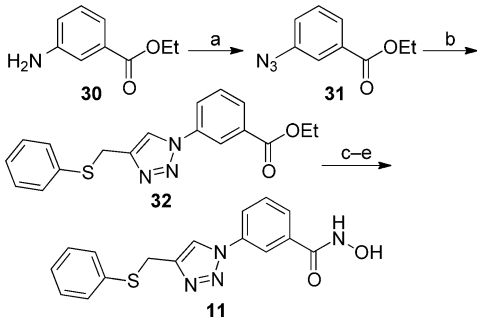
Scheme 4 illustrates the synthesis of reversed triazole **11**. Treatment of aniline **30** with $NaNO_2$ under acidic conditions, followed by addition of $NaN_3$, yielded azide **31**. The copper-catalyzed coupling of phenyl propargyl sulfide with azide **31** provided reversed triazole **32**. Compound **32** was converted into compound **11** using the procedure described above.

The preparation of thiophene **12** is shown in Scheme 5. Suzuki coupling of 2-iodo-3-methylthiophene **33** with 3-(methoxycarbonyl)phenylboronic acid **14** gave thiophenylbenzene compound **34**. Bromination of the 2-methyl group of **34** with *N*-bromosuccinimide in the presence of AIBN afforded bromide **35**. Coupling of **35** and thiophenol yielded sulfide **36**. Compound **36** was converted into hydroxamate **12** in three steps using the procedure described above.

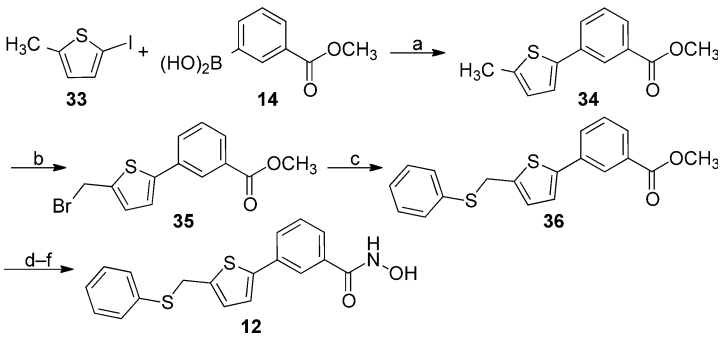
## Biological evaluation

### Enzyme assays

The synthesized compounds were examined for inhibitory effects toward total HDACs, HDAC1, HDAC2, HDAC3, HDAC4, HDAC6, and HDAC8. The results of the enzyme assays are shown in Table 1. NCC149 (**7**) showed potent and selective inhibition of HDAC8 ($IC_{50}$ for HDAC8 = 0.070 μM; $IC_{50}$ for HDACs = 54 μM; $IC_{50}$ for HDAC1 = 38 μM; $IC_{50}$ for HDAC2 > 100 μM; $IC_{50}$ for HDAC3 = 68 μM; $IC_{50}$ for HDAC4 = 44 μM; $IC_{50}$ for HDAC6 = 2.4 μM) as compared with vorinostat, a pan-HDAC inhibitor.[17] Compound **8**, in which the triazole ring of NCC149 (**7**) is replaced with a phenyl ring, displayed 20-fold less potent HDAC8 inhibition, although it showed HDAC8-selective inhibition. On the other hand, com-



**Scheme 4.** Synthesis of **11**. *Reagents and conditions*: a) 1. $NaNO_2$, $H_2O$, TFA, 0 °C, 1.5 h; 2. $NaN_3$, $H_2O$, RT, 2.5 h, 94%; b) phenyl propargyl sulfide, $CuSO_4$, Na ascorbate, MeOH, $H_2O$, RT, 21 h, 67%; c) NaOH, MeOH, $H_2O$, THF, RT, 5 h, (quant.); d) $NH_2OTHP$, EDCI, HOBt, DMF, RT, 30 h, 58%; e) TsOH, MeOH, RT, 5.5 h, 83%.



**Scheme 5.** Synthesis of **12**. *Reagents and conditions*: a) $Pd(OAc)_2$, $Na_2CO_3$, DMF, $H_2O$, 60 °C, 1.5 h, 69%; b) AIBN, NBS, $CCl_4$, reflux, 1 h, (quant.); c) PhSH, $Et_3N$, DMF, RT, 19.5 h, 73%; d) NaOH, MeOH, $H_2O$, THF, RT, 5 h, (quant.); e) $NH_2OTHP$, EDCI, HOBt, DMF, RT, 30 h, 79%; f) TsOH, MeOH, RT, 5.5 h, 63%.

**Table 1.** HDAC inhibitory activities of vorinostat, NCC149 (**7**), and compounds **8**–**12**.[a]

| Compd | $IC_{50}$ [μM] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nuclear Extract | Class I HDAC1 | HDAC2 | HDAC3 | HDAC8 | Class IIa HDAC4 | Class IIb HDAC6 |
| vorinostat | 0.17 | 0.27 | 0.78 | 0.68 | 1.5 | 28 | 0.21 |
| NCC149 (**7**) | 54 | 38 | >100 | 68 | 0.070 | 44 | 2.4 |
| **8** | >100 | >100 | >100 | 65 | 1.4 | >100 | 31 |
| **9** | >100 | >100 | >100 | 12 | 0.15 | >100 | 14 |
| **10** | >100 | >100 | >100 | >100 | 0.12 | 40 | 7.8 |
| **11** | >100 | >100 | >100 | >100 | 0.053 | >100 | 2.2 |
| **12** | >100 | >100 | >100 | 84 | 0.22 | >100 | 7.2 |

[a] Values are the mean of at least two experiments; in all cases data varied by 10% or less.

pounds **9**–**12** potently inhibited HDAC8. These results suggest that the five-membered ring of compounds **9**–**12** is important to fix the orientation of the zinc-binding hydroxamate and the hydrophobic pocket-binding phenylthiomethyl group appropriately, thereby contributing to the potent HDAC8 inhibitory activity. Among compounds **9**–**12**, reversed triazole analogue **11** showed slightly more potent HDAC8 inhibitory activity than NCC149 (**7**) ($IC_{50}$ of **11** = 0.053 μM; $IC_{50}$ of **7** = 0.070 μM). Moreover, compounds **9**–**12** selectively inhibited HDAC8. In particular, compound **11** displayed greater HDAC8 selectivity than NCC149 (**7**) (**11**: HDAC1 $IC_{50}$/HDAC8 $IC_{50}$ > 1900; HDAC4 $IC_{50}$/HDAC8 $IC_{50}$ > 1900; HDAC6 $IC_{50}$/HDAC8 $IC_{50}$ = 42. **7**: HDAC1 $IC_{50}$/HDAC8 $IC_{50}$ = 540; HDAC4 $IC_{50}$/HDAC8 $IC_{50}$ = 630; HDAC6 $IC_{50}$/HDAC8 $IC_{50}$ = 34). These data suggest that small geometric differences in five-membered heteroaryl rings have an effect on not only HDAC8 inhibitory activity but also HDAC8 selectivity. As a result of these enzyme assays, compound **11** was found to be a more potent and selective inhibitor of HDAC8 than NCC149 (**7**).

### Cell-based assays

To examine HDAC8 selectivity in cells of compounds **7**, **9**, **10**, and **11**, which were potent HDAC8-selective inhibitors in the enzyme assays (Table 1), we performed a cellular assay using Western blot analysis. As HDAC8 is a deacetylase of cohesin,[3] HDAC8 inhibition was initially assessed by evaluating the accumulation of acetylated cohesin in HeLa cells. As shown in Figure 4, the test compounds induced an increase of acetylated cohesin in a dose-dependent manner, suggesting strong HDAC8 inhibition in the cells. Although it has been reported that trichostatin A, a representative pan-HDAC inhibitor, significantly increases acetylated H3K9 in HeLa cells,[18] compounds **7**, **9**, **10**, and **11** did not enhance the acetylation of H3K9, a major substrate of nuclear HDACs such as HDAC1 and HDAC2. As for acetylated α-tubulin, a substrate of HDAC6,[19] NCC149 (**7**) and compounds **10** and **11** induced acetylation of α-tubulin at a concentration of >10 μM, whereas compound **9** did not display a major increase in acetylated α-tubulin. These results indicate that compound **9** selectively inhibits HDAC8 over other HDACs in cells, in contrast to NCC149 (**7**) and compounds **10** and **11**.

Because it has been reported that HDAC8 inhibitors including PCI-34051 (**3**), NCC149 (**7**), and NCC149 derivatives exhibit T-cell lymphoma cell growth inhibitory activity without affecting other cancer cells and normal cells,[8,15b] compounds **7**, **9**, **10**, and **11** were tested in cell growth inhibition assays using human T-cell lymphoma cell lines. Results are shown in Table 2. All of the tested HDAC8-selective inhibitors had clear growth inhibitory effects on T-cell lymphoma cell lines, including Jurkat, HH, MT4, and HUT78. Although compound **11** showed the highest HDAC8 inhibitory activity in enzyme assays (Table 1), it did not show strong T-cell lymphoma cell growth inhibitory activity as compared with compounds **7**, **9**, and **10** (Table 2). The reason for this weak T-cell lymphoma cell

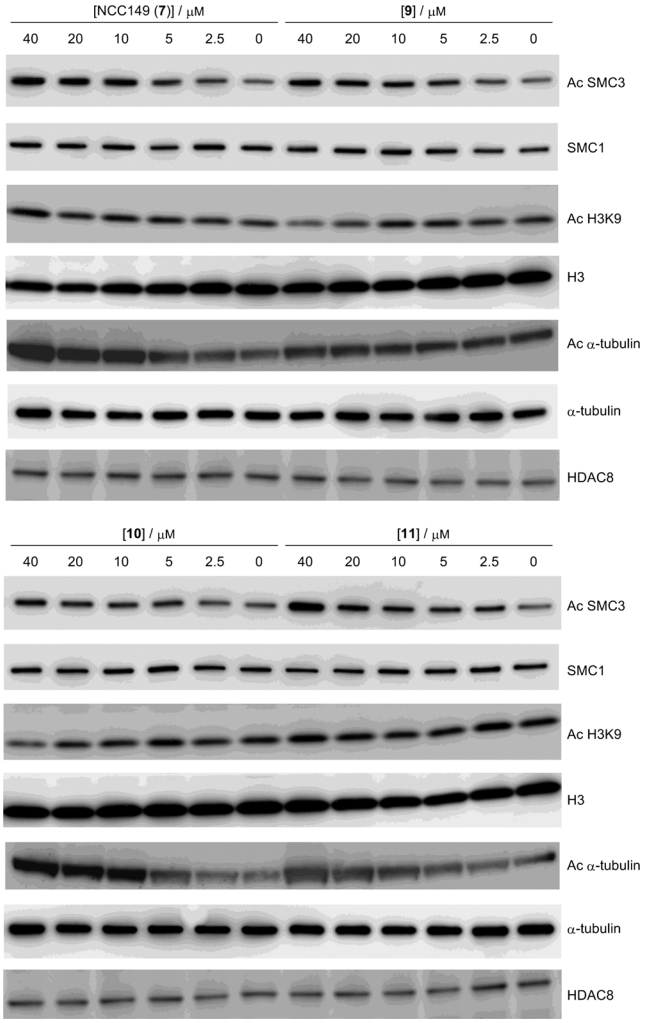



**Figure 4.** Western blot analysis of acetylated structural maintenance of chromosome 3 (SMC3, a subunit of cohesion), H3K9, and α-tubulin levels in HeLa cells after 4 h treatment with compounds **7**, **9**, **10**, and **11**.

**Table 2.** Growth inhibition of T-cell lymphoma cells by NCC149 (**7**) and compounds **9**–**11**.[a]

| Cell Line | $GI_{50}$ [μM] | | | |
|---|---|---|---|---|
| | **7** | **9** | **10** | **11** |
| Jurkat | 2.8 | 2.0 | 2.1 | 24 |
| HH | 21 | 7.4 | 12 | 40 |
| MT4 | 22 | 5.8 | 12 | 43 |
| HUT78 | 32 | 27 | 35 | 50 |

[a] Values are the mean of at least two experiments; in all cases data varied by 30% or less.

growth inhibitory activity of compound **11** is unclear, but it can be assumed that compound **11** has problems with plasma protein binding affinity, cell membrane permeability, metabolic stability, localization, and/or off-target effects in T-cell lymphoma cells. Among compounds **7**, **9**, **10**, and **11**, compound **9** showed greater growth inhibitory activity than NCC149 (**7**) against T-cell lymphoma cells. The results shown in Table 2 suggest that our HDAC8-selective inhibitors are useful for the treatment of T-cell lymphoma.

## Conclusions

We have identified novel, potent, HDAC8-selective inhibitors. Although in general, these inhibitors displayed a relatively small improvement in in vitro HDAC8 selectivity over HDAC6, compound **11** showed the most potent HDAC8 inhibitory activity. In vitro evaluation showed that, of the test compounds, only compound **9** increased cohesion (HDAC8 substrate) acetylation without also significantly enhancing acetylation of other HDAC substrates such as H3K9 and α-tubulin in a dose-dependent manner, indicating that this compound was the most highly HDAC8-selective. Compound **9** also exhibited more potent T-cell lymphoma cell growth inhibitory activity than NCC149. These findings should pave the way for the development of new anticancer drugs. Detailed studies of these HDAC8-selective inhibitors and further analogues are underway.

## Experimental Section

### *Chemistry*

**General**: Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus. Proton nuclear magnetic resonance spectra ($^1$H NMR) and carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded using a JEOL JNM-LA500 or a JEOL JNM-A500 spectrometer in solvent as indicated. Chemical shifts ($\delta$) are reported in parts per million relative to the internal standard tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within $\pm$0.4% of the calculated values, confirming $>$95% purity. EIMS analyses were performed on a JEOL JMS-SX102A mass spectrometer. FTIR spectra were measured on a Shimadzu FTIR-8400S spectrometer. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and were used without purification. Flash column chromatography was performed using silica gel 60 (particle size: 0.046–0.063 mm) from Merck.

**3-(3-Hydroxymethylphenyl)benzoic acid methyl ester (15)**: A mixture of 3-iodobenzyl alcohol (**13**) (2.72 g, 11.6 mmol), 3-(methoxycarbonyl)phenylboronic acid (**14**) (2.00 g, 11.1 mmol), and $Pd(OAc)_2$ (56.2 mg, 0.250 mmol) in DMF (7 mL) and 1 M aqueous $Na_2CO_3$ (34 mL) was heated at 55 °C for 1.5 h. The reaction mixture was diluted with $CHCl_3$ and filtered. The $CHCl_3$ layer was separated, washed with $H_2O$ and brine, and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 0–80% EtOAc/*n*-hexane gradient) gave 3.85 g (quant.) of **15** as a light brown oil: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.27 (t, *J* = 1.7 Hz, 1H), 8.00 (dt, *J* = 1.5, 7.5 Hz, 1H), 7.78 (dt, *J* = 1.6, 7.8 Hz, 1H), 7.63 (s, 1H), 7.57–7.48 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.37 (d, *J* = 7.0 Hz, 1H), 4.76 (d, *J* = 6.0 Hz, 2H), 3.94 ppm (s, 3H); EIMS: *m/z* 242 [$M^+$].

**3-(3-Bromomethylphenyl)benzoic acid methyl ester (16)**: $PBr_3$ (0.6 mL, 6.38 mmol) was added dropwise to a solution of alcohol **15** (3.85 g, 15.9 mmol) in 10 mL of anhydrous dioxane at 0 °C under $N_2$ atmosphere. The reaction mixture was stirred for 1.5 h at 0 °C. The mixture was poured into a mixture of ice and saturated $NaHCO_3$. The mixture was then extracted with $Et_2O$, and the $Et_2O$ layer was separated, washed with sat. $NaHCO_3$ and brine, and dried over $Na_2SO_4$. Filtration and concentration in vacuo gave 4.01 g (83%) of **16** as a yellow oil: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.26 (s, 1H), 8.02 (d, *J* = 7.5 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.63 (s, 1H), 7.56–7.47 (m, 2H), 7.44–7.38 (m, 2H), 4.55 (s, 2H), 3.94 ppm (s, 3H); EIMS: *m/z* 304, 306 [$M^+$].

**3-(3-Phenylthiomethylphenyl)benzoic acid methyl ester (17)**: Sodium thiophenol (2.07 g, 15.7 mmol) in $H_2O$ (15 mL) and $Et_3N$ (5 mL) was added to a solution of bromide **16** (3.70 g, 12.1 mmol) in acetone (35 mL). The mixture was stirred for 19.5 h at room temperature. After removal of the solvent, the residue was poured into $H_2O$ and extracted with $CHCl_3$. The organic layer was washed with $H_2O$ and brine and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 0–30% EtOAc/*n*-hexane gradient) gave 2.02 g (50%) of **17** as a colorless oil: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.20 (t, *J* = 1.7 Hz, 1H), 7.99 (dt, *J* = 1.4, 7.7 Hz, 1H), 7.68 (dt, *J* = 1.2, 7.2 Hz, 1H), 7.47–7.44 (m, 3H), 7.35–7.18 (m, 7H), 4.14 (s, 2H), 3.92 ppm (s, 3H); EIMS: *m/z* 334 [$M^+$].

**3-(3-Phenylthiomethylphenyl)benzoic acid (18)**: Aqueous NaOH (2 N, 12 mL, 24.0 mmol) was added to a solution of methyl ester **17** (2.02 g, 6.04 mmol) in MeOH (20 mL), THF (10 mL) and $CHCl_3$ (10 mL). The reaction mixture was stirred for 5 h at room temperature. After removal of the solvent, the residue was poured into 2 N aqueous NaOH and extracted with $CHCl_3$. The aqueous layer was neutralized by 2 N aqueous HCl and extracted with $CHCl_3$. The organic layer was separated, washed with brine, and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 10–40% EtOAc/*n*-hexane gradient, then a 10–40% MeOH/$CHCl_3$ gradient) gave 957 mg (50%) of **19** as a white solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.26 (s, 1H), 8.07 (d, *J* = 7.5 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.53–7.21 (m, 10H), 4.18 ppm (s, 2H); EIMS: *m/z* 320 [$M^+$].

**3-(3-Phenylthiomethylphenyl)-*N*-(tetrahydropyran-2-yloxy)benzamide (19)**: $NH_2OTHP$ (1.00 g, 8.55 mmol) was added to a solution of acid **18** (0.957 g, 2.99 mmol), EDCI·HCl (1.70 g, 8.85 mmol), and HOBt·$H_2O$ (1.39 g, 9.08 mmol) in DMF (25 mL), and the mixture was stirred for 30 h at room temperature. The mixture was poured into aqueous saturated $NaHCO_3$ and extracted with $Et_2O$. The organic

layer was washed with aqueous saturated $NaHCO_3$ and brine and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 10–50% EtOAc/*n*-hexane gradient) gave 1.06 g (84%) of **19** as a light yellow amorphous solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.90$ (s, 1H), 7.88 (s, 1H), 7.70 (d, $J = 7.5$ Hz, 1H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.49–7.45 (m, 3H), 7.37–7.27 (m, 6H), 7.21 (m, 1H), 5.11 (s, 1H), 4.16 (s, 2H), 4.02 (t, $J = 10.2$ Hz, 1H), 3.68–3.66 (m, 1H), 1.91–1.86 (m, 3H) 1.87–1.59 ppm (m, 3H); EIMS: *m/z* 419 [$M^+$].

**3-(3-Phenylthiomethylphenyl)-*N*-hydroxybenzamide (8)**: $TsOH \cdot H_2O$ (12.0 mg, 63 μmol) was added to a solution of compound **19** (264 mg, 0.630 mmol) in MeOH (30 mL), and the mixture was stirred for 5.5 h at room temperature. After removal of the solvent, the solid was suspended in EtOAc and collected by filtration to give 85.1 mg (40%) of **8** as a white solid. The solid was recrystallized from THF to give 51 mg of **8** as a colorless crystals: mp: 167–168 °C; 1H NMR (500 MHz, [$D_6$]DMSO): $\delta = 11.33$ (s, 1H), 9.12 (s, 1H), 7.98 (s, 1H), 7.76–7.73 (m, 2H), 7.69 (s, 1H), 7.59–7.53 (m, 2H), 7.44–7.37 (m, 4H), 7.31 (t, $J = 7.7$ Hz, 2H), 7.19 (t, $J = 7.2$ Hz, 1H), 4.33 ppm (s, 2H); $^{13}$C NMR (125 MHz, [$D_6$]DMSO): $\delta = 164.1$, 140.0, 139.5, 138.4, 136.0, 133.5, 129.3, 129.2, 129.1, 129.0, 128.5, 128.3, 127.4, 126.1, 126.0, 125.6, 125.1, 36.7 ppm; EIMS: *m/z* 335 [$M^+$]; Anal: calcd for $C_{20}H_{17}NO_2S \cdot 0.5H_2O$: C 69.74, H 5.27, N 4.07, found: C 69.72, H 5.12, N 4.17.

**3-Acetylbenzoic acid methyl ester (21)**: Concentrated $H_2SO_4$ (4 mL) was added to a solution of 3-acetylbenzoic acid (**20**) (2.02 g, 12.3 mmol) in MeOH (60 mL). The reaction mixture stirred at reflux for 26.5 h. After removal of the solvent, the residue was poured into $H_2O$ and extracted with EtOAc. The organic layer was washed with $H_2O$ and brine and dried over $Na_2SO_4$. Filtration and concentration in vacuo gave 2.12 g (97%) of **20** as a white solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.60$ (s, 1H), 8.24 (t, $J = 7.5$ Hz, 1H), 8.17 (d, $J = 7.5$ Hz, 1H), 7.57 (t, $J = 7.7$ Hz, 1H), 3.96 (s, 3H), 2.66 ppm (s, 3H); EIMS: *m/z* 178 [$M^+$].

**α-Bromo-3-acetylbenzoic acid methyl ester (22)**: $Br_2$ (0.3 mL, 5.82 mmol) and a catalytic amount of 25%HBr-AcOH were added to a solution of compound **21** (1.11 g, 6.24 mmol) in $CHCl_3$ (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The mixture was poured into aqueous saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration and concentration in vacuo gave 1.48 g (quant.) of **22** as a crude white solid: EIMS: *m/z* 256, 258 [$M^+$].

**2-(Phenylthio)acetamide (24)**: A catalytic amount of DMF was added to a solution of phenylthioacetic acid (**23**) (3.01 g, 17.9 mmol) and oxalyl chloride (4 mL, 46.6 mmol) in 20 mL of dry THF at 0 °C. The mixture was stirred at 0 °C for 0.5 h, then the solvent was removed in vacuo to give the corresponding acid chloride as a yellow solid. Aqueous 25% $NH_3$ (20 mL) was added dropwise to a solution of the acid chloride in 40 mL of THF at 0 °C. After 1.5 h, the reaction mixture was poured into aqueous saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with aqueous saturated$NaHCO_3$ and brine, and dried over $Na_2SO_4$. Filtration and concentration in vacuo gave 2.46 g (83%) of **24** as a white solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 7.34$–7.30 (m, 3H), 7.25–7.21 (m, 2H), 6.69 (broad s, 1H), 5.45 (broad s, 1H), 3.63 ppm (s, 2H); EIMS: *m/z* 167 [$M^+$].

**2-(Phenylthio)ethanethioamide (25)**: Lawesson's reagent (2.84 g, 7.02 mmol) was added to a solution of amide **24** (2.46 g, 14.7 mmol) in dry toluene (100 mL) under $N_2$ atmosphere. The mixture was stirred for 3.5 h at 80 °C. After removal of the solvent, the residue was purified by silica gel flash column chromatography (eluting with a 15–50% EtOAc/*n*-hexane gradient) to give 554 mg (21%) of **25** as a crude white solid: EIMS: *m/z* 183 [$M^+$].

**3-[2-(Phenylthiomethyl)thiazol-4-yl]benzoic acid methyl ester (26)**: A solution of thioamide **25** (555 mg, 2.16 mmol) in EtOH (10 mL) was added to a mixture of bromide **22** (1.52 g, 8.31 mmol) and activated 3 Å molecular sieves in EtOH (10 mL) and $CHCl_3$ (10 mL) under $N_2$ atmosphere. The mixture was stirred for 3 h at 70 °C. After filtration, the solvent was removed, and the residue was poured into aqueous saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with aqueous saturated $NaHCO_3$ and brine, and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 0–30% EtOAc/*n*-hexane gradient) gave 636 mg (78%) of **26** as a white solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.45$ (s, 2H), 8.11 (d, $J = 7.5$ Hz, 1H), 7.63–7.61 (m, 2H), 7.48 (d, $J = 7.5$ Hz, 2H), 7.34 (t, $J = 7.0$ Hz, 2H), 7.29–7.28 (m, 1H), 6.92 (s, 1H), 4.97 (s, 2H), 3.97 ppm (s, 3H); EIMS: *m/z* 341 [$M^+$].

**3-[2-(phenylthiomethyl)thiazol-4-yl]-*N*-hydroxybenzamide (9)**: Compound **9** (48% yield) was prepared from compound **26** according to the procedure for the preparation of compound **8**: mp: 56–60 °C; $^1$H NMR (500 MHz, $CD_3OD$): $\delta = 8.25$ (s, 1H), 8.02 (d, $J = 8.0$ Hz, 1H), 7.76 (s, 1H), 7.69 (d, $J = 7.5$ Hz, 1H), 7.50 (t, $J = 7.7$ Hz, 1H), 7.41 (d, $J = 8.0$ Hz, 2H), 7.28 (t, $J = 7.5$ Hz, 2H), 7.21 (t, $J = 8.0$ Hz, 1H), 4.53 ppm (s, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta = 171.1$, 168.0, 155.2, 136.1, 135.9, 134.2, 131.3, 130.4, 130.2, 130.1, 128.2, 127.6, 126.0, 116.3, 36.5 ppm; EIMS: *m/z* 343 [$M^+$]; Anal: calcd for $C_{17}H_{14}N_2O_2S_2 \cdot H_2O$: C 56.65, H 4.47, N 7.77, found: C 56.65, H 4.42, N 7.76.

**2-(Phenylthio)-*N*-hydroxyethanimidamide (28)**: A mixture of $NH_2OH \cdot HCl$ (3.70 g, 53.3 mmol) and KOH (3.00 g, 53.5 mmol) in MeOH (10 mL) was stirred for 30 min at room temperature. A solution of (phenylthio)acetonitrile **27** (2.00 g, 13.4 mmol) in MeOH (14 mL) was added, and the reaction mixture was stirred for an additional 20 h at room temperature. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash column chromatography (eluting with a 30–50% EtOAc/*n*-hexane gradient) to give 1.04 g (43%) of **28** as a brown oil: $^1$H NMR (500 MHz, [$D_6$]DMSO): $\delta = 9.18$ (s, 1H), 7.39 (d, $J = 8.5$ Hz, 2H), 7.29 (t, $J = 7.7$ Hz, 2H), 7.17 (t, $J = 6.7$ Hz, 1H), 5.50 (s, 2H), 3.56 ppm (s, 2H); EIMS: *m/z* 182 [$M^+$].

**3-[3-(phenylthiomethyl)-1,2,4-oxadiazol-5-yl]benzoic acid methyl ester (29)**: A solution of CDI (0.871 g, 5.37 mmol) in DMF (3 mL) was added to a solution of monomethyl isophthalate (0.937 g, 5.26 mmol) in DMF (4 mL). The mixture was stirred at room temperature for 30 min, and then a solution of **28** (0.979 g, 5.37 mmol) in DMF (7 mL) was added, and the mixture was stirred at room temperature overnight. The mixture was then heated in a microwave oven (140 °C, 2 min, 450 W). The mixture was poured into $H_2O$ and extracted with $Et_2O$. The organic layer was washed with $H_2O$ and brine and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 5–50% EtOAc/*n*-hexane gradient) gave 557 mg (33%) of **29** as a yellow solid:$^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.79$ (s, 1H), 8.30 (d, $J = 7.5$ Hz, 1H), 8.26 (d, $J = 7.0$ Hz, 1H), 7.62 (t, $J = 8.0$ Hz, 1H), 7.45 (d, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.7$ Hz, 2H), 7.26–7.24 (m, 1H), 4.22 (s, 2H), 3.97 ppm (s, 3H); EIMS: *m/z* 326 [$M^+$].

**3-[3-(Phenylthiomethyl)-1,2,4-oxadiazol-5-yl]-*N*-hydroxybenzamide (10)**: Compound **10** (17% yield) was prepared from compound **29** according to the procedure for the preparation of compound **8**: mp: 157–158 °C: $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.48$ (t,

$J$=1.5 Hz, 1H), 8.26 (d, $J$=8.0 Hz, 1H), 8.01 (dt, $J$=1.5, 8.0 Hz, 1H), 7.69 (t, $J$=8.0 Hz, 1H), 7.44 (d, $J$=7.0 Hz, 2H), 7.30 (t, $J$=7.5 Hz, 2H), 7.23 (t, $J$=7.5 Hz, 1H), 4.26 ppm (s, 2H); $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$=176.6, 170.3, 166.6, 135.8, 135.0, 132.5, 131.9, 131.8, 130.9, 130.2, 128.3, 127.7, 125.7, 29.8 ppm; EIMS: $m/z$ 327 [$M^+$]; Anal: calcd for $C_{16}H_{13}N_3O_3S$: C 58.70, H 4.00, N 12.84, found: C 58.31, H 4.14, N 12.53.

**3-Azido benzoic acid ethyl ester (31)**: An aqueous solution (30 mL) of $NaNO_2$ (3.69 g, 53.4 mmol) was added to a solution of 3-amino benzoic acid ethyl ester **30** (2.02 g, 12.2 mmol) in TFA (17 mL) at 0 °C. The mixture was stirred at 0 °C for 1.5 h, then an aqueous solution (14 mL) of $NaN_3$ (4.05 g, 62.3 mmol) was added. The solution was stirred at room temperature for 2.5 h. The mixture was poured into 2 N aqueous HCl and extracted with EtOAc. The organic layer was washed with brine and dried over $Na_2SO_4$. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (eluting with a 2–20% EtOAc/*n*-hexane gradient) gave 2.20 g (94%) of **31** as a yellow oil: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=7.82 (dt, $J$=1.0, 8.5 Hz, 1H), 7.70 (t, $J$=1.7 Hz, 1H), 7.68 (d, $J$=8.0 Hz, 1H), 7.42 (t, $J$=8.0 Hz, 1H), 7.19 (ddd, $J$=0.9, 2.4, 6.4 Hz, 1H), 4.39 (q, $J$=7.3 Hz, 2H), 1.40 ppm (t, $J$=7.0 Hz, 3H); FTIR (neat): $\tilde{\nu}$=2102 cm$^{-1}$.

**3-[4-(Phenylthiomethyl)-1H-1,2,3-triazol-1-yl]benzoic acid ethyl ester (32)**: An aqueous solution (10 mL) of $CuSO_4$ (83.7 mg, 523 μmol) and sodium ascorbate (0.585 g, 2.95 mmol) was added to a solution of phenyl propargyl sulfide (0.775 g, 5.23 mmol) and azide **31** (0.980 g, 5.12 mmol) in MeOH (10 mL). The reaction mixture was stirred for 21 h at room temperature, then filtered through Celite. After removal of the solvent, the residue was purified by silica gel flash column chromatography (eluting with a 5–40% EtOAc/*n*-hexane gradient) to give 1.17 g (67%) of **32** as a white solid: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=8.27 (t, $J$=1.7 Hz, 1H), 8.10 (dt, $J$=1.2, 8.0 Hz, 1H), 7.95 (ddd, $J$=1.0, 2.2, 6.2 Hz, 1H), 7.84 (s, 1H), 7.60 (t, $J$=8.0 Hz, 1H), 7.38 (d, $J$=7.5 Hz, 2H),7.30 (t, $J$=7.7 Hz, 2H), 7.22 (t, $J$=7.5 Hz, 1H), 4.43 (q, $J$=7.3 Hz, 2H), 4.33 (s, 2H), 1.43 ppm (t, $J$=7.2 Hz, 3H); EIMS: $m/z$ 339 [$M^+$].

**3-[4-(Phenylthiomethyl)-1H-1,2,3-triazol-1-yl]-*N*-hydroxybenzamide (11)**: Compound **11** (48% yield) was prepared from compound **32** according to the procedure for the preparation of compound **8**: mp: 179–180 °C: $^1$H NMR (500 MHz, [D$_6$]DMSO): $\delta$=11.40 (s, 1H), 9.22 (s, 1H), 8.74 (s, 1H), 8.22 (s, 1H), 8.01 (d, $J$=8.0 Hz, 1H), 7.84 (d, $J$=7.5 Hz, 1H), 7.67 (d, $J$=8.0 Hz, 1H), 7.41 (d, $J$=8.0 Hz, 2H), 7.33 (t, $J$=7.7 Hz, 2H), 7.20 (t, $J$=7.2 Hz, 1H), 4.39 ppm (s, 2H); $^{13}$C NMR (125 MHz, [D$_6$]DMSO): $\delta$=162.8, 144.9, 136.4, 135.5, 134.3, 130.1, 129.0, 128.2, 126.8, 126.0, 122.4, 121.5, 118.3, 27.1 ppm; EIMS: $m/z$ 326 [$M^+$]; Anal: calcd for $C_{16}H_{14}N_4O_2S$: C 58.88, H 4.32, N 17.17, found: C 58.69, H 4.54, N 17.00.

**3-(5-Methylthiophen-2-yl)benzoic acid methyl ester (34)**: Compound **34** (69% yield) was prepared from 3-(methoxycarbonyl)phenylboronic acid (**14**) and 2-iodo-3-methylthiophene (**33**) according to the procedure for the preparation of compound **15**: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=8.22 (s, 1H), 7.90 (d, $J$=8.0 Hz, 1H), 7.72 (d, $J$=7.5 Hz, 1H), 7.42 (t, $J$=7.7 Hz, 1H), 7.18 (d, $J$=3.5 Hz, 1H), 6.75–6.74 (m, 1H), 3.94 (s, 3H), 2.52 ppm (s, 3H); EIMS: $m/z$ 232 [$M^+$].

**3-(5-Bromomethylthiophen-2-yl)benzoic acid methyl ester (35)**: NBS (0.554 g, 3.11 mmol) and AIBN (19.2 mg, 0.116 mmol) were added to a solution of compound **34** (0.601 g, 2.61 mmol) in $CCl_4$ (20 mL). The mixture was stirred at reflux for 1 h, then diluted with $CCl_4$ and filtered. The filtrate was evaporated, poured into $H_2O$, and extracted with EtOAc. The organic layer was separated, washed with $H_2O$ and brine, and dried over $Na_2SO_4$. Filtration and concentration in vacuo gave 811 mg (quant.) of **35** as a brown oil: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$=8.24 (s, 1H), 7.96 (dt, $J$=1.4, 7.7 Hz, 1H), 7.74 (ddd, $J$=1.2, 2.0, 6.1 Hz, 1H), 7.21 (d, $J$=4.0 Hz, 1H), 7.10 (d, $J$=3.5 Hz, 1H), 4.75 (s, 2H), 3.95 ppm (s, 3H); EIMS: $m/z$ 310, 312 [$M^+$].

**3-(5-Phenylthiomethylthiophen-2-yl)-*N*-hydroxybenzamide (12)**: Compound **12** (36% yield) was prepared from compound **35** according to the procedure for the preparation of compound **8**: mp: 154–155 °C: $^1$H NMR (500 MHz, [D$_6$]DMSO): $\delta$=11.32 (s, 1H), 7.92 (s, 1H), 7.72 (d, $J$=7.5 Hz, 1H), 7.65 (d, $J$=8.5 Hz, 1H), 7.47 (t, $J$=7.5 Hz, 1H), 7.40–7.36 (m, 3H), 7.32 (t, $J$=7.7 Hz, 2H), 7.21 (t, $J$=7.2 Hz, 1H), 7.01 (d, $J$=3.5 Hz, 1H), 4.51 ppm (s, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$=163.6, 141.5, 141.5, 135.2, 133.7, 133.4, 129.2, 128.9, 128.6, 127.9, 127.5, 126.2, 125.8, 123.7, 123.2, 31.6 ppm; EIMS: $m/z$ 341 [$M^+$]; Anal: calcd for $C_{18}H_{15}NO_2S_2 \cdot 0.5H_2O$: C 61.19, H 4.60, N 4.00, found: C 61.77, H 4.76, N 4.10.

### *Biology*

*Enzyme assays*: HDAC activity assays were performed using an HDACs/HDAC8 deacetylase fluorimetric assay kit (CY-1150/CY-1158, Cyclex Company Limited), an HDAC1/HDAC6 fluorescent activity drug discovery kit (AK-511/AK-516, BIOMOL Research Laboratories), a Fluorescent SIRT1 Activity Assay/Drug Discovery Kit (AK-555, BIOMOL Research Laboratories) or and a Fluorogenic HDAC Class2α Assay Kit (BPS Bioscience Incorporated), with total HDACs (CY-1150, Cyclex Company Limited), HDAC1 (SE-456, BIOMOL Research Laboratories), HDAC2 (SE-500, BIOMOL Research Laboratories), HDAC3/NCOR1 complex (SE-515, BIOMOL Research Laboratories), HDAC4 (BPS Bioscience Incorporated), HDAC6 (SE-508, BIOMOL Research Laboratories), and HDAC8 (CY-1158, Cyclex Company Limited), according to the supplier's instructions. Fluorescence of the wells was measured on a fluorimetric reader with excitation set at 360 nm and emission detection set at 460 nm, and the percent inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The concentration of a compound that results in 50% inhibition ($IC_{50}$) was determined by plotting log[inhibitor] versus the log function of percent inhibition.

*Western blot analysis*: The cohesin, α-tubulin, or H3K9 acetylating activities of the test compounds were assayed according to published methods.[3,19].

*Cell growth inhibition assay*: Cells were plated at an initial density of $2\times10^5$ cells per well (50 μL per well) in 96-well plates in RPMI 1640 medium with 10% fetal bovine serum, and were exposed to inhibitors for 72 h at 37 °C in a 5% $CO_2$ incubator. A solution of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium inner salt (MTS) was then added (20 μL per well), and incubation was continued for 2 h. The solubilized dye was quantified by colorimetric reading at 490 nm using a reference wavelength of 650 nm. The absorbance values of control wells ($C$) and test wells ($T$) were measured. The absorbance of the test wells was also measured at time 0 ($T_0$, addition of compounds). Using these measurements, cell growth inhibition (percentage of growth) by a test inhibitor at each concentration used was calculated as: % growth = $100\times[(T-T_0)/(C-T_0)]$, when $T>T_0$ and % growth = $100\times[(T-T_0)/T]$, when $T<T_0$. Computer analysis of the percent growth values afforded a 50% growth inhibition parameter ($GI_{50}$). The $GI_{50}$ was calculated as $100\times[(T-T_0)/(C-T_0)]=50$.

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