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Effects of *E*/*Z* Configuration of Fluoroalkene-containing HDAC Inhibitors on Selectivity for HDAC Isoforms

Yoshiro Chuman,[1] Mariko Ueyama,[1] Satoshi Sano,[2] Fei Wu,[1,†] Yuhei Kiyota,[1] Takayoshi Higashi,[1] Satoshi Osada,[*2] and Kazuyasu Sakaguchi[*1]
[1]*Department of Biological Chemistry, Division of Chemistry, Graduate School of Science, Hokkaido University, Kita 8 Nishi 5, Sapporo, Hokkaido 060-0808*
[2]*Department of Chemistry and Applied Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo, Saga 840-8502*

(Received March 21, 2013; CL-130243; E-mail: kazuyasu@sci.hokudai.ac.jp)

Histone deacetylase (HDAC) inhibitors belong to a new class of potential anticancer agents. It may be possible to reduce some of the toxicity by specifically targeting only the HDAC isoform. Here, stereoisomeric HDAC inhibitors containing fluoroalkene were analyzed for their specificity toward HDAC isoforms. *Z*-Form **1(*Z*)** showed high affinity to HDACs whereas *E*-isoform **1(*E*)** had lower affinity to HDAC1 and HDAC4. These data suggested that introduction of alkene with *E*/*Z* configuration to HDAC inhibitor can be a new strategy to develop the isoform-selective HDAC inhibitors.

Histone deacetylases (HDACs) have crucial roles in numerous biological processes. There are eighteen encoded human HDACs, which are classified as class I (HDAC1, -2, -3, and -8), class II (HDAC4, -5, -6, -7, -9, and -10), class III (SIRT1-7), and class IV (HDAC11) enzymes. Recent studies of knockout mice lacking HDAC genes have revealed highly specific functions for individual HDAC isoforms.[1] These facts emphasize the importance of developing HDAC-isoform specific inhibitors.

A number of HDAC inhibitors have been reported to date, and they were used as anticancer drugs and reagents for serial animal cloning without clone-specific abnormalities.[2,3] HDAC inhibitors, including SAHA, typically consist of a zinc-binding group that coordinates to the catalytic metal atom within the HDAC active site, a linker that accommodates the tubular access of the active site, and a capping group that interacts with the residues at the entrance of the active site. It is very important to have moieties to enhance isoform selectivity to reduce side effects. Replacement of hydroxamic acid as the zinc-binding group has been carried out to produce new types of HDAC isozyme-selective inhibitors.[2] Recently, we developed HDAC inhibitors containing a sulfanylmethyl group attached with fluoroalkene. As the fluoroalkene is known for nonhydrolyzable amide mimetic, it is stable with respect to hydrolytic cleavage.[4] The thiol group may interact with zinc ion in a chelating manner[5] (Figure 1). The *Z*-isomer **1(*Z*)** compound showed the stronger general HDAC inhibitory activity using HeLa extract than SAHA.[6] Here, we designed and synthesized the stereoisomer, *E*-isomer **1(*E*)** to control the special configuration of thiol group. The effects of *E*- and *Z*-isomers on HDAC inhibitory activity and selectivity were analyzed against various HDAC-isoforms.

The synthesis of **1(*E*)** is outlined in Scheme 1. As outlined in Scheme 1, synthesis of the complementary **1(*E*)** was started from bromide **A**, which was used for preparation of *Z*-isomer.[7]

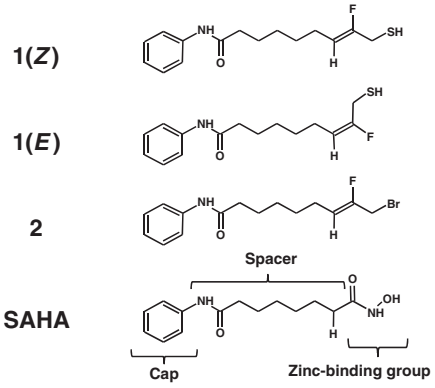
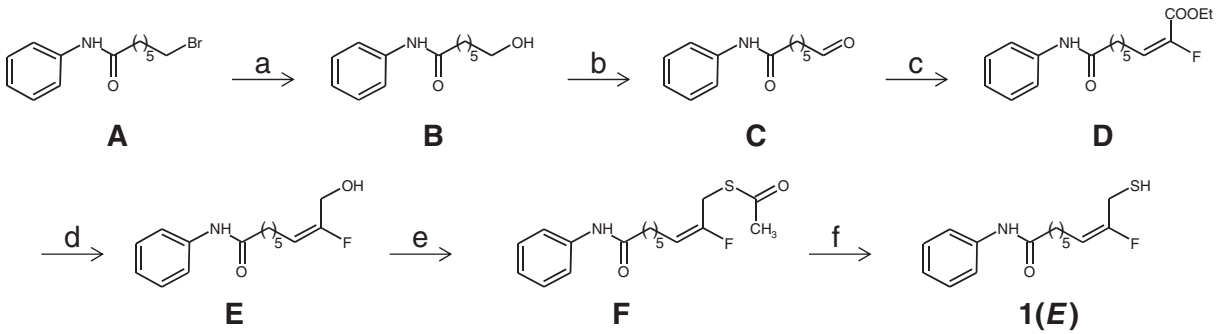


Figure 1. Structure of HDAC inhibitors.



Scheme 1. Reagents and conditions: (a) (i) AcOK, 18-crown-6, CH_3CN, (ii) NaOH, H_2O–MeOH, 82%; (b) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, 87%; (c) $(EtO)_2P(O)CHFCOOEt$, *n*-BuLi, THF, 87%; (d) $NaBH_4$, LiCl, THF–EtOH, 62%; (e) (i) MsCl, Et_3N, THF, (ii) AcSK, EtOH, 82%; (f) K_2CO_3, MeOH, 88%.

Table 1. HDAC inhibitor and their inhibitory activities[a]

	IC_{50}/μM				
	HeLa extract	Class I		Class IIa	Class IIb
		HDAC1	HDAC8	HDAC4	HDAC6
1(*Z*)	0.4	2.9	1.9	1.3	0.8
1(*E*)	0.8	8.6	4.5	9.1	2.3
2	N.A.	N.D.	N.D.	N.D.	N.D.
SAHA	1.1	5.5	5.8	6	1.7

[a]N.A.: not active, N.D.: not determined.

At first, bromide **A** was converted to acetate by treating with potassium acetate, and the acetate was saponified to give alcohol **B**.[8] The obtained alcohol **B** was converted to aldehyde **C** by the Swern oxidation, and the aldehyde **C** was subjected to the Horner–Wadsworth–Emmons (HWE) olefination.[9] In contrast to the syn-elimination of sulfoxide to form fluoroalkene with *Z*-selectivity,[4] the kinetically controlled HWE reaction by the lithium salt of triethyl 2-fluoro-2-phosphonoacetate resulted in the formation of fluoroalkene **D** with *E*-selectivity. Further transformations to construct a thiol moiety followed procedures described for *Z*-isomer.[10] Thus, *E*-fluoroalkene **D** was reduced to the allyl alcohol **E** using in situ formed $LiBH_4$;[11] the alcohol **E** was activated as mesylate, and treatment of **E** with AcSK gave thioacetate **F**.[12] Finally, alkaline hydrolysis gave the desired thiol **1(*E*)** in satisfactory yield.[13] Purities of **1(*E*)** and **1(*Z*)** were confirmed to be more than 95% by ^{1}H NMR analysis.

In order to evaluate the effect of stereoisomerization on HDAC inhibitory activity, synthetic compounds were analyzed for HDAC inhibitory activity in vitro using a HeLa nuclear extract as described.[6] The inhibitory activity of **1(*E*)** was very strong and comparable with that of SAHA. **1(*Z*)** showed almost threefold stronger activity than SAHA (Table 1). No inhibitory activity was detected in compound **2**, in which the bromo group was substituted for thiol group in **1(*Z*)**, indicating that the thiol group, not the bromine group, was essential for HDAC inhibitory activity. These data suggested that the difference of stereoisomer of fluoroalkene affected the inhibitory activity for HDACs.

The stereoisomeric inhibitors were analyzed for their specificity to various subtypes of HDACs (HDAC1 and HDAC8 for class I, HDAC4 for class IIa, and HDAC6 for class IIb). For all subtypes, **1(*Z*)** showed much stronger inhibitory activity than SAHA (Table 1). However, the selectivity of **1(*Z*)** among the subtypes was very low. **1(*E*)** also showed strong activity for the subtypes analyzed. Interestingly, inhibitory activities of **1(*E*)** against HDAC1 and HDAC4 decreased compared with that of **1(*Z*)**, suggesting that the inhibitory activity against HDAC1 and HDAC4 was effectively decreased by *E*-isomer. SAHA is known to take a stick form when it binds with class-II-type HDAC.[14] These data suggested that the particular configuration of sulfur and fluorine should be important for chelating with Zn^{2+} ion.

Next, we analyzed the effects of the compounds on HDAC inhibitory activity in the cells. The HDAC inhibitors were known to induce the cell cycle arrest and apoptosis through the induction of p21.[15] In order to evaluate the biological functions of the stereoisomer, the compounds were applied to HeLa human cervical cancer cells to analyze the induction of p21.

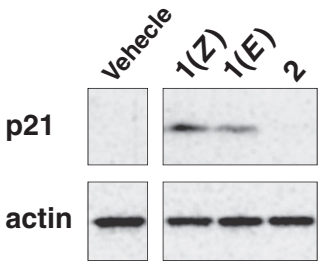


Figure 2. Induction of p21 in HeLa cells by HDAC inhibitors (25 μM).

After 24 h of incubation, induction of p21 was observed with the treatment of **1(*Z*)**- or **1(*E*)**-treated HeLa cells, whereas no induction was observed with compound **2** (Figure 2). **1(*Z*)** showed stronger induction of p21 than **1(*E*)**. This data was well consistent with that of their in vitro HDAC inhibitory activity.

The compound **1(*Z*)** with *Z*-configuration showed significantly higher activities to HDACs, including HDAC6 compared to SAHA. On the other hand, *E*-form **1(*E*)** decreased the inhibitory activities to HDAC1 and especially to HDAC4, resulting in preference of *E*-form **1(*E*)** to HDAC6. Although SAHA showed higher affinity to HDAC6 as previously reported, the HDAC6-selectivity over HDAC4 by **1(*E*)** is higher than that by SAHA.[16,17] These suggested that the configuration of thiol and/or fluorine in **1(*E*)** is suitable for HDAC6 but not HDAC4. The zinc-binding region connected with the linker region in **1(*E*)** is estimated to form a wide bended structure by *E*-configuration, although **1(*Z*)** forms a thin straight structure by *Z*-configuration (Figure 1). Butler et al. reported that a homology model of HDAC6 showed a wider channel rim than that of HDAC1 did.[18] The crystal structure of HDAC4 (PDB: 2VQM) also showed that the channel rim of HDAC4 was narrower than those of HDAC1 and HDAC6.[19] These suggested that **1(*E*)** with wide and bended structure might provide a possible binding conformation to HDAC6 with a wider channel rim, resulting in HDAC6 selectivity by **1(*E*)**.

In summary, the introduction of the sulfanylmethyl group with *E*- or *Z*-form into HDAC inhibitors should be a useful strategy to develop the isoform-selective HDAC inhibitors through the different configuration of zinc-binding position in inhibitors. Although several HDAC6 selective inhibitors has been reported,[17,20] further optimization of the compound might lead to the development of useful HDAC6-specific inhibitors as not only anticancer drugs but also antidepression drugs.

References and Notes

† Present address: College of Food Sciences, Northeast Agricultural University, Harbin 150030, P. R. China
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7 Melting points (uncorrected) were recorded on a Yanako MPS3 micro-melting point apparatus. All commercial reagents were used without further purification unless otherwise noted. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were obtained using a JEOL AL-300 spectrometer (300, 75, and 283 MHz, respectively). All chemical shifts are reported in ppm as δ values relative to internal tetramethylsilane (^{1}H and ^{13}C) or benzotrifluoride (^{19}F) in $CDCl_3$ unless otherwise noted. Multiplicities are described using the abbreviations s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. HRMS spectra were acquired using a JEOL GCmateII mass spectrometer or a JEOL JMS-700 mass spectrometer. Tetrahydrofuran was distilled from sodium benzophenone ketyl prior to use. All the manipulations with air-sensitive reagents were performed under a dry argon atmosphere. Analytical TLC was performed using Merck Silica Gel 60 F_{254} plates (0.25 mm on glass). Flash column chromatography was performed using either Wakogel C-300 (45–75 mm).

8 To a solution of compound **A** (4.16 g, 14.6 mmol) dissolved in CH_3CN (70 mL) was added 18-crown-6 (0.20 g, 0.76 mmol) and AcOK (5.74 g, 58.5 mmol). The mixture was refluxed for 20 h, then cooled to rt. Solvent was removed in vacuo and the residue was dissolved in AcOEt. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in MeOH (140 mL), 1 M aqueous NaOH solution (20 mL) was slowly added. After stirring for 30 min, the mixture was concentrated and extracted with AcOEt. The organic solution was washed with water and brine, and dried over Na_2SO_4. Solvent was removed in vacuo to give B as white crystals, which is pure enough for the next reaction (2.64 g, 82%). An analytical sample was chromatographically purified. Mp 72–74 °C; ^{1}H NMR: δ 7.51 (d, $J = 7.8$ Hz, 2H), 7.32–7.27 (m, 3H), 7.08 (t, $J = 7.5$ Hz, 1H), 3.63 (t, $J = 6.5$ Hz, 2H), 2.34 (t, $J = 7.4$ Hz, 2H), 1.73 (quin, $J = 7.1$ Hz, 2H), 1.63 (br s, 1H), 1.56 (quin, $J = 6.6$ Hz, 2H), 1.42–1.37 (m, 4H); ^{13}C NMR: δ 172.0, 138.1, 128.8, 124.1, 119.9, 62.5, 37.3, 32.3, 28.8, 25.5, 25.3; HRMS (FAB) m/z: $(M + H)^+$ calcd for $C_{13}H_{20}NO_2$ 222.1494, found 222.1486.

9 A solution of DMSO (1.2 g, 15 mmol) in CH_2Cl_2 (2.5 mL) was added to a solution of oxalyl chloride (0.74 mL, 8.0 mmol) in CH_2Cl_2 (10 mL) at −78 °C. The reaction mixture was kept for 15 min at −78 °C, and a solution of compound **B** (1.0 g, 4.6 mmol) in CH_2Cl_2 (20 mL) was slowly added over 50 min. The reaction mixture was stirred for 15 min at −60 °C. Et_3N (4.5 mL, 32 mmol) was then added at −60 °C, the cooling bath was removed, and the reaction mixture was stirred for 2 h at rt. The reaction mixture was quenched by the addition of 1 M aqueous HCl solution (10 mL), and the aqueous solution was extracted with CH_2Cl_2. The combined organic phases were washed with aqueous $NaHCO_3$, water, and brine. After drying the organic phase over Na_2SO_4, solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate = 1/1) to give **C** as white crystals (0.88 g, 87%). Mp 51–52 °C; ^{1}H NMR analysis is consistent with published data.[3]

10 *n*-Butyllithium (2.5 mL of 1.54 M solution in hexane, 3.8 mmol) was added slowly to a stirred solution of triethyl 2-fluoro-2-phosphonoacetate (0.71 mL, 3.5 mmol) in THF (11 mL) at −78 °C. The reaction mixture was then stirred at the same temperature for further 20 min. A solution of compound **C** (0.70 g, 3.2 mmol) in THF (8 mL) was added to the above reaction mixture, stirred at −78 °C for 30 min, allowed to warm slowly to rt, and maintained for 2 h. The reaction mixture was quenched by the addition of 1 M aqueous HCl solution (10 mL), and the aqueous solution was extracted with AcOEt. The organic extracts were washed with aqueous $NaHCO_3$, water, and brine. After drying the organic phase over Na_2SO_4, solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate = 3/2) to give D as a colorless oil (0.86 g, 87%). ^{1}H NMR: δ 7.54 (br s, 1H), 7.46 (d, $J = 7.5$ Hz, 2H), 7.22 (t, $J = 7.7$ Hz, 2H), 7.01 (t, $J = 7.4$ Hz, 1H), 5.87 (dt, $J = 22$, 8.3 Hz, 1H), 4.22 (q, $J = 7.2$ Hz, 2H), 2.48–2.40 (m, 2H), 2.27 (t, $J = 7.5$ Hz, 2H), 1.66 (quin, $J = 7.2$ Hz, 2H), 1.42–1.23 (m, 4H), 1.26 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR: δ 171.4, 160.8 (d, $J = 36$ Hz), 145.3 (d, $J = 252$ Hz), 138.0, 128.8, 124.1, 123.3 (d, $J = 18$ Hz), 119.8, 61.3, 37.3, 28.7, 28.5, 25.2, 25.1, 14.0; ^{19}F NMR: δ −123.7 (d, $J = 22$ Hz); HRMS (FAB) m/z: $(M + H)^+$ calcd for $C_{17}H_{23}FNO_3$ 308.1662, found 308.1658.

11 $NaBH_4$ (0.25 g, 6.6 mmol) was slowly added to a solution of LiCl (0.28 g, 6.6 mmol) dissolved in EtOH (3.7 mL). A solution of **D** (0.40 g, 1.3 mmol) dissolved in THF (1.5 mL) was added to this suspension at 0 °C, and this mixture was stirred for 48 h at room temperature. The reaction was quenched with aqueous NH_4Cl and the solution was evaporated to remove EtOH. The residue was extracted with AcOEt, and the organic extracts were successively washed with saturated $NaHCO_3$ solution and brine. After drying the organic phase over Na_2SO_4, solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate = 1/1) yielding E as colorless crystals (0.22 g, 62%). Mp 60–61 °C; ^{1}H NMR: δ 7.47 (d, $J = 7.8$ Hz, 2H), 7.32–7.27 (m, 3H), 7.08 (t, $J = 7.4$ Hz, 1H), 5.11 (dt, $J = 21$, 8.3 Hz, 1H), 4.19 (dd, $J = 21$, 5.6 Hz, 2H), 2.36–2.31 (m, 3H), 2.09 (t, $J = 7.2$ Hz, 2H), 1.70 (quin, $J = 7.4$ Hz, 2H), 1.44–1.32 (m, 4H); ^{13}C NMR: δ 172.2, 156.2 (d, $J = 249$ Hz), 138.0, 128.8, 124.1, 120.0, 108.6 (d, $J = 19$ Hz), 56.5 (d, $J = 31$ Hz), 37.2, 29.0, 27.9, 24.9, 24.4; ^{19}F NMR: δ −115.0 (dt, $J = 21$, 21 Hz); HRMS (FAB) m/z: $(M + H)^+$ calcd for $C_{15}H_{21}NO_2F$ 266.1556, found 266.1559.

12 To a solution of **E** (0.21 g, 0.79 mmol) and Et_3N (0.18 mL, 1.3 mmol) dissolved in THF (7.9 mL) was slowly added $MeSO_2Cl$ (0.10 mL, 1.3 mmol) at 0 °C. After the addition, the mixture was stirred for 6 h at room temperature. The precipitate was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved in ethanol (3.2 mL), AcSK (0.35 g, 3.1 mmol) was added to the solution, and the mixture was stirred for 13 h at room temperature. This mixture was diluted with AcOEt, washed with water and brine. After drying the organic phase over Na_2SO_4, solvent was removed in vacuo and the residue purified by flash chromatography (hexane/ethyl acetate = 3/2) yielding F (0.25 g, 96%) as a white powder. Mp 61–62 °C; ^{1}H NMR: δ 7.46 (d, $J = 7.8$ Hz, 2H), 7.27–7.22 (m, 3H), 7.03 (t, $J = 7.5$ Hz, 1H), 5.11 (dt, $J = 20$, 8.1 Hz, 1H), 3.72 (d, $J = 22$ Hz, 2H), 2.31–2.26 (m, 5H), 1.99 (q, $J = 6.9$ Hz, 2H), 1.71–1.66 (m, 2H), 1.36–1.32 (m, 4H); ^{13}C NMR: δ 194.8, 171.4, 152.9 (d, $J = 245$ Hz), 138.0, 128.9, 124.1, 119.8, 108.8 (d, $J = 20$ Hz), 37.4, 30.3, 29.1, 28.4, 26.5, 26.1, 25.2; ^{19}F NMR: δ −106.8 (dt, $J_{H-F} = 21$, 21 Hz); HRMS (FAB) m/z: $(M + H)^+$ calcd for $C_{17}H_{23}NO_2FS$ 324.1434, found 324.1437.

13 To a solution of compound **1(*E*)** (0.14 g, 0.40 mmol) dissolved in MeOH (4 mL) was added K_2CO_3 (290 mg, 0.21 mmol), and this mixture was stirred for 1 h at room temperature. The mixture was diluted with AcOEt, washed with water and brine, and dried over Na_2SO_4. Solvent was removed in vacuo and the residue was purified by flash chromatography (hexane/ethyl acetate = 3/2), yielding **1(*E*)** (0.10 g, 88%) as a white solid. Mp 47–48 °C; ^{1}H NMR: δ 7.48 (d, $J = 7.8$ Hz, 2H), 7.30 (t, $J = 7.8$ Hz, 2H), 7.11–7.06 (m, 3H), 5.01 (dt, $J = 20$, 8.1 Hz, 1H), 3.23 (dd, $J = 22$, 7.8 Hz, 2H), 2.34 (t, $J = 8.0$ Hz, 2H), 1.95 (q, $J = 7.2$ Hz, 2H), 1.83 (t, $J = 8.0$ Hz, 1H), 1.73 (quin, $J = 7.2$ Hz, 2H), 1.41–1.38 (m, 4H); ^{13}C NMR: δ 171.2, 155.8 (d, $J = 245$ Hz), 137.8, 128.9, 124.2, 119.8, 106.6 (d, $J = 21$ Hz), 37.5, 29.4, 28.6, 25.3, 21.3, 20.9; ^{19}F NMR: δ −109.9 (dt, $J = 21$, 21 Hz); HRMS (FAB) m/z: $(M + H)^+$ calcd for $C_{15}H_{21}NOFS$ 282.1328, found 282.1319.

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