of **15** as a colorless crystal: mp 130–133 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.53 (2H, d, J = 7.3 Hz), 7.32 (2H, t, J = 7.9 Hz), 7.25 (1H, broad s), 7.10 (1H, t, J = 7.2 Hz), 6.25 (1H, broad s), 3.51 (2H, s), 3.25 (2H, q, J = 6.6 Hz), 2.39 (3H, s), 2.37 (2H, t, J = 7.4 Hz), 1.75 (2H, quintet, J = 7.6 Hz), 1.55 (2H, quintet, J = 7.1 Hz), 1.39 (2H, quintet, J = 7.3 Hz); MS (EI) *m/z*: 322 (M^+); Anal. ($C_{16}H_{22}N_2O_3S$) C, H, N.

6-(2-Mercaptoacetylamino)hexanoic Acid Phenylamide (14). To a solution of **15** (190 mg, 0.59 mmol) obtained above in MeOH (5 mL) was added K_2CO_3 (141 mg, 1.02 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with AcOEt and THF, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography ($CHCl_3$/MeOH = 20/1) gave 103 mg (62%) of **14** as a white solid. The solid was recrystallized from $CHCl_3$–MeOH to give 38 mg of **14** as a colorless crystal: mp 171–173 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 9.84 (1H, s), 8.08 (1H, broad s), 7.57 (2H, d, J = 8.2 Hz), 7.27 (2H, t, J = 7.9 Hz), 7.00 (1H, t, J = 7.3 Hz), 3.90 (1H, s), 3.44 (2H, s), 3.07 (2H, q, J = 6.5 Hz), 2.28 (2H, t, J = 7.5 Hz), 1.59 (2H, quintet, J = 7.3 Hz), 1.45 (2H, quintet, J = 7 Hz), 1.31 (2H, quintet, J = 7.5 Hz); MS (EI) *m/z*: 280 (M^+); Anal. ($C_{14}H_{20}N_2O_2S$) C, H, N.

6-(2-Propynylamino)hexanoic Acid Phenylamide Hydrochloride Salt (16·HCl) and 6-(2-Dipropynylamino)-hexanoic Acid Phenylamide (17). To a solution of **58** (230 mg, 1.12 mmol) obtained above and K_2CO_3 (39 mg, 0.28 mmol) in MeOH (1 mL) was added propargyl bromide (38 mg, 0.32 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel flash chromatography ($CHCl_3$/MeOH = 15/1) to give 40 mg (51%) of **16** as a pale yellow oil and 12 mg (23%) of **17** as a pale yellow solid. To a solution of **16** in MeOH was added 1 N aqueous HCl (0.5 mL), and the solution was concentrated in vacuo. The residue was recrystallized from MeOH–AcOEt to give 16 mg of **16**·HCl as colorless needles: mp 161–165 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm) 9.91 (1H, broad s), 9.12 (2H, broad s), 7.59 (2H, d, J = 7.6 Hz), 7.28 (2H, t, J = 7.9 Hz), 7.01 (1H, t, J = 7.3 Hz), 3.89 (2H, d, J = 3.4 Hz), 3.70 (1H, t, J = 2.6 Hz), 2.94 (2H, t, J = 7.8 Hz), 2.32 (2H, t, J = 7.3 Hz), 1.64–1.56 (4H, m), 1.36–1.25 (2H, m); MS (EI) *m/z*: 244 (M^+–HCl); Anal. ($C_{15}H_{20}N_2O$·HCl·1/8H_2O) C, H, N.

The crude solid of **17** was recrystallized from $CHCl_3$–*n*-hexane to give 12 mg of **17** as colorless needles: mp 56–57 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8.3 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.11–7.10 (2H, m), 3.43 (4H, d, J = 2.4 Hz), 2.55 (2H, t, J = 7.3 Hz), 2.37 (2H, t, J = 7.4 Hz), 2.22 (2H, t, J = 2.3 Hz), 1.79–1.75 (2H, m), 1.54–1.52 (2H, m), 1.45–1.43 (2H, m); MS (EI) *m/z*: 281 (M^+); Anal. ($C_{18}H_{22}N_2O$) C, H, N.

7-Hydroxysulfamoylheptanoic Acid Phenylamide (6). Steps 1 and 2: Preparation of 7-Chlorosulfonylheptanoic Acid Ethyl Ester (60). To an aqueous solution (7 mL) of anhydrous sodium sulfite (2.03 g, 16.1 mmol) was added a solution of 7-bromoheptanoic acid ethyl ester (**59**, 2.0 g, 8.43 mmol) in EtOH (5 mL), and the solution was boiled under reflux with stirring for 2 h. The solution was evaporated to dryness, and the solid was dried in vacuo at 60 °C. This white solid was placed in a flask, toluene (30 mL) was added followed by a catalytic amount of DMF, and then thionyl chloride (6.2 mL, 85.0 mmol) was added dropwise. The mixture was boiled under reflux with stirring for 5 h, diluted with AcOEt, washed with aqueous saturated cold water and brine, and dried over $MgSO_4$. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1) gave 2.02 g (93%) of **60**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 4.13 (2H, q, J = 7.1 Hz), 3.66 (2H, t, J = 7.8 Hz), 2.31 (2H, t, J = 7.3 Hz), 2.06 (2H, quintet, J = 7.8 Hz), 1.66 (2H, quintet, J = 7.3 Hz), 1.53 (2H, quintet, J = 7.8 Hz), 1.41 (2H, quintet, J = 7.1 Hz), 1.26 (2H, quintet, J = 7.1 Hz).

Steps 3, 4, and 5: Preparation of 7-(2-Tetrahydropyranyloxysulfamoyl)heptanoic Acid Phenylamide (61). To a mixture of *O*-(2-tetrahydropyranyl)hydroxylamine (251 mg, 2.14 mmol), a catalytic amount of 4-(dimethylamino)pyridine, pyridine (1 mL), and CH_2Cl_2 (10 mL) was added a solution of **60** (500 mg, 1.95 mmol) obtained above in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water, saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 2/1) gave 618 mg (94%) of the sulfonamide as a crude oil.

To a solution of the sulfonamide(615 mg, 1.82 mmol) obtained above in EtOH (3 mL) was added 2 N aqueous NaOH (3.0 mL, 6.0 mmol). The mixture was stirred overnight at room temperature. The solvent was removed by evaporation in vacuo, and water was added to the residue. The mixture was neutralized with 2 N aqueous HCl (3.0 mL, 6.0 mmol) with cooling in an ice–water bath, and the mixture was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo gave 482 mg (86%) of the carboxylic acid as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.40 (1H, broad s), 5.08 (1H, m), 3.93 (1H, m), 3.66 (1H, m), 3.21 (2H, m), 2.37 (2H, t, J = 7.3 Hz), 1.90–1.35 (14H, m).

Compound **61** was prepared from the carboxylic acid obtained above and aniline using the procedure described for **13** in 88% yield: ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm) 10.04 (1H, broad s), 9.85 (1H, broad s), 7.58 (2H, d, J = 8 Hz), 7.28 (2H, t, J = 7.8 Hz), 7.01 (1H, t, J = 7.6 Hz), 4.88 (1H, m), 3.81 (1H, m), 3.52 (1H, m), 3.19–3.09 (2H, m), 2.30 (2H, t, J = 7.3 Hz), 1.80–1.25 (14H, m).

Step 6: Preparation of 7-Hydroxysulfamoylheptanoic Acid Phenylamide (6). Compound **6** was prepared from **61** obtained above using the procedure described for **12** (step 2) in 61% yield: mp 137–139 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm) 9.85 (1H, broad s), 9.51 (1H, d, J = 3.2 Hz), 9.13 (1H, d, J = 3.2 Hz), 7.58 (2H, d, J = 8 Hz), 7.28 (2H, t, J = 7.8 Hz), 7.01 (1H, t, J = 7.3 Hz), 3.09 (2H, t, J = 7.6 Hz), 2.30 (2H, t, J = 7.3 Hz), 1.68 (2H, quintet, J = 8 Hz), 1.59 (2H, quintet, J = 7.6 Hz), 1.41 (2H, quintet, J = 7.8 Hz), 1.32 (2H, quintet, J = 7.1 Hz); Anal. ($C_{13}H_{20}N_2O_4S$·1/20H_2O) C, H, N.

Thioacetic acid S–(6-phenylcarbamoylhexyl) Ester (8a). Steps 1, 2, and 3: Preparation of 7-Bromoheptanoic Acid Phenylamide (64c). 7-Bromoheptanoic acid was prepared from **59** using the procedure described for **6** (step 4) in 99% yield. In this case, LiOH was used instead of NaOH: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 3.41 (2H, t, J = 6.8 Hz), 2.37 (2H, t, J = 7.3 Hz), 1.87 (2H, quintet, J = 6.8 Hz), 1.66 (2H, quintet, J = 7.6 Hz), 1.54–1.32 (4H, m).

To a suspension of 7-bromoheptanoic acid (2.64 g, 12.6 mmol) obtained above in CH_2Cl_2 (30 mL) were added oxalyl chloride (1.65 mL, 18.9 mmol) and a catalytic amount of DMF. The mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation in vacuo to give acid chloride **62c**.

To a solution of aniline (3.50 g, 37.6 mmol) and triethylamine (5.30 mL, 38.1 mmol) in CH_2Cl_2 (40 mL) was added a solution of **62c** obtained above in CH_2Cl_2 (10 mL) dropwise cooling in an ice–water bath. The mixture was stirred at room temperature for 1 h. It was diluted with AcOEt and washed with aqueous saturated $NaHCO_3$, water, and brine, before being dried over $MgSO_4$. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 3/1) gave 3.13 g (87%) of **64c**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.15 (1H, broad s), 7.10 (1H, t, J = 7.6 Hz), 3.41 (2H, t, J = 6.8 Hz), 2.36 (2H, t, J = 7.3 Hz), 1.87 (2H, quintet, J = 7.1 Hz), 1.75 (2H, quintet, J = 7.3 Hz), 1.49 (2H, quintet, J = 7.6 Hz), 1.41 (2H, quintet, J = 6.8 Hz).

Step 4: Preparation of Thioacetic acid *S*-(6-Phenylcarbamoylhexyl) Ester (8a). Compound **8a** was prepared from **64c** obtained above using the procedure described for **15** in 98% yield: mp 80–81 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.3 Hz), 7.22

(1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.86 (2H, t, J = 7.1 Hz), 2.35 (2H, t, J = 7.3 Hz), 2.32 (3H, s), 1.73 (2H, quintet, J = 7.1 Hz), 1.59 (2H, quintet, J = 7.1 Hz), 1.40 (4H, m); MS (EI) *m/z*: 279 (M^+); Anal. ($C_{15}H_{21}NO_2S$) C, H, N.

7-Mercaptoheptanoic Acid Phenylamide (7) and 7-(6-Phenylcarbamoylhexyldisulfanyl)heptanoic Acid Phenylamide (37). Compounds **7** and **37** were prepared from **8a** using the procedure described for **6** (step 4) in 87% and 4% yield, respectively.

7: mp 88–89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.12 (1H, broad s), 7.10 (1H, t, J = 7.1 Hz), 2.53 (2H, q, J = 7.3 Hz), 2.36 (2H, t, J = 7.6 Hz), 1.74 (2H, quintet, J = 7.1 Hz), 1.63 (2H, quintet, J = 7.1 Hz), 1.42 (4H, m), 1.33 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 237 (M^+); Anal. ($C_{13}H_{19}NOS$) C, H, N.

37: mp 105–107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (4H, d, J = 8 Hz), 7.41 (2H, broad s), 7.30 (4H, t, J = 7.8 Hz), 7.09 (2H, t, J = 7.3 Hz), 2.68 (4H, t, J = 7.3 Hz), 2.36 (4H, t, J = 7.6 Hz), 1.74 (4H, quintet, J = 7.3 Hz), 1.69 (4H, quintet, J = 7.1 Hz), 1.50–1.34 (8H, m); MS (EI) *m/z*: 472 (M^+); Anal. ($C_{26}H_{36}N_2O_2S_2$) C, H, N.

Compounds **19–21, 24, 26–31,** and **32** were prepared from **62** and an appropriate aromatic amine using the procedure described for **8a** and **7**.

8-Mercaptooctanoic acid phenylamide (19): mp 84–86 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.14 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.52 (2H, q, J = 7.3 Hz), 2.35 (2H, t, J = 7.6 Hz), 1.73 (2H, quintet, J = 7.3 Hz), 1.61 (2H, quintet, J = 7.1 Hz), 1.46–1.34 (6H, m), 1.33 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 251 (M^+); Anal. ($C_{14}H_{21}NOS$) C, H, N.

6-Mercaptohexanoic acid phenylamide (20): mp 84–85 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.16 (1H, broad s), 7.11 (1H, t, J = 7.8 Hz), 2.55 (2H, q, J = 7.1 Hz), 2.37 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.8 Hz), 1.68 (2H, quintet, J = 7.6 Hz), 1.56–1.40 (2H, m), 1.35 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 223 (M^+); Anal. ($C_{12}H_{17}NOS$) C, H, N.

5-Mercaptopentanoic acid phenylamide (21): mp 120–121 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 7.6 Hz), 7.33 (2H, t, J = 8 Hz), 7.16 (1H, broad s), 7.11 (1H, t, J = 7.8 Hz), 2.58 (2H, q, J = 6.4 Hz), 2.39 (2H, t, J = 6.8 Hz), 1.85 (2H, quintet, J = 7.8 Hz), 1.71 (2H, quintet, J = 7.6 Hz), 1.39 (1H, t, J = 8 Hz); MS (EI) *m/z*: 209 (M^+); Anal. ($C_{11}H_{15}NOS \cdot 1/12H_2O$) C, H, N.

7-Mercaptoheptanoic acid (4-dimethylaminophenyl)amide (24): mp 121–122 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 9 Hz), 6.96 (1H, broad s), 6.70 (2H, d, J = 9 Hz), 2.91 (6H, s), 2.53 (2H, q, J = 7.3 Hz), 2.32 (2H, t, J = 7.3 Hz), 1.73 (2H, quintet, J = 7.4 Hz), 1.63 (2H, quintet, J = 7.6 Hz), 1.50–1.35 (4H, m), 1.33 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 280 (M^+); Anal. ($C_{15}H_{24}N_2OS$) C, H, N.

7-Mercaptoheptanoic acid 3-biphenylylamide (26): mp 91–92 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.78 (1H, s), 7.59 (2H, d, J = 7.6 Hz), 7.49 (1H, d, J = 7.4 Hz), 7.47–7.30 (5H, m), 7.18 (1H, broad s), 2.53 (2H, q, J = 7.3 Hz), 2.39 (2H, t, J = 7.3 Hz), 1.76 (2H, quintet, J = 7.1 Hz), 1.64 (2H, quintet, J = 7.3 Hz), 1.50–1.37 (4H, m), 1.33 (1H, t, J = 7.6 Hz); MS (EI) *m/z*: 313 (M^+); Anal. ($C_{19}H_{23}NOS$) C, H, N.

7-Mercaptoheptanoic acid (4-phenoxyphenyl)amide (27): mp 87–89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.47 (2H, d, J = 8.8 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.12 (1H, broad s), 7.08 (1H, t, J = 7.3 Hz), 6.98 (4H, d, J = 8.8 Hz), 2.53 (2H, q, J = 7.3 Hz), 2.36 (2H, t, J = 7.6 Hz), 1.75 (2H, quintet, J = 7.1 Hz), 1.64 (2H, quintet, J = 7.1 Hz), 1.50–1.37 (4H, m), 1.33 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 329 (M^+); Anal. ($C_{19}H_{23}NO_2S$) C, H, N.

7-Mercaptoheptanoic acid (3-phenoxyphenyl)amide (28): mp 68–69 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.34 (2H, t, J = 7.6 Hz), 7.30–7.18 (3H, m), 7.16 (1H, broad s), 7.11 (1H, t, J = 7.2 Hz), 7.02 (2H, d, J = 8.5 Hz), 6.74 (1H, s), 2.52 (2H, q, J = 7.3 Hz), 2.33 (2H, t, J = 7.3 Hz), 1.71 (2H, quintet, J = 7.3 Hz), 1.62 (2H, quintet, J = 7.1 Hz), 1.50–1.34 (4H, m), 1.32 (1H, t, J = 7.6 Hz); MS (EI) *m/z*: 329 (M^-); Anal. ($C_{19}H_{23}NO_2S$) C, H, N.

7-Mercaptoheptanoic acid 3-pyridinylamide (29): mp 74–76 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.54 (1H, d, J = 2.4 Hz), 8.35 (1H, d, J = 4.4 Hz), 8.19 (1H, d, J = 8.3 Hz), 7.31 (1H, broad s), 7.28 (1H, dd, J = 4.4, 8.3 Hz), 2.53 (2H, q, J = 7.1 Hz), 2.40 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.6 Hz), 1.64 (2H, quintet, J = 7.1 Hz), 1.50–1.36 (4H, m), 1.33 (1H, t, J = 7.6 Hz); MS (EI) *m/z*: 237 (M^+); Anal. ($C_{12}H_{18}N_2OS$) C, H, N.

7-Mercaptoheptanoic acid 3-quinolinylamide (30): mp 75–76 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.79 (1H, d, J = 2.7 Hz), 8.72 (1H, d, J = 2.7 Hz), 8.04 (1H, d, J = 8.3 Hz), 7.80 (1H, d, J = 8.3 Hz), 7.64 (1H, t, J = 7.1 Hz), 7.54 (1H, t, J = 7.1 Hz), 7.50 (1H, broad s), 2.54 (2H, q, J = 7.1 Hz), 2.47 (2H, t, J = 7.3 Hz), 1.80 (2H, quintet, J = 7.3 Hz), 1.64 (2H, quintet, J = 7.3 Hz), 1.53–1.37 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 288 (M^+); Anal. ($C_{16}H_{20}N_2OS$) C, H, N.

7-Mercaptoheptanoic acid (4-phenyl-2-thiazolyl)amide (31): mp 149–150 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.36 (1H, broad s), 7.83 (2H, d, J = 7.1 Hz), 7.43 (2H, t, J = 7.3 Hz), 7.16 (1H, s), 2.49 (2H, q, J = 7.1 Hz), 2.14 (2H, t, J = 7.6 Hz), 1.65–1.50 (4H, m), 1.32 (1H, t, J = 7.6 Hz), 1.30 (2H, quintet, J = 7.3 Hz), 1.15 (2H, quintet, J = 7.1 Hz); MS (EI) *m/z*: 320 (M^+); Anal. ($C_{16}H_{20}N_2OS_2 \cdot 1/10H_2O$) C, H, N.

7-Mercaptoheptanoic acid 2-benzothiazolylamide (32): mp 141–142 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 10.71 (1H, broad s), 7.86 (1H, d, J = 7.9 Hz), 7.77 (1H, d, J = 8 Hz), 7.46 (1H, t, J = 8.3 Hz), 7.34 (1H, t, J = 8.3 Hz), 2.49 (2H, t, J = 7.1 Hz), 2.48 (2H, q, J = 7.3 Hz), 1.72 (2H, quint, J = 7.6 Hz), 1.57 (2H, quint, J = 7.3 Hz), 1.40–1.25 (5H, m); MS (EI) *m/z*: 294 (M^+); Anal. ($C_{14}H_{18}N_2OS_2$) C, H, N.

7-Mercaptoheptanoic Acid 4-Biphenylylamide (25). Step 1: Preparation of 7-Bromoheptanoic Acid (4-Bromophenyl)amide (64a). Compound **64a** was prepared from **62c** and 4-bromoaniline using the procedure described for **8a** (step 3) in 86% yield: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.42 (4H, s), 7.14 (1H, broad s), 3.41 (2H, t, J = 6.6 Hz), 2.36 (2H, t, J = 7.6 Hz), 1.87 (2H, quintet, J = 7.1 Hz), 1.74 (2H, quintet, J = 7.3 Hz), 1.49 (2H, quintet, J = 7.3 Hz), 1.40 (2H, quintet, J = 6.8 Hz).

Step 2: Preparation of 7-Bromoheptanoic Acid 4-Biphenylylamide (64b). To a suspension of **64a** (500 mg, 1.38 mmol) obtained above in 1-methyl-2-pyrrolidinone (8 mL) and water (4 mL) were added phenylboronic acid (252 mg, 2.07 mmol), tetrakis(triphenylphosphine)palladium(0) (160 mg, 0.14 mmol), and $NaHCO_3$ (235 mg, 2.80 mmol). The mixture was heated at 80 °C for 1 h. The solution was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$, water, and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 3/1) gave 91 mg (18%) of **64b** as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.65–7.50 (6H, m), 7.43 (2H, t, J = 7.6 Hz), 7.33 (1H, t, J = 7.1 Hz), 7.20 (1H, broad s), 3.42 (2H, t, J = 6.6 Hz), 2.39 (2H, t, J = 7.3 Hz), 1.88 (2H, quintet, J = 7.1 Hz), 1.77 (2H, quintet, J = 7.3 Hz), 1.50 (2H, quintet, J = 7.1 Hz), 1.43 (2H, quintet, J = 6.4 Hz).

Steps 3 and 4: Preparation of 7-Mercaptoheptanoic Acid 4-Biphenylylamide (25). Compound **25** was prepared from **64b** obtained above using the procedure described for **15** and **6** (step 4) in 48% yield: mp 114–115 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.64–7.52 (6H, m), 7.43 (2H, t, J = 7.6 Hz), 7.33 (1H, t, J = 7.3 Hz), 7.17 (1H, broad s), 2.54 (2H, q, J = 7.4 Hz), 2.39 (2H, t, J = 7.3 Hz), 1.76 (2H, quintet, J = 7.3 Hz), 1.64 (2H, quintet, J = 7.3 Hz), 1.52–1.37 (4H, m), 1.34 (1H, t, J = 7.6 Hz); MS (EI) *m/z*: 313 (M^+); Anal. ($C_{19}H_{23}NOS \cdot 1/5H_2O$) C, H, N.

7-Methylsulfanylheptanoic Acid Phenylamide (9). To a solution of **64c** (300 mg, 1.06 mmol) in EtOH (10 mL) was added methylmercaptan sodium salt (15% in water, 1.50 g, 3.21 mmol), and the solution was stirred at room temperature for 5 h. The reaction mixture was diluted with AcOEt, washed with water and brine, and dried over $MgSO_4$. Filtration and concentration in vacuo and purification by silica gel flash

chromatography (*n*-hexane/AcOEt = 2/1) gave 262 mg (99%) of **9** as a crude solid. The solid was recrystallized from *n*-hexane–AcOEt and collected by filtration to give 217 mg of **9** as a colorless crystal: mp 50–51 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.16 (1H, broad s), 7.10 (1H, t, J = 7.6 Hz), 2.49 (2H, t, J = 7.1 Hz), 2.36 (2H, t, J = 7.3 Hz), 2.09 (3H, s), 1.74 (2H, quintet, J = 7.3 Hz), 1.61 (2H, quintet, J = 7.3 Hz), 1.42 (4H, m); MS (EI) *m/z*: 251 (M^+); Anal. ($C_{14}H_{21}NOS$) C, H, N.

7-Methanesulfonylheptanoic Acid Phenylamide (11). To a solution of **9** (80 mg, 0.32 mmol) in CH_2Cl_2 (3 mL) was added 3-chloroperoxybenzoic acid (65%, 180 mg, 0.68 mmol). The mixture was stirred overnight at room temperature. Next, saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ were added, and the mixture was stirred at room temperature for 1 h. It was then poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and separation by silica gel flash chromatography (*n*-hexane/AcOEt = 1/3) gave 63 mg (70%) of **11** as a crude solid. The solid was recrystallized from *n*-hexane-AcOEt and collected by filtration to give 50 mg of **11** as a colorless crystal: mp 121–123 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 7.8 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.17 (1H, brs), 7.11 (1H, t, J = 7.3 Hz), 3.01 (2H, t, J = 7.8 Hz), 2.89 (3H, s), 2.37 (2H, t, J = 7.3 Hz), 1.88 (2H, quint, J = 7.6 Hz), 1.76 (2H, quint, J = 7.6 Hz), 1.60–1.35 (4H, m); MS (EI) *m/z*: 283 (M^+); Anal. ($C_{14}H_{21}NO_3S$) C, H, N.

6-Phenoxy-1-hexanethiol (22). Step 1: Preparation of 6-Phenoxy-1-hexanol (67). To a solution of phenol (2.10 g, 22.31 mmol) and 6-bromo-1-hexanol (**65**, 2.00 g, 11.05 mmol) in DMF (30 mL) was added K_2CO_3 (3.10 g, 22.4 mmol), and the mixture was stirred at 80 °C for 1 h. The reaction mixture was diluted with AcOEt and washed with water and brine, before being dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 2/1) gave 2.06 g (96%) of **67** as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.28 (2H, t, J = 7.8 Hz), 6.93 (1H, t, J = 7.3 Hz), 6.89 (2H, d, J = 8.6 Hz), 3.96 (2H, t, J = 6.6 Hz), 3.67 (2H, m), 1.80 (2H, quintet, J = 6.8 Hz), 1.61 (2H, quintet, J = 7.3 Hz), 1.56–1.36 (4H, m), 1.27 (1H, m).

Step 2: Preparation of (6-Bromohexyloxy)benzene. To a solution of **67** (1.75 g, 9.01 mmol) obtained above and carbon tetrabromide (3.00 g, 9.05 mmol) in CH_2Cl_2 (50 mL) was added triphenylphosphine (2.60 g, 9.91 mmol) with cooling in an ice–water bath. The solution was stirred at room temperature for 1 h and concentrated in vacuo. To the residue was added *n*-hexane (30 mL), and the slurry was filtered. After the solid was washed with *n*-hexane (10 mL), the combined filtrates were concentrated in vacuo. The residue was purified by silica gel flash chromatography (*n*-hexane/AcOEt = 1/30) to give 1.45 g (63%) of (6-bromohexyloxy)benzene as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.28 (2H, t, J = 7.6 Hz), 6.93 (1H, t, J = 7.3 Hz), 6.89 (2H, d, J = 8.5 Hz), 3.96 (2H, t, J = 6.3 Hz), 3.43 (2H, t, J = 6.8 Hz), 1.90 (2H, quintet, J = 6.8 Hz), 1.80 (2H, quintet, J = 6.4 Hz), 1.56–1.46 (4H, m).

Steps 3 and 4: Preparation of 6-Phenoxy-1-hexanethiol (22). Compound **22** was prepared from (6-bromohexyloxy)-benzene obtained above using the procedure described for **15** and **6** (step 4) in 45% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.28 (2H, t, J = 7.3 Hz), 6.93 (1H, t, J = 7.6 Hz), 6.89 (2H, d, J = 7.8 Hz), 3.96 (2H, t, J = 6.4 Hz), 2.54 (2H, q, J = 7.1 Hz), 1.79 (2H, quintet, J = 6.6 Hz), 1.65 (2H, quintet, J = 6.8 Hz), 1.54–1.44 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 210 (M^+); HRMS calcd for $C_{12}H_{18}OS$ 210.108, found 210.108.

Compounds**23**, **33–35**, and **36** were prepared from an appropriate aromatic carboxylic acid and 6-amino-1-hexanol (**66**) using the procedure described for **13**, **22** (step 2), **15**, and **6** (step 4).

***N*-(6-Mercaptohexyl)benzamide (23):** mp 43–44 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.77 (2H, d, J = 7.2 Hz), 7.50 (1H, t, J = 7.2 Hz), 7.43 (2H, t, J = 6.8 Hz), 6.20 (1H, broad s), 3.47 (2H, q, J = 6.4 Hz), 2.54 (2H, q, J = 7.6 Hz), 1.68–1.58 (4H, m), 1.50–1.36 (4H, m), 1.52–1.37 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 237 (M^+); Anal. ($C_{13}H_{19}NOS \cdot 1/6H_2O$) C, H, N.

4-Dimethylamino-*N*-(6-mercaptohexyl)benzamide (33): mp 103–104 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.66 (2H, t, J = 8.8 Hz), 6.67 (2H, d, J = 8.8 Hz), 5.95 (1H, s), 3.43 (2H, q, J = 6.4 Hz), 3.02 (1H, s), 2.53 (2H, q, J = 7.2 Hz), 1.67–1.54 (4H, m), 1.49–1.36 (4H, m), 1.32 (1H, t, J = 8 Hz); MS (EI) *m/z*: 280 (M^+); Anal. ($C_{15}H_{24}N_2OS$) C, H, N.

Naphthalene-2-carboxylic acid (6-mercaptohexyl)amide (34): mp 76–78 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.28 (1H, s), 7.94–7.85 (3H, m), 7.82 (1H, d, J = 6.8 Hz), 7.58–7.53 (2H, m), 6.27 (1H, s), 3.52 (2H, q, J = 6.8 Hz), 2.54 (2H, q, J = 7.6 Hz), 1.70–1.62 (4H, m), 1.52–1.36 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 287 (M^+); Anal. ($C_{17}H_{21}NOS$) C, H, N.

Benzofuran-2-carboxylic acid (6-mercaptohexyl)amide (35): mp 72–73 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.68 (1H, d, J = 8 Hz), 7.50 (1H, d, J = 8.4 Hz), 7.46 (1H, s), 7.41 (1H, t, J = 8.4 Hz), 7.30 (1H, t, J = 8 Hz), 6.64 (1H, s), 3.49 (2H, q, J = 7.2 Hz), 2.54 (2H, q, J = 7.2 Hz), 1.72–1.58 (4H, m), 1.52–1.38 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 277 (M^+); Anal. ($C_{15}H_{19}NO_2S$) C, H, N.

Indole-2-carboxylic acid (6-mercaptohexyl)amide (36): mp 128–130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 9.12 (1H, broad s), 7.65 (1H, d, J = 8 Hz), 7.44 (1H, d, J = 8.4 Hz), 7.29 (1H, t, J = 8 Hz), 7.14 (1H, t, J = 6.6 Hz), 6.82 (1H, s), 6.13 (1H, broad s), 3.49 (2H, q, J = 6.8 Hz), 2.54 (2H, q, J = 7.6 Hz), 1.70–1.60 (4H, m), 1.45–1.40 (4H, m), 1.34 (1H, t, J = 7.8 Hz); MS (EI) *m/z*: 276 (M^+); Anal. ($C_{15}H_{20}N_2OS$) C, H, N.

Thiopropionic Acid *S*-(6-Phenylcarbamoylhexyl) Ester (38). To a solution of **7** (200 mg, 0.84 mmol) and a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 (2 mL) and pyridine (0.5 mL) was added propionyl chloride (220 μL, 2.53 mmol). The mixture was stirred at room temperature for 30 min and then diluted with AcOEt. The solution was washed with water and brine and dried over Na_2SO_4. Filtration and concentration in vacuo and separation by silica gel flash chromatography (*n*-hexane/AcOEt = 3/1) gave 238 mg (96%) of **38** as a crude solid. The solid was recrystallized from *n*-hexane-AcOEt and collected by filtration to give 184 mg of **38** as a colorless crystal: mp 54–55 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 7.9 Hz), 7.32 (2H, t, J = 7.9 Hz), 7.21 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.86 (2H, t, J = 7.4 Hz), 2.57 (2H, q, J = 7.7 Hz), 2.35 (2H, t, J = 7.6 Hz), 1.74 (2H, quintet, J = 7.3 Hz), 1.59 (2H, quintet, J = 7.3 Hz), 1.46–1.33 (4H, m), 1.18 (3H, t, J = 7.7 Hz); MS (EI) *m/z*: 293 (M^+); Anal. ($C_{16}H_{23}NO_2S$) C, H, N.

Compounds **39–45**, **47–54**, and **55** were prepared from the corresponding thiols and an appropriate acid chloride using the procedure described for **38**.

Thiobutyric acid *S*-(6-phenylcarbamoylhexyl) ester (39): mp 45–46 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.21 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.86 (2H, t, J = 7 Hz), 2.52 (2H, t, J = 7.3 Hz), 2.35 (2H, t, J = 7.4 Hz), 1.73 (2H, quintet, J = 7.4 Hz), 1.69 (2H, sextet, J = 7.7 Hz), 1.59 (2H, quintet, J = 7.4 Hz), 1.48–1.33 (4H, m), 0.95 (3H, t, J = 7.3 Hz); MS (EI) *m/z*: 307 (M^+); Anal. ($C_{17}H_{25}NO_2S$) C, H, N.

Thioisobutyric acid *S*-(6-phenylcarbamoylhexyl) ester (40): mp 44–45 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.52 (2H, d, J = 8 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.22 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.85 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 7 Hz), 2.35 (2H, t, J = 7.3 Hz), 1.73 (2H, quintet, J = 7.3 Hz), 1.59 (2H, quintet, J = 7.3 Hz), 1.46–1.36 (4H, m), 1.19 (6H, d, J = 7.6 Hz); MS (EI) *m/z*: 307 (M^+); Anal. ($C_{17}H_{25}NO_2S$) C, H, N.

2,2-Dimethylthiopropionic acid *S*-(6-phenylcarbamoylhexyl) ester (41): mp 57–59 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.52 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.20 (1H, broad s), 7.10 (1H, t, J = 7.6 Hz), 2.82 (2H, t, J = 7.3 Hz), 2.35 (2H, t, J = 7.3 Hz), 1.73 (2H, quintet, J = 7.3

Hz), 1.58 (2H, quintet, J = 7.3 Hz), 1.46−1.36 (4H, m), 1.23 (9H, s); MS (EI) *m/z*: 321 (M^+); Anal. ($C_{18}H_{27}NO_2S$) C, H, N.

Cyclopropanecarbothioic acid *S*-(6-phenylcarbamoylhexyl) ester (42): mp 64−65 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 8.3 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.22 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.89 (2H, t, J = 7.3 Hz), 2.35 (2H, t, J = 7.3 Hz), 2.01 (1H, m), 1.73 (2H, quintet, J = 7 Hz), 1.59 (2H, quintet, J = 7.3 Hz), 1.45−1.35 (4H, m), 1.15 (2H, m), 0.94 (2H, m); MS (EI) *m/z*: 305 (M^+); Anal. ($C_{17}H_{23}NO_2S$) C, H, N.

Cyclopentanecarbothioic acid *S*-(6-phenylcarbamoylhexyl) ester (43): mp 59−60 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.52 (2H, d, J = 7.9 Hz), 7.32 (2H, t, J = 7.9 Hz), 7.21 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 2.97 (1H, quintet, J = 8 Hz), 2.85 (2H, t, J = 7.4 Hz), 2.35 (2H, t, J = 7.7 Hz), 1.93−1.67 (8H, m), 1.63−1.52 (4H, m), 1.47−1.33 (4H, m); MS (EI) *m/z*: 333 (M^+); Anal. ($C_{19}H_{27}NO_2S$) C, H, N.

Thiobenzoic acid *S*-(6-phenylcarbamoylhexyl) ester (44): mp 107−109 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.97 (2H, d, J = 7.3 Hz), 7.57 (1H, t, J = 7.3 Hz), 7.52 (2H, d, J = 7.8 Hz), 7.45 (2H, t, J = 7.8 Hz), 7.31 (2H, t, J = 7.6 Hz), 7.21 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 3.07 (2H, t, J = 7.3 Hz), 2.36 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.3 Hz), 1.70 (2H, quintet, J = 7.3 Hz), 1.54−1.36 (4H, m); MS (EI) *m/z*: 341 (M^+); Anal. ($C_{20}H_{23}NO_2S$) C, H, N.

4-Nitrothiobenzoic acid *S*-(6-phenylcarbamoylhexyl) ester (45): mp 117−118 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 8.30 (2H, d, J = 8.8 Hz), 8.11 (2H, d, J = 8.6 Hz), 7.51 (2H, d, J = 8.1 Hz), 7.32 (2H, t, J = 7.8 Hz), 7.16 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 3.12 (2H, t, J = 7.3 Hz), 2.37 (2H, t, J = 7.3 Hz), 1.76 (2H, quintet, J = 7.6 Hz), 1.72 (2H, quintet, J = 7.3 Hz), 1.54−1.38 (4H, m); MS (EI) *m/z*: 386 (M^+); Anal. ($C_{20}H_{22}N_2O_4S$) C, H, N.

Thioisobutyric acid *S*-[6-(3-biphenylylcarbamoyl)-hexyl] ester (47): mp 73−74 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.79 (1H, s), 7.59 (2H, d, J = 7.4 Hz), 7.50 (1H, d, J = 8.3 Hz), 7.43 (2H, t, J = 7.3 Hz), 7.39 (1H, t, J = 8 Hz), 7.35 (1H, t, J = 7.3 Hz), 7.34 (1H, d, J = 7.3 Hz), 7.28 (1H, broad s), 2.85 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.8 Hz), 2.38 (2H, t, J = 7.3 Hz), 1.75 (2H, quintet, J = 7.6 Hz), 1.58 (2H, quintet, J = 7.3 Hz), 1.49−1.35 (4H, m), 1.18 (6H, d, J = 7.1 Hz); MS (EI) *m/z*: 390 (M^+); Anal. ($C_{23}H_{29}NO_2S$) C, H, N.

Thioisobutyric acid *S*-[6-(3-phenoxyphenylcarbamoyl)hexyl] ester (48): colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.34 (2H, t, J = 7.6 Hz), 7.30−7.15 (4H, m), 7.11 (1H, t, J = 7.4 Hz), 7.02 (2H, d, J = 7.6 Hz), 6.74 (1H, d, J = 7.3 Hz), 2.84 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 7 Hz), 2.32 (2H, t, J = 7.3 Hz), 1.71 (2H, quintet, J = 7.4 Hz), 1.57 (2H, quintet, J = 7.4 Hz), 1.45−1.33 (4H, m), 1.18 (6H, d, J = 7 Hz); MS (EI) *m/z*: 399 (M^+); HRMS calcd for $C_{23}H_{29}NO_3S$ 399.187, found 399.191.

Thioisobutyric acid *S*-[6-(3-pyridinylcarbamoyl)hexyl] ester (49): mp 47−48 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.55 (1H, d, J = 2.8 Hz), 8.34 (1H, d, J = 4.6 Hz), 8.21 (1H, d, J = 8.5 Hz), 7.56 (1H, broad s), 7.28 (1H, dd, J = 4.6, 8.3 Hz), 2.85 (2H, t, J = 7 Hz), 2.74 (1H, septet, J = 7 Hz), 2.39 (2H, t, J = 7.6 Hz), 1.75 (2H, quintet, J = 7.4 Hz), 1.59 (2H, quintet, J = 7.1 Hz), 1.45−1.35 (4H, m), 1.19 (6H, d, J = 6.8 Hz); MS (EI) *m/z*: 308 (M^+); Anal. ($C_{16}H_{24}N_2O_2S$) C, H, N.

Thioisobutyric acid *S*-[6-(3-quinolinylcarbamoyl)-hexyl] ester (50): mp 67−68 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.81 (1H, s), 8.73 (1H, d, J = 2.8 Hz), 8.03 (1H, d, J = 8.6 Hz), 7.80 (1H, d, J = 8.2 Hz), 7.70 (1H, broad s), 7.63 (1H, t, J = 7.1 Hz), 7.54 (1H, t, J = 7.3 Hz), 2.86 (2H, t, J = 7.3 Hz), 2.74 (1H, septet, J = 7 Hz), 2.46 (2H, t, J = 7.6 Hz), 1.79 (2H, quintet, J = 7.3 Hz), 1.60 (2H, quintet, J = 7.3 Hz), 1.50−1.35 (4H, m), 1.19 (6H, d, J = 6.7 Hz); MS (EI) *m/z*: 358 (M^+); Anal. ($C_{20}H_{26}N_2O_2S$) C, H, N.

Thioisobutyric acid *S*-[6-(4-phenyl-2-thiazolylcarbamoyl)hexyl] ester (51): mp 127−128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 10.48 (1H, broad s), 7.83 (2H, d, J = 7.3 Hz), 7.43 (2H, t, J = 7.3 Hz), 7.34 (1H, t, J = 7.4 Hz), 7.16 (1H, s), 2.81 (2H, t, J = 7.3 Hz), 2.74 (1H, septet, J = 7 Hz), 2.11 (2H, t, J = 7.6 Hz), 1.56 (2H, quintet, J = 7.6 Hz), 1.50 (2H, quintet, J = 7.3 Hz), 1.25 (2H, quintet, J = 7.6 Hz), 1.19 (6H, d, J = 7 Hz), 1.13 (2H, quintet, J = 7.3 Hz); MS (EI) *m/z*: 383 (M^+); Anal. ($C_{20}H_{26}N_2O_2S_2$) C, H, N.

Thioisobutyric acid *S*-[6-(2-benzothiazolylcarbamoyl)-hexyl] ester (52): mp 106−107 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 10.41 (1H, broad s), 7.85 (1H, d, J = 7.4 Hz), 7.77 (1H, d, J = 7.9 Hz), 7.46 (1H, dt, J = 1.2, 7.1 Hz), 7.34 (1H, dt, J = 1, 7.3 Hz), 2.81 (2H, t, J = 7.4 Hz), 2.73 (1H, septet, J = 7.1 Hz), 2.47 (2H, t, J = 7.7 Hz), 1.72 (2H, quintet, J = 7.3 Hz), 1.53 (2H, quintet, J = 7.1 Hz), 1.38−1.27 (4H, m), 1.18 (6H, d, J = 7 Hz); MS (EI) *m/z*: 364 (M^+); Anal. ($C_{18}H_{24}N_2O_2S_2$) C, H, N.

Thioisobutyric acid *S*-{6-[(2-naphthalenecarbonyl)-amino]hexyl} ester (53): mp 70−71 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.29 (1H, s), 7.93 (1H, d, J = 7.1 Hz), 7.90 (1H, d, J = 7.3 Hz), 7.88 (1H, d, J = 7.3 Hz), 7.84 (1H, d, J = 7 Hz), 7.57 (1H, t, J = 6.7 Hz), 7.54 (1H, t, J = 6.7 Hz), 6.36 (1H, broad s), 3.51 (2H, q, J = 6.4 Hz), 2.87 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.7 Hz), 1.67 (2H, quintet, J = 7.1 Hz), 1.60 (2H, quintet, J = 6.7 Hz), 1.50−1.38 (4H, m), 1.18 (6H, d, J = 6.8 Hz); MS (EI) *m/z*: 357 (M^+); Anal. ($C_{21}H_{27}NO_2S$) C, H, N.

Thioisobutyric acid *S*-{6-[(2-benzofurancarbonyl)amino]hexyl} ester (54): mp 67−68 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.67 (1H, d, J = 7.7 Hz), 7.50 (1H, d, J = 7.6 Hz), 7.46 (1H, d, J = 1 Hz), 7.41 (1H, dt, J = 1.2, 7.3 Hz), 7.29 (1H, t, J = 7.6 Hz), 6.66 (1H, broad s), 3.48 (2H, q, J = 7 Hz), 2.86 (2H, t, J = 7.4 Hz), 2.73 (1H, septet, J = 7.1 Hz), 1.66 (2H, quintet, J = 7 Hz), 1.59 (2H, quintet, J = 7 Hz), 1.48−1.37 (4H, m), 1.18 (6H, d, J = 6.7 Hz); MS (EI) *m/z*: 347 (M^+); Anal. ($C_{19}H_{25}NO_3S$) C, H, N.

Thioisobutyric acid *S*-{6-[(1*H*-2-indolecarbonyl)amino]hexyl} ester (55): mp 142−143 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.37 (1H, broad s), 7.65 (1H, d, J = 7.3 Hz), 7.44 (1H, d, J = 7.6 Hz), 7.29 (1H, t, J = 7 Hz), 7.14 (1H, t, J = 7.9 Hz), 6.86 (1H, s), 6.30 (1H, broad s), 3.49 (2H, q, J = 6.1 Hz), 2.87 (2H, t, J = 7.1 Hz), 2.74 (1H, septet, J = 7 Hz), 1.65 (2H, quintet, J = 7 Hz), 1.60 (2H, quintet, J = 7 Hz), 1.50−1.36 (4H, m), 1.19 (6H, d, J = 7 Hz); MS (EI) *m/z*: 346 (M^+); Anal. ($C_{19}H_{26}N_2O_2S$) C, H, N.

2,2-Dimethylpropionic Acid 6-Phenylcarbamoylhexylsulfanylmethyl Ester (46). To a suspension of sodium hydride (60%, 40.0 mg, 1.00 mmol) in DMF (2 mL) was added a solution of 7 (200 mg, 0.84 mmol) in DMF (3 mL) dropwise with cooling in an ice−water bath. The mixture was stirred for 30 min at 0 °C, and a solution of chloromethyl pivalate (134 μL, 0.93 mmol) in DMF (2 mL) was added at 0 °C. The solution was stirred at room temperature for 1 h. The reaction mixture was poured into ice−water and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration and concentration in vacuo and purification by silica gel flash chromatography (*n*-hexane/AcOEt = 4/1) gave 93 mg (32%) of **46** as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.51 (2H, d, J = 7.8 Hz), 7.32 (2H, t, J = 7.6 Hz), 7.16 (1H, broad s), 7.10 (1H, t, J = 7.3 Hz), 5.41 (2H, s), 2.65 (2H, t, J = 7.3 Hz), 2.36 (2H, t, J = 7.6 Hz), 1.74 (2H, quintet, J = 7.1 Hz), 1.66 (2H, quintet, J = 7.1 Hz), 1.50−1.36 (4H, m), 1.21 (9H, s); MS (EI) *m/z*: 351 (M^+); HRMS calcd for $C_{19}H_{29}NO_3S$ 351.187, found 351.189.

Biology. Enzyme Assays. The assay of HDAC activity was performed using an HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories). HeLa nuclear extracts (0.5 μL/well) were incubated at 37 °C with 25 μM of Fluor de Lys substrate and various concentrations of samples. Reactions were stopped after 30 min by adding Fluor de Lys Developer with trichostatin A which stops further deacetylation. Then, 15 min after addition of this developer, the fluorescence of the wells was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm, and the % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The concentration of compound which results in 50% inhibition was determined by plotting the log[Inh] versus the logit function of the % inhibition. IC_{50} values are

determined using a regression analysis of the concentration/inhibition data.

Lineweaver–Burk Double-Reciprocal Plot Analysis. The assay of HDAC activity was performed using an HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories). HeLa nuclear extracts (0.5 μL/well) were incubated at 37 °C with Fluor de Lys substrate (50, 100, 200, or 400 μM) in the presence of 0, 0.03, 0.1, or 0.3 μM of compound **7**. Reactions were stopped after 10 min by adding Fluor de Lys Developer with trichostatin A which stops further deacetylation. Then, 15 min after addition of this developer, the fluorescence of the wells was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm.

Monolayer Growth Inhibition Assay. Cancer cells were plated in 96-well plates at initial densities of 1500 cells/well and incubated at 37 °C. After 24 h, cells were exposed to test compounds at various concentrations in 10% FBS-supplemented RPMI-1640 medium at 37 °C in 5% CO_2 for 48 h. The medium was removed and replaced with 200 μL of 0.5 mg/mL of Methylene Blue in RPMI-1640 medium, and cells were incubated at room temperature for 30 min. Supernatants were removed from the wells, and Methylene Blue dye was dissolved in 100 μL/well of 3% aqueous HCl. Absorbance was determined on a microplate reader (BioRad) at 660 nm.

Western Blot Analysis. HCT-116 cells (purchased from ATCC) (1×10^6) treated for 8 h with SAHA and compound **51** at the indicated concentrations in 10% FBS-supplemented McCoy's 5A medium were collected and sonicated. Protein concentrations of the lysates were determined by using a Bradford protein assay kit (Bio-Rad Laboratories); equivalent amounts of proteins from each lysate were resolved in 15% SDS-polyacrylamide gel and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After blocking with Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST) containing 3% skim milk for 30 min, the transblotted membrane was incubated with hyperacetylated histone H4 antibody (Upstate Biotechnology) (1:2000) or $p21^{WAF1/CIP1}$ antibody (Medical and Biological Laboratories) (1: 200) in TBST containing 3% skim milk at 4 °C overnight. After treatment with the primary antibody, the membrane was washed twice with water for anti- hyperacetylated histone H4, or three times with TBS for anti-$p21^{WAF1/CIP1}$, then incubated with goat anti-rabbit or anti-mouse IgG-horseradish peroxidase conjugates (1:10000 or 1:5000) for 1.5 h at room temperature and washed twice with water for anti- hyperacetylated histone H4, or three times with TBS for anti-$p21^{WAF1/CIP1}$. The immunoblots were visualized by enhanced chemiluminescence.

Molecular Modeling. Docking and subsequent scoring were performed using Macromodel 8.1 software. Coordinates of HDAC8 complexed with MS344 were taken from the Brookhaven Protein Data Bank (PDB code 1T67) and hydrogen atoms were added computationally at appropriate positions. The structures of SAHA and compound **7** bound to HDAC8 were constructed by molecular mechanics (MM) energy minimization. The starting positions of SAHA and compound **7** were determined manually: the benzene ring and the linker parts were superimposed in the active site onto its crystallographic MS344 counterpart. The conformations of SAHA and compound **7** in the active site were minimized by a MM calculation based upon the OPLS-AA force field with each parameter set as follows: solvent: water, method: LBFGS, max. no. iterations: 10 000, converge on: gradient, convergence threshold: 0.05.

Acknowledgment. This work was supported in part by grants from the Health Sciences Foundation of the Ministry of Health, Labor and Welfare of Japan, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research. We thank the Screening Committee of New Anticancer Agents, supported by a Grant-in-Aid for Scientific Research on Priority Area "Cancer" from the Ministry of Education, Culture, Sports, Science and Technology of Japan, for cancer cell growth inhibition assay results.

Supporting Information Available: Results of the elemental analysis of **4–21**, **23–45**, **47**, **49–54**, and **55** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Grozinger, C. M.; Schreiber, S. L. Deacetylase Enzymes: Biological Functions and the Use of Small-Molecule Inhibitors. *Chem. Biol.* **2002**, *9*, 3–16. (b) Kouzarides, T. Histone acetylases and deacetylases in cell proliferation. *Curr. Opin. Genet. Dev.* **1999**, *9*, 40–48. (c) Hassig, C. A.; Schreiber, S. L. Nuclear histone acetylases and deacetylases and transcriptional regulation: HATs off to HDACs. *Curr. Opin. Chem. Biol.* **1997**, *1*, 300–308.

(2) Taunton, J.; Hassig, C. A.; Schreiber, S. L. A Mammalian Histone Deacetylase Related to the Yeast Transcriptional Regulator Rpd3p. *Science* **1996**, *272*, 408–411.

(3) Sambucetti, L. C.; Fischer, D. D.; Zabludoff, S.; Kwon, P. O.; Chamberlin, H. Trogani, N.; Xu, H.; Cohen, D. Histone Deacetylase Inhibition Selectively Alters the Activity and Expression of Cell Cycle Proteins Leading to Specific Chromatin Acetylation and Antiproliferative Effects. *J. Biol. Chem.* **1999**, *274*, 34940–34947.

(4) (a) Yoshida, M.; Horinouchi, S.; Beppu, T.Trichostatin A and trapoxin: Novel chemical probes for the role of histone acetylation in chromatin structure and function. *BioEssays* **1995**, *17*, 423–430. (b) Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705–5708. (c) Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003–3007.

(5) Cohen, L. A.; Amin, S.; Marks, P. A.; Rifkind, R. A.; Desai, D.; Richon, V. M. Chemoprevention of carcinogen-induced mammary tumorigenesis by the hybrid polar cytodifferentiation agent, suberanilohydroxamic acid (SAHA). *Anticancer Res.* **1999**, *19*, 4999–5005.

(6) (a) For a review, see: Miller, T. A.; Witter, D. J.; Belvedere, S. Histone Deacetylase Inhibitors. *J. Med. Chem.* **2003**, *46*, 5097–5116. (b) For a review, see: Yoshida, M.; Matsuyama, A.; Komatsu, Y.; Nishino, N. From discovery to the coming generation of histone deacetylase inhibitors. *Curr. Med. Chem.* **2003**, *10*, 2351–2358. (c) For a review, see: Miller, T. A. Patent status of histone deacetylase inhibitors. *Expert Opin. Ther. Pat.* **2004**, *14*, 791–804. (d) Remiszewski, S. W.; Sambucetti, L. C.; Bair, K. W.; Bontempo, J.; Cesarz, D.; Chandramouli, N.; Chen, R.; Cheung, M.; Cornell-Kennon, S.; Dean, K.; Diamantidis, G.; France, D.; Green, M. A.; Howell, K. L.; Kashi, R.; Kwon, P.; Lassota, P.; Martin, M. S.; Mou, Y.; Perez, L. B.; Sharma, S.; Smith, T.; Sorensen, E.; Taplin, F.; Trogani, N.; Versace, R.; Walker, H.; Weltchek-Engler, S.; Wood, A.; Wu, A.; Atadja, P. *N*-Hydroxy-3-phenyl-2-propenamides as Novel Inhibitors of Human Histone Deacetylase with in Vivo Antitumor Activity: Discovery of (2*E*)-*N*-Hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (NVP-LAQ824). *J. Med. Chem.* **2003**, *46*, 4609–4624. (e) Kim, D.-K. Lee, J. Y.; Kim, J.-S.; Ryu, J.-H.; Choi, J.-Y.; Lee, J. W.; Im, G.-J.; Kim, T.-K.; Seo, J. W.; Park, H.-J.; Yoo, J.; Park, J.-H.; Kim, T.-Y.; Bang, Y.-J. Synthesis and Biological Evaluation of 3-(4-Substituted-phenyl)-*N*-hydroxy-2-propenamides, a New Class of Histone Deacetylase Inhibitors. *J. Med. Chem.* **2003**, *46*, 5745–5751. (f) Lu, Q.; Yang, Y.-T.; Chen, C.-S.; Davis, M.; Byrd, J. C.; Etherton, M. R.; Umar, A.; Chen, C.-S. Zn^{2+}-Chelating, Motif-Tethered, Short-Chain Fatty Acids as a Novel Class of Histone Deacetylase Inhibitors. *J. Med. Chem.* **2004**, *47*, 467–474. (g) Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Ragno, R.; Bottoni, P.; Scatena, R.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1*H*-2-pyrrolyl)-*N*-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 2. Effect of Pyrrole-C_2 and/or $-C_4$ Substitutions on Biological Activity. *J. Med. Chem.* **2004**, *47*, 1098–1109. (h) Ragno, R.; Mai, A.; Massa, S.; Cerbara, I.; Valente, S.; Bottoni, P.; Scatena, R.; Jesacher, F.; Loidl, P.; Brosch, G. 3-(4-Aroyl-1-methyl-1*H*-pyrrol-2-yl)-*N*-hydroxy-2-propenamides as a New Class of Synthetic Histone Deacetylase Inhibitors. 3. Discovery of Novel Lead Compounds through Structure-Based Drug Design and Docking Studies. *J. Med. Chem.* **2004**, *47*, 1351–1359. (i) Glenn, M. P.; Kahnberg, P.; Boyle, G. M.; Hansford, K. A.; Hans, D.; Martyn, A. C.; Parsons, P. G.; Fairlie, D. P. Antiproliferative and Phenotype-Transforming Antitumor Agents Derived from Cysteine. *J. Med. Chem.* **2004**, *47*, 2984–2994.

(7) (a) Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* **1999**, *401*, 188−193. (b) Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B.-C.; Verner, E,; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. Structural Snapshots of Human HDAC8 Provide Insights into the Class I Histone Deacetylases. *Structure* **2004**, *12*, 1325−1334.

(8) (a) Mulder, G. J.; Meerman, J. H. Sulfation and glucuronidation as competing pathways in the metabolism of hydroxamic acids: the role of *N,O*-sulfonation in chemical carcinogenesis of aromatic amines. *Environ. Health Perspect.* **1983**, *49*, 27−32. (b) Vassiliou, S.; Mucha, A.; Cuniasse, P.; Georgiadis, D.; Lucet-Levannier, K.; Beau, F.; Kannan, R.; Murphy, G.; Knaeuper, V.; Rio, M. C.; Basset, P.; Yiotakis, A.; Dive, V. Phosphinic Pseudo-Tripeptides as Potent Inhibitors of Matrix Metalloproteinases: A Structure−Activity Study. *J. Med. Chem.* **1999**, *42*, 2610−2620.

(9) (a) Wong, J. C.; Hong, R.; Schreiber, S. L. Structural Biasing Elements for In−Cell Histone Deacetylase Paralog Selectivity. *J. Am. Chem. Soc.* **2003**, *125*, 5586−5587. (b) Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Butcher, R. A.; Schreiber, S. L. Multidimensional Chemical Genetic Analysis of Diversity-Oriented Synthesis-Derived Deacetylase Inhibitors Using Cell-Based Assays. *Chem. Biol.* **2003**, *10*, 383−396.

(10) (a) Suzuki, T.; Ando, T.; Tsuchiya, K.; Fukazawa, N.; Saito, A.; Mariko, Y.; Yamashita, T.; Nakanishi, O.Synthesis and Histone Deacetylase Inhibitory Activity of New Benzamide Derivatives. *J. Med. Chem.* **1999**, *42*, 3001−3003. (b) Vaisburg, A.; Bernstein, N.; Frechette, S.; Allan, M.; Abou-Khalil, E.; Leit, S.; Moradei, O.; Bouchain, G.; Wang, J.; Woo, S. H.; Fournel, M.; Yan, P. T.; Trachy-Bourget, M.-C.; Kalita, A.; Beaulieu, C.; Li, Z.; MacLeod, A. R.; Besterman, J. M.; Delorme, D. (2-Amino-phenyl)-amides of ω-substituted alkanoic acids as new histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 283−287.

(11) (a) Frey, R. R.; Wada, C. K.; Garland, R. B.; Curtin, M. L.; Michaelides, M. R.; Li, J.; Pease, L. J.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J.; Murphy, S. S.; Davidsen, S. K. Trifluoromethyl Ketones as Inhibitors of Histone Deacetylase. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3443−3447. (b) Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. α-Keto Amides as Inhibitors of Histone Deacetylase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3331−3335. (c) Vasudevan, A.; Ji, Z.; Frey, R. R.; Wada, C. K.; Steinman, D.; Heyman, H. R.; Guo, Y.; Curtin, M. L.; Guo, J.; Li, J.; Pease, L.; Glaser, K. B.; Marcotte, P. A.; Bouska, J. J.; Davidsen, S. K.; Michaelides, M. R. Heterocyclic Ketones as Inhibitors of Histone Deacetylase. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3909−3913.

(12) Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. Design, synthesis, and activity of HDAC inhibitors with a *N*-formyl hydroxylamine head group. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 449−453.

(13) (a) Suzuki, T.; Nagano, Y.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. Novel Histone Deacetylase Inhibitors: Design, Synthesis, Enzyme Inhibition, and Binding Mode Study of SAHA-Based Non-hydroxamates. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4321−4326. (b) Suzuki, T.; Kouketsu, A.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. Thiol-based SAHA analogues as potent histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3313−3317.

(14) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reaction of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457−2483.

(15) Ondetti, M. A.; Rubin, B.; Cushman, D. W. Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* **1977**, *196*, 441−444.

(16) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.

(17) (a) Furumai, R.; Matsuyama, A.; Kobashi, N.; Lee, K.-H.; Nishiyama, M.; Nakajima, H.; Tanaka, A.; Komatsu, Y.; Nishino, N.; Yoshida, M.; Horinouchi, S. FK228 (Depsipeptide) as a Natural Prodrug That Inhibits Class I Histone Deacetylases. *Cancer Res.* **2002**, *62*, 4916−4921. (b) Nishino, N.; Jose, B.; Okamura, S.; Ebisusaki, S.; Kato, T.; Sumida, Y.; Yoshida, M. Cyclic Tetrapeptides Bearing a Sulfhydryl Group Potently Inhibit Histone Deacetylases. *Org. Lett.* **2003**, *5*, 5079−5082.

(18) Christianson, D. W.; Lipscomb, W. N. Carboxypeptidase A.*Acc. Chem. Res.* **1989**, *22*, 62−69.

(19) Moree, W. J.; van der Marel, G. A.; Liskamp, R. M. J.Peptides containing a sulfinamide or a sulfonamide moiety: New transition-state analogues. *Tetrahedron Lett.* **1991**, *32*, 409−412.

(20) Kijima, M.; Yoshida, M.; Sugita, K.; Horinouchi, S.; Beppu, T. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.* **1993**, *268*, 22429−22435.

(21) Mercaptoacetamide **14** is also attractive for further study. The results will be reported soon.

(22) (a) Jung, M.; Brosch, G.; Kölle, D.; Scherf, H.; Gerhäuser, C.; Loidl, P. Amide Analogues of Trichostatin A as Inhibitors of Histone Deacetylase and Inducers of Terminal Cell Differentiation. *J. Med. Chem.* **1999**, *42*, 4669−4679. (b) Remiszewski, S. W.; Sambucetti, L. C.; Atadja, P.; Bair, K. W.; Cornell, W. D.; Green, M. A.; Howell, K. L.; Jung, M.; Kwon, P.; Trogani, N.; Walker, H. Inhibitors of Human Histone Deacetylase: Synthesis and Enzyme and Cellular Activity of Straight Chain Hydroxamates. *J. Med. Chem.* **2002**, *45*, 753−757.

(23) Gagnard, V.; Leydet, A.; Morere, A.; Montero, J.-L.; Lefebvre, I.; Gosselin, G.; Pannecouque, C.; De Clercq, E.Synthesis and in vitro evaluation of *S*-acyl-3-thiopropyl prodrugs of Foscarnet. *Bioorg. Med. Chem.* **2004**, *12*, 1393−1402.

(24) Barber, I.; Rayner, B.; Imbach, J.-L. The prooligonucleotide approach. I. Esterase-mediated reversibility of dithymidine *S*-alkyl-phosphorothioates to dithymidine phosphorothioates. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 563−568.

(25) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MACROMODEL − an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

(26) Mai, A.; Esposito, M.; Sbardella, G.; Massa, S.A new facile and expeditious synthesis of *N*-hydroxy-*N'*-phenyloctanediamide, a potent inducer of terminal cytodifferentiation. *Org. Prep. Proced. Int.* **2001**, *33*, 391−394.

JM049207J

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1547–1551

Design, synthesis, and biological activity of novel PPARγ ligands based on rosiglitazone and 15d-PGJ_2

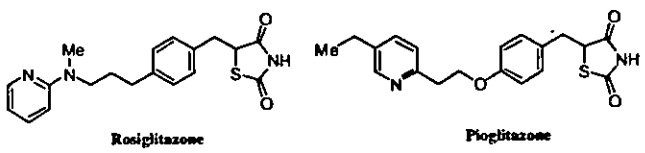
Shinya Usui, Takayoshi Suzuki, Yoshifumi Hattori, Kazuma Etoh, Hiroki Fujieda, Makoto Nishizuka, Masayoshi Imagawa, Hidehiko Nakagawa, Kohfuku Kohda† and Naoki Miyata*

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

Received 25 December 2004; revised 30 January 2005; accepted 31 January 2005

Abstract—To develop novel PPARγ ligands, we synthesized thirteen 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives, which are designed based on the structures of rosiglitazone and 15d-PGJ_2. Among these compounds, compound **9** was found to be as potent as rosiglitazone in a binding assay and a preadipocyte differentiation test. Molecular modeling suggested that the nonyl group of **9** interacted with hydrophobic amino acid residues constructing the hydrophobic region of PPARγ protein where the alkyl chain of 15d-PGJ_2 is expected to be located.

Peroxisome proliferator-activated receptors (PPARα, PPARγ, and PPARδ) belong to the nuclear receptor superfamily and act as ligand-activated transcription factors.[1–3] These receptors play a pivotal roles in regulating the expression of a large number of genes involved in lipid metabolism and energy balance.[4] Many studies on PPARs have been performed, and these efforts led to the discovery of the clinically useful thiazolidinedione (TZD) class of insulin sensitizers such as rosiglitazone[5] and pioglitazone[6] (Fig. 1), which are potent PPARγ agonists used in the treatment of Type 2 diabetes. However, the use of TZDs has been limited because of their poor safety profiles. For example, troglitazone, which came out to the market first, disappeared from the market due to its severe hepatic toxicity in 1999,[7] and rosiglitazone is reported to be associated with liver, cardiovascular, and hematological toxicities.[8] We therefore initiated a search for non-TZD PPARγ ligands with the goal of finding novel insulin sensitizers. In this letter, we report the design, synthesis, and biological activity of non-TZD PPARγ ligands based on the structure of rosiglitazone and 15-deoxy-Δ-12,14-prostaglandin J_2 (15d-PGJ_2).



Rosiglitazone Pioglitazone

Figure 1. Structures of rosiglitazone (GSK) and pioglitazone (Takeda).

The compounds prepared for this study are shown in Figure 2, and the routes used for their synthesis are illustrated in Schemes 1–4. Scheme 1 shows the preparation of *N*-(pyridin-2-yl)-*N*-alkyl derivatives **1–3** bearing a methyl, ethyl, and propyl group, respectively, on their nitrogen atom as an alkyl chain.[9] The protection of dialkylamine **14a–c** by $(Boc)_2O$ gave **15a–c**.[10] The Mitsunobu reaction[11] was applied to the conversion of **15a–c** into 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives **16a–c**: treatment of **15a–c** with diethylazodicarboxylate, PPh_3, and 3-(4-hydroxyphenyl)propanoic acid methyl ester **21** gave ethers **16a–c**. The *N*-Boc groups of **16a–c** were removed with trifluoroacetic acid to give amines **17a–c**. Treatment of **17a–c** with 2-fluoropyridine, or 2-chloropyridine gave *N*-(pyridin-2-yl)-*N*-alkyl compounds **18a–c** via nucleophilic aromatic substitution, and subsequent hydrolysis afforded carboxylic acids **1–3**.

Keywords: PPARγ ligand; 15d-PGJ_2; Insulin sensitizer; Agonist.
* Corresponding author. Tel./fax: +81 52 836 3407; e-mail: miyata-n@phar.nagoya-cu.ac.jp
† Current address: Faculty of Pharmaceutical Sciences, Musashino University, 1-1-20 Shin-machi, Nishi-Tokyo City, Tokyo 202-8585, Japan.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.01.074

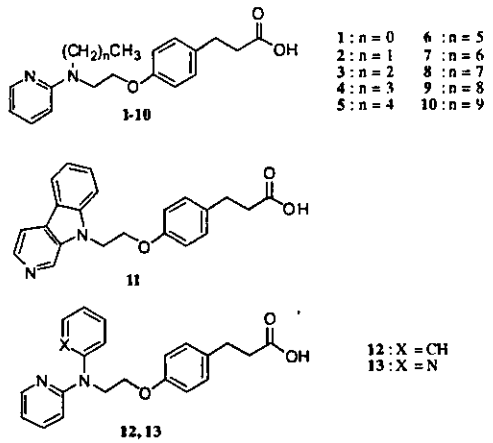
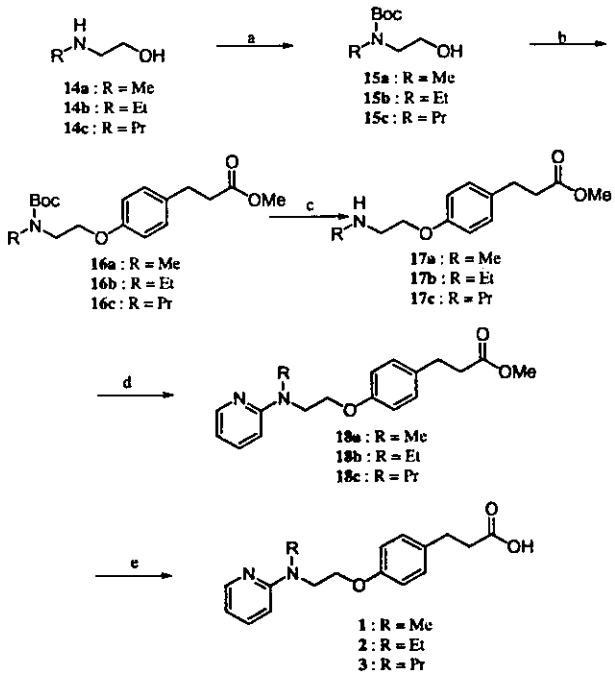


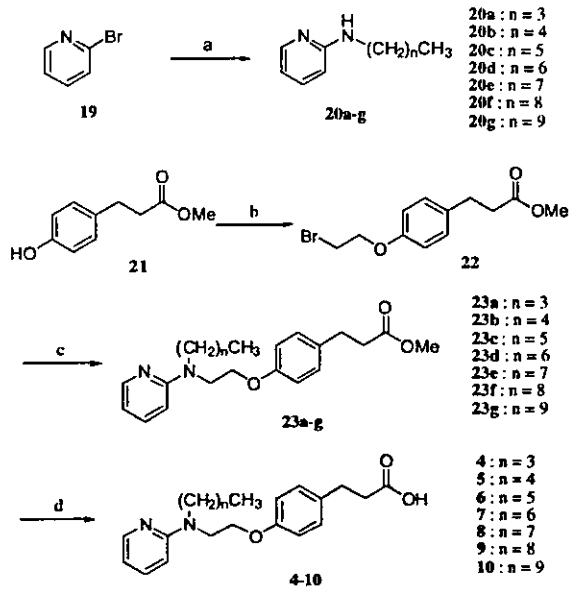
Figure 2. Structures of compounds **1–13**.



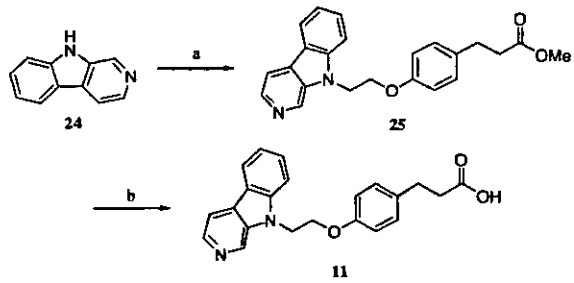
Scheme 1. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, 0 °C to rt, 91–100%; (b) DEAD, PPh_3, 3-(4-hydroxyphenyl)propanoic acid methyl ester **21**, THF, 0 °C to rt, 49–63%; (c) TFA, CH_2Cl_2, 0 °C to rt, 81–94%; (d) 2-fluoropyridine or 2-chloropyridine, DMF, reflux, 7–38%; (e) aq NaOH, THF/MeOH, rt, 78–92%.

The preparation of the other *N*-(pyridin-2-yl)-*N*-alkyl derivatives **4–10** is outlined in Scheme 2. The preparation of 2-alkylamino pyridine **20a–g** was achieved by the method of Buchwald:[12] treatment of **19** with *n*-alkylamine, $Pd_2(DBA)_3$, BINAP, and *t*-BuONa in toluene under reflux. Propanoic acid methyl ester **21** was allowed to react with 1,2-dibromoethane to give ether **22**. Coupling between amines **20a–g** and ether **22** afforded *N*-(pyridin-2-yl)-*N*-alkyl compounds **23a–g**, and subsequent hydrolysis afforded carboxylic acids **4–10**.

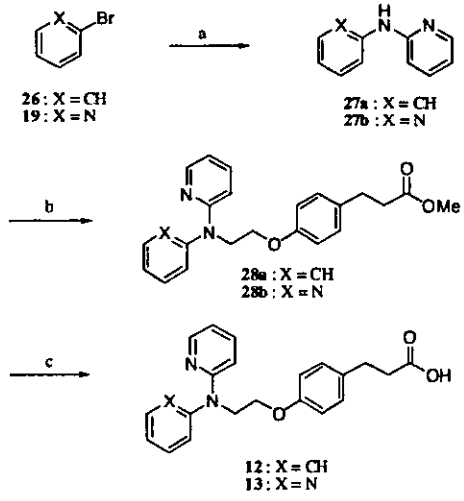
N-(2-Pyridin-2-yl)-*N*-aryl derivatives **11–13** were prepared by the procedure outlined in Schemes 3 and 4.



Scheme 2. Reagents and conditions: (a) $CH_3(CH_2)_nNH_2$, $Pd_2(DBA)_3$, BINAP, *t*-BuOH, toluene, 80 °C, 7–53%; (b) 1,2-dibromoethane, K_2CO_3, THF, 115 °C, 25%; (c) **20a–g**, Et_3N, KI, THF, 120 °C, 2–25%; (d) aq NaOH, THF, rt, 81–96%.



Scheme 3. Reagents and conditions: (a) (i) NaH, DMF, rt; (ii) **22**, KI, 90 °C, 72%; (b) aq NaOH, THF, rt, 89%.



Scheme 4. Reagents and conditions: (a) 2-aminopyridine, $Pd_2(DBA)_3$, BINAP, *t*-BuONa, toluene, 80 °C, 70–88%; (b) (i) NaH, DMF, rt, (ii) **22**, KI, 90 °C, 18–30%; (c) aq NaOH, THF, rt, 70–80%.

Norharman **24** was reacted with bromide **22** in the presence of sodium hydride in DMF to give 9*H*-β-carboline compound **25**, and subsequent hydrolysis gave compound **11** (Scheme 3). Compounds **27a** and **b** were prepared in the same way as 2-alkylamino pyridines **20a–g** (Scheme 4). Compounds **27a** and **b** were allowed to react with bromide **22** in the presence of sodium hydride in DMF to give compounds **28a** and **b**. Treatment of **28a** and **b** with aqueous NaOH gave *N*-(pyridin-2-yl)-*N*-phenyl derivative **12** and dipyridinyl derivative **13**.

The binding affinity of the compounds for PPARγ was evaluated with a CoA–BAP system (Microsystems).[13] In this system, the alkaline phosphatase (AP) activity is directly proportional to the PPARγ-binding affinity of the ligands.

Since it has been revealed that the TZD ring can be replaced by a carboxyl group,[14] we initially examined the binding affinity for PPARγ of compound **1**, in which the TZD group of rosiglitazone is replaced by a carboxyl group. Although compound **1** did not show any activity at 0.1 and 1 μM, a certain level of activity was observed at 10 μM (Table 1, line 1). For the further design of PPARγ ligands, we focused on the alkyl chain of 15d-PGJ_2,[15,16] an endogenous ligand of PPARγ. Since certain fatty acids with a long alkyl chain are known to be natural PPARγ ligands,[17] the hydrophobic moiety is assumed to be critical for the binding affinity for PPARγ. Our study regarding the binding mode of 15d-PGJ_2 in PPARγ protein (PDB code 1FM6) by computer calculation (Macromodel 8.1)[18] also suggested that the alkyl chain of 15d-PGJ_2 is located in the wide hydrophobic region of the PPARγ ligand-binding cavity (Fig. 3). However, the crystal structure of a PPARγ/rosiglitazone complex[19] revealed that rosiglitazone does not have any hydrophobic groups interacting with the hydrophobic amino acid residues of PPARγ. We hypothesized that the introduction of a hydrophobic group into compound **1** may increase the affinity for PPARγ (Fig. 4). We therefore designed compounds **2–10** in which alkyl groups of various lengths were introduced at the 2-aminopyridinyl moiety of compound **1**, and evaluated their ability to bind PPARγ. It was found that the affinity of compounds **1–10** was closely related to chain length, and the most potent compounds were heptyl **7**, octyl **8**, and nonyl **9**. In addition, *N*,*N*-diaryl compounds **11–13** exhibited weak activity compared with compounds **7–9** (Table 1, lines 11–13). We next compared the binding affinity of compounds **7–9** with that of rosiglitazone at lower concentrations. As shown in Figure 5, compound **9** showed the highest activity among the three, and had only slightly less affinity for PPARγ than did rosiglitazone.

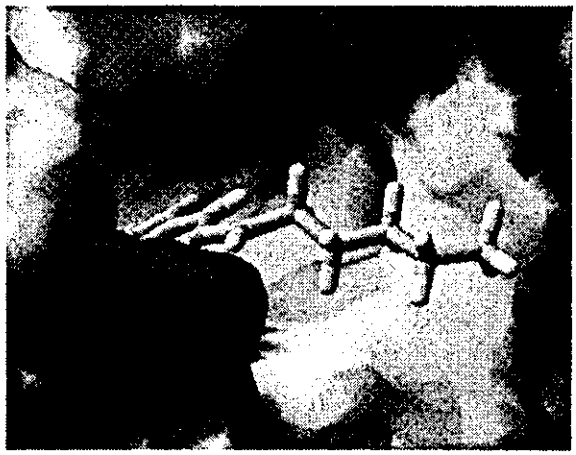


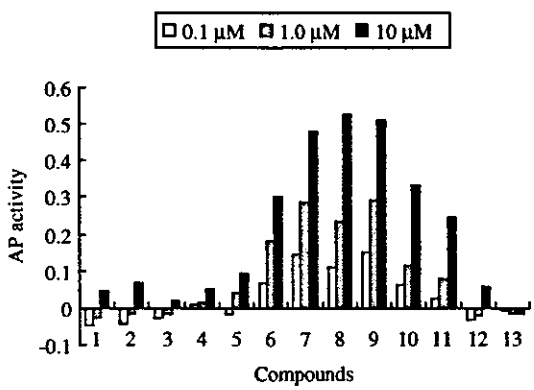
Figure 3. View of the conformation of 15d-PGJ_2 (tube) docked in PPARγ. The hydrophobic and hydrophilic regions are shown in yellow and blue, respectively.

As compound **9** was most active in our study, we used it for further evaluation. Since it has been reported that activation of PPARγ enhances adipocyte differentiation[20] and increases insulin sensitivity, compound **9** was subjected to a rat abdominal preadipocyte differentiation test.[21,22] The accumulation of neutral fat in the cells was observed after the administration of compound **9** at concentrations of 1, 2.5, and 5 μM, and the activity of compound **9** was found to be comparable to that of rosiglitazone (Fig. 6).

Since *N*-nonyl carboxylic acid **9** had a high level of activity, we studied its mode of binding to PPARγ. A low energy conformation was calculated when **9** was docked in a model based on the crystal structure of PPARγ using Macromodel 8.1 software.[18] An inspection of the simulated PPARγ/**9** complex suggested that oxygen atoms of compound **9** form hydrogen bonds with Ser 289, Tyr 327, and Tyr 473 (Fig. 7). In addition, it was shown that the nonyl group of **9** is located in the hydrophobic region formed by Phe 287, Gly 284, Ile 281, Ile 341, and Met 348 (Fig. 8) where the alkyl chain of 15d-PGJ_2 is calculated to be located (Fig. 3).

In summary, in order to explore novel PPARγ ligands, we prepared several 3-{4-(2-aminoethoxy)phenyl}propanoic acid derivatives designed based on the structures of rosiglitazone and 15d-PGJ_2. Among them, *N*-(pyridin-2-yl)-*N*-nonyl compound **9** was found to be as

Table 1. Binding affinity for PPARγ of compounds **1–13** at 0.1, 1.0, and 10 μM.[a]



[a] Values are means of at least three experiments.

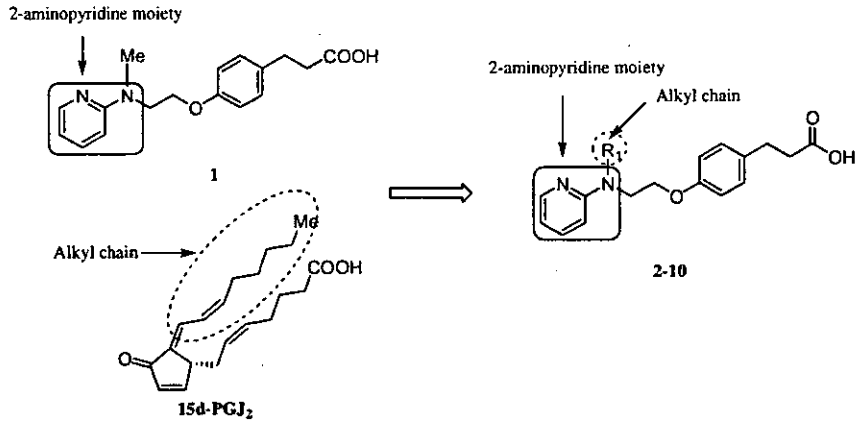


Figure 4. Structures of compounds **2–10** designed on the basis of the structure of 15d-PGJ_2 and rosiglitazone.

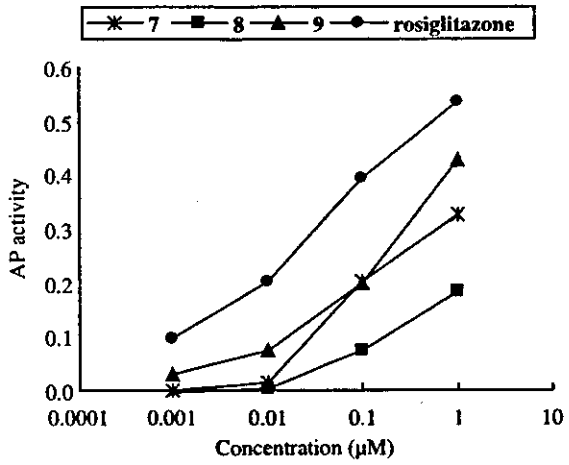


Figure 5. Binding affinity for PPARγ of rosiglitazone and compounds **7–9** at 0.001, 0.01, 0.1, and 1.0 μM. Values are means of at least three experiments.

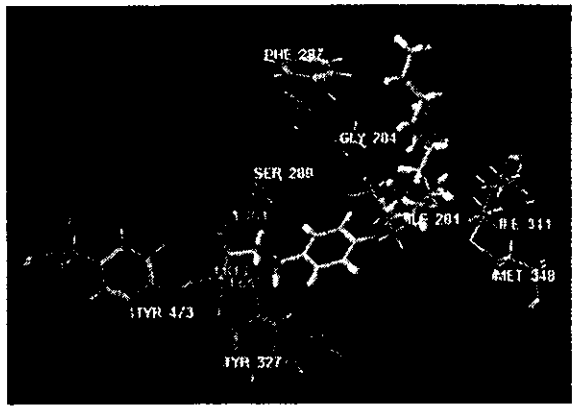


Figure 7. View of the conformation of **9** (tube) docked in PPARγ. Residues around compound **9** and hydrogen bonds are displayed as wires, and dotted lines, respectively. Figures represent distances in angstroms.

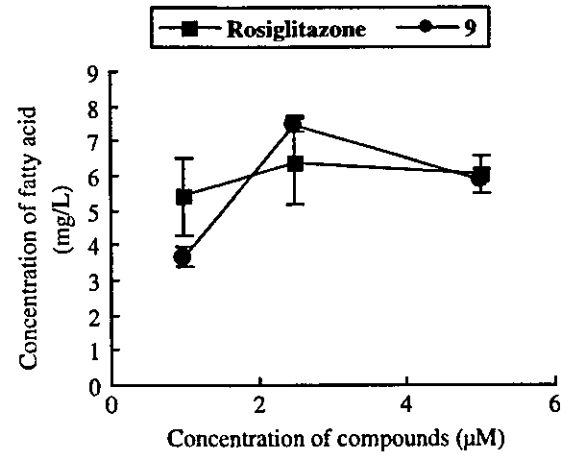


Figure 6. Accumulation of fatty acid in rat preadipocytes by rosiglitazone and compound **9**. Values are means of at least three experiments.

Figure 8. View of the conformation of **9** (tube) docked in PPARγ. The hydrophobic and hydrophilic regions are shown in yellow and blue, respectively.

potent as rosiglitazone in the binding assay and the preadipocyte differentiation test. Molecular modeling suggested that the carboxylate anion of **9** forms hydrogen bonds with some hydrophilic amino acid residues, and the nonyl group appropriately interacts with hydrophobic amino acid residues. The findings of this study will help provide an effective agent for Type 2 diabetes.

Currently, further detailed studies on compound **9** are under way.

Acknowledgements

This work was supported in part by grants from the Health Sciences Foundation of the Ministry of Health, Labor, and Welfare of Japan.

References and notes

1. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
2. Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D.; Moore, L. B.; Kliewer, S. A.; Lehmann, J. M. *J. Med. Chem.* **1996**, *39*, 665.
3. Kersten, S.; Desvergne, B.; Wahli, W. *Nature* **2000**, *405*, 421.
4. Bogacka, I.; Xie, H.; Bray, G. A.; Smith, S. R. *Diabetes Care* **2004**, *27*, 1660.
5. Cantello, B. C. C.; Cawthorone, M. A.; Cottam, G. P.; Duff, P. T.; Haigh, D.; Hindley, R. M.; Lister, C. A.; Smith, S. A.; Thurlby, P. L. *J. Med. Chem.* **1994**, *37*, 3977.
6. Momose, Y.; Meguro, K.; Ikeda, H.; Hatanaka, C.; Oi, S.; Sohda, T. *Chem. Pharm. Bull.* **1991**, *39*, 1440.
7. Liver damage warning for Troglitazone *Scrip.* **1997**, *2282*, 21.
8. Patel, J.; Miller, E.; Hu, J.; Granett, J. Diabetes **1997**, *46* (Suppl. 1), Abstr. 0578.
9. Henke, B. R.; Blanchard, S. G.; Brackeen, M. F.; Brown, K. K.; Cobb, J. E.; Collins, J. L.; Harrington, W. W.; Hashim, M. A.; Hull-Ryde, E. A.; Kaldor, I.; Kliewer, S. A.; Lake, D. H.; Leesenitzer, L. M.; Lehmann, J. M.; Lenhard, J. M.; Orband-Miller, L. A.; Miller, J. F.; Mook, R. A.; Noble, S. A.; Oliver, W.; Parks, D. J.; Plunket, K. D.; Szewczyk, J. R.; Willson, T. M. *J. Med. Chem.* **1998**, *41*, 5020.
10. Krapcho, A. P.; Maresch, M. J.; Lunn, J. *Synth. Commun.* **1993**, *23*, 2443.
11. For a review see: Mitsunobu, O. *Synthesis* **1981**, 1.
12. Wagaw, S.; Buchwald, S. L. *J. Org. Chem.* **1996**, *61*, 7240.
13. Kanayama, T.; Mamiya, S.; Nishihara, T.; Nishikawa, J. *J. Biochem.* **2003**, *133*, 791.
14. Rybczynski, P. J.; Zeck, R. E.; Dudash, J., Jr.; Combs, D. W.; Burris, T. P.; Yang, M.; Osborne, M. C.; Chen, X.; Demarest, K. T. *J. Med. Chem.* **2004**, *47*, 196.
15. Forman, B. M.; Tontonoz, P.; Chen, J.; Brun, R. P.; Spiegelman, B. M.; Evans, R. M. *Cell* **1995**, *83*, 803.
16. Kliewer, S. A.; Lenhard, J. M.; Willson, T. M.; Patel, I.; Morris, D. C.; Lehmann, J. M. *Cell* **1995**, *83*, 813.
17. Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. *Nature* **1992**, *358*, 771.
18. Docking and subsequent scoring were performed using Macromodel 8.1 software. Coordinates of PPARγ complexed with rosiglitazone were taken from the Brookhaven Protein Data Bank (PDB code 1FM6) and hydrogen atoms were added computationally at appropriate positions. The structure of a ligand bound to PPARγ was constructed by molecular mechanics (MM) energy minimization. The starting position of a ligand was determined manually: its carboxyl group was superimposed onto the TZD ring of crystallographic rosiglitazone. The conformation of the ligand in the active site was minimized by a MM calculation based upon the OPLS-AA force field with each parameter set as follows; solvent: water, method: LBFGS, Max # Iterations: 10,000, converge on: gradient, convergence threshold: 0.05.
19. Gampe, R. T., Jr.; Montana, V. G.; Lambert, M. H.; Miller, A. B.; Bledsoe, R. K.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M.; Xu, H. E. *Mol. Cell* **2000**, *5*, 545.
20. Henke, B. R. *J. Med. Chem.* **2004**, *47*, 4118.
21. Lagace, D. C.; Nachtigal, M. W. *J. Biol. Chem.* **2004**, *278*, 18851.
22. In rat preadipocyte differentiation experiments, we basically followed the protocol of the preadipocyte Total Kit (TOYOBO. Co., Ltd, Osaka, Japan). Rat preadipocytes obtained from the abdominal tissue (Wistar, male, 8 weeks old) were cultured for 10 days in a humidified incubator at 37 °C and 5% CO_2 in a preadipocyte growth medium. The medium was renewed every other day. After the preadipocytes reached confluence, they were treated with preadipocyte differentiation medium containing compound **9** or rosiglitazone. The cells were cultured for 15 days with the differentiation medium renewed every three days. Accumulating neutral fat in the cells was measured as absorbance at 590 nm with a 1420 ARVO™ multilabel-counter (PerkinElmer, Boston, MA, U.S.A.) after staining with lipidos liquid (TOYOBO. Co., Ltd, Osaka, Japan).

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 15 (2005) 331–335

Identification of a potent non-hydroxamate histone deacetylase inhibitor by mechanism-based drug design

Takayoshi Suzuki,* Azusa Matsuura, Akiyasu Kouketsu, Hidehiko Nakagawa and Naoki Miyata*

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

Received 10 September 2004; revised 25 October 2004; accepted 25 October 2004
Available online 13 November 2004

Abstract—In order to find novel non-hydroxamate histone deacetylase (HDAC) inhibitors, we synthesized several suberoylanilide hydroxamic acid (SAHA)-based compounds designed on the basis of the catalytic mechanism of HDACs. Among these compounds, **5b** was found to be as potent as SAHA. Kinetic enzyme assays and molecular modeling suggested that the mercaptoacetamide moiety of **5b** interacts with the zinc in the active site of HDACs and removes a water molecule from the reactive site of the deacetylation.

Histone deacetylases (HDACs) catalyze the deacetylation of the acetylated ε-amino groups of specific histone lysine residues,[1,2] and are involved in the expression of a number of genes.[3] In addition, HDACs have also been implicated in certain disease states such as cancer.[4–7] For this reason, there is a growing interest in the generation of potent small-molecule inhibitors of HDACs. Thus far, several classes of small-molecule HDAC inhibitors have been recognized.[8] Most of these are hydroxamic acid derivatives, typified by suberoylanilide hydroxamic acid (SAHA) (Fig. 3), and are thought to chelate the zinc ion in the active site.[9,10] Although hydroxamic acids are responsible for various potent inhibitors, they generally have many problems associated with their use such as low oral availability, poor in vivo stability, and undesirable side effects.[11,12] Thus, it has become increasingly desirable to find replacement groups that possess strong inhibitory action against HDACs. We and other groups have searched for a suitable hydroxamic acid replacement for HDAC inhibitors by structure-based drug design (SBDD)[13–15] ever since the crystal structure of an archaebacterial HDAC homologue (HDAC-like protein, HDLP)/SAHA complex was first reported.[9] However, SBDD has not yet led to the discovery of potent non-hydroxamate HDAC inhibitors, and the non-hydroxamates found with SBDD are approximately 10–1000-fold less potent than their corresponding hydroxamates. We therefore decided to search for hydroxamic acid replacements by an alternative approach, namely, mechanism-based drug design. In this paper, we report the mechanism-based design, synthesis, enzyme inhibition, and binding mode of non-hydroxamate HDAC inhibitors.

The crystal structures of the HDLP/hydroxamates and HDAC8/hydroxamates complexes have led to a solid understanding of not only the three dimensional structure of the active site of HDACs but also the catalytic mechanism for the deacetylation of acetylated lysine substrate.[9,10] It is proposed that the carbonyl oxygen of this substrate could bind the zinc, and the carbonyl could be attacked by a zinc-chelating water molecule (Fig. 2a), which would result in the production of deacetylated lysine via a tetrahedral carbon-containing transition state (Fig. 1a). On the basis of the proposed catalytic mechanism, we attempted to design non-hydroxamate HDAC inhibitors. First, we designed transition-state (TS) analogues. The TS of HDAC deacetylation was estimated to include a tetrahedral carbon (Fig. 1a) as with other zinc proteases.[16] To date, there has been only one report on TS analogue inhibitors of HDACs, namely, phosphorus-based SAHA analogues.[17] However, these analogues have a potency about 1000-fold less than that of SAHA. We focused attention on sulfone derivative TS analogues because it has been suggested that the sulfonamide moiety has strong similarity with the TS of amide bond hydrolysis,

Keywords: Histone deacetylase inhibitor; Non-hydroxamate.
* Corresponding authors. Tel./fax: +81 52 836 3407; e-mail addresses: suzuki@phar.nagoya-cu.ac.jp; miyata-n@phar.nagoya-cu.ac.jp

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.074

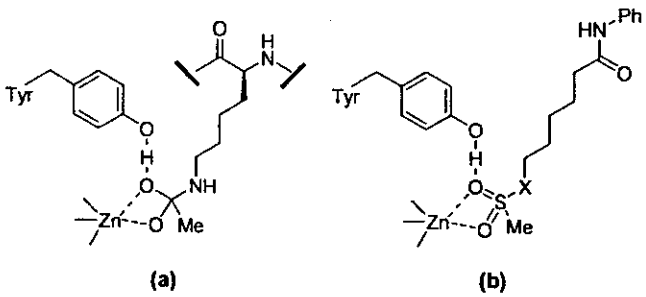
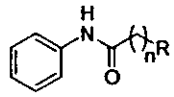


Figure 1. The transition state proposed for HDACs (a), and models for the binding of sulfone derivatives (b).

both from a steric and an electronic point of view.[18] Compounds **1** and **2**, in which a hydroxamic acid of SAHA was replaced by a sulfonamide and a sulfone, respectively, were designed as TS analogues (Figs. 1b, and 3). Our second approach was based on the proposed deacetylation mechanism whereby a zinc-chelating water molecule activated by His142 and His143 (HDAC8 numbering) makes a nucleophilic attack on the carbonyl carbon of acetylated lysine substrate (Fig. 2a). With this mechanism, if the water molecule is forcibly removed from the zinc ion, the HDACs would supposedly be inhibited. We then designed hetero atom containing substrate analogues **3**–**5** (Fig. 3). These analogues would be recognized as substrates by HDACs and would be easily taken into the active site where they could force the water molecule off the zinc ion and the reactive site of the deacetylation by chelation of the hetero atom to the zinc ion, and might behave as HDAC inhibitors (Fig. 2b).

The compounds prepared for this study are shown in Table 1. The routes used for synthesis of the compounds are indicated in Schemes 1–3. Scheme 1 shows the preparation of sulfonamide **1**, a TS analogue. The condensation of dicarboxylic acids **8a–c** with an equivalent amount of aniline gave mono-anilides **9a–c**. Carboxylic

Table 1. HDAC inhibition data for SAHA, SAHA-based transition state analogues, and substrate analogues[a]



Compd	R	*n*	% Inhbtn at 100 μM	IC_{50} (μM)
SAHA[b]	–CONHOH	6	100	0.28
1	$-NHSO_2Me$	5	10	7500
2	$-SO_2Me$	6	33	230
3	$-NHCOCH_2NH_2$	5	6	>100
4	$-NHCOCH_2OH$	5	0	>100
5a	$-NHCOCH_2SH$	6	96	3.0
5b	$-NHCOCH_2SH$	5	99	0.39
5c	$-NHCOCH_2SH$	4	88	11
6	$-NHCOCH_2SAc$	5	72	22
7	$-NHCOCH_2CH_2SH$	5	78	24

[a] Values are means of at least three experiments.
[b] Prepared as described in Ref. 25.

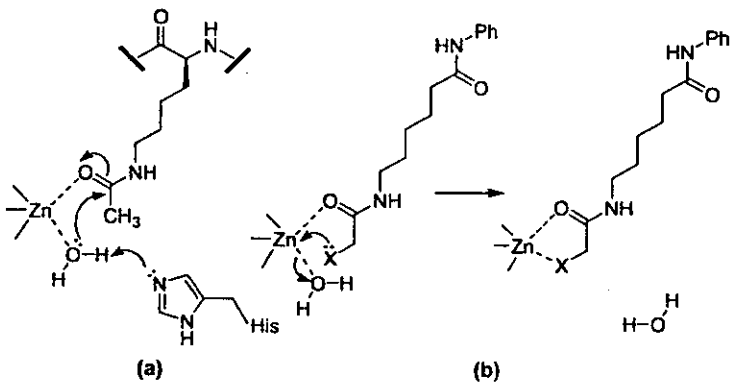
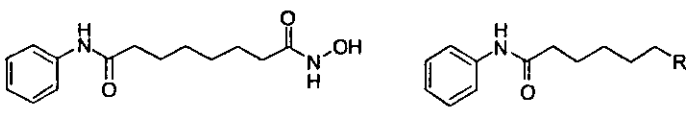


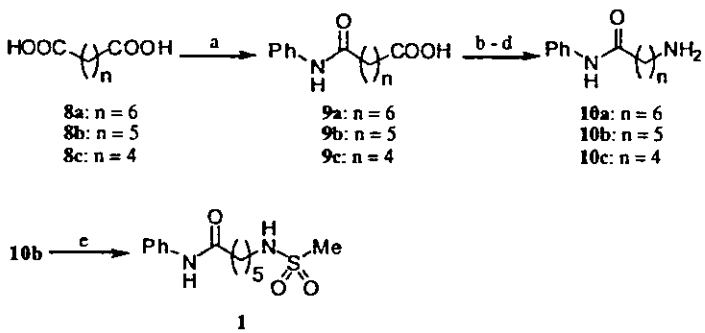
Figure 2. The mechanism proposed for the deacetylation of acetylated lysine substrate (a), and a model for the binding of hetero atom containing substrate analogues to zinc ion (b).



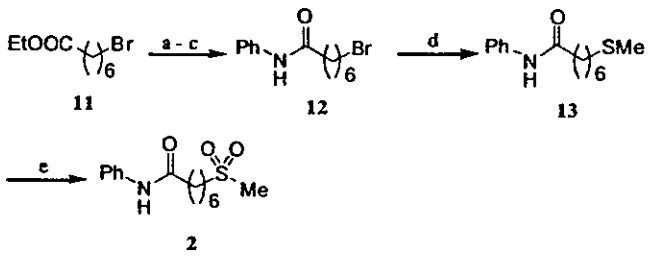
SAHA

1: R = $-NHSO_2Me$ **3**: R = $-NHCOCH_2NH_2$
2: R = $-CH_2SO_2Me$ **4**: R = $-NHCOCH_2OH$
5: R = $-NHCOCH_2SH$

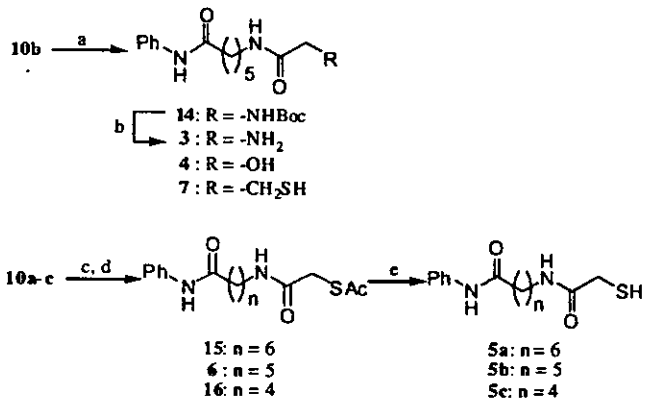
Figure 3. Structures of SAHA, SAHA-based transition state analogues **1** and **2**, and hetero atom containing substrate analogues **3**–**5** designed on the basis of the deacetylation mechanism.



Scheme 1. Reagents and conditions: (a) aniline, 180 °C, 46–54%; (b) diphenylphosphoryl azide, Et_3N, benzene, reflux; (c) BnOH, reflux, 63–94% (two steps); (d) H_2, 5% Pd–C, MeOH, rt, 72–96%; (e) MsCl, pyridine, rt, 71%.



Scheme 2. Reagents and conditions: (a) $LiOH \cdot H_2O$, THF, EtOH, H_2O, rt, 99%; (b) $(COCl)_2$, DMF, CH_2Cl_2, rt; (c) aniline, Et_3N, CH_2Cl_2, rt, 87%; (d) 15% aq NaSMe, EtOH, rt, 99%; (e) *m*-chloroperoxybenzoic acid, CH_2Cl_2, rt, 70%.



Scheme 3. Reagents and conditions: (a) RCH_2COOH, EDCI, HOBt, DMF, rt, 35–99%; (b) trifluoroacetic acid, CH_2Cl_2, rt, 84%; (c) bromoacetyl chloride, Et_3N, CH_2Cl_2, rt, 23–56%; (d) AcSK, EtOH, rt, 50–89%; (e) K_2CO_3, MeOH, rt, 28–75%.

acids **9a–c** were converted to amines **10a–c** in three steps: Curtius rearrangement of carboxylic acids **9a–c**, treatment of the resulting isocyanates with benzyl alcohol, and cleavage of the *Z* group by hydrogenolysis. Coupling between amine **10b** and methanesulfonyl chloride afforded sulfonamide **1**. Preparation of **2**, the other TS analogue, is shown in Scheme 2. 7-Bromoheptanoic acid ethyl ester **11** was converted to **12** in three steps by hydrolysis of the ester of **11**, acid chloride formation by oxalyl chloride, and condensation with aniline. Bromide **12** was allowed to react with sodium methanethiolate to give sulfide **13**, after which treatment with two equivalents of *m*-chloroperoxybenzoic acid gave sulfone **2**. Hetero atom containing substrate analogues **3–7** were prepared from amines **10** obtained above by the procedure outlined in Scheme 3. The amine **10b** was reacted with an appropriate carboxylic acid in the presence of EDCI and HOBt in DMF to give compounds **4**, **7** and **14**. The *N*-Boc group of compound **14** was removed by treating with trifluoroacetic acid to give aminoacetamide **3**. Coupling between amines **10a–c** and bromoacetyl chloride and subsequent treatment with potassium thioacetate afforded compounds **6**, **15**, and **16** and the deacetylation of these compounds in the presence of K_2CO_3 in MeOH gave mercaptoacetamides **5a–c**.

The compounds prepared for this study were evaluated using an HDAC enzyme inhibition assay[19] (Table 1). In the case of TS analogues, sulfone **2** showed anti-HDAC activity and the IC_{50} value was 230 μM, which was greater than those of phosphorus-based SAHA analogues.[17] However, sulfone **2** was approximately 820-fold less effective than SAHA. Next, we examined hetero atom containing substrate analogues. While **3** and **4** did not possess HDAC inhibitory activities,[20] potent inhibition was observed with mercaptoacetamide **5b**. Compound **5b** exhibited an IC_{50} of 0.39 μM, and its activity largely surpassed those of phosphorus compounds[17] and was comparable to those of SAHA and previously reported non-hydroxamates.[21,22] The potency of mercaptoacetamide **5a–c** was directly related to chain length, and the most potent compound was **5b**, where $n = 5$. As expected, thiol transformation into thioacetate (**6**) led to a 55-fold less potent inhibitor. This result suggests that thiolate anion generated under physiological conditions has an intimate involvement in the interaction with the zinc ion in the active site. The conversion of mercaptoacetamide to mercaptopropionamide (**7**) reduced potency as compared to compound **5b**.

Next, we studied the inhibition mechanism of mercaptoacetamide **5b**. Although the mercaptoacetamide group of **5b** was designed to make use of its chelation of the zinc ion in the active site, there is a possibility that mercaptoacetamide **5b** inhibits HDACs by forming a covalent disulfide bond with cysteine residues of these enzymes. We examined this possibility using a Lineweaver–Burk plot (a double reciprocal plot of $1/V$ versus 1/[substrate] at varying concentrations of inhibitor **5b**)

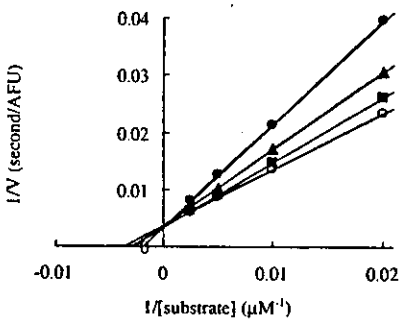


Figure 4. Reciprocal rate versus reciprocal acetyl lysine substrate concentration in the presence of 1 (●), 0.3 (▲), 0.1 (■), and 0 (○) μM of **5b**.

(Fig. 4), and the data from this study established that mercaptoacetamide **5b** engages in competitive inhibition versus acetylated lysine substrate, with an inhibition constant (K_i) of 0.78 μM. Since cysteine is not a component in the construction of the active site of HDACs, the mercaptoacetamide group of **5b** likely interacts with the zinc in the active site.

Since mercaptoacetamide **5b** was proven to act in the HDAC active center, we studied its binding mode in this site. The low energy conformation of **5b** was calculated when docked in the model based on the crystal structure of HDAC8 (PDB code 1T64, 1T67, 1T69, and 1VKG) using Macromodel 8.1 software. An inspection of the HDAC8/**5b** complex showed that the sulfur atom and oxygen atom of **5b** were located 2.44 Å and 2.04 Å from the zinc ion, respectively, and that a water molecule, which is required for the deacetylation of acetylated lysine substrate, was positioned 4.95 Å apart from the zinc ion (Fig. 5). This calculation suggests that **5b** inhibits HDACs by chelating the zinc ion in a bidentate fashion through its sulfur and oxygen atoms, and by removing a water molecule from the zinc and the reactive site of the deacetylation, without being hydrolyzed by HDACs.

In summary, in order to find novel non-hydroxamate HDAC inhibitors, we prepared several SAHA-based compounds whose designs were based on the proposed HDAC catalytic mechanism. Although transition state analogues were weakly active against HDACs, mercaptoacetamide **5b**, one of the hetero atom containing substrate analogues, was found to be as potent as SAHA. Mercaptoacetamide **5b** exhibits strong competitive inhibition versus acetylated lysine substrate. As far as we could determine, this is the first report of HDAC inhibitors with mercaptoacetamide. Since mercaptoacetamides are reported as potent, long-lived, and low-toxic matrix metalloproteinase inhibitors,[23,24] we believe that our findings in this study will provide the basis for the development of ideal HDAC inhibitors free of the problems associated with hydroxamates. Further detailed structure–activity relationship studies are currently under way and the next stage of evaluations pertaining to mercaptoacetamides **5** has begun.

Acknowledgements

This work was supported in part by grants from the Health Sciences Foundation of the Ministry of Health, Labor and Welfare of Japan, and the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

References and notes

1. Grozinger, C. M.; Schreiber, S. L. *Chem. Biol.* **2002**, *9*, 3.
2. Hassig, C. A.; Schreiber, S. L. *Curr. Opin. Chem. Biol.* **1997**, *1*, 300.
3. Taunton, J.; Hassig, C. A.; Schreiber, S. L. *Science* **1996**, *272*, 408.
4. Yoshida, M.; Horinouchi, S.; Beppu, T. *BioEssays* **1995**, *17*, 423.

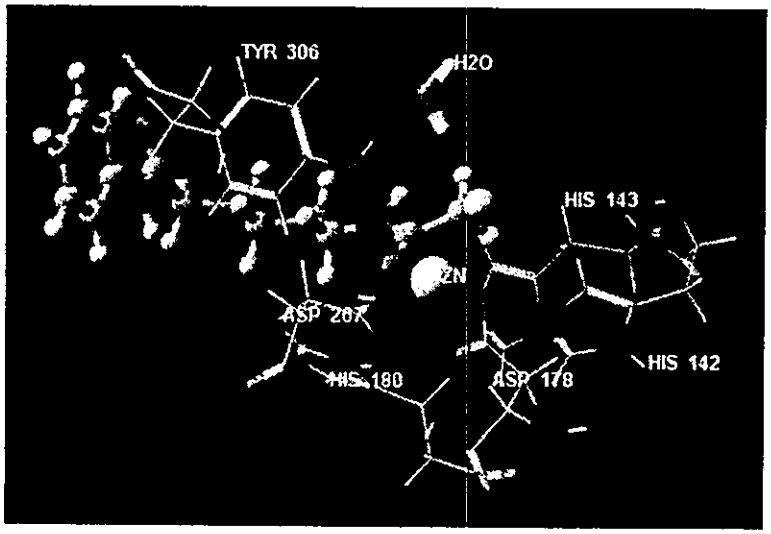


Figure 5. View of the conformation of **5b** (ball and stick) docked in the HDAC8 catalytic core. Residues around the zinc ion and a water molecule are displayed as wires and tubes, respectively.

5. Richon, V. M.; Webb, Y.; Merger, R.; Sheppard, T.; Jursic, B.; Ngo, L.; Civoli, F.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5705.
6. Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3003.
7. Cohen, L. A.; Amin, S.; Marks, P. A.; Rifkind, R. A.; Desai, D.; Richon, V. M. *Anticancer Res.* **1999**, *19*, 4999.
8. For a review, see: Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097.
9. Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188.
10. Somoza, J. R.; Skene, R. J.; Katz, B. A.; Mol, C.; Ho, J. D.; Jennings, A. J.; Luong, C.; Arvai, A.; Buggy, J. J.; Chi, E.; Tang, J.; Sang, B.-C.; Verner, E.; Wynands, R.; Leahy, E. M.; Dougan, D. R.; Snell, G.; Navre, M.; Knuth, M. W.; Swanson, R. V.; McRee, D. E.; Tari, L. W. *Structure* **2004**, *12*, 1325.
11. Mulder, G. J.; Meerman, J. H. *Environ. Health Perspect.* **1983**, *49*, 27.
12. Vassiliou, S.; Mucha, A.; Cuniasse, P.; Georgiadis, D.; Lucet-Levannier, K.; Beau, F.; Kannan, R.; Murphy, G.; Knaeuper, V.; Rio, M. C.; Basset, P.; Yiotakis, A.; Dive, V. *J. Med. Chem.* **1999**, *42*, 2610.
13. Mai, A.; Massa, S.; Rango, R.; Cerbara, I.; Jesacher, F.; Loidl, P.; Brosch, G. *J. Med. Chem.* **2003**, *46*, 512.
14. Suzuki, T.; Nagano, Y.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4321.
15. Wu, T. Y. H.; Hassig, C.; Wu, Y.; Ding, S.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 449.
16. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62.
17. Kapstin, G. V.; Fejer, G.; Gronlund, J. L.; McCafferty, D. G.; Seto, E.; Etzkorn, F. A. *Org. Lett.* **2003**, *5*, 3053.
18. Moree, W. J.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, *32*, 409.
19. The HDAC activity assay was performed using an HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories): HeLa Nuclear Extracts (0.5 μl/well) were incubated (37 °C) with 25 μM of Fluor de Lys™ substrate and various concentrations of samples. Reactions were stopped after 30 min. with Fluor de Lys™ Developer and fluorescence was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm.
20. The reason that compounds **3** and **4** were inactive is unclear, but it is probably because the amino group of **3** is not able to chelate zinc ion due to the protonation under the assay conditions, and because the zinc-chelating ability of hydroxyl group (**4**) is less than that of sulfhydryl group (**5b**).
21. Wada, C. K.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Garland, R. B.; Holms, J. H.; Li, J.; Pease, L. J.; Guo, J.; Glaser, K. B.; Marcotte, P. A.; Richardson, P. L.; Murphy, S. S.; Bouska, J. J.; Tapang, P.; Magoc, T. J.; Albert, D. H.; Davidsen, S. K.; Michaelides, M. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3331.
22. Suzuki, T.; Kouketsu, A.; Matsuura, A.; Kohara, A.; Ninomiya, S.; Kohda, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3313.
23. Rizvi, N. A.; Humphrey, J. S.; Ness, E. A.; Johnson, M. D.; Gupta, E.; Williams, K.; Daly, D. J.; Sonnichsen, D.; Conway, D.; Marshall, J.; Hurwitz, H. *Clin. Cancer Res.* **2004**, *10*, 1963.
24. Baxter, A. D.; Bird, J.; Bhogal, R.; Massil, T.; Minton, K. J.; Montana, J.; Owen, D. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 897.
25. Mai, A.; Esposito, M.; Sbardella, G.; Massa, S. *Org. Prep. Proced. Int.* **2001**, *33*, 391.

ヒストン脱アセチル化酵素阻害剤開発の最前線

鈴木　孝禎

Takayoshi SUZUKI

名古屋市立大学大学院薬学研究科助手

宮田　直樹

Naoki MIYATA

名古屋市立大学大学院薬学研究科教授

1.はじめに

2003年4月にヒトゲノム配列の解読完了が宣言された。DNA情報がRNAに転写され、さらにタンパク質へ翻訳されるというセントラルドグマに従うと、ヒトゲノム配列の読解により生命機能を担うすべてのタンパク質が分かれば生命現象がすべて理解できるはずである。しかし、実際にはDNAの塩基配列だけでは理解できない生命現象は多く存在する。例えば、一つの受精卵からの細胞の分化である。すべての細胞は同じ塩基配列のDNAを持っているにも関わらず、異なる形態や機能を持つ細胞へと分化していく。これは、DNAの塩基配列が同じであっても発現する遺伝子の種類が異なるためである。最近の研究により、ヒストンのメチル化、アセチル化、リン酸化、ユビキチン化やDNAのメチル化などの修飾が塩基配列に依存せず遺伝子の発現を制御することが明らかになってきた。この様な後天的な修飾により遺伝子発現が制御されることに起因する遺伝学あるいは分子生物学の研究分野は「エピジェネティクス」と呼ばれる。エピジェネティックな制御を行う化合物は、生命現象を理解するための重要なツールとなるであろうし、エピジェネティックな異常は癌などの疾病をもたらすことも明らかになっていることから、治療薬として応用できる可能性もある。本稿では、エピジェネティックな制御を行う物質の一つとして最近注目されているヒストン脱アセチル化酵素（HDAC）阻害剤の開発について最近の動向を概説する。

2.HDACの機能と構造

HDACはヒストンのN末端テールのアセチル化されたリシン残基からアセチル基を除去する反応を触媒している（図1）。その逆反応、すなわちヒストンのリシン残基をアセチル化する反応は、ヒストンアセチル化酵素（HAT）が担っている。HDACとHATによるヒストンリシン残基の可逆的アセチル化により多くの遺伝子の発現が制御されている。[1]一般に、ヒストンが脱アセチル化された状態では転写は抑制され、ヒストンのアセチル化が亢進すると転写は活性化される。いかにしてヒストンのアセチル化が転写を促進しているかについては、不明な点が多かった。しかし最近、転写基本因子TFIIDの構成成分である$TAF_{II}250$とヒストンH4のアセチル化部分の結合がX線結晶構造解析により明らかにされたことから、転写因子がヒストンのアセチル化されたリシン残基を認識し、特定の遺伝子のプロモーターに近づきやすくなることが転写の活性化に寄与していると考えられるようになった（図2）。[2]

1996年にSchreiberらによって、ヒトヒストン脱アセチル化酵素の精製、クローニングが行われ、それが出芽酵母の転写因子Rpd3のホモログであることが明らかにされた。[3]それ以来、今日までに複数のヒトHDACが見つかっており、構造上、酵母の転写因子であるRpd3に近いクラスI(HDAC1, 2, 3, 8, 11)、酵母のHda1に類似しているクラスII(HDAC4, 5, 6, 7, 9, 10)、酵母のタンパクsilent information regulator 2 (Sir 2)に相同性を示すクラスIII（SIRT1~7）の3つのグループに分類されている。[4]これら各HDACの機能については、細胞内局在、標的選択性などに焦点が当てられ、盛んに研究が進められている。

近年、HDACの構造研究に関しては、大きな進展が見られた。1999年、Finninらによりヒト HDACホモログであるHDLP(HDAC-like protein)とヒドロキサム酸系HDAC阻害剤として知られるトリコスタチンA（TSA、1）及びスベロイルアニリドヒドロキサム酸（SAHA、2）（図3）の複合体の結晶構造が、[5]2004年にはヒトHDACのアイソザイムの一つであるHDAC8とヒドロキサム酸系阻害剤の複合体の結晶構造が発表された。[6]酵素活性ポケットの内壁は疎水性残基によって形成され、ポケットの底部にはZn^{2+}イオンが存在していることが明らかとなった。ヒドロキサム酸系HDAC阻害剤のヒドロキサム酸部分のカルボニル基とヒドロキシル基の酸素が

Zn^{2+}に配位しており、さらにカルボニル基の酸素は Tyr306（HDAC8 の番号）と、窒素原子に付いた水素は His143 と、ヒドロキシル基の水素は His142 とそれぞれ水素結合を形成していることが明らかにされている（図 4）。また、この結晶構造から図 4 に示すようなHDAC によるヒストン脱アセチル化のメカニズムが推定されている。まず、Zn^{2+}及び His により活性化され求核性を増した水分子が、Zn^{2+}に配位することにより求電子性の高まったヒストンリシン残基のアセタミドのカルボニル基を求核攻撃する。ここでカルボニルの炭素は四面体構造をとるが、最終的に C-N 結合の開裂が起きて脱アセチル化が完結する。HDAC の三次元構造が明らかになり作用機序が推定されたことにより、その後の HDAC 阻害剤の開発研究は加速した。

3.HDAC 阻害剤

3-1.HDAC 阻害剤の抗癌作用

HDAC阻害剤により HDACの機能を停止させると相対的に HAT の働きが強くなり、ヒストンは過剰にアセチル化された状態となる。ヒストンのアセチル化により、多くの遺伝子の転写活性化が起こるが、その中で最も注目されている遺伝子が癌抑制に関わる p21 遺伝子である。[7]p21 は、cyclin-dependent kinase-2 を阻害し、細胞周期を停止することが知られている。また、caspase-3 や FAS などアポトーシス誘導に関与する遺伝子の発現も上昇することが報告されている。[8]実際に、TSA(1)や SAHA(2)などの HDAC 阻害剤は、癌細胞の細胞周期の停止、アポトーシスの誘導などの生物活性を示し、動物実験のレベルでも癌の増殖を抑えることが分かっている。[9]それゆえに HDAC 阻害剤は、HDAC の機能を調べるためのプローブとしてだけではなく、新たな作用機序の抗癌剤としても期待されている。以下に HDAC 阻害剤の最近の進展について述べるが、SIRT（Sir2）阻害剤については参考文献を参照されたい。[10]

3-2.ヒドロキサム酸系 HDAC 阻害剤

Streptomyces 属の真菌から単離された TSA(1)が HDAC を阻害することが吉田らにより報告されて以来、[11]TSA(1)をモデルに SAHA(2)など、多くの HDAC 阻害剤が開発されてきた。HDAC 阻害剤は、酵素の活性中心において亜鉛にキレートする zinc-binding group（ZBG）部位、ポケットの入り口付近で疎水性相互作用をすると考えられている cap 部位、その二つをつなぐ linker 部位の三つのパーツから構成される（図 3）。

これまでに最も多く報告されているのが、ZBG としてヒドロキサム酸を持つ HDAC 阻害剤である（図 6）。TSA、SAHA の linker 部位、cap 部位を変換することにより活性や安全性の改善された阻害剤が見出されている。吉田らにより開発された CHAP31(3)、[12]上里、長岡らにより見出された *N*-ヒドロキシベンズアミド(4)、[13]Richon らにより報告された Pyroxamide(5)、[14]Novartis 社で研究開発された NVP-LAQ824(6)、[15]Schreiber らによりコンビナトリアルライブラリーから見出された tubacin(7)[16]などが知られている。ヒドロキサム酸系 HDAC 阻害剤の中では、SAHA(2)、Pyroxamide(5)、NVP-LAQ824(6)が、現在抗癌剤として臨床試験が進められている。また、tubacin(7)は細胞内で HDAC6 を選択的に阻害することが報告されており、HDAC の機能をより詳しく解明するのに役立つことが期待されている。

3-3.非ヒドロキサム酸系 HDAC 阻害剤

ZBG としてヒドロキサム酸を持たない HDAC 阻害剤もいくつか報告されている（図 7）。三井製薬（現・日本シェーリング）で創製され、現在臨床開発中である MS-275(8)は、ZBG としてアミノアニリド構造を持つ。この構造の HDAC 阻害剤もいくつか開発されており、MethylGene 社のスルホンアミド化合物(9)は動物実験レベルでも高い抗癌活性を示すこと、[17]また、Schreiber らにより見出された histacin(10)及び PAOA(11)は、細胞内の HDAC6 に対し阻害活性を示さない HDAC 阻害剤であることが報告されている。[18]

Abbott 社により、α-ケトアミド(12)などの求電子性ケトンを有する化合物が HDAC 阻害活性を持つことが報告された。[19]求電子性ケトンは容易に水和され、1,1-ジオールとなり、その２つの酸素原子が酵素の活性部位の亜鉛に配位することにより HDAC 阻害活性を示すと考えられている。しかし、これらの求電子性ケトンは、生体内で容易に還元され不活性なアルコール体に代謝されてしまうという欠点がある。

Schultz らは、酵素の三次元構造を基にした設計により SAHA のヒドロキサム酸が *N*-ホルミルヒドロキシルアミンに置換された TWZ105(13)を見出した。[20]TWZ105(13)は、ホルミル基の酸素原子とヒドロキシルアミノ基の酸素原子が酵素活性部位の亜鉛に配位して、HDAC 阻害活性を示すと考えられている。*N*-ホルミルヒドロキシルアミン誘導体は、マトリックスメタロプロテイナーゼ阻害剤においては、ヒドロキサム酸に比べ体内動態が優れていることから、HDAC 阻害剤においても同様の効果が期待される。

我々は、酵素活性中心に存在する亜鉛イオンが高い硫黄親和性を持つことに着目し、新規 HDAC 阻害剤としてチオール(14)を見出した。[21]さらに linker 部位、cap 部位の構造最適化及びプロドラッグ化の検討により見出されたチオエステル(15)は、癌細胞増殖抑制試験で SAHA に匹敵する強い活性を示した。また、西野らはマクロサイクリックジスルフィド(16)が還元条件で強い HDAC 阻害作用を示すことを報告している。[22]藤沢薬品（現・アス

テラス製薬）は、シュードモナス菌から単離した FK228(17)を臨床試験に進めているが、FK228(17)でも細胞内の還元条件で生成したチオールが HDAC を阻害すると考えられている。[23]

HDAC の触媒メカニズムを基にした HDAC 阻害剤の設計も行われており、現在までにホスホネート、スルホンメチル、メルカプトアセタミドを ZBG に持つ化合物が HDAC 阻害能を有することが分かっている。特に我々が基質アナログとして見出したメルカプトアセタミド(18)は、SAHA と同等の HDAC 阻害活性を示すことから、新たなバイオプローブ、抗癌剤開発に向けたリード化合物として期待できる。[24]

4.展望

現在までに 18 種類の HDAC が同定されているが、それぞれの HDAC のアイソザイムの詳しい機能は不明な点も多い。HDAC のアイソザイムに特異性の高い阻害薬は、あらゆる生命現象を理解するために必要な生物学研究の重要なツールとして利用できるであろうし、副作用の少ない抗癌剤としても期待できる。すでにアイソザイム特異的な HDAC 阻害剤の探索研究も始まっている。[12),16),18),25)]興味深いことに、非ヒドロキサム酸系 HDAC 阻害薬は HDAC 6 に対する感受性が低いことが最近報告され、[25)]非ヒドロキサム酸系 ZBG のアイソザイム特異性における重要性が示された。今後、これらの知見を基にアイソザイム特異的な HDAC 阻害剤が見出され、生物学研究及び癌治療の分野に大きく貢献することが期待される。

参考文献

1) Grozinger, C. M. *et al.*, *Chem. Biol.*, **9**, 3(2002).
2) Jacobson, R. H. *et al.*, *Science*, **288**, 1422(2000).
3) Taunton, J. *et al.*, *Science*, **272**, 408(1996).
4) 小杉浩史，医学のあゆみ，**208**, 264(2004).
5) Finnin, M. S. *et al.*, *Nature*, **401**, 188(1999).
6) Somoza, J. R. *et al.*, *Structure*, **12**, 1325(2004).
7) Sambucetti, L. C. *et al.*, *J. Biol. Chem.*, **274**, 34940(1999).
8) Klisovic, D. D. *et al.*, *Invest. Ophth. Vis. Sci.*, **44**, 2390(2003).
9) Yoshida, M. *et al.*, *BioEssays*, **17**, 423(1995).
10) Posakony, J. *et al.*, *J. Med. Chem.*, **47**, 2635(2004).
11) Yoshida, M. *et al.*, *J. Biol. Chem.*, **265**, 17174(1990).
12) Furumai, R. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 87(2001).
13) Maeda, T. et al., *Bioorg. Med. Chem.*, **12**, 4351(2004).
14) Butler, L. M. et al., *Clin. Cancer Res.*, **7**, 962(2001).
15) Remiszewski, S. W. *et al.*, *J. Med. Chem.*, **46**, 4609(2003).
16) Haggarty, S. J. *et al.*, *Chem. Biol.*, **10**, 383(2003).
17) Fournel, M. *et al.*, *Cancer Res.*, **62**, 4325(2002).
18) Wong, J. C. *et al.*, *J. Am. Chem. Soc.*, **125**, 5586(2003).
19) Wada, C. K. *et al.*, *Bioorg. Med. Chem. Lett.*, **13**, 3331(2003).
20) Wu, T. Y. *et al.*, *Bioorg. Med. Chem. Lett.*, **14**, 449(2004).
21) Suzuki,T. *et al.*, *J. Med. Chem.*, **48**, 1019(2005).
22) Nishino, N. *et al.*, *Org. Lett.*, **5**, 5079(2003).
23) Furumai, R. *et al.*, *Cancer Res.*, **62**, 4916(2002).
24) Suzuki, T. *et al.*, *Bioorg. Med. Chem. Lett.*, **15**, 331(2005).
25) Glaser, K.B. *et al.*, *Biochem. Biophys. Res. Commun.*, **325**, 683(2004).

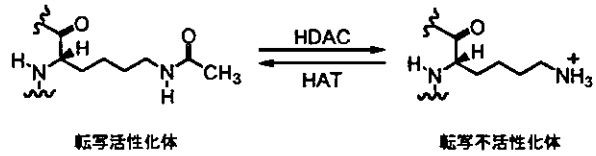


図1　ヒストンの可逆的アセチル化による遺伝子発現制御機構

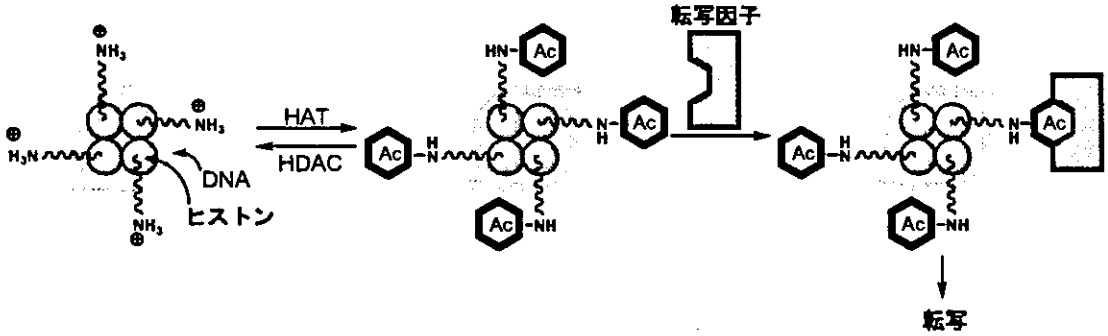


図2　ヒストンのアセチル化による転写活性化の機構
転写因子はアセチル基を認識し、特定遺伝子の転写を活性化する。

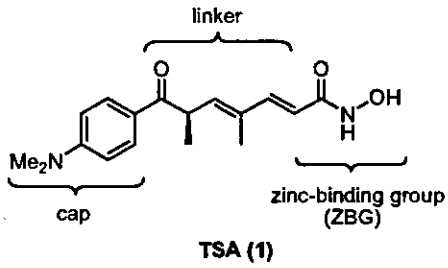


図3　TSA と SAHA の構造

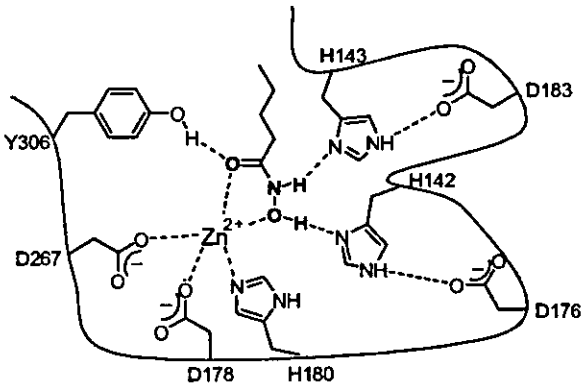


図4　ヒドロキサム酸と HDAC 活性中心を構成するアミノ酸、亜鉛イオンとの相互作用