4.18 (1H, sextet, J = 6.9 Hz), 3.94 (1H, s), 2.83 (2H, t, J = 7.2 Hz), 2.73 (1H, septet, J = 6.9 Hz), 1.97–1.94 (2H, m), 1.83–1.78 (1H, m), 1.68–1.53 (5H, m), 1.44 (9H, s), 1.47–1.37 (8H, m), 1.19 (6H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 170.59, 155.72, 77.23, 50.37, 43.11, 35.04, 34.95, 29.40, 28.43, 28.32, 28.30, 27.99, 25.06, 24.36, 19.42; Anal. ($C_{21}H_{38}N_2O_4S\cdot 1/3H_2O$) C, H, N.

Compounds **17b** and **19b–25b** were prepared from **43** and an appropriate amine using the procedure described for **16b**.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclohexylamino)-7-oxoheptyl 2-Methylpropanethioate (17b). Yield 19%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.89 (1H, d, J = 8.2 Hz), 4.99 (1H, s), 3.94 (1H, s), 3.75 (1H, s), 2.83 (2H, t, J = 7.4 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 1.90–1.78 (3H, m), 1.72–1.68 (2H, m), 1.65–1.50 (4H, m), 1.47–1.28 (15H, m), 1.21–1.10 (9H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.35, 170.87, 155.69, 77.23, 48.13, 43.11, 33.04, 29.39, 28.41, 28.32, 25.49, 25.03, 24.72, 19.42; MS (EI) m/z 428 (M^+); HRMS calcd for $C_{22}H_{40}O_4N_2S$, 428.270; found, 428.276.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(*tert*-butylamino)-7-oxoheptyl 2-Methylpropanethioate (19b). Yield 33%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.80 (1H, s), 4.97 (1H, s), 3.88 (1H, s), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.9 Hz), 1.81–1.74 (1H, m), 1.59–1.50 (2H, m), 1.48–1.40 (11H, m), 1.39–1.34 (11H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 171.11, 77.23, 51.32, 43.11, 32.33, 29.41, 28.71, 28.46, 28.31, 25.02, 19.42; Anal. ($C_{20}H_{38}N_2O_4S\cdot 2/3H_2O$) C, H, N.

(*S*)-*S*-7-(Adamant-1-ylamino)-6-(*tert*-Butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (20b). Yield 66%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm), 5.62 (1H, s), 4.99 (1H, m), 3.88 (1H, m), 2.83 (2H, t, J = 7.3 Hz), 2.72 (1H, septet, J = 6.7 Hz), 2.07 (3H, s), 1.98 (6H, s), 1.77 (1H, sextet, J = 7.0 Hz), 1.67 (6H,s), 1.56–1.30 (16H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$,500 MHz, δ, ppm), 204.27, 170.87, 155.69, 79.90, 55.01, 43.12, 41.58, 36.33, 36.20, 32.51, 29.55, 29.50, 29.43, 28.49, 28.34, 25.00, 19.42; MS (FAB) m/z 481 (MH^+); Anal. ($C_{26}H_{44}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2,3-dihydro-1*H*-inden-2-ylamino)-7-oxoheptyl 2-Methylpropanethioate (21b). Yield 87%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.23–7.14 (4H, m), 6.24 (1H, d, J = 7.6 Hz), 4.94 (1H, m), 4.71 (1H, m), 3.94 (1H, m), 3.30 (2H, m), 2.76 (4H, m), 2.69 (2H, septet, J = 6.7 Hz), 1.79 (1H, m), 1.58–1.51 (3H, m), 1.48–1.51 (13H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.32, 171.76, 155.68, 140.71, 126.81, 124.79, 80.09, 54.54, 50.57, 43.11, 40.09, 39.99, 32.24, 29.70, 28.37, 28.28, 25.05, 19.41; MS (FAB) m/z 463 (MH^+); Anal. ($C_{25}H_{38}N_2O_4S\cdot 1/2\ H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-hydroxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (22b). Yield 46%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.43 (1H, m), 4.98 (1H, m), 3.99 (1H, m), 3.72 (2H, q, J = 4.8 Hz), 3.43 (2H, m), 2.61 (1H, broad s) 1.82 (1H, m), 1.57–1.30 (16H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.68, 173.06, 155.57, 80.37, 61.96, 54.89, 43.13, 42.34, 32.12, 29.35, 28.32, 28.26, 28.39, 28.18, 24.95, 19.42; MS (FAB) m/z 391 (MH^+); Anal. ($C_{18}H_{34}N_2O_5S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-methoxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (23b). Yield 72%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.30 (1H, s), 4.98 (1H, m), 4.02 (1H, m), 3.45 (4H, m), 3.35 (3H, m), 2.83 (2H, t, J = 7.3 Hz), 2.72 (1H, septet, J = 6.9 Hz), 1.82 (1H, sextet, J = 7.0 Hz), 1.60–1.55 (2H, m), 1.50–1.29 (14H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.28, 172.02, 155.61, 80.02, 71.06, 58.77, 54.59, 43.12, 39.20, 32.57, 29.42, 28.43, 28.32, 25.03, 19.41; MS (FAB) m/z 495 (MH^+); Anal. ($C_{19}H_{36}N_2O_5S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-7-Amino-6-(*tert*-butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (24b). Yield 36%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.04 (1H, broad s), 5.36 (1H, broad s), 4.97 (1H, m), 4.10 (1H, m), 2.83 (2H, t, J = 7.6 Hz), 2.72 (1H, septet, J = 6.7 Hz), 1.84 (1H, m), 1.60–1.31 (16H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.37, 174.46, 155.74, 80.23, 54.03, 43.13, 32.10, 29.38, 28.33, 28.26, 25.94, 19.33; MS (FAB) m/z 347 (MH^+); Anal. ($C_{16}H_{30}N_2O_4S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(pyrrolidin-1-yl)heptyl 2-Methylpropanethioate (25b). Yield 74%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.32 (1H, d, J = 8.5 Hz), 4.40 (1H, m), 3.63 (1H, m), 3.53 (1H, m), 3.41 (2H, m), 2.82 (2H, t, J = 7.3 Hz), 2.72 (2H, quintet, J = 6.7 Hz), 1.97 (2H, quintet, J = 6.7 Hz), 1.87 (2H, m), 1.66 (1H, m), 1.60–1.29 (16H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.86, 155.58, 79.48, 51.84, 46.45, 45.94, 33.83, 33.11, 28.40, 28.10, 26.07, 24.48, 24.17; MS (FAB) m/z 401 (MH^+); MS (EI) m/z 400 (M^+); HRMS calcd for $C_{20}H_{36}N_2O_4S$, 400.240; found, 400.240.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cycloheptylamino)-7-oxoheptyl 2-Methylpropanethioate (18b). Compound **18b** was prepared from **43** using the procedure described for **11b** (step 2) and **16b** (step 2) in 63% yield: yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.98 (1H, d, J = 8.2 Hz), 4.98 (1H, s), 3.95–3.91 (2H, m), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.88–1.87 (2H, m), 1.81–1.78 (1H, m), 1.61–1.36 (26H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.31, 171.16, 77.23, 60.40, 50.37, 43.11, 32.27, 29.40, 28.32, 28.30, 27.99, 25.06, 24.03, 21.06, 19.42, 14.21; Anal. ($C_{23}H_{42}N_2O_4S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-Amino-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate Hydrochloride (26b·HCl). To a solution of **16b** (962 mg, 2.32 mmol) in AcOEt (12 mL) was added 4 N HCl/AcOEt (6 mL), and the mixture was stirred at room temperature for 2 h. Then, the solvent was removed in vacuo to give 813 mg (100%) of **26b**·HCl as a colorless oil: ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.59 (1H, d, J = 7.3 Hz), 8.19 (3H, broad s), 4.03 (1H, sextet, J = 6.7 Hz), 3.67 (1H, m), 2.81 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.7 Hz), 1.85–1.76 (2H, m), 1.69–1.58 (4H, m), 1.52–1.20 (10H, m), 1.10 (6H, t, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 203.17, 167.70, 52.14, 50.55, 42.40, 32.32, 31.83, 30.87, 28.82, 27.61, 27.59, 23.60, 23.38, 23.33, 19.09; MS (FAB) m/z 315 (MH^+ − HCl); Anal. ($C_{16}H_{30}N_2O_2S\cdot HCl\cdot H_2O$) C, H, N.

(*S*)-*S*-6-Benzamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (27b). To a suspension of **26b**·HCl (250 mg, 0.712 mmol) and Et_3N (1 mL, 13.6 mmol) in CH_2Cl_2 (2 mL) was added a solution of benzoyl chloride (248 μL mg, 2.13 mmol) in CH_2Cl_2 (3 mL) dropwise. The reaction mixture was poured into water and was extracted with AOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid and brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by flash column chromatography (AcOEt/*n*-hexane = 1/4 to 1/2) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 208 mg (70%) of **27b** as colorless crystals: mp 116–117 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.78 (2H, m), 7.51 (1H, t, J = 7.3 Hz), 7.43 (2H, t, J = 7.6 Hz), 6.89 (1H, d, J = 7.9 Hz), 6.24 (1H, d, J = 7.6 Hz), 4.57 (1H, q, J = 7.3 Hz), 4.19 (1H, sextet, J = 7.0 Hz), 2.83 (2H, m), 2.72 (1H, septet, J = 6.7 Hz), 2.02–1.90 (3H, m), 1.80–1.65 (7H, m), 1.45–1.35 (6H, m), 1.18 (6H, dd, J = 6.9, 1.2 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.36, 170.98, 167.24, 133.95, 131.73, 128.57, 127.06, 53.50, 51.34, 43.09, 33.10, 32.94, 32.57, 29.35, 28.35, 28.20, 24.85, 23.75, 23.72, 19.40; MS (EI) m/z 418 (M^+); HRMS calcd for $C_{23}H_{34}N_2O_3S$, 418.229; found, 418.229; Anal. ($C_{23}H_{34}N_2O_3S$) C, H, N.

Compounds **28b** and **29b** were prepared from **26b**·HCl and an appropriate acid chloride using the procedure described for **27b**.

(*S*)-*S*-6-(4-Chlorobenzamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (28b). Yield 95%; mp 119–121 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.74 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz), 6.89 (1H, d, J = 7.9 Hz), 6.04 (1H, d, J = 7.6 Hz), 4.51 (1H, q, J = 7.0 Hz), 4.20 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.72 (1H, septet, J = 7.0 Hz), 2.04–1.87 (3H, m), 1.80–1.53 (9H, m), 1.40 (6H, m), 1.18 (6H, dd, J = 7.0, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.48, 170.88, 166.13,

138.03, 132.32, 128.85, 128.55, 53.37, 51.44, 43.13, 33.13, 32.97, 32.64, 29.64, 28.25, 28.13, 24.76, 23.75, 23.72, 19.43, 19.41; Anal. ($C_{23}H_{33}ClN_2O_3S$) C, H, N.

(*S*)-*S*-7-(Cyclopentylamino)-6-(furan-2-carboxamido)-7-oxoheptyl 2-Methylpropanethioate (29b). Yield 37%; mp 88−89 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 7.46 (1H, d, *J* = 0.9 Hz), 7.11 (1H, d, *J* = 3.6 Hz), 6.88 (1H, d, *J* = 7.9 Hz), 6.50 (1H, dd, *J* = 3.5, 1.8 Hz), 6.03 (1H, d, *J* = 7.0 Hz), 4.46 (1H, q, *J* = 7.6 Hz), 4.19 (1H, sextet, *J* = 6.7 Hz), 2.84 (2H, td, *J* = 7.5, 2.1 Hz), 2.72 (1H, septet, *J* = 7.0 Hz), 2.02−1.87 (3H, m), 1.74−1.53 (7H, m), 1.45−1.34 (6H, m), 1.18 (6H, dd, *J* = 7.0, 0.9 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.39, 170.66, 158.20, 147.48, 144.28, 114.66, 112.15, 52.83, 51.39, 43.13, 33.13, 32.98, 32.43, 29.37, 28.35, 28.26, 24.93, 23.76, 23.73, 19.42; MS (EI) *m/z* 408 (M$^+$); HRMS calcd for $C_{21}H_{32}N_2O_4S$, 408.208; found, 408.209; Anal. ($C_{21}H_{32}N_2O_4S$) C, H, N.

Compounds **30b** and **32b−34b** were prepared from **26b**·HCl and an appropriate carboxylic acid using the procedure described for **16b** (step 1).

(*S*)-*S*-7-(Cyclopentylamino)-6-(2-hydroxyacetamido)-7-oxoheptyl 2-Methylpropanethioate (30b). Yield 28%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.97 (1H, d, *J* = 8.2 Hz), 5.99 (1H, d, *J* = 7.3 Hz), 4.34 (1H, m), 4.21−4.13 (3H, m), 2.91 (1H, broad s), 2.83 (2H, m), 2.74 (1H, septet, *J* = 7.0 Hz), 1.98 (2H, m), 1.85 (1H, m), 1.71−1.53 (7H, m), 1.44−1.31 (6H, m), 1.18 (6H, dd, *J* = 7.0, 1.4 Hz); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 204.91, 171.85, 170.86, 62.21, 52.78, 51.38, 43.14, 33.08, 32.92, 32.25, 29.28, 29.24, 29.06, 28.04, 24.73, 23.74, 19.44, 19.42; MS (EI) *m/z* 372 (M$^+$); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208.

(*S*)-*S*-6-(Cyclohexanecarboxamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (32b). Yield 62%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.34 (2H, m), 4.29 (1H, q, *J* = 6.7 Hz), 4.16 (1H, sextet, *J* = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, *J* = 7.0 Hz), 2.10 (1H, tt, *J* = 7.0, 3.3 Hz), 1.94 (2H, m), 1.90−1.75 (5H, m), 1.70−1.56 (9H, m), 1.49−1.22 (10H, m), 1.18 (6H, d, *J* = 7.0 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.39, 176.22, 171.14, 52.75, 51.22, 45.42, 43.13, 33.07, 33.01, 32.03, 29.70, 29.35, 28.33, 28.22, 25.72, 25.68, 25.64, 24.87, 23.74, 19.42; MS (EI) *m/z* 424 (M$^+$); HRMS calcd for $C_{23}H_{40}N_2O_3S$, 424.276; found, 424.275.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-pivalamidoheptyl 2-Methylpropanethioate (33b). Yield 62%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.21 (1H, d, *J* = 7.6 Hz), 6.08 (1H, d, *J* = 7.3 Hz), 4.29 (1H, q, *J* = 6.7 Hz), 4.17 (1H, sextet, *J* = 7.0 Hz), 2.82 (2H, m), 2.72 (1H, septet, *J* = 7.0 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.70−1.53 (7H, m), 1.42−1.29 (6H, m), 1.20 (9H, m), 1.18 (6H, d, *J* = 7.0 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.34, 178.62, 171.17, 52.89, 51.22, 43.13, 38.75, 33.07, 31.98, 29.37, 28.36, 28.23, 27.48, 24.90, 23.74, 23.69, 19.41; MS (EI) *m/z* 398 (M$^+$); HRMS calcd for $C_{21}H_{38}N_2O_3S$, 398.260; found, 398.261.

(*S*)-*S*-7-(Cyclopentylamino)-6-(3,3-dimethylbutanamido)-7-oxoheptyl 2-Methylpropanethioate (34b). Yield 59%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.21 (1H, d, *J* = 7.3 Hz), 6.05 (1H, d, *J* = 8.2 Hz), 4.30 (1H, q, *J* = 6.7 Hz), 4.16 (1H, sextet, *J* = 6.9 Hz), 2.81 (2H, m), 2.73 (1H, septet, *J* = 6.7 Hz), 2.07 (2H, m), 1.96 (2H, m), 1.80 (1H, m), 1.70−1.52 (7H, m), 1.42−1.30 (6H, m), 1.18 (6H, d, *J* = 6.7 Hz), 1.02 (9H, s); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.41, 171.89, 171.10, 53.02, 52.94, 51.23, 50.46, 43.13, 33.09, 32.97, 32.34, 31.90, 30.99, 29.85, 29.33, 28.31, 28.19, 23.75, 23.73, 19.43, 19.42; MS (EI) *m/z* 412 (M$^+$); HRMS calcd for $C_{22}H_{40}N_2O_3S$, 412.276; found, 412.276.

(*S*)-*S*-6-Acetamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (31b). To a solution of **26b**·HCl (102 mg, 0.291 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (3 mL) were added acetic acid anhydride (55 μL, 0.582 mmol) and Et_3N (200 μL, 2.72 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 3/1 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 54 mg (52%) of **31b** as colorless crystals: mp 130−131 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.10 (1H, d, *J* = 8.2 Hz), 5.94 (1H, d, *J* = 7.6 Hz), 4.28 (1H, q, *J* = 7.3 Hz), 4.17 (1H, sextet, *J* = 7.3 Hz), 2.82 (2H, m), 2.73 (1H, septet, *J* = 6.7 Hz), 2.02−1.93 (5H, m), 1.80 (1H, m), 1.74−1.49 (7H, m), 1.46−1.29 (6H, m), 1.18 (6H, dd, *J* = 7.0, 0.9 Hz); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 204.46, 170.02, 170.02, 53.17, 51.30, 43.14, 33.12, 32.97, 32.34, 29.32, 28.26, 28.16, 24.77, 23.73, 23.71, 23.26, 19.43; MS (EI) *m/z* 356 (M$^+$); HRMS calcd for $C_{18}H_{32}N_2O_3S$, 356.213; found, 356.213; Anal. ($C_{18}H_{32}N_2O_3S$) C, H, N.

(*S*)-*S*-6-(3-*tert*-Butylureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (35b). To a solution of **26b**·HCl (99 mg, 0.282 mmol) and Et_3N (400 μL, 5.44 mmol) in CH_2Cl_2 (4 mL) was added *tert*-butyl isocyanate (132 μL, 1.14 mmol), and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/1) gave 89 mg (76%) of **35b** as a colorless oil: ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.27 (1H, d, *J* = 7.9 Hz), 4.81 (1H, d, *J* = 7.9 Hz), 4.48 (1H, s), 4.16 (1H, sextet, *J* = 6.7 Hz), 4.07 (1H, q, *J* = 5.8 Hz), 2.92−2.70 (3H, m), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.55 (7H, m), 1.43−1.25 (15H, m), 1.18 (6H, dd, *J* = 6.7, 0.9 Hz); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 204.82, 172.39, 157.10, 53.85, 51.12, 50.57, 43.14, 33.08, 33.00, 32.07, 29.43, 29.22, 28.21, 28.12, 24.78, 23.73, 23.69, 19.46, 19.44; MS (EI) *m/z* 413 (M$^+$); HRMS calcd for $C_{21}H_{39}N_3O_3S$, 413.271; found, 413.273.

(*S*)-*S*-6-(3-*tert*-Butylthioureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (36b). Compound **36b** was prepared from **26b**·HCl and *tert*-thioisocyanate using the procedure described for **35b** in 24% yield: colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.53 (1H, d, *J* = 7.0 Hz), 6.09 (1H, m), 5.97 (1H, d, *J* = 7.0 Hz), 4.85 (1H, m), 4.19 (1H, sextet, *J* = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, *J* = 7.0 Hz), 1.98 (3H, m), 1.78−1.52 (7H, m), 1.48−1.29 (15H, m), 1.19 (6H, dd, *J* = 7.0, 1.5 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.47, 179.78, 170.93, 58.73, 52.94, 51.53, 43.12, 33.10, 32.94, 32.64, 29.46, 29.34, 28.33, 28.15, 24.21, 23.69, 23.64, 19.44, 19.42; MS (EI) *m/z* 429 (M$^+$); HRMS calcd for $C_{21}H_{39}N_3O_2S_2$, 429.248; found, 429.248.

(*S*)-*S*-7-(Cyclopentylamino)-6-(methoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (37b). To a solution of **26b**·HCl (177 mg, 0.504 mmol) in CH_2Cl_2 (3 mL) were added methyl chloroformate (39 μL, 0.505 mmol) and Et_3N (500 μL, 6.80 mmol), and the mixture was stirred at room temperature for 15 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/2 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 138 mg (74%) of **37b** as colorless crystals: mp 88−89 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 5.90 (1H, d, *J* = 6.7 Hz), 5.24 (1H, broad s), 4.18 (1H, sextet, *J* = 7.0 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.82 (2H, td, *J* = 7.6, 2.4 Hz), 2.73 (1H, septet, *J* = 7.0 Hz), 1.89 (2H, m), 1.81 (1H, m), 1.73−1.53 (7H, m), 1.42−1.31 (6H, m), 1.18 (6H, d, *J* = 7.0 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 204.38, 171.08, 156.86, 54.99, 52.38, 51.29, 43.13, 33.12, 33.01, 32.61, 29.71, 29.36, 28.30, 28.23, 24.82, 23.74, 19.42; MS (EI) *m/z* 372 (M$^+$); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208; Anal. ($C_{18}H_{32}N_2O_4S$) C, H, N.

Compounds **39b−41b** were prepared from **26b**·HCl and an appropriate chloroformate using the procedure described for **37b**.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-(phenoxycarbonyl)heptyl 2-Methylpropanethioate (39b). Yield 17%; colorless oil; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 7.36 (2H, t, *J* = 7.9 Hz), 7.20 (1H, t, *J* = 7.6 Hz), 7.12 (2H, d, *J* = 7.9 Hz), 5.85 (1H, d, *J* = 7.0 Hz), 5.66 (1H, d, *J* = 8.2 Hz), 4.22 (1H, sextet, *J* = 7.3 Hz), 4.09

(1H, q, J = 7.3 Hz), 2.83 (2H, td, J = 7.0, 2.4 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.99 (2H, m), 1.86 (1H, m), 1.72−1.53 (7H, m), 1.46−1.36 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.42, 170.69, 154.82, 150.92, 129.33, 125.47, 121.54, 55.06, 51.42, 43.14, 33.15, 33.02, 32.71, 29.37, 28.26, 28.21, 24.76, 23.74, 19.42; MS (FAB) m/z 435 (MH^+); Anal. ($C_{23}H_{34}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(Benzyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (40b). Yield 24%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.89 (1H, m), 5.31 (1H, m), 5.10 (2H, s), 4.17 (1H, sextet, J = 6.7 Hz), 4.04 (1H, m), 2.82 (2H, td, J = 7.2, 2.1 Hz), 2.72 (1H, septet, J = 7.0 Hz), 1.94 (2H, s), 1.81 (1H, m), 1.70−1.51 (7H, m), 1.42−1.23 (6H, m), 1.17 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.39, 170.98, 156.19, 136.27, 128.56, 128.22, 128.06, 67.04, 55.00, 51.28, 43.12, 33.09, 32.99, 32.52, 29.36, 28.30, 28.23, 24.84, 23.71, 19.42; MS (EI) m/z 448 (M^+); HRMS calcd for $C_{24}H_{36}N_2O_4S$, 448.240; found, 448.239; Anal. ($C_{24}H_{36}N_2O_4S$) C, H, N.

(*S*)-*S*-6-[{(9*H*-Fluoren-9-yl)methoxy}carbonyl]-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (41b). Yield 11%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.76 (2H, d, J = 7.6 Hz), 7.58 (2H, d, J = 7.6 Hz), 7.40 (2H, t, J = 7.6 Hz), 7.31 (2H, td, J = 7.7, 0.9 Hz), 5.82 (1H, m), 5.34 (1H, d, J = 7.7 Hz), 4.40 (2H, m), 4.23−4.15 (1H, m), 4.02 (1H, m), 2.84 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 1.97 (2H, m), 1.82 (1H, m), 1.68−1.52 (7H, m), 1.45−1.25 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 170.95, 156.19, 143.80, 141.35, 127.75, 127.10, 125.04, 120.02, 120.00, 67.01, 54.99, 51.32, 47.21, 43.14, 33.12, 33.02, 32.59, 29.37, 28.28, 28.23, 24.80, 23.74, 23.72, 19.42; MS (FAB) m/z 537 (MH^+); Anal. ($C_{31}H_{40}N_2O_4S \cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(Cyclohexyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (38b). To a solution of triphosgene (654 mg, 2.20 mmol) in dry Et_2O (4 mL) was added dropwise a mixture of cyclohexanol (682 μL, 6.46 mmol) and pyridine (626 μL, 7.74 mmol) at −78 °C. The mixture was stirred for 1 h at −78 °C and then 1.5 h at room temperature. The reaction mixture was poured into 1 N aqueuos HCl and was extracted with AcOEt, washed with brine, and dried over Na_2SO_4. Filtration and evaporation in vacuo gave a colorless oil. To a solution of **26b**·HCl (94 mg, 0.268 mmol) and Et_3N (300 μL, 4.08 mmol) in CH_2Cl_2 (4 mL) was added the colorless oil, and the mixture was stirred at room temperature for 13 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 30 mg (26%) of **38b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.91 (1H, d, J = 7.3 Hz), 5.08 (1H, m), 4.61 (1H, m), 4.18 (1H, sextet, J = 7.6 Hz), 3.98 (1H, m), 2.82 (2H, td, J = 7.0, 1.8 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.98 (2H, m), 1.84 (3H, m), 1.75−1.50 (11H, m), 1.37 (10H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 171.20, 63.46, 54.80, 51.20, 43.11, 33.11, 33.03, 32.41, 31.92, 29.37, 28.34, 28.25, 25.36, 24.92, 23.72, 23.70, 23.69, 19.42; MS (FAB) m/z 441 (MH^+); MS (EI) m/z 440 (M^+); HRMS calcd for $C_{23}H_{40}N_2O_4S$, 440.271; found, 440.270.

(*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). Step 1: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl Ethanethioate (16d). A solution of **16c** (410 mg, 1.05 mmol) obtained above and KSAc (183 mg, 1.60 mmol) in EtOH (3 mL) was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo. The residue was subjected to silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) to give 303 mg (75%) of **16d** as a yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.3 Hz), 4.97 (1H, s), 4.18 (1H, m), 3.94 (1H, m), 2.85 (2H, t, J = 7.3 Hz), 2.32 (3H, s), 1.97−1.96 (2H, m), 1.81−1.80 (1H, m), 1.67−1.55 (8H, m), 1.44 (9H, s), 1.39−1.36 (5H, m).

Step 2: Preparation of (*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). To a solution of **16d** (300 mg, 0.778 mmol) obtained above in EtOH (5 mL) was added 2 N aqueous NaOH (2 mL, 4.00 mmol), and the solution was stirred at room temperature for 10 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) gave 200 mg (74%) of **16a** as a colorless solid. The solid (106 mg) was recrystallized from AcOEt/*n*-hexane to give 60 mg of **16a** as colorless crystals: mp 108−110 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.93 (1H, d, J = 7.0 Hz), 4.95 (1H, s), 4.19−4.16 (1H, m), 3.94 (1H, s), 2.52 (2H, q, J = 7.3 Hz), 1.98−1.96 (2H, m), 1.85−1.80 (1H, m), 1.67−1.52 (7H, m), 1.44 (9H, s), 1.42−1.31 (7H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.89, 77.23, 48.04, 36.96, 33.79, 33.31, 28.65, 28.04, 25.68, 25.56, 24.88, 24.56; Anal. ($C_{17}H_{32}N_2O_3S$) C, H, N.

Compounds **13a**, **15a**, and **17a**−**20a** were prepared from the corresponding bromide using the procedure described for **16a**.

(*S*)-*tert*-Butyl 1-(Biphenyl-3-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (13a). Yield 34%; colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.23 (1H, broad s), 7.79 (1H, s), 7.58 (2H, d, J = 7.3 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.44−7.33 (5H, m), 4.97 (1H, s), 4.18 (1H, s), 2.53 (2H, q, J = 7.3 Hz), 2.00−1.96 (1H, m), 1.71−1.59 (3H, m), 1.47−1.43 (13H, m), 1.33 (1H, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.39, 156.27, 142.14, 140.64, 138.12, 129.35, 128.73, 127.48, 127.18, 123.18, 118.65, 118.61, 80.71, 55.27, 33.70, 30.93, 28.33, 27.99, 25.25, 24.46; MS (EI) m/z 428 (M^+); HRMS calcd for $C_{24}H_{34}N_2O_3S$, 428.213; found, 428.213.

(*S*)-*tert*-Butyl 7-Mercapto-1-oxo-1-(quinolin-3-ylamino)heptan-2-ylcarbamate (15a). Yield 39%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.79 (1H, broad s), 8.74 (2H, s), 8.03 (1H, d, J = 8.5 Hz), 7.78 (1H, d, J = 7.9 Hz), 7.62 (1H, t, J = 7.0 Hz), 7.52 (1H, t, J = 7.8 Hz), 5.00 (1H, d, J = 6.7 Hz), 4.26 (1H, m), 2.53 (2H, q, J = 7.6 Hz), 2.00 (1H, m), 1.73−1.63 (3H, m), 1.50−1.36 (13H, m), 1.34 (1H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.17, 145.18, 143.89, 131.37, 128.97, 128.20, 128.07, 127.68, 127.14, 123.66, 81.13, 77.23, 33.65, 30.94, 28.35, 27.97, 25.35, 24.43; MS (EI) m/z 403 (M^+); HRMS calcd for $C_{21}H_{29}N_3O_3S$, 403.193; found, 403.194.

(*S*)-*tert*-Butyl 1-(cyclohexylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (17a). Yield 72%; mp 125−127 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.85 (1H, d, J = 7.9 Hz), 4.96 (1H, s), 3.95 (1H, s), 3.75 (1H, s), 2.51 (2H, q, J = 7.4 Hz), 1.90−1.78 (3H, m), 1.72−1.68 (2H, m), 1.64−1.56 (4H, m), 1.44 (9H, s), 1.43−1.31 (7H, m), 1.19−1.13 (3H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.84, 77.23, 48.13, 33.74, 33.08, 32.93, 30.94, 28.33, 27.99, 25.49, 25.07, 24.71, 24.46; MS (EI) m/z 358 (M^+); HRMS calcd for $C_{18}H_{34}O_3N_2S$, 358.229; found, 358.229; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Cycloheptylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (18a). Yield 62%; mp 76−78 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.92 (1H, d, J = 7.6 Hz), 4.96 (1H, s), 3.95−3.91 (2H, m), 2.52 (2H, q, J = 7.3 Hz), 1.88−1.79 (3H, m), 1.62−1.58 (7H, m), 1.54−1.33 (20H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.55, 77.21, 54.60, 50.38, 35.06, 34.96, 33.74, 32.34, 28.33, 27.99, 25.08, 24.47, 24.02; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(*tert*-Butylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (19a). Yield 58%; mp 90−91 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.78 (1H, s), 4.97 (1H, m), 3.89 (1H, m), 2.52 (2H, q, J = 7.6 Hz), 1.79 (1H, sextet, J = 8.2 Hz), 1.66−1.50 (3H, m), 1.49−1.38 (11H, m), 1.36−1.30 (12H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.05, 155.69, 79.90, 54.91, 51.26, 33.68, 32.36, 28.66, 28.26, 27.94, 24.96, 24.39; MS (FAB) m/z 333 (MH^+); Anal. ($C_{16}H_{32}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Adamant-1-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (20a). Yield 41%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.60 (1H, s), 4.97 (1H, m), 3.88 (1H, m), 2.81 (2H, q, J = 7.3 Hz), 2.07 (3H, s), 1.98 (6H, d, J = 2.7 Hz), 1.78 (1H, sextet, J = 7.8 Hz), 1.67 (3H, s), 1.65−1.30 (17H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.87, 155.71, 79.93,

54.98, 41.60, 36.32, 33.76, 32.60, 30.92, 29.55, 29.43, 29.22, 28.35, 28.02, 25.00, 24.47; MS (FAB) *m/z* 411 (MH^+); Anal. ($C_{22}H_{38}N_2O_3S\cdot 1/4H_2O$) C, H, N.

(*S*)-2-Amino-*N*-cyclopentyl-7-mercaptoheptanamide Hydrochloride (26a·HCl). Compound **26a**·HCl was prepared from **16a** using the procedure described for **26b**·HCl in 94% yield: colorless oil; ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.43 (1H, d, J = 7.3 Hz), 8.13 (3H, broad s), 4.03 (1H, sextet, J = 6.7 Hz), 3.65 (1H, t, J = 6.7 Hz), 2.46 (2H, q, J = 7.3 Hz), 2.28 (1H, t, J = 7.6 Hz), 1.82 (2H, m), 1.66 (4H, m), 1.58−1.23 (10H, m); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 167.65, 52.08, 50.46, 32.89, 32.24, 31.75, 30.85, 27.08, 23.50, 23.41, 23.30, 23.24; MS (FAB) *m/z* 245 (MH^+ − HCl); Anal. ($C_{12}H_{24}N_2OS\cdot HCl\cdot 3/4H_2O$) C, H, N.

Compounds **27a** and **28a** were prepared from **16d** using the procedure described for **26b**·HCl, **27b**, and **16a** (step 2).

(*S*)-*N*-(1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl)-benzamide (27a). Yield 69%; mp 171−172 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 7.79 (2H, m), 7.52 (1H, t, J = 7.3 Hz), 7.44 (2H, t, J = 7.6 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.01 (1H, d, J = 7.0 Hz), 4.54 (1H, q, J = 7.0 Hz), 4.19 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.6 Hz), 1.98 (3H, m), 1.77−1.56 (7H, m), 1.48−1.35 (6H, m), 1.32 (1H, t, J = 7.6 Hz); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 170.94, 167.23, 133.94, 131.81, 128.65, 127.04, 53.50, 51.41, 33.71, 33.17, 33.00, 32.76, 28.04, 25.01, 24.46, 23.75, 23.72; MS (EI) *m/z* 348 (M^+); HRMS calcd for $C_{19}H_{28}N_2O_2S$, 348.187; found, 348.187; Anal. ($C_{19}H_{28}N_2O_2S$) C, H, N.

(*S*)-4-Chloro-*N*-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]benzamide (28a). Yield 54%; mp 178−180 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 7.73 (2H, d, J = 8.5 Hz), 7.41 (2H, d, J = 8.5 Hz), 6.84 (1H, d, J = 7.6 Hz), 5.95 (1H, d, J = 7.3 Hz), 4.53 (1H, q, J = 7.0 Hz), 4.20 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q J = 7.0 Hz), 1.96 (3H, m), 1.74−1.54 (7H, m), 1.41 (6H, m), 1.32 (1H, t, J = 7.9 Hz); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 170.84, 166.10, 138.08, 132.29, 128.88, 128.51, 53.55, 51.44, 33.66, 33.17, 32.96, 32.91, 28.01, 24.94, 24.45, 23.74, 23.71; Anal. ($C_{19}H_{27}ClN_2O_2S$) C, H, N.

Compounds **30a** and **33a** were prepared from **16d** using the procedure described for **26b**·HCl, **16b** (step 1), and **16a** (step 2).

(*S*)-*N*-Cyclopentyl-2-(2-hydroxyacetamido)-7-mercaptoheptanamide (30a). Yield 55%; mp 85−86 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.93 (1H, d, J = 8.5 Hz), 5.90 (1H, d, J = 6.7 Hz), 4.34 (1H, q, J = 7.9 Hz), 4.21−4.10 (3H, m), 2.69 (1H, t, J = 5.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.97 (2H, m), 1.83 (1H, m), 1.72−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 171.82, 170.99, 62.16, 52.97, 51.40, 33.65, 33.10, 32.89, 32.48, 27.93, 25.02, 24.45, 23.73; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.167; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-*N*-Cyclopentyl-7-mercapto-2-pivalamidoheptanamide (33a). Yield 60%; mp 134−139 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.20 (1H, d, J = 7.9 Hz), 6.08 (1H, d, J = 7.3 Hz), 4.30 (1H, q, J = 7.3 Hz), 4.17 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.71−1.55 (8H, m), 1.45−1.25 (6H, m), 1.20 (9H, s); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 178.60, 171.13, 52.83, 51.22, 38.75, 33.70, 33.08, 33.03, 32.13, 28.01, 27.47, 24.97, 24.45, 23.73, 23.68; MS (EI) *m/z* 328 (M^+); HRMS calcd for $C_{17}H_{32}N_2O_2S$, 328.218; found, 328.219; Anal. ($C_{17}H_{32}N_2O_2S$) C, H, N.

Compounds **35a** and **36a** were prepared from **16d** using the procedure described for **26b**·HCl, **35b**, and **16a** (step 2).

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]urea (35a). Yield 71%; mp 188−191 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.19 (1H, d, J = 6.7 Hz), 4.92 (1H, d, J = 8.5 Hz), 4.49 (1H, s), 4.16 (1H, sextet, J = 7.3 Hz), 4.09 (1H, q, J = 7.6 Hz), 2.52 (2H, q, J = 7.6 Hz), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.50 (7H, m), 1.46−1.22 (16H, m); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 172.45, 157.02, 53.83, 51.16, 50.57, 33.75, 33.10, 32.98, 32.64, 29.44, 28.07, 25.20, 24.47, 23.72, 23.67; Anal. ($C_{17}H_{33}N_3O_2S\cdot 1/6H_2O$) C, H, N.

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl)thiourea (36a). Yield 25%; mp 125−127 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 6.48 (1H, d, J = 7.6 Hz), 6.06 (1H, s), 5.85 (1H, d, J = 7.6 Hz), 4.87 (1H, m), 4.19 (1H, sextet, J = 7.0 Hz), 2.50 (2H, q, J = 7.0 Hz), 2.02 (3H, m), 1.76−1.58 (7H, m), 1.48−1.36 (16H, m); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 179.92, 171.32, 58.04, 53.03, 51.49, 33.71, 33.10, 32.87, 32.74, 29.42, 28.08, 24.50, 24.45, 23.70, 23.66; MS (EI) *m/z* 359 (M^+); HRMS calcd for $C_{17}H_{33}N_3O_2S$, 359.207; found, 359.205; Anal. ($C_{17}H_{33}N_3O_2S$) C, H, N.

Compounds **37a** and **40a** were prepared from **16d** using the procedure described for **26b**·HCl, **37b**, and **16a** (step 2).

(*S*)-Methyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (37a). Yield 89%; mp 81−82 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 5.79 (1H, d, J = 7.0 Hz), 5.18 (1H, m), 4.18 (1H, sextet, J = 6.7 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.51 (2H, q, J = 7.6 Hz), 1.98 (2H, m), 1.81 (1H, m), 1.71−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 171.02, 54.97, 52.41, 51.30, 33.68, 33.14, 33.03, 32.73, 27.93, 24.91, 24.45, 23.71; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.169; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-Benzyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (40a). Yield 77%; mp 119−122 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.80 (1H, m), 5.27 (1H, m), 5.11 (2H, s), 4.17 (1H, q, J = 6.7 Hz), 4.03 (1H, m), 2.50 (2H, q, J = 7.3 Hz), 1.96 (2H, s), 1.84 (1H, m), 1.70−1.53 (7H, m), 1.45−1.26 (7H, m); ^{13}C NMR (CDCl$_3$, 500 MHz, δ, ppm) 170.98, 156.18, 136.22, 128.57, 128.24, 128.06, 67.04, 54.17, 51.26, 33.68, 33.11, 32.98, 32.66, 27.93, 24.92, 24.44, 23.71; MS (EI) *m/z* 378 (M^+); HRMS calcd for $C_{20}H_{30}N_2O_3S$, 378.198; found, 378.197; Anal. ($C_{20}H_{30}N_2O_3S\cdot 1/3H_2O$) C, H, N.

(*S*)-Cyclohexyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (38a). Compound **38a** was prepared from **16d** using the procedure described for **26b**·HCl, **38b**, and **16a** (step 2) in 78% yield: mp 110−111 °C; ^{1}H NMR (CDCl$_3$, 500 MHz, δ, ppm) 5.85 (1H, m), 5.06 (1H, m), 4.62 (1H, m), 4.18 (1H, sextet, J = 7.0 Hz), 3.99 (1H, m), 2.51 (2H, q, J = 7.0 Hz), 1.97 (2H, m), 1.85 (3H, m), 1.74−1.46 (11H, m), 1.45−1.30 (11H, m); ^{13}C NMR (CDCl$_3$, 600 MHz, δ, ppm) 171.21, 156.06, 73.63, 54.75, 51.20, 33.70, 33.12, 33.03, 32.53, 31.91, 29.70, 27.96, 25.35, 24.99, 24.46, 23.76, 23.71, 23.39; MS (FAB) *m/z* 371 (MH^+); Anal. ($C_{19}H_{34}N_2O_3S\cdot 1/4H_2O$) C, H, N.

Biology. Western Blot Analysis. Human colon cancer HCT116 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in McCoy5A culture medium containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions. HCT-116 cells (5 × 10^5) were treated for 8 h with samples at the indicated concentrations in 10% FBS supplemented with McCoy's 5A medium and were then collected and extracted with SDS buffer. Protein concentrations of the lysates were determined using a Bradford protein assay kit (Bio-Rad Laboratories) with which equivalent amounts of protein from each lysate were resolved in 15% SDS-polyacrylamide gels and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After blocking for 30 min with Tris-buffered saline (TBS) containing 3% skimmed milk, the transblotted membranes were incubated overnight at 4 °C with hyperacetylated histone H4 antibody (Upstate Biotechnology; 1:4000 dilution), acetylated α-tubulin antibody (SIGMA; 1:4000 dilution), or β-actin antibody (Abcam; 1:500 dilution) in TBS containing 3% skimmed milk. After probing with the primary antibody, the membrane was washed twice with water and then incubated with goat, antirabbit, or antimouse IgG-horseradish peroxidase conjugates (diluted 1:5000) for 2 h at room temperature and washed twice more with water. The immunoblots were visualized by enhanced chemiluminescence.

Enzyme Assays. The inhibitory activities of the test compounds against partially purified HDAC1, HDAC4, and HDAC6 were assayed according to a method reported in ref 14c.

Cell Growth Inhibition Assay. Human colon cancer HCT116 cells and ERα-positive breast cancer MCF-7 cells, which were purchased from American Type Culture Collection (ATCC, Manassas, VA), were cultured in McCoy5A and Dulbecco's Modified

Eagle's Medium (DMEM) containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions, respectively. HCT116 and MCF-7 cells were plated in 96-well plates at initial densities of 5000 (HCT116) or 1500 (MCF-7) cells/well (50 μL/well) and incubated at 37 °C. After 24 h, cells were exposed to test compounds by adding solutions (50 μL/well) of compounds at various concentrations in McCoy5A (HCT116) and DMEM (MCF-7) medium at 37 °C at 5% CO_2 for 72 h. The mixtures were then treated with 10 μL of alamarBlue, and cells were further incubated at 37 °C for 3 h. The fluorescence in each well was measured on a fluorometric plate reader, with excitation set at 530 nm and emission detection set at 590 nm, and the percentage of cell growth was calculated from the fluorescence readings.

Acknowledgment. This research was partly supported by Grants-in-Aid for Young Scientists (B) from the Ministry of Education, Science, Culture, Sports, Science, Technology, Japan, and grants from Takeda Science Foundation and the Program for the Promotion of Fundamental studies in Health Science of the National Institute of Biomedical Innovation (NIBIO), Japan. We thank the Screening Committee of Anticancer Drugs, 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 HDAC1 inhibition assay results.

Supporting Information Available: Results of the elemental analysis of **11b**, **16b**, **18b–24b**, **26b–29b**, **31b**, **37b**, **39b–41b**, **16a–20a**, **26a–28a**, **30a**, **33a**, **35a–38a**, and **40a** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Sterner, D. E.; Berger, S. L. Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* **2000**, *64*, 435–459. (b) Yoshida, M.; Shimazu, T.; Matsuyama, A. Protein deacetylases: Enzymes with functional diversity as novel therapeutic targets. *Prog. Cell Cycle Res.* **2003**, *5*, 269–278. (c) Glozak, M. A.; Sengupta, N.; Zhang, X.; Seto, E. Acetylation and deacetylation of non-histone proteins. *Gene* **2005**, *363*, 15–23. (d) Konstantinopoulos, P. A.; Karamouzis, M. V.; Papavassiliou, A. G. Focus on acetylation: The role of histone deacetylase inhibitors in cancer therapy and beyond. *Expert Opin. Investig. Drugs* **2007**, *16*, 569–571.

(2) (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. Acetylation: A regulatory modification to rival phosphorylation? *EMBO J.* **2000**, *19*, 1176–1179. (c) Kouzarides, T. Histone acetylases and deacetylases in cell proliferation. *Curr. Opin. Genet. Dev.* **1999**, *9*, 40–48. (d) 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.

(3) (a) 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. (b) Hirose, T.; Sowa, Y.; Takahashi, S.; Saito, S.; Yasuda, C.; Shindo, N.; Furuichi, K.; Sakai, T. p53-Independent induction of Gadd45 by histone deacetylase inhibitor: Coordinate regulation by transcription factors Oct-1 and NF-Y. *Oncogene* **2003**, *22*, 7762–7773.

(4) (a) Biel, M.; Wascholowski, V.; Giannis, A. Epigenetics—An epicenter of gene regulation: Histones and histone-modifying enzymes. *Angew. Chem., Int. Ed.* **2005**, *44*, 3186–3216. (b) Mai, A.; Massa, S.; Rotili, D.; Cerbara, I.; Valente, S.; Pezzi, R.; Simeoni, S.; Ragno, R. Histone deacetylation in epigenetics: An attractive target for anticancer therapy. *Med. Res. Rev.* **2005**, *25*, 261–309. (c) Suzuki, T.; Miyata, N. Epigenetic control using natural products and synthetic molecules. *Curr. Med. Chem.* **2006**, *13*, 935–958. (d) Schaefer, S.; Jung, M. Chromatin modifications as targets for new anticancer drugs. *Arch. Pharm.* **2005**, *338*, 347–357.

(5) (a) Bali, P.; Pranpat, M.; Bradner, J.; Balasis, M.; Fiskus, W.; Guo, F.; Rocha, K.; Kumaraswamy, S.; Boyapalle, S.; Atadja, P.; Seto, E.; Bhalla, K. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: A novel basis for antileukemia activity of histone deacetylase inhibitors. *J. Biol. Chem.* **2005**, *280*, 26729–26734. (b) Hubbert, C.; Guardiola, A.; Shao, R.; Kawaguchi, Y.; Ito, A.; Nixon, A.; Yoshida, M.; Wang, X.; Yao, T. HDAC6 is a microtubule-associated deacetylase. *Nature* **2002**, *417*, 455–458. (c) Matsuyama, A.; Shimazu, T.; Sumida, Y.; Saito, A.; Yoshimatsu, Y.; Seigneurin-Berny, D.; Osada, H.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Horinouchi, S.; Yoshida, M. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. *EMBO J.* **2002**, *21*, 6820–6831. (d) Kovacs, J. J.; Murphy, P. J.; Gaillard, S.; Zhao, X.; Wu, J. T.; Nicchitta, C. V.; Yoshida, M.; Toft, D. O.; Pratt, W. B.; Yao, T. P. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol. Cell* **2005**, *18*, 601–607. (e) Zhang, X.; Yuan, Z.; Zhang, Y.; Youg, S.;Salas-Burgos, A.; John, K.; Olashaw, N.; Parsons, J. T.; Yang, X.; Dent, S. R.; Yao, T.; Lane, W. S.; Seto, E. HDAC6 modulates cell motility by altering the acetylation level of cortactin. *Mol. Cell* **2007**, *27*, 197–213.

(6) Hideshima, T.; Bradner, J. E.; Wong, J.; Chauhan, D.; Richardson, P.; Schreiber, S. L.; Anderson, K. C. Small-molecule inhibition of proteasome and aggresome function induces synergistic antitumor activity in multiple myeloma. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8567–8572.

(7) Saji, S.; Kawakami, M.; Hayashi, S.; Yoshida, N.; Hirose, M.; Horiguchi, S.; Itoh, A.; Funata, N.; Schreiber, S. L.; Yoshida, M.; Toi, M. Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. *Oncogene* **2005**, *24*, 4531–4539.

(8) (a) Dompierre, J. P.; Godin, J. D.; Charrin, B. C.; Cordelieres, F. P.; King, S. J.; Humbert, S.; Saudou, F. Histone deacetylase-6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J. Neurosci.* **2007**, *27*, 3571–3583. (b) Kozikoeski, A. P.; Chen, Y.; Gaysin, A.; Chen, B.; D'Annibale, M. A.; Suto, C. M.; Langley, B. C. Functional differences in epigenetic modulators—superiority of mercaptoacetamide-based histone deacetylase inhibitors relative to hydroxamates in cortical neuron neuroprotection studies. *J. Med. Chem.* **2007**, *50*, 3054–3061.

(9) (a) Miller, T. A.; Witter, D. J.; Belvedere, S. Histone deacetylase inhibitors. *J. Med. Chem.* **2003**, *46*, 5097–5116. (b) 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) Miller, T. A. Patent status of histone deacetylase inhibitors. *Expert Opin. Ther. Pat.* **2004**, *14*, 791–804. (d) Weinmann, H.; Ottow, E. Histone deacetylase inhibitors: a survey of recent patents. *Expert Opin. Ther. Pat.* **2005**, *15*, 1677–1690.

(10) (a) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by Trichostatin A. *J. Biol. Chem.* **1990**, *265*, 17174–17179. (b) 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.

(11) (a) 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. (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.

(12) 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.

(13) 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.

(14) (a) Suzuki, T.; Miyata, N. Non-hydroxamate histone deacetylase inhibitors. *Curr. Med. Chem.* **2005**, *12*, 2867–2880. (b) 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. (c) Furumai, R.; Komatsu, Y.; Nishino, N.; Khochbin, S.; Yoshida, M.; Horinouchi, S. Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 87–92. (d) Glaser, K. B.; Li, J.; Pease, L. J.; Staver, M. J.; Marcotte, P. A.; Guo, J.; Frey, R. R.; Garland, R. B.; Heyman, H. R.; Wada, C. K.; Vasudevan, A.; Michaelides, M. R.; Davidsen, S. K.; Curtin, M. L. Differential protein acetylation induced by novel histone deacetylase inhibitors. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 683–690. (e) Suzuki,

T.; Matsuura, A.; Kouketsu, A.; Hisakawa, S.; Nakagawa, H.; Miyata, N. Design and synthesis of nonhydroxamate histone deacetylase inhibitors: Identification of a selective histone acetylating agent. *Bioorg. Med. Chem.* **2005**, *13*, 4332–4342.

(15) (a) 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. (b) Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Domain-selective small-molecule inhibitor of histone deacetylase-6 (HDAC6)-mediated tubulin deacetylation. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4389–4394. (c) Sternson, S. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L. Synthesis of 7200 small molecules based on a substructural analysis of the histone deacetylase inhibitors trichostatin and trapoxin. *Org. Lett.* **2001**, *3*, 4239–4242.

(16) (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. (c) Suzuki, T.; Matsuura, A.; Kouketsu, A.; Nakagawa, H.; Miyata, N. Identification of a potent nonhydroxamate histone deacetylase inhibitor by mechanism-based drug design. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 331–335. (d) Suzuki, T.; Hisakawa, S.; Itoh, Y.; Suzuki, N.; Takahashi, K.; Kawahata, M.; Yamaguchi, K.; Nakagawa, H.; Miyata, N. Design, synthesis, and biological activity of folate receptor-targeted prodrugs of thiolate histone deacetylase inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4208–4212.

(17) (a) Suzuki, T.; Nagano, Y.; Kouketsu, A.; Matsuura, A.; Maruyama, S.; Kuronaki, M.; Nakagawa, H.; Miyata, N. Novel inhibitors of human histone deacetylases: Design, synthesis, enzyme inhibition, and cancer cell growth inhibition of SAHA-based nonhydroxamates. *J. Med Chem.* **2005**, *48*, 1019–1032. (b) Suzuki, T.; Hisakawa, S.; Itoh, Y.; Maruyama, S.; Kurotaki, M.; Nakagawa, H.; Miyata, N. Identification of a potent and stable antiproliferative agent by the prodrug formation of a thiolate histone deacetylase inhibitor. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1558–1561.

(18) Suzuki, T.; Kouketsu, A.; Itoh, Y.; Hisakawa, S.; Maeda, S.; Yoshida, M.; Nakagawa, H.; Miyata, N. Highly potent and selective histone deacetylase-6 inhibitors designed based on a small-molecular substrate. *J. Med. Chem.* **2006**, *49*, 4809–4812.

(19) Watanabe, L. A.; Jose, B.; Kato, T.; Nishino, N.; Yoshida, M. Synthesis of L-α-amino-ω-bromoalkanoic acid for side chain modification. *Tetrahedron Lett.* **2004**, *45*, 491–494.

(20) Heltweg, B.; Dequiedt, F.; Marshall, B. L.; Brauch, C.; Yoshida, M.; Nishino, N.; Verdin, E.; Jung, M. Subtype selective substrates for histone deacetylases. *J. Med. Chem.* **2004**, *47*, 5235–5243.

(21) Belvedere, S.; Witter, D. J.; Yan, J.; Secrist, J. P.; Richon, V.; Miller, T. A. Aminosuberoyl hydroxamic acids (ASHAs): A potent new class of HDAC inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3969–3971.

(22) Mai, A.; Massa, S.; Pezzi, R.; Simeoni, S.; Rotili, D.; Nebbioso, A.; Scognamiglio, A.; Altucci, L.; Loidl, P.; Brosch, G. Class II (IIa)-selective histone deacetylase inhibitors. 1. Synthesis and biological evaluation of novel (aryloxopropenyl)pyrrolyl hydroxyamides. *J. Med. Chem.* **2005**, *48*, 3344–3353.

(23) (a) Schiff, P. B.; Fant, J.; Horwitz, S. B. Promotion of microtubule assembly in vitro by taxol. *Nature* **1979**, *277*, 665–667. (b) Marcus, A. I.; Zhou, J.; O'Brate, A.; Hamel, E.; Wong, J.; Nivens, M.; El-Naggar, A.; Yao, T.; Khuri, F. R.; Giannakakou, P. The synergistic combination of the farnesyl transferase inhibitor lonafarnib and paclitaxel enhances tubulin acetylation and requires a functional tubulin deacetylase. *Cancer Res.* **2005**, *65*, 3883–3893.

JM7009217

J. Med. Chem. **2007**, *50*, 5053–5056

New Series of Antiprion Compounds: Pyrazolone Derivatives Have the Potent Activity of Inhibiting Protease-Resistant Prion Protein Accumulation

Ayako Kimata, Hidehiko Nakagawa,* Ryo Ohyama, Tomoko Fukuuchi, Shigeru Ohta, Takayoshi Suzuki, and Naoki Miyata*

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan, and Faculty of Pharmaceutical Sciences, Hiroshima International University, 5-1-1 Koshingai, Hiro, Kure, Hiroshima 737-0112, Japan

Received June 13, 2007

Abstract: To find effective antiprion compounds, we synthesized and evaluated various pyrazolone derivatives. Seven of 19 compounds showed inhibition of PrP-res accumulation and the remarkably active compound **13** showed an IC_{50} value of 3 nM in both ScN2a and F3 cell lines. Findings from studies on physicochemical and biochemical properties suggest that the action mechanism of these compounds does not correlate with any antioxidant activities, any of hydroxyl radical scavenging activities, or any SOD-like activities.

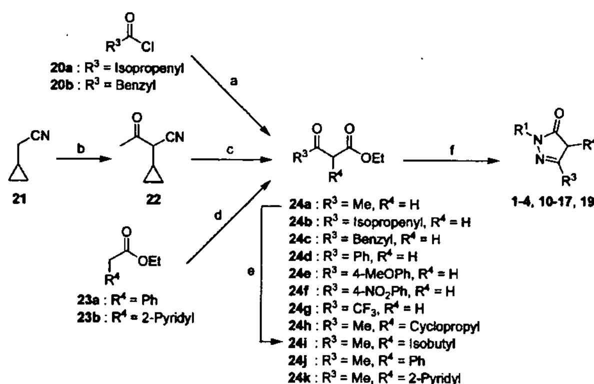
Prion diseases or transmissible spongiform encephalopathyies (TSEs[a]) are invariably fatal neurodegenerative diseases that include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), familial fatal insomnia (FFI), and kuru in humans, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. These diseases are characterized by deposition of the protease-resistant isoform of prion protein (PrP^{Sc}), which is thought to be the main component responsible for the pathogenesis. PrP^{Sc} is known to be an abnormally folded β-rich conformation of cellular prion protein (PrP^{C}) and is resistant to digestion with proteinase K.[1]

Natural and constitutive prion protein, PrP^{C}, is a GPI-anchored membrane glycoprotein. The biological significance of this protein is unclear, but it is reported that the N-terminal octapeptide repeat region of PrP^{C} binds several copper ions with a femtomolar dissociation range (K_d).[2,3] PrP^{C} has copper-dependent superoxide dismutase (SOD) activity[4] and may also be involved in copper uptake into cells.[5,6] Recently, there has been increasing interest in the role of copper in prion diseases.[7,8] In 2003, it was reported that a copper chelator, D-penicillamine, delayed the onset of prion disease in infected mice and suggested that chelator-based therapy might attenuate the disease.[9] Copper has been implicated in the pathogenesis of prion disease, but numerous studies have only succeeded in demonstrating the complexity of the effects of copper on the development of prion diseases, and it remains unclear whether this ion promotes or inhibits disease progression.

Although there are no suitable therapies for this disorder, outbreaks of variant CJD and iatrogenic CJD through the use of cadaveric growth hormone or dural grafts in younger people have necessitated their development. Furthermore, screening for antiprion compounds in a cell culture model of prion disease has led to the identification of many antiprion compounds,[10] such as quinoline derivatives,[11,12] Congo red and analogues,[13,14] and 2-aminopyridine-3,5-dicarbonitrile compounds;[15] however, their activity is thought to be insufficient to develop therapeutic agents.

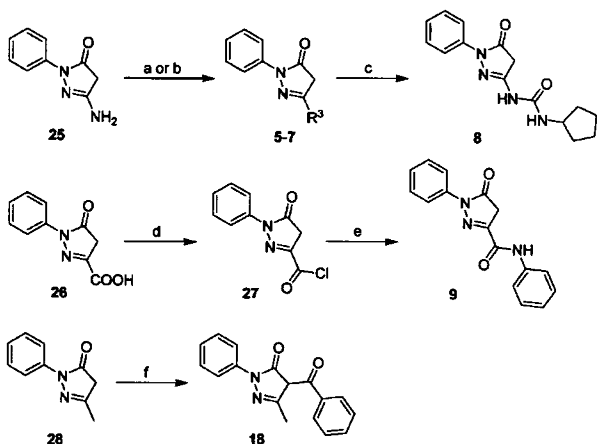
Recently, a new pyrazolone compound, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, also known as MCI-186), has been developed as a medical drug for brain ischemia[16,17] and has also been reported to be effective for myocardial ischemia.[18] In this

Scheme 1. Synthesis of Pyrazolone Derivatives **1–4**, **10–17**, and **19**[a]



[a] Reagants and conditions: (a) (i) malonic acid monoethyl ester potassium salt, $MgCl_2$, Et_3N, MeCN; (ii) 2 M HCl aq, 0 °C, 39–43%; (b) (i) LDA, THF; (ii) Ac_2O, THF, −78 °C, 78%; (c) (i) AcCl, EtOH; (ii) c-HCl, EtOH, 40 °C, 89%; (d) (i) NaH, THF, 60 °C; (ii) Ac_2O, THF, rt, 7–51%; (e) (i) NaOEt, EtOH; (ii) isobutyl iodide, THF, 80 °C, 43%; (f) R^1NHNH_2, EtOH or AcOH, reflux, 11–85%.

Scheme 2. Synthesis of Pyrazolone Derivatives **5–9**, **18**[a]



[a] Reagants and conditions: (a) for **5** and **6**, R^1OCOCl, pyridine, 50 °C, 16–23%; (b) for **7**, benzoyl chloride, dioxane, rt, 15%; (c) **6**, cyclopentylamine, xylene reflux, 42%; (d) oxalyl chloride, DMF, CH_2Cl_2; (e) aniline, CH_2Cl_2, rt, 74% (two steps); (f) benzoyl chloride, $Ca(OH)_2$, dioxane, reflux, 79%.

* To whom correspondence should be addressed. Phone: 81-52-836-3408 (H.N.); 81-52-836-3407 (N.M.). Fax: 81-52-836-3407 (H.N. and N.M.). E-mail: deco@phar.nagoya-cu.ac.jp (H.N.); miyata-n@phar.nagoya-cu.ac.jp (N.M.).

[a] Abbreviations: TSEs, transmissible spongiform encephalopathies; CJD, Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler–Scheinker syndrome; FFI, familial fatal insomnia; CWD, chronic wasting disease; BSE, bovine spongiform encephalopathy; PrP^{Sc}, infectious conformational form of prion protein; PrP^{C}, normal cellular prion protein; GPI, glycosylphosphatidylinositol; SOD, superoxide dismutase; PrP-res, protease-resistant form of prion protein; RML, Rocky Mountain Laboratory; PBS, phosphate-buffered saline; Tris, tris(hydroxymethyl) aminomethane; SDS, sodium dodecyl sulfate.

10.1021/jm070688r CCC: $37.00 © 2007 American Chemical Society
Published on Web 09/13/2007

Table 1. Inhibition of PrP-res Accumulation in ScN2a Cells and F3 Cells, Oxidation Potentials (E_{pa}), and Hydroxyl Radical Scavenging Activity

cmpd	R^1	R^3	R^4	inhibition PrP-res IC_{50}[a,c,d] (nM) ScN2a cells	inhibition PrP-res IC_{50}[a,c,d] (nM) F3 cells	E_{pa}[e,f] (mV)	pH[g]	scavenging activity IC_{50} (mM)[h]
edaravone	Ph	CH_3	H	>1000	N.E.[b]	483	7.0	0.25
1	cyclohexyl	CH_3	H	13	25	549	7.4	
2	4-CH_3OPh–	CH_3	H	N.E.[b]	N.E.[b]	678	7.8	
3	4-ClPh–	CH_3	H	0.5	N.E.[b]	473	7.4	
4	Ph	isopropenyl	H	158	794	387	7.4	
5	Ph	CH_3OCONH–	H	6	501	454	7.8	
6	Ph	PhOCONH–	H	N.E.[b]	N.E.[b]	397	7.0	0.38
7	Ph	PhCONH–	H	2000	1260	458	7.8	0.22
8	Ph	cyclopentylNHCONH–	H	126	158	372	7.8	
9	Ph	PhNHCO–	H	398	1580	478	7.8	
10	Ph	PhCH_2–	H	N.E.[b]	N.E.[b]	269	>8.0	
11	Ph	Ph	H	N.E.[b]	N.E.[b]	397	7.6	
12	Ph	4-CH_3OPh–	H	N.E.[b]	N.E.[b]	397	7.8	
13	Ph	4-NO_2Ph–	H	3	3	419	7.4	0.09
14	Ph	CF_3	H	398	631	673	7.6	0.81
15	Ph	CH_3	cyclopropyl	N.E.[b]	N.E.[b]	275	7.8	0.72
16	Ph	CH_3	isobutyl	N.E.[b]	16	262	>8.0	
17	Ph	CH_3	Ph	40	1	227	7.6	0.79
18	Ph	CH_3	PhCO–	6	1000	640	7.0	0.61
19	Ph	CH_3	2-pyridyl	79	631	403	>8.0	

[a] IC_{50}, concentration of a compound causing 50% inhibition of PrP-res accumulation relative to the control. [b] N.E., no effect. [c] At the concentration range for antiprion activity assay (10^{-10}–10^{-7} M for **2**, **3**, **11**, **12**, and **17** and 10^{-10}–10^{-6} M for the others), no cytotoxicity was observed against both of the two cell lines (Supporting Information). [d] In our system, IC_{50} values of quinine and quinidine in ScN2a cells are 10 μM and 5 μM, respectively. Those values are consistent with a previously report.[11] [e] Conditions for measurement: 10 mM sample in 50 mM NaCl; working electrode, Pt; reference electrode, Ag^+/AgCl; counter electrode, Pt; scan speed, 50mV/s; scan range, −0.2 to 1.0 V. [f] Oxidation potentials were expressed versus Ag^+/ AgCl. [g] Oxidation properties (E_{pa}) were measured at indicated pH because of their poor solubility in acidic and neutral aqueous solutions. [h] Conditions for measurement: a mixture of 25 mM H_2O_2, 25 mM DMPO, and a compound was irradiated with UV. ESR spectrometer parameters were as follows: microwave power, 10 mW; modulation width, 0.063 mT; time constant, 0.03 s; sweep width, 7.5 mT; sweep time, 1 min; gain, 320.

study, we focused on and explored the pyrazolone compounds derived from edaravone, as antiprion agents, and found new and highly active antiprion compounds.

Our initial goal was to prepare a small focused library of edaravone derivatives. The preparation of pyrazolone compounds was achieved by refluxing the corresponding β-ketoester and hydrazine compound in ethanol or acetic acid. β-Ketoesters **24b,c,h–k**, which are not commercially available, were synthesized from acyl chlorides **20a,b**, nitrile **21**, ethyl acetoacetate **24a**, or ethyl esters **23a,b** (Scheme 1). Treatment of amine **25** with chloroformic acid ester or benzoyl chloride gave carbamates **5** or **6** or amide **7** (Scheme 2). Carbamate **6** was then converted to urea **8** by treatment with cyclopentylamine. Amide **9** was synthesized from carboxylic acid **26** via acyl chloride **27**. Compound **18** was prepared from edaravone **28** with benzoyl chloride in the presence of $Ca(OH)_2$.

The antiprion activity of each compound was evaluated as the ability to inhibit the accumulation of the abnormal protease-resistant form of prion protein (PrP-res), as described in previous reports.[11,19,20] In this study, two types of prion-infected mouse neuroblastoma (N2a) cell lines, ScN2a and F3, were used. N2a cells that were infected with the RML strain are called ScN2a,[21] and N2a#58 cells that were infected with the Fukuoka-1 strain are called F3. N2a#58 cells are known to express five times more normal PrP than N2a cells. Both ScN2a cells and F3 cells were grown in six-well culture plates in Opti-MEM (Invitrogen) supplemented with 10% fetal bovine serum. Compounds were added at the designated concentration to the medium when cells were passaged at 10% confluency. The cells were allowed to grow to confluence (3 or 4 days) and lysed with lysis buffer (0.5% sodium deoxycholate, 0.5% Nonidet P-40, and PBS). The lysates were digested with 10 μg/mL proteinase K for 30 min at 37 °C and centrifuged at 15 000 rpm for 5 min at 24 °C with GLASSFOG (Q-bio gene, CA). The pellets were resuspended in sample loading buffer and boiled. Samples were separated by electrophoresis on 15% Tris-glycine-SDS-polyacryl-amide gel and electroblotted. PrP-res was detected using an antibody, SAF83 (1:5000; SPI-Bio, Montigny-le-Bretonneux, France), followed by an alkaline phosphatases-conjugated secondary antibody. Immunoreactive signals were visualized using CDP-Star detection reagent (Amersham Biosciences Corp., NJ) and were analyzed densitometrically. At least three independent experiments were performed to determine the IC_{50} value of each compound.

The original lead compound, edaravone, showed weak antiprion activity in ScN2a cells. The pyrazolone compounds **3** and **16** were effective in one of two cell lines (Table 1). Compounds **1**, **4**, **5**, **7**, **8**, **9**, **13**, **14**, **17**, **18**, and **19** inhibited PrP-res accumulation in both ScN2a cells and F3 cells, but the others did not (within a nontoxic dose range). Among the synthesized pyrazolone derivatives, 3-(4-nitrophenyl) compound **13** showed the highest activity for inhibiting PrP-res accumulation (IC_{50} = 3 nM), which is 130 times more active than quinacrine (IC_{50} = 400 nM)[19] and was one of the most potent compounds reported so far.[12,14] Although there are no reports that pyrazolone derivatives inhibit PrP-res accumulation in

prion-infected cells, compounds having a pyrazolone ring might be a new series of antiprion activity substances.

Because various types of compounds, such as 1-cyclohexyl compound **1**, 3-isopropenyl compound **4**, 3-(4-nitrophenyl) compound **13**, and 4-benzoyl compound **18**, showed relatively high antiprion activity, the position and class of substituents were not directly correlated with the activity of inhibiting PrP-res accumulation; therefore, we searched for the properties of synthesized compounds.

We had previously determined the oxidation potential and hydroxyl radical scavenging activity of edaravone-related derivatives.[22] Briefly, one-electron oxidation potentials (E_{pa}) of all synthesized derivatives were measured in a 50 mM NaCl solution by cyclic voltammetry (CV). Oxidation currents were observed with all the tested compounds but were irreversible, probably because the one-electron oxidation products were unstable and converted to degraded compounds as reported.[23] Because of the poor solubility of several derivatives in the neutral aqueous solution, the solutions were slightly basified using aqueous NaOH to solubilize these compounds.

Although the derivatives showed a wide variety of oxidation potentials (Table 1), no correlations were observed between oxidation potentials and antiprion activity.

Radical scavenging activity, which is known as the main action of edaravone as a brain-protecting drug,[23] was evaluated using the electron spin resonance (ESR) spin-trapping method with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap.[22] Hydroxyl radicals were generated by UV irradiation (200 mJ/cm^2) of hydrogen peroxide solution containing DMPO and edaravone derivatives. The inhibitory effect of the derivatives on the formation of hydroxyl radical adducts of DMPO was used as a measure of radical scavenging activity.

IC_{50} values were determined for 7 of 19 derivatives with diverse antiprion activity (**6**, **7**, **13**, **14**, **15**, **17**, and **18**). Compounds **15** and **17** exhibited efficient inhibition of the hydroxyl radical adduct formation to a similar extent, but **15** did not inhibit PrP-res accumulation in contrast with **17**, which showed antiprion activity in the nanomolar range. It was found that there is poor correlation between hydroxyl radical scavenging activity and antiprion activity.

Recently, Fukuuchi et al. found that compounds that have copper-selective chelating ability and whose copper complexes have high SOD-like activity are candidates for antiprion drugs.[24] For example, D-penicillamine has been reported to show moderate antiprion activity[9] and its copper complex exhibits SOD-like activity with an IC_{50} value of 28 μM.[24] The copper complex of 2,2′-biquinoline, whose IC_{50} value of antiprion activity has been reported to be 5 nM,[24] also exhibits SOD-like activity, with an IC_{50} value of 3 μM.[24] We therefore considered if our compounds might show SOD-like activity itself or in the form of a copper complex.

To investigate this idea, we first examined whether the synthesized derivatives could chelate with Cu(II). The chelation study was carried out using Job's method.[25] Solutions of each compound and $Cu(ClO_4)_2$ at a ratio (compound/Cu(II)) of 1:0 to 0:1 were prepared in 95% ethanol, and absorption spectra were measured. Spectrophotometric complexation studies showed that **1**, **15**, and **18** bound with Cu(II) at a 2:1 ratio and **6**, **7**, and **16** bound with Cu(II) at a 1:1 ratio. Compounds **9**, **14**, and edaravone showed no spectral changes in the presence of Cu(II) (**18**, Figure 1A; others, data not shown). It was unclear whether other compounds, such as **3**, **13**, and **17**, can bind with Cu(II) because they showed little spectral shift in the presence of Cu(II) (**13**, Figure 1B; others, data not shown).

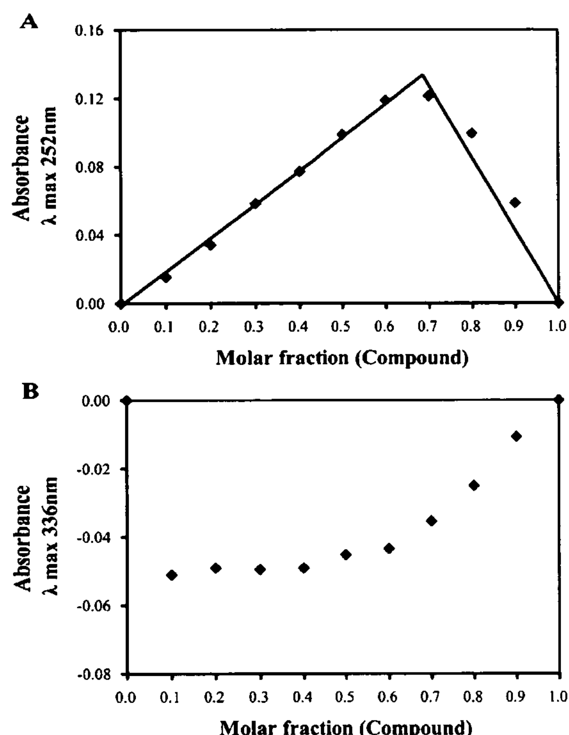


Figure 1. Continuous variation plots for compound **18** and Cu(II) (A) and compound **13** and Cu(II) (B). A: A 2:1 binding ratio between compound **18** and Cu(II); B: binding of compound **13** to Cu(II) was uncertain. Plots were obtained by Job's method in ethanol solution.

Table 2. SOD-Like Activities of Edaravone Derivatives

cmpd	R^3	R^4	SOD-like activity[a] (%) cmpd	with Cu(II)[b]
edaravone	CH_3	H	0.6	5.7
6	PhOCONH–	H	5.6	11.6
7	PhCONH–	H	9.2	3.2
13	4-NO_2Ph–	H	1.8	11.9
14	CF_3	H	13.4	7.4
15	CH_3	cyclopropyl	15.9	35.4

[a] Activity, percentage of inhibition of WST-1 tetrazorium formation by a compound at 1 mM. [b] All compounds were measured at 1 mM and 2 mM of Cu(II).

Findings from these experiments suggest that copper-chelating ability was not essential for antiprion activity, as previously reported.[24,26]

SOD-like activity of synthesized compounds (edaravone, **6**, **7**, **13**, **14**, and **15**) was measured in vitro using SOD-like assay kit-WST (Dojindo Laboratories, Kumamoto, Japan). This method is a xanthine-based photometric assay using tetrazolium salt WST-1. SOD-like activities of derivatives were evaluated at 1 mM (Table 2). Although it was uncertain whether some compounds, such as **13**, bind with Cu(II), SOD-like activities of derivatives in the presence of Cu(II) were also investigated using a solution of 1 mM $Cu(ClO_4)_2$ and 0.5 mM compound in a 0.9% NaCl solution. Because it is known that Cu(II) has superoxide scavenging activity, we also evaluated the SOD-

like activity of Cu(II) itself. A total of 1 mM of Cu(II) inhibited WST-1 formazan formation by 6.7%.

For all measured compounds, SOD-like activity was found to be very weak. Furthermore, because nonantiprion compounds, such as **6** and **15**, showed comparable activity with **13** and were more effective than compounds **7** and **14**, SOD-like activity may not be correlated with antiprion activity of these compounds.

In conclusion, we found that some pyrazolone compounds derivatized from edaravone have the ability to inhibit the accumulation of PrP-res, and 3-(4-nitrophenyl) compound **13** had remarkable activity (IC_{50} = 3 nM). To obtain information about their action mechanism, we investigated their oxidation potentials, copper-complexing, and SOD-like activity. Findings from these experiments suggest that these properties have little correlation with activity.

Further active antiprion derivatives and the mechanistic studies are under investigation.

Acknowledgment. We thank Dr. K. Doh-ura for kindly providing ScN2a and F3 cell lines for us. This work was supported in part by Grants-in-Aid for Scientific Research (No. 17590089 for H.N.) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Supporting Information Available: Experimental details of the synthesis and characterization data for all compounds and analytical methodologies. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Prusiner, S. B. Molecular biology of prion diseases. *Science* **1991**, *252*, 1515–1522.
(2) Brown, D. R.; Qin, K.; Herms, J. W.; Madlung, A.; Manson, J.; Strome, R.; Fraser, P. E.; Kruck, T.; von Bohlen, A.; Schulz-Schaeffer, W.; Giese, A.; Westaway, D.; Kretzschmar, H. The cellular prion protein binds copper in vivo. *Nature* **1997**, *390*, 684–687.
(3) Jackson, G. S.; Murray, I.; Hosszu, L. L.; Gibbs, N.; Waltho, J. P.; Clarke, A. R.; Collinge, J. Location and properties of metal-binding sites on the human prion protein. *Proc. Natl. Acad. Sci U.S.A.* **2001**, *98*, 8531–8535.
(4) Brown, D. R.; Wong, B. S.; Hafiz, F.; Clive, C.; Haswell, S. J.; Jones, I. M. Normal prion protein has an activity like that of superoxide dismutase. *Biochem. J.* **1999**, *344*, 1–5.
(5) Pauly, P. C.; Harris, D. A. J. Copper stimulates endocytosis of the prion protein. *J. Biol. Chem.* **1998**, *273*, 33107–33110.
(6) Miura, T.; Sasaki, S.; Toyama, A.; Takeuchi, H. Copper reduction by the octapeptide repeat region of prion protein: pH dependence and implications in cellular copper uptake. *Biochemistry* **2005**, *44*, 8712–8720.
(7) Hijazi, N.; Shaked, Y.; Rosenmann, H.; Ben-Hur, T.; Gabizon, R. Copper binding to PrP^C may inhibit prion disease propagation. *Brain Res.* **2003**, *993*, 192–200.
(8) McKenzie, D.; Bartz, J.; Mirwald, J.; Olander, D.; Marsh, R.; Aiken, J. Reversibility of scrapie inactivation is enhanced by copper. *J. Biol. Chem.* **1998**, *273*, 25545–25547.
(9) Sigurdsson, E. M.; Brown, D. R.; Alim, M. A.; Scholtzova, H.; Carp, R.; Meeker, H. C.; Prelli, F.; Frangione, B.; Wisniewski, T. Copper chelation delays the onset of prion disease. *J. Biol. Chem.* **2003**, *278*, 46199–46202.
(10) Kocisko, D. A.; Baron, G. S.; Rubenstein, R.; Chen, J.; Kuizon, S.; Caughey, B. New inhibitors of scrapie-associated prion protein formation in a library of 2000 drugs and natural products. *J. Virol.* **2003**, *77* 10288–10294.
(11) Murakami-Kubo, I.; Doh-ura, K.; Ishikawa, K.; Kawatake, S.; Sasaki, K.; Kira, J.; Ohta, S.; Iwaki, T. Quinoline derivatives are therapeutic candidates for transmissible spongiform encephalopathies. *J. Virol.* **2004**, *78*, 1281–1288.
(12) Kilingenstein, R.; Melnyk, P.; Leliveld, R.; Ryckebusch, A.; Korth, C. Similar structure–activity relationships of quinoline derivatives for antiprion and antimalarial effects. *J. Med. Chem.* **2006**, *49*, 5300–5308.
(13) Ingrosso, L.; Ladogana, A.; Pocchiari, M. Congo red prolongs the incubation period in scrapie-infected hamsters. *J. Virol.* **1995**, *69*, 506–508.
(14) Sellarajha, S.; Lekishvili, T.; Bowring, C.; Thompsett. A. R.; Rudyk, H.; Birkett, C. R.; Brown, D. R.; Gilbert, I. H. Synthesis of analogues of Congo red and evaluation of their anti-prion activity. *J. Med. Chem.* **2004**, *47*, 5515–5534.
(15) May, B. C. H.; Zorn, J. A.; Witkop, J.; Sherrill, J.; Wallace, A. C.; Legname, G.; Prusiner, S. B.; Cohen, F. E. Structure–activity relationship study of prion inhibition by 2-aminopyridine-3,5-dicarbonitrile-based compounds: parallel synthesis, bioactivity, and in vitro pharmacokinetics. *J. Med. Chem.* **2007**, *50*, 65–73.
(16) Watanabe, T.; Yuki, S.; Egawa, M.; Nishi, H. Protective effects of MCI-186 on cerebral ischemia: Possible involvement of free radical scavenging and antioxidant actions. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1597–1604.
(17) Kawai, H.; Nakai, H.; Suga, M.; Yuki, S.; Watanabe, T.; Saito, K. I. Effects of a novel free radical scavenger, MCI-186, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 921–927.
(18) Wu, T. W.; Zeng, L. H.; Wu, J.; Fung, K. P. Myocardial protection of MCI-186 in rabbit ischemia-reperfusion. *Life Sci.* **2002**, *71*, 2249–2255.
(19) Doh-ura, K.; Iwaki, T.; Caughey, B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. *J. Virol.* **2000**, *74*, 4894–4897.
(20) Ishikawa, K.; Doh-ura, K.; Kudo, Y.; Nishida, N.; Murakami-Kubo, I.; Ando, Y.; Sawada, T.; Iwaki, T. Amyloid imaging probes are useful for detection of prion plaques and treatment of transmissible spongiform encephalopathies. *J. Gen. Virol.* **2004**, *85*, 1785–1790.
(21) Milhavet, O.; McMahon, H. E.; Rachidi, W.; Nishida, N.; Katamine, S.; Mange, A.; Arlotto, M.; Casanova, D.; Riondel, J.; Favier, A.; Lehmann, S. Prion infection impairs the cellular response to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13937–13942.
(22) Nakagawa, H.; Ohyama, R.; Kimata, A.; Suzuki, T.; Miyata, N. Hydroxyl radical scavenging by edaravone derivatives: Efficient scavenging by 3-methyl-1-(pyridine-2-yl)-5-pyrazolone with an intramolecular base. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5939–5942.
(23) Ono, S.; Okazaki, K.; Sakurai, M.; Inoue, Y.; Density functional study of the radical reactions of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186): implication for the biological function of MCI-186 as a highly potent antioxidative radical scavenger. *J. Phys. Chem. A* **1997**, *101*, 3769–3775.
(24) Fukuuchi, T.; Doh-ura, K.; Yoshihara, S.; Ohta, S. Metal complexes with superoxide dismutase-like activity as candidates for anti-prion drug. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5982–5987.
(25) Vosburgh, W. C.; Cooper, G. R. Complex ions. I. The identification of complex ions in solution by spectrophotometric measurements. *J. Am. Chem. Soc.* **1941**, *63*, 437–442.
(26) Doh-ura, K.; Tamura, K.; Karube, Y.; Naito, M.; Tsuruo, T.; Kataoka, Y. Chelating compound, chrysoidine, is more effective in both antiprion activity and brain endothelial permeability than quinacrine. *Cell. Mol. Neurobiol.* **2007**, *27*, 303–316.

JM070688R

Synthesis and Evaluation of 2-Nonylaminopyridine Derivatives as PPAR Ligands

Shinya USUI,[a] Hiroki FUJIEDA,[a] Takayoshi SUZUKI,[a] Naoaki YOSHIDA,[a] Hidehiko NAKAGAWA,[a] Michitaka OGURA,[b] Makoto MAKISHIMA,[b] and Naoki MIYATA*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Nagoya City University; 3–1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467–8603, Japan: and* [b] *Nihon University School of Medicine; 30–1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173–8610, Japan.* Received March 26, 2007; accepted May 7, 2007; published online May 9, 2007

To find novel PPAR ligands, we prepared several 3-{3 or 4-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}-propanoic acid derivatives which were designed based on the structure of our previous PPARγ ligand 1. In PPAR binding affinity assays, compound 4, which had an ethoxy group at the C-2 position of the propanoic acid of 1, showed selective binding affinity for PPARγ. Compound 3, with an ethyl group at the C-2 position, was found to be a PPARα/γ dual ligand. Compound 6, the meta isomer of 1, has been shown to be a PPARα ligand. The introduction of methyl (7) and ethyl (8) groups to the C-2 position of the propanoic acid of 6 further improved PPARα-binding potency. In cell-based transactivation assay, compounds 3 and 4 showed dual-agonist activity toward PPARα and PPARγ. Compound 6 was found to be a triple agonist and compound 8 proved to be a selective PPARα agonist. In the human hypodermic preadipocyte differentiation test, it was demonstrated that the maximal activity of compounds 3 and 4 was higher than that of rosiglitazone.

Key words peroxisome proliferator-activated receptor (PPAR); agonist; selectivity

The peroxisome proliferator-activated receptors (PPARα, PPARγ and PPARδ) are a set of ligand-activated transcription factors in the nuclear hormone receptor superfamily.[1—4] These receptors regulate the expression of a large number of genes involved in lipid metabolism and energy balance by binding to a DNA sequence termed PPAR response elements.[5] The PPARγ is predominantly expressed in adipose tissues, and plays a pivotal role in adipose differentiation, and the regulation of glucose and lipid homeostasis. The clinically useful thiazolidinedione (TZD) class of insulin sensitizers such as rosiglitazone[6] and pioglitazone[7] (Fig. 1) are potent PPARγ agonists used in the treatment of Type 2 diabetes. TZDs are known to improve insulin resistance, which is a key underlying feature of Type 2 diabetes[8]; however, the use of TZDs has been limited because of their serious side effects such as hepatic toxicity, weight gain and edema. Meanwhile, PPARα is highly expressed in metabolically active tissues such as the liver, heart and muscle, and regulates lipid homeostasis. PPARα agonists such as clofibrate (Fig. 1) have demonstrated the ability to reduce serum triglyceride and increase HDL cholesterol levels,[9] and are being utilized as hypolipidemic agents. In addition, recent studies revealed that dual agonists of PPARα/γ decrease the free triglyceride plasma concentration and increase plasma HDL concentration in an insulin-resistant animal model.[10,11] Thus, many groups have ongoing research programs to identify more potent and less toxic PPARα agonists, PPARγ agonists and PPARα/γ dual agonists.

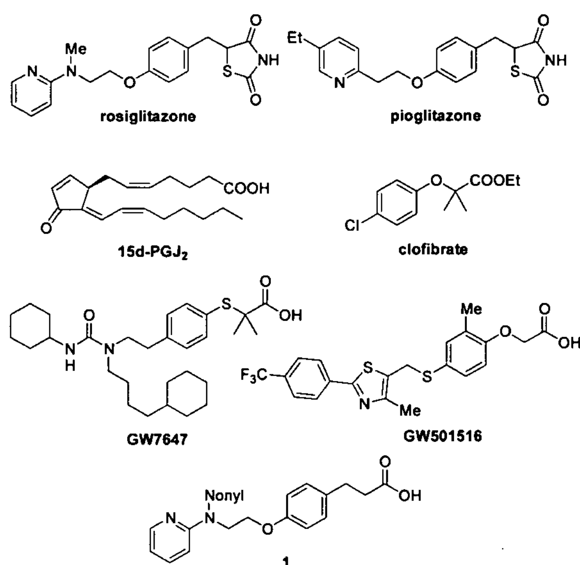


Fig. 1. Structures of Rosiglitazone, Pioglitazone, 15d-PGJ$_2$, Clofibrate, GW7647, GW501516 and Compound 1

We previously reported compound **1** (Fig. 1), which was designed based on the structure of rosiglitazone and 15d-PGJ$_2$,[12,13] as a potent PPARγ ligand.[14] To find more potent PPARγ agonists and novel PPARα agonists, we chose compound **1** as the lead structure, because recent reports indicated that PPARγ affinity can be increased by the introduction of substituents into the C-2 position of propanoic acid,[15—19] and minor structural modifications can convert PPAR subtype selectivity.[20—22] In this article, we report the synthesis, binding affinity and biological activity of PPAR ligands based on the structure of compound **1**.

Chemistry The compounds prepared for this study are shown in Fig. 2, and the routes used for synthesis are shown in Charts 1—3. Chart 1 shows the preparation of compounds **1**, **3**, **4**, **6**—**10**, **12** and **13**. The 2-nonylaminopyridine **18** was prepared by the method of Buchwald[23]: treatment of *n*-nonylamine **17** with 2-bromopyridine, Pd$_2$(DBA)$_3$, BINAP, and *t*-BuONa in toluene at 105 °C. *p*-Hydroxybenzaldehyde **19a**, *m*-hydroxybenzaldehyde **19b** and isovaniline **19c** were allowed to react with 1,2-dibromoethane to give ethers **20a**—**c**. The Horner–Wadsworth–Emmons reaction[24] was applied to the conversion of **20a**—**c** into acrylic acid ethyl esters **21a**—

* To whom correspondence should be addressed. e-mail: miyata-n@phar.nagoya-cu.ac.jp © 2007 Pharmaceutical Society of Japan

j. The double bonds of **21a**—**j** were hydrogenated to yield compounds **22a**—**j**. Coupling between 2-nonylaminopyridine **18** and propanoic acid ethyl esters **22a**—**j** afforded *N*-(2-pyridinyl)-*N*-nonylpropanoic acid ethyl esters **23a**—**j**. The subsequent hydrolysis of **23a**—**j** gave the desired carboxylic acids **1**, **3**, **4**, **6**—**10**, **12** and **13**.

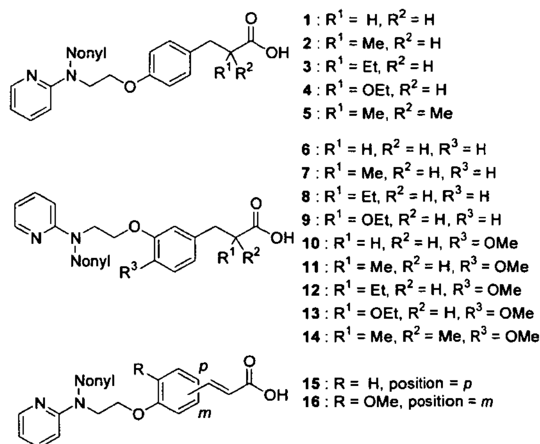
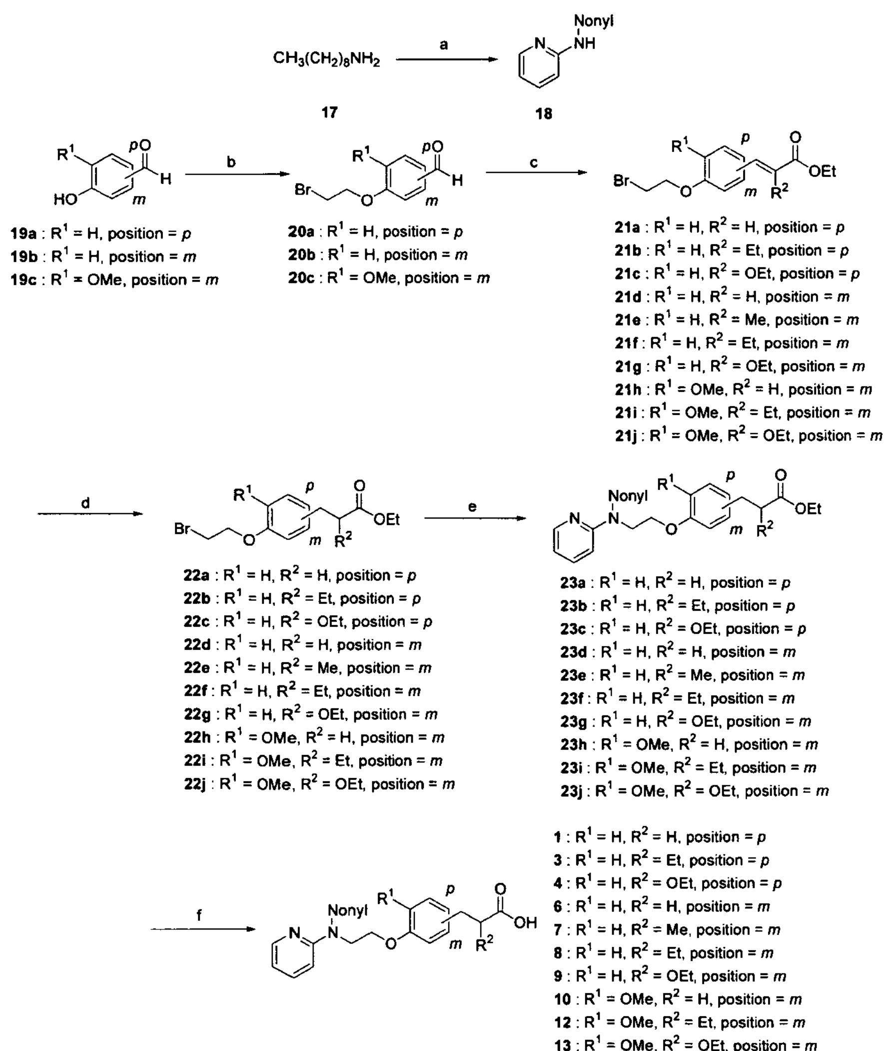


Fig. 2. Structures of Compounds **1**—**16**

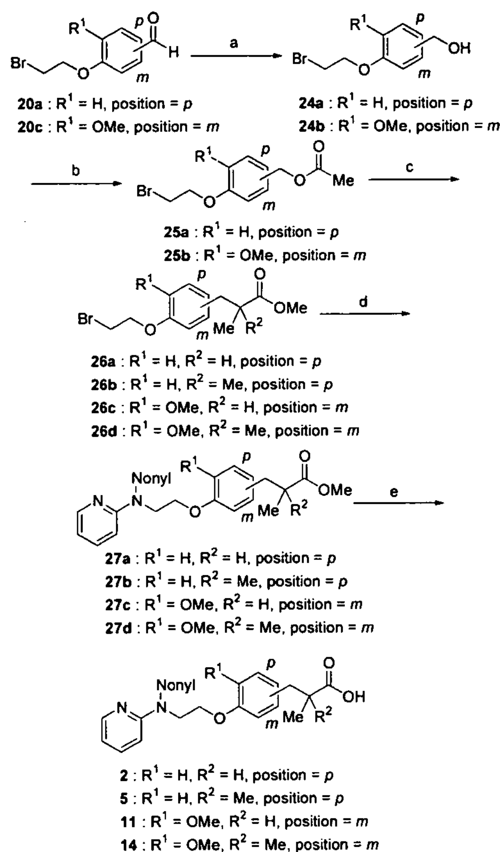
The preparation of compounds **2**, **5**, **11** and **14**, which have one or two methyl groups at the C-2 position of propanoic acid, is outlined in Chart 2. Aldehydes **20a** and **20c** were reduced by $NaBH_4$ and allowed to react with acetic anhydride to give **25a** and **25b**. Compounds **25a** and **25b** were treated with 1-methoxy-1-trimethylsilyloxypropene or dimethylketene methyltrimethylsilyl acetal in the presence of magnesium perchlorate in anhydrous CH_2Cl_2 to give esters **26a**—**d**.[25] Coupling between 2-nonylaminopyridine **18** and propanoic acid methyl esters **26a**—**d** afforded *N*-(2-pyridinyl)-*N*-nonyl compounds **27a**—**d** and subsequent hydrolysis gave carboxylic acids **2**, **5**, **11** and **14**.

Preparation of the acrylic acid derivatives **15** and **16** is shown in Chart 3. Acrylic acid ethyl esters **21a** and **21h** were allowed to react with 2-nonylaminopyridine **18** to give compounds **28a** and **28b**. Treatment of **28a** and **28b** with aqueous NaOH gave *N*-(2-pyridinyl)-*N*-nonyl acrylic acids **15** and **16**.



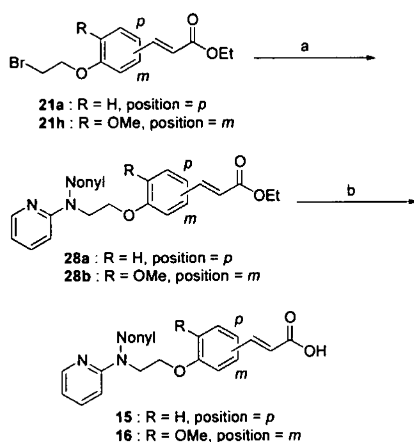
(a) 2-bromopyridine, $Pd_2(DBA)_3$, BINAP, *t*-BuOH, toluene, 105 °C, 70%; (b) 1,2-dibromoethane, Cs_2CO_3, THF, 65 °C, 33—57%; (c) $(EtO)_2P(O)CH(R)CO_2Et$, NaH, anhydrous THF, 0 °C to rt, 47—95%; (d) H_2, Pd/C, EtOH, 79—97%; (e) **18**, Et_3N, KI, 105 °C, 9—17%; (f) 2 N aq. NaOH, EtOH, THF, rt, 82—100%.

Chart 1



(a) $NaBH_4$, EtOH, rt, 81—95%; (b) Ac_2O, DMAP, rt, 96—97%; (c) 1-methoxy-1-trimethylsilyloxypropene, or dimethylketene methyltrimethylsilyl acetal, $Mg(ClO_4)_2$, rt, 90—94%; (d) **18**, Et_3N, KI, 105 °C, 5—13%; (e) 2 N aq. NaOH, EtOH, rt, 75—97%.

Chart 2



(a) **18**, Et_3N, KI, 105 °C, 9—13%; (b) 2 N aq. NaOH, EtOH, THF, rt, 82—99%.

Chart 3

Results and Discussion

The binding affinity of the compounds for PPARs was evaluated with the CoA-BAP System (Microsystems).[26] In this system, alkaline phosphatase (AP) activity is directly proportional to the affinity of the ligands for PPARs. The abilities of compounds **1**—**16** to bind PPARα, PPARγ and PPARδ were evaluated and the results are shown in Figs. 3, 4

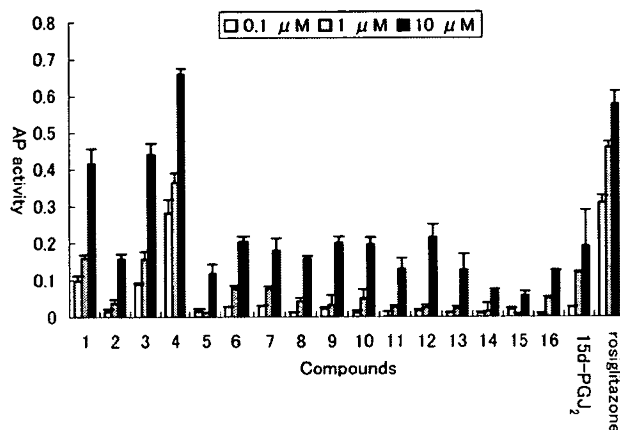


Fig. 3. Binding Affinity for PPARγ of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

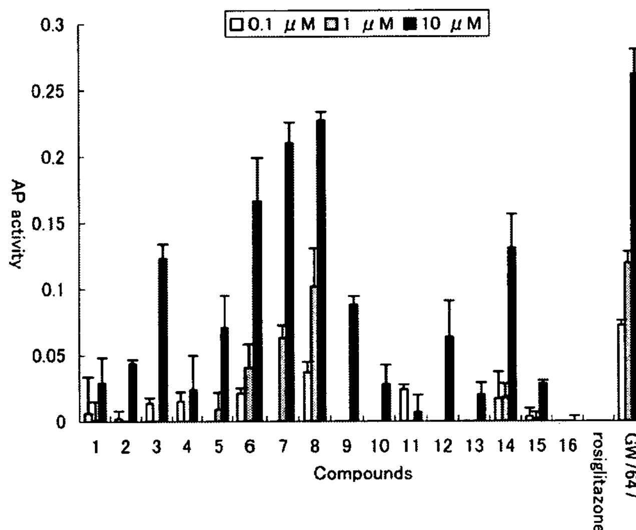


Fig. 4. Binding Affinity for PPARα of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

and 5, respectively. GW7647[27] (PPARα), rosiglitazone[6] (PPARγ) and GW501516[28] (PPARδ) were used as reference compounds (Fig. 1).

The lead compound **1** showed relatively high affinity for PPARγ and little affinity for PPARα and PPARδ (Fig. 3—5). We initially examined the effect of substituents at the C-2 position of the propanoic acid of **1**, because it has been reported that the introduction of an alkyl or an alkoxy group at this position increases the activity of PPARγ and PPARα.[15—19] Among compound **1**, methyl **2**, ethyl **3**, ethoxy **4**, and dimethyl **5**, compound **4** showed the strongest affinity for PPARγ, so compound **4** was founded to be a potent and selective PPARγ ligand (Figs. 3—5). In addition, the PPARγ affinity of compound **3** was comparable to that of compound **1**, whereas compound **3** displayed strong affinity for PPARα as compared with **1** (Figs. 3, 4).

Next, we investigated the PPARs affinity of compound **6**, the *meta* isomer of compound **1**.[29] Compound **6** showed

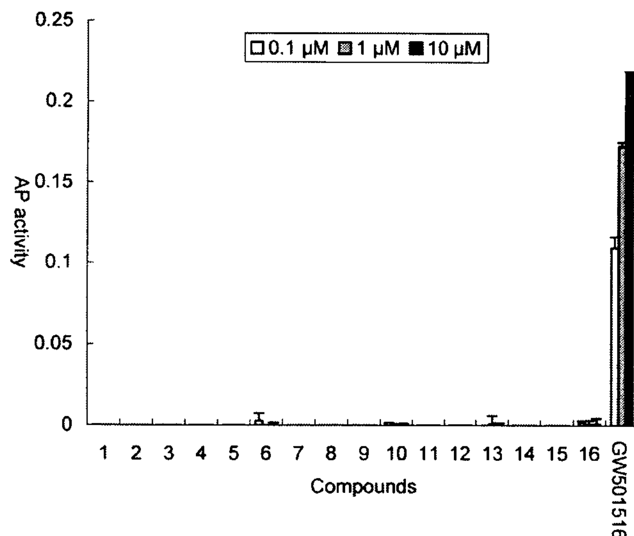


Fig. 5. Binding Affinity for PPARδ of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

much higher affinity for PPARα than **1** (Fig. 4). Furthermore, the affinity for PPARγ of **6** was lower than that of **1** (Fig. 3), and compound **6** exhibited little affinity for PPARδ (Fig. 5). To find more potent PPARα ligands, we prepared and tested compounds **7**, **8** and **9** in which a methyl, ethyl and ethoxy group was substituted at the C-2 position of the propanoic acid of **6**, respectively. Compounds **7** and **8** showed strong affinity for PPARα and compound **8** displayed slightly weak affinity for PPARγ as compared with the parent compound **6**. The effect of the introduction of a methoxy group at the C-4 position of the benzene ring of **6** was also investigated; however, compounds **10**—**14** showed no pronounced affinity for PPARs as compared to compound **6** (Figs. 3—5).

The acrylic acid derivatives **15** and **16** did not show notable affinity for PPARs (Figs. 3—5).

Based on the findings in the binding assay, we next investigated the PPAR transactivation activity of compounds **1**, **3**, **4**, **6**, **7**, and **8** by reporter gene assay[30] (Table 1). We initially tested the activity of compound **1**, which did not show significant transcriptional activity for PPARα, δ, and γ. We then examined the activity of compounds **3** and **4**, which have an ethyl or ethoxy group at the C-2 position of the propanoic acid of **1**. As expected from the binding assay, compound **3** was a PPARα/γ dual agonist. Although the binding assay indicated that compound **4** is a selective PPARγ ligand, it showed potent dual-agonist activity toward PPARα and PPARγ. Compound **6**, the *meta* isomer of compound **1**, was found to be a triple agonist. Compound **7**, which had an introduced methyl group at the C-2 position of the propanoic acid moiety of **6**, improved the EC_{50} values and selectivity for PPARα and PPARγ, and the introduction of ethyl group (compound **8**) increased the activity and selectivity for PPARα. Among these compounds, compound **8** showed the highest selectivity for PPARα.

As compounds **3** and **4** were found to have relatively high PPARγ agonist activity in our study, we used them for further evaluation. Since it has been reported that the activation

Table 1. *In Vitro* Functional PPAR Transactivation Activity of Compounds

Compound	R	Position	EC_{50}[a] (μM)		
			PPARγ	PPARδ	PPARα
1	H	*p*	>10	>10	>10
3	Et	*p*	2.62	>10	3.68
4	OEt	*p*	0.32	>10	0.74
6	H	*m*	7.76	6.42	3.20
7	Me	*m*	4.72	>10	2.65
8	Et	*m*	>8	>10	1.81

a) Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells as described. EC_{50} value is the molar concentration of the test compound that affords 50% of maximal reporter activity.[33]

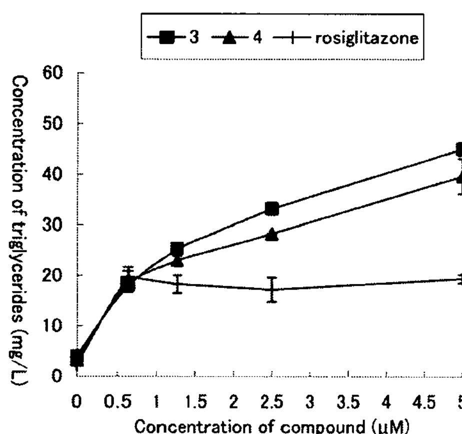


Fig. 6. Accumulation of Fatty Acid in Human Preadipocytes by Compounds **3**, **4** and Rosiglitazone

Values are the means of at least three experiments.

of PPARγ enhances adipocyte differentiation[31] and increases insulin sensitivity, compounds **3** and **4** were subjected to a human hypodermic preadipocyte differentiation test.[32] The accumulation of triglycerides in the cells was observed after the administration of compounds **3** and **4** at concentrations of 0, 0.613, 1.25, 2.5 and 5 μM, and the activity of compounds **3** and **4** was found to be higher than that of rosiglitazone at 1 μM and higher concentrations (Fig. 6).

Conclusion

To find novel PPAR ligands, we prepared several 2-nonylaminopyridine derivatives which were designed based on the structure of a PPARγ ligand **1**. In PPAR binding affinity assays, compound **4**, which had an ethoxy group at the C-2 position of the propanoic acid of **1**, showed high binding affinity for PPARγ and little affinity for PPARα and PPARδ. Compound **3**, which had an ethyl group at the C-2 position of propanoic acid, was found to be a PPARα/γ dual ligand. Compound **6**, the *meta* isomer of **1**, has been shown to be a PPARα ligand. The introduction of methyl (**7**) and ethyl (**8**) groups at the C-2 position of the propanoic acid of **6** further improved PPARα-binding potency. In cell-based transactivation assay, compounds **3** and **4** showed dual-agonist activity toward PPARα and PPARγ. Compound **6** was found to be a

triple agonist and compound **8** proved to be a selective PPARα agonist. In the human hypodermic preadipocyte differentiation test, it was demonstrated that the activity of compounds **3** and **4** was higher than that of rosiglitazone. The findings of this study will help provide an effective agent for Type 2 diabetes and hyperlipidemia.

Experimental

Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a JEOL JNM-LA500 spectrometer in solvent as indicated. Chemical shifts (δ) were reported in parts per million relative to the internal standard tetramethylsilane. High-resolution mass spectra (HR-MS) were recorded on a JEOL JMS-SX102A mass spectrometer. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku, and used without purification. Flash column chromatography was performed using Silica Gel 60 (particle size 0.046—0.063 mm) supplied by Merck.

2-Nonylaminopyridine (18) To a solution of 2-bromopyridine (0.9 ml, 9.20 mmol) in 10 ml of anhydrous toluene were added *n*-nonylamine (**17**, 10.0 ml, 55.2 mmol), $Pd_2(DBA)_3$ (0.169 g, 0.18 mmol), *rac*-BINAP (0.229 g, 0.37 mmol) and sodium *tert*-butoxide (1.24 g, 12.9 mmol). The reaction mixture was stirred at 105 °C for 3 d under Ar, and poured into water. The whole was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/5) gave 1.43 g (70%) of **18** as a yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.07 (1H, dd, J=4.9, 1.5 Hz), 7.41 (1H, ddd, J=8.5, 6.7, 1.8 Hz), 6.55 (1H, dd, J=6.7, 5.5 Hz), 6.37 (1H, d, J=8.2 Hz), 4.45 (1H, m), 3.24 (2H, m), 1.65—1.59 (2H, m), 1.41—1.27 (12H, m), 0.88 (3H, t, J=6.9 Hz).

4-(2-Bromoethoxy)benzaldehyde (20a) To a solution of *p*-hydroxybenzaldehyde (**19a**, 1.25 g, 10.0 mmol) in 13 ml of THF were added Cs_2CO_3 (4.28 g, 13.0 mmol) and 1,2-dibromoethane (1.76 ml, 20.0 mmol). The mixture was stirred at 65 °C for 14 h. The mixture was poured into 2 N aqueous NaOH. The mixture was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/3) gave 0.758 g (33%) of **21a** as a light yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 9.90 (1H, s), 7.85 (2H, d, J=8.5 Hz), 7.02 (2H, d, J=8.8 Hz), 4.38 (2H, t, J=6.1 Hz), 3.67 (2H, t, J=6.1 Hz).

3-[4-(2-Bromoethoxy)phenyl]acrylic Acid Ethyl Ester (21a) To a suspension of NaH (191 mg, 4.78 mmol) in 4 ml of anhydrous THF was added dropwise a solution of ethyl diethylphosphono acetate (1.07 ml, 5.21 mmol) in 5 ml of anhydrous THF at 0 °C under Ar. The mixture was stirred for 1 h at 0 °C. To the mixture was added dropwise a solution of **20a** (916 mg, 4.00 mmol) in 8 ml of anhydrous THF. The reaction mixture was stirred for 16 h at 0 °C. The reaction mixture was poured into ice-water and extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/5) gave 901 mg (76%) of **20a** as a white solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.64 (1H, d, J=15.8 Hz), 7.48 (2H, d, J=8.8 Hz), 6.92 (2H, d, J=8.5 Hz), 6.32 (1H, d, J=15.8 Hz), 4.32 (2H, t, J=6.1 Hz), 4.26 (2H, q, J=7.3 Hz), 3.65 (2H, t, J=6.1 Hz), 1.34 (3H, t, J=7.0 Hz).

3-[4-(2-Bromoethoxy)phenyl]propanoic Acid Ethyl Ester (22a) To a solution of **21a** (445 mg, 1.49 mmol) in 5 ml of EtOH was added 64 mg of 7% Pd/C. The reaction mixture was stirred for 1 d under H_2 at room temperature. The catalyst was removed by filtration, and the solvent was removed by evaporation *in vacuo*. 359 mg (80%) of **22a** was obtained as a light yellow oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.12 (2H, dt, J=8.8, 3.0 Hz), 6.83 (2H, dt, J=8.5, 3.0 Hz), 4.26 (2H, t, J=6.1 Hz), 4.12 (2H, q, J=7.0 Hz), 3.62 (2H, t, J=6.1 Hz), 2.89 (2H, t, J=7.6 Hz), 2.58 (2H, t, J=8.2 Hz), 1.23 (3H, t, J=7.0 Hz).

3-{4-[2-(Nonyl-pyridin-2-ylamino)ethoxy]phenyl}propanoic Acid Ethyl Ester (23a) To a solution of **22a** (0.343 g, 1.14 mmol) in 1 ml of THF were added 2-nonylaminopyridine (1.01 g, 4.56 mmol), KI (0.189 g, 1.14 mmol) and Et_3N (0.45 ml, 2.30 mmol). The reaction mixture was stirred for 28 h at 105 °C. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Purification by silica gel flash chromatography (toluene : $CHCl_3$: AcOEt=45 : 5 : 3) gave 55 mg (11%) of **23a** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J=4.8, 1.8, 0.9 Hz), 7.40 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.09 (2H, d, J=8.5 Hz), 6.83 (2H, dt, J=8.5, 2.7 Hz), 6.50 (2H, m), 4.14 (2H, t, J=6.1 Hz), 4.11 (2H, q, J=7.3 Hz), 3.91 (2H, t, J=6.1 Hz), 3.47 (2H, t, J=7.9 Hz), 2.87 (2H, t, J=7.6 Hz), 2.57 (2H, t, J=7.9 Hz), 1.66—1.59 (2H, m), 1.36—1.20 (12H, m), 1.23 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3036.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (1) (Chart 1) To a solution of **23a** (49 mg, 0.119 mmol) in 1.0 ml of THF and 1.0 ml of EtOH was added a solution of 2 N aqueous NaOH (0.60 ml, 1.19 mmol). The reaction mixture was stirred for 1 d at room temperature, and neutralized to pH 7 with 2 N aqueous HCl. The mixture was subjected to silica gel flash chromatography ($CHCl_3$/MeOH=19/1) to give 44 mg (90%) of **1** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, dd J=4.9, 1.2 Hz), 7.45 (1H, t, J=7.1 Hz), 7.10 (2H, d, J=8.5 Hz), 6.81 (2H, d, J=8.5 Hz), 6.54 (2H, m), 4.12 (2H, t, J=5.6 Hz), 3.93 (2H, t, J=5.6 Hz), 3.50 (2H, t, J=7.9 Hz), 2.88 (2H, t, J=7.8 Hz), 2.61 (2H, t, J=7.8 Hz), 1.66—1.62 (2H, m), 1.35—1.25 (12H, m), 0.88 (3H, t, J=6.8 Hz); MS (EI) *m/z*: 412 (M^+); HR-MS Calcd for $C_{25}H_{36}N_2O_3$ 412.2727, Found 412.2726.

2-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (3) Compound **3** was prepared from **19a** using the procedure described for **1** in 2.5% yield. In the step of the synthesis of **21b**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.11 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.41 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.08 (2H, d, J=8.5 Hz), 6.79 (2H, d, J=8.5 Hz), 6.50 (1H, dd, J=7.0, 4.8 Hz), 6.48 (1H, d, J=7.9 Hz), 4.05 (1H, m), 3.99 (1H, m), 3.85 (2H, m), 3.46 (2H, t, J=7.9 Hz), 2.88 (1H, dd, J=13.7, 8.5 Hz), 2.70 (1H, dd, J=13.7, 6.4 Hz), 2.58 (1H, m), 1.68—1.55 (4H, m), 1.31—1.21 (12H, m), 0.96 (3H, t, J=7.6 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3029.

2-Ethoxy-3-{4-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (4) Compound **4** was prepared from **19a** using the procedure described for **1** in 2.1% yield. In the step of the synthesis of **21c**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.12 (1H, dd, J=4.9, 1.2 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.28 (2H, d, J=8.5 Hz), 6.80 (2H, d, J=8.5 Hz), 6.51 (2H, m), 4.05 (2H, m), 3.90 (2H, m), 3.60 (1H, m), 3.47 (2H, t, J=7.6 Hz), 3.05 (1H, dd, J=14.0, 4.5 Hz), 2.94 (1H, dd, 14.3, 7.6 Hz), 1.65—1.59 (2H, m), 1.35—1.20 (12H, m), 1.17 (3H, t, J=7.0 Hz), 1.17 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.2999.

3-{3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (6) Compound **6** was prepared from **19b** using the procedure described for **1** in 7.7% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.9, 2.0, 0.8 Hz), 7.42 (1H, ddd, J=8.5, 6.7, 2.1 Hz), 7.18 (1H, t, J=7.9 Hz), 6.75 (3H, m), 6.5 (2H, m), 4.15 (2H, t, J=6.1 Hz), 3.91 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.6 Hz), 2.91 (2H, t, J=7.6 Hz), 2.65 (2H, t, J=7.9 Hz), 1.65—1.61 (2H, m), 1.35—1.25 (12H, m), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{25}H_{36}N_2O_3$ 412.2726, Found 412.2729.

2-Methyl-3-{3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (7) Compound **7** was prepared from **19b** using the procedure described for **1** in 3.5% yield. In the step of the synthesis of **21e**, 2-(diethoxyphosphoryl)propanoic acid ethyl ester was used instead of ethyl diethylphosphono acetate: light yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.8, 1.8, 0.9 Hz), 7.42 (1H, ddd, J=8.8, 6.7, 2.1 Hz), 7.17 (1H, t, J=7.9 Hz), 6.74—6.78 (3H, m), 6.52 (1H, m), 6.50 (1H, d, J=8.8 Hz), 4.14 (2H, t, J=6.1 Hz), 3.90 (2H, m), 3.46 (3H, t, J=7.6 Hz), 3.02 (1H, dd, J=13.4, 6.7 Hz), 2.75 (1H, m), 2.64 (1H, dd, J=13.4, 7.6 Hz), 1.66—1.58 (2H, m), 1.35—1.25 (12H, m), 1.17 (3H, d, J=7.0 Hz), 0.88 (3H, t, J=7.3 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_3$ 426.2882, Found; M^+ 426.2877.

2-{3-[2-(Nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (8) Compound **8** was prepared from **19b** using the procedure described for **1** in 4.9% yield. In the step of the synthesis of **21f**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J =5.2, 1.2 Hz), 7.42 (1H, ddd, J=8.8, 6.7, 2.1 Hz), 7.15 (1H, t, J=7.9 Hz), 6.71—6.79 (3H, m), 6.52 (1H, dd, J=6.7, 5.2 Hz), 6.50 (1H, d, J=8.8 Hz), 4.12 (2H, t, J=5.5 Hz), 3.83—3.94 (2H, m), 3.46 (3H, t, J=7.6 Hz), 2.93 (1H, dd, J=13.7, 8.2 Hz), 2.72 (1H, dd, J=13.7, 6.7 Hz), 2.60 (1H, m), 1.69—1.55 (4H, m), 1.35—1.25 (12H, m), 0.96 (3H, t, J=7.3 Hz), 0.82 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3043.

2-Ethoxy-3-{3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (9) Compound **9** was prepared from **19b** using the procedure described for **1** in 2.5% yield. In the step of the synthesis of **21g**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, dd, J=4.9, 1.2 Hz), 7.44 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.17 (1H, t, J=7.6 Hz), 6.83 (2H, m), 6.77 (1H, dd, J=7.9, 1.8 Hz), 6.53 (2H, m), 4.15 (2H, t, J=5.5 Hz), 4.07 (1H, dd, J=7.3, 4.9 Hz), 3.90 (2H, m), 3.59 (1H, m), 3.47 (2H, t, J=7.9 Hz), 3.43 (1H, m), 3.09 (1H, dd, J=13.7, 4.6 Hz), 2.98 (1H, dd, J=13.7, 7.3 Hz), 1.67—1.59 (2H, m), 1.36—1.23 (12H, m), 1.16 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.2985.

3-{4-Methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (10) Compound **10** was prepared from **19c** using the procedure described for **1** in 2.7% yield: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.93 (1H, d, J=1.5 Hz), 6.79 (1H, d, J=8.2 Hz), 6.74 (1H, dd, J=8.2, 1.8 Hz), 6.53 (1H, dd, J=7.0, 5.2 Hz), 6.51 (1H, d, J=8.8 Hz), 4.24 (2H, t, J=6.7 Hz), 3.94 (2H, t, J=6.4 Hz), 3.83 (3H, s), 3.45 (2H, t, J=7.6 Hz), 2.88 (2H, t, J=7.6 Hz), 2.61 (2H, t, J=7.6 Hz), 1.64—1.58 (2H, m), 1.31—1.25 (12H, m), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_4$ 442.2832, Found 442.2846.

2-{4-Methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (12) Compound **12** was prepared from **19c** using the procedure described for **1** in 3.0% yield. In the step of the synthesis of **21i**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.8, 1.8, 0.6 Hz), 7.43 (1H, ddd, J=9.1, 7.3, 2.1 Hz), 6.91 (1H, d, J=1.8 Hz), 6.76 (1H, d, J=8.2 Hz), 6.71 (1H, dd, J=8.2, 2.1 Hz), 6.53 (1H, dd, J=6.4, 5.1 Hz), 6.50 (1H, d, J=8.8 Hz), 4.23 (2H, m), 3.98 (2H, m), 3.86 (1H, m), 3.83 (3H, s), 3.43 (2H, m), 2.87 (1H, dd, J=13.7, 8.5 Hz), 2.71 (1H, dd, J=13.6, 6.1 Hz), 2.56—2.50 (1H, m), 1.70—1.51 (4H, m), 1.34—1.23 (12H, m), 0.95 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_4$ 470.3145, Found 470.3142.

2-Ethoxy-3-{4-methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl-propanoic Acid (13) Compound **13** was prepared from **19c** using the procedure described for **1** in 3.8% yield. In the step of the synthesis of **21j**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, dd, J=4.9, 1.8 Hz), 7.45 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.96 (1H, s), 6.78 (2H, m), 6.54 (1H, dd, J=6.7, 5.5 Hz), 6.52 (1H, d, J=8.5 Hz), 4.25 (2H, m), 4.04 (1H, m), 3.93 (2H, m), 3.83 (3H, s), 3.59 (1H, m), 3.44 (2H, t, J=7.6 Hz), 3.05 (1H, dd, J=13.7, 4.9 Hz), 2.97 (1H, dd, J=13.7, 6.4 Hz), 1.65—1.58 (2H, m), 1.35—1.21 (12H, br), 1.17 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_5$ 486.3094, Found 486.3111.

{4-(2-Bromoethoxy)phenyl}methanol (24a) To a solution of **20a** (890 mg, 3.89 mmol) in 10 ml of EtOH was added $NaBH_4$ (163 mg, 3.89 mmol). The reaction mixture was stirred for 6 h at room temperature. The reaction mixture was poured into water, and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. 763 mg (81%) of **24a** was obtained as a white solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.30 (2H, d, J=8.5 Hz), 6.91 (2H, d, J=8.8 Hz), 4.62 (2H, d, J=5.1 Hz), 4.29 (2H, t, J=6.1 Hz), 3.64 (2H, t, J=6.4 Hz), 1.58 (1H, t, J=5.5 Hz).

Acetic Acid 4-(2-Bromoethoxy)benzyl Ester (25a) To a solution of **24a** (2.31 g, 10.0 mmol) in 20 ml of CH_2Cl_2 were added Ac_2O (3.10 ml, 30.0 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 1 d at room temperature and diluted with AcOEt. The AcOEt solution was washed with water and brine, and dried over Na_2SO_4. Purification by silica gel flash chromatography (AcOEt/*n*-hexane =1/5) gave 2.62 g (96%) of **25a** as a light yellow oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.29 (2H, d, J=8.8 Hz), 6.90 (2H, d, J=8.8 Hz), 5.04 (2H, s), 4.28 (2H, t, J=6.1 Hz), 3.63 (2H, t, J=6.1 Hz), 2.07 (3H, s).

3-{4-(2-Bromoethoxy)phenyl}-2-methylpropionic Acid Methyl Ester (26a) To a solution of **25a** (0.948 g, 3.47 mmol) and 1-methoxy-1-trimethylsilyloxypropene (1.00 g, 6.24 mmol) in 35 ml of anhydrous CH_2Cl_2 was added magnesium perchlorate (0.129 g, 0.694 mmol) under Ar. The reaction mixture was stirred for 1 d at room temperature. The reaction mixture was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/10) gave 0.935 g (90%) of **26a** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.07 (2H, dt, J=8.5, 3.0 Hz), 6.82 (2H, dt, J=8.5, 3.0 Hz), 4.26 (2H, t, J=6.4 Hz), 3.63 (3H, s), 3.62 (2H, t, J=6.1 Hz), 2.95 (1H, dd, J=13.4, 7.1 Hz), 2.69 (1H, m), 2.61 (1H, dd, J=13.4, 7.6 Hz), 1.14 (3H, d, J=7.0 Hz).

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethyl-propanoic Acid Methyl Ester (27a) Compound **27a** was prepared from **26a** using the procedure described for **23a** in 11% yield: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.41 (1H, ddd, J=8.8, 7.0, 2.1 Hz), 7.04 (2H, d, J=8.5 Hz), 6.82 (2H, dt, J=8.5, 1.8 Hz), 6.5 (2H, m), 4.14 (2H, t, J=6.1 Hz), 3.91 (2H, t, J=5.8 Hz), 3.63 (3H, s), 3.47 (2H, t, J=7.9 Hz), 2.94 (1H, dd, J=13.4, 6.7 Hz), 2.68 (1H, m), 2.59 (1H, dd, J=13.4, 7.6 Hz), 1.64—1.57 (2H, m), 1.35—1.25 (12H, m), 1.13 (3H, d, J=7.0 Hz), 0.88 (3H, t, J=7.2 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3046.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2-methylpropanoic Acid (2) Compound **2** was prepared from **27a** using the procedure described for **1** in 97% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (2H, d, J=4.8 Hz), 7.41 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 7.08 (2H, d, J=8.5 Hz), 6.82 (2H, d, J=8.5 Hz), 6.5 (2H, m), 4.12 (2H, m), 3.90 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.9 Hz), 2.98 (1H, dd, J=13.4, 7.0 Hz), 2.73 (1H, m), 2.63 (1H, dd, J=13.4, 7.6 Hz), 1.65—1.58 (2H, br), 1.35—1.25 (12H, m), 1.17 (3H, d, J=6.7 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_3$ 426.2882, Found 426.2889.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethyl-propanoic Acid (5) Compound **5** was prepared from **20a** using the procedure described for **2** in 4.2% yield. In the step of the synthesis of **26b**, dimethylketene methyltrimethylsilyl acetal was used instead of 1-methoxy-1-trimethylsilyloxypropene: light yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (2H, dd, J=4.9, 1.5 Hz), 7.41 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 7.05 (2H, d, J=8.5 Hz), 6.80 (2H, d, J=8.5 Hz), 6.50 (2H, m), 4.12 (2H, t, J=5.8 Hz), 3.90 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.6 Hz), 2.81 (2H, s), 1.65—1.59 (2H, m), 1.35—1.25 (12H, m), 1.17 (6H, s), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3040.

3-{4-Methoxy-3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2-methylpropanoic Acid (11) Compound **11** was prepared from **20c** using the procedure described for **2** in 3.3% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.44 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 6.95 (1H, d, J=1.8 Hz), 6.78 (1H, d, J=7.9 Hz), 6.71 (1H, dd, J=8.2, 1.8 Hz), 6.54 (1H, dd, J=7.0, 5.1 Hz), 6.50 (1H, d, J=8.8 Hz), 4.25 (2H, m), 3.92 (2H, m), 3.83 (3H, s), 3.43 (2H, t, J=7.3 Hz), 2.92 (1H, m), 2.70 (2H, m), 1.64—1.54 (2H, m), 1.31—1.20 (12H, m), 1.16 (3H, d, J=6.7 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.3007.

3-{4-Methoxy-3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethylpropanoic Acid (14) Compound **14** was prepared from **20c** using the procedure described for **2** in 8.4% yield. In the step of the synthesis of **26d**, dimethylketene methyltrimethylsilyl acetal was used instead of 1-methoxy-1-trimethylsilyloxypropene: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=5.1, 1.8, 0.6 Hz), 7.45 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.00 (1H, d, J=2.1 Hz), 6.76 (1H, d, J=8.2 Hz), 6.68 (1H, dd, J=8.2, 1.8 Hz), 6.55 (1H, dd, J=7.0, 5.2 Hz), 6.50 (1H, d, J=8.8 Hz), 4.27 (2H, t, J=7.0 Hz), 3.90 (2H, t, J=7.3 Hz), 3.84 (3H, s), 3.40 (2H, t, J=7.6 Hz), 2.79 (2H, s), 1.63—1.57 (2H, m), 1.35—1.25 (12H, m), 1.20 (6H, s), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_4$ 470.3145, Found 470.3144.

3-{4-[3-(Nonylpyridin-2-ylamino)propyl]phenyl}acrylic Acid Ethyl Ester (28a) (Chart 3) Compound **28a** was prepared from **21a** using the procedure described for **23a** in 13% yield: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.8, 2.1, 0.9 Hz), 7.63 (1H, d, J=16.2 Hz), 7.45 (2H, d, J=8.8 Hz), 7.42 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.91 (2H, d, J=8.5 Hz), 6.52 (1H, dd, J=7.0, 5.5 Hz), 6.50 (1H, d, J=8.8 Hz), 6.30 (1H, d, J=15.8 Hz), 4.25 (2H, q, J=7.0 Hz), 4.21 (2H, t, J=5.8 Hz), 3.94 (2H, t, J=5.8 Hz), 3.46 (2H, t, J=7.9 Hz), 1.64—1.60 (2H, m), 1.36—1.30 (3H, t, J=7.0 Hz), 1.33—1.23 (12H, br), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{38}N_2O_3$ 438.2882, Found 438.2855.

3-{4-[3-(Nonylpyridin-2-ylamino)propyl]phenyl}acrylic Acid (15) To a solution of **28a** (84 mg, 0.192 mmol) in 2 ml of THF and 2 ml of EtOH was added a solution of 2 N aqueous NaOH (0.29 ml, 0.580 mmol). The reaction mixture was stirred for 18 h at room temperature, and neutralized to pH 7 with aqueous 2 N HCl. Purification by silica gel flash chromatography ($CHCl_3$/MeOH=19/1) gave 78 mg (99%) of white solid. The 41 mg of the white solid was recrystallized from AcOEt–*n*-hexane and collected by filtration to give 22 mg (52%) of **15** as a white solid: mp 142—143 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.15 (1H, dd, J=4.9, 1.8 Hz), 7.70 (1H, d, J=15.9 Hz), 7.47 (2H, d, J=8.8 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.93 (1H, d, J=8.8 Hz), 6.53 (1H, dd, J=6.4, 5.5 Hz), 6.50 (1H, d,

J=8.5 Hz), 6.29 (1H, d, J=16.1 Hz), 4.22 (2H, t, J=5.8 Hz), 3.95 (2H, t, J=5.8 Hz), 3.46 (2H, t, J=8.2 Hz), 1.65—1.61 (2H, m), 1.34—1.24 (12H, m), 0.88 (3H, t, J=7.0 Hz); *Anal.* Calcd for $C_{25}H_{34}N_2O_3$: C, 73.14; H, 8.35; N, 6.82. Found: C, 73.23; H, 8.46; N, 6.89.

3-{4-Methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}acrylic Acid (16) Compound **16** was prepared from **21h** using the procedure described for **15** in 7.4% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.22 (1H, dd, J=4.9, 1.8 Hz), 7.69 (1H, d, J=15.8 Hz), 7.44 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.31 (1H, d, J=1.5 Hz), 7.09 (1H, dd, J=8.2, 1.8 Hz), 6.86 (1H, d, J=8.2 Hz), 6.56 (1H, dd, J=7.0, 5.2 Hz), 6.51 (1H, d, J=8.5 Hz), 6.31 (1H, d, J=15.8 Hz), 4.31 (2H, t, J=6.4 Hz), 3.99 (2H, t, J=6.1 Hz), 3.90 (3H, s), 3.45 (2H, t, J=7.6 Hz), 1.65—1.61 (2H, m), 1.32—1.22 (12H, m), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{26}H_{36}N_2O_4$ 440.2675, Found 440.2716.

Binding Assay The assay for PPAR binding activity was performed using a CoA-BAP System kit (NuLigand series, Microsystems). Briefly, glutathione-*S*-transferase-human nuclear receptor ligand binding domain (GST-hNR LBD) fusion proteins were incubated in a glutathione-fixed micro-well plate at 4 °C overnight. After excessive proteins were removed, human transcriptional intermediary factor 2 (hTIF2)-bacterial alkaline phosphatase (BAP) fusion proteins were added to the well with test chemicals. After 1 h incubation at 4 °C, excessive proteins were removed carefully. The enzyme reaction was allowed to start with the addition of *p*-nitrophenylphosphoric acid (NPP) as a substrate, and incubated at 30 °C. After 3 h, the reaction was stopped by the addition of 0.5 N aqueous NaOH. The product was measured by reading absorbance at 405 nm with a 1420 ARVO™ multilabel counter (ParkinElmer, Boston, MA, U.S.A.). AP activity was determined by subtracting background absorption from the reading at 405 nm.

Cell-Based Transactivation Assay[30] Human embryonic kidney (HEK) 293 cells were cultured in DMEM containing 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections of PPAR and reporter gene constructs were performed by calcium phosphate coprecipitation. Eight hours after transfection, ligands were added. Cells were harvested 12—16 h after treatment, and luciferase and β-galactosidase activities were assayed using a 1420 ARVO™ MX multilabel counter (ParkinElmer, Boston, MA, U.S.A.). DNA cotransfection experiments included 58 ng of reporter plasmid, 12 ng of CMX-β-galactosidase, and 18 ng of each receptor expression plasmid per well in a 96-well plate. Luciferase data were normalized to an internal β-galactosidase control and reported values are the means of triplicate assays.

Preadipocyte Differentiation Test Human preadipocytes from hypodermic tissues, a preadipocyte growth medium and a preadipocyte differentiation medium were purchased from TOYOBO. Co., Ltd (Osaka, Japan). Human preadipocytes were cultured for 8 d in preadipocyte growth medium in a humidified incubator at 37 °C and 5% CO_2. The medium was renewed every other day. When the preadipocytes reached confluence, the cells were treated with preadipocyte differentiation medium containing compounds **3**, **4**, or rosiglitazone. The cells were cultured for a further 7 d with the differentiation medium renewed every 3 d. The accumulation of triglycerides was evaluated by measuring the absorbance at 570 nm with a 1420 ARVO™ multilabel counter (ParkinElmer, Boston, MA, U.S.A.) after staining with Lipidos Liquid® (TOYOBO Co., Ltd, Osaka, Japan).

References and Notes

1) Nuclear Receptors Nomenclature Committee. *Cell*, **97**, 161—163 (1999).
2) Willson T. M., Brown P. J., Sternbach D. D., Henke B. R., *J. Med. Chem.*, **43**, 527—550 (2000).
4) Kersten S., Desvergne B., Wahli W., *Nature* (London), **405**, 421—424 (2000).
6) 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.*, **37**, 3977—3985 (1994).
7) Momose Y., Meguro K., Ikeda H., Hatanaka C., Oi S., Sohda T., *Chem. Pharm. Bull.*, **39**, 1440—1445 (1991).
8) Hauner H., *Diabetes Metab. Res. Rev.*, **18**, S10—S15 (2002).
9) Francis G. A., Annicotte J. S., Auwerx J., *Curr. Opin. Parmacol.*, **3**, 186—191 (2003).
10) Lohray B. B., Lohray V. B., Bajji A. C., Kalchar S., Poondra R. R., Padakanti S., Chakrabarti R., Vikramadithyan R. K., Misra P., Juluri S., Mamidi N. V., Rajagopalan R., *J. Med. Chem.*, **44**, 2675—2678 (2001).
11) Duran-Sandoval D., Thomas A. C., Bailleul B., Fruchart J. C., Staels B., *Med. Sci.*, **19**, 819—825 (2003).
12) Forman B. M., Tontonoz P., Chen J., Brun R. P., Spiegelman B. M., Evans R. M., *Cell*, **83**, 803—812 (1995).
13) Kliewer S. A., Lenhard J. M., Willson T. M., Patel I., Morris D. C., Lehmann J. M., *Cell*, **83**, 813—819 (1995).
14) Usui S., Suzuki T., Hattori Y., Etoh K., Fujieda H., Nishizuka M., Imagawa M., Nakagawa H., Kohda K., Miyata N., *Bioorg. Med. Chem. Lett.*, **15**, 1547—1551 (2005).
15) Buckle D. R., Cantello B. C. C., Cawthorne M. A., Coyle P. J., Dean D. K., Faller A., Haigh D., Hindley R. M., Jefcott L. J., Lister C. A., Pinto I. L., Rami H. K., Smith D. G., Smith S. A., *Bioorg. Med. Chem. Lett.*, **6**, 2121—2126 (1996).
16) Buckle D. R., Cantello B. C. C., Cawthorne M. A., Coyle P. J., Dean D. K., Faller A., Haigh D., Hindley R. M., Jefcott L. J., Lister C. A., Pinto I. L., Rami H. K., Smith D. G., Smith S. A., *Bioorg. Med. Chem. Lett.*, **6**, 2127—2130 (1996).
17) Hulin B., Newton L. S., Lewis D. M., Genereux P. E., Gibbs E. M., Clark D. A., *J. Med. Chem.*, **39**, 3897—3907 (1996).
18) Sauerberg P., Pettersson I., Jeppesen L., Bury P. S., Mogensen J. P., Wassermann K., Brand C. L., Sturis J., Woldike H. F., Fleckner J., Andersen A. S., Mortensen S. B., Svensson L. A., Rasmussen H. B., Lehmann S. V., Polivka Z., Sindelar K., Panajotova V., Ynddal L., Wulff E. M., *J. Med. Chem.*, **45**, 789—804 (2002).
19) Cronet P., Petersen J. F., Folmer R., Blomberg N., Sjoblom K., Karlsson U., Lindstedt E. L., Bamberg K., *Structure*, **9**, 699—706 (2001).
20) Nomura M., Tanase T., Ide T., Tsunoda M., Suzuki M., Uchiki H., Murakami K., Miyachi H., *J. Med. Chem.*, **46**, 3581—3599 (2003).
21) Weigand S., Bischoff H., Dittrich-Wengenroth E., Heckroth H., Lang D., Vaupel A., Woltering M., *Bioorg. Med. Chem. Lett.*, **15**, 4619—4623 (2005).
22) Kasuga J., Makishima M., Hashimoto Y., Miyachi H., *Bioorg. Med. Chem. Lett.*, **16**, 554—558 (2006).
23) Wagaw S., Buchwald S. L., *J. Org. Chem.*, **61**, 7240—7241 (1996).
24) Maryanoff B. E., Reitz A. B., *Chem. Rev.*, **89**, 863—927 (1989).
25) Grieco P. A., Handy S. T., *Tetrahedron Lett.*, **38**, 2645—2648 (1997).
26) Kanayama T., Mamiya S., Nishihara T., Nishikawa J., *J. Biochem.* (Tokyo), **133**, 791—797 (2003).
27) Brown P. J., Stuart L. W., Hurley K. P., Lewis M. C., Winegar D. A., Wilson J. G., Wilkison W. O., Ittoop O. R., Willson T. M., *Bioorg. Med. Chem. Lett.*, **11**, 1225—1227 (2001).
28) Sznaidman M. L., Haffner C. D., Maloney P. R., Fivush A., Chao E., Goreham D., Sierra M. L., LeGrumelec C., Xu H. E., Montana V. G., Lambert M. H., Willson T. M., Oliver W. R., Jr., Sternbach D. D., *Bioorg. Med. Chem. Lett.*, **13**, 1517—1521 (2003).
29) Usui S., Fujieda H., Suzuki T., Yoshida N., Nakagawa H., Miyata N., *Bioorg. Med. Chem. Lett.*, **16**, 3249—3254 (2006).
30) Fukuen S., Iwaki M., Yasui A., Makishima M., Matsuda M., Shimomura I., *J. Biol. Chem*, **280**, 23653—23659 (2005).
31) Henke B. R., *J. Med. Chem.*, **47**, 4118—4127 (2004).
32) Lagace D. C., Nachtigal M. W., *J. Biol. Chem.*, **279**, 18851—18860 (2004).
33) Relative transactivation activity at 10 μM: compound **4**, 163% of WY14643 for α-activity, and 88% of rosiglitazone for γ-activity, compound **8**, 275% of WY14643 for α-activity.

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Bioorganic & Medicinal Chemistry Letters

Bioorganic & Medicinal Chemistry Letters 17 (2007) 4208–4212

Design, synthesis, and biological activity of folate receptor-targeted prodrugs of thiolate histone deacetylase inhibitors

Takayoshi Suzuki,[a,*] Shinya Hisakawa,[a] Yukihiro Itoh,[a] Nobuaki Suzuki,[a] Katsumasa Takahashi,[a] Masatoshi Kawahata,[b] Kentaro Yamaguchi,[b] Hidehiko Nakagawa[a] and Naoki Miyata[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan*
[b]*Evaluation Group, Drug Research Department, R&D Division, Pharmaceuticals Group, Nippon Kayaku Co., Ltd, 31-12, Shimo 3-chome, Kita-ku, Tokyo 115-8588, Japan*

Received 22 April 2007; revised 10 May 2007; accepted 11 May 2007
Available online 17 May 2007

Abstract—Aiming to develop selective anticancer drugs, we designed and synthesized three disulfides bearing a folic acid moiety as candidate folate receptor (FR)-targeted prodrugs of thiolate histone deacetylase inhibitors. Among them, compound **1** displayed growth-inhibitory activity toward folate receptor-positive MCF-7 breast cancer cells. The activity of **1** was significantly reduced by free folic acid, suggesting that cellular uptake of **1** is mediated by FR.
© 2007 Elsevier Ltd. All rights reserved.

Histone deacetylases (HDACs) have recently emerged as a new target for the development of anticancer drugs, and some small-molecular HDAC inhibitors, including suberoylanilide hydroxamic acid (SAHA) (also known as vorinostat) and MS-275, have been developed as anticancer drugs (Fig. 1).[1–6] Inhibition of HDACs causes histone hyperacetylation which leads to the disruption of the chromatin structure and the transcriptional activation of genes associated with cancer. Indeed, HDAC inhibitors have shown anticancer activity in vitro, in animal models and in patients with solid tumors and hematological malignancies.[7] Nevertheless, they have been reported to cause adverse events, such as nausea, vomiting, anorexia, anemia, thrombocytopenia, and fatigue, in the course of clinical trials.[7–9] Therefore, it is necessary to find HDAC inhibitors that show selective anticancer activity.

We have focused on folate receptor (FR)-targeted prodrugs for selectively targeting cancer cells. The vitamin folic acid and its analogues display extremely high affinity for the folate receptor on the cell surface, and are internalized via receptor-mediated endocytosis.[10] Since the FR is overexpressed on certain malignant cell types and is undetectable or present only at low levels in most normal tissues,[11–13] targeting of the FR has been proposed as a potential mechanism for delivery of drugs to treat cancer.[14] In addition, small molecules including folate-drug conjugates may avoid the limitations associated with antibody-mediated targeting.[15,16] Here, we report on the design, synthesis, and biological activity of FR-targeted prodrugs of HDAC inhibitors.

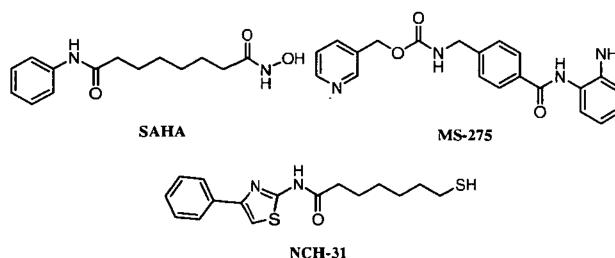


Figure 1. Structures of SAHA, MS-275, and the thiolate HDAC inhibitor NCH-31.

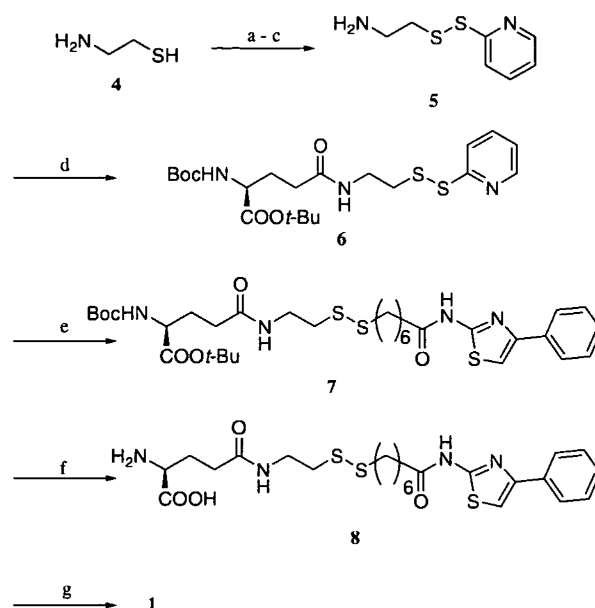
We previously reported that thiol-based analogues, including NCH-31 (Fig. 1), are potent HDAC inhibitors.[17–19] Thiols are thought to inhibit HDACs by coordinating the zinc ion, which is required for deacetylation

Keywords: Histone deacetylase inhibitor; Selective anticancer activity; Folate conjugate; Prodrug.
* 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 © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.040

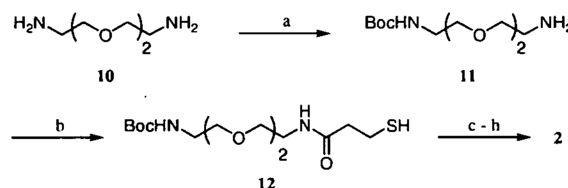
of the acetylated lysine substrate. Further, thiolate analogues showed potent cancer cell growth-inhibitory activities.[20,21] Based on these findings, we designed FR-targeted prodrugs of HDAC inhibitors. Unlike hydroxamates and *o*-aminoanilides, such as SAHA and MS-275, thiolate HDAC inhibitors can be conjugated with a folic acid moiety via a disulfide bond, which would be reduced under reductive conditions, releasing the free thiol as an active species. We designed the folic acid-NCH-31 conjugates **1** and **2** (Fig. 2), which are expected to be recognized by the FR located on the cell surface, to enter cells via receptor-mediated endocytosis, and then to release the HDAC inhibitor NCH-31 upon cleavage of the disulfide bond in the cellular environment. We also designed the symmetrical disulfide **3** bearing a folic acid moiety. The reduced form of compound **3** itself could behave as an HDAC inhibitor.

The synthesis of the folic acid-NCH-31 conjugate **1** is outlined in Scheme 1. Mercaptoethylamine **4** was converted into 2-(2-(2-pyridinyl)disulfanyl)ethylamine **5** by the Boc protection of **4**, followed by treatment with 2,2′-dithiopyridine and Boc deprotection. Compound **5** was then coupled with *N*-Boc-L-glutamic acid α-*tert*-butyl ester to give the amide **6**. Treatment of compound **6** with NCH-31[17,20] in DMF afforded the sulfur-exchanged product **7**. Universal deprotection of **7** using hydrochloric acid yielded the NCH-31-glutamic acid linked compound **8**. The folic acid-NCH-31 conjugate **1** was obtained in 92% yield by the reaction of **8** with pteroyl azide **9**[22] in DMSO in the presence of tetramethylguanidine.



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt; (b) 2,2′-dithiopyridine MeOH, rt; (c) HCl, AcOEt, rt, 39% (three steps); (d) Boc-Glu-O-*t*-Bu, EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 76%; (e) NCH-31, DMF, rt, 86%; (f) HCl, AcOEt, rt, quant; (g) pteroyl azide (**9**), tetramethylguanidine, DMSO, rt, 92%.

Scheme 2 shows the preparation of the other folic acid-NCH-31 conjugate **2**. Compound **2** was synthesized from 2,2′-(ethylenedioxy)diethylamine **10**. Reaction of the diamine **10** with an equivalent amount of $(Boc)_2O$ gave the mono-Boc-protected compound **11**. Coupling between amine **11** and 3-mercaptopropanoic acid in the presence of EDCI and HOBt afforded **12**. The folic acid-NCH-31 conjugate **2** was prepared from the thiol **12** in the same manner as described for the synthesis of **1**.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, CH_2Cl_2, 0 °C to rt, quant; (b) 3-mercaptopropanoic acid, EDCI, HOBt, CH_2Cl_2, rt, 53%; (c) 2,2′-dithiopyridine MeOH, rt; (d) HCl, AcOEt, rt; (e) Boc-Glu-O-*t*-Bu, EDCI, HOBt, Et_3N, CH_2Cl_2, rt; (f) NCH-31, DMF, rt; (g) HCl, AcOEt, rt; (h) **9**, tetramethylguanidine, DMSO, rt, 59% (six steps).

The attempted route to **3** is shown in Scheme 3. In this scheme, we anticipated that disulfide dimer **3** would be obtained from the corresponding thiol monomer by reaction with I_2. The 7-bromoheptanoic acid ethyl ester **13** was hydrolyzed to give the carboxylic acid **14**, after which Curtius rearrangement of the acyl azide prepared from **14** using diphenylphosphoryl azide (DPPA) provided the isocyanate. This, on treatment with *tert*-butanol, gave the *N*-Boc compound **15**. Treatment of **15** with triphenylmethanethiol in the presence of NaOMe afforded compound **16**. Deprotection of the Boc group of **16** and coupling with *N*-Fmoc-L-glutamic acid α-*tert*-butyl ester gave the amide **17**. The Fmoc group of **17** was removed using piperidine and coupling with pteroyl azide **9** afforded compound **18**. Removal of the *tert*-butyl group and the triphenylmethyl group of **18** under acidic conditions gave the thiol **19**. Although **19** was successfully obtained from **13** in eight steps, it was poorly soluble. We examined a variety of solvents for the dimerization of **19** using I_2, but a suspension of **19** with I_2 failed to provide the disulfide dimer **3**.

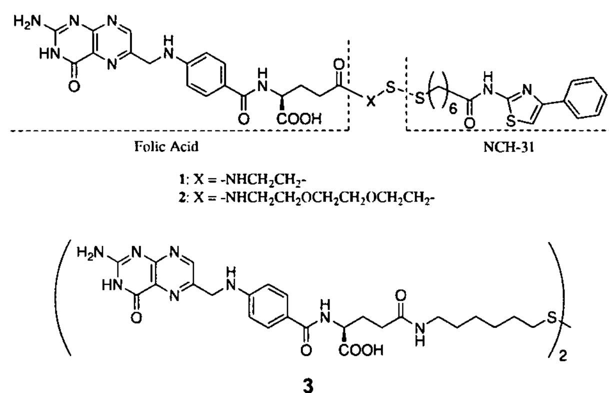
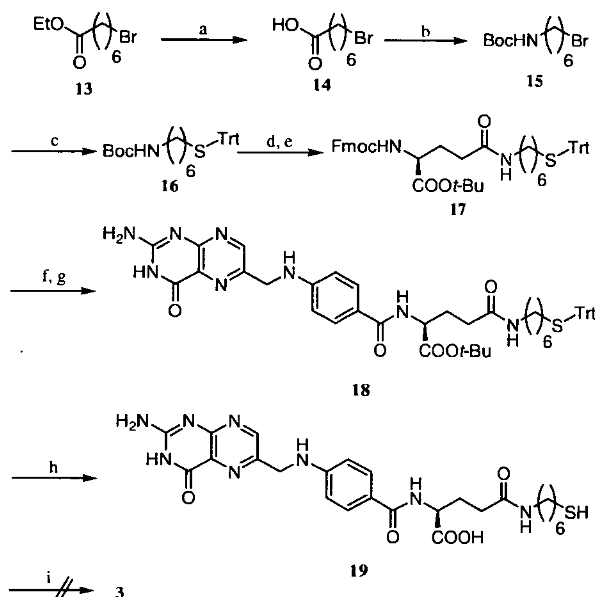


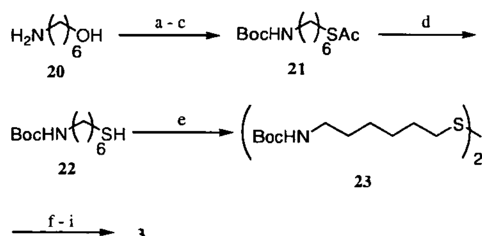
Figure 2. Candidate FR-targeted prodrugs of thiolate HDAC inhibitors.



Scheme 3. Reagents and conditions: (a) LiOH, THF, EtOH, H_2O, rt, quant; (b) 1—DPPA, Et_3N, reflux, 2—t-BuOH, toluene, reflux, 44%; (c) NaOMe, HS-Trt, toluene, EtOH, 60 °C, 94%; (d) TFA, CH_2Cl_2, rt; (e) Fmoc-Glu-O-t-Bu, EDCI, HOBt, CH_2Cl_2, rt, 76% (two steps); (f) piperidine, DMF, rt, 87%; (g) **9**, tetramethylguanidine, DMSO, rt, 21%; (h) TFA, CH_2Cl_2, rt, 94%; (i) I_2.

We succeeded in obtaining **3** through the route outlined in Scheme 4. In this route, a disulfide bond was formed in the early stage. Initially, 6-aminohexanol **20** was converted to compound **21** by N-Boc protection, O-tosylation, and treatment with potassium thioacetate. The acetyl group of **21** was then removed to give the thiol **22**, and the disulfide dimer **23** was obtained by the reaction of the thiol monomer **22** with I_2. The desired disulfide **3** was successfully obtained from **23** in 74% yield using the same procedure as described for the synthesis of **1**.

Compounds **1–3** were initially tested in an in vitro HDAC inhibition assay under reductive conditions (Table 1).[23] Among these compounds, compound **1** showed the most potent activity inhibiting HDACs with an IC_{50} of 0.27 μM, and the activity was comparable with that of NCH-31. This result suggested that the



Scheme 4. Reagents and conditions: (a) $(Boc)_2O$, THF, rt; (b) TsCl, Et_3N, THF, rt; (c) KSAc, acetone, rt, 75% (three steps); (d) aq NaOH, MeOH, THF, rt, 71%; (e) I_2, MeOH, rt, 95%; (f) HCl, AcOEt, rt; (g) Boc-Glu-O-t-Bu, EDCI, HOBt, Et_3N, CH_2Cl_2, rt; (h) HCl, AcOEt, rt; (i) **9**, tetramethylguanidine, DMSO, rt, 74% (four steps).

Table 1. HDAC inhibition data for NCH-31, **1–3**, and **19**[a]

Entry	Compound	IC_{50} (μM)
1	NCH-31	0.17[b]
2	**1**[c]	0.27
3	**2**[c]	3.0
4	**3**[c]	9.0
5	**19**	21

[a] Values are means of at least three experiments.
[b] Data taken from the literature (Ref. 20).
[c] Incubated with DTT (250 μM).

disulfide bond of compound **1** was reduced to release NCH-31 under the reductive conditions. On the other hand, the HDAC-inhibitory activities of compounds **2** and **3** were weaker than that of **1**. The reason for the weaker activity of **2** is unclear, but it may be because compound **2** is resistant to reduction as compared with compound **1**.

To confirm the effectiveness of the folic acid-based prodrugs of HDAC inhibitors, compounds **1** and **2** were tested in a cancer cell growth inhibition assay[24] using FR-positive human breast cancer MCF-7 cells.[25] Consistent with the results in the enzyme assay, compound **1** displayed dose-dependent cell growth-inhibitory activity (Fig. 3). Further, a competition experiment with 100 μM free folic acid significantly reduced the cell growth-inhibitory activity of **1**, demonstrating that the FR is responsible for the cellular entry of **1** (Fig. 4). In addition, treatment of MCF-7 cells with compound **1** produced an increase in the accumulation of acetylated histone H4 (Fig. 5),[26] which indicated that the cell growth-inhibitory activity of compound **1** significantly correlates with the inhibition of HDACs. Furthermore, the activity of **1** to cause histone hyperacetylation was significantly reduced by 100 μM free folic acid (Fig. 5). These results also suggested that the uptake of compound **1** is FR mediated.

In summary, we have designed and synthesized FR-targeted prodrugs of thiolate HDAC inhibitors that possess a cleavable disulfide bond. The folic acid-NCH-31 conjugate **1** showed potent HDAC-inhibitory activity under reductive conditions. Furthermore, compound **1** exerted growth-inhibitory activity against FR-positive breast cancer MCF-7 cells, and the cellular uptake of **1** was considered to be FR mediated, based on a competition experiment with free folic acid. Our strategy of utilizing

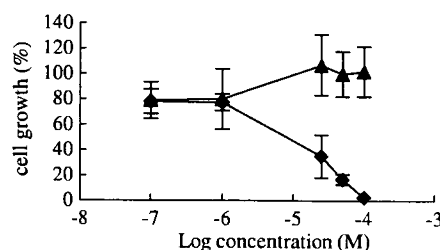


Figure 3. Growth inhibition of FR-positive MCF-7 cells by compounds **1** (●) and **2** (▲).