Supporting Information

Synthesis and Evaluation of Diaryl Thiazole Derivatives That Inhibit Activation of Sterol Regulatory Element-Binding Protein

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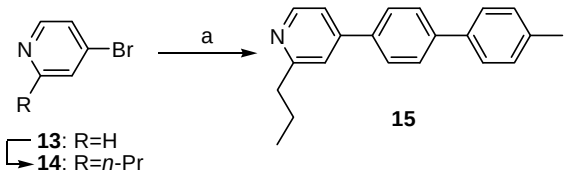
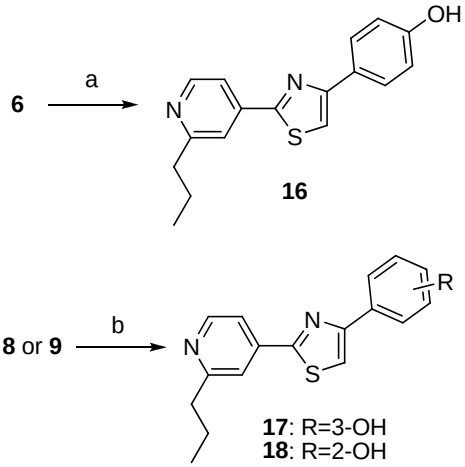
Scheme S1. Synthesis of Analogue **15**[a]



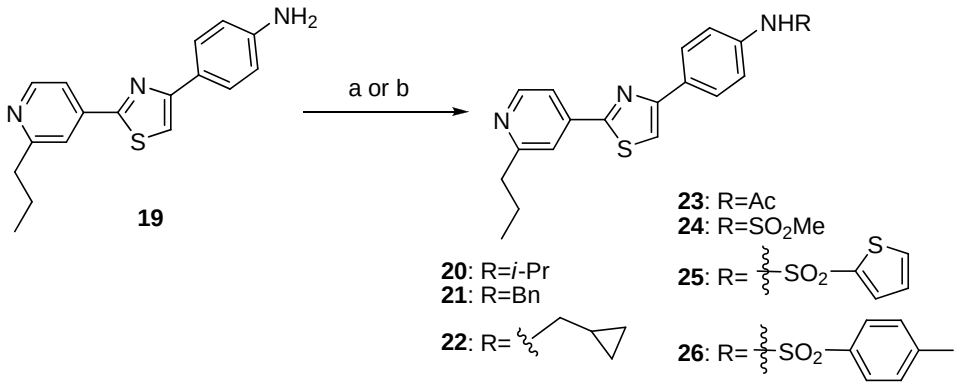
[a]Reagents and conditions: (a) 4'-methyl-4-biphenylboronic acid, $(Ph_3P)_4Pd$, K_2CO_3, DMF, H_2O, 80 °C, 18 h, 52%.

Scheme S2. Synthesis of Analogues **16**─**18**[a]



[a]Reagents and conditions: (a) KOH aq, THF, MeOH, 60 °C, 0.5 h, 80%; (b) BBr_3, CH_2Cl_2, 0 °C to room temp, 6─14 h, 16─40%.

Scheme S3. Synthesis of Analogues **20**─**26**[a]



[a]Reagents and conditions: (a) acetone, benzaldehyde or cyclopropanecarboxaldehyde, AcOH, $Na(AcO)_3BH$, CH_2Cl_2, room temp, 19─21 h, 52─73%; (b) acetyl chloride, methanesulfonyl chloride, thiophenesulfonyl chloride or tosyl chloride, pyridine, CH_2Cl_2, 0 °C to room temp, 0.5─72 h, 14─87%.

Table S1. *In silico* properties of fatostatin (**1**) and analogues **20** and **24.**

Analogue	**1**		**20**		**24**	
Property	Value	Error	Value	Error	Value	Error
clogP	5.72	0.41	5.82	0.43	3.97	0.58
Molecular weight	294.41	-	337.48	-	373.49	-
PSA (polar surface area) $Å^2$	54.02	-	66.05	-	108.57	-
Number of freely rotatable bonds	4	-	6	-	5	-
Number of H-bond donors	0	-	1	-	1	-
Number of H-bond acceptors	2	-	3	-	5	-
Number of "Rule Of 5" violations	1	-	1	-	0	-

Table S2. HPLC retention time and purity data for the fatostatin analogues used in this study. Purity was assessed by integration of peak areas.

Compound	LC purity	Retention time (min.)
1	98.7	12.3
4a	99.3	10.3
4b	99.8	9.1
5a	99.1	9.7
5d	98.8	14.4
6	95.6	14.7
7	99.4	11.1
8	98.5	10.0
10	98.7	8.3
15	98.2	12.2
16	99.4	4.8
18	96.9	8.5
22	95.1	3.9
23	95.7	4.9
24	97.7	4.3
26	98.6	10.2
27	98.5	12.6

Table S3. Combustion analyses for the fatostatin analogues synthesized in this study.

Compound	Anal.	Calculated C; H; N; S, X.	Found C; H; N; S, X.
5b	$C_{17}H_{15}FN_2S$	68.43; 5.07; 9.39; 10.75, 6.37.	68.32; 5.10; 9.17; 10.65, 6.19.
5c	$C_{17}H_{15}ClN_2S$	64.85; 4.80; 8.90; 10.18, 11.26.	64.81; 4.83; 8.97; 10.09, 11.26.
9	$C_{18}H_{18}N_2OS$	69.65; 5.84; 9.02; 10.33.	69.43; 5.85; 8.86; 10.20.
12	$C_{16}H_{13}NS$	76.46; 5.21; 5.57; 12.76.	76.46; 5.28; 5.52; 12.71.
17	$C_{17}H_{16}N_2OS$	68.89; 5.44; 9.45; 10.82.	68.59; 5.43; 9.43; 10.69.
19	$C_{17}H_{17}N_3S$	69.12; 5.80; 14.22; 10.85.	69.17; 5.86; 14.10; 10.75.
20	$C_{20}H_{23}N_3S$	71.18; 6.87; 12.45; 9.50.	71.21; 6.92; 12.42; 9.40.
21	$C_{24}H_{23}N_3S$	74.77; 6.01; 10.90; 8.32.	74.82; 5.99; 10.93; 8.27.
24	$C_{18}H_{19}N_3O_2S_2$	57.88; 5.13; 11.25; 17.17.	57.95; 5.14; 11.18; 16.88.
25	$C_{21}H_{19}N_3O_2S_3$	57.12; 4.34; 9.52; 21.78.	57.05; 4.40; 9.55; 21.57.

Experimental Section

General. All materials, including compounds **4b, 5a, 5c** and **5d**, were obtained from commercial suppliers and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Column chromatography was performed on Fuji Silysia Chemical silica gel BW820MH. Thin-layer chromatography was carried out with glass TLC plates, precoated with Merck silica gel 60 F254. ^{1}H NMR spectra were recorded at 300 MHz with a JEOL DELTA 300 spectrometer. Low-resolution mass spectra were obtained on a SHIMADZU LCMS-2010 spectrometer, using electron spray ionization (ESI). High-resolution mass spectra were obtained on a JEOL JMS 700 Mass Spectrometer, using fast atom bombardment (FAB) ionization. Purity of each fatostatin analogue was estimated by combustion analysis (**Table S3**) and/or HPLC analysis (**Table S2**). Combustion analyses were performed using a J-Science Lab Micro Corder JM10 and a DIA Instruments SX-100, and were within 0.4% of calculated values. HPLC analyses used the following conditions: column, GL Science Inc. Inertsil ODS-3 (150 × 4.6 mm); particle size, 5 μm; mobile phase, 0.1% TFA in MeOH:0.1% TFA in H_2O; gradient, 50:50 to 100:0 over 30 min, then 100:0 isocratic for 5 min; flow rate, 1.0 mL min^{-1}; detection wavelength, λ = 235 nm.

2-(2-Ethylpyridin-4-yl)-4-*p*-tolylthiazole (4a). A mixture of prothionamide (**3**) (83 mg, 0.5 mmol) and 2-bromo-4'-methylacetophenone (117 mg, 0.55 mmol) in ethanol (4 ml) was stirred for 18 h at 80 °C, then cooled to 0 °C. The yellow precipitate was filtered, washed with cold ethanol, and dried to produce compound **4a** HBr salt (35 mg, 19%) as yellow needles. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.82 (d, J = 5.5 Hz, 1H), 8.48 (s, 1H),

8.31 (s, 1H), 8.23 (d, J = 5.5 Hz, 1H), 8.00 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 3.02 (q, J = 7.5 Hz, 2H), 2.36 (s, 3H), 1.34 (t, J = 7.6 Hz, 3H); HRMS (m/z): $[M+H]^+$ calcd for $C_{17}H_{17}N_2S$, 281.1112; found, 281.1111.

4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenyl benzoate (6). Compound **6** (85%) was prepared using methods similar to that described for compound **4a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (d, J = 5.8 Hz, 1H), 8.25-8.22 (m, 4H), 8.08 (d, J = 8.8 Hz, 2H), 7.88 (s, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.55 (t, J = 7.4 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H), 3.31 (t, J = 7.7 Hz, 2H), 2.04 (m, 2H), 1.12 (t, J = 7.4 Hz, 3H); HRMS (m/z): $[M+H]^+$ calcd for $C_{24}H_{21}N_2O_2S$, 401.1324; found, 401.1323.

2-(2-Propylpyridin-4-yl)-4-(thiophen-2-yl)thiazole (10). Compound **10** (78%) was prepared using methods similar to that described for compound **4a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (d, J = 6.0 Hz, 1H), 8.43 (s, 1H), 8.33 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 7.74 (d, J = 3.6 Hz, 1H), 7.64 (d, J = 5.2 Hz, 1H), 7.18 (dd, J = 3.6, 5.2 Hz, 1H), 3.00 (t, J = 7.6 Hz, 2H), 1.78 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H); HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{15}N_2S_2$, 287.0677; found, 287.0682.

4-(4-Methoxyphenyl)-2-(2-propylpyridin-4-yl)thiazole (7). A mixture of prothionamide (**3**) (700 mg, 3.88 mmol) and 2-bromo-1-(4-methoxyphenyl)ethanone (890 mg, 3.89 mmol) in ethanol (15 ml) was stirred for 0.5 h at 80 °C, then cooled to 0 °C. The yellow precipitate was filtered and washed with cold ethanol. The residue was partitioned between EtOAc and saturated $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc. The combined extracts were dried over Na_2SO_4, and concentrated to produce compound **7** (721 mg, 60%) as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, J =

5.2 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 5.2 Hz, 1H), 7.47 (s, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H), 2.91 (t, *J* = 7.7 Hz, 2H), 1.84 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{19}N_2OS$, 311.1218; found, 311.1213.

4-(4-Fluorophenyl)-2-(2-propylpyridin-4-yl)thiazole (5b). Compound **5b** (60%) was prepared by a method similar to that described for compound **7**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (d, *J* = 5.2 Hz, 1H), 7.98 (dd, *J* = 5.5, 8.8 Hz, 2H), 7.76 (d, *J* = 1.7 Hz, 1H), 7.68 (dd, *J* = 1.7, 5.2 Hz, 1H), 7.53 (s, 1H), 7.16 (t, *J* = 8.8 Hz, 2H), 2.87 (t, *J* = 7.6 Hz, 2H), 1.84 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); *m*/*z* = 299 $[M+H]^+$. Anal. ($C_{17}H_{15}FN_2S$) C, H, N, S, F.

4-(3-Methoxyphenyl)-2-(2-propylpyridin-4-yl)thiazole (8). Compound **8** (82%) was prepared by a method similar to that described for compound **7.** ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (d, *J* = 5.2 Hz, 1H), 7.77 (d, *J* = 1.5 Hz, 1H), 7.69 (dd, *J* = 1.5, 5.2 Hz, 1H), 7.59 (s, 1H), 7.59-7.55 (m, 2H), 7.38 (t, *J* = 8.1 Hz, 1H), 6.94 (dd, *J* = 2.6, 8.1 Hz, 1H), 3.91 (s, 3H), 2.87 (t, *J* = 7.7 Hz, 2H), 1.84 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{18}H_{19}N_2OS$, 311.1218; found, 311.1214.

2-Phenyl-4-*p*-tolylthiazole (12). Compound **12** (60%) was prepared by a method similar to that described for compound **7.** ^{1}H NMR (300 MHz, CD_3OD): δ 8.03-8.00 (m, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.70 (s, 1H), 7.51-7.44 (m, 3H), 7.24 (d, *J* = 8.0 Hz, 2H), 2.37 (s, 3H); *m*/*z* = 252 $[M+H]^+$. Anal. ($C_{16}H_{13}NS$) C, H, N, S.

4-(4'-Methylbiphenyl-4-yl)-2-propylpyridine (15). 4'-Methyl-4-biphenylboronic acid (106 mg, 0.5 mmol), tetrakis(triphenylphosphine)palladium(0) (57 mg, 0.05 mmol), and K_2CO_3 (276 mg, 2.0 mmol) were added to a solution of 4-bromo-2-propylpridine

(compound **14**, 100 mg, 0.5 mmol) in DMF (6 mL) and H_2O (2 mL). The mixture was degassed and stirred for 18 h under an N_2 atmosphere at 80 °C. After being cooled to room temperature, the reaction mixture was extracted with EtOAc, and the combined EtOAc layers were dried over Na_2SO_4, and concentrated. Chromatography of the crude product (SiO_2, 1:1 hexane: EtOAc) produced compound **15** (38 mg, 52%) as a white crystal. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (d, *J* = 5.2 Hz, 1H), 7.71 (s, 4H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.41 (d, *J* = 1.7 Hz, 1H), 7.37 (dd, *J* = 1.7, 5.2 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 2H), 2.85 (t, *J* = 7.7 Hz, 2H), 2.42 (s, 3H), 1.83 (m, 2H), 1.01 (t, *J* = 7.3 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{22}N$, 288.1752; found, 288.1748.

4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenol (16). A 2 N aqueous KOH solution (30 mL) was added to a solution of compound **6** (628 mg, 1.57 mmol) in THF (30 mL) and MeOH (90 mL). After stirring for 0.5 h at 60 °C, the reaction mixture was extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated to produce compound **16** (371 mg, 80%) as a yellow foam. ^{1}H NMR (300 MHz, CD_3OD): δ 8.53 (dd, *J* = 0.8, 5.2 Hz, 1H), 7.88 (m, 1H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.82 (dd, *J* = 1.7, 5.2 Hz, 1H), 7.77 (s, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 2.85 (t, *J* = 7.7 Hz, 2H), 1.80 (m, 2H), 1.01 (t, *J* = 7.3 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{17}H_{17}N_2OS$, 297.1062; found, 297.1055.

3-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenol (17). A 1.0 M solution of boron tribromide in CH_2Cl_2 (4 mL) was added dropwise to a stirred solution of compound **8** (730 mg, 2.35 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was slowly warmed to room temperature, then stirred for 14 h. The reaction mixture was poured into saturated $NaHCO_3$ solution, and extracted with EtOAc. The combined extracts were dried over Na_2SO_4, and

concentrated to produce compound **17** (113 mg, 16%) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 8.54 (dd, *J* = 0.8, 5.2 Hz, 1H), 7.92 (s, 1H), 7.89 (m, 1H), 7.83 (dd, *J* = 1.7, 7.8 Hz, 1H), 7.49-7.45 (m, 2H), 7.26 (t, *J* = 8.1 Hz, 1H), 6.80 (m, 1H), 2.85 (t, *J* = 7.5 Hz, 2H), 1.80 (m, 2H), 1.01 (t, *J* = 7.3 Hz, 3H); *m*/*z* = 297 $[M+H]^+$. Anal. ($C_{17}H_{16}N_2OS$) C, H, N, S.

2-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenol (18). A 1.0 M solution of boron tribromide in CH_2Cl_2 (2 mL) was added dropwise to a stirred solution of compound **9** (352 mg, 1.13 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was slowly warmed to room temperature, then stirred for 6 h. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with EtOAc. The combined extracts were dried over Na_2SO_4 and concentrated to produce compound **18** (135 mg, 40%) as a white foam. ^{1}H NMR (300 MHz, CD_3OD): δ 8.76 (d, *J* = 6.3 Hz, 1H), 8.56 (s, 1H), 8.52 (d, *J* = 1.7 Hz, 1H), 8.47 (dd, *J* = 1.7, 6.3 Hz, 1H), 8.19 (dd, *J* = 1.7, 8.1 Hz, 1H), 7.25 (dt, *J* = 1.7, 7.7 Hz, 1H), 6.99-6.93 (m, 2H), 3.01 (t, *J* = 7.8 Hz, 2H), 1.91 (m, 2H), 1.10 (t, *J* = 7.3 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{17}H_{17}N_2OS$, 297.1062; found, 297.1057.

***N*-Benzyl-4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)aniline (21).** Benzaldehyde (20 μl, 0.2 mmol) and acetic acid (60 μl, 1.0 mmol) were added to a stirred solution of compound **19** (60 mg, 0.2 mmol) in CH_2Cl_2 (0.5 mL). After stirring for 1 h, $Na(AcO)_3BH$ (133 mg, 0.6 mmol) was added to the reaction mixture and stirred for 20 h. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with EtOAc. The combined extracts were dried over Na_2SO_4 and concentrated. Chromatography of the crude product (SiO_2, 4:1 hexane: EtOAc) produced compound **21** (44 mg, 57%) as a white foam. ^{1}H NMR (300

MHz, $CDCl_3$): δ 8.60 (d, *J* = 5.2 Hz, 1H), 7.82 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 5.2 Hz, 1H), 7.39-7.33 (m, 5H), 7.32-7.27 (m, 1H), 6.69 (d, *J* = 8.8 Hz, 2H), 4.39 (s, 2H), 2.92 (t, *J* = 7.7 Hz, 2H), 1.85 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H); *m*/*z* = 386 $[M+H]^+$. Anal. ($C_{24}H_{23}N_3S$) C, H, N, S.

***N*-(Cyclopropylmethyl)-4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)aniline (22).** Cyclopropanecarboxaldehyde (15 μl, 0.2 mmol) and acetic acid (114 μl, 2.0 mmol) were added to a stirred solution of compound **19** (59 mg, 0.2 mmol) in CH_2Cl_2 (10 mL). After stirring for 1 h, $Na(AcO)_3BH$ (130 mg, 0.6 mmol) was added to the reaction mixture and stirred for 18 h. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with EtOAc. The combined extracts were dried over Na_2SO_4 and concentrated. Chromatography of the crude product (SiO_2, 5:1 hexane: EtOAc) produced compound **22** (36 mg, 52%) as a white foam. ^1H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, *J* = 5.2 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 2H), 7.76 (s, 1H), 7.69 (d, *J* = 5.2 Hz, 1H), 7.35 (s, 1H), 6.68 (d, *J* = 8.3 Hz, 2H), 3.03 (d, *J* = 6.9 Hz, 2H), 2.86 (t, *J* = 7.7 Hz, 2H), 1.83 (m, 2H), 1.12 (m, 1H), 1.02 (t, *J* = 7.3 Hz, 3H), 0.58 (m, 2H), 0.28 (m, 2H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{21}H_{24}N_3S$, 350.1691; found, 350.1687.

***N*-(4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenyl)acetamide (23).** Acetyl chloride (14 mg, 0.18 mmol) was added to a stirred solution of compound **19** (51.4 mg, 0.174 mmol) and pyridine (27 mg, 0.34 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After stirring for 0.5 h, the reaction mixture was poured into 2 M citric acid solution and extracted with EtOAc. The combined extracts were washed with saturated $NaHCO_3$ solution, washed with brine, dried over Na_2SO_4, and concentrated to produce compound **23** (8.5 mg, 14%) as a yellow foam.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J* = 5.1 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.76 (d, *J* = 1.5 Hz, 1H), 7.68 (dd, *J* = 1.5, 5.1 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.53 (s, 1H), 2.87 (t, *J* = 7.7 Hz, 2H), 2.22 (s, 3H), 1.84 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H); *m*/*z* = 338 $[M+H]^+$.

***N*-(4-(2-(2-Propylpyridin-4-yl)thiazol-4-yl)phenyl)thiophene-2-sulfonamide (25).** Pyridine (50 μL, 0.6 mmol) was added to a solution of compound **19** (59 mg, 0.2 mmol) in 2 mL of CH_2Cl_2 at 0 °C. Thiophene sulfonylchloride (37 mg, 0.2 mmol) at 0 °C was then added, and the reaction mixture was stirred for 48 h at room temperature. The reaction mixture was evaporated *in vacuo*, and the residue was crystallized in hexane. The product was washed with water and air dried to give compound **25** (71 mg, 80%) as brown-red solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.54 (dd, *J* = 0.7, 5.4 Hz, 1H), 7.93 (d, *J* = 8.9 Hz, 2H), 7.93 (s, 1H), 7.88 (m, 1H), 7.83 (dd, *J* = 1.8, 5.4 Hz, 1H), 7.71 (dd, *J* = 1.4, 5.0 Hz, 1H), 7.53 (dd, *J* = 1.4, 3.8 Hz, 1H), 7.24 (d, *J* = 8.9 Hz, 2H), 7.05 (dd, *J* = 3.8, 5.0 Hz, 1H), 2.85 (t, *J* = 7.7 Hz, 2H), 1.80 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H); *m*/*z* = 442 $[M+H]^+$. Anal. ($C_{21}H_{19}N_3O_2S_3$) C, H, N, S.

4-Methyl-*N*-(4-(2-(2-propylpyridin-4-yl)thiazol-4-yl)phenyl)benzenesulfonamide (26). Pyridine (50 μL, 0.6 mmol) was added to a solution of compound **19** (59 mg, 0.2 mmol) in 2 mL of CH_2Cl_2 at 0 °C. Tosyl chloride (38 mg, 0.2 mmol) at 0 °C was then added, and the reaction mixture was stirred for 72 h at room temperature. The reaction was quenched by adding 2 M citric acid solution, and the product was extracted with EtOAc. The EtOAc extract was washed with brine and dried over Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (SiO_2, 3:1 hexane: EtOAc) to give the compound **26** (61 mg, 68%) as yellow solid. ^{1}H NMR (300 MHz,

CD_3OD): δ 8.58 (d, *J* = 5.2 Hz, 1H), 8.00 (d, *J* = 1.7 Hz, 1H), 7.95 (s, 1H), 7.94 (dd, *J* = 1.7, 5.2 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.9 Hz, 2H), 2.89 (t, *J* = 7.7 Hz, 2H), 2.35 (s, 3H), 1.81 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H); HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{24}H_{24}N_3O_2S_2$, 450.1310; found, 450.1311.

Supplementary Biological Assay Methods

Luciferase reporter assay. On day 0, CHO-K1 cells were plated out onto a 96-well plate in medium A (a 1:1 mixture of Ham's F-12 medium and Dulbecco's modified Eagle's medium, with 5% fetal bovine serum, 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin sulfate). On day 2, the cells were transiently co-transfected with pSRE-Luc (an SRE-1-driven luciferase reporter construct) and pAc-β-gal (β-gal reporter, in which the expression of β-gal is controlled by an actin promoter), using Lipofectamine reagent (Invitrogen). After a 5 h incubation, the cells were washed with phosphate-buffered saline (PBS), then incubated, in the absence or presence of specific compounds, in medium B (a 1:1 mixture of Ham's F-12 medium and Dulbecco's modified Eagle's medium, with 5% lipid-depleted serum, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin sulfate, 50 μM compactin, and 50 μM sodium mevalonate). After a 20 h incubation, the cells in each well were lysed, and aliquots were used to measure luciferase and β-galactosidase activities. Luciferase activity was normalized using the activity of β-galactosidase.

Western blot analysis of SREBP processing. On day 0, CHO-K1 cells were plated out onto a 24-well plate of medium A. On day 2, the cells were pre-treated for 2 h with fatostatin or compound **24** in medium A, then incubated in medium B in the absence or

presence of fatostatin or compound **24**. After 6 h, the cells were washed once with cold PBS, then collected with SDS sample buffer and heated for 7 min at 95°C. The samples were separated on a 10% SDS-PAGE gel and blotted, using mouse monoclonal antibodies against SREBP-2 (IgG-7D4)[1]. The specific bands were visualized using enhanced chemiluminescent (ECL) detection reagents (Amersham).

Quantitative Real-Time PCR. Total RNA was isolated from DU145 cells with QIAshredder (Qiagen) and further isolated with an RNeasy Mini Kit (Qiagen). First-strand cDNA was synthesized from 3 μg of total RNA, using SuperScript II reverse transcriptase (Invitrogen) with oligo(dT) primers. Quantitative real-time PCR was performed using 7500 Fast and Fast SYBR Green PCR Master Mix (Applied Biosystems), according to the manufacturer's instructions. Each RNA sample was analyzed in duplicate, and relative RNA expression was normalized to endogenous glyceraldehyde-3-phosphate dehydrogenase, according to the Δ Δ Ct method. The primer pairs were: 5'-CAACGGCTCAGACGAGCAAG and 5'-AGTCACAGACGAACTGCCGAGA for LDL receptor (LDLR); 5'-CACTTGGGAGCCCTGTATGG and 5'-AGCCGAGCTTTGTAAGAGCG for stearoyl-CoA desaturase (SCD1); 5'-TGTAACAGAGCCAGGAACCC and 5'-CTGTACCCCAGTGGCTGTTT for ATP citrate lyase (ACL); 5'-ACAGGCTTGAATGAAGCTTTGCCC and 5'-GACATGCAGCCAAAGCAGCACATA for HMG-CoA reductase (HMGCR); 5'-ACCACGGGGACACCAAGGT and 5'-CCACACAGCAGCCACAAACTC for mevalonate pyrophosphate decarboxylase (MVD); and 5'-GGACGACAGTTAGCTATGGGTGTT and 5'- GAGTCATTTGTACAGTCAGCCCGA

for insulin induced gene 1 (INSIG1); 5'- GAGGGCTTCGTGGGACACATA and 5'-GCCACTGGGCATGGATCTTT for HMG-CoA synthase 1 (HMGCS1); 5'-CACTAACCACCTCGACAAGCAAC and 5'- GACAATTCTTCTTGGTCTCAGCTCC for isopentenyl-diphosphate delta isomerase 1 (IDI1); 5'-CCACTGTGGCTCCGGAAGAT and 5'-GGACTGCAAGGCACACAGCTT for Acetyl-CoA acetyltransferase 2 (ACAT2); 5'- CCTTGTGGCTGGCGTCAGAAA and 5'- CGAGGGCATTCAGATGGTGCT for mevalonate kinase (MVK); 5'-AATTATGGACAGGACTGAACGTCTTGCT and 5'-TCCAGCAGGTCAGCAAAGAATTTATAGC for hypoxanthine phosphoribosyltransferase 1 (HPRT1); 5'-CCTGGAGGAGAAGAGGAAAGAGA and 5'-TTGAGGACCTCTGTGTATTTGTCAA for ribosomal protein L13a (RPL13A); 5'-CTCCGTGGCCTTAGCTGTG and 5'-TTTGGAGTACGCTGGATAGCCT for Beta-2 microglobulin (B2M); 5'-TGGGCTACACTGAGCACCAG and 5'-CAGCGTCAAAGGTGGAGGAG for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Solubility at pH 3 and 7. Each compound stock solution in DMSO was diluted to a concentration of 250 μM in aqueous buffer at pH 3 or 7. After 24 h, the solution was filtered through a 0.22 μm membrane (MSGVN2250-Millipore), and concentration of the compound in the filtrate was determined using LC-MS/MS.

Metabolic stability in mouse hepatocytes. To determine the intrinsic clearance rate, each compound was incubated at 1 μM concentration and 37 °C in Leibovitz L-15 medium (Sigma) at a final volume of 0.75 mL with mouse hepatocytes (3×10^5 cells mL^{-1}).

Incubations were performed in duplicate in a 48-well plate with shaking. Sample handling was performed using an automatic liquid handling system (Multiprobe II EX, Packard). At 0, 10, 20, 30, 60, and 90 min, 50 μL aliquots of the incubates were removed, 80 μL of ice-cold acetonitrile and 20 μL of 1 μM warfarin in acetonitrile (internal standard) were added, and samples were centrifuged at 2000 rpm for 20 min. The supernatant was analyzed by LC-MS/MS. Negative controls were run with the compound incubated in only medium for 0 and 90 min; positive control incubations included 1 μM of 7-ethoxycoumarin and 30 μM of 7-hydroxycoumarin, to determine phase I and phase II activity of hepatocytes.

Permeability by passive diffusion (Parallel Artificial Membrane Permeability Assay, PAMPA). PAMPA experiments were performed in 96-well acceptor and donor plates, using 15% soy lecithin in *n*-dodecane artificial membranes. The acceptor plate (96-well hydrophobic filter plate, MAIP N45, Millipore) was prepared by adding 4 μL of artificial membrane material on the top of the filter, and the plate was filled with 250 μL of HEPES buffered Hanks' Balanced Salt Solution (HBSS, Gibco BRL). The donor plate (an indented 96-well plate MultiScreen, Millipore) was filled with 275 μL of HEPES buffered HBSS (pH 7.4) containing 10 μM of the compound, or 10 μM Warfarin as a reference compound. The acceptor plate was placed onto the donor plate to form a "sandwich" and was incubated for 4 h at 37 °C. The process was performed with a Multiprobe II (Perkin-Elmer). After the incubation period, the acceptor, donor, and initial donor solutions (reference) were analyzed via LC-MS/MS. The apparent permeability coefficient (Papp) and % retention in the membrane were calculated.

Animal study procedures. Five-week-old homozygous male obese (*ob/ob*) mice (C57BL/6J, The Jackson Laboratory, Bar Harbor, ME) were housed under controlled conditions (12-h light/dark cycle; 25 °C) in the Animal Care Center at Baylor College of Medicine. Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The animals were housed five per cage, and had *ad libitum* access to normal chow (Research Diets, Inc., New Brunswick, NJ) and water for 1 wk after their arrival. On day 1 of the experiment, the animals (10 per group) were fed normal chow (control diet) or chow that contained 200 mg/kg of analogue **24** (treated). These doses were estimated to provide approximately 0.7 mg analogue **24** per day (~23mg/kg body weight per day). Daily food intake and body weight were carefully monitored and recorded between 3:00 and 5:00 p.m. Serum constituents, and TG levels in livers were determined as we previously described.[2]

Reference

1. Yang, J.; Brown, M. S.; Ho, Y. K.; Goldstein, J. L. Three different rearrangements in a single intron truncate sterol regulatory element binding protein-2 and produce sterol-resistant phenotype in three cell lines. Role of introns in protein evolution. *J Biol Chem* **1995,** 270, 12152-12161.

2. Kamisuki, S.; Mao, Q.; Abu-Elheiga, L.; Gu, Z.; Kugimiya, A.; Kwon, Y.; Shinohara, T.; Kawazoe, Y.; Sato, S.; Asakura, K.; Choo, H. Y.; Sakai, J.; Wakil, S. J.;

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