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Novel spiropiperidine-based stearoyl-CoA desaturase-1 inhibitors: Identification of 1′-{6-[5-(pyridin-3-ylmethyl)-1,3,4-oxadiazol-2-yl]-pyridazin-3-yl}-5-(trifluoromethyl)-3,4-dihydrospiro[chromene-2,4′-piperidine]

Yoshikazu Uto [a,*], Yohei Kiyotsuka [a], Yuko Ueno [a], Yuriko Miyazawa [a], Hitoshi Kurata [a], Tsuneaki Ogata [b], Tsuneo Deguchi [c], Makiko Yamada [c], Nobuaki Watanabe [c], Masahiro Konishi [d], Nobuya Kurikawa [d], Toshiyuki Takagi [e], Satoko Wakimoto [d], Keita Kono [d], Jun Ohsumi [d]

[a] Medicinal Chemistry Research Laboratories I, Daiichi Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Global Project Management Department, Daiichi Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[c] Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[d] Biological Research Laboratories II, Daiichi Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[e] Clinical Development Department I, Daiichi Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

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ABSTRACT

Cyclization of the benzoylpiperidine in lead compound **2** generated a series of novel and highly potent spiropiperidine-based stearoyl-CoA desaturase (SCD)-1 inhibitors. Among them, 1′-{6-[5-(pyridin-3-ylmethyl)-1,3,4-oxadiazol-2-yl]pyridazin-3-yl}-5-(trifluoromethyl)-3,4-dihydrospiro[chromene-2,4′-piperidine] (**19**) demonstrated the most powerful inhibitory activity against SCD-1, not only in vitro but also in vivo (C57BL/6 J mice). With regard to the pharmacological evaluation, **19** showed powerful reduction of the desaturation index in the plasma of C57BL/6 J mice on a non-fat diet after a 7-day oral administration (q.d.) without causing notable abnormalities in the eyes or skin up to the highest dose (3 mg/kg) in our preliminary analysis.

As part of our research program to investigate novel treatments for metabolic disorders, we have recently reported the thiazole-based (**1**) and benzoylpiperidine-based (**2**) stearoyl-CoA desaturase-1 (SCD-1) inhibitors (Fig. 1).[1,2] SCD-1 is a rate-limiting enzyme in the synthesis of monounsaturated fatty acids from their saturated fatty acid precursors.[3,4] The products of SCD-1, oleic (C18:1 n-9) and palmitoleic acids (C16:1 n-7), are the most abundant fatty acids found in phospholipids, cholesterol esters, and triglycerides. While the detailed mechanism by which SCD-1 deficiency affects body weight and adiposity is not completely understood, inhibition of SCD-1 may represent a novel approach for the treatment of metabolic syndrome.[5,6]

In our continuing investigation of the novel SCD-1 inhibitors as a new treatment for metabolic disorders such as dyslipidemia, obesity and diabetes, we tried to improve the potency of benzoylpiperidine-based SCD-1 inhibitors such as **2**. We envisioned that simple cyclization of the benzoylpiperidine to form a spirocyclic system, such as in **3** (Fig. 2), would reduce the number of free rotatable bonds and make the entire molecule more rigid, potentially resulting in a more efficient interaction with the enzyme and possibly better pharmacokinetic (PK) profile. Herein, we would like to disclose our efforts to optimize the spiropiperidines in order to obtain more potent SCD-1 inhibitors.

The synthetic routes to the spiropiperidines (**3**–**21**)[7] are outlined in Schemes 1–8. As shown in Scheme 1, the spiropiperidine intermediates for **3** and **4** were synthesized[8] via piperidine formation at the α-carbon of the ketone (indanone (**24a**) or 3,4-dihydro-2*H*-naphthalen-1-one (**24b**)) with dibromide (**23**). The intermediate with a spiro[1-benzofuran-2,4′-piperidin]-3-one scaffold (**29**) was prepared in accord with the procedures known in literature.[9] The intermediates for the synthesis of **7** and related analogs were prepared[10] by way of condensation between various *o*-hydroxyacetophenones and *N*-protected-4-piperidones in the presence of pyrrolidine. Although some of the substituted *o*-hydroxyacetophenones were not commercially available, they were readily accessible as shown in Scheme 2. The pivotal step for the preparation of **31a**–**c** was the ortho-lithiation of the corresponding substrates with appropriate directing groups. The methyl substituted *o*-hydroxyacetophenone (**31d**) was prepared by a transformation from the ethyl ester. 2-Fluorophenol (**34**) was acetylated and subjected to the Fries rearrangement conditions to provide 3-fluoro-2-hydroxy analog (**31f**). After spirocyclization, subsequent manipulation (Scheme 3) was necessary for the appropriate functional group

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8563.
E-mail address: uto.yoshikazu.pw@daiichisankyo.co.jp (Y. Uto).

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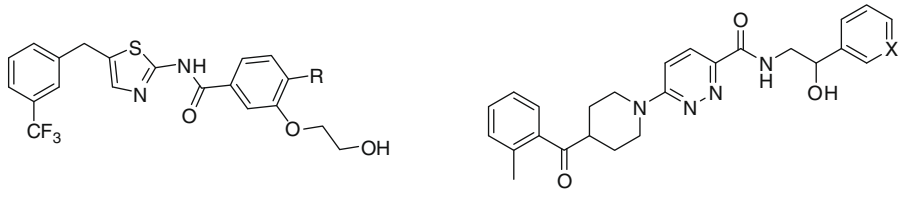


(1)
1a: R = OMe
1b: R = NHEt

(2)
2a: X = H
2b: X = N

Figure 1. Structures of previously reported small molecule SCD-1 inhibitors.

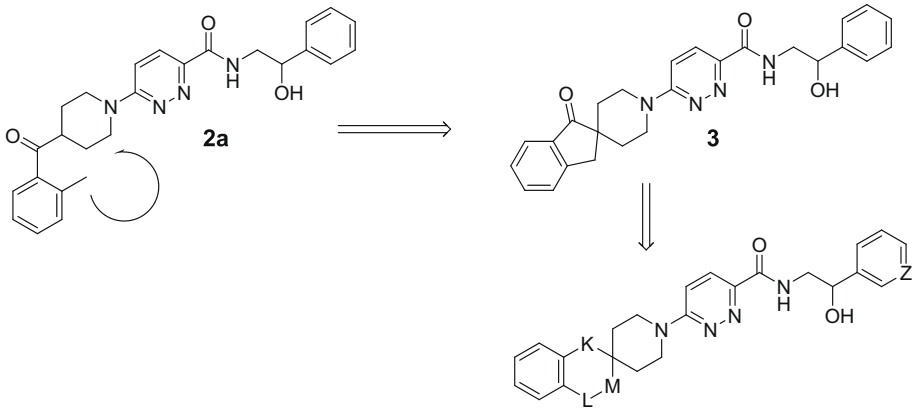
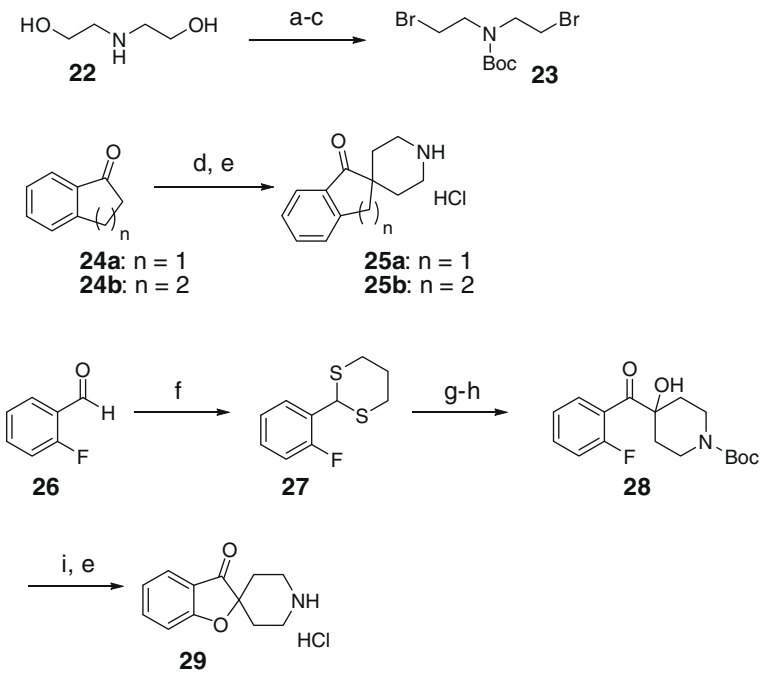
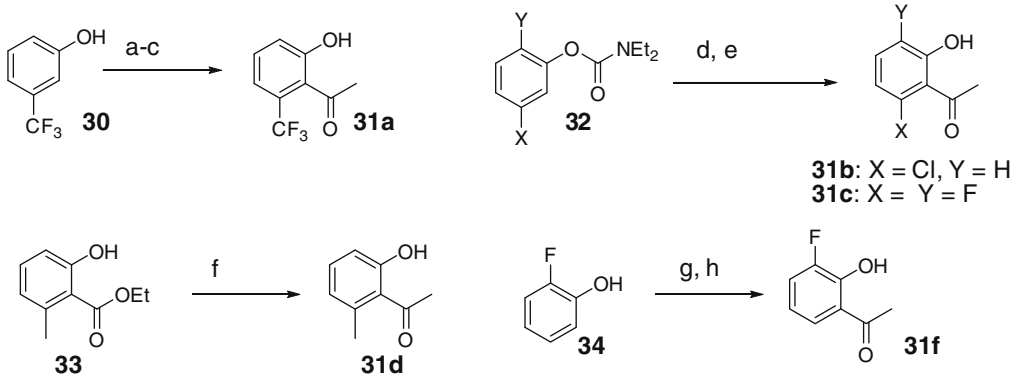


Figure 2. Transition from benzoylpiperidine to spiropiperidine.

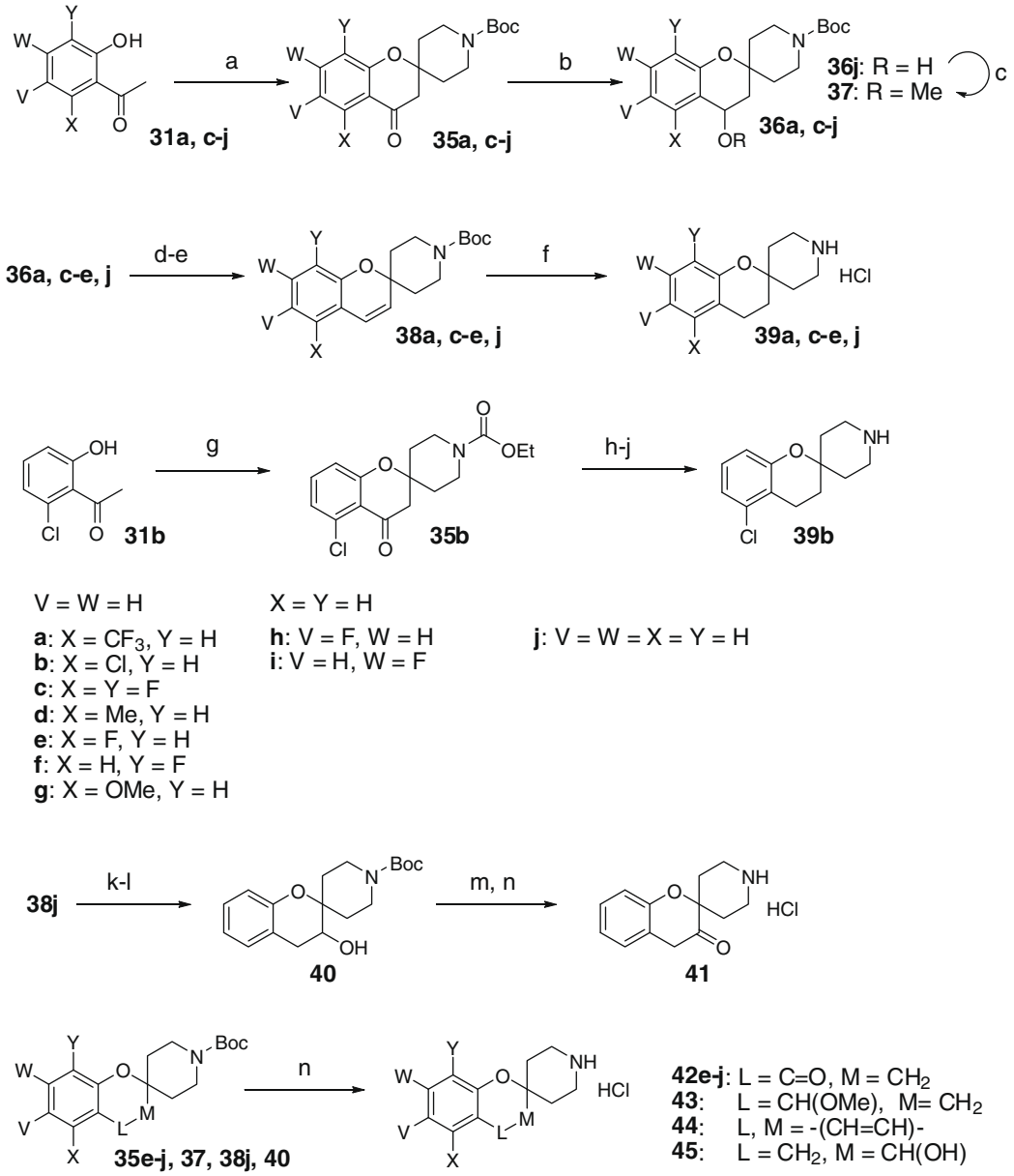


Scheme 1. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, rt, 96%; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, −78 °C; (c) LiBr, THF, rt (61%, two steps); (d) NaH, **23**, DMF, 50 °C, 30% from **24a** or 14% from **24b** (−25 °C to rt); (e) 4 N HCl in 1,4-dioxane, rt 94% for **25a** or 83% for **25b**; (f) 1,3-propanedithiol, I_2, $CHCl_3$, quantitative; (g) diisopropylamine, *n*-BuLi, −78 °C, then 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester, 93%; (h) pyridinium tribromide, tetrabutylammonium bromide, pyridine/CH_2Cl_2/H_2O, 84%; (i) *tert*-BuOK, 1,4-dioxane, reflux, 63%.

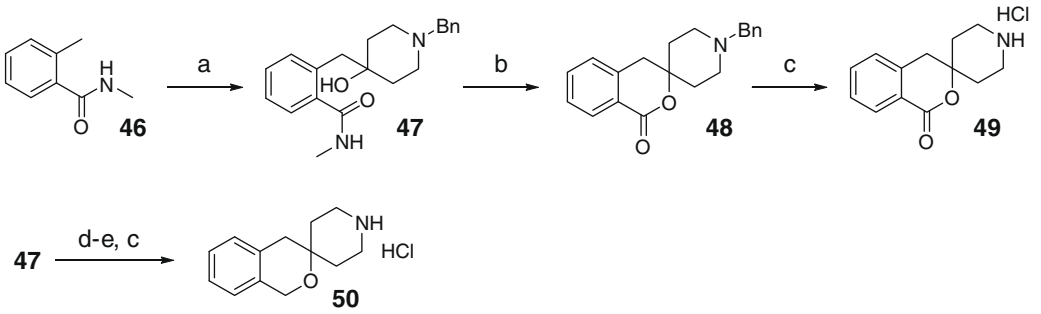
setting to provide the spiropiperidines (**39a–j**, **41–45**). The intermediates for **8** and **14** are shown in Scheme 4. The spiropiperidines (**49** and **50**) are prepared in accord with the procedures reported in literature.[11] Preparation of the chloropyridazines (**51**) for the final condensation step (Scheme 5) has already been disclosed in our previous publication.[2]



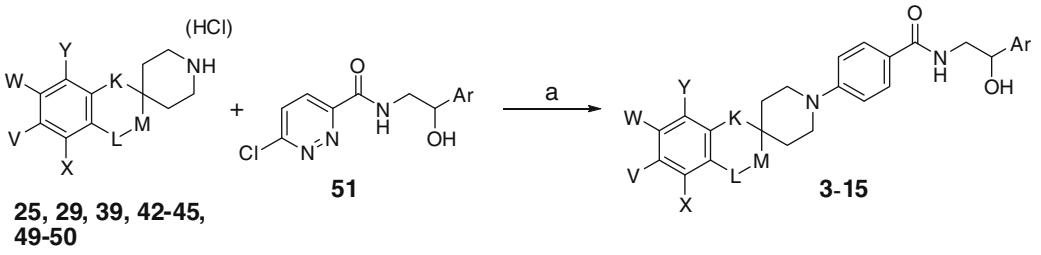
Scheme 2. Reagents and conditions: (a) *n*-BuLi, *tert*-BuOK, TMEDA, then dry ice, −78 °C–0 °C, 61%; (b) trimethylsilyldiazomethane, MeOH–EtOAc–hexane, 0 °C to rt, 93%; (c) MeMgBr, Et_3N, toluene, 0 °C to rt, 43%; (d) *n*-BuLi, THF, then Ac_2O, 41% (X = Y = F) or 37% (X = Cl, Y = H); (e) $TiCl_4$, CH_2Cl_2, 63% (**31**) or quantitative yield (**31c**); (f) MeMgBr, Et_3N, toluene, 0 °C to rt, 75%; (g) AcCl, pyridine, CH_2Cl_2, 82%; (h) $AlCl_3$, 1,2-dichlorobenzene.



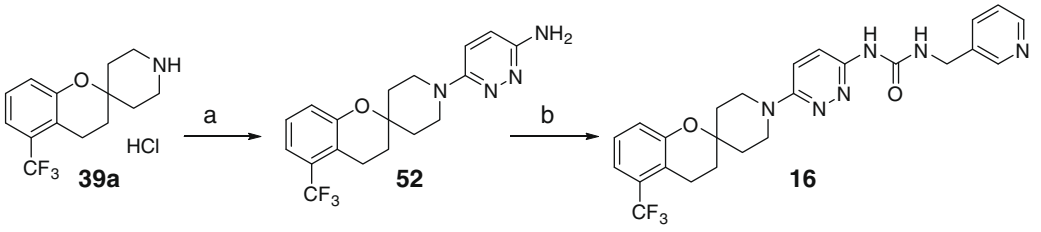
Scheme 3. Reagents and conditions: (a) *N*-Boc-4-piperidone, pyrrolidine, 1-PrOH or EtOH, rt–reflux; (b) $NaBH_4$, MeOH, rt; (c) NaH, MeI, DMA, rt, 55%; (d) MsCl, Et_3N, CH_2Cl_2; (e) DBU, NMP, 100 °C; (f) Pd/C (10wt%), H_2, 1 N HCl, (if necessary, acidic deprotection (4 N HCl, 1,4-dioxane) was followed.); (g) pyrrolidine, 4-oxo-piperidine-1-carboxylic acid ethyl ester, EtOH, rt, 34%; (h) $NaBH_4$, MeOH/THF, rt, 68%; (i) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 40 °C, 95%; (j) KOH, EtOH/H_2O, 100 °C, 95%; (k) *m*-CPBA, 0.5 N $NaHCO_3$(aq), CH_2Cl_2, 58%; (l) $LiAlH_4$, THF, 98%; Dess–Martin periodinane, CH_2Cl_2, 86%; (n) 4 N HCl, 1,4-dioxane, rt, 94% (**41**).



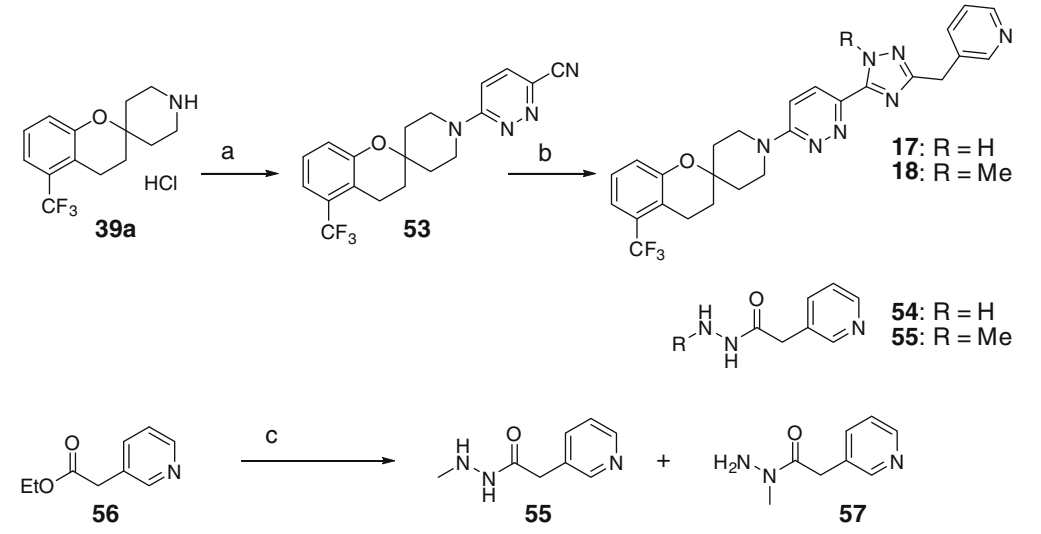
Scheme 4. Reagents and conditions: (a) *n*-BuLi, THF, 0 °C to rt, then 1-benzyl-piperidin-4-one, Et_2O, rt; (b) 50% AcOH, concd H_2SO_4, reflux, 12% (two steps); (c) H_2, Pd/C (10 wt %), 1 N HCl, EtOH, 38% (**49**) or quantitative (**50**); (d) $LiAlH_4$, THF, 0 °C; (e) H_3PO_4, 100 °C, 49% (two steps).



Scheme 5. Reagents and conditions: (a) Et_3N (or *i*PrNEt$_2$), *n*-BuOH, 100 °C.

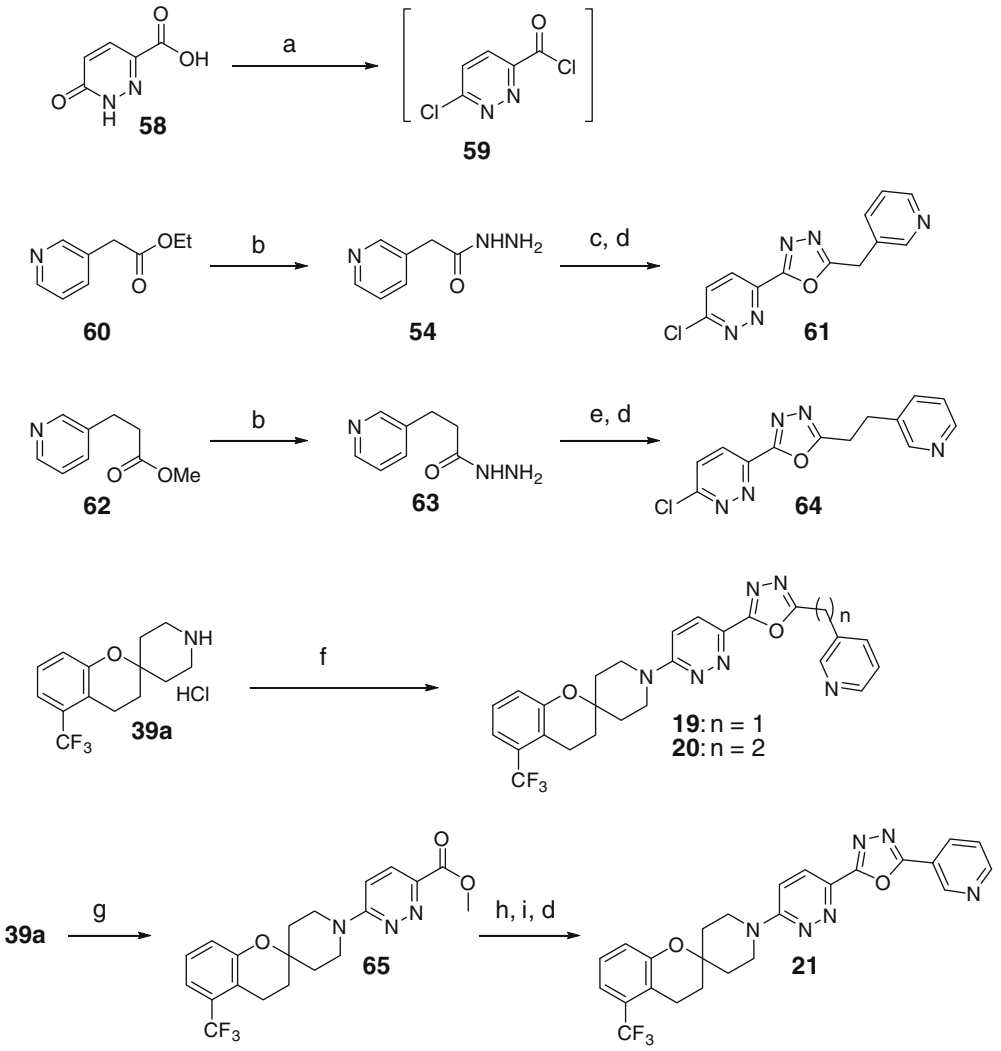


Scheme 6. Reagents and conditions: (a) 6-chloropyridazin-3-ylamine, Et_3N, *n*-BuOH, 120 °C, 68%; (b) 3-isocyanatomethylpyridine, CH_2Cl_2, reflux, 62%.



Scheme 7. Reagents and conditions: (a) 6-chloro-pyridazine-3-carbonitrile, Et_3N, NMP, 100 °C, 96%; (b) **54** or **55**, NaOMe, MeOH, rt to reflux, then AcOH, reflux: 80% for **17** and 23% for **18**; (c) methylhydrazine, EtOH, 80 °C, **55** (61%), **57** (13%).

Schemes 6–8 describe the synthetic routes for various right-hand amide modifications. For the urea analog (**16**, Scheme 6), the spiropiperidine (**39a**)[12] was condensed with 6-chloropyridazine-3-ylamine to provide **52** that was subsequently reacted with 3-isocyanatomethylpyridine to give the desired product. Scheme 7 outlines the synthesis of the triazole analogs (**17** and



Scheme 8. Reagents and conditions: (a) $SOCl_2$, DMF, $CHCl_3$; (b) hydrazine monohydrate, EtOH, quant; (c) **59**, Et_3N, DMF, 57%; (d) TsCl, DMAP, CH_3CN, 32% (**61**) or 71% (**64**); (e) 6-chloropyridazine-3-carboxylic acid, T3P, Et_3N, DMF, rt, 43%; (f) **61** or **64**, Et_3N, *n*-BuOH, 100 °C, 65% (**19**), 72% (**20**); (g) 6-chloropyridazine-3-carboxylic acid methyl ester, Et_3N, NMP, 100 °C, quantitative; (h) NaOH (aq), 1,4-dioxane, 60 °C, 92%; (i) BOP reagent, Et_3N, nicotinic acid hydrazide, quantitative.

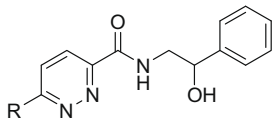
18). The nitrile derivative (**53**) was separately condensed with corresponding hydrazides to provide the desired triazoles (**17** from **54** and **18** from **55**). The hydrazide (**57**), which was obtained as a minor regioisomer in the preparation of **55**, failed to provide a corresponding triazole under the same reaction conditions. In order to synthesize the oxadiazole analog (**19**), the hydrazide (**54**) was acylated with **59** and readily cyclized in the presence of TsCl and Et_3N to provide the pyridazyloxadiazole (**61**)[12] that was coupled with the spiropipridine (**39a**) in the analogous conditions shown in Scheme 5.

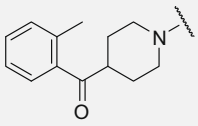
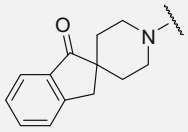
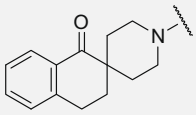
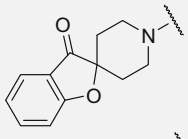
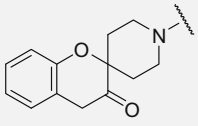
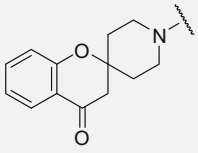
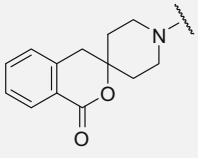
As shown in Table 1, simple cyclization of the benzoylpiperidine (**2a**) generated the five-membered spiro (**3**) that showed much weaker inhibitory activity[12] against human SCD-1. Ring expansion to the six-membered ring (**4**) regained inhibitory activity against human SCD-1 with sub-micro molar IC_{50}. Replacement of the methylene linkage in the spiro[indene-2,4′-piperidin]-1(3*H*)-one of **3** to the ether as shown in **5** did not improve the potency in SCD-1 inhibition. Insertion of a methylene between the phenyl and the carbonyl in the five-membered ring of **5** was unproductive as shown in **6** (IC_{50} (human) >10 μM). By contrast, insertion of a methylene between the piperidine and the carbonyl of **5** produced **7**, which showed reasonable potency, with IC_{50} values of 0.050 μM for murine SCD-1 and 0.25 μM for human enzyme. With the position of the *O*-linker of **7** being changed within the six-membered spirocyclic system, the lactone (**8**) exhibited sub-micro molar IC_{50} against human SCD-1. Among the three spiro-ring systems (**4**, **7**, and **8**) that presented comparable inhibitory activity against human SCD-1, **7** was chosen for further structural optimization because this spirocyclic system is much easier to prepare and more suitable for robust structural transformation.

Further optimization of the spirocyclic system of **7** is displayed in Table 2. Reduction of the ketone to hydroxyl as in **9j** resulted in a threefold improvement in the potency against murine SCD-1. The methoxy analog (**10**) exhibited much weaker inhibitory activity against murine SCD-1 while the compound showed comparable activity against human enzyme. Changing the position of the hydroxyl of **9j** to the adjacent carbon as shown in **11** or introducing the olefin as shown in **12** did not make significant improvements in terms of potency.

Significant improvement was actually provided, to our surprise, by simply reducing the olefin of **12**. Compound **13**, featured with a 3,4-dihydrospiro[chromene-2,4′-piperidine]-motif, presented more than a 20-fold increase in inhibitory activity against murine SCD-1 and a 100-fold increase in that against human enzyme.

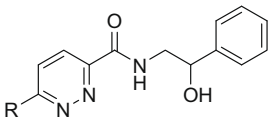
Table 1
Evaluation of the spiropiperidines[a]

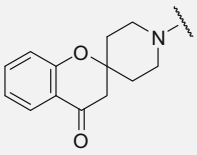
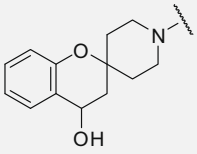
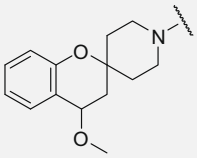
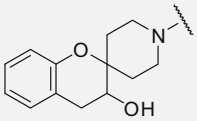
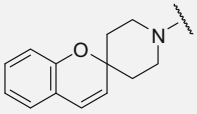
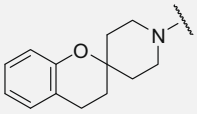
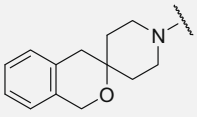


Compound	R	Enzymatic assay[b]	
		IC_{50} (μM) Mouse liver microsomal Δ9	IC_{50} (μM) Human cell (293A) microsomal Δ9
2a		0.007	0.015
3		NT[c]	2.0
4		0.020	0.57
5		>10	NT[c]
6		NT[c]	>10
7		0.050	0.25
8		0.23	0.32

[a] Values are the arithmetic mean of at least two experiments.
[b] See Supplementary data for the experimental procedures for the desaturase assay.
[c] NT = not tested.

Table 2
Evaluation of the spiropiperidines (**7** and related compounds)[a]



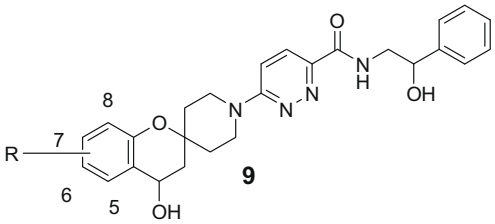
Compound	R	Enzymatic assay[b]		Cellular assay[b]
		IC_{50}[b] (μM) Mouse liver microsomal Δ9	IC_{50} (μM) Human cell (293A) microsomal Δ9	IC_{50} (μM) Human cell (293A) Δ9
7		0.050	0.25	0.69
9j		0.016	0.34	0.48
10		0.060	0.60	NT[c]
11		0.049	0.30	0.56
12		0.061	0.79	NT[c]
13		0.0027	0.007	0.068
14		0.076	>0.40[d]	NT[c]

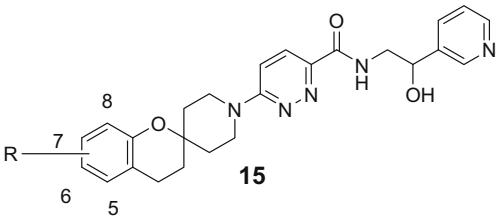
[a] Values are the arithmetic mean of at least two experiments.
[b] See Supplementary data for the experimental procedures for the desaturase assay and cellular assay.
[c] NT = not tested.
[d] 29% inhibition at 0.40 μM.

Compound **13** also showed strong potency in the cellular assay with an IC_{50} value of 0.068 μM. A similar analog, **14**, with the only difference from **13** being the position of the ether in the ring system, caused a significant decrease in inhibitory activity.

With the optimized spirocyclic system in hand, the substitution on the west side phenyl was investigated. For synthetic ease, the spirocyclic structure of **9** was utilized for various substitutions. As exemplified in Table 3, the available positions for substitution on the phenyl were numbered as 5, 6, 7 and 8. Fluorine was used in the search for a favorable substitution position because the corresponding starting materials were either commercially available (**9e**, **h**, **i**) or readily accessible by simple synthetic procedures (**9f**). While the enzymatic inhibitory activity of the 5- or 8-substituted compounds (**9e** or **9f**) were comparable to that of the unsubstituted **9j**, the 6- or 7-substituted ones (**9h** or **9i**) showed only marginal inhibitory activity. Between the 5- and 8-substitution, the 5-substituted **9e** was preferred over the 8-substituted **9f** because **9e** demonstrated three times better activity against human enzyme than that of **9f**. Even more, in terms of in vivo potency, as evaluated by inhibitory activity in the liver SCD-1 of db/db mice and described as ID_{50} (mg/kg),[12] **9e** presented a twofold increase in the in vivo potency, with an ID_{50} value of 7 mg/kg, compared to that of the unsubstituted **9j** (ID_{50} = 15 mg/kg). As exemplified by the results of **9g**, an electron-donating substituent such as methoxy is not ideal for strong SCD-1 inhibition; **9g** showed almost no inhibitory activity against human SCD-1 under the standard assay conditions. In the next round of optimization with the more potent spirosystem of **13**, the east side phenyl was replaced with 3-pyridyl because the 3-pyridyl substitution was proved to be more favorable for

Table 3
Evaluation of the substituents of the spiropiperidines[a]





Compound	R	Enzymatic assay[b]		Cellular assay[b]	db/db mice[b]
		IC_{50} (nM) Mouse liver microsomal Δ9	IC_{50} (nM) Human cell (293A) microsomal Δ9	IC_{50} (nM) Human cell (293A) Δ9	ID_{50} (mg/kg)
9j	—	16	338	481	15
9e	5-F	17	97	157	7
9h	6-F	NT[c]	>1000	NT[c]	NT[c]
9i	7-F	156	548	NT[c]	20
9f	8-F	33	315	NT[c]	NT[c]
9g	5-OMe	NT[c]	>1000	NT[c]	NT[c]
15e	5-F	6	12	7	2
15b	5-Cl	1	0.6	2	3
15d	5-Me	1	2	6	4
15a	5-CF_3	0.5	0.3	3	0.8
15c	5,8-Di-F	2	2	7	5

[a] Values are the arithmetic means of at least two experiments.
[b] See Supplementary data for the experimental procedures for the desaturase assay, cellular assay, and the determination of ID_{50}.
[c] NT = not tested.

in vivo potency in our previous investigation.[2] As showcased in Table 3, the halogens (5-F in **15e** and 5-Cl in **15b**), alkyl (5-Me in **15d**), and haloalkyl (5-CF_3 in **15a**)[12] exhibited very strong potency in all of the enzymatic (murine and human), cellular, and in vivo assay. As expected from the combined results of the mono-substitution, the 5,8-difluoro substitution (**15c**) also showed strong potency with an IC_{50} value of 7 nM in the cellular assay. Among the cluster of strong SCD-1 inhibitors, **15a**, the most potent compound, was chosen for further investigation.

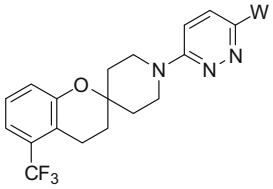
Table 4 summarizes the optimization of the right-hand moiety of **15a**, particularly the amide bond replacement. The hydroxyl group was removed in this series for synthetic simplicity and our prior studies[2] indicated that the inhibitory activity against SCD-1 was stronger without the hydroxyl. The first example, the urea (**16**) showed a 20-fold decrease in murine SCD-1 inhibition and more than a 30-fold decrease in the cellular assay. The triazole analog (**17**) demonstrated comparable inhibitory activity against both SCD-1s to **15a**. However, simple methylation of the nitrogen of the triazole (**18**) resulted in an evident loss of inhibitory activity, especially more than a >100-fold loss against murine SCD-1. The oxadiazole (**19**)[12] was most powerful in this series, presenting about a 10-fold increase in inhibitory activity against human SCD-1 (IC_{50} = 0.03 nM). The length of the linker between the oxadiazole and the terminal pyridine turned out to be critical since elongation of the linker to the ethylene (**20**) resulted in a significant loss of inhibition (>200-fold against murine SCD-1) and removal of the linker (**21**) resulted in a complete loss of inhibitory activity against SCD-1.

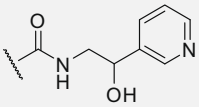
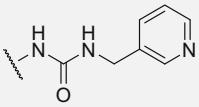
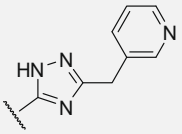
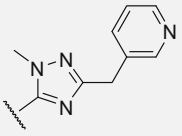
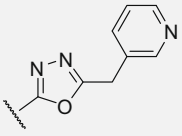
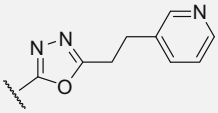
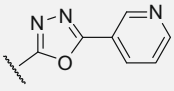
The representative spiropiperidines (**15a** and **19**) were evaluated for PK profiles in C57BL/6 J mice (Table 5). The plasma half-life of **15a** ($t_{1/2}$ = 0.9 h) was as short as that of lead compound **2**[2] and **15a** was quickly eliminated from plasma (Cl = 17 mL/min/kg). After oral administration, **15a** was readily absorbed and detected in the plasma through the tested time course with fair values of concentration (C_{max} = 3.5 μg/mL and AUC = 13 μg h/mL) and bioavailability (F = 67%). The iv data for the oxadiazole analog **19** was not taken at this point because we could make sure that the compound showed enough plasma exposure after oral administration (C_{max} = 2.6 μg/mL and AUC = 7.8 μg h/mL).

For the analysis of the in vivo efficacy of SCD-1 inhibitors, we took note that Attie and co-workers reported that the hepatic triglyceride levels of mice on a very low-fat diet increased by 240%.[13] We assumed that the SCD-1 activity in the liver of these mice was very high and were interested in the inhibitory effect of the spiropiperidine-based SCD-1 inhibitors against the liver SCD-1 in C57BL/6 J mice on a non-fat diet.[12,14] In comparison with the prior model (db/db mice on a chow diet), utilized in the evaluation of the compounds in Table 3, **15a** showed strong potency and an almost identical ID_{50} value (Table 6) in the C57BL/6 J on a non-fat diet at the same time-point (ID_{50} = 0.9 mg/kg at 2–3 h). Similarly, **19** demonstrated very strong potency in liver SCD-1 inhibition (ID_{50} (2–3 h) = 0.5 mg/kg) resulting from a combination of strong enzymatic inhibitory activity (IC_{50} (mouse) = 0.06 nM) and good oral exposure right after oral administration (C_{max} = 2.6 μg/mL and T_{max} = 1 h).

For multiple dosing studies of SCD-1 inhibitors, **19** was tested in a 7-day efficacy study using C57BL/6 J mice on a non-fat diet.[14] The desaturation index, calculated as the ratio of C18:1/C18:0, was used as an in vivo biomarker. After once-daily administration for seven days,[12] **19** dose-dependently reduced the desaturation index, with a 75% reduction at 3 mg/kg (Fig. 3). In the preliminary analysis, we did not observe notable abnormalities in the skin or eyes of the C57BL/6 J mice at 3 mg/kg (cutaneous abnormalities and narrow eye fissure have been reported in studies on SCD-1 deficient mice).[15] We assume that the balanced combination of the strong potency and short plasma half-life of **19** resulted in pharmacological efficacy in vivo and may be beneficial in preventing adverse events. While this is a preliminary speculation, the relatively short plasma half-life of **19** may help to accomplish favorable tissue selectivity (liver over eyes or skin). Currently, we do not know the long term effect of **19**. However, some abnormalities[15] may be observable with an extended administration period. Histopathological analysis of the key tissues (eyes, skin, and liver) of the C57BL/6 J mice after a 7-day treatment with the SCD-1 inhibitor **19** is currently in progress and will be reported elsewhere along with more details about the pharmacological studies of the spiropiperidine-based SCD-1 inhibitors.

Table 4
Evaluation of the spiropiperidines (right-hand structure)[a]



Compound	W	Enzymatic assay[b]		Cellular assay[b]
		IC_{50} (nM) Mouse liver microsomal Δ9	IC_{50} (nM) Human cell (293A) microsomal Δ9	IC_{50} (nM) Human cell (293A) Δ9
15d		0.45	0.28	3
16		10	2	106
17		1	0.13	4
18		65	4	38
19		0.06	0.03	0.8
20		16	2	35
21		>10,000	NT[c]	NT[c]

[a] Values are the arithmetic means of at least two experiments.
[b] See Supplementary data for the experimental procedures for the desaturase assay and the cellular assay.
[c] NT = not tested.

Table 5
PK parameters of **15a** and **19** in C57BL/6 J mice[a]

No	PK[b] (iv, 5 mg/kg)			PK[b] (po, 20 mg/kg)				*F* (%)
	$t_{1/2}$ (h)	Cl (mL/min/kg)	V_d (L/kg)	C_{max} (μg/mL)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{(0-8\ h)}$ (μg h/mL)	
15a	0.9	17	1.0	3.5	2.0	1.0	13	67
19	NT[c]	NT[c]	NT[c]	2.6	1.4	1.0	7.8	NA

[a] A dose of each compound was either intravenously (5 mg/kg, DMA/Tween80/saline = 10/10/80) injected into the tail vein of C57BL/6 J mice ($n = 2$) or orally (20 mg/kg, PG/Tween = 4/1, $n = 3$) administered using an intubation tube. Plasma samples (20 μL) were collected up to 8 h after intravenous or oral administration. The plasma concentrations of compounds were determined by LC/MS.
[b] Values are the arithmetic means of at least two experiments.
[c] Not tested.

In summary, cyclization of the benzoylpiperidine in lead compound **2** generated a series of spiropiperidines. Comprehensive optimization of the spiropiperidines led to a series of powerful SCD-1 inhibitors. Among them, 1′-{6-[5-(pyridin-3-ylmethyl)-1,3,4-oxadiazol-2-yl]pyridazin-3-yl}-5-(trifluoromethyl)-3,4-dihydrospiro[chromene-2,4′-piperidine] (**19**) demonstrated strong inhibitory activity against SCD-1 not only in vitro but also in vivo (C57BL/6 J mice). As for pharmacological evaluation, **19** showed powerful reduction of the desaturation index in the plasma of C57BL/6 J mice on a non-fat diet after a 7-day oral administration (q.d.) without causing notable abnormalities in the eyes or skin up to the highest dose (3 mg/kg). Further evaluation of the novel

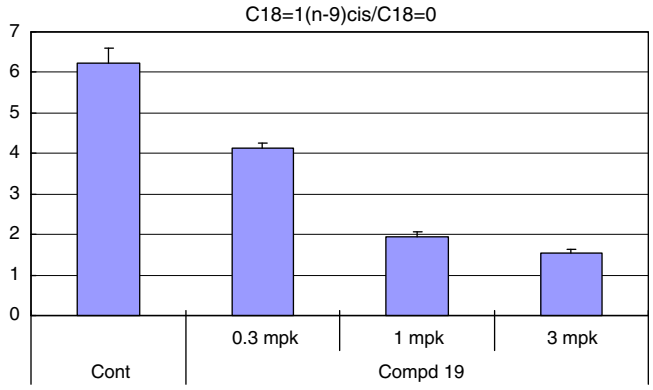


Figure 3. Plasma desaturation index lowering effect of the treatment with SCD-1 inhibitor **19** for 7 days (q.d.) in C57BL/6 J mice ($n = 4$) fed a non-fat diet.

Table 6
The liver SCD-1 inhibition by **15a** and **19** in C57BL/6 J mice on a non-fat diet[a]

Compound	ID_{50}[b] (mg/kg)	
	at 2–3 h[c]	at 6–7 h[c]
15a	0.9	2.5
19	0.5	0.8

[a] See Supplementary data for the experimental protocols.
[b] Values are the arithmetic means of at least two experiments.
[c] SCD-1 inhibitors were administered to the mice ($n = 2$) 2 h or 6 h prior to the administration of [^{14}C] stearate. One hour after the injection of [^{14}C] stearate, the mice were sacrificed and their livers were removed.

spiropiperidine-based SCD-1 inhibitors reported in this article will be disclosed in future.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.043.

References and notes

1. (a) Uto, Y.; Ogata, T.; Harada, J.; Kiyotsuka, Y.; Ueno, Y.; Miyazawa, Y.; Kurata, H.; Deguchi, T.; Watanabe, N.; Takagi, T.; Wakimoto, S.; Okuyama, R.; Abe, M.; Kurikawa, N.; Kawamura, S.; Yamato, M.; Ohsumi, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4151; (b) Uto, Y.; Ogata, T.; Kiyotsuka, Y.; Miyazawa, Y.; Ueno, Y.; Kurata, H.; Deguchi, T.; Yamada, M.; Watanabe, N.; Takagi, T.; Wakimoto, S.; Okuyama, R.; Konishi, M.; Kurikawa, N.; Kono, K.; Ohsumi, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4159.
2. Uto, Y.; Ogata, T.; Kiyotsuka, Y.; Miyazawa, Y.; Ueno, Y.; Kurata, H.; Deguchi, T.; Watanabe, N.; Konishi, M.; Okuyama, R.; Kurikawa, N.; Takagi, T.; Wakimoto, S.; Ohsumi, J. *Bioorg. Med. Chem. Lett.*, in press, doi:10.1016/j.bmcl.2009. 10. 101.
3. Dobrzyn, A.; Ntambi, J. M. *Obes. Rev.* **2005**, *6*, 169.
4. Ntambi, J. M.; Miyazaki, M. *Curr. Opin. Lipidol.* **2003**, *14*, 255.
5. Jiang, G.; Li, Z.; Liu, F.; Ellsworth, K.; Dallas-Yang, Q.; Wu, M.; Ronan, J.; Esau, C.; Murphy, C.; Szalkowski, D.; Bergeron, R.; Doebber, T.; Zhang, B. B. *J. Clin. Invest.* **2005**, *115*, 1030.
6. Gutierrez-Juarez, G.; Pocai, A.; Mulas, C.; Ono, H.; Bhanot, S.; Monia, B. P.; Rossetti, L. *J. Clin. Invest.* **2006**, *116*, 1686.
7. All the final compounds were characterized by ^{1}H NMR and mass spectroscopy.
8. Yang, L.; Morriello, G.; Prendergast, K.; Cheng, K.; Jacks, T.; Chan, W. W.-S.; Schleim, K. D.; Smith, R. G.; Patchett, A. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 107.
9. Wilson, R. A.; Chan, L.; Wood, R.; Brown, R. C. D. *Org. Biomol. Chem.* **2005**, *3*, 3228.
10. (a) Nerenberg, J. B.; Erb, J. M.; Bergman, J. M.; O'Malley, S.; Chang, R. S. L.; Scott, A. L.; Broten, T. P.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 291; (b) Fletcher, S. F.; Burkamp, F.; Blurton, P.; Cheng, S. K. F.; Clarkson, R.; O'Connor, D.; Spinks, D.; Tudge, M.; van Niel, M. B.; Patel, S.; Chapman, K.; Marwood, R.; Shepheard, S.; Bentley, G.; Cook, G. P.; Bristow, L. J.; Castro, J. L.; Hutsaon, P. H.; MacLeod, A. M. *J. Med. Chem.* **2002**, *45*, 492.
11. Butcher, J. W.; Claremon, D. A. *Synthesis* **1993**, 211.
12. For the experimental procedures, please see Supplementary data.
13. (a) Flowers, M. T.; Groen, A. K.; Oler, A. T.; Keller, M. P.; Choi, YJ.; Schueler, K. L.; Richards, O. C.; Lan, H.; Miyazaki, M.; Kuipers, F.; Kendziorski, C. M.; Ntambi, J. M.; Attie, A. E. *J. Lipid Res.* **2006**, *47*, 2668; The association of high-carbohydrate diets with high SCD activity was recently reviewed (b) Flowers, M. T.; Ntambi, J. M. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids* **2009**, *1791*, 85.
14. The non-fat diet was purchased from Research Diets, Inc. (D05052506). The diet is composed of protein (22.2 kcal%) and carbohydrate (77.8 kcal%).
15. (a) Miyazaki, M.; Man, W. C.; Ntambi, J. M. *J. Nutr.* **2001**, *131*, 2260; (b) Binczek, E.; Jenke, B.; Holtz, B.; Gunter, R. H.; Thevis, M.; Stoffel, W. *Biol. Chem.* **2007**, *388*, 405.