Bioorganic & Medicinal Chemistry Letters 19 (2009) 4151–4158

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel and potent inhibitors of stearoyl-CoA desaturase-1. Part I: Discovery of 3-(2-hydroxyethoxy)-4-methoxy-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide

Yoshikazu Uto [a,*], Tsuneaki Ogata [b], Jun Harada [c], Yohei Kiyotsuka [a], Yuko Ueno [a], Yuriko Miyazawa [a], Hitoshi Kurata [a], Tsuneo Deguchi [d], Nobuaki Watanabe [d], Toshiyuki Takagi [e], Satoko Wakimoto [f], Ryo Okuyama [f], Manabu Abe [f], Nobuya Kurikawa [f], Sayako Kawamura [g], Michiko Yamato [h], Jun Osumi [f]

[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] *Exploratory Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*
[d] *Drug Metabolism & Pharmacokinetics Research Laboratories, 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*
[f] *Biological Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[g] *Exploratory Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*
[h] *Biological Research Laboratories IV, Daiichi Sankyo Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*

ARTICLE INFO

Article history:
Received 17 March 2009
Revised 27 May 2009
Accepted 29 May 2009
Available online 2 June 2009

Keywords:
SCD1 inhibitors
Thiazole

ABSTRACT

A series of structurally novel stearoyl-CoA desaturase-1 (SCD-1) inhibitors has been identified by optimizing a hit from our corporate library. Preliminary structure–activity relationship (SAR) studies led to the discovery of the highly potent and orally bioavailable thiazole-based SCD-1 inhibitor, 3-(2-hydroxyethoxy)-4-methoxy-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide (**23a**).

Stearoyl-CoA desaturase-1 (SCD-1), a microsomal enzyme, is a rate-limiting enzyme in the synthesis of monounsaturated fatty acids from their saturated fatty acid precursors.[1,2] SCD-1, in conjunction with the cofactors NADPH, cytochrome b5 reductase, and cytochrome b5 introduces a double bond at the Δ9 position of the stearoyl (C18:0) and palmitoyl-CoA (C16:0).[1,2] The products of SCD-1, oleic (C18:1 N9) and palmitoleic acids (C16:1 N7), are the most abundant fatty acids found in phospholipids, cholesterol esters, and triglycerides. SCD-1, which is widely expressed, is predominantly located in the endoplasmic reticulum and one of four characterized SCD genes in mice.[3a] There are two isoforms of SCD in the human genome (1 and 5)[3b] and the human SCD-1 gene shows 85% homology to murine SCD-1.[4]

Numerous scientific experiments and reports suggest that SCD-1 plays a crucial role in lipid metabolism and body weight control. In adult mice, SCD-1 isoform is expressed in lipogenic tissues, including the liver, and in adipose tissue. Deficiency of SCD-1 has been shown to cause defective hepatic cholesterol ester and triglyceride synthesis,[5] resistance against obesity,[5] and reduced liver steatosis in rodents.[6] In humans, the higher desaturation index (the ratio of oleate to stearate or 18:1/18:0) is strongly correlated with higher plasma triglyceride levels.[7] Even though 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 syndromes.

Initially, Xenon Pharmaceuticals published the first example of small molecule-based SCD-1 inhibitors and Abbott Laboratories reported piperidine-based small molecule SCD-1 inhibitors (**1–4**, Fig. 1.).[8] By high-throughput screening of our corporate library, we identified our own lead compound (**5a**, Fig. 2), which has phenyl-*N*-(5-benzylthiazol-2-yl)acrylamide pharmacophore.[9] This lead compound exhibits very strong inhibitory activity against SCD-1 but has very poor pharmacokinetics. One of the major problems with compound **5a** is that it was not detected at all at any time point in the plasma of the C57BL/6J mice after oral administration (20 mg/kg, 0.5% MC). In this report, our current efforts to optimize the structures of SCD-1 inhibitors are disclosed. The main goal of our project at this stage is to improve the pharmacokinetic

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

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.119

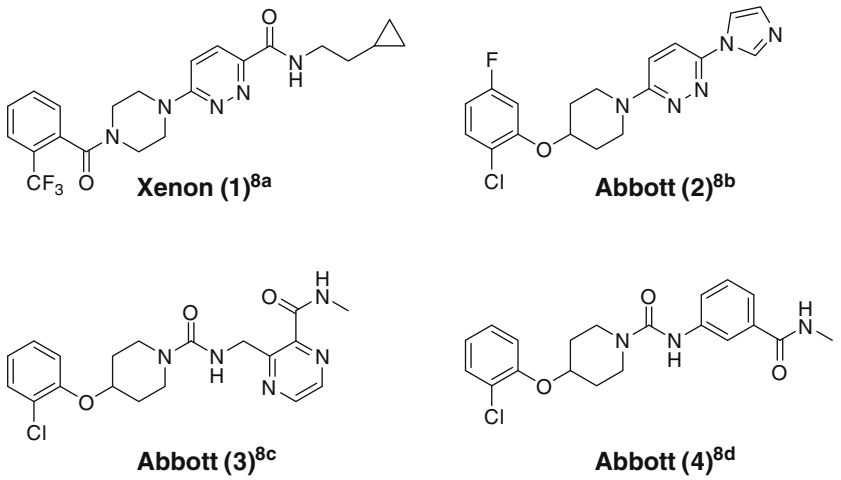


Figure 1. Structures of piperazine and piperidine-based small molecule SCD-1 inhibitors reported by Xenon and Abbott.

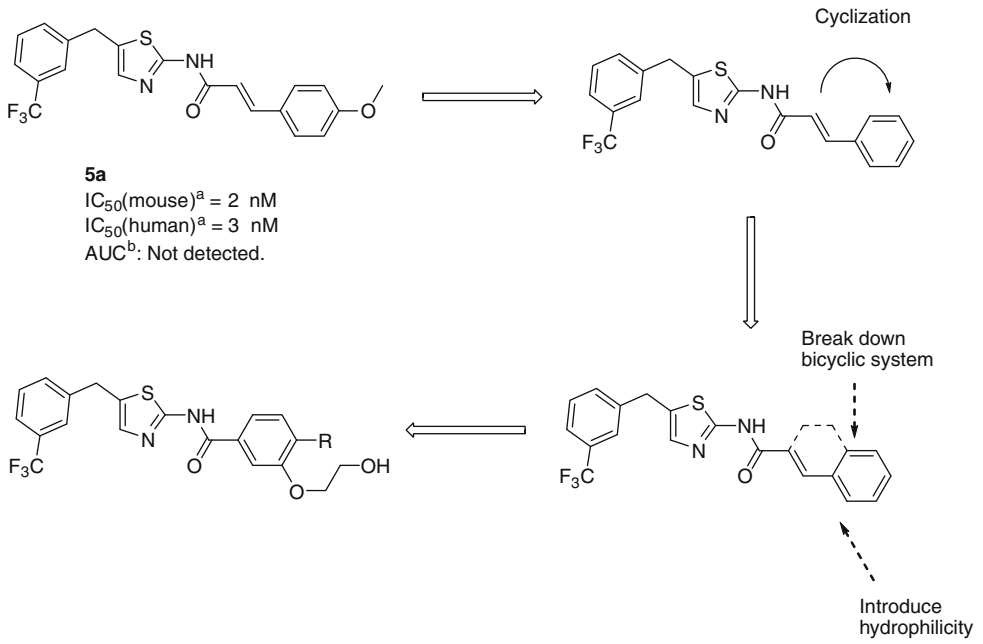


Figure 2. Structures of lead compound and strategies to improve pharmacokinetic profiles. [a] Values are the geometric means of at least two experiments. [b] A 20 mg/kg dose of each compound was administered to C57BL.6 J mice ($n = 3$) orally (0.5% MC) using an incubation tube. Plasma samples (20 μL) were collected up to 8 h after intravenous or oral administration. The plasma concentrations of compounds were determined by LC/MS.

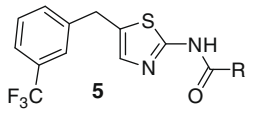
properties while retaining or improving the SCD-1 inhibitory activity of the lead compound.

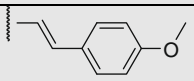
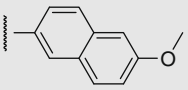
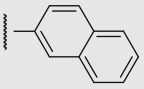
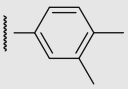
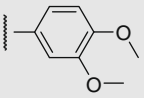
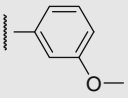
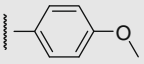
The strategies to optimize the structure of **5a** with an emphasis on improving the pharmacokinetic (PK) profiles are outlined in Figure 2. The acrylamide substructure, which is supposed to be labile for metabolism, was cyclized to form a bicyclic ring system. To make derivatization more efficient, we tried to mimic the bicyclic ring system by introducing appropriate substituents on a simple monocyclic ring system. In addition, we tried to put hydrophilic functional groups, such as hydroxy, at the appropriate positions to improve the PK profiles.

The representative synthetic routes for the compounds in Tables 1–4[10] are outlined in Schemes 1–4. Syntheses of the key intermediate aminothiazole are shown in Scheme 1. 5-(3-Trifluoromethylbenzyl)thiazol-2-ylamine (**7a**) was initially synthesized in accord with the protocols reported in the literature,[11] utilizing a Meerwein reaction of arenediazonium chloride prepared from **6** with acrolein. These procedures gave poor yields and it was difficult to provide the desired aminothiazole in a scalable or reproducible manner. The improved synthesis of aminothiazole was initiated by a palladium catalyzed arylation of allylic alcohol[12] with **8a**, which afforded 3-phenylpropionaldehyde derivative (**9a**) in 89% yield. The aldehyde was chlorinated by NCS[13] and then used for the condensation with thiourea after a simple work-up. The desired aminothiazole (**7a**) was efficiently prepared in 57% yield over two steps (Scheme 1). This protocol provided the key aminothiazole intermediate reproducibly.[14] The commercially available 4-methoxycinnamic acid (**10a**), 6-methoxy-2-naphthoic acid (**10b**), naphthalene-2-carbonyl chloride (**10c**), 3,4-dimethylbenzoic acid (**10d**), 3,4-dimethoxybenzoic acid (**10e**), 3-methoxybenzoic acid (**10f**), 4-methoxybenzoyl chloride (**10g**) were condensed with **7a** under standard coupling conditions to provide **5a–g**.

The synthetic procedures of 3,4-dialkoxybenzoic acids are summarized in Schemes 2 and 3. For example, the synthesis of **12**[15a–c]

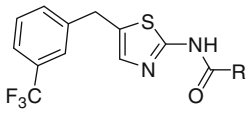
Table 1
Evaluation of different right-hand parts of **5**

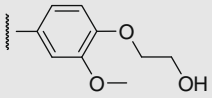
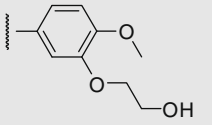
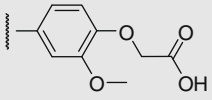
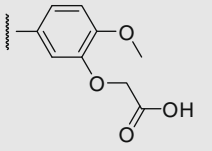
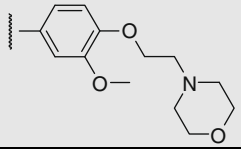


No.	R	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	Inhibition%[a] at 10 μM human Δ6
5a		2	3	21
5b		5	9	<5
5c		>10,000	NT[b]	NT[b]
5d		8218	NT[b]	NT[b]
5e		76	145	<5
5f		193	915	53
5g		NT[b]	1218	NT[b]

[a] Values are the geometric means of at least two experiments.
[b] NT = not tested.

Table 2
Introduction of hydrophilic functional groups in the right hand phenyl



No.	R	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	Inhibition % at 10 μM human Δ6
21		3	10	16
23a		2	3	15
26a		NT[b]	4377	<5
26b		>9999	NT[b]	NT[b]
22		528	1402	<5

[a] Values are the geometric means of at least two experiments.
[b] NT = not tested.

is outlined in Scheme 2. Alkylation of **11** with 2-bromoethanol, protection of the primary alcohol with THP, and saponification of the methyl ester provided a convenient method for the synthesis of **12**. The other related 3,4-dialkoxybenzoic acids (**13–15**, **17**, **18**, **20**) were prepared under slightly modified conditions or basic functional group manipulation. As shown in Scheme 4, condensation between **7** and the benzoic acids which were prepared in Schemes 2 and 3 was mediated with HATU. Acylation of the 2-aminothiazoles was very sluggish and gave modest to good yields at higher temperatures. The subsequent deprotection or manipulation of the ketone as in **28** provided the desired final products.[10]

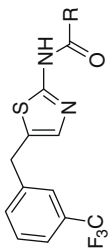
The results of the initial optimization of the right hand fragment are summarized in Table 1. The simple cyclization of cinnamic amide to the naphthyl ring (**5b**) retained strong SCD-1 inhibition in both mice (IC_{50} = 5 nM)[16] and humans (IC_{50} = 9 nM). The removal of methoxy resulted in a complete loss of activity (**5c**). At this point, we assumed that it might be more efficient and easier to regain SCD-1 inhibitory activity by optimizing the substitution patterns on a simple phenyl ring system than on the naphthyl or other bicyclic ring systems. Interestingly, a change of naphthalene to 3,4-dimethylphenyl (**5d**) regained weak SCD-1 inhibitory activity and the 3,4-dimethoxyphenyl (**5e**) showed improved SCD-1 inhibition. Simple mono-methoxy substitution at the 3- or 4-position was not enough to effect strong SCD-1 inhibition (**5f** and **5g**). We thought that the 3,4-dimethoxy substitution pattern was a good scaffold to introduce hydrophilic substituents on the phenyl.

The modification of the 3,4-alkoxy substituents is summarized in Table 2. Elongation of the methoxy to the hydroxyethoxy at the 4-position gave **21** and at the 3-position provided **23a**.[15] Both compounds showed very strong SCD-1 inhibition. An acidic functional group at the terminal of the 3-alkoxy or the 4-alkoxy, such as **26a** or **26b** substantially decreased SCD-1 inhibition. A basic functional group such as the morpholine in **22** was also very detrimental to SCD-1 inhibition. As for selectivity towards other desaturase species, all of the compounds tested in this report (except **5f**) exhibited very weak inhibitory activity, less than 50% inhibition at 10 μM, against Δ6 isozyme of the desaturase (Tables 1 and 2).

We then investigated the PK profiles of the 5-benzyl-thiazole derived SCD-1 inhibitors in mice. The selected compounds were orally administered to C57BL/6J mice (20 mg/kg) and the results are summarized in Table 3. The lead compound **5a** was rapidly cleared from the plasma after iv administration and was not detected at all at any time point after oral administration. The 3-methoxy-4-hydroxyethoxy-benzoyl derivative (**21**) showed modest plasma exposure (AUC = 0.15 μg h/mL, from 0 to 8 h) with marginal bioavailability. Remarkably, a change in the position of the methoxy and the hydroxyethoxy dramatically improved plasma exposure and **23a** demonstrated more than 50 times improvement in AUC (8.2 μg h/mL, from 0 to 8 h) with modest bioavailability.

Further investigation of the pivotal 3-hydroxyethoxy group is summarized in Table 4. Simple elongation of the hydroxyethoxy to the hydroxypropoxy (**24**) resulted in a >1000-fold decrease in

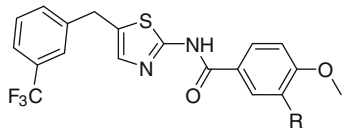
Table 3
PK parameters of selected compounds after oral administration to C57BL/6J mice (20 mg/kg)[a]

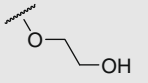
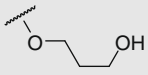
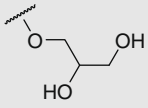
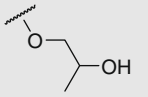
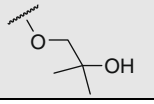


	R	$t_{1/2}$[b] (h)	PK profiles[a] (iv, 5 mg/kg)			PK profiles[a] (po, 20 mg/kg)				F (%)
			Cl[b] (mL/min/kg)	Vd[b] (L/kg)	$AUC_{(0\text{-}8\ h)}$[b] (μg h/mL)	C_{max}[b] (μg/mL)	$t_{1/2}$[b] (h)	T_{max}[b] (h)	$AUC_{(0\text{-}8\ h)}$[b] (μg h/mL)	
5a		0.4	32	0.30	2.7	ND[c]	ND[c]	ND[c]	ND[c]	–
21		1.4	3.4	0.42	24	0.07	1.3	0.5	0.15	0.2
23a		0.9	4.7	0.42	17	1.7	3.5	0.7	8.2	12

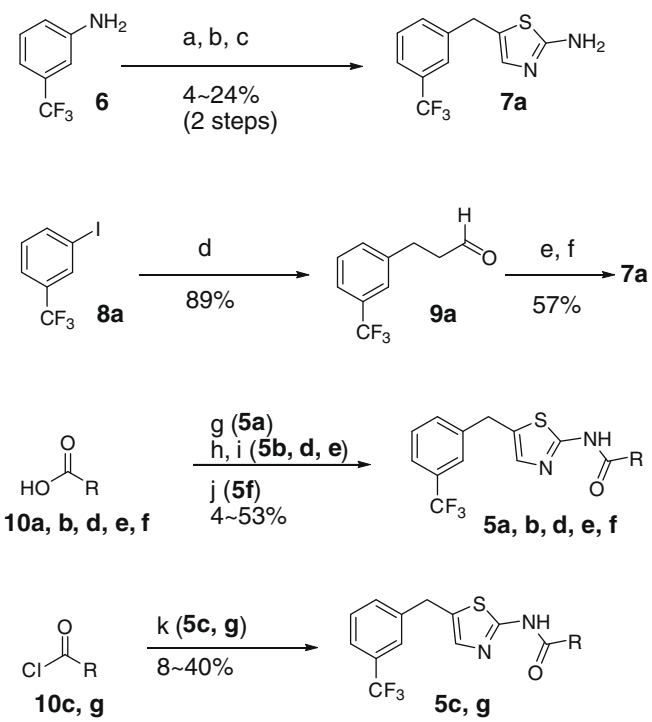
[a] A dose of each compound was either intravenously (5 mg/kg, DMA/Tween80/saline = 10/10/80) injected into the tail vein of C57BL/6 J mice ($n = 2$) or orally (20 mg/kg, 0.5% MC, n = 3) administered using an intubation tube. Plasma samples (20 μL) were collected up to 8 h after intravenous or oral administration. The plasma concentrations of the compounds were determined by LC/MS.
[b] Values are the geometric means of at least two experiments.
[c] Not detected.

Table 4
Modification of 3-hydroxyethoxy group



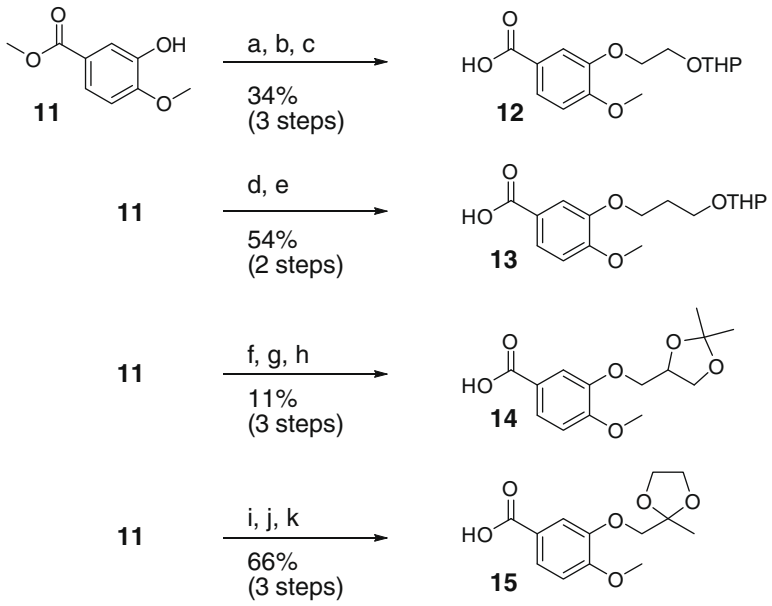
No.	R	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9
23a		2	3
24		NT[b]	>9999
25		NT[b]	>9999
28		2170	2162
29		>9999	NT[b]

[a] Values are the geometric means of at least two experiments.
[b] NT = not tested.

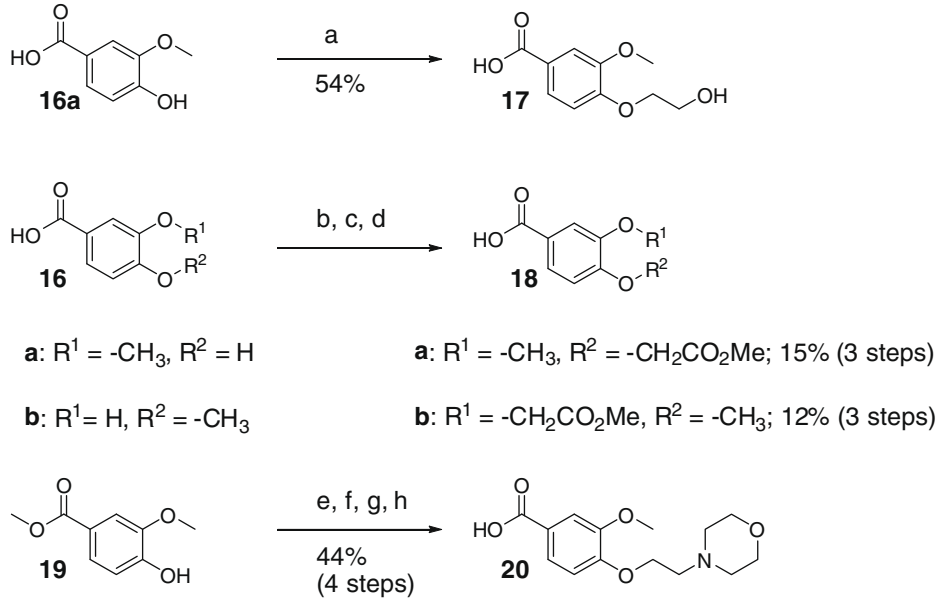


Scheme 1. Reagents and conditions: (a) concd HCl, $NaNO_2$; (b) MgO, $CuCl_2{\cdot}2H_2O$, acrolein/acetone, 0 °C; (c) thiourea, EtOH, reflux; (d) allyl alcohol, $Pd(OAC)_2$, TBACl, $NAHCO_3$, DMF, 50 °C; (e) NCS, proline, CH_2Cl_2, rt; (f) thiourea, EtOH, reflux; (g) **7a**, HATU, Et_3N, *N*,*N*-dimethylacetamide (DMA); (h) oxalyl chloride, DMF, CH_2Cl_2; (i) **7a**, Et_3N, CH_2Cl_2; (j) **7a**, HOBt, EDCl, DMA; (k) **7a**, Et_3N, CH_2Cl_2 (**5c**) or DMA (**5g**).

SCD-1 inhibitory activity. Replacement of the hydroxyethoxy with the 2,3-dihydroxypropoxy led to a complete loss of SCD-1 inhibition. Introduction of α-branch at the terminal hydroxy (**28**)



Scheme 2. Reagents and conditions: (a) 2-bromoethanol, K_2CO_3, DMA, reflux; (b) DHP, PPTS, CH_2Cl_2, rt; (c) 1 N NaOH, dioxane, 70 °C; (d) 2-(3-bromopropoxy)tetrahydro-2*H*-pyran; (e) 1 N NaOH, THF, 50 °C; (f) 3-bromo-1,2-propanediol, K_2CO_3, CH_3CN; (g) 2,2-dimethoxypropane, PPTS, DMF; (h) 1 N NaOH, dioxane; (i) bromoacetone, K_2CO_3, CH_3CN, reflux; (j) ethyleneglycol, TsOH, trimethylorthoformate, THF, reflux; (k) 1 N NaOH, THF, 50 °C.

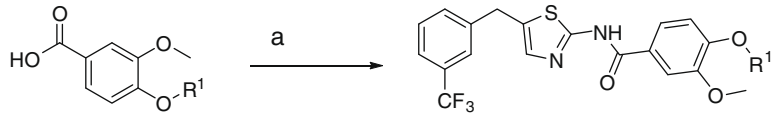


Scheme 3. Reagents and conditions: (a) 2-bromoethanol, KOH, EtOH/H_2O (2:1), 54%; (b) benzyl bromide, K_2CO_3; (c) methyl bromoacetate, K_2CO_3; (d) H_2, Pd/C; (e) 2-bromoethanol, KI, K_2CO_3, cyclohexanone, 0 °C to reflux; (f) MsCl, Et_3N, THF; (g) morpholine, K_2CO_3, CH_3CN, reflux; (h) 1 N NaOH, THF/MeOH.

also decreased SCD-1 inhibitory activity to a great extent and further decrease was observed in the dimethylated analog (**29**). These results persuasively indicate that the hydroxyethoxy is a crucial structural element for strong SCD-1 inhibition.

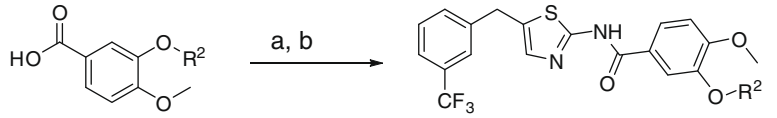
We then derivatized **23a** further to investigate the influence of substituents in the left-hand phenyl on SCD-1 inhibitory activity (Tables 5–7). The compounds (**23b**)–(**23n**) were prepared in accord with the protocols described for the synthesis of **23a**.[14,15] The yields for the synthesis of **7**, **9**, and **23** are summarized in Table 5. For the preparation of **7**, palladium catalyzed aldehyde synthesis and the subsequent 2-aminothiazole formation generally worked well for most of the substrates except for the 3,5-CF_3 disubstituted one (**7i**). As for the inhibitory activity, halogen or haloalkyl substitution in the 3(5)-position on the left-side phenyl ring was favorable. Alkyl substitution caused substantial decrease in SCD-1 inhibition (**23d** and **23n**). Haloalkoxy substitution was also tolerated (**23e**: IC_{50} (h) = 4 nM). Disubstitution with halogens or CF_3 on the 3, 4- or 3, 5-position also led to very strong SCD-1 inhibitory activity (**23g–23m**). Replacement of the left-side phenyl ring with an unsubstituted 3-pyridyl ring (structure not shown) resulted in a 1000-fold decrease (IC_{50} (hSCD-1) >3000 nM).

These compounds were further evaluated in a cell-based assay system using a transfected 293 cell line and stably expressing human SCD-1 (Table 6).[17] 3-CF_3-4-F Substitution resulted in one of the most active compounds (**23j**; IC_{50} = 8 nM). 3-Chloro



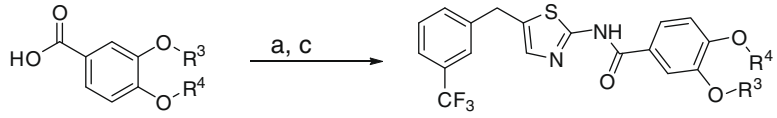
17: R^1 = -CH_2CH_2OH
20: R^1 = 2-Morpholin-4-yl-ethyl

21: R^1 = -CH_2CH_2OH; 19%
22: R^1 = 2-Morpholin-4-yl-ethyl; 28%



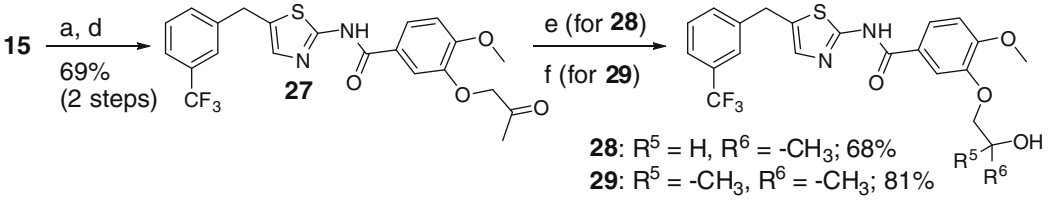
12: R^2 = -CH_2CH_2OTHP
13: R^2 = -$(CH_2)_3$-OTHP
14: R^2 = -(2,2-Dimethyl-[1,3]dioxolan-4-yl)-methyl

23a: R^2 = -CH_2CH_2OH; 66%
24 : R^2 = -$(CH_2)_3$-OH; 18%
25 : R^2 = -$CH_2CH(OH)CH_2OH$; 45%



16a: R^3 = -CH_3, R^4 = -CH_2CO_2Me
16b: R^3 = -CH_2CO_2Me, R^4 = -CH_3

26a: R^3 = -CH_3, R^4 = -CH_2CO_2H; 49%
26b: R^3 = -CH_2CO_2H, R^4 = -CH_3; 35%



28: R^5 = H, R^6 = -CH_3; 68%
29: R^5 = -CH_3, R^6 = -CH_3; 81%

Scheme 4. Reagents and conditions: (a) **7a**, HATU, Et_3N, DMA, rt to 70 °C; (b) 1 N HCl, MeOH; (c) 1 N NaOH, 1,4-dioxane/MeOH; (d) 1 N HCl, CH_3CN; (e) $NaBH_4$, MeOH; (f) MeMgBr, 1,4-dioxane.

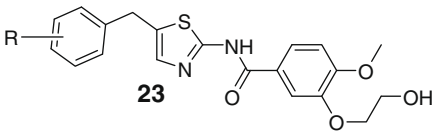
Table 5
The yields for the synthesis of **7**, **9**, and **23**

	R	**9** (yield%)	**7** (yield%, two steps)	**23** (yield%, two steps)
a	3-CF_3	89	57	66
b	3-F	91	43	43
c	3-Cl	Quantitative	50	49
d	3-*t*-Bu	98	53	45
e	3-OCF_3	95	33	37
f	2,5-Di-Cl	Quantitative	34	58
g	3,5-Di-Cl	55	26	34
h	3,5-Di-F	81	47	26
i	3,5-Di-CF_3	17	14	41
j	3-CF_3, 4-F	79	44	47
k	3-Cl-4-F	79	55	52
l	3,4-Di-F	83	55	40
m	3,4-Di-Cl	68	44	49
n	3-F, 4-Me	80	32	50

substitution displayed very strong activity (**23c**; IC_{50} = 9 nM) as well. Disubstitution with chloro and other halogens at the 3-, 4- or 5-position also provided prominently potent compounds (**23g, 23k**, and **23m**).

The inhibitory activity of these compounds against SCD-1 in vivo was determined by measuring the ratio of [^{14}C] stearate

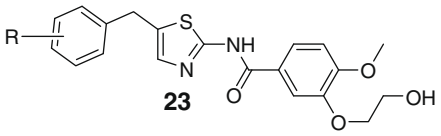
Table 6
SAR of the left-side phenyl ring of **23**



No.	R	IC_{50}[a] (nM) mouse	IC_{50}[a] (nM) human	Cellular IC_{50}[a] (nM) human 293A cell
23a	3-CF_3	2	3	15
23b	3-F	2	11	34
23c	3-Cl	1	4	9
23d	3-*t*-Bu	1307	NT[b]	NT[b]
23e	3-OCF_3	3	4	59
23f	2,5-Di-Cl	9	125	429
23g	3,5-Di-Cl	0.7	1	13
23h	3,5-Di-F	0.1	2.9	116
23i	3,5-Di-CF_3	0.6	0.2	26
23j	3-CF_3, 4-F	0.2	0.6	8
23k	3-Cl, 4-F	1	2	17
231	3,4-Di-F	2	9	34
23m	3,4-Di-Cl	0.8	1	13
23n	3-F, 4-Me	22	82	639

[a] Values are the geometric means of at least two experiments.
[b] NT = not tested.

Table 7
Summaries of SCD-1 inhibition, PK profiles[a], and SCD-1 inhibition in mice



23

No.	R	IC_{50}[b] (nM) mouse	C_{max}[b] (μg/mL)	AUC (0–8 h)[b] (μg h/mL)	$t_{1/2}$[b] (h)	T_{max}[b] (h)	F[b] (%)	ID_{50}[b] (mg/kg)
23a	3-CF_3	2	1.7	8.2	3.5	1.7	12	2
23c	3-Cl	1	1.8	10	5.4	2.0	12	1
23e	3-OCF_3	3	1.2	4.9	1.5	1.3	8	8
23j	3-CF_3, 4-F	0.2	0.55	2.0	1.3	1.3	3	5
23k	3-Cl, 4-F	1	3.6	14	2.1	1.0	38	2

[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, 0.5% MC, $n = 3$) administered using an intubation tube. Plasma samples (20 μL) were collected up to 8 h after intravenous or oral administration. The plasma concentrations of the compounds were determined by LC/MS.
[b] Values are the geometric means of at least two experiments.

and [^{14}C] oleate in the liver of db/db mice.[18] The dose at which 50% of the conversion is inhibited is described as ID_{50}. The results of this in vivo assay are summarized in Table 7 with enzymatic inhibitory activity and PK parameters. Most of the compounds showed very strong potency but **23e** and **23j** were relatively weak in terms of in vivo SCD-1 inhibition. It is hypothesized that the lower in vivo activity is correlated to the lower plasma concentration and shorter plasma half life of these compounds compared to **23a**. 3-Cl Substitution on the left-hand phenyl ring, as in **23c** and **23k**, demonstrated potency equal to that of **23a**. **23k** showed a shorter plasma half-life but an improvement in bioavailability. **23c** demonstrated very strong enzymatic murine SCD-1 inhibition, good plasma exposure, and a long plasma half-life, indicating that improved pharmacokinetic profiles contribute to the enhancement of the in vivo potency of these SCD-1 inhibitors.

In summary, we discovered the very potent and orally bioavailable SCD-1 inhibitor, 3-(2-hydroxyethoxy)-4-methoxy-*N*-[5-(3-trifluoromethylbenzyl)-thiazol-2-yl]benzamide (**23a**), by optimizing the structure of the lead compound **5a**, which was identified from our in-house corporate library. Modification of the left-hand phenyl of **23a** resulted in the identification of robust SCD-1 inhibitors such as **23c** and **23k**, which demonstrated powerful liver SCD-1 inhibition in db/db mice. Detailed pharmacology and further optimization of this structural motif will be reported in the following article.[19]

References and notes

1. Dobrzyn, A.; Ntambi, J. M. *Obesity Rev.* **2005**, *6*, 169.
2. Ntambi, J. M.; Miyazaki, M. *Curr. Opin. Lipidol.* **2003**, *14*, 255.
3. (a) Miyazaki, M.; Jacobsen, M. J.; Man, W. C.; Cohen, P.; Asilmaz, E.; Friedman, J. M.; Ntambi, J. M. *J. Biol. Chem.* **2003**, *278*, 33904; (b) Wang, J.; Yu, L.; Schmidt, R. E.; Su, C.; Huang, X.; Gould, K.; Cao, G. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 735.
4. Zhang, L.; Ge, L.; Parimoo, S.; Stenn, K.; Prouty, S. M. *Biochem. J.* **1999**, *340*, 255.
5. (a) Miyazaki, M.; Kim, Y.-C.; Gray-Keller, M. P.; Attie, A. D.; Ntambi, J. M. *J. Biol. Chem.* **2000**, *275*, 30132; (b) Flowers, M. T.; Ntambi, J. M. *Curr. Opin. Lipidol.* **2008**, *19*, 248.
6. Dobrzyn, P.; Dobrzyn, A. *Drug Development Res.* **2006**, *67*, 643.
7. Attie, A. D.; Krauss, R. M.; Gray-Keller, M. P.; Brownlie, A.; Miyazaki, M.; Kastelein, J. J.; Lusis, A. J.; Stalenhoef, A. F. H.; Stoehr, J. P.; Hayden, M. R.; Ntambi, J. M. *J. Lipid Res.* **2002**, *43*, 1899.
8. (a) For the typical structures of Xenon's SCD-1 inhibitors, see: Abreo, M.; Chafeev, M.; Chakka, N.; Chowdhury, S.; Fu, J.-M.; Gschwend, H. W.; Holladay, M. W.; Hou, D.; Kamboj, R.; Kodumuru, V.; Li, W.; Liu, S.: Raina, V.; Sun, S.; Sun, S.; Sviridov, S.; Tu, C.; Winther, M. D.; Zhang, Z. WO2005011655A2, February 10, 2005.; (b) Liu, G.; Lynch, J. K.; Freeman, J.; Liu, B.; Xin, Z.; Zhao, H.; Serby, M. D.; Kym, P. R.; Suhar, T. S.; Smith, H. T.; Cao, N.; Yang, R.; Janis, R. S.; Krauser, J. A.; Cepa, S. P.; Beno, D. W. A.; Sham, H. L.; Collins, C. A.; Surowy, T. K.; Camp, H. S. *J. Med. Chem.* **2007**, *50*, 3086; (c) Zhao, H.; Serby, M. D.; Smith, H. T.; Cao, N.; Suhar, T. S.; Surowy, T. K.; Camp, H. S.; Collins, C. A.; Sham, H. L.; Liu, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3388; (d) Xin, Z.; Zhao, H.; Serby, M. D.; Liu, B.; Liu, M.; Szczepankiewicz, B. G.; Nelson, L. T. J.; Smith, H. T.; Suhar, T. S.; Janis, R. S.; Cao, N.; Camp, H. S.; Collins, C. A.; Sham, H. L.; Surowy, T. K.; Liu, G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4298; Recently, a group from CV Therapeutics reported 2-oxo-2*H*-quinoxalin-based SCD-1 inhibitors: (e) Koltun, D. O.; Parkhill, E. Q.; Vasilevich, N. I.; Glushkov, A. I.; Zilbershtein, T. M.; Ivanov, A. V.; Cole, A. G.; Henderson, I.; Zautke, N. A.; Brunn, S. A.; Mollova, N.; Leung, K.; Chisholm, J. W.; Zablocki, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2048; (f) Koltun, D. O.; Vasilevich, N. I.; Parkhill, E. Q.; Glushkov, A. I.; Zilbershtein, T. M.; Mayboroda, E. I.; Boze, M. A.; Cole, A. G.; Henderson, I.; Zautke, N. A.; Brunn, S. A.; Chu, N.; Hao, J.; Mollova, N.; Leung, K.; Chisholm, J. W.; Zablocki, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3050.
9. During the preparation of this manuscript, a group at Dainippon Sumitomo Pharmaceutical reported 4-aminoimidazole based SCD-1 inhibitors, which were derived from a hit compound structurally similar to **5a** (Yamaguchi, H. The 27th Medicinal Chemistry Symposium, Osaka, Japan, November, 2008).
10. All the final compounds were characterized by ^{1}H NMR and mass spectroscopy.
11. Obushak, N. D.; Matiichuk, V. S.; Vasylyshin, R. Y.; Ostapyuk, Y. V. *Russ. J. Org. Chem.* **2004**, *40*, 383.
12. Jeffery, T. *Tetrahedron Lett.* **1991**, *32*, 2121.
13. Halland, N. *J. Am. Chem. Soc.* **2004**, *126*, 4790.
14. (a) *The aminothiazole intermediate* (**7a**) *was prepared as follows*: 3-(3-Trifluoromethylphenyl)propionaldehyde (**9a**). To a solution of 3-iodobenzotrifluoride (**8a**, 10.8 g) in DMF (150 mL) were added allyl alcohol (4.1 mL), tetrabutylammonium chloride (16.6 g), sodium bicarbonate (8.34 g), and palladium acetate (485 mg) at 0 °C. The reaction mixture was warmed to room temperature, heated at 50 °C for 2 h, diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed on SiO_2 (hexanes/EtOAc, 10:1–4:1) to give 7.17 g (89%) of **9a** as a pale yellow oil: ^{1}H NMR(400 MHz,$CDCl_3$):δ 9.84(1H, s), 7.49–7.39(4H, m), 3.02(2H, t, $J = 7.4$ Hz), 2.83(2H, t, $J = 7.6$ Hz).; (b) *5-(3-Trifluoromethylbenzyl)thiazol-2-ylamine* (**7a**). To a solution of **9a** (14.0 g) in CH_2Cl_2 (150 mL) were added L-proline (1.6 g) and *N*-chlorosuccinimide (12.1 g) at 0 °C. The reaction mixture was warmed to room temperature, stirred for 1.5 h, and diluted with hexane (300 mL). The resulting suspension was vigorously stirred, and filtered. The filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue (17.4 g) was mixed with thiourea (5.28 g) in EtOH (200 mL). The mixture was heated to reflux for 17 h, concentrated, diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, and concentrated. Chromatography of the residue on SiO_2 (Chromatorex NH 100–200 mesh FUJISILYSIA CHEMICAL LTD, CH_2Cl_2/EtOAC 10:1–3:1) gave 10.2 g (57%) of **7a** as a beige solid: ^{1}H NMR (400 MHz,$CDCl_3$): δ 7.48–7.46(2H, m), 7.43–7.38(2H, m), 6.81(1H, s), 4.87(2H, br s), 4.01(2H, s); MS (ESI) *m/z*:259 $(M+H)^+$.
15. (a) *Compound* **23a** *was prepared as follows*: 3-(2-Hydroxyethoxy)-4-methoxybenzoic acid methyl ester. A suspension of 3-hydroxy-4-methoxybenzoic acid methyl ester (**11**, 27.8 g), 2-bromoethanol (27 mL), and K_2CO_3 (42.3 g) in DMA (280 mL) was heated at 80–100 °C for 8 h. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (hexanes/EtOAc 7:1–1:4) gave an impure product, which was triturated in hexanes/iPr$_2$O, collected by filtration, and dried in vacuo to give 23.7 g (69%) of 3-(2-hydroxyethoxy)-4-methoxybenzoic acid methyl ester as a pale yellow solid: MS (ESI) *m/z*:227 $(M+H)^+$; (b) *4-Methoxy-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid methyl ester*: A solution of 3-(2-hydroxyethoxy)-4-methoxybenzoic acid methyl ester (14.8 g), DHP (18 mL), and PPTS (1.64 g) in CH_2Cl_2 (150 mL) was stirred at room temperature for 32 h, concentrated, diluted with EtOAc, washed with water, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (hexanes/EtOAc) gave 15.1 g (74%) of 4-methoxy-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid methyl ester as a colorless oil: MS (FAB^+) *m/z*:333 $(M+Na)^+$; (c) *4-Methoxy-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid* (**12**). A solution of 4-methoxy-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid methyl ester (14.4 g) in 1 N NaOH (51 mL) and dioxane (140 mL) was heated at 70 °C. The organic solvent was removed by evaporation. The aqueous mixture was acidified with 10% citric acid (aq), and extracted with EtOAc. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (CH_2Cl_2/MeOH 1:0–10:1) gave an impure product, which was vigorously stirred in hexanes/iPr$_2$O (5:1) at 60 °C for 30 min, collected by filtration, and dried in vacuo to give 9.24 g (67%) of **12** as a white solid: MS (FAB^+) *m/z*: 319 $(M+Na)^+$; (d) *3-(2-Hydroxyethoxy)-4-methoxy-N-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide* (**23a**): A solution of 5-(3-trifluoromethylbenzyl)thiazol-2-ylamine (**7a**, 4.35 g), 4-methoxy-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid (**12**, 5.49 g), HATU (7.03 g), and Et_3N (4.7 mL) in DMA (90 mL) was stirred at room temperature for 4 days, and heated at 70 °C for 3 h. The reaction mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (CH_2Cl_2/MeOH) gave 9.26 g of the amide. The amide (9.26 g) was mixed with 1 N HCl (17 mL) and MeOH (90 mL), and heated at 50 °C for 2 h. The reaction mixture was neutralized with 2 N NaOH, and the organic solvent was removed by

evaporation. The aqueous mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 ($CHCl_3$/MeOH, 1:0–30:1) gave an impure product, which was washed with CH_2Cl_2, and recrystallized (2-PrOH) to give 5.04 g (66%, over two steps) of **23a** as a white solid: ^{1}H NMR (400 MHz,DMSO-d_6): δ 12.4(1H, br s), 7.73–7.71(3H, m), 7.64–7.57(3H, m), 7.38(1H, s), 7.10(1H, d, J = 9.0 Hz), 4.88(1H, br s), 4.25(2H, s), 4.08(2H, t, J = 5.2 Hz), 3.85(3H, s), 3.76(2H, t, J = 4.9 Hz); MS (ESI) m/z:453 $(M+H)^+$.

16. Desaturase enzymatic assay:
The SCD-1 activity was determined by measuring the conversion of stearate to oleate. In each reaction tube, test compounds were preincubated with 10 μL microsomes for 10 min at room temperature. The SCD-1 reaction was started by the addition of 40 μL of a mixture containing 250 mM sucrose, 150 mM KCl, 40 mM NaF, 5 mM $MgCl_2$, 100 mM sodium phosphate, pH 7.4, 1 mM ATP, 1.5 mM reduced glutathione, 0.06 mM reduced coenzyme A, 0.33 mM nicotinamide, 1.25 mM NADH and 0.01 μCi [^{14}C] stearate. After 60 min incubation at 37 °C, the reaction was stopped by adding 50 μL methanol containing 10% KOH and then the mixture was saponified at 80 °C for 30 min. The free fatty acids in the reaction were protonated by the addition of 5 N HCl (15 μL) and extracted with 100 μL ethyl acetate. 30 μL of the ethyl acetate extracts of each reaction was charged to an $AgNO_3$-TLC plate (20 × 20 cm LK5D plates, 150 Å pore diameter, 250 μm thick) and differentiated in a solvent consisting of chloroform: methanol: acetate: water (90:8:1:0.8). [^{14}C] stearate and [^{14}C] oleate were quantified with BAS2500 (Fujifilm) and SCD-1 activity was determined as the ratio of [^{14}C] oleate to [^{14}C] stearate. The IC_{50} values were calculated by linear regression using the straight line portions of the concentration–response curve. To measure the delta-6 desaturase activity, [^{14}C] linolenic acid was used as the substrate and the delta-6 desaturase activity was determined as the ratio of [^{14}C] C18:3$n-3$ to [^{14}C] C18:4$n-3$.

17. A 293A cell-based desaturase assay was performed in a 96-well plate. Human SCD-1 gene was cloned into the expression vector pCMV-script (Stratagene). 293A cells stably expressing human SCD-1 were obtained by transfecting the expression vector to 293 cells and selected with G418. 293A cells in 100 μL media (DMEM + 10% FBS) were seeded to each well of the 96-well plate and grown overnight to be confluent. The cells were preincubated with test compound in fresh media for 30 min, after which 10 μL media containing 0.1 μCi [^{14}C] stearate was added to each well and incubated for another 4 h. Then the cells in each well were washed with cold PBS and the cellular lipids were saponified directly by adding 100 μL of 5% KOH in methanol/H_2O (1:1). The samples were processed as described for the SCD-1 enzymatic assay to determine the SCD-1 activity by quantifying the ratio of [^{14}C] oleate to [^{14}C] stearate.
18. SCD-1 inhibitors were administered to 7-week-old db/db male mice by oral gavage 2 hours prior to the administration of [^{14}C] stearate. Then the mice were injected ip with 5 mL/kg of 20 μCi/mL [^{14}C] stearate solution in saline containing 2% BSA, resulting in a bolus amount of 100 μCi/kg. One hour after the injection of [^{14}C] stearate, the mice were sacrificed and their livers were removed and frozen quickly in liquid nitrogen. The livers were homogenized in 9× volume of cold PBS, and 250 μL of homogenate was mixed with equal volume of methanol containing 10% KOH. Then the samples were processed as described for the SCD-1 enzymatic assay to determine the SCD-1 activity by quantifing the ratio of [^{14}C] oleate to [^{14}C] stearate. The dose at which 50% of SCD-1 activity is inhibited is described as ID_{50}.
19. 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.; Osumi, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, in press.