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Novel and potent inhibitors of stearoyl-CoA desaturase-1. Part II: Identification of 4-ethylamino-3-(2-hydroxyethoxy)-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide and its biological evaluation

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ABSTRACT

The continuing investigation of SAR studies of 3-(2-hydroxyethoxy)-*N*-(5-benzylthiazol-2-yl)-benzamides as stearoyl-CoA desaturase-1 (SCD-1) inhibitors is reported. Our prior hit-to-lead effort resulted in the identification of **1a** as a potent and orally efficacious SCD-1 inhibitor. Further optimization of the structural motif resulted in the identification of 4-ethylamino-3-(2-hydroxyethoxy)-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide (**37c**) with sub nano molar IC_{50} in both murine and human SCD-1 inhibitory assays. This compound demonstrated a dose-dependent decrease in the plasma desaturation index in C57BL/6J mice on a non-fat diet after 7 days of oral administration.

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] In adult mice, SCD-1 isoform is expressed in lipogenic tissues including the liver and adipose tissue. Deficiency of SCD-1 has been shown to cause defective hepatic cholesterol ester and triglyceride synthesis,[3] resistance against obesity,[3] and reduced liver steatosis in rodents.[4] In humans, a higher desaturation index (the ratio of oleate to stearate or 18:1/18:0) is strongly correlated with higher plasma triglyceride levels.[5] 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. In the preceding article,[6] we reported optimization of the HTS hit compound to identify potent and orally bioavailable SCD-1 inhibitors, such as **1** (Fig. 1). The improvement in oral bioavailability that was accomplished in the course of the optimization was significant, however, more improvement in bioavailability is desirable to achieve more potency in pharmacological studies in vivo. In this article, we would like to report further exploration in SAR of the lead compound to improve bioavailability and in vivo potency. Our plans to modify **1** are summarized in Figure 1. Structurally, the hydroxyethoxy functional group in the 3-position of the right-hand phenyl is considered to be essential for both strong SCD-1 inhibitory

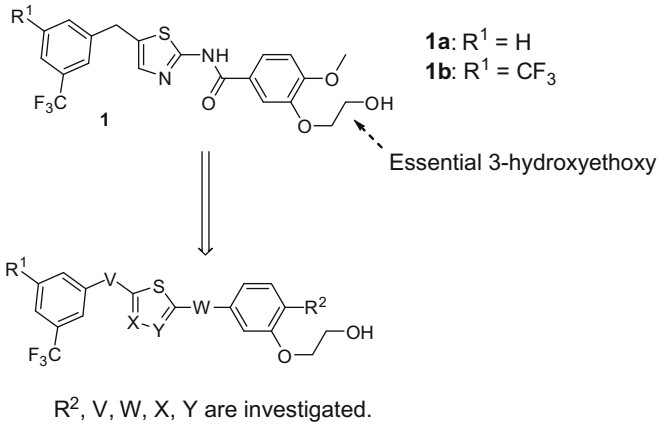

Figure 1. Plans for SAR studies of **1**.

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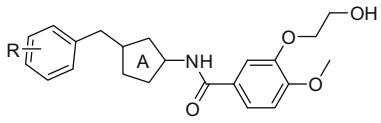
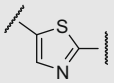
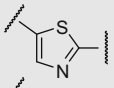
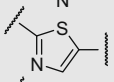
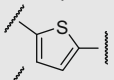
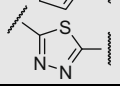
activity and good oral exposure,[6] whereas the methoxy on the 4-position is presumed to be modifiable (R^2 in Fig. 1). In regard to the central pharmacophore, we tried to replace the thiazole with other *S*-containing heteroaryls (X and Y in Fig. 1). The linkers between the thiazole core and the terminal phenyls on both ends (V and W in Fig. 1) were investigated as well. As for the substituents on the left-hand phenyl, we reported in the preceding manuscript[6] that halogens and haloalkyls were preferred on the 3-, 3,4- or 3,5-positions. We assumed it was not necessary to further investigate substitutions on the left hand phenyl at this point.

The synthetic routes for the compounds in Tables 1–3 are outlined in Schemes 1–3.[7] Condensation of the commercially available 2-(3,5-bis-trifluoromethylphenyl)thioacetamide (**2**) and 2-chloro-3-oxo-propionic acid ethyl ester (**3**)[8] efficiently provided the desired 2-benzylthiazole (**4**) in 97% yield. Saponification of the ethyl ester, Curtius rearrangement in *t*-BuOH, and deprotection of the *N*-Boc with TFA provided the 5-aminothaizole (**5**) in 38% yield over three steps. Coupling between **5** and the benzoic acid (**10**) in the presence of HATU was extremely sluggish and the desired product **11** was obtained in only 6% yield after THP deprotection (Scheme 1). For the synthesis of thiophene analogs, nucleophilic addition of 2-lithiothiophene to 3-trifluoromethylbenzaldehyde and reduction of the resulting alcohol in the presence of an excess amount of TMS–Cl and NaI[9] provided 2-(3-trifluoromethylbenzyl)thiophene (**6**) in 85% yield over two steps. Regioselective nitration by $Cu(NO_3)_2$ in acetic anhydride gave the desired nitrothiophene (**7**) in 83% yield. Reduction, condensation with **10**, and THP deprotection gave **12**. The thiadiazole analog (**13**)[10] was prepared from 5-(3-trifluoromethylbenzyl)-[1,3,4]thiadiazol-2-ylamine (**9**) and **10** in the analogous procedures utilized for the preparation of **11**.

For optimization of the V linkers (Fig. 1), the synthetic routes are outlined in Scheme 2. Lithium–halogen exchange on (5-bromothiazol-2-yl)-carbamic acid *tert*-butyl ester (**14**)[11] with *n*-BuLi, addition of 3-trifluoromethylbenzaldehyde, Dess–Martin oxidation, and acidic deprotection of the *N*-Boc group gave **15** in 42% yield over three steps. The aminothiazole (**15**) was coupled with **10** and subsequent THP deprotection provided the 5-benzoylthiazole (**16**) in 54% yield over two steps. The ketone in **16** was reduced with $NaBH_4$ to produce the thiazole–phenyl–methanol analog (**17**).

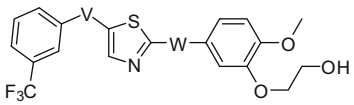
Table 1
Evaluation of heteroaryl cores

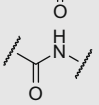


No.	R	A	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	Inhibition %[a] at 10 μM human Δ6
1a	3-CF_3		2	3	15
1b	3,5-Di-CF_3		0.6	0.3	NT[b]
11	3,5-Di-CF_3		44	32	22
12	3-CF_3		76	145	22
13	3-CF_3		10	8	<5

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

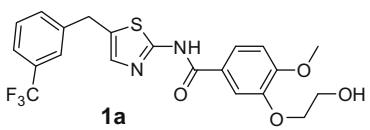
Table 3
Evaluation of linkers to the thiazole core (V and W)

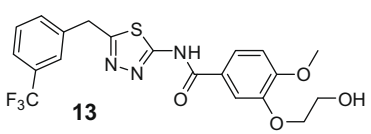


No.	V	W	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	Inhibition %[a] at 10 μM human Δ6
1a			2	3	22
16			>1000	NT[b]	NT[b]
17			276	884	8
20			>1000	>1000	NT[b]
24			836	828	16
29			49	32	<5

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

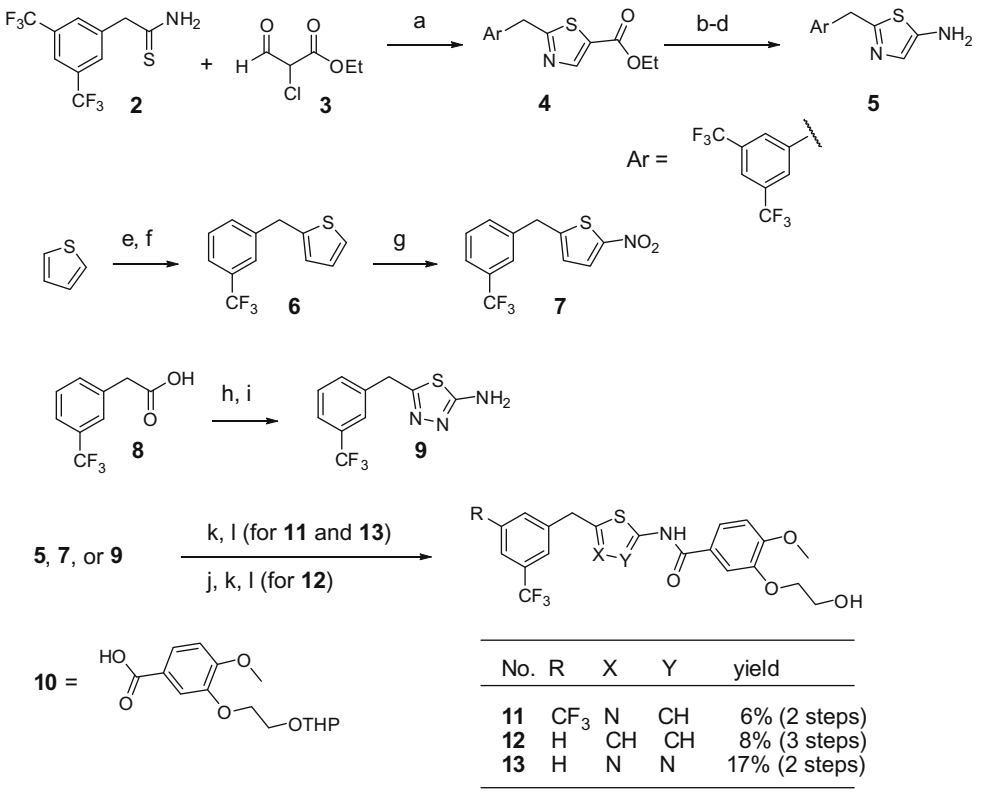
Table 2
Summaries of PK profiles[a] in C57BL/6J mice


1a


13

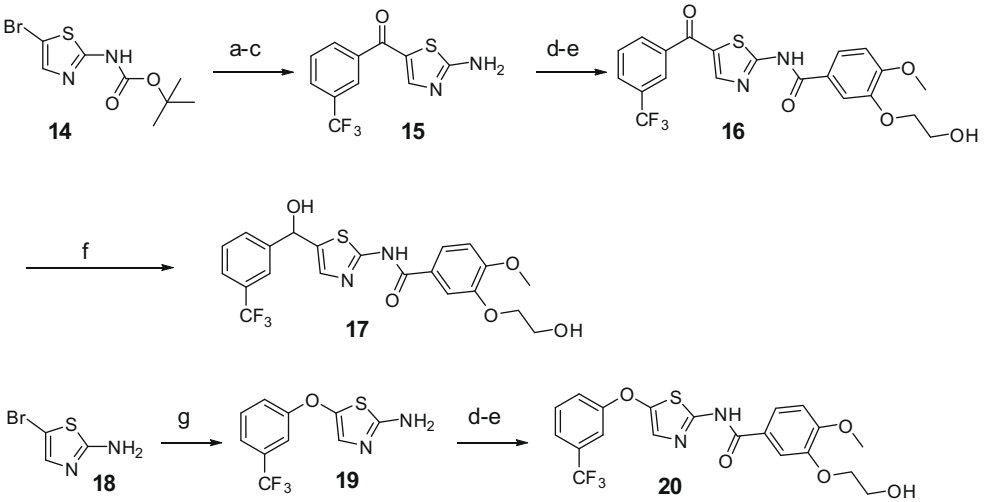
No.	PK profiles[a] (po, 20 mg/kg)				
	C_{max}[b] (μg/mL)	$t_{1/2}$[b] (h)	T_{max}[b] (h)	$AUC_{(0-8\ h)}$[b] (μg h/mL)	*F* (%)
1a	1.7	3.5	0.7	8.2	12
13	0.12	2.5	1.0	0.4	2

[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/6Jmice ($n = 2$) 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.

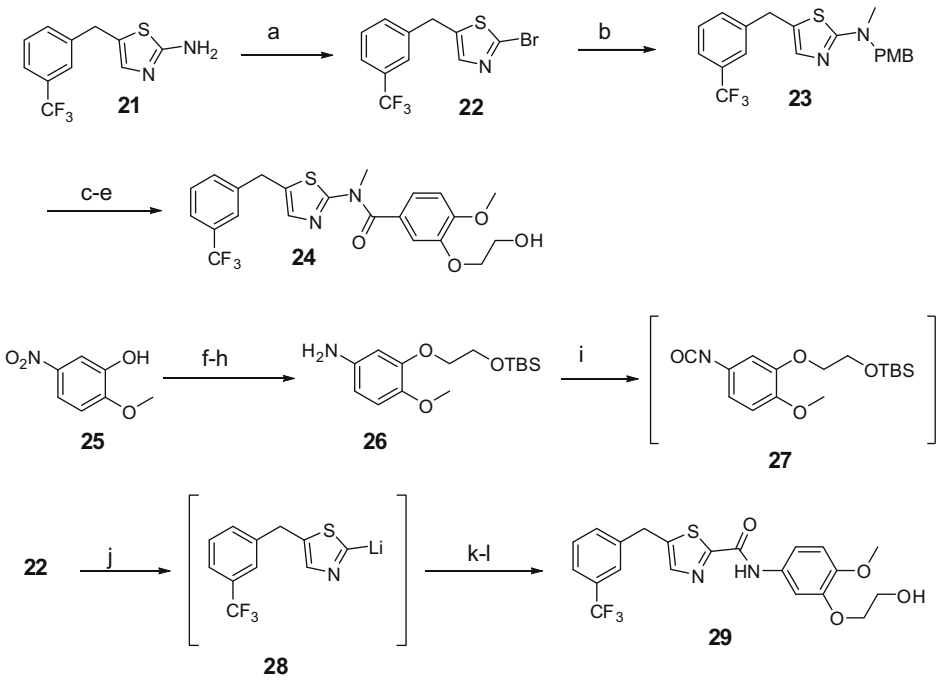


Scheme 1. Reagents and conditions: (a) toluene, reflux, 97%; (b) 1 N NaOH, 1,4-dioxane, 60 °C, 94%; (c) DPPA, Et_3N, *t*-BuOH, rt to reflux, 40%; (d) TFA, CH_2Cl_2, rt, quantitative yield; (e) *n*-BuLi/THF, then 3-trifluoromethylbenzaldehyde, 97%; (f) TMS–Cl, NaI, MeCN, 87%; (g) $Cu(NO_3)_2$, Ac_2O, 83%; (h) thiosemicarbazide, BOP reagent, Et_3N, THF, (i) CH_3SO_3H, toluene, reflux, 48% (two steps); (j) Zn Powder, 1 N HCl/2-PrOH; (k) HATU, **10**, Et_3N, DMA, rt to 70–80 °C; (l) 1 N HCl, MeOH, rt.

Condensation of 2-amino-5-bromothiazole (**18**) and 3-trifluoromethylphenol provided **19**. Amide bond formation and deprotection gave **20**. As for the amide linker (W in Fig. 1), synthetic routes for the methylated amide analogs and the reverse amide analogs are shown in Scheme 3. Since it was presumed that alkylation of acylated aminothiazoles would provide undesired alkylation products (i.e., alkylation at the 3-position of thiazole),[12] we tried to aminate the 2-bromothiazole (**22**) with (4-methoxybenzyl)methylamine. The synthesis was initiated with diazonium formation and subsequent bromination on the aminothiazole (**21**) under Sandmeyer reaction conditions gave the 2-bromothiazole (**22**). Palladium catalyzed amination with (4-methoxybenzyl)methylamine produced **23**. Deprotection of the PMB group in TFA, HATU mediated amide bond formation and subsequent THP deprotection gave the *N*-methylated thiazole analog (**24**). Synthesis of the reverse amide analog (**29**) was initiated with alkylation of 2-methoxy-5-nitrophenol (**25**) with 2-bromoethanol, followed by TBS protection, and reduction of the nitro group to provide the aniline (**26**) in 70% yield over three steps. The isocyanate (**27**), prepared from **26** and triphosgene, was immediately reacted



Scheme 2. Reagents and conditions: (a) *n*-BuLi/THF, then 3-trifluoromethylbenzaldehyde, −78 to −5 °C, 68%; (b) Dess–Martin periodinane, CH_2Cl_2, 71%; (c) TFA, CH_2Cl_2, 87%; (d) HATU, **10**, Et_3N, DMA, rt to 80 °C; (e) 1 N HCl, MeOH, rt, (**16**; 54% over two steps), (**20**, 38% over two steps); (f) $NaBH_4$, MeOH/THF, 0 °C to rt, 48%; (g) NaH, 3-trifluoromethylphenol, THF/DMF (1:9), 39%.

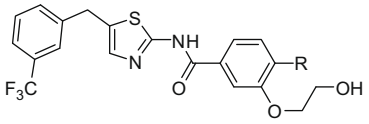


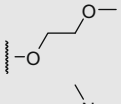
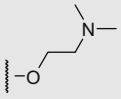
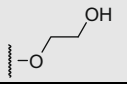
Scheme 3. Reagents and conditions: (a) *tert*-butyl nitrite, $CuBr_2$, CH_3CN, rt, 23%; (b) (4-methoxybenzyl)methylamine, K_3PO_4, $Pd(OCOCF_3)_2$, P(*t*-Bu)$_3$, toluene, 80 °C, 85%; (c) TFA, rt; (d) HATU, **10**, Et_3N, DMA, rt to 80 °C; (e) 1 N HCl, MeOH, rt, 18% (three steps); (f) 2-bromoethanol, K_2CO_3, CH_3CN, reflux, 79%; (g) TBS–Cl, imidazole, DMF, rt; (h) H_2, Pd/C, THF, rt, 88% (two steps); (i) triphosgene, Et_3N, CH_2Cl_2, rt; (j) *n*-BuLi, THF, −78 °C; (k) THF, −78 °C to rt; (l) 1 N HCl, MeOH, rt, 22% (four steps).

with lithiated thiazole (**28**), which was freshly prepared from **22**. After acidic deprotection, **29** was obtained.

The SCD-1 inhibitory activity[13] of the compounds prepared in Scheme 1 is summarized in Table 1. The data suggest that the nitrogen atom at the 3-position of the thiazole core should play a pivotal role in causing powerful SCD-1 inhibition because the 2-benzylthiazole core (**11**) and the thiophene core (**12**) exhibited significant decrease in SCD-1 inhibition. The sulfur atom in the core also turned out to be very important because the 5-benzyloxazole analog was >100 times weaker in SCD-1 inhibition than the

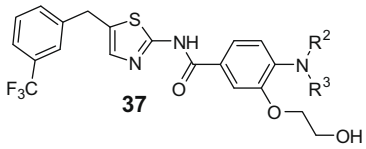
Table 4
Modification of 4-methoxy group (R)



No.	R	IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	IC_{50}[a] (nM) human cell
1a	OMe	2	3	15
36a		2	2	22
36b		3	0.6	6
36c		NT[b]	>9999	NT[b]
36d		22	16	NT[b]

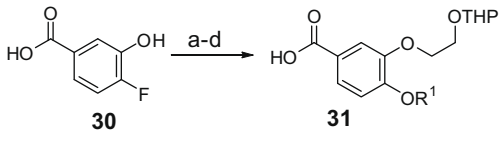
[a] Values are the geometric means of at least two experiments.
[b] NT—not tested.

Table 5
Evaluation of 4-amino(–NR^2R^3) analogs

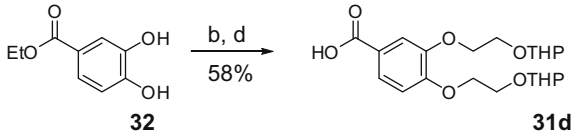


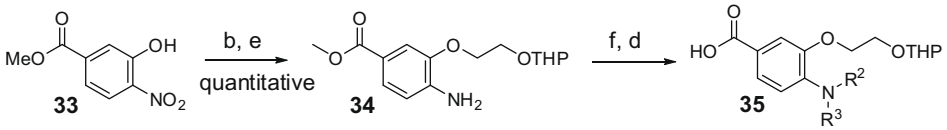
No.		IC_{50}[a] (nM) mouse Δ9	IC_{50}[a] (nM) human Δ9	IC_{50}[a] (nM) human cell
37a		7	15	23
37b		4	4	5
37c		0.4	0.04	2
37d		6	12	44
37e		8	11	119
37f		>9999	NT[b]	NT[b]\

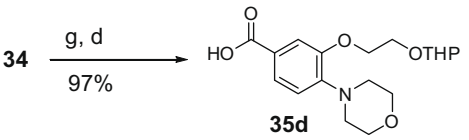
[a] Values are the geometric means of at least two experiments.
[b] NT—not tested.



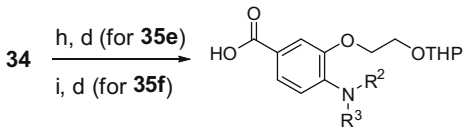
No.	R^1	yield (4 steps)
31a	Et	43%
31b	$-(CH_2)_2-OCH_3$	33%
31c	$-(CH_2)_2-N(CH_3)_2$	33%





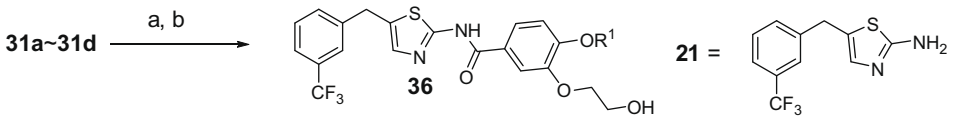


No.	R^2	R^3	yield (2 steps)
35a	Me	Me	86%
35b	Et	Et	23%
35c	H	Et	50%

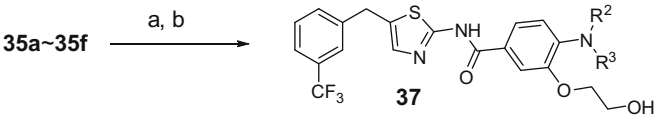


No.	R^2	R^3	yield (2 steps)
35e	Ac	H	48%
35f	Ms	H	66%

Scheme 4. Reagents and conditions: (a) H_2SO_4, ROH (R = Et for **31a**, R = Me for **31b** and **31c**), reflux; (b)2-(2-bromoethoxy)tetrahydro-2*H*-pyran, K_2CO_3, CH_3CN, reflux; (c) NaOEt, EtOH, reflux (for **31a**); or R^1OH, *t*-BuOK, 100 °C (for **31b** and **31c**); (d) 1–2 N NaOH; (e) H_2, Pd/C, EtOAc, rt; (f) HCHO (for **35a**) or CH_3CHO (for **35b** and **35c**), $NaBH_3CN$, AcOH, THF; (g) bis(2-bromoethyl)ether, K_2CO_3, NaI, DMF; (h) acetic anhydride, Et_3N, THF; (i) MsCl, pyridine.



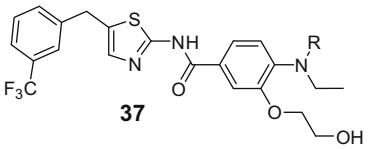
No.	R^1	yield (2 steps)
36a	Et	40%
36b	$-(CH_2)_2OCH_3$	47%
36c	$-(CH_2)_2N(CH_3)_2$	23%
36d	$-(CH_2)_2OH$	39%



No.	R^2	R^3	yield (2 steps)
37a	Me	Me	58%
37b	Et	Et	47%
37c	H	Et	36%
37d	$-(CH_2)_2O(CH_2)_2-$		14%
37e	Ac	H	23%
37f	Ms	H	76%

Scheme 5. Reagents and conditions: (a) **21**, HATU, Et_3N, DMA, rt to 70 °C; (b) 1 N HCl, MeOH, rt to 50 °C.

Table 6
PK parameters of **37b** and **37c** in C57BL/6J mice[a]



37

No.	R	PK profiles[a] (iv, 5 mg/kg)				PK profiles[a] (po, 20 mg/kg)				
		$t_{1/2}$[b] (h)	Cl[b] (mL/min/kg)	Vd[b] (L/kg)	$AUC_{(0-8\ h)}$[b] (μg h/mL)	C_{max}[b]	$t_{1/2}$	T_{max}[b]	$AUC_{(0-8\ h)}$[b] (μg h/mL)	F (%)
37b	Et	0.7	83	2.7	1.0	0.8	1.5	0.8	2.0	51
37c	H	1.1	21	1.2	4.0	1.8	1.4	1.0	4.3	27

[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/6J 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.

corresponding 5-benzylthiazole analog (data not shown). The thiadiazole core (**13**) demonstrated comparable enzymatic SCD-1 inhibitory activity (IC_{50} (human) = 8 nM) and selectivity toward Δ6 isozyme of the desaturase (<5% inhibition at 10 μM) as the 5-benzylthiazole core (**1a**). The pharmacokinetic profiles of **1a** and **13** are compared in Table 2. **13** exhibited only marginal plasma exposure (AUC = 0.4 μg h/mL) and bioavailability (F = 2%) after oral administration, indicating that the thiazole core is important not only for potency but also for desirable pharmacokinetics.

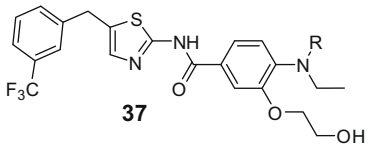
The results of the linker modification are summarized in Table 3. As for linker V, modifications of methylene in **1a** to carbonyl (**16**), carbinol (**17**), and ether (**20**), resulted in a significant decrease of activity, indicating that the methylene linker in **1a** is crucial for robust SCD-1 inhibition. In the case of amide linker W, methylation of the amide proton (**24**) caused a >100-fold loss of activity, whereas the reverse amide (**29**) retained weaker SCD-1 inhibitory activity (IC_{50} (human) = 32 nM). At this point, we were quite convinced that the *N*-(5-benzylthiazol-2-yl) benzamide system is a critical structural requirement for the development of potent SCD-1 inhibitors. In the next phase of the optimization, we turned to the right-hand portion of the lead compound.

The synthetic procedures for the compounds in Tables 4 and 5 are outlined in Schemes 4 and 5.[7] Synthesis of the 4-alkoxy analog was initiated by esterification of 4-fluoro-3-hydroxybenzoic acid (**30**), followed by alkylation with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran, an S_N2Ar reaction with a corresponding alcohol and subsequent saponification to provide **31a**–**31c**. Compound **31d** was prepared by dialkylation of **32** and saponification. To prepare 4-amino derivatives, the commercially available 3-hydroxy-4-nitrobenzoic acid methyl ester (**33**) was alkylated with 2-(2-bromoethoxy)tetrahydro-2*H*-pyran and reduced under a hydrogen atmosphere to generate aniline (**34**). The aniline was alkylated with a corresponding aldehyde in the presence of $NaBH_3CN$ and saponified to provide **35a**–**35c**. Preparation of morpholino (**35d**), acetylamino (**35e**), and methylsulfonylamino (**35f**) are also depicted in Scheme 4. As shown in Scheme 5, the 4-alkoxy or 4-amino benzoic acids thus prepared were condensed with aminothiazole **21** in the presence of HATU. Subsequent THP deprotection gave 4-alkoxy analogs (**36a**–**36d**) and 4-amino series (**37a**–**37f**).

As shown in Table 4, elongation of the methoxy to ethoxy in **36a** retained strong potency (IC_{50} (human) = 2 nM).[13,14] The methoxyethoxy analog (**36b**) also displayed potency equal to that of lead compound **1a**, while the terminal dimethylamino (**36c**) completely lost activity and the hydroxyethoxy group at the 4-position (**36d**) resulted in a slight decrease in activity. It is fair to say there is a steric tolerance at this position and that lipophilic groups are preferred. The alkoxy groups were replaced with amines such as those in **37a**–**37f** (Table 5). As indicated in the SAR of the 4-alkoxy analogs, a variety of alkyl amines were tolerated, dimethylamino (**37a**), diethylamino (**37b**), and morpholino (**37d**) retained strong inhibitory activity against SCD-1. Remarkably, ethylamino (**37c**)[15] was found to be the most active SCD-1 inhibitor in this series, with IC_{50} (human) = 0.04 nM. In the case of acylated and sulfonylated analogs, acetylamino (**37e**) retained potency for both murine and human SCD-1, while methylsulfonylamino analog (**37f**) showed only marginal inhibitory activity.

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%.[16] 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 most potent SCD-1 inhibitor (**37c**) and its structurally related analog (**37b**) against the liver SCD-1 in C57BL/6J mice on a non-fat diet. The inhibitory activity was determined by measuring the ratio of [^{14}C] stearate and [^{14}C] oleate in the liver.[17] The dose at which 50% of the conversion is inhibited is described as ID_{50}. As shown in Tables 6 and 7, both compounds showed similar PK profiles and in vivo activity. A combination of strong enzymatic inhibitory activity (IC_{50} (mouse) = 0.4 nM) and good oral exposure right after oral administration (C_{max} = 1.8 μg/mL and T_{max} = 1 h) of **37c** contributed to strong potency in liver SCD-1 inhibition (ID_{50} (2–3 h) = 0.8 mg/kg). The decrease in potency at 6–7 h (ID_{50} = 2 mg/kg) could be attributed to the relatively short plasma half-life ($t_{1/2}$ = 1.4 h) and fast clearance (Cl = 21 mL/min/kg). While showing about 10-fold weaker enzymatic SCD-1 inhibition and relatively lower plasma exposure, **37b** demonstrated potency equal to that of **37c** in liver SCD-1 inhibition in mice. It is assumed that the concentration of **37b** in the liver was higher than that of **37c**, though no data on their hepatic concentrations were available at this

Table 7
The liver SCD-1 inhibition by **37b** and **37c** in C57BL/6J mice on a non-fat diet[a]



37

No.	R	ID_{50} (mg/kg)[a]	
		At 2–3 h	At 6–7 h
37b	Et	1.0	2.0
37c	H	0.8	2.0

[a] Values are the geometric means of at least two experiments.

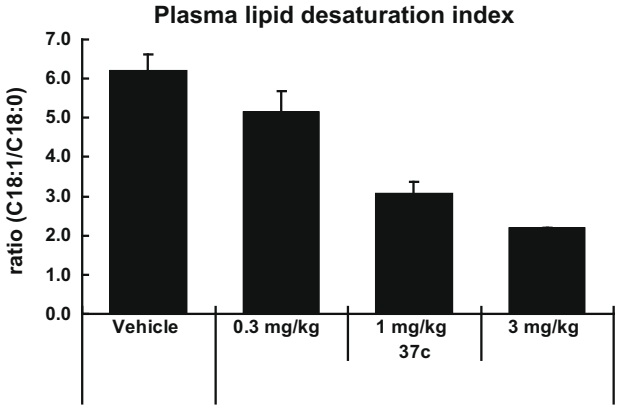


Figure 2. Plasma desaturation index lowering effect of the treatment with SCD-1 inhibitor **37c** for 7 days (q.d.) in C57BL/6J mice fed with a non-fat diet.

point. For multiple dosing studies of SCD-1 inhibitors, **37c** was tested in a 7-day efficacy study using C57BL/6J mice on a non-fat diet.[18] The desaturation index, calculated as the ratio of C18:1N9(*cis*)/C18:0, was used as an in vivo biomarker. After once-daily administration for 7 days, **37c** dose-dependently reduced the desaturation index, with a 65% reduction at 3 mg/kg (Fig. 2). In the preliminary analysis, we did not observe any abnormalities in the skin or eyes of the C57BL/6J mice at 3 mg/kg (Cutaneous abnormalities and narrow eye fissure have been reported in studies on SCD-1 deficient mice[19]). We assume that the balanced combination of the strong potency and short plasma half life of **37c** resulted in pharmacological efficacy in vivo and may be beneficial in ameliorating adverse events. The liver can be a key tissue for metabolizing xenobiotics, and orally administered drugs can obtain significant concentrations in this tissue. Since SCD-1 is expressed in the liver, significant systemic exposure of SCD-1 inhibitors may not be necessary to realize a pharmacodynamic response. While this is a preliminary speculation, the relatively short plasma half-life of **37c** may help to accomplish favorable tissue selectivity (liver over eyes or skin). Histopathological analysis of the key tissues (eyes, skin, and liver) of the C57BL/6J mice after a 7-day treatment with the SCD-1 inhibitor **37c** is currently in progress and will be reported elsewhere along with more details about the pharmacological studies of the 3-(2-hydroxyethoxy)-*N*-(5-benzylthiazol-2-yl)benzamide-based SCD-1 inhibitors.

In summary, we prepared and assayed compounds derived from **1**, which was identified as a potent SCD-1 inhibitor in the preceding article. SAR studies of this lead compound proved that the *N*-(5-benzylthiazol-2-yl)benzamide system is a critical structural requirement for the development of potent SCD-1 inhibitors. Further delineation of SAR studies of this lead compound, especially in the right-hand portion, resulted in the identification of 4-ethylamino-3-(2-hydroxyethoxy)-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl] benzamide (**37c**) with sub nano molar IC_{50} in both murine and human SCD-1 inhibitory assays. Compound **37c** demonstrated a dose-dependent decrease in the plasma desaturation index in C57BL/6J mice on a non-fat diet after once-daily 7-day oral administration. Further optimization and pharmacological and toxicological evaluation of this series of compounds will be reported in due course.

References and notes

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7. All the final compounds were characterized by ^{1}H NMR and mass spectroscopy.
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10. (a) *Compound **13** was prepared as follows*: *5-(3-Trifluoromethylbenzyl)-[1,3,4]thiadiazol-2-ylamine* (**9**): A mixture of (α,α,α-trifluoro-*m*-tolyl)acetic acid (**8**, 1.15 g, 6.12 mmol), thiosemicarbazide (1.15 g, 12.6 mmol), BOP reagent (3.24 g, 7.33 mmol), and Et_3N (1.7 mL, 12 mmol) in anhydrous THF (20 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated, diluted with H_2O and extracted with EtOAc (twice). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The off-white solid thus obtained (1.72 g) was mixed with methanesulfonic acid (0.48 mL, 7.4 mmol) and toluene (20 mL). The suspension was heated to reflux for 4 h and cooled to room temperature. The reaction mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ (×2) and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (CH_2Cl_2/MeOH 30:1–10:1) gave 760 mg (48%) of **9** as a white solid. MS (ESI) *m/z*: 260 $(M+H)^+$.; (b) *3-(2-Hydroxyethoxy)-4-methoxy-N-[5-(3-trifluoromethylbenzyl)-[1,3,4]thiadiazol-2-yl]benzamide* (**13**). A solution of **9** (100 mg, 0.386 mmol), 3-(2-hydroxyethoxy)-4-methoxybenzoic acid (83 mg, 0.39 mmol), HATU (178 mg, 0.468 mmol), and Et_3N (90 μL, 0.65 mmol) in DMA (2 mL) was stirred at room temperature for four days. After work-up, the crude product was purified by chromatography on SiO_2 (CH_2Cl_2/MeOH 20:1) and subsequent recrystallization (2-PrOH) to give 29 mg (17%) of **13** as a white solid:^{1}H NMR(400 MHz, DMSO-d_6): δ 12.8 (1H, br s), 7.76 (3H, d, *J* = 9.8 Hz), 7.68 (2H, dd, *J* = 8.1 and 8.1 Hz), 7.61 (1H, dd, *J* = 7.8 and 7.8 Hz), 7.62 (1H, d, *J* = 7.9 Hz), 4.88 (1H, t, *J* = 5.2 Hz), 4.52 (2H, s), 4.07 (2H, t, *J* = 4.9 Hz), 3.85 (3H, s), 3.76 (2H, dt, *J* = 4.8 and 4.9 Hz); MS (ESI) *m/z*: 454 $(M+H)^+$.
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13. *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, pH7.4, 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$n-3$.
14. 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.
15. (a) *Compound **37c** was prepared as follows*: 4-Amino-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid methyl ester (**34**). A suspension of 3-hydroxy-4-nitro-benzoic acid ethyl ester (**33**, 5.02 g, 25.5 mmol), 2-(2-bromo-ethoxy)tetrahydro-2*H*-pyran (5.8 mL, 38 mmol), K_2CO_3 (7.04 g, 50.9 mmol) in DMA (70 mL) was heated at 80 °C for 5 h, cooled to room temperature, and extracted with EtOAc. The organic layer was washed with H_2O (×4) and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (hexanes/EtOAc 17:3–1:1) to give 8.50 g of the alkylated compound as a yellow oil: ^{1}H NMR(400MHz,$CDCl_3$): δ 7.84 (1H, s), 7.83 (1H, d, *J* = 9.8 Hz), 7.71 (1H, d, *J* = 9.7 Hz), 4.73 (1H, t, *J* = 3.3 Hz), 4.42–4.34 (2H, m), 4.16–4.08 (1H, m), 3.97 (3H, s), 3.92–3.83 (2H, m), 3.57–3.52 (1H, m), 1.86–1.71 (2H, m), 1.65–1.51 (4H, m). A suspension of the yellow oil (8.50 g, 25.5 mmol) and Pd/C (10 wt %,

1.28 g) in EtOAc (100 mL) was treated with H_2 gas for 3 h. The reaction mixture was filtered through a plug of Celite, concentrated, and dried in vacuo to give 7.78 g (quantitative yield) of **34** as a colorless oil: MS(ESI) *m/z*: 296 $(M+H)^+$.; (b) *4-Ethylamino-3-[2-(tetrahydropyran-2-yloxy)ethoxy]benzoic acid* (**35c**). To a solution of **34** (4.45 g, 15.3 mmol) in THF/MeOH (2:1, 90 mL) were successively added acetic acid (0.88 mL, 15 mmol), acetaldehyde (4.3 mL, 77 mmol) and sodium cyanoborohydride (2.89 g, 46.0 mmol) at 0 °C. To complete the reaction, additional amounts of the reagents were added and the reaction was heated to 60 °C. The reaction mixture was concentrated, diluted with EtOAc and saturated aqueous $NaHCO_3$. The separated organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (CH_2Cl_2/EtOAc, and then CH_2Cl_2/MeOH) gave 3.19 g (64%) of the monoalkylated compound as a colorless oil and 1.63 g (30%) of the dialkylated compound as a colorless oil. To a solution of the monoalkylated compound (514 mg, 1.59 mmol) in 1,4-dioxane (10 mL) was added 1 N NaOH (2.4 mL) at room temperature. The reaction mixture was heated to reflux for 16 h. To complete the conversion, an additional amount of 1 N NaOH was added during the reaction. The reaction mixture was concentrated, and diluted with EtOAc and citric acid (aq). The separated organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was triturated in hexanes/iPr_2O, collected by filtration, and dried in vacuo to give 385 mg (78%) of **35c** as an off-white solid: MS (ESI) *m/z*: 310 $(M+H)^+$.; (c) 4-Ethylamino-3-(2-hydroxyethoxy)-*N*-[5-(3-trifluoromethylbenzyl)thiazol-2-yl]benzamide (**37c**). A solution of **21** (95 mg, 0.37 mmol), **35c** (114 mg, 0.369 mmol), HATU (154 mg, 0.405 mmol), and Et_3N (0.10 mL, 0.74 mmol) in DMA (6 mL) was heated at 70 °C for 3 days. The reaction mixture was diluted with EtOAc and citric acid (aq). The separated organic layer was washed with H_2O (×3) and brine, dried (Na_2SO_4), and concentrated. Chromatography of the residue on SiO_2 (KP-NH, 40–75 μm, 100A, Biotage, hexanes/CH_2Cl_2 3:7 to 0:1) gave 122 mg (60%) of the amide compound as a pale yellow oil. To a solution of the amide (122 mg, 0.221 mmol) in MeOH (10 mL) was added 1 N HCl (0.55 mL). The reaction mixture was heated at 50 °C for 1.5 h and concentrated. The residue was diluted with CH_2Cl_2, iPr_2O, hexanes and saturated aqueous $NaHCO_3$. The resulting precipitate was collected by filtration, washed with iPr_2O and H_2O, and dried in vacuo to give 62 mg (61%) of **37c** as a white solid: ^1H NMR(400MHz, DMSO-d_6): δ 12.1 (1H, s), 7.67–7.56 (6H, m), 7.32 (1H, s), 6.58 (1H, d, J = 8.6 Hz), 5.78 (1H, t, J = 5.6 Hz), 4.98 (1H, t, J = 6.3 Hz), 4.23 (2H, s), 4.03 (2H, t, J = 4.7 Hz), 3.79–3.75 (2H, m), 3.24–3.17 (2H, m), 1.19 (3H, t, J = 7.2 Hz). MS (ESI) *m/z*: 466 $(M+H)^+$.

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17. 9-Week-old C57BL/6J mice grown with a normal chow diet were fed a non-fat diet (Research Diets, Inc., D05052506) for 7 days. SCD-1 inhibitors were administered to the mice (n = 2) 2 h or 6 h 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 quickly frozen in liquid nitrogen. The livers were homogenized in 9× volume of cold PBS, and 250 μL of homogenate was mixed with an 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 quantifying 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}.
18. 9-Week-old male C57BL6J mice grown with a normal chow diet were fed with a non-fat diet (Research Diets, Inc., D05052506) for 7 days. Compound **37c** was administered daily at doses of 0.3, 1, and 3 mg/kg to male C57BL6J mice on a non-fat diet for 7 days in the evening by oral gavage in propylene glycol/Tween 80 (4/1) formulation (dosing vehicle). The animals were allowed free access to the non-fat diet and water throughout the study. After the seventh dose, the animals were sacrificed in the morning and the blood serum was assayed for the desaturation index as follows. 30 μL serum was mixed with 4 mL 0.5 N KOH in methanol and saponified at 100 °C for 30 min. The free fatty acids in the reaction were protonated by the addition of 2 mL of 1 N HCl and extracted with 3 mL of *n*-hexane. Two milliliters of the hexane extracts were dried up and dissolved with 1 mL of BF_3–methanol and esterified at 100 °C for 15 min. The samples were mixed with 1 mL of H_2O and extracted with 1 mL *n*-hexane. One microliter of the final hexane extracts was loaded to a gas chromatogram to determine the stearate and oleate content. The desaturation index was calculated as the ratio of oleate to stearate.
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