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# Thiazole analog as stearoyl-CoA desaturase 1 inhibitor

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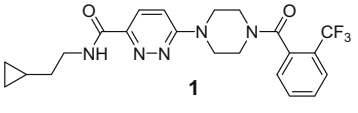
ABSTRACT

SCD1 inhibition may represent a novel treatment for obesity, type-2 diabetes and related metabolic disorders. A prototype thiazole amide analog **13** (**MF-152**) was identified as an excellent tool in the study of SCD biology in animals.



Stearoyl-CoA desaturase-1 (SCD1), also known as delta-9 desaturase (Δ9D), is a critical enzyme in the lipogenic pathway. It converts saturated fatty acids to mono-unsaturated fatty acids with the formation of a cis-double bond at the C-9 position.[1] The preferred substrates are the C-16 palmitoyl-CoA and the C-18 stearoyl-CoA which are transformed into palmitoleoyl- and oleoyl-CoA, respectively.[2] The resulting mono-unsaturated acyl-CoAs are major building blocks of lipids including phospholipid, triglyceride, cholesterol ester and wax ester. Four SCD isoforms have been characterized in rodents and two in human. SCD1, with about 85% identity across species, is the major isoform found in lipogenic tissues including liver and adipose tissues. Evidence from rodent and human strongly supports the key roles of SCD1 in lipid and carbohydrate metabolism. SCD1-deficient mice from natural mutation or targeted deletion are resistant to high fat diet-induced obesity and show improved insulin sensitivity as well as increased energy expenditure.[3,4] This phenotype is also observed in high fat diet-induced obese (DIO) mouse treated with anti-sense oligonucleotide (ASO)[5] or small molecule inhibitors.[6,7] In human, elevated SCD activity is positively correlated with high triglyceride in familial hypertriglyceridemia subjects,[8] increased body mass index (BMI) and high plasma insulin levels.[9] Therefore, SCD1 inhibition may represent a novel treatment for obesity, type-2 diabetes, and related metabolic disorders.

Two additional fatty acyl-CoA specific desaturases, delta-5 and delta-6 desaturases (Δ5D and Δ6D), are required to catalyze the formation of highly unsaturated fatty acids (HUFAs) such as arachidonic acid from dietary essential fatty acids (polyunsaturated fatty acids, PUFAs) in human. The primary role of HUFAs in mammals is cell signaling. An imbalance of Δ5D and Δ6D activities will lead to various disorders.[10] Therefore, selectivity against Δ5D and Δ6D is required for SCD1 inhibitors. In addition to the favorable metabolic profile, global SCD1 deficiency also causes alopecia and dry eyes in mice,[3] although this effect is not observed in mice treated with ASO.[5a] Thus, our initial challenge in the SCD inhibitor development program was to identify an orally active SCD inhibitor that is suitable for evaluating the efficacy and potential adverse events of SCD inhibition in animal models.[11] In 2005, several SCD1 inhibitors were disclosed[12] and followed by some recent SAR studies.[13–15] One of the key features of these SCD1 inhibitors is a relatively rigid linear core template with substituents at both ends. A representative compound **1** was prepared and its SCD inhibitory activity was confirmed (Fig. 1). However, circulating metabolites **2** and **3** derived from the metabolism of the cyclopropylethyl amide were ob-




**1**

rSCD $IC_{50}$: 0.3μM

**Figure 1.** A representative SCD example from Xenon.

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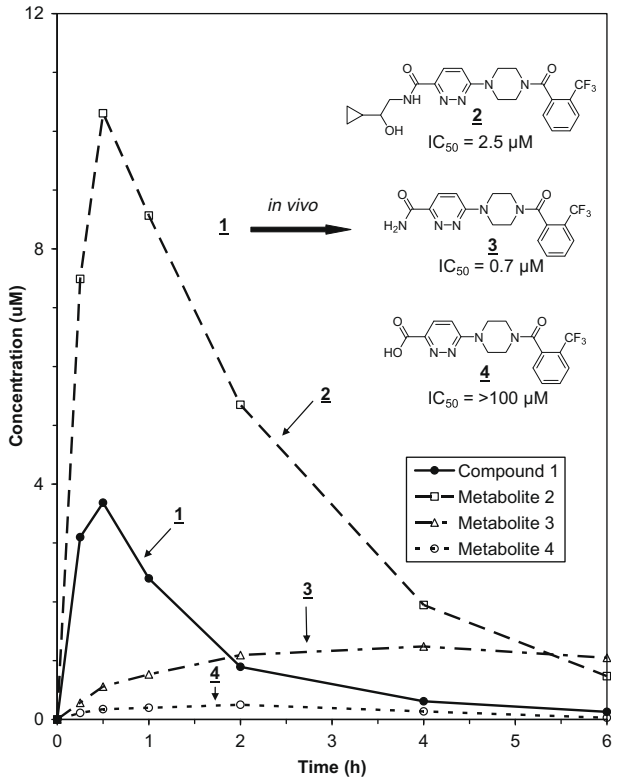



**Figure 2.** Levels of compound **1** and its metabolites after oral dosing of 50 mg/kg in mice over a 6 h period. Their $IC_{50}$ in rat liver microsome assay are indicated below the structures.

served at high levels following oral dosing in mice, along with low level of the corresponding acid metabolite **4** (Fig. 2). The two amide metabolites are also relatively active against SCD1 and thus would complicate data interpretation from animal studies using **1**. Herein, we report our study to identify a compound with a simpler metabolic profile for in vivo SCD inhibition studies.

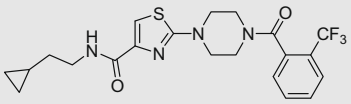
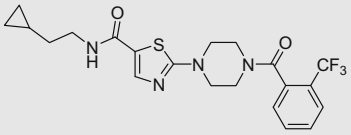
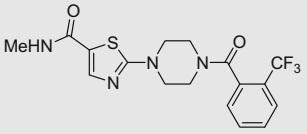
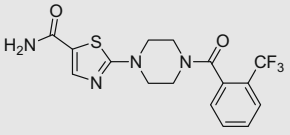
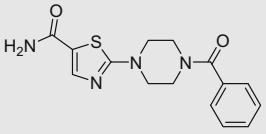
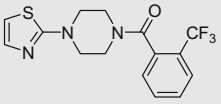
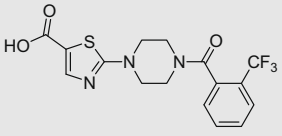
One of our early modifications focused on diverse structures by replacing the six-membered pyridazine in compound **1** with a five-membered thiazole.[16] Synthesis of these thiazole analogs is described in Scheme 1. A halo-substituted thiazole was reacted with the piperazine intermediate **6**, which could be obtained from 1-Boc-piperazine **5** and the corresponding benzoyl chloride. The resulting ester intermediate **7** was then converted to the corresponding amide analogs **9** in a standard manner.

To measure the effect of modification on the intrinsic potency against SCD enzyme, compounds were tested in a rat liver microsomal assay which measured the release of tritiated water in the formation of oleoyl-CoA from 9,10-[$^3$H]-stearoyl-CoA.[17] Their cellular potency on SCD1 and selectivity against the delta-5 and delta-6 desaturases for selected compounds were evaluated using a HepG2-based whole cell assay, which measures the cellular activities of SCD, delta-5 and delta-6 activities simultaneously.[18]

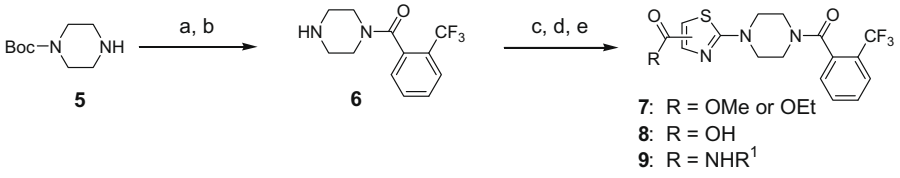
The potencies of representative compounds are summarized in Table 1.

The initial thiazole analogs **10** and **11** with the amide substituent at either the 4 or 5-position of the thiazole were not potent. However, it appeared that amide substituent at the 5-position of **11** was more favorable. When the size of the amide substituent was reduced from cyclopropylethyl to a methyl group as shown in **12**, an 18-fold improvement in potency was observed. The best analog in the series turned out to be the unsubstituted amide **13**,

**Table 1**
Potency of thiazole SCD inhibitors

| Compound | Structure | Rat SCD $IC_{50}$[a] (μm) | HepG2 $IC_{50}$[a] (μm) |
|---|---|---|---|
| **10** |  | >100 | |
| **11** |  | 37 | |
| **12** |  | 2 | 4 |
| **13 (MF-152)** |  | 0.1 | 0.3 |
| **14** |  | 2 | |
| **15** |  | 3 | 13 |
| **16** |  | 7 | 68 |

[a] $IC_{50}$s are an average of at least two independent titrations.




**Scheme 1.** (a) 2-(Trifluoromethyl)benzoyl chloride, $Et_3N$, $CH_2Cl_2$, room temperature, 2 h, 94%; (b) TFA, $CH_2Cl_2$, room temperature, overnight, 93%; (c) 2-halothiazole, DBU, 80 °C, 5 h to overnight, 50–90%; (d) NaOH, THF, MeOH, 60 °C, 1 h, quantitative; (e) oxalyl chloride, then $RNH_2$, or HATU, $RNH_2$, DIPEA, DMF, 70–90%.

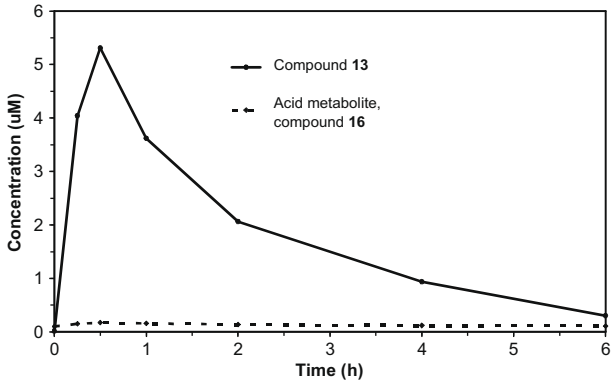



**Figure 3.** Levels of compound **13** and its acid metabolite **16** after oral dosing of 10 mg/kg in mice over a 6 h period.

which showed comparable in vitro potency to compound **1**. In the whole cell assay, compound **13** displayed an $IC_{50}$ of 0.3 μM against the hSCD1 (delta-9 desaturase) and was selective against both delta-5 and delta-6 desaturases with $IC_{50}$'s >50 μM and >10 μM, respectively. Removal of the 2-trifluoromethyl group on the benzoyl moiety resulted in a 20-fold loss of potency in **14**. The unsubstituted thiazole analog **15** was not very potent. In vitro metabolism studies showed that compound **13** has a much cleaner metabolic profile than **1** with amide hydrolysis being the major metabolic pathway.[19] The resulting acid metabolite **16** is a much weaker SCD1 inhibitor and circulates in low levels after oral dosing of **13** in mice (Fig. 3). The simpler metabolic profile rendered compound **13** a more desirable compound for in vivo studies.

To measure the in vivo potency, compound **13** was dosed orally to mice on high carbohydrate diet. The SCD activity was indexed by following the conversion of intravenously administrated [1-$^{14}$C]-stearic acid tracer to the SCD-derived $^{14}$C-oleic acid in liver lipids. As illustrated in Figure 4, the in vivo SCD activity index (ratio of $^{14}$C-oleic acid/$^{14}$C-stearic acid in the saponified liver lipids) decreased dose-dependently with an increased dose of compound **13**. An $ED_{50}$ of ~3 mg/kg was observed, demonstrating compound **13** is highly effective at suppressing the SCD activity in vivo. To determine the consequences from chronic SCD inhibition in a rodent model, a diet formulation containing 0.05% (w/w) of

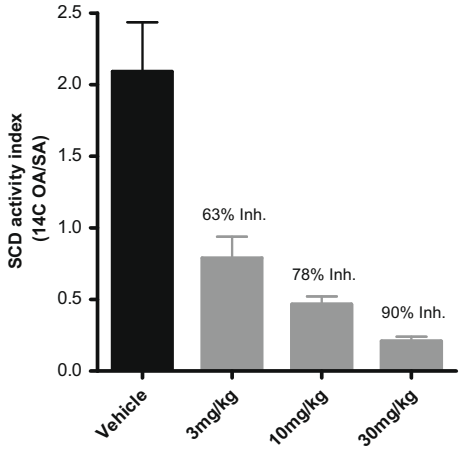



**Figure 4.** In vivo SCD inhibition following compound **13** treatment—compound **13** was dosed orally via 0.5% methocel vehicle in C57B6 mice. One hour later, $^{14}$C-stearic acid in 60% aqueous PEG 200 was administrated intravenously and livers were harvested at two hours post tracer. The SCD activity index [ratio of $^{14}$C-oleic acid (OA)/$^{14}$C-stearic acid (SA) in hydrolyzed liver lipids] decreased with increasing doses of compound **13** with an $ED_{50}$ of ~3 mg/kg compared with the vehicle group. Mean (±SE, $n$ = 5/group, $p$ <0.001 for all treated groups).

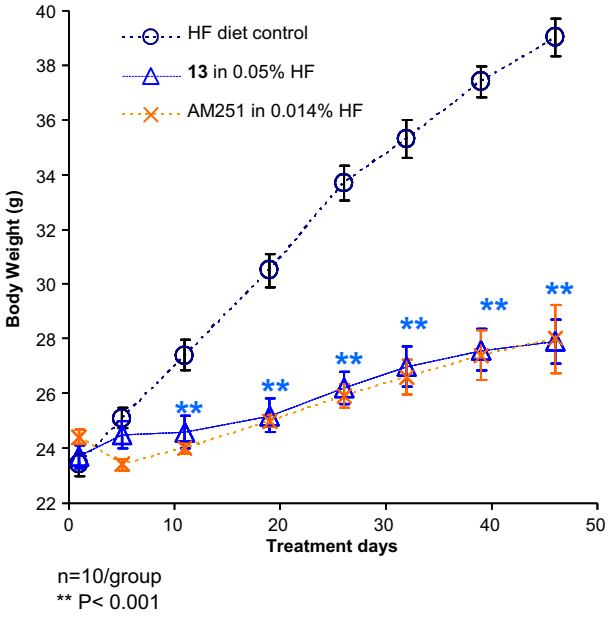



**Figure 5.** Effect of compound **13** on body weight gain of C57BL6 mice. In comparison to the control diet, a 73% reduction in body weight gained was observed when compound **13** was formulated in high fat diet (0.05%, w/w) over a 7 week study. The effect on body weight was comparable to the positive control AM251, formulated in high fat diet (0.014%, w/w).

compound **13** in a high fat diet (Bio-Serv F3282) was prepared to circumvent its short half-life of ~1 h. In comparison to a group on the control diet, C57BL6 mice on the drug-containing diet showed a 73% reduced body weight gain throughout a 7-week treatment duration,[20] with the effect on body weight being comparable to a parallel group treated with the CB1 inverse agonist AM-251[21] which was formulated at 0.014% (w/w) in the high fat diet (Fig. 5). The resistance to HFD-induced body weight gain following compound **13** treatment was associated with a decreased fat accumulation in inguinal and epididymal adipose tissues. In addition, an improved metabolic profile was detected as exemplified by the reduction on plasma insulin level by 69% ($p$ <0.01), plasma cholesterol level by 41% ($p$ <0.001), and plasma triglyceride level by 22% ($p$ = 0.02), respectively. However, partial eye closure and progressive alopecia emerged after ~14 days of drug treatment, with features resembling those reported in the SCD1 knockout mice and in mice treated with other SCD inhibitors,[6b] suggesting these events are likely mechanism-based. However, these adverse events reversed rapidly upon the discontinuation of drug treatment, with new hair emerged and recovery from eye closure after ~10 days.

In conclusion, we have identified compound **13** (designated **MF-152**), a specific SCD inhibitor with an excellent in vivo potency and no cross activity against the delta-5 and delta-6 desaturases. It is an excellent tool in the study of SCD biology in animals. However, further optimization is needed to develop a SCD inhibitor suitable for therapeutic potential evaluation in human.

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19. Unpublished results: Both **1** and **13** at 20 μM showed comparable parent remaining (42–50%) after incubation at 37 °C with cryoperserved rat hepatocytes (2 million cells/mL of Krebs–Henseleit buffer) for 2 h under 95% $O_2$/5% $CO_2$ atmosphere. However, compound **1** has a total of 10 metabolites with the hydroxylation metabolite **2** as major product whereas compound **13** has only three metabolites with the acid **16** as the major metabolite. Some of these metabolites from both compounds were confirmed with synthetic standard and not all metabolites were fully characterized.
20. Food consumption was not vigorously measured for this exploratory experiment. There were ~5% less food consumptions for both compound **13** and AM251, but they were not statistically significant in this study. Subsequent studies showed that compound **13** does not appear to have effect on food consumption (unpublished results).
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