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SAR and optimization of thiazole analogs as potent stearoyl-CoA desaturase inhibitors

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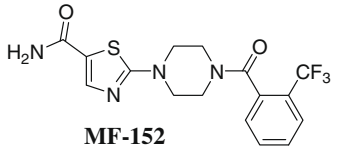
ABSTRACT

Elevated stearoyl-CoA desaturase (SCD) activity has been linked to a number of metabolic disorders including obesity and type II diabetes. Compound **3j**, a potent SCD inhibitor (human HepG2 IC_{50} = 1 nM) was identified from the optimization of a lead thiazole compound **MF-152** with over 100-fold improvement in potency. In a 4-week chronic oral dosing at 0.2 mg/kg, **3j** gave a robust 24% prevention of body weight gain in mice fed on a high fat diet accompanied with an improved metabolic profile on insulin and glucose levels.

Stearoyl-CoA desaturase (SCD) is a microsomal enzyme that catalyses the initial desaturation of long-chain fatty acyl-coenzyme A esters (LCFA-CoA), primarily stearoyl-CoA and palmitoyl-CoA, at the Δ9 (C_9–C_{10}) position to produce monounsaturated oleoyl-CoA and palmitoleoyl-CoA, respectively.[1] These monounsaturated LCFA-CoAs, are major building blocks for de novo lipid synthesis and therefore play an important role in the lipogenic pathway.[2] Four SCD isoforms (SCD1–4) have been characterized in rodents and two in human (SCD1 and SCD5). SCD1 with about 85% homology across all murine SCDs, is the major isoform present in lipogenic tissues (including liver and adipose tissues) and is a key regulator of lipid and carbohydrate metabolism. In rodents, SCD-null mice display a beneficial metabolic phenotype characterised by resistance to high fat diet-induced obesity, improved insulin sensitivity and reduced body adiposity.[3,4] These beneficial phenotypes are also observed in high fat diet-induced obese (DIO) mice treated with anti-sense oligonucleotide (ASO)[5] or small molecule inhibitors.[6a,7–9] In human, an elevated SCD activity is positively correlated with high triglyceride in familial hypertriglyceridemia subjects,[11] increased body mass index (BMI) and high plasma insulin levels.[12] Therefore, the SCD1 enzyme represents an attractive target for the treatment of obesity, type-II diabetes, and related metabolic disorders.

In human, there are two additional fatty acyl-CoA specific desaturases, Δ5D and Δ6D which are involved in the biosynthesis of long-chain polyunsaturated fatty acids and are crucial in cell signaling.[13] Therefore, it is important to identify inhibitors which are selective for SCD1 over these desaturases. Recently, a number of reports have been published on small molecule inhibitors of SCD1.[6–10,14] In our previous communication,[9a] we reported a lead thiazole amide inhibitor **MF-152** (Fig. 1) which was suitable for in vivo SCD inhibition studies in rodents. However this compound was moderately potent (rat SCD IC_{50} = 0.1 μM and human HepG2 IC_{50} = 0.3 μM), the primary amide moiety was metabolically labile and to circumvent its short half-life a diet formulation had to be used for in vivo studies. Herein we wish to report further SAR in the five-membered ring thiazole series to improve potency and to identify a suitable compound for in vivo studies using oral dosing.[15]



MF-152

rSCD IC_{50} : 106 nM
hHepG2 IC_{50} : 253 nM

Figure 1. SCD1 lead inhibitor for in vivo studies.

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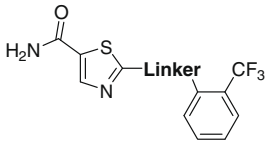
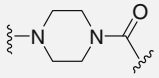
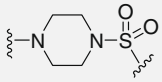
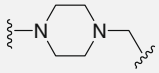
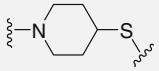
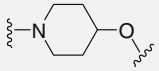
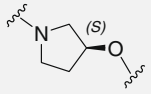
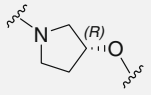
In the absence of an SCD1 enzyme X-ray crystal structure, we chose to proceed with a systematic SAR study to guide our optimization efforts. We first set out to investigate the effect of the piperazine ring modification on the SCD1 inhibition. The representative general procedure for the synthesis of the compounds in Table 1 is depicted in Scheme 1.[16] Reaction of the 2-bromo-thiazole **4** with an appropriately substituted cyclic amine **5** affords a thiazole-ester adduct which can be converted to the corresponding primary amide by reaction with ammonia in a sealed tube.

Table 1
SAR on the piperazine ring



Compound	Linker	Rat SCD IC_{50}[a] (nM)	hHepG2 IC_{50}[a] (nM)
MF-152		106	254
1a		>3000	nd
1b		76	153
1c		4	24
1d		3	10
1e		12	84
1f		>20,000	nd

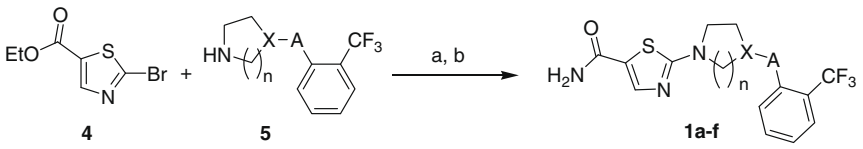
[a] IC_{50}s are an average of at least two independent titrations; nd—not determined.

The compounds were tested against the SCD1 enzyme in an SCD1-induced rat liver microsomal assay[16] and their cellular potencies were evaluated in a human HepG2-based whole cell assay.[17] As shown in Table 1, replacement of the benzamide functionality in **MF-152** with a with a more polar sulfonamide **1a** led to a significant loss in potency. However, the potency could be recovered with benzylamine **1b** and a further ~30-fold improvement in potency was achieved with the piperidine thioether **1c** as well as the piperidine ether **1d**.[18] The six-membered piperidine ring in **1d** can be substituted by a five-membered pyrolidine ring in **1e** possessing the *S*-configuration with a modest fourfold loss in potency, and in contrast the *R*-enantiomer **1f** was inactive. Overall, the piperidine ether moiety in **1d** was clearly an excellent replacement of the piperazine amide in **MF-152**.

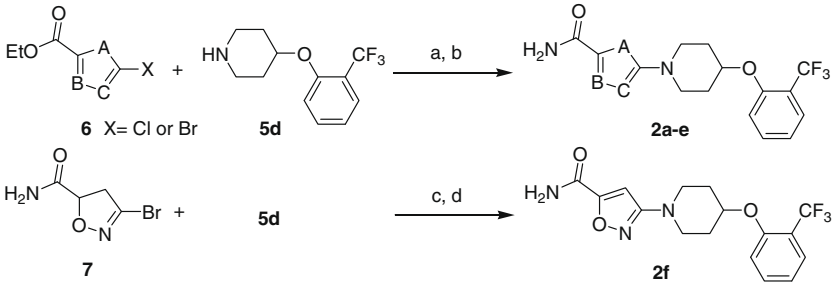
Having identified the piperidine ether **1d** as the optimal linker, we next examined the effect of five-membered heteroaromatic ring substitution on potency. The general synthetic route is summarized in Scheme 2.[16] Compounds **2a–e** can be prepared via displacement of a halo-heteroaromatic ring **6** with the phenoxypiperidine **5d** followed by conversion of the ester group to the corresponding primary amide with ammonia. However, for the isoxazole derivative **2f**, this strategy was unsuccessful and instead the bromo-dihydroisoxazole **7** was treated with the phenoxypiperidine **5d** followed by oxidation with iodine to furnish the isoxazole compound **2f**.[19]

The results showed that potency was highly dependent on subtle changes in this heterocycle (Table 2). For example, inversion of the thiazole ring from 1,3-thiazole-5-carboxamide **1d** to the regioisomer 1,3-thiazole-4-carboxamide **2a** led to a 225-fold loss in potency. The 1,3,4-thiadiazole **2b** was comparable in potency as the 1,3-thiazole **1d**. However, substitution of the 1,3,4-thiadiazole **2b** with a 1,3,4-oxadiazole **2c** led to a 27-fold loss in potency. Likewise, 1,2,4-oxadiazole **2d** and 1,2,4-triazole **2e** are less potent. Finally, the isoxazole ring **2f** showed similar potency to the thiadiazole. These modifications indicate that choosing the correct five-membered heteroaromatic ring is important to maximize the inhibitory activity. Given the equipotency of the thiazole **1d** and the thiadiazole **2b**, we chose to utilize the simpler thiazole ring for further SAR.

Our next approach was to replace the metabolically labile primary amide group in **MF-152** with more stable isosteres. As illustrated in Table 3.[16] conversion of the primary amide **1d** into a more stable primary sulfonamide led to a 13-fold loss in the enzyme

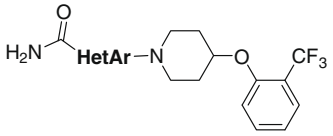


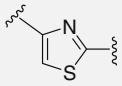
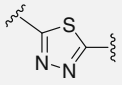
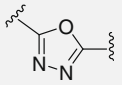
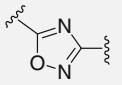
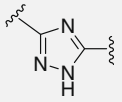
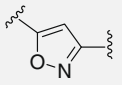
Scheme 1. Reagents: (a) DBU, THF, rt-Δ; (b) ammonia, MeOH, Δ, sealed tube.



Scheme 2. Reagents: (a) DBU, THF, rt-Δ; (b) ammonia, MeOH, Δ, sealed tube; (c) *N*,*N*-diisopropylethylamine, EtOH, Δ; (d) I_2, NaOAc, toluene, Δ.

Table 2
SAR on the five-membered heteroaromatic ring



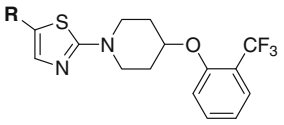
Compound	HetAr	Rat SCD IC_{50}[a] (nM)	hHepG2 IC_{50}[a] (nM)
2a		676	6523
2b		3	7
2c		83	199
2d		1634	nd
2e		127	2823
2f		8	14

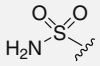
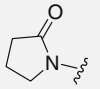
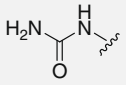
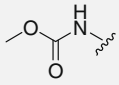
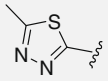
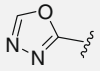
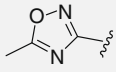
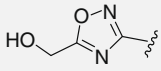
[a] IC_{50}s are an average of at least two independent titrations; nd—not determined.

potency. Substitution with a cyclic reversed amide **3b**, urea **3c** and carbamate **3d**, all afforded less potent compounds. Interestingly, replacement of the primary amide with five-membered ring oxazole **3f**, or oxadiazole **3g–i** retained potency, with **3h** being the most potent (IC_{50} = 4 nM). Pharmacokinetic (PK) profiles for the potent compounds were determined in C57BL6 mice following oral dosing at 10 mg/kg in 0.5% methocel as the vehicle. Compound **3h** had an acceptable bioavailability with a modest whole blood exposure (F = 33%, $AUC_{0-24\ h}$ = 2.9 μM h). The methyl-substituted oxadiazole **3i** showed comparable exposure (F = 24%, $AUC_{0-24\ h}$ = 3.5 μM h) as **3h**. However, it exhibited a high level of a circulating metabolite which we speculate is the hydroxymethyl analog **3j** arising from in vivo oxidation of the methyl group. An authentic sample of **3j** was prepared and further confirmed that it was indeed the circulating metabolite of **3i**. The synthesis is described in Scheme 3.[16] Dehydration of the primary amide **1d** afforded the corresponding cyano derivative which upon treatment with hydroxylamine yielded the carboximidamide **8**. Reaction of **8** with ethyl glycolate in the presence of sodium ethoxide (formed in situ with Na in EtOH) furnished **3j**. Interestingly, this compound was found to be more potent than its parent methyl analog **3i** with an IC_{50} = 1 nM against both the rat SCD enzyme and the hSCD in HepG2 whole cell assay. In addition, it was selective against both Δ5D and Δ6D desaturases with IC_{50}'s >2 μM. Furthermore, compound **3j** showed an improved bioavailability and whole blood exposure (F = 48%, $AUC_{0-24\ h}$ = 16.8 μM h) when dosed orally at 10 mg/kg. Because of its high potency and improved PK, **3j** was chosen for further in vivo profiling.

Tissue distribution of **3j**, 6 h post oral dosing at 10 mg/kg in C57BL6 mice (Fig. 2) indicates high exposure in the liver (6.7 μM) and adipose tissues (8.9 μM) with levels ~6–8-fold higher than found in plasma. Furthermore, to assess the in vivo potency, **3j** was dosed orally in mice fed on a high carbohydrate diet and the SCD activity was indexed 3 h later by following the conversion of

Table 3
SAR on the primary amide moiety

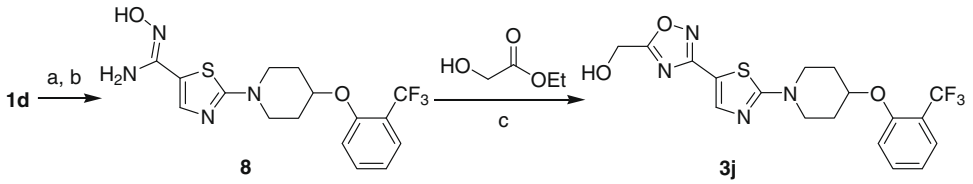


Compound	R	Rat SCD IC_{50}[a] (nM)	hHepG2 IC_{50}[a] (nM)
3a		41	1019
3b		164	174
3c		150	nd
3d		750	775
3e		750	nd
3f		14	55
3g		34	65
3h		4	16
3i		15	39
3j		1	1

[a] IC_{50}s are an average of at least two independent titrations; nd—not determined.

intravenously administrated [1-^{14}C]-stearic acid tracer to the SCD-derived [1-^{14}C]-oleic acid in liver lipids. As illustrated in Figure 3, **3j** dose-dependently decreased the liver SCD activity index (ratio of ^{14}C-oleic acid/^{14}C-stearic acid) with an ED_{50} of ~0.3 mg/kg at a liver concentration of ~1.9 μM, demonstrating its high efficacy in suppressing the liver SCD activity.

To evaluate the in vivo efficacy from chronic SCD inhibition, we first carried out a pharmacokinetic study in C57BL6 mice fed on a high fat diet for dose selection. The mice were dosed once daily with compound **3j** for 3 days at 0.3 mg/kg and analysis of the terminal-24 h blood plasma level on day-3 indicates a concentration of 0.18 μM which correlates to almost full SCD inhibition in the liver (~89% inhibition, Fig. 3). Also, taking into consideration compound accumulation after multiple dosing, we chose to dose compound **3j** at 0.2 mg/kg orally, once daily for 4 weeks which should give ~24 h inhibition of SCD in the liver. Two control arms with mice on high fat and normal chow diets were also included in the study. As shown in Figure 4, the mice treated with **3j** showed a robust 24% (p <0.001) reduction in body weight gain with no significant change in food consumption as compared to the control mice on high fat diet. In addition, the mice treated with **3j** had body weight gain comparable to that of mice fed on a normal chow diet during the study. Furthermore, the resistance to HFD-induced body weight gain with **3j** was associated with an improved metabolic profile as exemplified by a ~24% (p >0.05) reduction in plasma glucose and a ~4% (p >0.05) reduction in insulin levels. However, as



Scheme 3. Reagents (a) NEt_3, TFAA, THF, 0 °C–rt; (b) $NH_2OH{\cdot}HCl$, Na_2CO_3, $EtOH/H_2O$, Δ; (c) Na, EtOH, Δ.

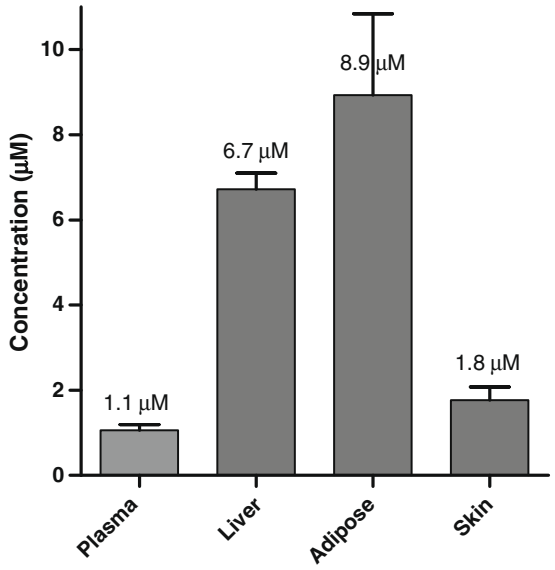


Figure 2. Tissue distribution of **3j,** 6 h post oral dosing at 10 mg/kg in C57BL6 mice fed on normal chow diet.

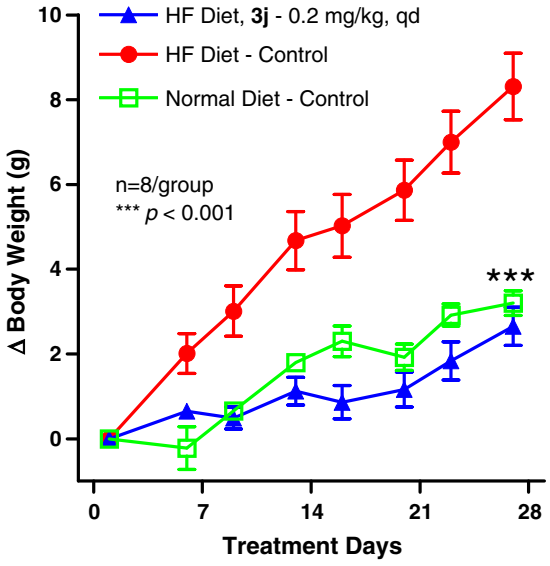


Figure 4. Change in body weight gain of C57BL6 mice fed on a high fat diet dosed orally once daily with **3j** at 0.2 mg/kg compared to control arms with mice fed on a high fat and normal chow diets after 4 weeks.

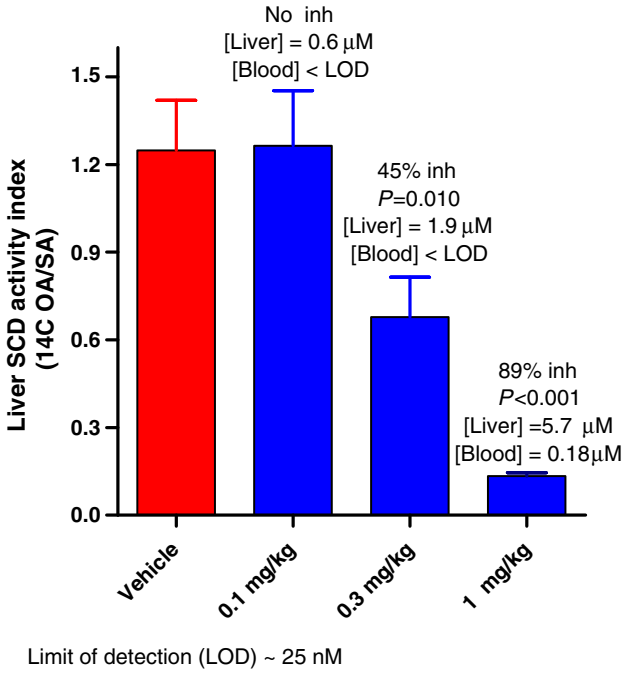


Figure 3. In vivo SCD inhibition of **3j** dosed orally in 0.5% methocel vehicle in C57BL6 mice fed on a high carbohydrate diet. ^{14}C-stearic acid in 60% aqueous PEG 200 was administrated intravenously 1 h later and livers were harvested at 2 hs post tracer. The SCD activity index [ratio of ^{14}C-oleic acid (OA)/^{14}C-stearic acid (SA) in hydrolyzed liver lipids] were measured (n = 5/group).

observed previously with **MF-152**,[9a] the mice treated with **3j** also developed partial eye closure and progressive alopecia after ~7 days of drug treatment. These are presumably due to SCD inhibition in the eyes and skin, since the SCD1 knockout mice ($SCD^{-/-}$) also displayed alopecia and dry eyes.[3] Additionally, we have previously shown that these AEs are reversible upon discontinuation of drug treatment.[9a]

In conclusion, we have carried out a systematic SAR study on the thiazole lead **MF-152** and identified a superior compound **3j**, displaying over 100-fold improvement in potency with suitable pharmacokinetic profile for oral dosing. It demonstrated a dose dependent reduction of liver SCD activity index in C57BL6 mice with an ED_{50} of ~0.3 mg/kg, a 10-fold improvement over **MF-152** (ED_{50} of ~3 mg/kg). In a 4-week chronic dosing study at 0.2 mg/kg, **3j** afforded a robust 24% prevention of body weight gain in mice fed on a high fat diet accompanied with an improved metabolic profile on insulin and glucose levels. However, this compound also caused eye and skin adverse effects and our efforts continue to identify an efficacious SCD inhibitor devoid of these toxicities.

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