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Synthesis and biological activity of a potent and orally bioavailable SCD inhibitor (MF-438)

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ABSTRACT

A series of stearoyl-CoA desaturase 1 (SCD1) inhibitors were developed. Investigations of enzyme potency and metabolism led to the identification of the thiadiazole–pyridazine derivative MF-438 as a potent SCD1 inhibitor. MF-438 exhibits good pharmacokinetics and metabolic stability, thereby serving as a valuable tool for further understanding the role of SCD inhibition in biological and pharmacological models of diseases related to metabolic disorders.

Obesity, fatty liver disease, type 2 diabetes and atherosclerosis are occurring with increasing frequency, a phenomenon generally attributed to physical inactivity and nutrient oversupply. Dysregulation of lipid metabolism has been identified as a critical contributor to the pathogenesis of these related disorders. Stearoyl-CoA desaturase (SCD) is a long chain fatty acyl-CoA specific desaturase with a putative di-iron-oxo-containing active site.[1] SCD catalyzes the formation of a cis-double bond at the Δ9-position of the preferred substrate stearoyl-CoA. The resulting oleoyl-CoA is a major fuel intermediate for β-oxidation and a key substrate in triglyceride, cholesterol ester, phospholipid and lipid signaling molecule production. Four SCD isoforms have been characterized in rodents and two in humans.[2] SCD1, with ~85% identity across species, is the major isoform found in lipogenic tissues including liver and adipose. Emerging evidence supports the hypothesis that elevated SCD1 activity is a key player in the development of obesity, fatty liver, insulin resistance and related metabolic disorders.[3–5] In humans, elevated SCD activity is positively associated with a high body mass index, hyperinsulinemia, hypertriglyceridemia and liver steatosis.[5–7] Therefore, SCD1 inhibition may represent a novel treatment for obesity, diabetes, atherosclerosis and related metabolic disorders and to date, many groups have published on various SCD inhibitor scaffolds.[8–12]

Small molecule SCD inhibitors recently described in the literature, which are derived from pyridazine amides, (e.g., compound **1**) were found to be extensively metabolized on the alkyl chain of the amide.[13] To provide compounds suitable for in vivo models, it is necessary to transform the secondary amide into a metabolically more resistant chemical entity. As depicted in Table 1, removal of the alkyl chain (**2**) resulted in a modest potency loss when tested in a SCD1-induced rat liver microsomal assay.[14]

Potency can be improved by changing the linker between the piperazine and the trifluoromethylphenyl moiety. Replacement of the amide linker with an ether (**3**) improves the SCD1 potency by more than 20-fold in the rat microsomal assay. Additionally, the potency gain observed with the ether linker is retained when the metabolically labile alkyl chain of the secondary amide is removed (**4**).

Having metabolically stabilized two potentially vulnerable areas of the molecule, an evaluation of the pharmacokinetic profile in rats was performed with the primary amide **4**. Poor pharmacokinetic properties were still observed ($F = 10\%$, $t_{½} = 0.54$ h). This was attributed to hydrolysis of the primary amide to form the inactive acid metabolite **5**. This metabolite was shown to be the major circulating species with an AUC for compound **5** being 200-fold greater than the AUC of compound **4** (Fig. 1).

From this pharmacokinetic study, it became clear that the amide required replacement with a metabolically more robust moiety. We explored the use of an imidazole as an amide surrogate, a small heterocycle providing the required polarity. Replacement of the primary amide of **4** with an imidazole afforded **8p** (Table 2), which was demonstrated to be metabolically robust as determined by pharmacokinetic analysis in rats ($F = 53\%$,

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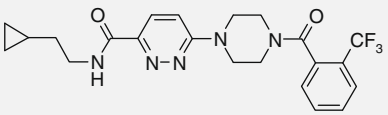
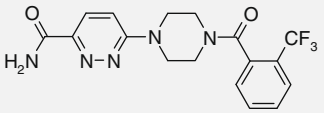
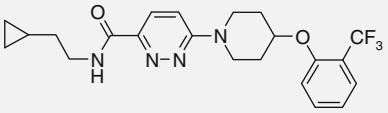
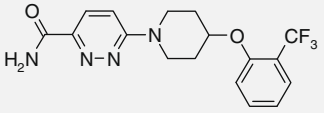
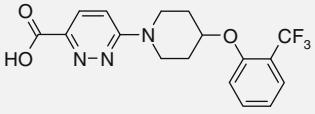
Table 1
SAR summary of amide-pyridazine compounds

		rSCD1 IC_{50} (nM)
1		322
2		692
3		14
4		16
5		~10,000

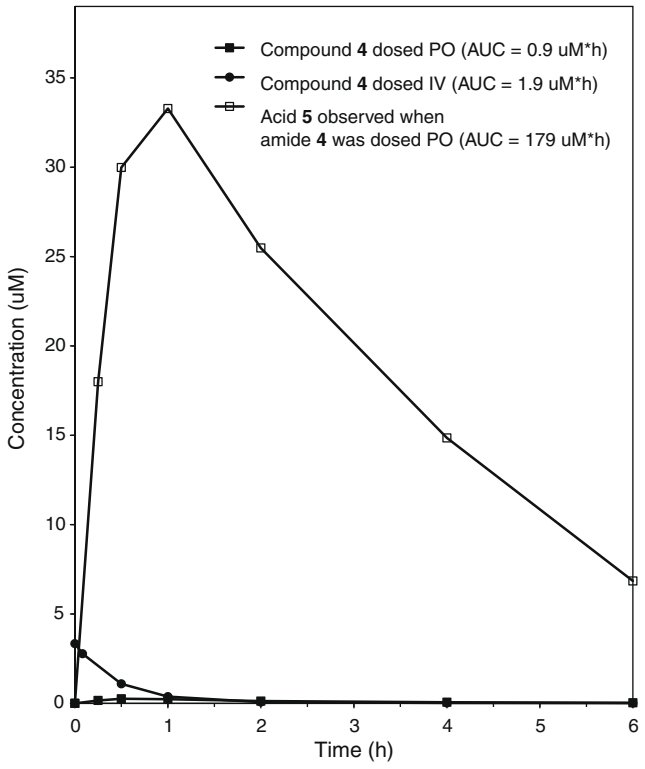
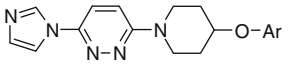


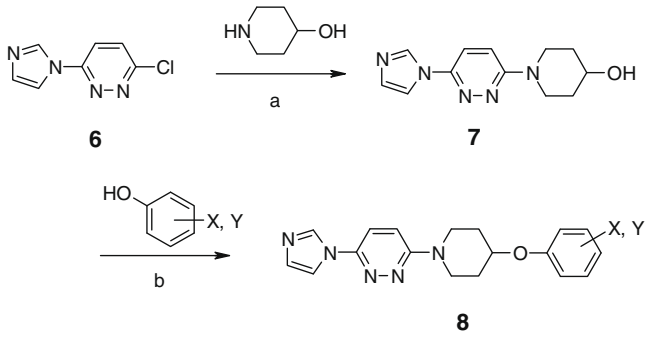
Figure 1. Plasma concentration versus time profile of compounds **4** (closed squares) and **5** (opened squares) when compound **4** is dosed in rats at 10 mg/kg PO and 2 mg/kg IV (closed circles). Concentrations are the average of two animals per time point.

$t_{½}$ = 1.2 h, data not shown). Having addressed the metabolic issues with this analog, we sought to explore the SAR around the aryl ring of the phenoxy-piperidine moiety. A series of phenyl ethers were rapidly accessed from the 4-piperidinol derivative **7**, prepared via a nucleophilic aromatic substitution from the commercially available 3-chloro-6-(1*H*-imidazol-1-yl)pyridazine **6** (Scheme 1). This was followed by a Mitsunobu reaction, which afforded a series of

Table 2
SAR summary of substitution on the phenoxy moiety in the imidazole–pyridazine series



Compound	Ar	rSCD1 IC_{50} (nM)
8a	Phenyl	1890
8b	2-Tolyl	39
8c	3-Tolyl	251
8d	4-Tolyl	527
8e	2-Ethylphenyl	4
8f	3-Ethylphenyl	38
8g	4-Indanyl	10
8h	5-Indanyl	62
8i	4-Indolyl	162
8j	2-Methoxyphenyl	543
8a	2-Cyanophenyl	236
8b	2-Acetylphenyl	34
8c	2-Bromophenyl	5
8d	3-Bromophenyl	23
8e	2-CF_3-phenyl	4
8f	3-CF_3-phenyl	19
8g	4-CF_3-phenyl	371



Scheme 1. Reagents and conditions: (a) TBAI, K_2CO_3, 1,4-dioxane, reflux 16 h. (b) DEAD, PPh_3, THF, room temperature, 16 h.

phenyl ethers (**8a–r**) required to establish the SAR of the phenoxy portion.

The potency of this class of inhibitors is strongly influenced by the substitution pattern on the phenyl ether portion (Table 2). This is clearly illustrated by comparing the unsubstituted phenyl ether (**8a**), with a compound containing even a simple methyl substituent (**8b**). In general *ortho* substituted aryl ethers were preferred over *meta* or *para* substituted analogs, regardless of the nature of the substituent. In addition, increasing the bulkiness of the substituent is also desirable in term of intrinsic potency. Bicyclic systems are also tolerated on the aromatic portion of these compounds, although introduction of polar heteroatoms appears to compromise their potency (**8g** vs **8i**). The introduction of heteroatoms on monocyclic systems was also evaluated: electron-donating (**8j**) or electron-withdrawing (**8k**) groups improve the potency relative to the unsubstituted phenyl but were less desirable compared to lipophilic groups. Halides (**8n**) and haloalkyls (**8p**) were found to be the preferred substituents; generally this type of substitution also affords metabolic robustness.

Following this analysis, compound **8p** was for further profiled in a variety of in vitro and in vivo assays. Unfortunately, it was found that **8p** inhibits cytochrome P450 enzymes (IC_{50} of 0.4 μM vs CYP3A4 and 0.3 μM vs CYP2D6). Based on literature precedent showing that imidazoles are often responsible for CYP inhibition,[15] the replacement of the imidazole heterocycle was investigated.

Ring systems of different size and polarity were evaluated for their ability to retain potency while reducing the potential for CYP inhibition. When bicyclic systems like benzothiazoles or benzimidazoles were used to replace the imidazole, the resultant loss in SCD potency prompted us to focus on small heterocycles (data not shown). Several small heterocycles were evaluated; some were not pursued due to potential metabolic stability issues like the 1,2,4-oxadiazoles,[16] while others did not provide the required potency profile. Table 3 illustrates a series of 1,3,4-oxa and thia-diazoles which demonstrated the targeted profile. Substitution at the 5-position was evaluated in order to optimize potency. Comparison of the unsubstituted oxadiazole **9a** with other 5-alkyl analogs reveals that substitution is beneficial for SCD potency.

However, comparison of analogs **b** with **d** and **e**, reveals that the size of the aliphatic substituent cannot be large. The potency is not significantly affected by addition of polarity in this area of the molecule and a hydroxymethyl group is well tolerated (**9g**).

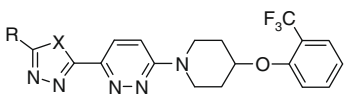
In the thiadiazole series the methyl analog **10b**, or MF-438, was identified as the most promising compound in this series. MF-438 is readily accessible from the methyl ester analog **11** of acid **5**[16] in three steps. First, conversion of the ester to a hydrazide followed by acetylation afforded compound **12**. Direct formation of **12** from ester **11** using acethydrazide consistently gave unsatisfactory yields and a stepwise approach was found to be more efficient. Finally the formation of the thiazole was achieved by refluxing compound **12** with phosphorus pentasulfide in a high boiling, non-polar solvent[17] (Scheme 2).

MF-438 has an overall good pharmacokinetic profile in rodents. Oral bioavailability was 73% in mice and 38% in rats with half-lives of 6.4 and 6.0 h, respectively. A circulating metabolite resulting from the oxidation of the methyl of the thiazole to the hydroxymethyl (**10g**) was observed in both species at all time points, but was present at only approximately 10% of the concentration of the parent compound.

The CYP inhibition issues encountered with the imidazole compound **8p** was resolved with the thiadiazole analog. MF-438 does not inhibit either CYP3A4 or CYP2D6 at concentrations up to 30 μM.

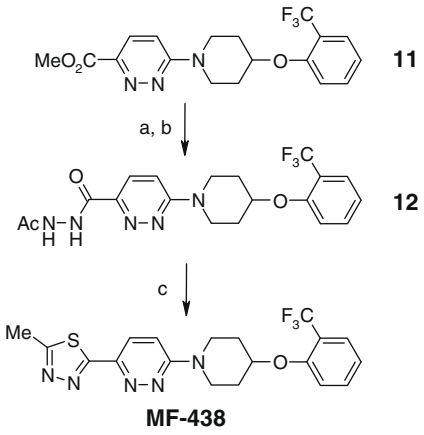
The long half-lives observed in rodents make this compound suitable for once-daily dosing and as such MF-438 became an excellent tool for in vivo assessment of SCD inhibition. Indeed, this compound was found to be very potent in vivo in a mouse liver PD assay which measures SCD inhibition in the liver of mice on a high carbohydrate diet.[18] The studied compound was administered PO followed by an IV administration of a ^{14}C labeled stearic acid tracer, 1 h later. After an additional 2 h, mouse livers were harvested and analyzed for their lipid content. Inhibition of SCD activity in the liver was determined by comparing the conversion of ^{14}C-stearic acid to ^{14}C-oleic acid of treated animals versus a vehicle control group. MF-438 exhibited an ED_{50} between 1 and 3 mg/kg in this mouse model.

Table 3
SAR summary of heterocyclic-pyridazine compounds



9 X = O
10 X = S

Compound	R	rSCD1 IC_{50} (nM)	
		X = O	X = S
a	H	22	—
b	Me	6.0	2.3 MF-438
c	*i*-Pr	3.9	8.8
d	*i*-Bu	15	37
e	*t*-Bu	980	710
f	OH	—	6.1
g	CH_2OH	2.9	3.2



Scheme 2. Reagents and conditions: (a) hydrazine hydrate, MeOH, reflux 2 h; (b) AcCl, Hunig's base, CH_2Cl_2, 1 h; (c) P_2S_5, xylene, 160 °C, 16 h.

Upon chronic dosing in animal models, this compound displayed adverse effects similar to other SCD inhibitors recently reported.[13,19] After approximately one week of qd dosing with MF-438 at 5 mg/kg in DIO mice, the first symptoms of alopecia and partial eye closure began to appear. The severity and the time at which these adverse effects were observed were directly related to the dose being administered. Similar adverse effect patterns were observed in other rodent models such as the obese diabetic Zucker rats.[20] Importantly, these adverse effects were shown to be reversible upon cessation of treatment.

In conclusion we have described the SAR which led to the identification of MF-438, a potent SCD inhibitor. The in vivo metabolic and pharmacokinetic profiles of MF-438 are greatly improved over previously described amide-based inhibitors, thus enabling this compound to serve as a valuable tool for in vivo assessment of SCD inhibition.

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