Discovery of Potent, Selective, Orally Bioavailable Stearoyl-CoA Desaturase 1 Inhibitors

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Received February 25, 2007

Stearoyl-CoA desaturase 1 (SCD1) catalyzes the committed step in the biosynthesis of monounsaturated fatty acids from saturated, long-chain fatty acids. Studies with SCD1 knockout mice have established that these animals are lean and protected from leptin deficiency-induced and diet-induced obesity, with greater whole body insulin sensitivity than wild-type animals. In this work, we have discovered a series of potent, selective, orally bioavailable SCD1 inhibitors based on a known pyridazine carboxamide template. The representative lead inhibitor **28c** also demonstrates excellent cellular activity in blocking the conversion of saturated long-chain fatty acid-CoAs (LCFA-CoAs) to monounsaturated LCFA-CoAs in HepG2 cells.

Introduction

Obesity is quickly becoming a global epidemic. According to the World Health Organization, there are more than one billion overweight adults, of which at least 300 million are clinically obese. Approximately two-thirds of U.S. adults are overweight or obese.[1] Obesity is a complex condition associated with an increased risk of developing numerous diseases, including type 2 diabetes, hypertension, cardiovascular diseases, and certain cancers. An increasing body of evidence indicates that alterations in intracellular lipid metabolism play a prominent role in the pathology of obesity.[2] Storage of even a modest caloric surplus in lean tissues, such as liver, muscle, and other peripheral tissues, leads to lipid-induced dysfunction in those tissues. Reducing fat accumulation in nonadipose sites appears to be of benefit in the treatment of lipotoxicity and can halt or reverse the progression of complications related to obesity.[3]

Stearoyl-CoA desaturase (SCD), also commonly known as Δ^9-desaturase, is a microsomal enzyme that catalyzes the committed step in the biosynthesis of mono-unsaturated fatty acids from saturated fatty acids. This oxidative reaction introduces a *cis*-double bond to a fatty acyl-CoAs.[4] The preferred substrates are palmitoyl- and stearoyl-CoAs, which yield palmitoleoyl- and oleoyl-CoA, respectively.[5] Other enzymes required for turnover of the catalytic cycle are NADH-cytochrome b_5 (cyt b_5) and cytochrome b_5 reductase (cyt b_5R).[5] The monounsaturated fatty acyl-CoAs produced by SCD are substrates for incorporation into a variety of lipids, including phospholipids, triglycerides, cholesterol esters, wax esters, and diacylglycerols.[6] Monounsaturated fatty acids may also serve as mediators of signal transduction, cellular differentiation, and apoptosis.[7]

Four SCD genes (SCD1, SCD2, SCD3, and SCD4) have been identified and characterized in mice.[8] SCD1 and SCD2 are the main isoforms expressed in mouse liver and brain, respectively. SCD3 is expressed exclusively in skin, whereas SCD4 is expressed predominantly in the heart. In certain tissues, such as adipose, skin, and eyelids, multiple SCD isoforms are expressed. The physiological relevance for having multiple SCD isoform expression in the same tissue remains elusive.

SCD expression is regulated by a number of nutritional and hormonal factors, including insulin, cholesterol, and polyunsaturated fatty acids.[9] Targeted deletion of the SCD1 gene in mice has shown that this enzyme is an important control point in lipid homeostasis[9] and body weight regulation.[10] SCD1 knockout mice showed increased energy expenditure,[11] reduced body adiposity,[12] and increased insulin sensitivity and are resistant to diet-induced obesity.[13] Such findings also corroborated with the phenotype observed in asebia mice carrying natural mutations in the SCD1 gene. The SCD1 knockout has also been crossed with genetically leptin-deficient *ob*/*ob* mice. SCD1 deficiency completely corrected the hypometabolic phenotype, hepatic steatosis, increased liver triglycerides, and VLDL production of *ob*/*ob* mice.[13] More recently, diet-induced obese (DIO) mice injected with antisense oligonucleotides against SCD1 have been shown to have reduced body adiposity and increased energy expenditure.[14]

Despite its involvement in regulating lipid metabolism, human SCD1 has been the only human SCD gene identified and characterized until recently. It shows 85% homology to the murine SCD1.[15] Reports of a second hSCD gene only appeared in the literature lately.[16] Information on the involvement of human SCD1 in obesity and related diseases is preliminary. For example, a positive correlation between SCD1 activity and plasma triglycerides in human hypertriglyceridemia was established,[17] and recently, a link between high muscle SCD1 activity and severe obesity in humans has been revealed.[18]

The genetic validation experiments suggest that reducing SCD1 activity might serve as a therapy for type 2 diabetes, dyslipidemia, and obesity. Small molecule SCD1 inhibitors would provide important pharmacological tools for fully assessing the therapeutic potential of modulating the SCD1 pathway. At the onset of our effort, there were very few reports of SCD1 inhibitors in the public domain. The first small molecule SCD1 inhibitors appeared in a series of patent applications published by Xenon Pharmaceuticals in 2005.[19,20] Compound **1** was highlighted in one of the patent applications with biological activity reported (Figure 1).[20] Because of our interest in evaluating SCD1 as a drug discovery target and our desire to use small molecules for in vivo target validation, we

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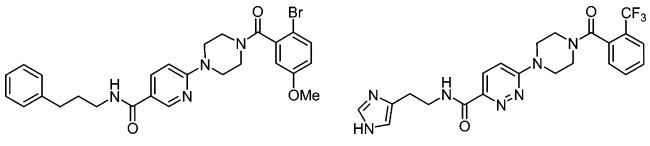
10.1021/jm070219p CCC: $37.00
Published on Web 05/27/2007



1
mSCD1 IC_{50} = 100 nM (US2005/0119251)
mSCD1 IC_{50} = 505 nM (Abbott)
0% remaining (30 min/MLM)
0% remaining (30 min/RLM)

2
mSCD1 IC_{50} = 950 nM (Abbott)
Cyp3A4 IC_{50} = 2900 nM (Abbott)
69% remaining (30 min/MLM)
88% remaining (30 min/RLM)

Figure 1. Small molecule SCD1 inhibitor and derivative disclosed in patent applications.

synthesized compound **1**. Compound **2** was also prepared based on the common fragments utilized in the patent application.[19a]

Our own profiling showed that both **1** and **2** inhibited mouse SCD1 with submicromolar IC_{50} values. Compound **1** was also shown to have very poor stability upon incubation with mouse (MLM) or rat (RLM) liver microsomes, making it a poor candidate for in vivo studies.[21] Compound **2** had much better microsomal stability, but it also showed fairly potent cytochrome p450 (Cyp) isoform 3A4 inhibition ($IC_{50} = 2.9\ \mu M$), hampering its utility in probing SCD1 inhibition in vivo. Our subsequent medicinal chemistry strategy focused on the creation of novel SCD1 inhibitors based on the pyridazine carboxamide template represented by **2** and the identification of suitable tool compounds for in vivo target validation. This paper will describe the discovery of highly potent, selective, orally bioavailable SCD1 inhibitors with robust cellular activity in HepG2 cells.

Chemistry

Starting from piperazine-1-carboxylic acid *tert*-butyl ester and 2-trifluoromethyl-benzoyl chloride, 1-(2-trifluoromethyl-benzoyl)-piperazine **3** could be prepared by a sequence of acylation and deprotection, as shown in Scheme 1. Analogously, 4-(2-trifluoromethyl-benzyl)-piperazine **4** was synthesized by sequential alkylation and deprotection using the 2-trifluoromethylbenzyl bromide. The preparation of pyridazine carboxamide analogs started from the previously known 6-chloropyridazine-3-carbonyl chloride **5**.[19a] Coupling of **5** with isoamyl amine yielded amide **6**. Subsequent nucleophilic substitution (SNAr) of the 6-chloropyradiazine with piperazine derivatives (**3** and **4**) or 4-hydroxypiperidine in a microwave reactor provided the desired pyridazine carboxamide analogs (**7** and **8**). The piperidinol **8** was then converted to the final phenoxypiperidine analogs **9** under Mitsunobu conditions with various phenols.

The second general synthesis of pyridazine carboxamide analogs is described in Scheme 2. The requisite phenoxypiperidine building blocks could be readily accessed starting from *tert*-butyl 4-hydroxypiperidine-1-carboxylate. Mitsunobu reaction of the *sec*-alcohol with different phenols could be effected to give the phenoxyethers **10**. Subsequent removal of the Boc group provided phenoxypiperidines **11** in good yield. Displacing the chloride of methyl 6-chloropyridazine-3-carboxylate **12**[19a] with **11** afforded esters **13**, then hydrolysis with NaOH yielded the carboxylic acids **14**. Condensation of **14** with amines afforded the pyridazine carboxamides **15**. Alternatively, SNAr of pyridazine **6b** and **6c** with phenoxypiperidines **11** also led to carboxamides **15**.

Heterocycles were introduced as replacements for the pyridazine carboxamides. 1,2,4-Oxadiazole pyridazines were prepared from primary amide **16**, as shown in Scheme 3. Dehydration of **16** with trifluoroacetic anhydride yielded the cyanopyridazine **17**. SNAr of **17** with phenoxypiperidines **11** provided piperidinopyridazines **18**. Addition of hydroxylamine to the nitrile generated the *N*′-hydroxyamidines **19**.[22] Acylation and cyclization of **19** with acid chlorides afforded the 3-pyridazine-1,2,4-oxadiazole analogs **20**. The cyano analog **18** could also be converted to the corresponding tetrazole **21** under the standard conditions.

To prepare 5-pyridazine-1,2,4-oxadiazoles, monochlorination of 6-oxo-1,6-dihydropyridazine-3-carboxylic acid followed by coupling of the resulting acid chloride with commercially available N′-hydroxyacetamidines generated 5-pyridazine-1,2,4-oxadiazoles **22** (Scheme 4). Chlorination of **22** with $POCl_3$ created 6-chloro-pyridiazines **23**. SNAr of **23** with phenoxypiperidines **11** yielded 5-pyridazine-1,2,4-oxadiazole analogs **24**.

1,3,4-Oxadiazole analogs were synthesized starting from commercially available 1-(6-chloropyridazin-3-yl)piperidin-4-ol (Scheme 5). Conversion of the *sec*-alcohol to aryl ether **25** proceeded under Mitsunobu conditions. Palladium-mediated carbonylation created the methyl ester **26**. Hydrolysis of the ester and coupling of the resultant acid with an acyl hydrazide yielded **27**. Cyclization using $POCl_3$ generated the desired oxadiazole **28**.[23] Subsequent aminolysis of **28** in a pressure reactor yielded the 1,3,4-triazole **29**.

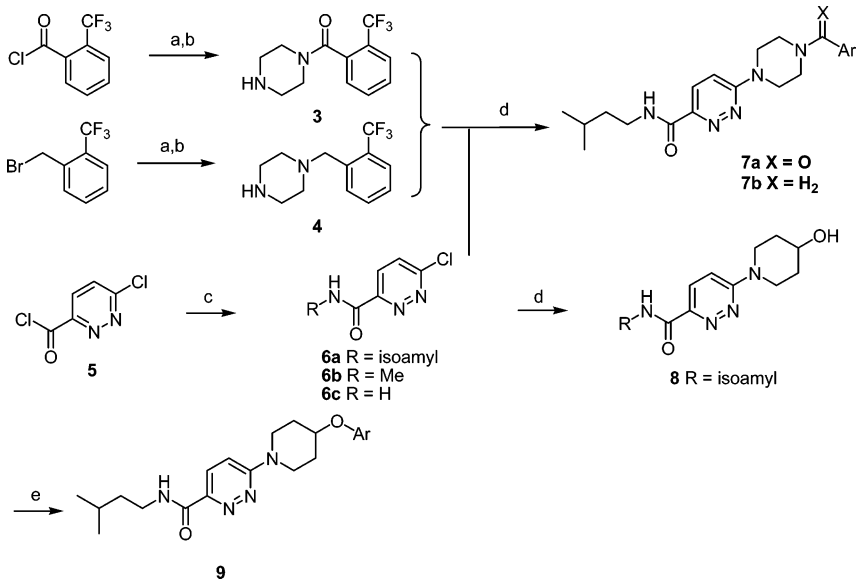
The synthesis of furan- or pyridine-substituted pyridazines could be realized starting from known 3-chloro-6-iodo-pyridazine **30** (Scheme 6).[24] Suzuki coupling of the iodide **30** with 3-furanboronic acid or 3-pyridineboronic acid afforded chloropyridazines **31**. Displacement of the chloride of **31** with phenoxypiperidines **11** provided SCD1 inhibitors **32**. Pyrrole-, pyrazole-, and imidazole-substituted pyridazines could be synthesized starting from the appropriately substituted pyridazine chlorides **33**, as described in the literature, respectively.[25−27] Nucleophilic substitution of the chloride of **33** with **11** led to the desired pyridazines **34**.

Results and Discussion

In our work, the basis for the primary biochemical assay was measuring the decreased production of tritiated water from the desaturation of [^{3}H]-(9,10) stearoyl-CoA substrate to oleoyl-CoA mediated by SCD1. Two sources of SCD1 were used: the human SCD1 was recombinantly expressed in insect cells in tandem with human cyt b_5 and human cyt b_5R; and the mouse version of SCD1 was derived from *ob*/*ob* mouse liver microsome preparation.

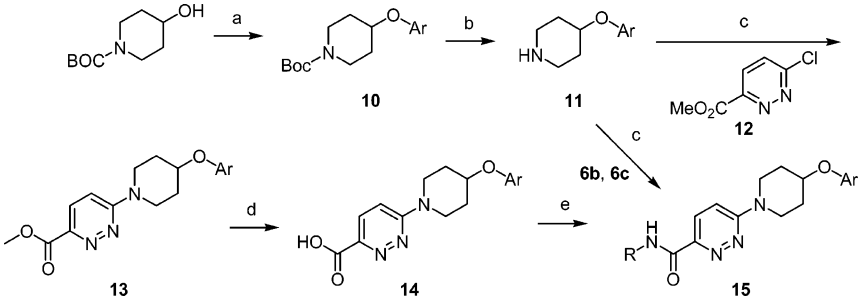
The initial structure−activity relationships (SAR) focus was on modification of the piperazine portion of **1** to boost the inhibitory activity against SCD1 and to generate analogs with improved metabolic stability. To simplify SAR comparison, pyridazine isoamyl carboxamide was selected as the common left-hand motif because it was the most frequently described motif in those patent applications.[19,20] As shown in Table 1, **7a** was synthesized based on template and was shown to have a similar mouse SCD1 IC_{50} as **2** although its human SCD1 IC_{50} was 8-fold weaker. Changing from the piperazine benzamide **7a** to the corresponding benzyl amine **7b** did not really affect the mouse SCD1 potency but did improve the IC_{50} versus human SCD1 by about 70-fold. The microsomal stability of **7b** only improved slightly compared to that of **1**. Moving away from the piperazine structure, we made the critical observation that changing the piperazine benzylamine of **7b** to piperidine ether (**9a**) boosted the mouse SCD1 IC_{50} 6-fold. We do not quite understand the reason behind the difference between human and mouse SCD1 IC_{50}s for **9a** and related compounds. The difference could be related to the different enzyme preparation (recombinant form for human vs liver microsome derived mouse form) or the sequence difference between human SCD1 and mouse ortholog (85% homology in the amino acid sequence).

Scheme 1[a]



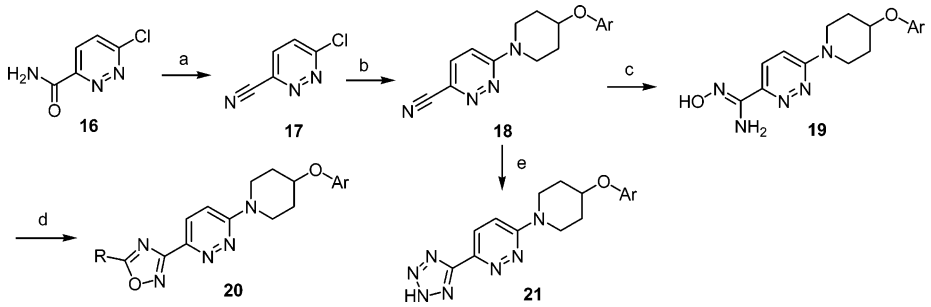
[a] Reagents and conditions: (a) piperazine-1-carboxylic acid *tert*-butyl ester, Et_3N, THF, 0−25 °C; (b) 4 N HCl/1,4-dioxane, 0−25 °C; (c) isoamyl amine, CH_2Cl_2, *i*-Pr_2NEt, 25 °C; (d) **3**, **4,** or 4-hydroxypiperidine, Et_3N, CH_3CN, 180 °C (μW); (e) ArOH, DBAD, Ph_3P, THF, 25 °C.

Scheme 2[a]



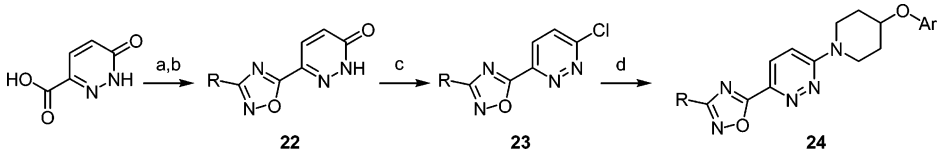
[a] Reagents and conditions: (a) ArOH, DEAD, Ph_3P, THF, 25 °C; (b) 4 N HCl, 1,4-dioxane, 25 °C; (c) *i*-Pr_2NEt, DMF, 125 °C; (d) NaOH, MeOH/THF, 25 °C; (e) R-NH_2, TBTU, Et_3N, DMF, 25 °C.

Scheme 3[a]



[a] Reagents and conditions: (a) TFAA, pyridine, −14 °C; (b) **11,** Et_3N, CH_3CN, 180 °C (μW); (c) NH_2OH HCl, Et_3N, EtOH/CH_2Cl_2, 25 °C; (d) RCOCl, pyridine, 90 °C; (e) NaN_3, *n*-Bu_3SnCl, toluene, 120 °C.

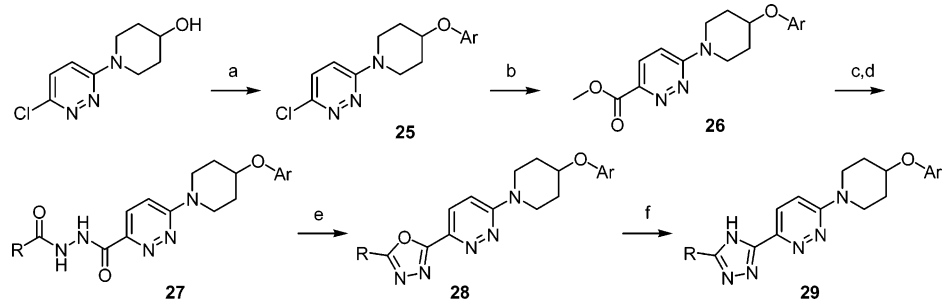
Scheme 4[a]



[a] Reagents and conditions: (a) $SOCl_2$, $CHCl_3$, reflux; (b) *N*′-hydroxyacetamidines, *i*-Pr_2NEt, CH_3CN, 125 °C, (μW); (c) $POCl_3$, 110 °C; (d) **11**, *i*-Pr_2NEt, CH_3CN, 160 °C.

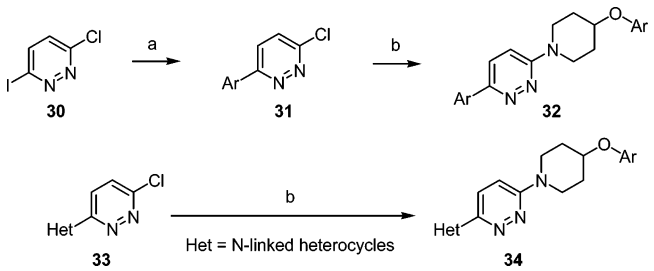
Encouraged by the new SAR information regarding **9a**, we explored the SAR of piperidine aryl ether more broadly. Both 2- and 3-fluorinated phenoxypiperidine derivatives (**9b**−**9c**) exhibited 3−5-fold potency improvement in mouse SCD1 IC_{50}s over the unsubstituted phenoxypiperidine **9a**, respectively, while the 4-fluorophenoxypiperidine (**9d**) was equipotent to **9a**. On the other hand, the IC_{50} values of **9b**−**d** against human SCD1 showed a 10−15-fold improvement over that of **9a**. Among the

Scheme 5[a]



[a] Reagents and conditions: (a) DEAD, Ph_3P, THF, 25 °C; (b) $PdCl_2(dppf)CH_2Cl_2$, CO (g), MeOH, 100 °C; (c) NaOH, MeOH/THF, 25 °C; (d) acyl hydrazide, TBTU, Et_3N, DMF, 25 °C; (e) $POCl_3$, 100 °C; (f) NH_3, MeOH, 100 °C.

Scheme 6[a]



[a] Reagents and conditions: (a) $ArB(OH)_2$, $PdCl_2(PPh_3)_2$, $NaHCO_3$, DME/H_2O/EtOH, 150 °C, (μW); (b) **11**, *i*-Pr_2NEt, CH_3CN, 125 °C, (μW).

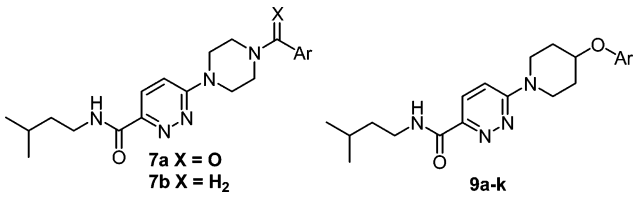
difluorinated phenoxypiperidine analogs, 2,3-difluoro (**9e**) and 2,5-difluoro (**9g**) phenoxypiperidine derivatives exhibited similar potency to 2-fluorinated **9b**. Interestingly, 2,4-difluorinated analog **9f** was inactive against SCD1, while 2,6-difluorination (**9h**) also reduced the inhibitory potency against SCD1. Consistent with the SAR trend observed for the 2-fluorinated analog **9b**, 2-trifluoromethyl- (**9i**) and 2-chloro- (**9j**) substituted phenoxypiperidine derivatives were also potent SCD1 inhibitors against mouse SCD1, with some deterioration in potency against human SCD1. 2,5-Dichlorophenoxypiperidine derivative **9k** was found to be slightly more potent than the 2-chloro analog **9j** against human SCD1, with virtually no difference in mouse SCD1 IC_{50}. Based on these observations, the ensuing SAR exploration was conducted using either 2- or 2,5-disubstituted phenoxypiperidine as the preferred structure for generating potent SCD1 inhibitors, which would also take advantage of the ability of the 2-substituent to block the potential phenoxy ring oxidation.

Despite the fact that the phenoxypiperidine analogs offered better in vitro potency, the metabolic stability of the resulting analogs did not improve at all. As shown in Table 1, among all the analogs tested (**9a**−**e**, **9g**, **9j**−**k**), less than five percent of parent compounds remained when incubated for 30 min with either mouse or rat liver microsomes. We suspected that the hydrophobic isoamyl carboxamide might be the culprit for the metabolic instability. Therefore, we sought to modify the left-hand portion of these inhibitors.

When isoamyl amide **9b** was replaced with methyl amide **15a**, the SCD1 activity decreased by 50−70-fold versus both mouse and human enzymes, respectively (Table 2). Fortunately, changing the 2-substituent of the phenoxypiperidine from fluorine (**15a**) to chlorine (**15b**) dramatically boosted the mouse IC_{50} by over 100-fold and the human IC_{50} by over 20-fold. More excitingly, the pyridazine methylamide **15b** was shown to exhibit much-improved metabolic stability in mouse/rat liver microsomes (Table 2). Consistent with the previously observed phenoxypiperidine preference for ortho substituents, 2-bromo analog **15c** showed a 2-fold improvement over **15b**, while the 2-cyano compound **15d** was substantially less active against mouse SCD1. The 2,5-disubstituted phenoxypiperidine analogs **15e**,**f** also yielded inhibitors with single digit nanomolar IC_{50}s against mouse SCD1 and double-digit nanomolar IC_{50}s versus human SCD1. The increased lipophilicity of **15c**−**f** did not affect their microsomal metabolic stability, suggesting that modulation of the physiochemical properties of this series of compounds was feasible without compromising the metabolic stability. We next demonstrated that the primary pyridazine carboxamides (**15g**,**h**) possessed the same degree of potent SCD1 inhibitory activity and high metabolic stability as the corresponding methyl amides **15b** and **15f**. Using the 2-chlorophenoxypiperidine as the constant motif, the SAR of a variety of different pyridazine amides was further explored. Small hydroxyethylamide **15i** retained most of the SCD1 potency, while larger, nonaromatic groups (**15j**,**k**) were generally not favored. The lipophilic thiophene-derived **15l** was shown to possess high SCD1 potency yet with poor microsomal stability. The histamine-based **15m** was equipotent as **15l** with substantially higher microsomal stability. Unfortunately, it was also found to inhibit Cyp3A4 with an IC_{50} of 0.19 μM. A large variation of potency and metabolic stability was observed with heteroarylmethylamine-based pyridazine carboxamides. 3-Pyridinylmethyl amide **15n** was quite potent against mouse SCD1, but the human SCD1 IC_{50} dropped by over 20-fold in comparison to the methyl amide **15b**. The loss of human SCD1 potency could be recovered by changing the 3-pyridinyl to the 3-methylisoxazolyl (**15o**), 3-methylpyrazolyl (**15p**), and 2-imidazolyl (**15q**) amides. However, the microsomal stability of these heteroaryl-based pyridazine carboxamides deteriorated in comparison to the smaller amides **15b** and **15g**.

Concurrent to the pyridazine carboxamide SAR exploration, we also investigated different heterocycles as amide replacements. Because the methyl ester **13b** (Ar = 2-chlorophenyl, IC_{50} < 40 nM versus mouse SCD1) was as potent as the corresponding methyl carboxamide **15b**, we postulated that the hydrogen-bond donating character of the amide might not be critical for retaining the SCD1 inhibitory activity. 1,2,4-Oxadiazole, a well-known ester mimic,[28] was then incorporated into the pyridazine-based SCD1 inhibitors. As shown in Table 3, 5-methyl-, 5-trifluoromethyl, and 5-propyl-1,2,4-oxadiazoles (**20a**−**c**) were substantially less potent than the corresponding 5-benzyl-1,2,4-oxadiazole **20d** in the context of unsubstituted phenoxypiperidine, suggesting that an additional hydrophobic interaction with SCD1 could be exploited. The addition of a 2-chloro substituent (**20e**) to **20a** boosted the SCD1 IC_{50} by more than 20-fold. Unfortunately, this series of 1,2,4-oxadiazole analogs appeared to have worse microsomal stability than the methyl amide **15b** (Table 3 **20a**−**e** vs Table 2 **15b**).

Table 1. SAR about Pyridazine Isoamyl Carboxamides



Compd	Ar	Mouse SCD1 IC_{50}[a] (nM)	Human SCD1 IC_{50}[a] (nM)	% Remaining (MLM)	% Remaining (RLM)
7a	2-trifluoromethylphenyl	627 ± 210	5261	NT[b]	NT
7b	2-trifluoromethylphenyl	394 ± 265	70	14	18
9a	Phenyl	63 ± 147	1518	0.85	0.65
9b	2-fluorophenyl	20 ± 11	95 ± 4	0.2	10
9c	3-fluorophenyl	12 ± 9	89 ± 36	0.4	2.8
9d	4-fluorophenyl	65 ± 47	174 ± 20	0.67	1.98
9e	2,3-difluorophenyl	19	119 ± 28	0.48	3.22
9f	2,4-difluorophenyl	>1000	NT	NT	NT
9g	2,5-difluorophenyl	14 ± 10	55 ± 5	0.9	17
9h	2,6-difluorophenyl	150	7093	NT	NT
9i	2-trifluoromethylphenyl	34	504 ± 220	NT	NT
9j	2-chlorophenyl	21 ± 4	315 ± 87	0.8	1.4
9k	2,5-dichlorophenyl	16 ± 2	165 ± 40	1.4	13

[a] Values are the geometric means of at least two experiments, with standard errors given. [b] NT = not tested.

5-Pyridazine-1,2,4-oxadiazoles were also prepared and evaluated. 3-Benzyl-1,2,4-oxadiazole **24a** showed similar mouse SCD1 potency to **20d** but with a dramatic decrease of human SCD1 potency (Table 3). The addition of a 2-fluoro (**24b**) or 2-chloro substituent (**24c**) recovered some of the lost human SCD1 potency without compromising the mouse SCD1 activity. 3-Methyl-5-pyridazine-1,2,4-oxadiazoles (**24d**,**e**) exhibited similar SCD1 potency to 5-methyl-1,2,4-oxadiazole **20e**, while methoxy-substituted **24f** was less potent. Among this series of oxadiazole analogs, only **24b** and **24f** reached the desired level of microsomal stability.

We then profiled the 1,3,4-oxadiazole regioisomer. As shown in Table 3, 5-benzyl-1,3,4-oxadiazole **28a** was a potent SCD1 inhibitor, although it still suffered from poor metabolic stability. The 5-methyl-1,3,4-oxadiazole **28b** showed a 5-fold decrease in human SCD1 inhibitory activity compared to **28a**, despite the much higher microsomal stability. Combination with 2-chloro-5-fluorophenoxypiperidine further improved the SCD1 potency of **28b** to give compound **28c**, which also possessed excellent mouse and rat microsomal stability.

Based on the favorable in vitro profiles exhibited by the oxadiazoles, we expanded our SAR effort to profile additional heterocycles in place of the carboxamides. As shown in Table 4, highly potent SCD1 inhibitors were obtained using different carbon-linked heterocycles, such as 5-methyl-1,3,4-triazole **29**, 3-furan **32a**, and 3-pyridine **32b**. The acidic tetrazolylpyridazine **21** was shown to be a weaker mouse SCD1 inhibitor. We also found that heterocycles, such as pyrole, imidazole, and pyrazole, linked to the pyridazine core via the nitrogen atom could afford equally potent SCD1 inhibitors (**34a**−**e**) against both mouse and human SCD1, with the exception of **34b** versus human SCD1. It was also encouraging to note that the presence of the imidazole moiety in **34c**−**e** not only preserved the good metabolic stability, but also resulted in no significant inhibition of the major isoforms of CypP450 (3A4, 2D6) when tested at 10 μM.

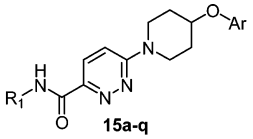
Pharmacokinetic (PK) profiles were determined for potent SCD1 inhibitors **15b**, **15g**, **24b**, **28c**, **34c**, and **34d** that also demonstrated good liver microsomal stability in Sprague−Dawley rats (Table 5). It is clear from this group of compounds that liver microsomal stability does not always predict in vivo clearance value (Clp). Inhibitors **15b**, **15g**, and **24b** demonstrated high intrinsic clearance from IV dosing, which contributed to the low oral exposure and the barely measurable oral bioavailability. 1,3,4-Oxadiazole **28c** and imidazole-N-linked pyridazine **34d** showed a much reduced clearance value in IV dosing and good oral area under the curve (AUC) and high oral bioavailability. For the imidazole-N-linked pyridazine-based compound **34c**, even though the absolute bioavailability is over 50%, the high IV clearance value was reflected in the lower oral Cmax and substantial lower oral AUC in comparison to **28c** and **34d**. The oral bioavailability and reasonable drug exposures demonstrated with **28c** and **34d** make them suitable candidates for further in vivo testing.

After demonstrating the in vitro high potency and favorable PK profiles for SCD1 inhibitor **28c**, we turned our attention to confirming that **28c** is selective for inhibiting SCD1. Cerep HTP screen[29] results for inhibitor **28c** were obtained. The cross-activity of **28c** was found to be minimal against 75 different receptors, transporters, and ion channels. The only "hits" (% inhibition values > 50% at 10 μM) were for human dopamine transporter and 5-HT transporter, with 92% and 51% inhibition of the control-specific binding at 10 μM, respectively.

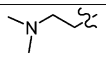
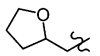
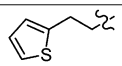
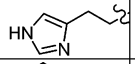
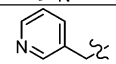
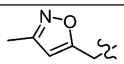
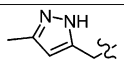
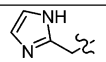
Because the SCD1 enzymatic activity depends on the activity of the co-enzymes cyt b_5 and cyt b_5R, we needed to rule out the possibility that **28c** may actually inhibit either reductase. To that end, an assay using the conversion of sulfamethoxazole hydroxylamine (SMX-HA) to sulfamethoxazole in human liver microsomes to measure cyt b_5/cyt b_5R activity was used.[30] Inhibitor **28c** was unable to inhibit the cyt b_5/cyt b_5R activity in this assay when tested at 10 and 50 μM (data not shown). The results support the specificity of this compound for direct SCD1 inhibition.

The cellular activity of SCD1 inhibitor **28c** was assessed by detecting the conversion of saturated LCFA-CoAs to monounsaturated LCFA-CoAs. HepG2 cells, a human hepatoma cell line, were incubated with compound **28c**, 0.1 mM heptadecanoic acid (17:0), and [^{13}C]-labeled palmitic acid (16:0) for 6 h. The LCFA-CoAs were then extracted from the cells as reported previously.[31] As the endogenous levels of C17:0 and [^{13}C]-labeled C16:0 fatty acids are extremely low, measurement of cellular levels of C17:0, [^{13}C]-C16:0, along with C17:1 and

Table 2. SAR about Other Pyridazine Carboxamides



15a-q

Compd	R_1	Ar	Mouse SCD1 IC_{50}[a] (nM)	Human SCD1 IC_{50}[a] (nM)	% Remaining (MLM)	% Remaining (RLM)
15a	Me	2-fluorophenyl	980	7384 ± 416	NT[b]	NT
15b	Me	2-chlorophenyl	8.5 ± 3.0	324 ± 58	75.8	64.1
15c	Me	2-bromophenyl	3.0	140	86	51
15d	Me	2-cyanophenyl	250	480	92	82
15e	Me	2,5-dichlorophenyl	7.0	55 ± 5	66	53.1
15f	Me	2-chloro-5-fluoro-phenyl	5.0	43 ± 7	78.9	62.5
15g	H	2-chlorophenyl	7.1 ± 2.5	65 ± 9	78.5	57.9
15h	H	2-chloro-5-fluoro-phenyl	1.0	25 ± 8	80.0	92.2
15i	Hydroxyethyl	2-chlorophenyl	57	785 ± 80	NT	NT
15j		2-chlorophenyl	>10,000	NT	NT	NT
15k		2-chlorophenyl	1,970	82% inh@10 μM	NT	NT
15l		2-chlorophenyl	10 ± 3	82 ± 2	6.4	6.1
15m		2-methoxyphenyl	6	71 ± 19	61.2	63.6
15n		2-chlorophenyl	24	8360	13.9	63.4
15o		2-chlorophenyl	10	155 ± 48	27.6	31
15p		2-chlorophenyl	70	271 ± 241	28.3	27.4
15q		2-chlorophenyl	60	478 ± 661	44	47.8

[a] Values are the geometric means of at least two experiments, with standard errors given. [b] NT = not tested.

[^{13}C]-C16:1 generated by intracellular SCD1 provided a sensitive means to detect cellular SCD1 activity inhibition with minimum background. Desaturation index (DI), defined as the ratio of C17:1/C17:0 or [^{13}C]-C16:1/[^{13}C]-C16:0 in this case, can be used as a distinct biomarker for gauging the cellular SCD activity. Inhibitor **28c** showed a dose-dependent inhibition of SCD1-mediated conversion of saturated LCFA-CoAs to monounsaturated LCFA-CoAs in HepG2 cells (Figure 2). The calculated cellular IC_{50}s of **28c** (7.9 nM as measured by C17:1/C17:0, or 6.8 nM as measured by [^{13}C]-C16:1/[^{13}C]-C16:0) for decreasing DI was very similar to the IC_{50} obtained in the human SCD1 enzymatic assay (IC_{50} = 26 nM).

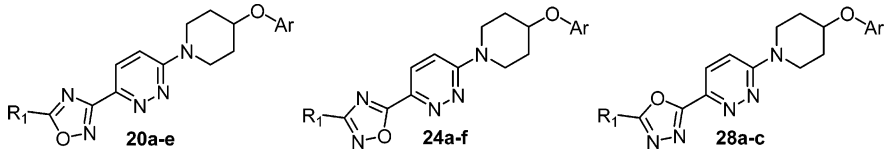
Conclusions

We have identified a series of pyridazine heteroaryl-based highly potent, selective, orally bioavailable SCD1 inhibitors.[32] Metabolically stable pyridazine analogs were realized by the replacement of carboxamides with heterocycles. The representative compound **28c** was shown to be selective for SCD1 and to possess a good PK profile. Inhibitor **28c** also showed robust cellular activity in inhibiting the conversion of saturated LCFA-CoA to monounsaturated LCFA-CoA in HepG2 cells. The combined in vitro potency/selectivity/cellular activity and PK profiles of **28c** rendered it an ideal pharmacological tool for elucidating the role SCD1 plays in obesity, diabetic, and dyslipidemia models.

Experimental Section

General. Column chromatography was performed under 5 psi N_2 using 230−400 mesh silica gel or with an Analogix Intelli Flash 280 MPLC system using prepacked, disposable silica gel columns. Analytical HPLC traces and compounds purified by HPLC were obtained by elution through a C_{18} reverse-phase column with a gradient of 5 mM aq ammonium acetate/ CH_3CN or 0.1% aq TFA/ CH_3CN as eluents. Concentration gradients started at 0% CH_3CN

Table 3. SAR about Oxadiazole Pyridazines



Compd	R_1	Ar	Mouse SCD1 IC_{50}[a] (nM)	Human SCD1 IC_{50}[a] (nM)	% Remaining (MLM)	% Remaining (RLM)
20a	Me-	phenyl	220	5805 ± 1074	NT[b]	NT
20b	CF_3-	phenyl	820	7050	27.7	27.1
20c	*n*-Pr-	phenyl	1330	NT	NT	NT
20d	$PhCH_2$-	phenyl	< 40	35 ± 25	1.2	0.6
20e	Me-	2-chlorophenyl	10	76 ± 29	33.1	NT
24a	$PhCH_2$-	phenyl	10	4860	NT	NT
24b	$PhCH_2$-	2-fluorophenyl	7.4 ± 1.3	397 ± 190	64.8	94.7
24c	$PhCH_2$-	2-chlorophenyl	26 ± 20	201 ± 75	62.9	64.3
24d	Me-	2-chlorophenyl	20	70	11.6	33.9
24e	Me-	2-methylphenyl	5.3 ± 1.5	140 ± 52	12.4	26.1
24f	Me-	2-methoxyphenyl	85	1706 ± 327	53.5	68.4
28a	$PhCH_2$-	2-chlorophenyl	12	41 ± 42	9.5	5.6
28b	Me-	2-chlorophenyl	NT	209 ± 277	80.2	73.1
28c	Me-	2-chloro-5-fluoro-phenyl	4.5 ± 4.0	26 ± 9	80.9	86.4

[a] Values are the geometric means of at least two experiments, with standard errors given. [b] NT = not tested.

and 100% aqueous medium. Microwave heating was performed using a Personal Chemistry Emrys Optimizer microwave apparatus, and reaction pressures were not allowed to exceed 20 atm. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons, coupling constants in Hertz. Where mixtures of stereoisomers or rotamers were obtained, the ^{1}H NMR resonances of the major species are reported, unless otherwise indicated.

1-(2-Trifluoromethyl-benzoyl)-piperazine Hydrochloride (3). To a solution of piperazine-1-carboxylic acid *tert*-butyl ester (2.50 g, 13.4 mmol) and triethylamine (2.83 mL, 20.0 mmol) in THF (40 mL) at 0 °C was added 2-trifluoromethyl-benzoyl chloride (1.98 mL, 13.4 mmol). The mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was concentrated in vacuo, extracted with EtOAc (80 mL), washed with satd $NaHCO_3$ (2 × 75 mL), and water (50 mL). The crude extract was concentrated in vacuo and then dissolved in THF (20 mL), cooled to 0 °C, and 4 M HCl (14 mL) was added. The mixture was warmed to room temperature and stirred until precipitate had formed (5 h). The crude reaction mixture was then concentrated and dried overnight in vacuo. The crude reaction mixture was then triturated with CH_3CN (20 mL) to provide **3** as a white solid (2.58 g, 65%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.29 (s, 2H), 7.87−7.73 (m, 2H), 7.72−7.56 (m, 2H), 4.07−3.92 (m, 1H), 3.79−3.65 (m, 1H), 3.35−3.02 (m, 5H), 3.00−2.85 (m, 1H); MS (ESI) *m*/*z* 259 (M + H)$^+$.

4-(2-Trifluoromethyl-benzyl)-piperazine Hydrochloride (4). To a solution of piperazine-1-carboxylic acid *tert*-butyl ester (652 mg, 3.5 mmol) and triethylamine (487 μL, 3.5 mmol) in THF (7 mL) was added 2-(trifluoromethyl) benzyl bromide (837 mg, 3.5 mmol) at room temperature. The reaction mixture was stirred at room temperature for 3 d and then partitioned between satd $NaHCO_3$ (20 mL) and EtOAc (50 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography eluting with 20% EtOAc in hexanes to provide 4-(2-trifluoromethyl-benzyl)-piperazine-1-carboxylic acid *tert*-butyl ester as a colorless oil. This compound was then treated with 4 N HCl in dioxane (10 mL) at room temperature for 3 h and concentrated in vacuo and coevaporated with CH_3CN (3 mL) to provide **4** as a white solid (1.04 g, yield 94%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.53 (s, 1H), 9.09 (s, 2H), 7.93 (s, 1H), 7.78−7.70 (m, 2H), 7.58−7.53 (m, 1H), 3.92−4.07 (m, 1H), 3.33 (s, 1H), 3.28−3.13 (m, 5H), 2.85−3.00 (m, 3 H); MS (ESI) *m*/*z* 245 (M + H)$^+$.

6-Chloro-*N*-isopentylpyridazine-3-carboxamide (6a). 6-Chloropyridazine-3-carbonyl chloride (3.54 g, 20 mmol) was dissolved in CH_2Cl_2 (70 mL) and added dropwise to a stirred solution of isoamylamine (92.7 mL, 30 mmol) and triethylamine (5.0 mL, 29 mmol) in CH_2Cl_2 (150 mL). After stirring at room temperature for 90 min, the black homogeneous solution was washed with 1 N HCl (200 mL), satd $NaHCO_3$ (200 mL), and brine (200 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was recrystallized with CH_3CN to provide **6** as a white solid (4.35 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.25 (t, J = 6.1 Hz, 1H), 8.22 (d, J = 9.0 Hz, 1H), 8.09 (m, 1H), 3.38−3.32 (m, 2H), 1.59 (dq, J = 13.3, 6.5 Hz, 1H), 1.49−1.42 (m, 2H), 0.90 (d, J = 6.8 Hz, 6H); MS (ESI) *m*/*z* 228 (M + H)$^+$.

Compounds 6b and 6c. The general procedure described for **6a** was followed for the preparation of these two compounds, using the appropriate amines for the coupling.

6-Chloro-*N*-methylpyridazine-3-carboxamide (6b). White solid (88%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (m, 1H), 9.22 (d, J = 9.0 Hz, 1H), 8.09 (d, J = 9.0 Hz, 1H), 2.86 (d, J = 4.8 Hz, 3H); MS (ESI) *m*/*z* 196 (M + H)$^+$.

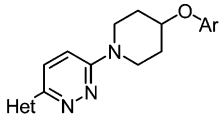
6-Chloropyridazine-3-carboxamide (6c). White powder (98%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (br s, 1H), 8.10 (d, J = 9.0 Hz, 1H), 8.23 (d, J = 9.0 Hz, 1H), 8.58 (br s, 1H); MS (ESI) *m*/*z* 158 (M + H)$^+$.

***N*-Isopentyl-6-(4-(2-(trifluoromethyl)benzoyl)piperazin-1-yl)-pyridazine-3-carboxamide (7a).** A mixture of **6a** (22.8 mg, 0.1 mmol), **3** (29.5 mg, 0.1 mmol), and *N*,*N*-diisopropylethylamine (50 μL, 0.29 mmol) in CH_3CN (1 mL) was heated in a microwave reactor at 160 °C for 20 min. The reaction mixture was dissolved in EtOAc (10 mL), washed with brine (5 mL), and then concentrated in vacuo. The residue was purified by silica gel flash chromatography, eluting with a gradient of 0−40% EtOAc in hexanes to yield **7a** as a white solid (24.3 mg, 54%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.79 (t, J = 6.0 Hz, 1H), 7.86 (t, J = 8.7 Hz, 2H), 7.78 (t, J = 7.5 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.36 (d, J = 9.5 Hz, 1H), 3.80−3.91 (m, 4H), 3.77−3.60 (m, 2H), 3.33−3.17 (m, 4H), 1.65−1.53 (m, 1H), 1.48−1.37 (m, 2H), 0.89 (d, J = 6.7 Hz, 6H); MS (ESI) *m*/*z* 436 (M + H)$^+$. Anal. ($C_{17}H_{18}ClFN_4O_2 \cdot 0.2H_2O$) C, H, N.

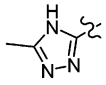
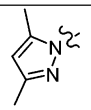
Compounds 7b and 9a. The general procedure described for **7a** was followed for the preparation of these compounds, using the appropriate piperazine or piperidine.

***N*-Isopentyl-6-(4-(2-(trifluoromethyl)benzyl)piperazin-1-yl)-pyridazine-3-carboxamide (7b).** White solid (56%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.80−8.71 (m, 1H), 7.89−7.80 (m, 2H), 7.75−7.64 (m, 2H), 7.49 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 9.8 Hz, 1H), 3.80−3.65 (m, 6H), 3.40−3.25 (m, 4H), 2.59−2.51 (m, J = 4.8

Table 4. SAR about Other Heteroaryl Pyridazines



21, 29, 32a-b, 34a-e

Compd	Het	Ar	Mouse SCD1 IC_{50}[a] (nM)	Human SCD1 IC_{50}[a] (nM)	% Remaining (MLM)	% Remaining (RLM)
21		2-chlorophenyl	370	NT[b]	NT	NT
29		2-chloro-5-fluoro-phenyl	4 ± 4	208 ± 21	64.4	94.7
32a		2-chlorophenyl	9.2 ± 0.2	88 ± 12	5.0	5.8
32b		2-chlorophenyl	< 4.0	28 ± 35	NT	NT
34a		2-chlorophenyl	10	76 ± 29	13.3	13.3
34b		2-chlorophenyl	32	712 ± 365	19.4	21.7
34c		2-chlorophenyl	20	91 ± 6	56.5	NT
34d		2-chloro-5-fluoro-phenyl	< 4.0	14 ± 5	71.3	64.5
34e		2-chloro-5-fluoro-phenyl	NT	17 ± 10	51.5	46.0

[a] Values are geometric means of at least two experiments with standard errors given. [b] NT = not tested.

Table 5. Pharmacokinetic Parameters for SCD1 Inhibitors in Sprague−Dawley Rats (5 mg/kg Dose)

	IV[a]					PO[a]				
cmpd	$t_{1/2}$[b]	V_{ss}[c]	V_b[d]	AUC[e]	CL_p[f]	$t_{1/2}$[g]	C_{max}[h]	T_{max}[i]	AUC[j]	F[k]
15b	0.4	0.6	0.7	3.78	1.3	1.7	0.11	0.5	0.37	9.8
15g	0.3	1.3	1.3	1.59	3.2	0.4	0.10	0.0	0.08	5.3
24b	1.6	2.9	8.6	1.37	3.74	nf[l]	0.00	ND[m]	0.00	0.0
28c	1.8	0.71	0.71	17.99	0.28	1.8	1.14	1.2	10.66	59.2
34c	1.1	2.3	2.4	3.40	1.5	1.4	0.72	0.5	1.84	54.1
34d	3.5	1.3	1.4	26.63	0.30	2.6	2.82	1.3	16.97	63.7

[a] Harmonic means based on six animals per compound. [b] Intravenous half-life (hours). [c] Intravenous volume of distribution at steady state (L/kg). [d] Intravenous volume of distribution (L/kg). [e] Area under the curve or total drug exposure after intravenous dosing from $t = 0$ to $t = \infty$ (μg·hr/mL). [f] Plasma clearance after intravenous dosing ((L/hr)/kg). [g] Oral half-life (hours). [h] Maximum plasma concentration after oral dosing (μg/mL). [i] Time from oral dosing to maximum plasma concentration (hours). [j] Area under the curve or total drug exposure after oral dosing from $t = 0$ to $t = \infty$ (μg·hr/mL). [k] Oral bioavailability (%). [l] nf = not found. [m] ND = not determined.

Hz, 2H), 1.67−1.52 (m, 1H), 1.43 (q, J = 6.9 Hz, 2H), 0.89 (d, J = 6.8 Hz, 6H); MS (ESI) m/z 436 $(M + H)^+$. Anal. ($C_{22}H_{28}F_3N_5O$) C, H, N.

6-[4-(Phenoxy)piperidin-1-yl]-*N*-(3-methylbutyl)pyridazine-3-carboxamide (9a). White solid (69%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 6.1 Hz, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 9.8 Hz, 1H), 7.33−7.26 (m, 2H), 7.01 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.74−4.66 (m, 1H), 4.12 (ddd, J = 13.2, 6.3, 3.9 Hz, 2H), 3.57 (ddd, J = 13.1, 9.2, 3.4 Hz, 2H), 3.32−3.29 (m, 2H), 2.04 (ddd, J = 9.8, 6.2, 3.1 Hz, 2H), 1.72−1.63 (m, 2H), 1.63−1.53 (m, 1H), 1.43 (q, J = 6.9 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 369 $(M + H)^+$. Anal. ($C_{21}H_{28}N4O_2$) C, H, N.

6-(4-Hydroxypiperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (8). To a solution of **6a** (228 mg, 1 mmol), 4-hydroxypiperidine (111 mg, 1.1 mmol) and CH_3CN (3 mL) in a microwave vial was added triethylamine (0.2 mL, 1.4 mmol). The reaction vial was heated in a microwave reactor at 160 °C for 20 min. The reaction mixture was partitioned between EtOAc (10 mL) and 1 N aq K_2CO_3. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to give **8** as a white powder (270 mg, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 (t, J = 5.9 Hz, 1H), 7.79 (d, J = 9.5 Hz, 1H), 7.34 (d, J = 9.8 Hz, 1H), 4.76 (d, J = 4.4 Hz, 1H), 4.25−4.05 (m, 2H), 3.86−3.66 (m, 1H), 3.1 (m, 2H), 1.92−1.72 (m, 2H), 1.67−1.50 (m, 1H), 1.43 (m, 4H), 1.18 (t, J = 7.3 Hz, 2H), 0.90 (d, J = 6.8 Hz, 6H); MS (ESI) m/z 293 $(M + H)^+$.

6-(4-(2-Fluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9b). To **8** (58.5 mg, 0.2 mmol), 2-fluorophenol (24.7 mg, 0. 22 mmol), and triphenylphosphine (78.7 mg, 0.3 mmol)

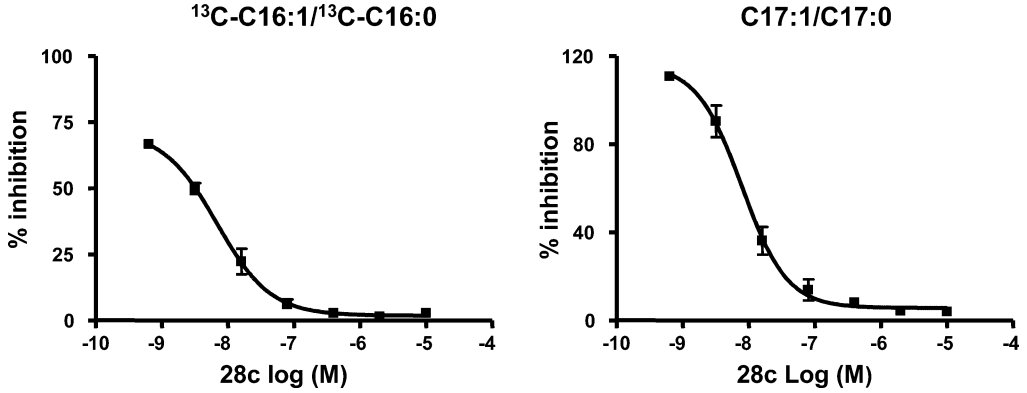


Figure 2. Dose−response of the inhibition of LCFA-CoA desaturation by SCD1 inhibitor **28c** in HepG2 cells. Results are expressed as the percentage of inhibition relative to the background in the absence of added SCD1 inhibitor. In each experiment, each value given is the mean from two separate experiments.

in THF (3 mL) was added di-*tert*-butyl azodicarboxylate (DBAD)[a] (69 mg, 0.3 mmol) in portions at room temperature. The mixture was stirred for 30 min and EtOAc was added (10 mL). The organic layer was then washed with 2 N aq NaOH and brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by silica gel flash chromatography, eluting with a gradient of 0−30% EtOAc in hexanes to provide **9b** as a white solid (49.5 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82−8.70 (m, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.34−7.09 (m, 3H), 7.03−6.92 (m, 1H), 4.77−4.63 (m, 1H), 4.18−4.03 (m, 2H), 3.65−3.52 (m, 2H), 3.30−3.25 (m, 2H), 2.12−1.97 (m, 2H), 1.77−1.65 (m, 2H), 1.62−1.52 (m, 1H), 1.43 (q, J = 7.0 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 387 (M + H)$^+$. Anal. ($C_{21}H_{28}FN_4O_2$) C, H, N.

Compounds 9c−9k. The general procedure described for **9b** was followed for the preparation of these compounds, using the appropriate phenols.

6-(4-(3-Fluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9c). White solid (61%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 5.9 Hz, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 9.5 Hz, 1H), 7.36−7.26 (m, 1H), 6.96−6.81 (m, 2H), 6.81−6.70 (m, 1H), 4.81−4.68 (m, 1H), 4.20−4.05 (m, 2H), 3.63−3.49 (m, 2H), 3.30−3.25 (m, 2H), 2.12−1.97 (m, 2H), 1.74−1.51 (m, 3H), 1.43 (q, J = 6.9 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 387 (M + H)$^+$. Anal. ($C_{21}H_{28}FN_4O_2$) C, H, N.

6-(4-(4-Fluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9d). White solid (67%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 5.9, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 9.5 Hz, 1H), 7.16−7.08 (m, 3H), 7.07−7.00 (m, 1H), 4.77−4.63 (m, 1H), 4.18−4.03 (m, 2H), 3.62−3.51 (m, 2H), 3.30−3.25 (m, 2H), 2.07−1.98 (m, 2H), 1.71−1.55 (m, 3H), 1.43 (q, J = 7.0 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 387 (M + H)$^+$. Anal. ($C_{21}H_{27}FN_4O_2$) C, H, N.

6-(4-(2,3-Difluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9e). White solid (65%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 5.9 Hz, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.22−7.09 (m, 2H), 7.08−6.94 (m, 1H), 4.86−4.68 (m, 1H), 4.20−4.02 (m, 2H), 3.68−3.49 (m, 2H), 3.49−3.31 (m, 2H), 2.14−1.96 (m, 2H), 1.79−1.51 (m, 3H), 1.43 (q, J = 6.8 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 405 (M + H)$^+$. Anal. ($C_{21}H_{26}F_2N_4O_2$) C, H, N.

6-(4-(2,4-Difluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9f). White solid (69%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82−8.65 (m, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 9.8 Hz, 1H), 7.36−7.23 (m, 2H), 7.09−6.96 (m, J = 1.7 Hz, 1H), 4.70−4.55 (m, J = 3.7 Hz, 1H), 4.18−3.98 (m, 2H), 3.66−3.47 (m, 2H), 3.36−3.23 (m, 2H), 2.11−1.92 (m, 2H), 1.77−1.49 (m, 3H), 1.43 (q, J = 6.8 Hz, 2H), 0.90 (d, J = 6.8 Hz, 6H); MS (ESI) m/z 405 (M + H)$^+$. Anal. ($C_{21}H_{26}F_2N_4O_2$) C, H, N.

6-(4-(2,5-Difluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9g). White solid (70%); ^{1}H NMR (300 MHz, DMSO-d_6) 8.67−8.82 (m, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.19−7.35 (m, 2H), 6.71−6.86 (m, 1H), 4.68−4.85 (m, 1H), 4.02−4.21 (m, 2H), 3.49−3.66 (m, 2H), 3.25−3.39 (m, 2H), 1.98−2.15 (m, 2H), 1.52−1.80 (m, 3H), 1.43 (q, J = 6.9 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6 H); MS (ESI) m/z 405 (M + H)$^+$. Anal. ($C_{21}H_{26}F_2N_4O_2$) C, H, N.

6-(4-(2,6-Difluorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9h). White solid (58%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.74 (t, J = 6.1 Hz, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.24−7.11 (m, 3H), 4.53−4.37 (m, 1H), 4.21−4.03 (m, 2H), 3.64−3.49 (m, 2H), 3.35−3.23 (m, 2H), 2.09−1.91 (m, 2H), 1.81−1.50 (m, 3H), 1.43 (q, J = 6.8 Hz, 2H), 0.90 (d, J = 6.8 Hz, 6H); MS (ESI) m/z 405 (M + H)$^+$. Anal. ($C_{21}H_{26}F_2N_4O_2$·0.1 H_2O) C, H, N.

***N*-Isopentyl-6-(4-(2-(trifluoromethyl)phenoxy)piperidin-1-yl)-pyridazine-3-carboxamide (9i).** A white solid purified by reverse phase HPLC eluting with 5−100% CH_3CN in 1% aqueous TFA (51%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 5.9 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.62 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 8.8 Hz, 2H), 7.09 (t, J = 7.6 Hz, 1H), 5.00−4.90 (m, 1H), 3.97−3.84 (m, 2H), 3.84−3.73 (m, 2H), 3.35−3.26 (m, 2H), 2.10−1.96 (m, 2H), 1.83−1.68 (m, 2H), 1.66−1.51 (m, 1H), 1.49−1.35 (m, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 437 (M + H)$^+$. Anal. ($C_{22}H_{27}F_3N_4O_2$·0.25 CF_3COOH) C, H, N.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9j). White solid (72%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 5.9 Hz, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.48−7.36 (m, 2H), 7.33−7.25 (m, 2H), 7.03−6.92 (m, 1H), 4.87−4.75 (m, 1H), 4.06−3.94 (m, 2H), 3.77−3.65 (m, 2H), 3.29−3.05 (m, 2H), 2.10−1.94 (m, 2H), 1.83−1.67 (m, 2H), 1.66−1.50 (m, 1H), 1.43 (q, J = 7.0 Hz, 2H), 0.90 (d, J = 6.4 Hz, 6H); MS (ESI) m/z 403 (M + H)$^+$. Anal. ($C_{21}H_{27}ClN_4O_2$) C, H, N.

6-(4-(2,5-Dichlorophenoxy)piperidin-1-yl)-*N*-isopentylpyridazine-3-carboxamide (9k). White solid (75%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (t, J = 6.1 Hz, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.47 (d, J = 9.5 Hz, 1H), 7.44 (d, J = 2.4 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.05 (dd, J = 8.7, 2.2 Hz, 1H), 4.95−4.68 (m, 1H), 4.07−3.97 (m, 2H), 3.75−3.65 (m, 2H), 2.09−1.97 (m, 2H), 1.79−1.66 (m, 2H), 1.67−1.53 (m, 1H), 1.43 (q, J = 7.1 Hz, 2H), 0.90 (d, J = 6.8 Hz, 6H); MS (ESI) m/z 438 (M + H)$^+$. Anal. ($C_{21}H_{26}Cl_2N_4O_2$·0.5H_2O) C, H, N.

General Procedure for the Synthesis of 4-(2-Chloro-phenoxy)-piperidine Hydrochloride 11b (Ar = 2-Chlorophenyl). To a mixture of *tert*-butyl 4-hydroxypiperidine-1-carboxylate (6.03 g, 30 mmol) in THF (50 mL) was added 2-chlorophenol (4.24 g, 33 mmol) and triphenylphosphine (10.2 g, 39 mmol), followed by the slow addition of DBAD (8.98 g, 39 mmol). EtOAc was added after 30 min and the mixture was washed with 2 N aq NaOH and brine. The organic layer was dried over $MgSO_4$ and concentrated under pressure. The residue was purified by silica gel column chromatography eluting with a gradient of 0−20% EtOAc in hexanes to give 4-(2-chloro-phenoxy)-piperidine-1-carboxylic acid *tert*-butyl ester **10b**. This material was dissolved in 4 N HCl in dioxane (30

mL). Solvents were removed in vacuo after 2 h, and the residue was triturated in CH_3CN. The precipitates were collected and washed with CH_3CN to give **11b** as a white solid (5.7 g, 76%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.81 (s, 2H), 7.45 (dd, J = 7.8, 1.36 Hz, 1H), 7.36−7.21 (m, 2H), 7.05−6.95 (m, 1H), 4.82−4.71 (m, 1H), 3.25−3.15 (m, 2H), 3.14−3.05 (m, 2H), 2.16−2.03 (m, 2H), 1.97−1.81 (m, 2H); MS (ESI) m/z 212, 214 $(M + H)^+$.

6-(4-(2-Fluorophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15a). A mixture of **6b** (17.2 mg, 0.1 mmol), 4-(2-fluorophenoxy)piperidine hydrochloride (23.1 mg, 0.1 mmol), and triethylamine (0.1 mL, 0.72 mmol) in CH_3CN (1 mL) was heated in a microwave reactor at 160 °C for 20 min. The reaction mixture was then partitioned between CH_2Cl_2 (20 mL) and water (10 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by reverse phase HPLC to give **15a** as a white solid (20.5 mg, 61%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (q, J = 4.2 Hz, 1H), 7.83 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 9.5 Hz, 1H), 7.35−7.08 (m, 3H), 7.04−6.92 (m, 1H), 4.78−4.61 (m, 1H), 4.23−4.03 (m, 2H), 3.69−3.50 (m, 2H), 2.81 (d, J = 4.7 Hz, 3H), 2.12−1.96 (m, 2H), 1.82−1.60 (m, 2H); MS (ESI) m/z 331 $(M + H)^+$. Anal. ($C_{17}H_{19}FN_4O_2 \cdot 0.05\ H_2O$) C, H, N.

Compounds 15b−15h. The general procedure described for **15a** was followed for the preparation of these compounds starting from either **6b** or **6c**, using the appropriate phenoxypiperidines **11**.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15b). White solid (60%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (q, J = 4.3 Hz, 1H), 7.83 (d, J = 9.8 Hz, 1H), 7.50−7.23 (m, 4H), 7.05−6.91 (m, 1H), 4.88−4.72 (m, 1H), 4.10−3.92 (m, 2H), 3.80−3.63 (m, 2H), 2.81 (d, J = 5.1 Hz, 3H), 2.12−1.95 (m, 2H), 1.83−1.67 (m, 2H); MS (ESI) m/z 347 $(M + H)^+$. Anal. ($C_{17}H_{19}ClN_4O_2$) C, H, N.

6-(4-(2-Bromophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15c). White solid (60%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83−8.73 (m, 1H), 7.84 (d, J = 9.8 Hz, 1H), 7.59 (dd, J = 8.0, 1.5 Hz, 1H), 7.41 (d, J = 9.5 Hz, 1H), 7.38−7.20 (m, 2H), 6.98−6.85 (m, 1H), 4.91−4.78 (m, 1H), 4.06−3.89 (m, 2H), 3.75−3.62 (m, 2H), 2.81 (d, J = 5.1 Hz, 3H), 2.09−1.91 (m, 2H), 1.86−1.66 (m, 2H); MS (ESI) m/z 393 $(M + H)^+$. Anal. ($C_{17}H_{19}BrN_4O_2$) C, H, N.

6-(4-(2-Cyanophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15d). White solid (65%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (d, J = 4.6 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.74 (dd, J = 7.7, 1.5 Hz, 1H), 7.71−7.62 (m, 1H), 7.42 (dd, J = 12.3, 9.2 Hz, 2H), 7.11 (t, J = 7.7 Hz, 1H), 5.05−4.79 (m, 1H), 4.12−3.95 (m, 2H), 3.79−3.66 (m, 2H), 2.82 (d, J = 4.9 Hz, 3H), 2.23−1.96 (m, 2H), 1.84−1.72 (m, 2H); MS (ESI) m/z 338 $(M + H)^+$. Anal. ($C_{18}H_{19}N_5O_2$) C, H, N.

6-(4-(2,5-Dichlorophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15e). White solid (70%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (t, J = 4.8 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.45−7.38 (m, 2H), 7.05 (dd, J = 8.7, 2.2 Hz, 1H), 4.96−4.86 (m, 1H), 4.08−3.96 (m, 2H), 3.79−3.70 (m, 2H), 2.81 (d, J = 4.8 Hz, 3H), 2.10−1.97 (m, 2H), 1.80−1.66 (m, 2H); MS (ESI) m/z 381 $(M + H)^+$. Anal. ($C_{17}H_{18}Cl_2N_4O_2$) C, H, N.

6-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)-*N*-methylpyridazine-3-carboxamide (15f). White solid (72%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.78 (t, J = 4.8 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.48 (dd, J = 9.0, 6.3 Hz, 1H), 7.40 (d, J = 9.5 Hz, 1H), 7.30 (dd, J = 11.0, 2.9 Hz, 1H), 6.90−6.76 (m, 1H), 4.92−4.80 (m, 1H), 4.08−3.93 (m, 2H), 3.74−3.62 (m, 2H), 2.81 (d, J = 4.8 Hz, 3H), 2.10−1.96 (m, 2H), 1.81−1.64 (m, 2H); MS (ESI) m/z 365 $(M + H)^+$. Anal. ($C_{17}H_{18}ClFN_4O_2$) C, H, N.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)pyridazine-3-carboxamide (15g). White solid (58%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.84 (d, J = 9.8 Hz, 1H), 7.51 (s, 1H), 7.47−7.35 (m, 1H), 7.34−7.25 (m, 2H), 7.01−6.95 (m, 1H), 4.87−4.77 (m, 1H), 4.10−3.96 (m, 2H), 3.78−3.67 (m, 2H), 2.08−1.96 (m, 2H), 1.81−1.69 (m, 2H); MS (ESI) m/z 333 $(M + H)^+$. Anal. ($C_{16}H_{17}ClN_4O_2$) C, H, N.

6-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)pyridazine-3-carboxamide (15h). White solid (62%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.52−7.44 (m, 2H), 7.39 (d, J = 9.8 Hz, 1H), 7.29 (dd, J = 10.9, 2.9 Hz, 1H), 6.88−6.80 (m, 1H), 4.92−4.82 (m, 1H), 4.00 (d, J = 3.38 Hz, 2H), 3.76−3.66 (m, 2H), 2.09−1.98 (m, 2H), 1.75 (d, J = 4.0 Hz, 2H); MS (ESI) m/z 351 $(M + H)^+$. Anal. ($C_{16}H_{16}ClFN_4O_2$) C, H, N.

Methyl 6-[4-(2-chlorophenoxy)piperidin-1-yl]pyridazine-3-carboxylate (13a). A mixture of 6-chloropyridazine-3-carboxylic acid methyl ester (1.7 g, 10 mmol), **11b** (2.1 g, 10 mmol), and *N*,*N*-diisopropylethylamine (1.7 mL, 10 mmol) in CH_3CN (50 mL) was refluxed at 120 °C for 12 h. The reaction mixture was partitioned between EtOAc (100 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel flash chromatography eluting with a gradient of 0−40% EtOAc in hexanes to provide **13a** (2.7 g, 78%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, J = 9.5 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.39−7.25 (m, 3H), 7.03−6.93 (m, 1H), 4.88−4.76 (m, 1H), 4.10−3.95 (m, 2H), 3.87 (s, 3H), 3.83−3.69 (m, 2H), 2.13−1.95 (m, 2H), 1.83−1.67 (m, 2H); MS (ESI) m/z 348 $(M + H)^+$.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)pyridazine-3-carboxylic Acid (14a). To a mixture of **13a** (2.6 g, 7.1 mmol) in methanol (50 mL) was added 2 N aq NaOH (10 mL). The reaction mixture was stirred at room temperature for 4 h, concentrated in vacuo to remove most of the methanol, and then water (10 mL) was added. To this water solution was added 2 N aq HCl slowly to adjust the pH to ∼3−4. The white precipitate was collected through filtration, washed with cold water, and dried in a vacuum oven to give **14a** as a white solid (2.3 g, 93%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 (d, J = 9.5 Hz, 1H), 7.48−7.41 (m, 1H), 7.38−7.27 (m, 3H), 7.03−6.93 (m, 1H), 4.88−4.77 (m, 1H), 4.10−3.96 (m, 2H), 3.81−3.69 (m, 2H), 2.10−1.96 (m, 2H), 1.82−1.67 (m, 2H); MS (ESI) m/z 334 $(M + H)^+$.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-(2-hydroxyethyl)pyridazine-3-carboxamide (15i). To a stirring mixture of **14a** (35 mg, 0.1 mmol) in DMF (1 mL) was added TBTU[a] (48 mg, 0.15 mmol) at room temperature. After 10 min, 2-aminoethanol (12.2 mg, 0.2 mmol) was added, following the addition of Et_3N to adjust the pH to ∼5−6. The reaction mixture was partitioned between EtOAc (20 mL) and water (10 mL) after 1 h. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by reverse phase HPLC to give **15i** as a white solid (17 mg, 45%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (t, J = 5.9 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.48−7.37 (m, 2H), 7.33−7.27 (m, 2H), 7.07−6.94 (m, 1H), 4.86−4.79 (m, 1H), 4.77 (t, J = 5.6 Hz, 1H), 4.07−3.95 (m, 2H), 3.77−3.66 (m, 2H), 3.56−3.48 (m, 2H), 3.37 (q, J = 6.2 Hz, 2H), 2.07−1.96 (m, 2 H), 1.81−1.67 (m, 2H); MS (ESI) m/z 377 $(M + H)^+$. Anal. ($C_{18}H_{21}ClN_4O_3$) C, H, N.

Compounds 15j−15q. The general procedure described for **15i** was followed for the preparation of these compounds starting from **14a**, using the appropriate amines for the condensation.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-(2-(dimethylamino)-ethyl)pyridazine-3-carboxamide (15j). White solid (46%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (t, J = 5.8 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.47−7.37 (m, 2H), 7.33−7.27 (m, 2H), 7.03−6.93 (m, 1H), 4.86−4.76 (m, 1H), 4.06−3.95 (m, 2H), 3.77−3.64 (m, 2H), 3.38−3.32 (m, 2H), 2.42 (t, J = 6.4 Hz, 2H), 2.18 (s, 6H), 2.07−1.95 (m, 2H), 1.81−1.67 (m, 2H); MS (ESI) m/z 404 $(M + H)^+$. FAB-HRMS calcd for $C_{20}H_{26}ClN_5O_2$ $(M + H)^+$, 404.1848; found, 404.1845.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-((tetrahydrofuran-2-yl)methyl)pyridazine-3-carboxamide (15k). White solid purified by reverse phase HPLC eluting with 5−100% CH_3CN in 1%

[a] Abbreviations: DBAD, di-*t*-butyl azodicarboxylate; TBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyl-uronium tetrafluoroborate.

aqueous TFA (43%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (t, J = 5.9 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.48−7.38 (m, 2H), 7.32−7.27 (m, 2H), 7.03−6.94 (m, 1H), 4.85−4.78 (m, 1H), 4.08−3.96 (m, 3H), 3.81−3.58 (m, 4H), 3.36 (t, J = 5.9 Hz, 2H), 2.09−1.96 (m, 2H), 1.91−1.69 (m, 5H), 1.67−1.54 (m, 1H); MS (ESI) m/z 417 (M + H)$^+$. Anal. ($C_{21}H_{25}ClN_4O_3$·1.2CF_3COOH) C, H, N.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-(2-(thiophen-2-yl)-ethyl)pyridazine-3-carboxamide (15l). White solid (61%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.93 (t, J = 5.9 Hz, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.49−7.36 (m, 2H), 7.35−7.24 (m, 3H), 7.05−6.88 (m, 3H), 4.88−4.75 (m, 1H), 4.06−3.92 (m, 2H), 3.77−3.66 (m, 2H), 3.61−3.49 (m, 2H), 3.13−3.04 (m, 2H), 2.12−1.95 (m, 2H), 1.81−1.67 (m, 2H); MS (ESI) m/z (M + H)$^+$. Anal. ($C_{22}H_{23}ClN_4O_2S$) C, H, N.

***N*-(2-(1*H*-Imidazol-4-yl)ethyl)-6-(4-(2-chlorophenoxy)piperidin-1-yl)pyridazine-3-carboxamide (15m).** White solid (68%) purified by reverse phase HPLC eluting with 5−100% CH_3CN in 1% aqueous TFA; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89 (s, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.56 (s, 1H), 7.46−7.36 (m, 2H), 7.33−7.26 (m, 3H), 7.02−6.94 (m, 1H), 6.91 (s, 1H), 4.86−4.75 (m, 1H), 4.08−3.92 (m, 2H), 3.77−3.66 (m, 2H), 3.60−3.52 (m, 2H), 2.87−2.69 (m, 2H), 2.07−1.95 (m, 2H), 1.81−1.76 (m, 2H); MS (ESI) m/z (M + H)$^+$. Anal. ($C_{21}H_{26}Cl_2N_4O_2$·0.3CF_3COOH) C, H, N.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-(pyridin-3-ylmethyl)pyridazine-3-carboxamide (15n). White solid (56%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.49 (t, J = 6.1 Hz, 1H), 8.57 (s, 1H), 8.46 (s, 1H), 7.84 (d, J = 9.5 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.46−7.36 (m, 3H), 7.32−7.26 (m, 2H), 7.02−6.94 (m, 1H), 4.86−4.77 (m, 1H), 4.52 (d, J = 6.4 Hz, 2H), 4.08−3.97 (m, 2H), 3.77−3.66 (m, 2H), 2.07−1.95 (m, 2H), 1.81−1.69 (m, 2H); MS (ESI) m/z (M + H)$^+$. FAB-HRMS calcd for $C_{22}H_{22}ClN_5O_2$ (M + H)$^+$, 424.1535; found, 424.1547.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-((3-methylisoxazol-5-yl)methyl)pyridazine-3-carboxamide (15o). White solid (52%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.46 (t, J = 5.9 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.48−7.38 (m, 2H), 7.36−7.24 (m, 2H), 7.01−6.94 (m, 1H), 6.16 (s, 1H), 4.83 (d, J = 3.4 Hz, 1H), 4.56 (d, J = 6.1 Hz, 2H), 4.02 (s, 2H), 3.80−3.66 (m, 2 H), 2.18 (s, 3H), 2.10−1.95 (m, 2H), 1.77 (d, J = 3.4 Hz, 2H); MS (ESI) m/z (M + H)$^+$. FAB-HRMS calcd for $C_{21}H_{22}ClN_5O_3$ (M + H)$^+$, 377.1375; found, 377.1377.

6-(4-(2-Chlorophenoxy)piperidin-1-yl)-*N*-((3-methyl-1*H*-pyrazol-5-yl)methyl)pyridazine-3-carboxamide (15p). White solid purified by reverse phase HPLC eluting with 5−100% CH_3CN in 1% aqueous TFA (50%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.98 (s, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.47−7.38 (m, 2H), 7.35−7.26 (m, 2H), 7.02−6.93 (m, 1H), 5.90 (s, 1H), 4.89−4.74 (m, 1H), 4.41 (d, J = 5.8 Hz, 2H), 4.09−3.93 (m, 2H), 3.79−3.65 (m, 2H), 2.16 (s, 3H), 2.05 (d, J = 16.3 Hz, 2H), 1.76 (s, 2H); MS (ESI) m/z (M + H)$^+$. Anal. ($C_{21}H_{23}ClN_6O_2$·0.35CF_3COOH) C, H, N.

***N*-((1*H*-Imidazol-2-yl)methyl)-6-(4-(2-chlorophenoxy)piperidin-1-yl)pyridazine-3-carboxamide (15q).** White solid purified by reverse phase HPLC eluting with 5−100% CH_3CN in 1% aqueous TFA (55%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.76 (s, 1H), 9.09 (t, J = 5.9 Hz, 1H), 7.90−7.83 (m, 1H), 7.46−7.38 (m, 2H), 7.32−7.21 (m, 2H), 7.07−6.92 (m, 2H), 6.80 (s, 1H), 4.91−4.71 (m, 1H), 4.52 (d, J = 6.1 Hz, 2H), 4.08−3.93 (m, 2H), 3.82−3.64 (m, 2H), 2.09−1.96 (m, 2H), 1.81−1.63 (m, 2H); MS (ESI) m/z (M + H)$^+$. Anal. ($C_{20}H_{21}ClN6O_2$·0.3CF_3COOH) C, H, N.

6-Chloropyridazine-3-carbonitrile (17). To a stirring mixture of **6c** (200 mg, 1.27 mmol) in pyridine (5 mL) was added trifluoroacetic anhydride (720 μL, 5.08 mmol) at −14 °C. After stirring for 1 h, the mixture was quenched with ice and diluted with EtOAc (20 mL). The organic layer was washed with saturated aq $NaHCO_3$ (40 mL) and brine (40 mL), dried over Na_2SO_4, and then filtered and concentrated to give a white solid (101 mg, 57%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (d, J = 8.8 Hz, 1 H), 8.47 (d, J = 8.8 Hz, 1 H); MS (ESI) m/z (M + H)$^+$.

6-(4-Phenoxypiperidin-1-yl)pyridazine-3-carbonitrile (18a). The title compound was made from **17** (101 mg, 0.72 mmol) and 4-phenoxypiperidine hydrochloride (155 mg, 0.72 mmol) according to the procedure described for **15a** as a white solid (151 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 9.8 Hz, 1H), 7.33−7.27 (m, 2H), 7.01 (d, J = 7.5 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.72 (tt, J = 7.7, 3.9 Hz, 1H), 4.18−4.08 (m, 2H), 3.72−3.59 (m, 2H), 2.09−1.99 (m, 2H), 1.74−1.62 (m, 2H); MS (ESI) m/z 281 (M + H)$^+$.

***N*′-Hydroxy-6-(4-phenoxypiperidin-1-yl)pyridazine-3-carboximidamide (19a).** To a stirring mixture of **18a** (145 mg, 0.52 mmol) in ethanol (6 mL) and CH_2Cl_2 (0.5 mL) were added hydroxylamine hydrochloride (55 mg, 0.78 mmol) and triethylamine (123 μL, 0.88 mmol). The heterogeneous mixture was stirred at room temperature for 14 h. The mixture was diluted with EtOAc (30 mL), washed with saturated aq $NaHCO_3$ (15 mL) and brine (10 mL), and filtered and concentrated to give **19a** as a white solid (114 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.85 (s, 1H), 7.70 (d, J = 9.8 Hz, 1H), 7.35−7.26 (m, 3H), 7.00 (d, J = 7.8 Hz, 2H), 6.93 (t, J = 7.29 Hz, 1H), 5.86 (s, 2H), 4.72−4.64 (m, 1H), 4.11−4.02 (m, 2H), 3.55−3.46 (m, 2H), 2.03 (ddd, J = 13.1, 6.5, 3.7 Hz, 2H), 1.72−1.60 (m, 2H); MS (ESI) m/z 314 (M + H)$^+$.

3-(5-Methyl-1,2,4-oxadiazol-3-yl)-6-(4-phenoxypiperidin-1-yl)-pyridazine (20a). To a mixture of **19a** (31.3 mg, 0.1 mmol) with CH_3CN (1.0 mL) in a microwave vial were added acetyl chloride (15.7 mg, 0.2 mmol) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.29 mmol). The mixture was heated in a microwave reactor at 150 °C for 15 min and then diluted with EtOAc (10 mL), washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel flash column chromatography, eluting with a gradient of 0−60% EtOAc in hexanes to provide **20a** as an off-white solid (16.8 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, J = 9.8 Hz, 1H), 7.40 (d, J = 9.8 Hz, 1H), 7.33−7.26 (m, 2H), 7.01 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.5 Hz, 1H), 4.72 (dt, J = 7.7, 3.8 Hz, 1H), 4.17−4.08 (m, 2H), 3.70−3.60 (m, 2H), 2.09−1.99 (m, 3H), 1.74−1.61 (m, 2H), 1.20 (s, 3 H); MS (ESI) m/z 338 (M + H)$^+$. FAB-HRMS calcd for $C_{18}H_{19}N_5O_2$ (M + H)$^+$, 338.1547; found, 338.1552.

Compounds 20b−20d. The general procedure described for **20a** was followed for the preparation of these compounds starting from **19a**, using the appropriate acyl chlorides for the cyclization.

3-(4-Phenoxypiperidin-1-yl)-6-[5-(trifluoromethyl)-1,2,4-oxadiazol-3-yl]pyridazine (20b). White solid (38%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.96 (d, J = 9.8 Hz, 1H), 7.47 (d, J = 9.5 Hz, 1H), 7.33−7.27 (m, 2H), 7.02 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.73 (dt, J = 7.9, 4.0 Hz, 1H), 4.21−4.12 (m, 2H), 3.69−3.60 (m, 2H), 2.12−2.02 (m, 2H), 1.64−1.76 (m, 2H); MS (ESI) m/z 392 (M + H)$^+$. FAB-HRMS calcd for $C_{18}H_{16}F_3N_5O_2$ (M + H)$^+$, 392.1264; found, 392.1260.

3-(6-(4-Phenoxypiperidin-1-yl)pyridazin-3-yl)-5-propyl-1,2,4-oxadiazole (20c). Pale yellow solid (42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (d, J = 9.5 Hz, 1H), 7.44 (d, J = 9.8 Hz, 1H), 7.36−7.26 (m, 2H), 7.02 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.78−4.64 (m, 1H), 4.21−4.08 (m, 2H), 3.66−3.53 (m, 2H), 3.00 (t, J = 7.5 Hz, 2H), 2.12−2.00 (m, 2H), 1.88−1.77 (m, 2H), 1.75−1.60 (m, 2H), 0.99 (t, J = 7.5 Hz, 3H); MS (ESI) m/z 366 (M + H)$^+$. Anal. ($C_{20}H_{23}N_5O_2$·0.25 H_2O) C, H, N.

3-(5-Benzyl-1,2,4-oxadiazol-3-yl)-6-(4-phenoxypiperidin-1-yl)-pyridazine (20d). White solid (42%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 9.83 Hz, 1H), 7.44−7.38 (m, 4H), 7.36−7.27 (m, 4H), 7.01 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.75−4.68 (m, 1H), 4.46 (s, 2H), 4.10 (d, J = 3.1 Hz, 2H), 3.64−3.55 (m, 2H), 2.04 (s, 2H), 1.72−1.65 (m, 2H); MS (ESI) m/z 414 (M + H)$^+$. Anal. ($C_{24}H_{23}N_5O_2$·0.5H_2O) C, H, N.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(5-methyl-1,2,4-oxadiazol-3-yl)pyridazine (20e). The title compound was prepared according to the procedures described for **20a**, substituting 4-(2-chlorophenoxy)-piperidine hydrochloride for 4-(phenoxy)-piperidine hydrochloride used in **18a** to provide **20e** as a white solid (19.3 mg, 52%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (d, J = 9.8 Hz, 1H), 7.49−7.39 (m, 2H), 7.33−7.26 (m, 2H), 7.03−6.94 (m, 1H), 4.89−4.76 (m, 1H), 4.11−3.96 (m, 2H), 3.80−3.67 (m, 2H), 2.68

(s, 3H), 2.13−1.96 (m, 2H), 1.86−1.69 (m, 2H); MS (ESI) *m*/*z* 372 (M + H)$^+$. Anal. ($C_{18}H_{18}ClN_5O_2$) C, H, N.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(2*H*-tetrazol-5-yl)pyridazine (21). A mixture of **18b** (Ar = 2-chlorophenyl) (31.5 mg, 0.1 mmol), NaN_3 (7.8 mg, 0.12 mmol), and tri-*n*-butyl stannyl chloride (39.0 mg, 0.12 mmol) in toluene (2 mL) was heated at 120 °C for 24 h. After solvent was removed under reduced pressure, the residue was purified by silica gel flash chromatography eluting with a gradient of 50−100% EtOAc in hexanes to provide **21** as a white solid (15 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 9.5 Hz, 1H), 7.49−7.45 (m, 1H), 7.44 (s, 1H), 7.34−7.28 (m, 2H), 7.23−7.03 (m, 1H), 7.03−6.92 (m, 1H), 4.88−4.75 (m, 1H), 4.10−3.93 (m, 2H), 3.77−3.63 (m, 2H), 2.14−1.94 (m, 2H), 1.86−1.68 (m, 2H); MS (ESI) *m*/*z* 358 (M + H)$^+$. Anal. ($C_{16}H_{16}ClN_7O\cdot 0.2H_2O$) C, H, N.

6-(3-Benzyl-1,2,4-oxadiazol-5-yl)-pyridazine-3-ol (22a). To 6-oxo-1,6-dihydropyridazine-3-carboxylic acid monohydrate (158 mg, 1.0 mmol) in chloroform (10 mL) was added $SOCl_2$ (501 mg, 4.0 mmol) and DMF (0.2 mL). The mixture was refluxed for 12 h, and then solvent was removed under reduced pressure to provide 6-hydroxy-pyridazine-3-carbonyl chloride as a yellow solid. This compound was mixed with *N*-hydroxy-2-phenylacetamidine (150 mg, 1.0 mmol) and *N*,*N*-diisopropylethylamine (0.2 mL, 1.2 mmol) in CH_3CN (2.5 mL) in a microwave vial. The mixture was heated in a microwave reactor at 150 °C for 20 min. After solvent was removed under reduced pressure, the residue was purified by silica gel flash chromatography, eluting with a gradient of 0−60% EtOAc in hexanes to provide **22a** as a white solid (216 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.79 (s, 1H), 7.95 (d, J = 9.8 Hz, 1H), 7.38−7.33 (m, 4H), 7.31−7.24 (m, 1H), 7.04 (d, J = 10.2 Hz, 1H), 4.18 (s, 2H); MS (ESI) *m*/*z* 255 (M + H)$^+$.

3-(3-Benzyl-1,2,4-oxadizole-5-yl)-6-chloropyridazine (23a). Compound **22a** (216 mg, 0.85 mmol) was refluxed in $POCl_3$ (10 mL) for 2 h. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc (20 mL) and water (10 mL). The organic layer was washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo to afford **23a** as a white solid (220 mg, 95%), which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 8.8 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.42−7.22 (m, 5H), 4.26 (s, 2H); MS (ESI) *m*/*z* 273 (M + H)$^+$.

3-(3-Benzyl-1,2,4-oxadiazol-5-yl)-6-(4-phenoxypiperidin-1-yl)-pyridazine (24a). To a mixture of **23a** (55 mg, 0.2 mmol) with CH_3CN (1.0 mL) in a microwave vial were added 4-phenoxypiperidine hydrochloride (17.7 mg, 0.1 mmol) and *N*,*N*-diisopropylethylamine (0.05 mL, 0.29 mmol). The mixture was heated in a microwave reactor at 160 °C for 20 min. The solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography, eluting with a gradient of 0−50% EtOAc in hexanes to provide **24a** as a white solid (22.7 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, J = 9.8 Hz, 1H), 7.42 (d, J = 9.8 Hz, 1H), 7.38−7.22 (m, 7H), 7.01 (d, J = 7.8 Hz, 2H), 6.94 (t, J = 7.5 Hz, 1H), 4.79−4.66 (m, 1H), 4.19 (s, 2H), 4.18−4.08 (m, 2H), 3.73−3.59 (m, 2H), 2.12−1.98 (m, 2H), 1.77−1.60 (m, 2H); MS (ESI) *m*/*z* 414 (M + H)$^+$. Anal. ($C_{24}H_{23}N_5O_2$) C, H, N.

Compounds 24b−24f. The general procedure described for **24a** was followed for the preparation of these compounds starting from **23a**, using the appropriate phenoxypiperidines **11** for the nucleophilic substitution.

3-(3-Benzyl-1,2,4-oxadiazol-5-yl)-6-[4-(2-fluorophenoxy)piperidin-1-yl]pyridazine (24b). White solid (67%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 9.8 Hz, 1H), 7.43 (d, J = 9.8 Hz, 1H), 7.40−7.21 (m, 7H), 7.14 (t, J = 7.8 Hz, 1H), 7.07−6.90 (m, 1H), 4.83−4.55 (m, 1H), 4.19 (s, 2H), 4.18−4.06 (m, 2H), 3.79−3.59 (m, 2H), 2.17−1.95 (m, 2H), 1.85−1.58 (m, 2H); MS (ESI) *m*/*z* 432 (M + H)$^+$. Anal. ($C_{24}H_{22}FN_5O_2$) C, H, N.

3-(3-Benzyl-1,2,4-oxadiazol-5-yl)-6-[4-(2-chlorophenoxy)piperidin-1-yl]pyridazine (24c). White solid (72%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 9.5 Hz, 1H), 7.48−7.39 (m, 2H), 7.39−7.23 (m, 7H), 7.03−6.94 (m, 1H), 4.89−4.77 (m, 1H), 4.19 (s, 2H), 3.98−4.12 (m, 2H), 3.87−3.72 (m, 2H), 2.12−1.96 (m, 2H), 1.85−1.69 (m, 2H); MS (ESI) *m*/*z* 448 (M + H)$^+$. Anal. ($C_{24}H_{22}ClN_5O_2$) C, H, N.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)pyridazine (24d). White solid (60%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00 (d, J = 9.8 Hz, 1H), 7.50−7.41 (m, 2H), 7.34−7.28 (m, 2H), 7.04−6.94 (m, 1H), 4.89−4.79 (m, 1H), 4.03 (s, 2H), 3.87−3.74 (m, 2H), 2.44 (s, 3H), 2.12−1.98 (m, 2H), 1.79 (s, 2H); MS (ESI) *m*/*z* 372 (M + H)$^+$. FAB-HRMS calcd for $C_{18}H_{18}ClN_5O_2$ (M + H)$^+$, 372.1222; found, 372.1220.

3-(3-Methyl-1,2,4-oxadiazol-5-yl)-6-[4-(2-methylphenoxy)piperidin-1-yl]pyridazine (24e). White solid (60%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00 (d, J = 9.5 Hz, 1H), 7.46 (d, J = 9.8 Hz, 1H), 7.21−7.11 (m, 2H), 7.09−7.01 (m, 1H), 6.85 (t, J = 6.8 Hz, 1H), 4.82−4.68 (m, 1H), 4.13−3.98 (m, 2H), 3.86−3.71 (m, 2H), 2.43 (s, 3H), 2.18 (s, 3H), 2.11−1.96 (m, 2H), 1.84−1.67 (m, 2H); MS (ESI) *m*/*z* 352 (M + H)$^+$. Anal. ($C_{18}H_{19}N_5O_2$) C, H, N.

3-[4-(2-Methoxyphenoxy)piperidin-1-yl]-6-(3-methyl-1,2,4-oxadiazol-5-yl)pyridazine (24f). White solid (51%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (d, J = 9.5 Hz, 1H), 7.45 (d, J = 9.8 Hz, 1H), 7.09 (dd, J = 7.6, 1.9 Hz, 1H), 7.05−6.99 (m, 1H), 6.98−6.92 (m, 1H), 6.92−6.84 (m, 1H), 4.78−4.54 (m, 1H), 4.32−4.06 (m, 2H), 3.76 (s, 3H), 3.73−3.56 (m, 2H), 2.43 (s, 3H), 2.16−1.91 (m, 2H), 1.83−1.56 (m, 2H); MS (ESI) *m*/*z* 368 (M + H)$^+$. Anal. ($C_{19}H_{21}N_5O_3\cdot 0.15H_2O$) C, H, N.

3-Chloro-6-(4-(2-chloro-5-fluorophenoxy)piperidin-1-yl)pyridazine (25c). To a mixture of 1-(6-chloropyridazin-3-yl)piperidin-4-ol (2.1 g, 10 mmol), 2-chloro-5-fluorophenol (1.6 g, 11 mmol), and triphenylphosphine (3.93 g, 15 mmol) in THF (10 mL) at 0 °C was added DBAD (3.45 g, 15 mmol) slowly. The mixture was then stirred at room temperature for 2 h before EtOAc was added (100 mL). The organic layer was washed with aqueous 2 N NaOH and brine, dried over Na_2SO_4, and concentrated under pressure, and the residue was purified by silica gel flash chromatography, eluting with a gradient of 0−10% EtOAc in hexanes to give **25c** as a white solid (1.75 g, 51%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.59−7.38 (m, 3H), 7.28 (dd, J = 11.2, 2.7 Hz, 1H), 6.90−6.75 (m, 1H), 4.91−4.77 (m, 1H), 3.98−3.83 (m, 2H), 3.67−3.51 (m, 2H), 2.08−1.93 (m, 2H), 1.79−1.63 (m, 2H); MS (ESI) *m*/*z* 341 (M + H)$^+$.

Methyl 6-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)pyridazine-3-carboxylate (26c). A mixture of **25c** (1.71 g, 5 mmol), [1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium(II) complex with dichloromethane (1:1, 203.5 mg, 5 mol %) and MeOH (24 mL) in a 100 mL pressure tube was charged with 200 psi CO. The mixture was heated at 100 °C for 3 h, cooled to room temperature, filtered, and concentrated in vacuo. The residue was purified by silica gel flash chromatography eluting with a gradient of 0−40% EtOAc in hexanes to give **26c** as a white solid (1.37 g, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, J = 9.8 Hz, 1H), 7.48 (dd, J = 8.8, 6.1 Hz, 1H), 7.39−7.26 (m, 2H), 6.91−6.77 (m, 1H), 4.96−4.78 (m, 1H), 4.13−3.97 (m, 2H), 3.87 (s, 3H), 3.81−3.67 (m, 2H), 2.13−1.96 (m, 2H), 1.84−1.62 (m, 2H); MS (ESI) *m*/*z* 366 (M + H)$^+$.

***N*′-Acetyl-6-(4-(2-chloro-5-fluorophenoxy)piperidin-1-yl)pyridazine-3-carbohydrazide (27c).** To a mixture of **26c** (1.1 g, 3 mmol) in MeOH (20 mL) was added 2 N aq NaOH (5 mL) and stirred at room temperature for 3 h. Solvent was then removed under reduced pressure and water (10 mL) was added. To this water solution was added 2 N HCl dropwise until pH = 3∼4. The white precipitate was collected and dried in a vacuum oven to give 6-(4-(2-chloro-5-fluorophenoxy)piperidin-1-yl)pyridazine-3-carboxylic acid (1.0 g, 95%). To this acid (0.95 g, 2.7 mmol) in DMF (10 mL) were added TBTU (1.3 g, 4.0 mmol) and acetic hydrazide (0.24 g, 3.24 mmol), following by the addition of triethylamine to adjust the pH to ∼6. The mixture was stirred at room temperature for 1 h and partitioned between EtOAc (30 mL) and water (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated, and the residue was purified by silica gel flash chromatography eluting with a gradient of 0−60% EtOAc in

hexanes to give **27** as a white solid (1.02 g, 92%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (s, 1H), 9.88 (s, 1H), 7.82 (d, J = 9.5 Hz, 1H), 7.52−7.37 (m, 2H), 7.30 (dd, J = 11.0, 2.9 Hz, 1H), 6.90 (d, 1H), 4.87 (d, J = 3.1 Hz, 1H), 4.11−3.96 (m, 2H), 3.83−3.64 (m, 2H), 2.12−1.94 (m, J = 7.5 Hz, 2H), 1.90 (s, 3H), 1.83−1.67 (m, 2H); MS (ESI) m/z 408 (M + H)$^+$.

2-(6-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)pyridazin-3-yl)-5-methyl-1,3,4-oxadiazole (28c). A mixture of **27c** (1.0 g, 2.45 mmol) in $POCl_3$ (20 mL) was heated at 100 °C for 6 h. After $POCl_3$ was removed under reduced pressure, EtOAc (40 mL) was added. The mixture was washed with 1 N NaOH and brine, and the organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by reverse phase HPLC to yield **28c** as a white solid (0.5 g, 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 9.5 Hz, 1H), 7.54−7.43 (m, 2H), 7.30 (dd, J = 11.0, 2.9 Hz, 1H), 6.90−6.78 (m, 1H), 4.95−4.82 (m, 1H), 4.11−3.97 (m, 2H), 3.82−3.68 (m, 2H), 2.61 (s, 3H), 2.13−1.98 (m, 2H), 1.83−1.68 (m, 2H); MS (ESI) m/z 390 (M + H)$^+$. Anal. ($C_{18}H_{17}ClFN_5O_2$) C, H, N.

Compounds 28a−28b. The general procedure described for **28c** was followed for the preparation of these compounds starting from **14a** (Ar = 2-chlorophenyl), using the appropriate acyl hydrazide for the coupling.

2-Benzyl-5-(6-(4-(2-chlorophenoxy)piperidin-1-yl)pyridazin-3-yl)-1,3,4-oxadiazole (28a). White solid (56%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.97 (d, J = 9.5 Hz, 1H), 7.50−7.41 (m, 2H), 7.40−7.34 (m, 4H), 7.32−7.26 (m, 3H), 7.03−6.93 (m, 1H), 4.88−4.76 (m, 1H), 4.39 (s, 2H), 4.03 (m, 2H), 3.82−3.67 (m, 2H), 2.11−1.96 (m, 2 H), 1.84−1.66 (m, 2H); MS (ESI) m/z 448 (M+H)$^+$. Anal. ($C_{24}H_{22}ClN_5O_2$) C, H, N.

2-(6-(4-(2-Chlorophenoxy)piperidin-1-yl)pyridazin-3-yl)-5-methyl-1,3,4-oxadiazole (28b). White solid (48%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 9.5 Hz, 1H), 7.53−7.41 (m, 2H), 7.34−7.29 (m, 2H), 7.04−6.93 (m, 1H), 4.89−4.76 (m, 1H), 4.11−3.96 (m, 2H), 3.83−3.68 (m, 2H), 2.61 (s, 3H), 2.12−1.96 (m, 2H), 1.86−1.68 (m, 2H); MS (ESI) m/z 372 (M + H)$^+$. Anal. ($C_{18}H_{18}ClN_5O_2$) C, H, N.

3-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)-6-(5-methyl-4*H*-1,2,4-triazol-3-yl)pyridazine (29). A mixture of **28c** (78 mg, 0.2 mmol) and 20% NH_3 in MeOH in a pressure tube was heated at 160 °C for 24 h and then solvent was removed under reduced pressure. The residue was partitioned between CH_2Cl_2 (20 mL) and water (10 mL), and the organic layer was washed with brine, dried with Na_2SO_4, and concentrated. The residue was purified by reverse phase HPLC to yield **29** as a white solid (27.3 mg, 35%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.81 (s, 1H), 7.89 (d, J = 9.5 Hz, 1H), 7.52−7.38 (m, 2H), 7.30 (dd, J = 11.2, 2.7 Hz, 1H), 6.90−6.78 (m, 1H), 4.93−4.82 (m, 1H), 4.06−3.94 (m, 2H), 3.76−3.61 (m, 2H), 2.40−2.31 (m, 3H), 2.12−1.98 (m, 2H), 1.82−1.67 (m, 2H); MS (ESI) m/z 389 (M + H)$^+$. Anal. ($C_{18}H_{18}ClFN_6O$) C, H, N.

3-Chloro-6-furan-3-yl-pyridazine (31a, Ar = 3-Furyl). A mixture of 3-furanboronic acid (25 mg, 0.22 mmol), 3-chloro-6-iodo-pyridazine (48 mg, 0.2 mmol), dichlorobis(triphenylphosphine)palladium(II) (1.4 mg, 0.002 mmol), and sodium carbonate (25 mg, 0.24 mmol) in a solvent of 1,2-dimethoxyethane/H_2O/EtOH (7:3:2) (0.8 mL) was heated in a microwave reactor at 150 °C for 10 min. The mixture was diluted with 2 mL water and extracted with EtOAc (2 × 2 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography eluting with 25% EtOAc in hexanes to provide **31a** as an off-white solid (18 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60−8.56 (m, 1H), 8.14 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.88 (m, 1H), 7.17 (m, 1H). MS (ESI) m/z 181 (M + H)$^+$.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(3-furyl)pyridazine (32a). A mixture of **31a** (18 mg, 0.1 mmol), 4-(2-chlorophenoxy)piperidine hydrochloride (24.8 mg, 0.1 mmol), and triethylamine (40 μL, 0.29 mmol) was heated in a microwave reactor at 160 °C for 20 min. The reaction mixture was partitioned with EtOAc (10 mL) and water (5 mL). The organic layer was washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by reverse phase HPLC to give **32a** as a white solid (18.5 mg, 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (dd, J = 1.5, 0.8 Hz, 1 H), 7.79−7.76 (m, 1H), 7.74 (d, J = 9.5 Hz, 1H), 7.48−7.25 (m, 4H), 7.06−7.04 (m, 1H), 7.02−6.94 (m, 1H), 4.85−4.73 (m, 1H), 4.03−3.88 (m, 2H), 3.66−3.53 (m, 2H), 2.07−1.94 (m, 2H), 1.80−1.66 (m, 2H); MS (ESI) m/z 356 (M + H)$^+$. FAB-HRMS calcd $C_{19}H_{18}ClN_3O_2$ (M + H)$^+$, 356.1160; found, 356.1164.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-pyridin-3-ylpyridazine (32b). The titled compound was prepared according to the procedures described for **32a**, substituting 3-pyridineboronic acid for 3-furanboronic acid used in **31a** to yield **32b** as a white solid (17.6 mg, 48%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.22 (d, J = 1.4 Hz, 1H), 8.61 (dd, J = 4.7, 1.7 Hz, 1H), 8.45−8.33 (m, 1H), 8.04 (d, J = 9.5 Hz, 1H), 7.56−7.41 (m, 3H), 7.36−7.25 (m, 2H), 7.05−6.92 (m, 1H), 4.89−4.75 (m, 1H), 4.08−3.93 (m, 2H), 3.76−3.59 (m, 2H), 2.15−1.96 (m, 2H), 1.83−1.64 (m, 2H); MS (ESI) m/z 367 (M + H)$^+$. Anal. ($C_{20}H_{19}ClN_4O\cdot0.1H_2O$) C, H, N.

Compounds 34a−34e. The general procedure described for **32a** was followed for the preparation of these compounds starting from the appropriate *N*-heteroaryl-substituted pyridazines, using the appropriate phenoxypiperidines **11**.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(1*H*-pyrrol-1-yl)pyridazine (34a). White solid (61%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.85 (d, J = 9.8 Hz, 1H), 7.63−7.60 (m, 2H), 7.54 (d, J = 9.8 Hz, 1H), 7.47−7.41 (m, 1H), 7.36−7.25 (m, 2H), 7.01−6.94 (m, 1H), 6.32−6.27 (m, 2H), 4.86−4.72 (m, 1H), 4.00−3.86 (m, 2H), 3.65−3.51 (m, 2H), 2.07−1.96 (m, 2H), 1.82−1.67 (m, 2H); MS (ESI) m/z 355 (M + H)$^+$. FAB-HRMS calcd for $C_{19}H_{19}ClN_4O$ (M + H)$^+$, 355.1320; found, 355.1326.

3-[4-(2-Chlorophenoxy)piperidin-1-yl]-6-(3,5-dimethyl-1*H*-pyrazol-1-yl)pyridazine (34b). White solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, J = 9.8 Hz, 1H), 7.53 (d, J = 9.8 Hz, 1H), 7.47−7.41 (m, 1H), 7.36−7.25 (m, 2H), 7.05−6.92 (m, 1H), 6.11 (s, 1H), 4.87−4.73 (m, 1H), 4.03−3.86 (m, 2H), 3.71−3.54 (m, 2H), 2.51 (s, 3H), 2.20 (s, 3H), 2.09−1.96 (m, 2H), 1.87−1.63 (m, 2H); MS (ESI) m/z 384 (M + H)$^+$. FAB-HRMS calcd for $C_{20}H_{22}ClN_5O$ (M + H)$^+$, 384.1586; found, 384.1578.

3-(4-(2-Chlorophenoxy)piperidin-1-yl)-6-(2-methyl-1*H*-imidazol-1-yl)pyridazine (34c). White solid (50%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72−7.65 (m, 1H), 7.61−7.52 (m, 2H), 7.48−7.42 (m, 1H), 7.34−7.29 (m, 2H), 7.07 (d, J = 1.4 Hz, 1H), 7.02−6.94 (m, 1H), 4.87−4.76 (m, 1H), 4.05−3.93 (m, 2H), 3.73−3.61 (m, 2H), 2.45 (s, 3H), 2.10−1.99 (m, 2H), 1.83−1.69 (m, 2H); MS (ESI) m/z 370 (M + H)$^+$. FAB-HRMS calcd for $C_{19}H_{20}ClN_5O$ (M + H)$^+$, 370.1429; found, 370.1437.

3-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)-6-(1*H*-imidazol-1-yl)pyridazine (34d). White solid (62%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.96−7.88 (m, 2H), 7.62 (d, J = 9.8 Hz, 1H), 7.48 (dd, J = 8.8, 6.1 Hz, 1H), 7.29 (dd, J = 11.0, 2.9 Hz, 1H), 7.13 (d, J = 1.4 Hz, 1H), 6.91−6.76 (m, 1H), 4.91−4.79 (m, 1H), 4.04−3.91 (m, 2H), 3.71−3.55 (m, 2H), 2.12−1.96 (m, 2H), 1.82−1.66 (m, 2H); MS (ESI) m/z 374 (M + H)$^+$. Anal. ($C_{24}H_{22}ClN_5O_2$) C, H, N.

3-(4-(2-Chloro-5-fluorophenoxy)piperidin-1-yl)-6-(2-methyl-1*H*-imidazol-1-yl)pyridazine (34e). White solid (60%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74−7.67 (m, 1H), 7.63−7.55 (m, 2H), 7.48 (dd, J = 8.8, 6.4 Hz, 1H), 7.30 (dd, J = 11.0, 2.9 Hz, 1H), 7.14 (s, 1H), 6.89−6.79 (m, 1H), 4.92−4.81 (m, 1H), 4.06−3.93 (m, 2H), 3.74−3.60 (m, 2H), 2.47 (s, 3H), 2.11−1.99 (m, 2H), 1.81−1.66 (m, 2H); MS (ESI) m/z 387 (M + H)$^+$. FAB-HRMS calcd for $C_{19}H_{19}ClFN_5O$ (M + H)$^+$, 388.1335; found, 388.1343.

Preparation of Mouse Liver Microsomes. Male *ob*/*ob* mice (B6.V-Lep^{ob}/J, Jackson Labs) on an 11% fat chow diet (5015, LabDiet, PMI) were sacrificed by CO_2 inhalation and then exsanguinated by cardiac puncture. The livers were rinsed with saline, weighed, and placed in homogenization buffer (250 mM sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, and Roche Complete-EDTA proteinase inhibitor cocktail). The livers were homogenized in this buffer (6 mL/liver) using a Dounce glass−glass homogenizer (four loose strokes and four tight strokes). The

homogenate was differentially centrifuged (600 × *g* for 10 min, 10 000 × *g* for 10 min, and 100 000 × *g* for 1 h) to eliminate mitochondria and cellular debris. The 100 000 × *g* microsomal pellet was gently resuspended with a Dounce glass homogenizer to between 10 μg/μL and 30 μg/μL protein. Protein determination was done with a detergent compatible kit (Biorad) using bovine serum albumin as the standard.

Preparation of Recombinant Human SCD1/cyt b_5/cyt b_5R in Insect Cells. Human recombinant SCD1/cyt b_5/cyt b_5R genes were cloned independently into the baculovirus expression vector pFASTBac1. After DNA sequence confirmation, the three independent constructs were used to generate recombinant baculoviruses using the Bac-to-Bac system (Invitrogen). High titer baculovirus stocks were generated following two successive rounds of viral amplification in Sf9 cells (Invitrogen) at a multiplicity of infection (MOI) of 0.1 for all three constructs. Expressions were carried out at a 10 liter scale in Wave Bioreactors (Wave Biotech). High Five (Invitrogen) cells were grown at 28 °C in Express Five serum-free media (Invitrogen). When the culture reached a density of 2.1 × 10^6 cell/mL, cells were infected simultaneously with all three recombinant baculoviruses, each at a multiplicity of infection (MOI) of 1. Additionally, at infection, the culture was supplemented with 5 μM $FeCl_3$ and 0.5 mM 5-aminolevulinic acid. Cells were harvested 48 h post-infection by centrifugation and lysates were prepared immediately. The thawed pellets were treated with hypotonic shock (resuspension in water for 20 min) and then adjusted with 5× homogenization buffer concentrate (250 mM sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, Roche Complete−EDTA proteinase inhibitor cocktail) to 5 mL buffer per each 100 mL original fermentation liquid. The resuspension was homogenized in 16 mL batches with an Ultra-Turrax T25 polytron (Ika-Werke, setting 6 for 3 min). The resultant homogenate was differentially centrifuged (600 × *g* for 10 min, 10 000 × *g* for 10 min, and 100 000 × *g* for 1 h) to eliminate cellular debris and mitochondria. The 100 000 × *g* microsomal pellet was gently resuspended with a Dounce glass homogenizer to between 10 μg/μL and 30 μg/μL protein. Protein determination was done with the Biorad detergent compatible kit using bovine serum albumin as the standard.

SCD1 In Vitro Enzymatic Assay. SCD1 activity was determined by measuring the production of tritiated water from the desaturation of [^{3}H]-(9,10) stearoyl-CoA substrate. One version of the assay uses *ob*/*ob* mouse liver microsomes as the source of SCD1 enzyme. Another used recombinant human SCD1 expressed in tandem with human cyt b_5/cyt b_5R as the source of the SCD1 enzyme.

The SCD1 activity assay was carried out in 96-well plates in a 50 μL total assay reaction volume appropriate for high throughput screening. Typically a 50 μL reaction volume contained the following: 5 μL of a diluted test compound in 10% DMSO and 10 μM stearoyl-CoA, 0.24 μM [^{3}H]-(9,10) stearoyl-CoA in 35 μL of assay buffer (250 mM sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, Roche Complete−EDTA proteinase inhibitor cocktail, 2 mM NADH, and 50 mM NaF). The reaction was then initiated with the addition of either human or mouse microsomes at a protein concentration of between 10 and 20 μg of protein per reaction, depending on the specific activity of the particular microsome preparation.

After a 30 min reaction time, the reactions were terminated by the addition of a stop solution, typically 30 μL of 4 N HCl. A set reaction volume, usually 65 μL, was then transferred from each well to 50 mg of methanol−prewetted Norit A charcoal (Sigma C-9157) in a Millipore Multiscreen Plate (Millipore catalog number MAFCNOB50). Plates were shaken for 1 min and allowed to sit for 5 min to allow for adsorption of the stearoyl-CoA and the oleoyl-CoA. The [^{3}H]-H_2O was separated from the charcoal adsorbed stearoyl-CoA and oleoyl-CoA by centrifugation into a collection plate (Perkin-Elmer Opti plate). The charcoal-filled Multiscreen Plate was removed, and scintillation fluid (Perkin-Elmer Optiphase Supermix) was added to each well. Radioactivity was counted, typically in a Microbeta TriLux (Wallac) radioactivity counter. SCD1 activity was expressed relative to control reactions with no inhibitor after background subtraction. Dose−response curve fitting and IC_{50} values were determined using nonlinear regression analysis (GraphPad Prism software 4.0).

NADH cyt b_5/cyt b_5R Inhibition Assay. The cyt b_5/cyt b_5R assay was described previously.[30] Briefly, 500 μL reaction buffer containing 500 μM sulfamethoxazole-hydroxyamine (SMX-HA), 1 mM NADH, 1 mM glutathione, and 1 mM ascorbate in PBS pH = 7.4 was incubated with 2 mg/mL human microsomes (BD gentest, cat# 452161) at 37 °C for 30 min. The reaction was stopped by adding 250 μL of cold methanol and kept on ice for 20 min. The samples were spun at 10 000 × *g* for 5 min, and about 200 μL of solution was filtered through Amicon Ultrafree-MC column (Millipore) for HPLC analysis. HPLC analysis was recorded on an Agilent 1100 system using a Phenomenex Luna C18 column (4.6 × 250 mm, 5 μ). The HPLC solvent system consisted of 50 mM ammonium acetate at pH ∼ 4 (solvent A) and CH_3CN containing 5% water (solvent B). The linear gradient at 1 mL/min ramped from 80% solvent B to 30% solvent B over 10 min, and held at 30% solvent B for an additional 3 min before returning to 80% solvent B. The SMX-HA cleavage product (sulfamethoxazole) from the assay was detected at 274 nm using an Agilent 1100 diode array detector.

Generation of LCFA-CoAs in HepG2 Cells by SCD1 and their Analysis. HepG2 cells were cultured for 6 h in MEM medium (Invitrogen, cat# 11095) containing 10% serum, 1 mM sodium pyruvate, 1 × nonessential amino acid (Invitrogen, cat# 11360 and 11140, respectively), 0.1 mM sodium heptadecanoic acid (Sigma, cat# H3500), and 0.1 mM sodium palmitic acid-1,2,3,4-[^{13}C]$_4$ (Sigma, cat# 489611). Cells were collected in 15 mL tubes and centrifuged at 100 000 × *g* for 10 min at 4 °C. Cell pellets were frozen and thawed in 1 mL of 100 mM KH_2PO_4 (pH = 4.9) containing 2 mM arachidoyl CoA (Avanti, cat# 117486) as an internal standard. Cells were homogenized and 1 mL of 2-propanol was added. The samples were homogenized again and 0.125 mL of saturated NH_4SO_4 and 2 mL of CH_3CN were added. The mixture was vortexed for 2 min. The samples were centrifuged at 150 000 × *g* and at 4 °C for 10 min. About 150 μL of solution was passed through Amicon Ultrafree-MC column (Millipore) for HPLC analysis, the rest of samples was stored at −80 °C for future analysis.

High-pressure liquid chromatography combined with mass spectrometry (LC/MS) was employed for the measurement of specific LCFA-CoA species. The HPLC instrument used consisted of three Shimadzu LC-10ADvp pumps and a SCL-10Avp Controller. The reversed phase column was a Zorbax Extended C18 (2.1 × 50 mm). Mobile phase A was 10:90 CH_3CN/(15 mM ammonium hydroxide); mobile phase B was 15 mM NH_4OH in CH_3CN; mobile phase C was 65:35 CH_3CN/(0.1% formic acid). A gradient was used consisting of 100% A for 2 min, followed by a linear ramp to 60% B over 3 min and held for 2 min; at 7 min, a wash cycle began, with the mobile phase switched to 100% C and held for 5 min; at 12 min, the mobile phase was again switched to 100% A and equilibrated for 3 min prior to injection of the next sample. The flow rate for the analysis portion was 200 μL/min and 400 μL/min for the wash portion. The autosampler was a CTC PAL with cooling trays kept at 8 °C. The various LCFA-CoAs eluted between 4 and 6 min on this system.

The mass spectrometer was a Sciex API-3000 with Turbo IonSpray, operated in positive ionization mode. Multiple reaction monitoring (MRM) experiments were performed. For each LCFA-CoA analyzed, loss of 506 from its molecular ion was monitored in this product ion MS/MS technique. This is assumed to arise from ATP loss in the gas-phase experiment.[33] Using the instrument's Analyst software, AUC was determined and transferred into Excel spreadsheets. Desaturation indices were determined either directly from the AUC data, or LCFA-CoA concentrations in μM calculated first and then the ratios determined.

Acknowledgment. We thank the R4EK exploratory kinetic staff for pharmacokinetic studies, the R46Y protein expression/purification group for providing insect cells coexpressing human SCD1, human cytochrome b_5 and human cytochrome b_5

reductase, as well as the HT-ADME group for the microsomal stability studies.

Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) World Health Organization. Global Strategy on Diet, Physical Activity and Health [online], <http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf> (accessed 2004).

(2) McGarry, J. D. Banting lecture 2001: Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* **2002**, *51*, 7−18.

(3) (a) Unger, R. H. Lipid overload and overflow: Metabolic trauma and the metabolic syndrome. *Trends Endocrinol. Metab.* **2003**, *14*, 398−403. (b) Unger, R. H. Minireview: Weapons of lean body mass destruction: The role of ectopic lipids in the metabolic syndrome. *Endocrinology* **2003**, *144*, 5159−5165.

(4) Enoch, H. G.; Catala, A.; Strittmatter, P. Mechanism of rat liver microsomal stearoyl-CoA desaturase: Studies of the substrate specificity, enzyme−substrate interactions, and the function of lipid. *J. Biol. Chem.* **1976**, *251*, 5095−5103.

(5) Ntambi, J. M. The regulation of stearoyl-CoA desaturase (SCD). *Prog. Lipid Res.* **1995**, *34*, 139−150.

(6) Miyazaki, M.; Gomez, F. E.; Ntambi, J. M. Lack of stearoyl-CoA desaturase-1 function induces a palmitoyl-CoA Delta6 desaturase and represses the stearoyl-CoA desaturase-3 gene in the preputial glands of the mouse. *J. Lipid Res.* **2002**, *43*, 2146−2154.

(7) Artwohl, M.; Roden, M.; Waldhausl, W.; Freudenthaler, A.; Baumgartner-Parzer, S. M. Free fatty acids trigger apoptosis and inhibit cell cycle progression in human vascular endothelial cells. *FASEB J.* **2004**, *18*, 146−148.

(8) Miyazaki, M.; Jacobson, M. J.; Man, W. C.; Cohen, P.; Asilmaz, E.; Friedman, J. M.; Ntambi, J. M. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. *J. Biol. Chem.* **2003**, *278*, 33904−33911 and the references cited therein.

(9) Ntambi, J. M.; Miyazaki, M. Recent insights into stearoyl-CoA desaturase-1. *Curr. Opin. Lipidol.* **2003**, *14*, 255−261.

(10) (a) Dobrzyn, A.; Ntambi, J. M. The role of stearoyl-CoA desaturase in body weight regulation. *Trends Cardiovasc. Med.* **2004**, *14*, 77−81. (b) Dobrzyn, A.; Ntambi, J. M. Stearoyl-CoA desaturase as a new drug target for obesity treatment. *Obes. Rev.* **2005**, *6*, 169−174.

(11) Lee, S. H.; Dobrzyn, A.; Dobrzyn, P.; Rahman, S. M.; Miyazaki, M.; Ntambi, J. M. Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment. *J. Lipid. Res.* **2004**, *45*, 1674−1682.

(12) Ntambi, J. M.; Miyazaki, M.; Stoehr, J. P.; Lan, H.; Kendziorski, C. M.; Yandell, B. S.; Song, Y.; Cohen, P.; Friedman, J. M.; Attie, A. D. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11482−11486.

(13) Rahman, S. M.; Dobrzyn, A.; Dobrzyn, P.; Lee, S. H.; Miyazaki, M.; Ntambi, J. M. Stearoyl-CoA desaturase 1 deficiency elevates insulin-signaling components and down-regulates protein-tyrosine phosphatase 1B in muscle. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11110−11115.

(14) (a) Jiang, G.; Li, Z.; Liu, F.; Ellsworth, K.; Dallas-Yang, Q.; Wu, M.; Ronan, J.; Esau, C.; Murphy, C.; Szalkowski, D.; Bergeron, R.; Doebber, T.; Zhang, B. B. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J. Clin. Invest.* **2005**, *115*, 1030−1038. (b) Gutierrez-Juarez, R.; Pocai, A.; Mulas, C.; Ono, H.; Bhanot, S.; Monia, B. P.; Rossetti, L. Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. *J. Clin. Invest.* **2006**, *116*, 1686−1695.

(15) Zhang, L.; Ge, L.; Parimoo, S.; Stenn, K.; Prouty, S. M. Human stearoyl-CoA desaturase: Alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. *Biochem. J.* **1999**, *340*, 255−264.

(16) (a) Wang, J.; Yu, L.; Schmidt, R. E.; Su, C.; Huang, X.; Gould, K.; Cao, G. Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 735−742. (b) Zhang, S.; Yang, Y.; Shi, Y. Characterization of human SCD2, an oligomeric desaturase with improved stability and enzyme activity by cross-linking in intact cells. *Biochem. J.* **2005**, *388* (Pt 1), 135−142.

(17) Attie, A. D.; Krauss, R. M.; Gray-Keller, M. P.; Brownlie, A.; Miyazaki, M.; Kastelein, J. J.; Lusis, A. J.; Stalenhoef, A. F.; Stoehr, J. P.; Hayden, M. R.; Ntambi, J. M. Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia. *J. Lipid Res.* **2002**, *43*, 1899−907.

(18) Hulver, M. W.; Berggren, J. R.; Carper, M. J.; Miyazaki, M.; Ntambi, J. M.; Hoffman, E. P.; Thyfault, J. P.; Stevens, R.; Dohm, G. L.; Houmard, J. A.; Muoio, D. M. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metab.* **2005**, *2*, 251−261.

(19) (a) 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. Pyridazine derivatives and their use as therapeutic agents. WO 2005/011655 A2, February 10, 2005. (b) Sviridov, S.; Kodumuru, V.; Liu, S.; Abreo, M.; Winther, M. D.; Gschwend, H. W.; Kamboj, R.; Sun, S.; Holladay, M. W.; Li, W.; Tu, C. WO 2005/011657 A2, February 10, 2005.

(20) Fu, J.-M.; Kodumuru, V.; Sun, S.; Winther, M. D.; Fine, R. M.; Harvey, D. F.; Klebansky, B.; Gray-Keller, M. P; Gschwend, H. W.; Li, W. Nicotinamide derivatives and their use as therapeutic agents. US 2005/0119251 A1, June 2, 2005.

(21) The SCD1 inhibitors were also tested for metabolic stability using human liver microsomes, and the compounds generally showed slightly better metabolic stability unless otherwise noted. The determination of the remaining fraction of the drug was done with LC-MS.

(22) Santilli, A. A.; Morris, R. L. Synthesis of 3-arylsulfonylmethyl-1,2,4-oxadiazole-5-carboxylic acid derivatives. *J. Heterocycl. Chem.* **1979**, *16*, 1197−1120.

(23) Rajapakse, H. A.; Zhu, H.; Young, M. B.; Mott, B. T. A mild and efficient one pot synthesis of 1,3,4-oxadiazoles from carboxylic acids and acyl hydrazides. *Tetrahedron Lett.* **2006**, *47*, 4827−4830.

(24) Goodman, A. J.; Stanforth, S. P.; Tarbit, B. Desymmetrization of dichloroazaheterocycles. *Tetrahedron* **1999**, *55*, 15067−15070.

(25) Steiner, G.; Gries, J.; Lenke, D. Synthesis and antihypertensive activity of new 6-heteroaryl-3-hydrazinopyridazine derivatives. *J. Med. Chem.* **1981**, *24*, 59−63.

(26) Blake, A. J.; Hubberstey, P.; Li, W.-S.; Russell, C. E.; Smith, B. J.; Wraith, L. D. Intramolecular hydrogen bonding between 2-cyanoguanidine and 3-chloro-6-(pyrazol-1-yl)pyridazines in copper(II) complexes. *J. Chem. Soc., Dalton Trans.* **1998**, *4*, 647−656.

(27) Steiner, G.; Gries, J.; Lenke, D. 6-Imidazol-1-yl-3-hydrazinopyridazines, their preparation and antihypertensive use. US4399137 A1, August 16, 1983.

(28) Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. E.; Wadsworth, H. J.; Wyman, P. Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor. *J. Med. Chem.* **1991**, *34*, 2726−2735.

(29) Compound **28c** was tested for % inhibition at 10 μM in a standard panel of 75 receptors, transporters, and ion channels offered from CEREP (www. cerep.com).

(30) Kurian, J. R.; Bajad, S. U.; Miller, J. L.; Chin, N. A.; Trepanier, L. A. NADH cytochrome b_5 reductase and cytochrome b_5 catalyze the microsomal reduction of xenobiotic hydroxylamines and amidoximes in humans. *J. Pharmacol. Exp. Ther.* **2004**, *311*, 1171−1178.

(31) Golovko, M. Y.; Murphy, E. J. An improved method for tissue long-chain acyl-CoA extraction and analysis. *J. Lipid Res.* **2004**, *45*, 1777−1782.

(32) At the time of submission, a patent application describing SCD1 inhibitors with similar structures was published. See: Black, C.; Deschenes, D.; Gagnon, M.; Lachance, N.; LeBlanc, Y.; Leger, S.; Li, C. S.; Oballa, R. M. Heteroaromatic compounds as inhibitors of stearoyl-coenzyme A delta-9 desaturase. WO2007/009236 A1, January 25, 2007.

(33) Zirrolli, J. A.; Wheelan, P.; Murphy, R. C. Analysis of long chain fatty acyl coenzyme A thioesters by negative ion fast-atom bombardment mass spectrometry and tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 416−424.

JM070219P