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Potent, orally bioavailable, liver-selective stearoyl-CoA desaturase (SCD) inhibitors

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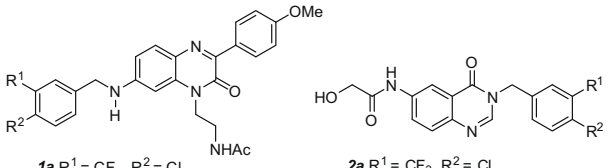
ABSTRACT

Two structurally distinct series of SCD (Δ9 desaturase) inhibitors (**1** and **2**) have been previously reported by our group. In the present work, we merged the structural features of the two series. This led to the discovery of compound **5b** (CVT-12,012) which is highly potent in a human cell-based (HEPG2) SCD assay (IC_{50} = 6 nM). This compound has 78% oral bioavailability in rats and is preferentially distributed into liver (76 times vs plasma) with relatively low brain penetration. In a five-day study (sucrose fed rats) compound **5b** significantly reduced SCD activity in a dose-dependent manner as determined by GC analysis of fatty acid composition in plasma and liver, and significantly reduced liver triglycerides versus the control group (~50%).

In the past decade, stearoyl-CoA desaturase (SCD, Δ9 desaturase) has emerged as a promising target for the potential treatment of metabolic diseases.[1–8] SCD catalyzes the Δ9-desaturation of long-chain fatty acids, leading to the biosynthesis of monounsaturated fatty acids: oleic ($18:1_{n-9}$), palmitoleic ($16:1_{n-7}$), and vaccenic ($18:1_{n-7}$, via the elongase). Monounsaturated fatty acids are major components of triglycerides, cholesterol esters, and phospholipids. SCD inhibition, based on studies using SCD knockdown with antisense RNA and in mice lacking functional SCD, is expected to be beneficial for the treatment of type II diabetes, obesity, and hypertriglyceridemia. In one such study, Ntambi and co-workers demonstrated that $SCD1^{-/-}$ mice fed a high-fat diet had reduced body weight and adiposity, increased oxygen consumption, energy expenditure and improved insulin sensitivity.[1] In another study, *ob/ob* mice lacking SCD1 were lean and had increased energy expenditure (Fig. 1).[2]

In humans, two isoforms of SCD, SCD1 and SCD5, have been identified. SCD1 is primarily found in liver, adipose and skeletal muscle[9] while SCD5 is found primarily in the brain.[10] The development of isoform-selective compounds may be challenging due to



1a $R^1 = CF_3$, $R^2 = Cl$
1b $R^1 = Cl$, $R^2 = Cl$
1c $R^1 = CF_3$, $R^2 = H$

2a $R^1 = CF_3$, $R^2 = Cl$
2b CVT-11,563, $R^1 = Cl$, $R^2 = Cl$

Figure 1. Structures of previously reported SCD inhibitors.

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the homology between the two SCD isoforms. Furthermore, mounting evidence suggests SCD1 inhibition in peripheral tissues like skin, pancreas and macrophages may lead to side-effects.[3,11,12] As a result our program focused on the discovery of tissue selective non-brain penetrating compounds that could inhibit SCD primarily in the liver and/or adipose tissue.

Δ9 Desaturases belong to a class of enzymes that includes the Δ5 and Δ6 desaturases. All 3 fatty acid desaturases are localized to the endoplasmic reticulum and are complexed with cytochrome *b5* and cytochrome *b5* reductase.[13] As a result, we needed to ensure that SCD inhibitors acted specifically on the SCD enzyme and not on cytochrome *b5* or cytochrome *b5* reductase. For this purpose we counter-screened selected molecules for Δ5 and Δ6 desaturase inhibitory activity.

A number of small-molecule SCD inhibitors have been published to date.[14–18] In previous communications, we have disclosed two chemically distinct classes of SCD inhibitors.[19,20] In series **1**,[19] compound **1a** has been shown to be extremely potent in the rat microsomal SCD assay (IC_{50} 0.6 nM) and in the human cell-based SCD assay[21] (HEPG2 IC_{50} 0.05 nM). The difference in potency may be explained by the structural difference between species-specific isoforms, and/or potential differences resulting from cell-membrane permeability. Representative compounds **1b** and **1c** also demonstrated single-digit nanomolar potency in the HEPG2 SCD assay, however that series exhibited poor oral bioavailability in rodents. For example, compound **1b** had no detectable plasma levels following oral administration to rats.

In the series **2**,[20] compounds with good oral absorption were identified by lowering the molecular weight and lipophilicity of the initial leads, in addition to incorporating a key hydroxyacetamide group. In rats, representative compounds **2a** and **2b** (CVT-11,563) were shown to have 29% and 90% oral bioavailability, respectively. Compound **2b** has also been found to be distributed into the liver with moderate selectivity (threefold versus both plasma and adipose tissue) and showed in vivo inhibition of SCD activity in a five-day study using rats fed a high sucrose diet.

In this work, we set out to modify the highly potent series **1** and exploit the features from the more bioavailable series **2**. A superposition of molecules **1b** and **2b** (Fig. 2) revealed a striking overlap between the bulky, lipophilic substituted benzyl groups containing Cl and/or CF_3 substituents. Another ovelapping feature was the presence of the NHAc group in series **1** that we modified to incorporate the OH group from CVT-11,563 (**2b**). We did not find a match for the 4-methoxyphenyl substituent. We experimented by replacing it with a much smaller methyl group (Table 1).

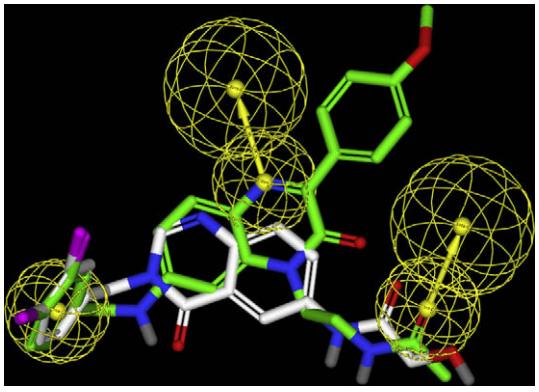
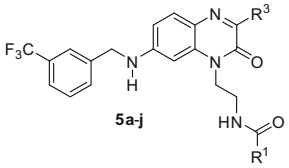


Figure 2. Three dimensional superimposition of **1b** (green) and **2b** (CVT-11, 563, white). This picture was produced using the 'Molecular Overlay' feature of Accelrys Discovery Studio v2.0, which gives an automated optimum overlay of a number of small molecules using equally weighted steric and electrostatic fields, and flexible rotatable bonds. Pharmacophore features were added manually to show possible common hydrogen bond acceptor atoms (arrows) and hydrophobic/aromatic regions (centroid).

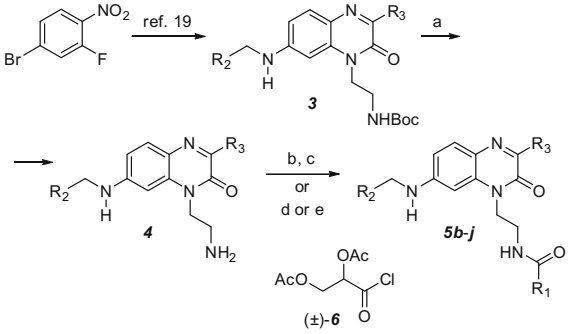
We synthesized an initial set of compounds that shared features of series **1** and **2** as shown in Schemes 1 and 2. The compound **5a** and all of the *N*-Boc protected intermediates (**3**) were prepared from 4-bromo-2-fluoronitrobenzene following the previously reported method.[19] The Boc group in **3** was removed to yield the amine **4** (Scheme 1). Reaction of **4** with the corresponding acid chlorides and the subsequent hydrolysis of the esters produced compounds **5b**,[22] **5f**, and **5h–j**. Compounds **5–e** were prepared by the direct HATU coupling[23] of the corresponding hydroxyacids. Compound **5g** was prepared by heating with phenyl carbamate in the presence of *N*-methylmorpholine.[24]

The synthesis of series **9** compounds was carried out as shown in Scheme 2 using 2-amino-4-nitrophenol as a starting material. Reaction with chloroacetyl chloride, 2-bromopropionyl bromide, or 2-bromo-2-methylpropionyl bromide followed by cyclization yields the intermediates **7a–c**.[25] Intermediate **10** (NPhth = phthalimide) was prepared in one step from commercial 2-(2-bromoethylethyl)-isoindoline-1,3-dione via Finkelstein reaction.[26] The

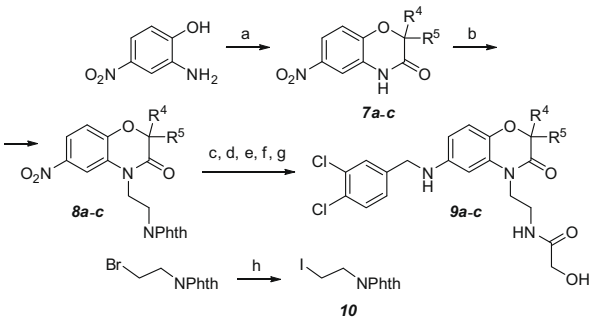
Table 1
Potency for SCD inhibition in **5a–j**



	R^1	R^3	SCD IC_{50} (nM)	
			Rat Microsomal	Human HEPG2
1a			0.6	0.05
1b			110	8.6
1c			93	33
2a			190	110
2b			261	119
5a	Me	Me	162	
5b	CH_2OH	Me	38	6.1
5c	1(*S*)-Hydroxyethyl	Me	8000	
5d	1(*R*)-Hydroxyethyl	Me	3900	
5e	2-Hydroxyethyl	Me	1100	
5f	(±)-$CH(OH)CH_2OH$	Me	268	68
5g	NH_2	Me	15	>1000
5h	CH_2OH	CF_3	1.9	2.7
5i	CH_2OH	*i*-Pr	2800	
5j	CH_2OH	*t*-Bu	>3000	

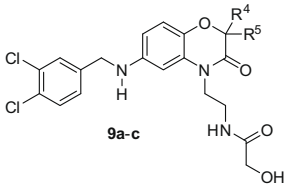


Scheme 1. Reagents and conditions: (a) TFA, CH_2Cl_2, rt, 24 h; (b) $AcOCH_2COCl$ or (±)-**6**, $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt, 24 h; (c) $LiOH{\cdot}H_2O$, H_2O/THF/MeOH, r.t., 24 h; (d) R^1CO_2H, HATU, $(i\text{-Pr})_2NEt$, $CHCl_3$, rt, 24 h; (e) $PhOCONH_2$, *N*-methylmorpholine, THF, reflux, 2 h.



Scheme 2. Reagents and conditions: (a) $ClCH_2COCl$, or $BrCH(Me)COBr$, or $BrCMe_2COBr$, acetone, 0 °C to rt, followed by Et_3N, H_2O, reflux, 3 h; (b) **10**, NaH, DMF, 0 °C to rt, 24 h; (c) $NH_2NH_2{\cdot}H_2O$, EtOH, 60 °C, 5 h; (d) $AcOCH_2CO_2H$, TBTU, $(i\text{-}Pr)_2NEt$, THF, rt; (e) Zn, AcOH, 55 °C, 4 h; (f) 3,4-dichlorobenzaldehyde, $Si(OEt)_4$, EtOH, reflux, 3 h, followed by $NaBH_4$, EtOH, 0 °C to r.t., 3 h; (g) K_2CO_3, H_2O/MeOH; (h) 4 equiv NaI, acetone, reflux, 5 h.

Table 2
Potency for SCD inhibition in **9a–c**



	R^4, R^5	SCD IC_{50}, nM	
		Rat microsomal	Human HEPG2
5b		38	6.1
9a	H, H	1.4	0.5
9b	(±)-H, Me	80	
9c	Me, Me	12	

coupling of intermediates **7a–c** and **10** provided compounds **8a–c** and was followed by treatment with hydrazine to remove the phthalimide protecting group, TBTU-assisted amide formation,[27] and nitro group reduction. The subsequent reductive amination was carried out in two steps (one pot). The aniline was heated with 3,4-dichlorobenzaldehyde in ethanol in the presence of silicon tetraethoxide, and then sodium borohydride was added. This was followed by the ester hydrolysis to yield compounds **9a–c**.

Compound **5b** displayed the highest potency in both the microsomal and the HEPG2 SCD assays (IC_{50} 38 nM and 6.1 nM, respectively) compared to the other methyl-substituted compounds (**5a–g**, Table 1). This suggests that, similarly to CVT-11,563 (**2b**)[20] the hydroxyacetamide functional group appears to be optimal for SCD inhibitory potency.

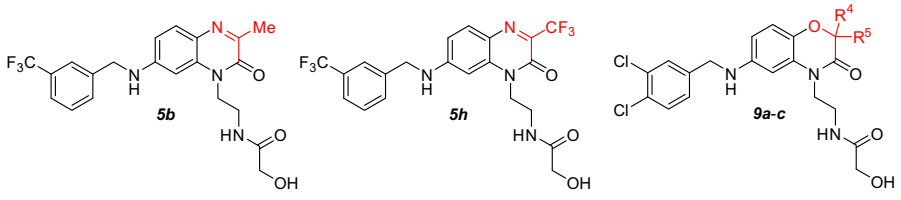
Having found that the methyl group is an acceptable substituent at the R^3 position, we proceeded to study the influence of the other R^3 substituents (**5h–j**, Table 1). This SAR study revealed that the trifluoromethyl group was preferred as compound **5h** showed superior potency in both assays (HEPG2 SCD IC_{50} 1.9 nM, miscosomal SCD IC_{50} 2.7 nM) while methyl (**5b**), isopropyl (**5i**), and *tert*-butyl (**5j**) analogs were significantly less potent.

The oxygen-containing compound **9a** (Table 2) led to an improvement in potency in both the microsomal and the HEPG2 SCD assays (IC_{50} 1.4 nM and 0.5 nM, respectively) when compared to series **5**. Introduction of the methyl substituents (**9b** and **c**) slightly decreased potency for SCD inhibition in the rat microsomal assay (IC_{50} 80 nM and 12 nM, respectively).

Compounds **5b**, **5h**, and **9a–c** were selected for the follow-up in the microsomal stability assays (Table 3), were only **5b** exhibited >50% stability after the 30 min incubation in both human and rat liver microsomal assay. Surprisingly, **9a** was the more stable compound among **9a–c**, since the introduction of the methyl groups next to the ether moiety was intended to improve metabolic stability. In a rat PK study, **5b** and **9a** both demonstrated good oral bioavailability (78% and 39%, respectively). It appears that the oral absorption of **5b** was not affected by a significant Pgp efflux, which was expected based on Caco-2 assay results (P_{app} A→B 6.8×10^6 cm/s, B→A 48.2×10^6 cm/s, efflux ratio of 7). The plasma clearance of both **5b** and **9a** was high (88 and 56 mL/min/kg, respectively) with elimination half-life of approximately 1 h for each compound.

We confirmed that **5b** was selective for Δ9 desaturase (Δ5 desaturase and Δ6 desaturase IC_{50} >30 μM in the rat microsomal assay).[13] Next, we investigated the tissue partitioning of compound **5b** (Fig. 3). Acute single dose PK experiments in rat (5 mg/kg) dem-

Table 3
In vitro, and in vivo DMPK parameters for the selected compounds



	R^4, R^5	Met. stability				Pharmacokinetics[a]			
		% After 30 min		dAUC (PO) (ng h/mL)	C_{max} (PO) (ng/mL)	%F	CL_p (IV) (mL/min/kg)	Vdβ (L/kg)	$t_{1/2}$ (IV) (h)
		Human	Rat						
1b		83	54	0	0	n.d.	n.d.	n.d.	n.d.
2a		78	88	451 ± 123	439 ± 118	29	12.9 ± 2.3	1.46 ± 0.13	1.38 ± 0.16
2b		85	75	935 ± 90	360 ± 111	90	16.6 ± 3.8	2.06 ± 0.24	1.46 ± 0.16
5b		56	54	148 ± 8.6	374 ± 147	78	88.1 ± 7.5	7.22 ± 0.96	0.95 ± 0.07
5h		19	77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9a	H, H	32	14	122 ± 85	427 ± 88	39	53.0 ± 6.4	4.89 ± 0.47	1.07 ± 0.13
9b	(±)-H, Me	2.3	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9c	Me, Me	3.1	9.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Mean and standard deviation, based on $n = 3$ (male Sprague–Dawley rats, 1.5 mg/kg PO dose, 1 mg/kg IV dose). dAUC—AUC (0–∞) adjusted to 1 mg/kg dose; CLp—plasma clearance (0–∞); Vdβ—terminal phase volume of distribution; *F*—oral bioavailability, $t_{1/2}$—elimination half-life (0–∞); n.d.—not determined.

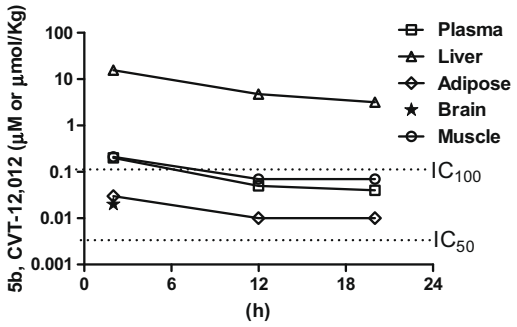


Figure 3. Time course for compound **5b** tissue partitioning (PO, rat, 5 mg/kg, $n = 3$, mean values, SEM omitted).

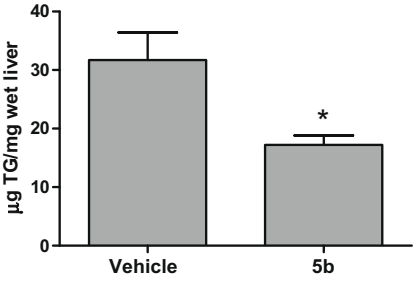


Figure 5. Effect of SCD inhibitor **5b** on liver triglycerides after 5 days of BID oral treatment. High-carbohydrate fed male Sprague–Dawley rats, vehicle ($n = 5$) compound **5b** (20 mg/kg, $n = 6$). Samples were collected 4 h after last dose. Mean ± SEM, $^{*}p$ <0.05 by unpaired t-test (actual $p = 0.012$).

onstrated selective partitioning into the liver. At 2 h post oral dose the **5b** levels measured in liver were 76, 75, 549 and 973-fold higher than those in plasma, muscle, adipose and brain, respectively. The concentration of **5b** remained above the IC_{100} (100% inhibition level) in the liver and above the IC_{50} level in plasma, muscle, and adipose tissue beyond 12 h (the IC_{100} and IC_{50} values are based on the HEPG2 data).

These tissue and plasma levels were deemed sufficient to enable further in vivo efficacy studies.

Sprague–Dawley rats were kept on a high sucrose diet for 4 weeks (Fig. 4) prior to being dosed orally (BID) with compound **5b** for 5 days. The Δ9 desaturation products [palmitoleic acid ($16:1_{n-7}$) and vaccenic acid ($18:1_{n-7}$)] were measured 4 h after the final dose following extraction, transmethylation, and GC analysis of the fatty acid methyl esters.[28,29]

Palmitoleic and vaccenic acids combined represent a better marker of SCD activity than oleic acid ($18:1_{n-9}$); as they are virtually absent from non- animal fat diets and originate from de novo liogenesis and SCD mediated desaturation. In this five-day study, we found a dose-dependent reduction in SCD product fatty acids in both plasma and liver at all three dose levels (5, 10, and 20 mg/kg) thus demonstrating that compound **5b** has in vivo SCD inhibitory properties.

In a separate five-day study (Fig. 5), compound **5b** (20 mg/kg PO, BID) was administered to rats that were kept on a high sucrose diet for 4 weeks and liver triglycerides were measured. Administration of **5b** conferred a 50% decrease in liver triglycerides compared to vehicle.

In conclusion, we have created a new series of potent and orally bioavailable SCD inhibitors. The highlights of the SAR study were the introduction of the hydroxyacetamide functional group into series **1**-like molecules and the replacement of bulky, lipophilic 4-methoxyphenyl substituent with smaller groups. This effort produced the investigational compound **5b**, a potent SCD inhibitor in the microsomal assay ($IC_{50} = 38$ nM) and the human cell-based assay (HEPG2 $IC_{50} = 6.1$ nM) that is orally bioavailable (78%) and strongly partitions to the liver. In 5 day in vivo efficacy studies, compound **5b** significantly reduced SCD products in the plasma and the liver as well as reducing liver TG's by ~50%. Long-term animal studies are under way to further investigate the effect of compound **5b** (CVT-12,012) in models of obesity, fatty liver diseases and diabetes.

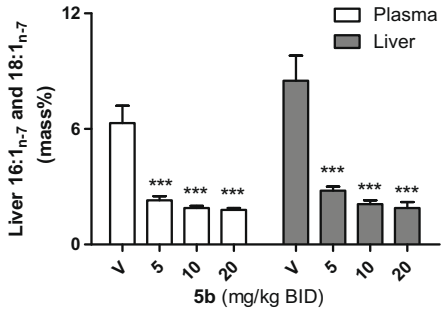


Figure 4. Effect of SCD inhibitor **5b** on plasma and liver desaturation index (5 day, PO BID, mean ± SEM). High-carbohydrate fed male Sprague–Dawley rats, vehicle (V, $n = 6$), compound **5b**, 5 mg/kg ($n = 6$), 10 mg/kg, ($n = 5$), 20 mg/kg ($n = 6$), Samples were collected 4 h after last dose. $^{***}p$ <0.001 by unpaired t-test.

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22. Compound **5b**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (t, J = 5.9 Hz, 1H), 7.87 (s, 1H), 7.76 (d, J = 7.4 Hz, 1H), 7.49–7.68 (m, 2H), 7.42 (d, J = 9.0 Hz, 1H), 7.15 (t, J = 6.1 Hz, 1H), 6.75 (br s, 1H), 6.71 (br d, J = 9.0 Hz, 1H), 5.54 (t, J = 5.7 Hz, 1H), 4.57 (d, J = 6.3 Hz, 2H), 4.14 (t, J = 7.0 Hz, 2H), 3.84 (d, J = 5.5 Hz, 2H), 3.28 - 3.37 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.44, 155.34, 150.23, 150.02, 141.72, 134.94, 132.19, 130.19, 129.81, 129.47 (q, J = 31.1 Hz), 125.00, 124.84 (q, J = 3.8 Hz), 124.79 (q, J = 272.3 Hz), 124.08 (q, J = 3.6 Hz), 111.70, 93.85, 61.91, 45.86, 40.98, 35.80, 21.01. Anal. C, H, N.
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25. Synthesis of 6-Nitro-4*H*-benzo[1,4]oxazin-3-one (**7a**). To a solution of 2-amino-4-nitrophenol (4 g, 26 mmol) in acetone (50 mL) at 0 °C chloroacetyl chloride (3.3 g, 29 mmol) was added dropwise and the reaction mixture was allowed to warm up to room temperature. At that time a precipitate was observed and Et_3N (5.8 g, 57 mmol) was added slowly. The reaction mixture was stirred for additional 30 min, diluted with water (50 mL), and heated to reflux for 3 h, then kept overnight at room temperature. The precipitate was filtered, and washed consequetively with water and methanol. The product 6-Nitro-4*H*-benzo[1,4]oxazin-3-one (**7a**) was obtained after drying as a grey solid (4.3 g, 85 %). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.07 (br s, 1H); 7.83 dd, J = 2.2, 8.8 Hz, 1H), 7.72 d, J = 2.2 Hz, 1H), 7.14 d, J = 8.8 Hz, 1H), 4.77 (s, 2H).
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