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

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ABSTRACT

We discovered a structurally novel SCD (Δ9 desaturase) inhibitor **4a** (CVT-11,563) that has 119 nM potency in a human cell-based (HEPG2) SCD assay and selectivity against Δ5 and Δ6 desaturases. This compound has 90% oral bioavailability (rat) and excellent plasma exposure (dAUC 935 ng h/mL). Additionally, **4a** shows moderately selective liver distribution (three times vs plasma and adipose tissue) and relatively low brain penetration. In a five-day study (high sucrose diet, rat) compound **4a** significantly reduced SCD activity as determined by GC analysis of fatty acid composition in plasma and liver. We describe the discovery of **4a** from HTS hit **1** followed by scaffold replacement and SAR studies focused on DMPK properties.

Obesity and Type II Diabetes continue to expand at epidemic rates and new medicines targeting novel mechanisms are urgently needed. Stearoyl CoA desaturases (SCD's), also known as Δ9 fatty acid desaturases are microsomal enzymes that convert coenzyme A conjugates of saturated long-chain fatty acids into monounsaturated fatty acids (primarily, stearic acid into oleic acid and palmitic acid into palmitoleic acid). Deletion, mutation or inhibition of SCD in mice and rats results in decreased hepatic triglyceride, resistance to weight gain and improvements in insulin sensitivity and glucose uptake.[1–5] Thus, SCD inhibitors may present a therapy for obesity, insulin resistance, and diabetes.[6–8]

Two isoforms of SCD, SCD1 and SCD5 (also known as SCD2 in rodents), have been identified in humans. SCD1 is primarily found in liver, adipose and skeletal muscle and is well characterized[9] compared to SCD2/5 which is found primarily in the brain.[10] The development of isoform-selective compounds may be challenging due to the high homology between SCD isoforms. In addition, SCD1 inhibition in peripheral tissues like skin, pancreas and macrophages may lead to side-effects.[3,11,12] In light of these observations, the development of tissue selective, non-brain penetrating compounds may be desirable. The compounds described in this letter were designed not to be SCD1 isoform selective, but rather to be tested later for appropriate in vivo tissue distribution (including brain penetration and pharmacodynamics).

Δ9 Desaturases are localized to the endoplasmic reticulum and complexed with cytochrome *b5* and cytochrome *b5* reductase. This feature is shared with Δ5 and Δ6 desaturases. Therefore, it was important to verify that our small-molecule inhibitors acted directly on SCD enzyme itself, and not on cytochrome *b5* or cytochrome *b5* reductase. As a result, selected molecules were screened against Δ5 and Δ6 desaturases as a part of our discovery paradigm.[13]

A large number of small-molecule SCD inhibitors have been published to date by our group[14] and others,[15–19] therefore we pursued an independent approach to discovering new structural series. Using a rat microsomal Δ9 desaturase assay derived from the literature,[20] we performed a screen of approximately 5.2 million proprietary compounds. Initial hits were confirmed in a human hepatocellular liver carcinoma cell (HEPG2) Δ9 desaturase assay. Several compounds showed differential activity in the rat microsomal and human HEPG2 assays. This was not surprising given the potential differences in binding between the different species-specific isoforms, in addition to potential differences in cell-membrane permeability. Consequently, we set out to find compounds with high inhibitory potency in both assays.

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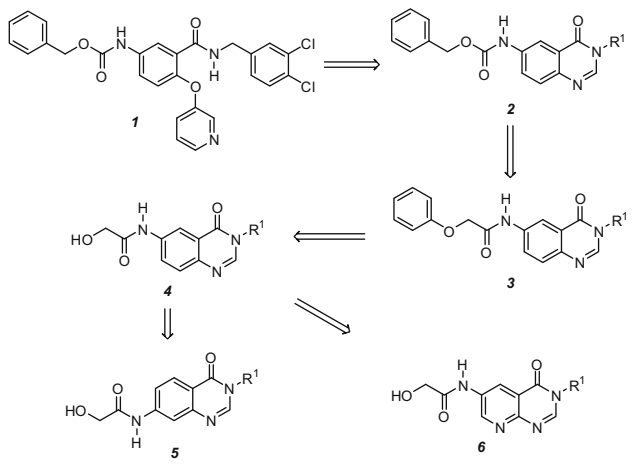


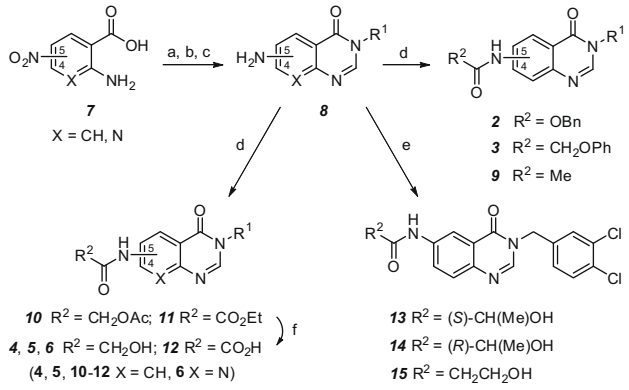
Figure 1. Screening hit **1** and the follow-up series **2**–**6**.

Table 1
Potency for SCD inhibition

	R^1	SCD IC_{50} (nM)	
		Rat Microsomal	Human HEPG2
1		3200	1150
2a	3,4-Dichlorobenzyl	251	193
2b	4-Chlorobenzyl	260	250
2c	3-Chloro-4-fluorobenzyl	865	220
2d	2,4-Dichlorobenzyl	>30,000	
2e	3,4-Dimethylbenzyl	250	790
3a	3,4-Dichlorobenzyl	268	68
3b	4-Chlorobenzyl	7970	
4a	3,4-Dichlorobenzyl	261	119
4b	4-Chlorobenzyl	1170	
4c	4-Chloro-3-(trifluoromethyl)benzyl	190	110
4d	3-(Trifluoromethyl)-benzyl	1360	
4e	4-(Trifluoromethyl)-benzyl	3420	
4f	3-Phenylbenzyl	3850	>1000
5a	3,4-Dichlorobenzyl	348	180
5b	4-Chlorobenzyl	3120	
5c	3-Chlorobenzyl	2690	
6a	3,4-Dichlorobenzyl	208	49
6b	4-Chlorobenzyl	2580	

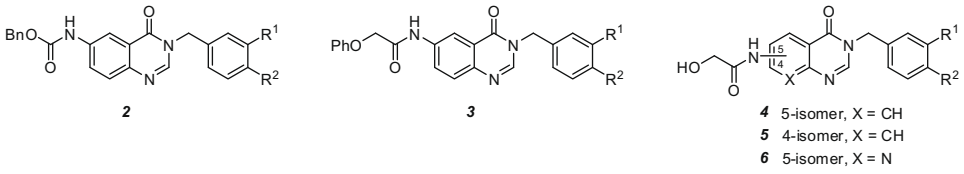
Compound **1** (Fig. 1) was identified from the screen as a modestly potent inhibitor of SCD (Table 1, IC_{50} 3.2 μM in rat microsomal/1.15 μM in HEPG2 SCD assays) and selective against Δ5 and Δ6 desaturases (IC_{50}'s > 30 μM). Unfortunately, compound **1** had poor metabolic stability (Table 2 and 1.0% and 0.2% remaining after a 30 min incubation with human and rat liver microsomes, respectively). In an effort to improve the metabolic stability of the hit, we replaced the central benzene core with a quinazolin-4-one, a known 'drug-like' scaffold (Fig. 1).

Quinazolin-4-one analogs **2**–**5**, **9**, and **12**–**15**, and related aza-analogs **6** were synthesized as shown in Scheme 1. Commercial carboxylic acids **7** were first coupled with the desired amine, cyclized to generate the quinazolin-4-one core,[21] and reduced to afford the corresponding anilines (**8**). Acylation of common intermediate **8** (5-isomer, X = CH, R^1 = 3,4-dichlorobenzyl) was performed with the following reagents and resulted in compounds: CbzCl (**2a–e**), phenoxyacetyl chloride (**3a**,**b**), acetyl chloride (**9**). Acylation of intermediate **8** (5-isomer, X = CH) bearing appropriate R^1 substituent with acetoxyacetyl chloride with subsequent hydrolysis of the ester provided compounds **4a–f**. Similarly compounds **5a–c** were obtained from appropriately substituted intermediates **8** (4-isomer, X = CH) and compounds **6a** and **b** were



Scheme 1. Reagents and conditions: (a) amine, EDC·HCl, HOBt, $(i\text{-Pr})_2NEt$, $CHCl_3$, rt, 24 h; (b) $HC(OEt)_3$ reflux, 24 h; (c) $NH_2NH_2{\cdot}H_2O$, Raney Ni, MeOH, 60 °C, 24 h; (d) R_2COCl, $(i\text{-Pr})_2NEt$, CH_2Cl_2, rt, 24 h; (e) carboxylic acid, HATU, $(i\text{-Pr})_2NEt$, $CHCl_3$, rt; (f) $LiOH{\cdot}H_2O$, H_2O/THF/MeOH, rt, 24 h.

Table 2
Calculated, in vitro, and in vivo DMPK parameters for the selected compounds



4 5-isomer, X = CH
5 4-isomer, X = CH
6 5-isomer, X = N

	R^1, R^2	MLogP[c]	Caco-2 P_{app}[a] $\times 10^6$ cm/s		Met. stability % after 30 min		Pharmacokinetics[b] dAUC (PO) (ng h/mL)	C_{max} (PO) (ng/mL)	%F	CLp (IV) (mL/min/kg)	$t_{1/2}$ (IV) (h)
			A→B	B→A	Human	Rat					
1		3.18	n.d.	n.d.	1.0	0.2	n.d.	n.d.	n.d.	n.d.	n.d.
2a	3,4-Cl_2	4.09	1.7	0.7	94	72	1.2 ± 1.2	1.05 ± 0.15	n.d.	n.d.	n.d.
2c	3-Cl-4-F	3.88	0.8	0.3	56	56	0	0	n.d.	n.d.	n.d.
3a	3,4-Cl_2	3.69	1.5	0.7	61	46	0.21 ± 0.02	1.29 ± 0.27	n.d.	n.d.	n.d.
4a	3,4-Cl_2	2.60	39.7	47.3	85	75	935 ± 90	360 ± 111	90	16.6 ± 3.8	1.46 ± 0.16
4c	3-CF_3-4-Cl	2.94	42.4	39.6	78	88	451 ± 123	439 ± 118	29	12.9 ± 2.3	1.38 ± 0.16
5a	3,4-Cl_2	2.60	33.3	40.8	82	42	195 ± 68	53 ± 20	23	29.0 ± 1.2	2.20 ± 0.21
6a	3,4-Cl_2	2.42	9.3	47.1	100	88	415 ± 54	53.5 ± 18	56	22.7 ± 3.1	1.44 ± 0.05

[a] Caco-2 permeability data was obtained by Absorption Systems, Inc., Exton, PA, USA.
[b] 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; F—oral bioavailability, $t_{1/2}$—elimination half-life (0–∞); n.d.—not determined.
[c] Calculated by Accord™ software, Accelrys, Inc., San Diego, CA, USA.

obtained from appropriately substituted intermediates **8** (5-isomer, X = N). Reaction of **8** (5-isomer, X = CH, R^1 = 3,4-dichlorobenzyl) with oxalic acid monoethyl ester chloride and subsequent hydrolysis provided analog **12**. Direct HATU coupling[22] of **8** (5-isomer, X = CH, R^1 = 3,4-dichlorobenzyl) with 1(*S*)-, 1(*R*)-, and 2-hydroxypropionic acids provided analogs **13-15**.

In a newly-designed scaffold **2** the Cbz-group on the western portion and the substituted benzyl group on the eastern portion were retained, and the oxy-pyridyl group on the southern end was left out. The resulting compound **2a** (IC_{50} 251 nM in the rat microsomal/193 nM in the HEPG2 SCD assays) not only exceeded the potency of the initial hit, but also had very good metabolic stability (94% and 72% remaining after 30 min incubation with human and rat liver microsomes, respectively, Table 2).

Next, we undertook an analoging effort around the R^1 substituent in scaffold **2** and looked at a number of substituted benzyl groups resembling the 3,4-dichlorobenzyl group, namely, 4-chlorobenzyl (**2b**), 3-chloro-4-fluorobenzyl (**2c**), 2,4-dichlorobenzyl (**2d**), and 3,4-dimethylbenzyl (**2e**) groups. Compound **2c** had potency for SCD inhibition somewhat comparable to **2a** (IC_{50} 865 nM in rat microsomal/220 nM in HEPG2 SCD assays) and was also moderately stable in liver microsomes (56% in each human and rat). Unfortunately both **2a** and **2c** showed low or no measurable plasma levels after oral dosing in rats (Table 2).

While the 3,4-dichlorobenzyl substituent on the eastern side appeared near-optimal, we turned our attention to the western side and attempted to modify the Cbz substituent. We postulated that Cbz group could be a site of metabolic cleavage and that by replacing it with a bioisostere we might be able to improve the pharmacokinetic properties. For that purpose, we switched the western portion from benzyloxycarbonyl in **2** to phenoxyacetyl in **3**. In **3a**, we were able to maintain potency (IC_{50} 268 nM in the rat microsomal/68 nM in the HEPG2 SCD assays) and microsomal stability (61% and 46% with human and rat liver microsomes, respectively) but failed to improve the oral exposure.

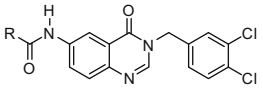
Since compounds of series **2** and **3** are highly lipophilic (M log*P* > 3.5) and were found to have low Caco-2 permeability (Table 2), we assumed that poor absorption rather than metabolism may be responsible for low oral exposure. In subsequent design, we proceeded to reduce the molecular weight and introduced a hydrophilic group. The phenyl group was removed from the phenoxyacetyl western portion of series **3** and replaced with a free hydroxyl resulting in series **4** (Fig. 1). The compound **4a**[23] retained good SCD inhibitory potency (IC_{50} 261 nM in rat microsomal/119 nM in HEPG2 SCD assays), good microsomal stability (85% and 75% with human and rat liver microsomes, respectively), and selectivity against Δ5 and Δ6 desaturases (IC_{50}'s > 30 μM). Caco-2 permeability of **4a** was dramatically improved compared to **2a**, **2c**, and **3a** (Table 2). The pharmacokinetic data of compound **4a** demonstrated good oral plasma exposure as expressed by the area under curve (dose-adjusted AUC = 935 ng h/mL) and C_{max} (360 ng/mL), and high oral bioavailability (90%). This suggests that GI absorbtion may have been indeed the limiting factor since Caco-2 screen was predictive of oral PK properties. The moderate intravenous plasma clearance (16.6 mL/min/kg) and terminal half-life of about 1.5 h were expected to be sufficient to enable a twice-a-day dosing regimen in a potential proof of concept study.

Within series **4**, we tested some additional benzyl substituents on the eastern portion, and found 4-chloro-3-(trifluoromethyl)benzyl analog (**4c**) to have SCD inhibitory activity (IC_{50} 190 nM in the rat microsomal/110 nM in the HEPG2 SCD assays) and good microsomal stability (85% and 75% with human and rat liver microsomes, respectively) similar to **4a**. However, the introduction of the bulkier, more lipophilic trifluoromethyl group (**4c**) reduced the oral exposure approximately 2-fold compared to the chloro substituent in **4a** (dose-adjusted AUC = 451 vs 935 ng h/mL).

As a part of our SAR effort we tested isomeric series **5** and aza-series **6**. In each case 3,4-dichlorobenzyl analogs (**5a** and **6a**) were similar to **4a** in terms of SCD activity and microsomal stability (Table 1). At the same time, both **5a** and **6a** showed higher IV clearance (29.0 and 22.7 mL/min/kg, respectively) and lower oral bioavailability (23% and 56%, respectively) than **4a** (Table 2).

We were also interested in further exploring the SAR around the hydroxyacetamide substituent. While keeping the 3,4-dichlorobenzyl substituent constant, we replaced the hydroxyacetyl group in the western portion with unsubstituted acetyl (**10**, R^2 = methyl, Table 3), oxalyl (**12**), 1(*S*)-hydroxypropionyl (**13**), 1(*R*)-hydroxypropionyl (**14**), and 2-hydroxypropionyl (**15**) derivatives. None of the above mentioned compounds presented any advantage over the parent analog **4a** in terms of potency of SCD inhibition.

Table 3
Elaboration of hydroxyacetyl motif in compound **4a**



	R	SCD IC_{50} (nM)	
		Rat Microsomal	Human HEPG2
4a	Hydroxymethyl	267	79
10	Methyl	3860	
12	Carboxylic acid	890	>1000
13	1(*S*)-Hydroxyethyl	>30,000	
14	1(*R*)-Hydroxyethyl	>30,000	
15	2-Hydroxyethyl	11,600	

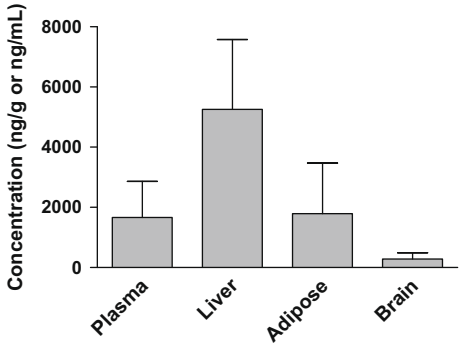


Figure 2. Tissue partitioning of compound **4a** (PO, rat, 10 mg/kg, 2 h post dose, mean ±SEM).

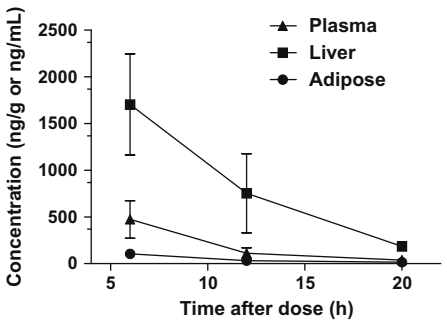


Figure 3. The time course of tissue partitioning for **4a** (PO, rat, 5 mg/kg, mean ±SEM).

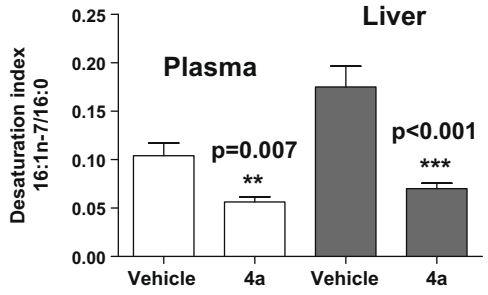


Figure 4. Effect of SCD inhibitor **4a** on plasma and liver desaturation index (5 day, PO BID, 10 mg/kg, mean ±SEM) (male Sprague–Dawley rats, high-carbohydrate diet, $n = 10$ (control) and $n = 8$ (treatment), tested for significance in unpaired t-test, data collected 4 h after last dose).

Next we investigated the tissue partitioning of compound **4a**. We found **4a** to selectively partition into the liver. At 2 h post oral dose (10 mg/kg) it had three times higher levels in liver than plasma and adipose tissue (Fig. 2). After oral dosing at 5 mg/kg (Fig. 3) we found that preferential hepatic partitioning of the compound was maintained to at least 24 h and the compound was cleared similarly from plasma ($t_{1/2} \sim 5$ h), liver, ($t_{1/2} \sim 5.6$ h) and adipose ($t_{1/2} \sim 6.4$ h). Based on the oral half-life and significant drug levels (plasma112 ng/mL and liver 753 ng/g) at 12 h post dose the compound appeared suitable for BID dosing. In brain, significantly lower levels of **4a** were detected the (283 ng/g, 2 h post 10 mg/kg oral dose) (Fig. 2). This finding at least partially alleviates the concern for potential inhibition of SCD2/5 in the brain.

We carried out an in vivo efficacy study in Sprague–Dawley rats that were kept on a high carbohydrate diet for 4 weeks (Fig. 4). Compound **4a** was dosed orally (BID) for 5 days and the desaturation index [SCD product/substrate, palmitoleic acid ($16{:}1_{n-7}$)/palmitic acid (16:0)] measured 4 h after the final dose by extraction, esterification, and GC analysis of methyl esters.[24,25] A similar trend was observed for other indexes of Δ9 desaturation, however our experience has been that the C16 ratio is less influenced by dietary and stored oleic acid ($18{:}1_{n-9}$), since $16{:}1_{n-7}$ is a low abundant fatty acid and absent from non-animal fat diets. In this 5-day study we found a highly significant reduction in SCD product fatty acids in both plasma and liver, thus demonstrating the in vivo SCD inhibitory properties of compound **4a**.

In conclusion, by changing the benzene ring core in hit **1** with quinazolin-4-one (**2**), we achieved good metabolic stability and improved SCD inhibitory properties. By reducing the molecular weight and introducing the hydroxyacetamide group, we created compounds with high oral bioavailability (90% in case of **4a**). Caco-2 has proven useful as a screening tool, since in series **2–4**, cell permeability was found to be predictive of oral exposure. In an in vivo efficacy study compound **4a** demonstrated the highly significant reduction in the amount of SCD product in plasma and liver. Long-term animal studies are under way to further investigate the effect of compound **4a** (CVT-11,563) in models of obesity and diabetes.

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23. Compound **4a** ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.6 4 d, $J = 2.4$ Hz, 1H), 8.52 (s, 1H), 8.10 (dd, $J = 8.8$, 2.4 Hz, 1H), 7.71 (d, $J = 1.6$ Hz, 1H), 7.68 (d, $J = 8.4$ Hz, 1H), 7.62 (d, $J = 8.8$ Hz, 1H), 7.37 (dd, $J = 8.0$,1.6 Hz, 1H), 5.19 (s, 2H), 4.06 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.35, 160.02, 146.46, 143.89, 137.89, 137.51, 131.10, 130.79, 130.34, 129.97, 128.15, 127.79, 126.69, 121.82, 114.99, 79.15, 61.91. Anal. C, H, N.
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