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Discovery of potent and liver-selective stearoyl-CoA desaturase (SCD) inhibitors in an acyclic linker series

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ABSTRACT

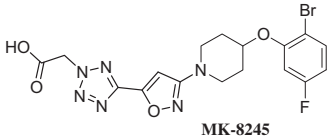
Elevated levels of stearoyl-CoA desaturase (SCD) activity have been implicated in metabolic disorders such as obesity and type II diabetes. To circumvent skin and eye adverse events observed in rodents with systemically-distributed inhibitors, our research efforts have been focused on the search for new liver-targeting compounds. This work has led to the discovery of novel, potent and liver-selective acyclic linker SCD inhibitors. These compounds possess suitable cellular activity and pharmacokinetic properties to inhibit liver SCD activity in a mouse pharmacodynamic model.

Inhibition of stearoyl-CoA desaturase (SCD) represents a potential novel mechanism of action for the treatment of obesity and type II diabetes, which continue to expand at epidemic rates.[1] The SCD enzyme is present in two isoforms in humans (SCD1 and SCD5) and four in rodents (SCD1-4) where the SCD1 isoform is predominantly found in liver (target organ for efficacy). In the literature, adverse events (AEs),[2] such as partial eye closure and progressive alopecia, were observed in mice after ~7 days of treatment with systemically-distributed SCD inhibitors. These AEs are likely due to the depletion of SCD-derived lubricating lipids in skin and eye, and the development of liver-targeted SCD inhibitors should circumvent these issues.[3]

The initial strategy employed in the design of liver-selective compounds centers on exploring the addition of polar acidic moieties which are recognized by organic anionic transporters (OATPs),[4] such as tetrazoles or carboxylic acids, on SCD inhibitors to obtain the desired in vivo properties: a high liver concentration (target organ for efficacy) and a low systemic concentration to minimize exposures in off-target tissues and cells associated with adverse events (skin and eye).

MK-8245 (Fig. 1), a phenoxy piperidine isoxazole derivative, has been identified as a potent and liver-selective SCD inhibitor with an enzymatic potency (IC_{50}) against the rat SCD of 3 nM.[5] In addition, MK-8245 possesses good in vivo potency in a mouse liver pharmacodynamic model (mLPD, ED_{50} ~1 mg/kg) and showed no AEs after 4 weeks of chronic dosing in mice.[5,6] Following the discovery of MK-8245, our group continued our efforts on the identification of structurally diverse liver-targeted SCD inhibitors for the selection of a back-up compound to our lead MK-8245 to support the development of SCD inhibitors in preclinical species and eventually in humans.

In vitro studies have demonstrated that the liver-targeted tissue distribution profile of MK-8245 is likely the result of substrate recognition by organic anionic transporter proteins (OATPs) which are highly expressed in hepatocytes.[4,5] An important feature in the structure of MK-8245 is the tetrazole acetic acid moiety, which is the key functionality for OATPs recognition and liver-targeting. In a search for new structural classes, we intentionally kept this group in place for active transporter recognition and modified other parts of the molecule. We envisioned that the replacement of the piperidine core present in MK-8245, and also frequently



MK-8245

Figure 1. MK-8245: a potent SCD inhibitor (Rat SCD IC_{50} = 3 nM).

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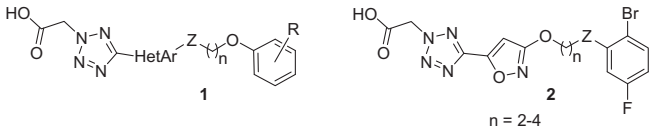


Figure 2. Core structure of acyclic linker series **1** and initial tether optimization in compound **2**.

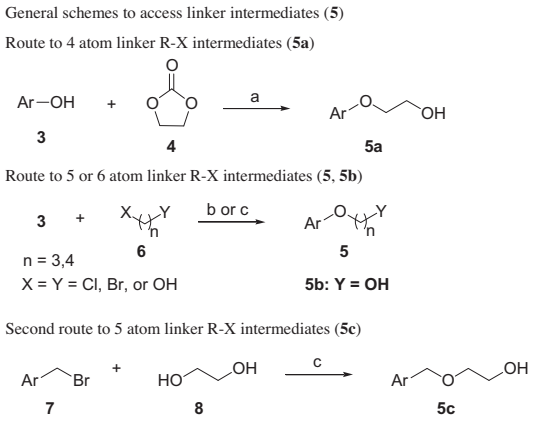
found in many SCD-inhibitors,[2a,7] with linkers such as an acyclic tether would provide a distinct and structurally diverse series of SCD1 inhibitors **1** (Fig. 2).[8]

In the absence of an SCD1 enzyme X-ray crystal structure, we chose to proceed with a systematic SAR study to guide our optimization efforts. Initially, we turned our attention to the synthesis of 4–6 atom linkers **2** (Fig. 2) while keeping the remaining structural elements of MK-8245 unchanged.

As depicted in Scheme 1, the construction of 4–6 atom linker counterparts involved ring opening of ethylene carbonate **4** with phenol **3**, Mitsunobu reaction between phenol **3** and alcohol chains **6** and S_N2 alkylation of the phenol or the ethylene glycol **8** with the alkyl halide **6** or **7** in presence of a base such as K_2CO_3.

Having in hand a suitable collection of phenoxy linkers **5** with different chain lengths, we next turned our attention to link these molecules to the isoxazole tetrazole acetic acid moiety. As illustrated in Scheme 2, the initial route consisted of a stepwise construction of the tetrazole acetic acid moiety contained in **13**.[9] To this end, reaction of hydroxyisoxazole **9** with **5** under Mitsunobu conditions or S_N2 alkylation with K_2CO_3 gave exclusively the O-alkylation product **10**. The ester compound **10** thus obtained was transformed to the nitrile **11** by conversion of the ester group to the corresponding primary amide with ammonia followed by dehydration with TFAA. The preparation of the tetrazole **12** was completed by reaction of the nitrile **11** with NaN_3 under slightly acidic conditions. Introduction of the acetate ester group on the tetrazole **12** gave two regioisomers **13** and **14**. Under several conditions evaluated (NaH in DMF, K_2CO_3 in DMF, Mitsunobu, Et_3N in THF, Hünig's base in 1,4-dioxane) the best ratio in favor of **13** was obtained with tertiary amine bases and ethereal solvents. Final hydrolysis of **13** with NaOH afforded **16**.

Subsequently, a simplified route was realized through the use of a highly functionalized intermediate **15**. Employing *p*-methoxybenzyl (PMB) alcohol **5** under the reaction conditions outlined in Scheme 2 and subsequent cleavage of the PMB under acidic conditions, then afforded the versatile synthetic intermediate **15**. By



Scheme 1. Preparation of phenoxy linker moieties **5**. Reagents and conditions: (a) imidazole (cat.), neat, 150 °C for 5 h; (b) (Y = OH); di-*tert*-butyl azodicarboxylate, PPh_3, CH_2Cl_2–THF, −78 °C to r.t. for 1–24 h; (c) (Y = Cl, Br); K_2CO_3, DMF, 60 °C or 100 °C for 1–5 h.

avoiding the repetitive tedious synthesis of the tetrazole acetic acid moiety for each compound, the coupling of **15** with an appropriately substituted phenoxy linker **5** represented an expedient method that allows the preparation of acyclic linker targets **16** in only two steps (Scheme 2).

The SCD inhibitors prepared were tested against the SCD1 enzyme in an SCD-induced rat liver microsomal assay.[10] Their cellular potencies were evaluated in a human HepG2-based whole cell assay which was devoid of OATPs,[11] and the ability to cross the cell membrane through active transporters was assessed in a rat hepatocyte (Rat Hep) assay which contains OATPs.[5] The goal was to qualitatively determine if an SCD inhibitor was actively transported into hepatocytes (potent Rat Hep IC_{50}) while maintaining poor cell permeability (poor HepG2 IC_{50}).

As shown in Table 1, the optimal replacement of the piperidine core in MK-8245 was with a 5-atom tether **16b**, whereas the presence of a shorter tether **16a** or longer one **16c** resulted in loss of potency. Interestingly, the position of the oxygen atom next to the phenyl group remains critical as illustrated with the loss of potency observed with the benzyl analog **16d**. The acyclic linker **16b** displayed a ~600-fold shift of potency in the HepG2 assay (IC_{50} = 7740 nM) and improved to 16-fold shift in the rat hepatocyte assay (IC_{50} = 213 nM), which highly suggests the involvement of active transporter systems. Overall, the acyclic linker **16b** showed 4 to 7-fold lower in vitro potency (Rat SCD, HepG2 and Rat Hep) when compared to MK-8245 (Table 1).

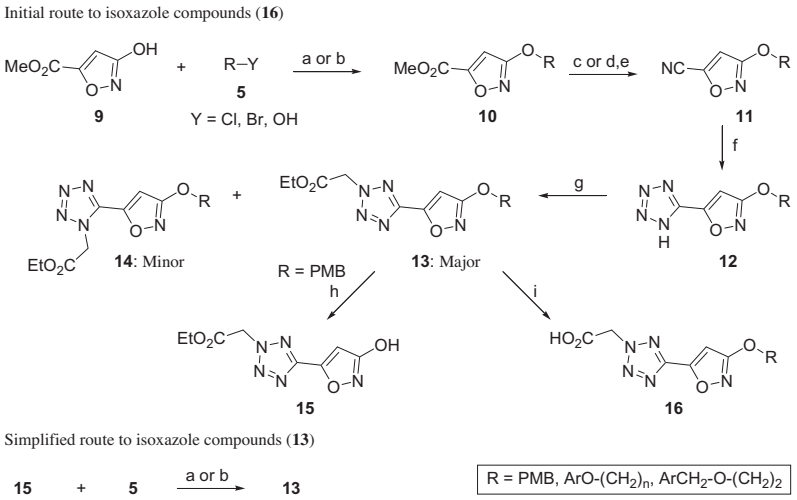
The enzymatic potency against the human SCD was also measured. In a human SCD1 enzyme assay (hSCD1) (delta-9 desaturase), compound **16b** displayed an IC_{50} of 41 nM compared to 1 nM for MK-8245.[5] The linker **16b** is almost equipotent at inhibiting the other isoform found in human (hSCD5 IC_{50} = 32 nM). In addition, it is selective against two other desaturase enzymes (delta-5 and delta-6 desaturases) present in human, with IC_{50}s >20 μM in a whole cell assay.[11]

Before pursuing further SAR, we decided to evaluate the in vivo potency of **16b** in a mouse liver pharmacodynamic model (mLPD).[6] To support this model, the activity for the linker **16b** was measured in a mouse SCD1 enzyme assay (IC_{50} = 10 nM) which showed a similar potency compared to the rat SCD enzyme. Also, the dose selection was based on the pharmacokinetic (PK) profile determined in C57BL6 mice following oral dosing at 10 mg/kg in 0.5% methocel as the vehicle. Under these conditions, the linker **16b** had a low liver concentration at a 6 h time-point (0.23 μM).[12] Consequently, a high dose (60 mg/kg) of **16b** was required to efficiently suppress liver SCD activity in the mLPD assay (85% inhibition at a liver concentration of 7.8 μM of **16b**). In contrast, MK-8245 required only a dose of 2 mg/kg in the mLPD model to afford 89% liver SCD inhibition at a liver exposure of 4.9 μM.

To further understand the underlying cause of the poor PK profile of **16b**, we performed in vitro metabolism studies. In standard mouse and rat hepatocyte incubations, compound **16b** showed two major metabolites: the oxidative defluorination followed by glutathione (GSH) conjugation on the phenyl ring and the reductive ring opening of the isoxazole heterocycle.[13,14]

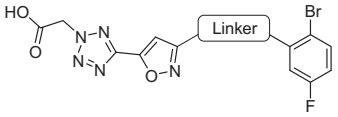
These data oriented our synthetic efforts on improving the in vivo potency by increasing the metabolic stability of this class of inhibitors.

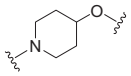
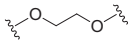
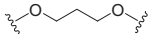
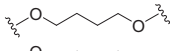
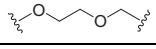
The identification of more metabolically stable heterocycles that may not suffer from the ring opening observed with the isoxazole core became our first priority. The method used to prepare isoxazole compounds **16** under O-alkylation conditions was unsuccessful for other heteroaromatic rings delivering exclusively the N-alkylation product. To circumvent this problem, compounds **22–24** were prepared via displacement of a halo-heteroaromatic ring **17** with the alkoxy compounds **5a–b** as reported in Scheme 3. Further elaboration to the tetrazole acetic acid group from ester **18a**, amide



Scheme 2. Preparation of analogs in Table 1. Reagents and conditions: (a) (Y = OH); di-*tert*-butyl azodicarboxylate, PPh_3, CH_2Cl_2–THF, −78 °C to r.t. for 1−24 h; (b) (Y = Cl, Br); K_2CO_3, DMF, 60 °C for 1–5 h; (c) NH_4OH (conc.), THF 0 °C to r.t. for 2 days; (d) NH_3, MeOH, sealed tube, 125 °C for 30–60 min; (e) TFAA, Hünig's base, CH_2Cl_2, −78 to 0 °C for 5–45 min; (f) NaN_3, Py·HCl, NMP, 125–140 °C for 30–90 min; (g) ethyl bromoacetate, Hünig's base, 1,4-dioxane, 90 °C for 1 h; (h) (PMB: *p*-methoxybenzyl); TFA, Me_2S, H_2O, CH_2Cl_2, r.t. for 3 h; (i) 1 N NaOH, MeOH, THF, r.t. for 5 min.

Table 1
Exploration of the piperidine replacement



Compound	Linker	IC_{50} (nM)[a]		
		Rat SCD	HepG2	Rat Hep
MK-8245		3	1070	68
16a		226	>40,000	n.d.
16b		13	7740	213
16c		248	>40,000	n.d.
16d		305	>100,000	n.d.

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

18b or nitrile **18c** employed similar conditions as described for the isoxazoles **16** (see Scheme 2).

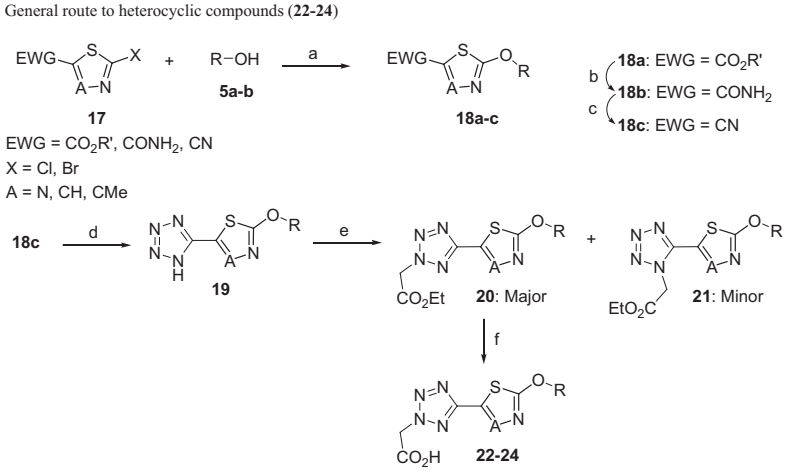
The activities on the SCD-induced rat liver microsomal assay for the heterocycles **22–24** prepared are reported in Table 2. Interestingly, SCD inhibitory activity was slightly improved with the 5-membered heterocyclic thiazole **23a**.[15] Also, this thiazole heterocyclic ring displayed an improved in vitro metabolic profile. Compound **23a**, when subjected to a standard rat hepatocyte incubation for 2 h, was metabolically stable, with 94% parent remaining.[13] The only metabolites observed resulted from the oxidative defluorination and GSH conjugation on the phenyl ring.

In comparison to **16b**, the data from the in vitro metabolism experiment was in agreement with a better PK profile measured for the thiazole **23a** in C57BL6 mice following typical 10 mg/kg oral dosing (F = 32%, $AUC_{0-6\ h}$ = 11.4 μM h). The liver concentration at a 6 h time-point (8.07 μM) was also improved. Evaluation of this compound **23a** in the mLPD model showed a 49% inhibition at a lower dose of 2 mg/kg with a liver exposure of 7.6 μM (total drug concentration, see Table 3).

Having in hand a good replacement for the central isoxazole ring, we turned our attention to minimize the oxidative defluorination metabolism observed in hepatocyte incubations with **23a**. Among the fluorine replacements explored,[16] we found that the phenyl ring substituted with an OCF_3 group instead of the fluorine was metabolically more stable under hepatocyte incubation conditions. In addition to the superior metabolic stability over the fluorine atom, the in vitro potency of **23b** was also slightly improved when compared to **23a** (Table 2). In the mLPD experiment, 81% inhibition of the SCD activity was measured following an oral dose of 2 mg/kg of **23b** with a liver exposure of 4.2 μM (Table 3). In summary, the acyclic linker **23b** possesses a comparable in vitro potency to MK-8245 (Table 1) and excellent in vivo efficacy in the mLPD at 2 mg/kg (Table 3).

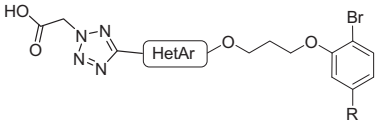
As expected, **23b** displayed a liver-selective tissue distribution profile. However, the liver/plasma and liver/Harderian glands ratios were slightly reduced when compared to MK-8245 (Table 3). The concentration of **23b** in the Harderian glands was in the micro-molar level, which would not be desirable, given the connection that inhibiting SCD in this tissue is linked to eye AEs.[5]

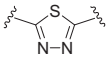
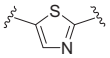
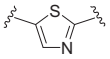
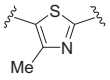
In conclusion, we have identified an acyclic linker series as a new structural class of SCD inhibitors. In this series, the piperidine core present in MK-8245, and in typical SCD-inhibitors,[7] is



Scheme 3. Preparation of analogs in Table 2. Reagents and conditions: (a) NaH, DMF, r.t. or 60 °C for 1 h; (b) NH_3, MeOH–THF, sealed tube, 125 °C for 5 h; (c) TFAA, Hünig's base, CH_2Cl_2, −78 to 0 °C for 5 min; (d) NaN_3, NH_4Cl, DMF, 100 °C for 1–2 h; (e) ethyl bromoacetate, Hünig's base, 1,4-dioxane, 90 °C for 1 h; (f) 1 N NaOH, THF, r.t. for 15–30 min.

Table 2
SAR on the heteroaromatic ring



Compound	HetAr	R	IC_{50} (nM)[a]		
			Rat SCD	HepG2	Rat Hep
22		F	26	>60,000	n.d.
23a		F	5	3870	176
23b		OCF_3	2	969	91
24		F	22	20,500	945

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

Table 3
In vitro and in vivo profiles of MK-8245, **23a** and **23b** in mice

	Mouse SCD IC_{50} (nM)[a]	In vivo	
		TD (μM)[b]	mLPD[c]
MK-8245	3	[Liver] = 2.70 [Plasma] = 0.03 [skin (shaved)] = 0.07 [Harderian glands] = 0.13	89% inh. at 2 mg/kg [Liver] = 4.9 μM 49% inh. at 0.4 mg/kg [Liver] = 1.6 μM
23a	13	[Liver] = 8.07 [Plasma] = 1.08 [skin (shaved)] = 0.24 [Harderian glands] = 0.59	79% inh. at 10 mg/kg [Liver] = 28.6 μM 49% inh. at 2 mg/kg [Liver] = 7.6 μM
23b	6	[Liver] = 13.7 [Plasma] = 0.77 [skin (shaved)] = 0.30 [Harderian glands] = 1.85	91% inh. at 10 mg/kg [Liver] = 18.3 μM 81% inh. at 2 mg/kg [Liver] = 4.2 μM

[a] IC_{50}s are an average of at least two independent titrations.
[b] TD—tissue distribution (PO, mouse (n = 2), 10 mg/kg; 6 h post dose).
[c] mLPD (PO, mouse (n = 5), 3 h post dose). inh.—inhibition.

replaced by an alkyl chain connecting the heterocycle ring via an oxygen atom. SAR and metabolism studies have led to the identification of the thiazole heterocycle and the 2-bromo-5-trifluoromethoxyphenol in **23b** as the optimal combination for in vitro potency and in vivo efficacy. Presently, the exploration of the acyclic linker series is suspended but any future work will need to focus on improving liver-selectivity of this series and reducing the Harderian gland drug exposure that may be linked to potential eye AEs.

Acknowledgments

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6. Mouse liver pharmacodynamic model (mLPD) is expressed in percentage (%) inhibition and is used to assess the in vivo potency. In the mLPD experiment, mice were fed on a high carbohydrate diet and the SCD activity was indexed 3 h post oral dose of SCD inhibitors by following the conversion of intravenously administered [1–^{14}C]-stearic acid tracer to the SCD-derived [1–^{14}C]-oleic acid in liver lipids. The percentage (%) of inhibition of an SCD inhibitor is calculated from the liver SCD activity index (ratio of ^{14}C-oleic acid/^{14}C-stearic acid) from drug treated animals compared to a vehicle group.
7. Leading references: (a) Zhao, H.; Serby, M. D.; Smith, H. T.; Cao, N.; Suhar, T. S.; Surowy, T. K.; Camp, H. S.; Collins, C. A.; Sham, H. L.; Liu, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3388; (b) Liu, G.; Lynch, J. K.; Freeman, J.; Liu, B.; Xin, Z.; Zhao, H.; Serby, M. D.; Kym, P. R.; Suhar, T. S.; Smith, H. T.; Cao, N.; Yang, R.; Janis, R. S.; Krauser, J. A.; Cepa, S. P.; Beno, D. W. A.; Sham, H. L.; Collins, C. A.; Surowy, T. K.; Camp, H. S. *J. Med. Chem.* **2007**, *50*, 3086.
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9. The regiochemistry of the acetic acid side chain on the tetrazole was confirmed by evaluation of the structures through NMR experiments: gHMBC, ^{15}N gHMBC and 1D NOESY.
10. Rat microsomal assay conditions: Li, C. S.; Ramtohul, Y.; Huang, Z.; Lachance, N. WO 2006/130986.
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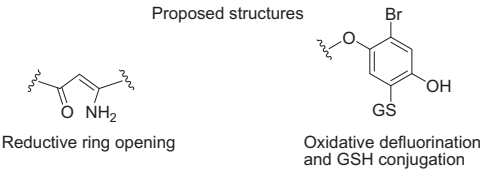
12. PK data of compound **16b** in C57BL6 mice following oral dosing at 10 mg/kg in 0.5% methocel: F = 10% and $AUC_{0-6\ h}$ = 1.0 μM h.
13. For compound **16b**, extensive metabolism was observed (mouse: 75% parent remaining; rat: 55% parent remaining) after incubation at 37 °C with fresh mouse hepatocytes and rat hepatocytes (2 million cells/mL of Krebs-Henseleit buffer) for 2 h under 95% O_2/5% CO_2 atmosphere. There were two major metabolites observed but not fully characterized. In mouse, the main metabolite is the ring opening of the isoxazole. However in rat, the major metabolite involves oxidation of the phenyl ring, defluorination and glutathione (GSH) adduct, in addition to the minor reductive ring-opened metabolite.



14. For comparison, the isoxazole ring-opened metabolite was observed at low level (4%) in vitro in rat hepatocytes with MK-8245.
15. Compounds containing a 6-membered heterocycle ring pyridazine or pyrazine were less potent. Data unpublished.
16. Several substituents (H, Cl, Br, CF_3, SO_2Me, OCyp, $NHCH_2CF_3$, Ar) were evaluated across numerous combinations of different tether lengths and various 5-membered ring heterocycles. Unpublished results.