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Synthesis and Biological Evaluation of a Novel 3-Sulfonyl-8-azabicyclo[3.2.1]octane Class of Long Chain Fatty Acid Elongase 6 (ELOVL6) Inhibitors

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Abstract: Long chain fatty acid elongase 6 (ELOVL6) catalyzes the elongation of long chain fatty acyl-CoAs and is a potential target for the treatment of metabolic disorders. The ultrahigh throughput screen of our corporate chemical collections resulted in the identification of a novel 3-sulfonyl-8-azabicyclo[3.2.1]octane class of ELOVL6 inhibitor **1a**. Optimization of lead **1a** led to the identification of the potent, selective, and orally available ELOVL6 inhibitor **1w**.

The incidence of type 2 diabetes has dramatically increased over the past decade. Accumulating evidence suggests a strong correlation between insulin resistance and the development of type 2 diabetes mellitus. An increase in fat storage in non-adipose tissues, such as liver, leads to dysfunction of those tissues (i.e., insulin resistance).[1] Although the mechanism by which increased intracellular lipid content exacerbates tissue and whole body insulin sensitivity is unclear, it has been suggested that increased levels of long chain fatty acyl-CoA[a] antagonize the metabolic actions of insulin.[2] Interestingly, recent reports have suggested that alteration of the specific fatty acid component (i.e., palmitoleate) has significant impact on the insulin sensitivity of the liver and whole body.[3,4] These observations indicate that modulation of fatty acid composition may lead to amelioration of insulin resistance.

Biosynthesis of fatty acids is sequentially catalyzed by acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), long chain fatty acid elongase 6 (ELOVL6), and stearoyl-CoA desaturase (SCD)-1.[5] Of these, ELOVL6 (known as LCE, FACE) is responsible for the elongation reaction of long chain fatty acyl-CoAs (C12−16 saturated and monounsaturated fatty acyl-CoAs) using a malonyl-CoA as a two-carbon donor.[6−10] ELOVL6 is a membrane protein and resides in the endoplasmic reticulum in cells.[6] ELOVL6 is abundantly expressed in lipogenic tissues such as liver and white adipose tissue (WAT).[6] ELOVL6 expression is directly and primarily regulated by sterol regulatory element-binding protein (SREBP)-1c.[11] Human and mouse ELOVL6 isoforms display 96% identity in amino acid sequence.[6]

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[a]Abbreviations: ACC, acetyl-CoA carboxylase; AUC, area under the curve; CoA, coenzyme A; ELOVL, long chain fatty acid elongase; FAS, fatty acid synthase, SCD-1, stearoyl-CoA desaturase-1; SD, Sprague−Dawley; SREBP, sterol regulatory element binding protein; UHTS, ultrahigh throughput screening; WAT, white adipose tissue.

So far, seven subtypes of ELOVL enzyme have been identified in mammals and are designated ELOVL1−7.[6,7,12−15] The ELOVL enzymes can be divided into two major groups in terms of substrate preference: (a) enzymes that elongate saturated and monounsaturated long-chain fatty acids (ELOVL1, -3, and -6) and (b) enzymes that elongate polyunsaturated fatty acids (ELOVL2, -4, and -5). In addition to these different fatty acid substrate preferences, each ELOVL enzyme is expressed in different tissues and their expression is differently regulated, indicating that they play different physiological roles in vivo.[7,16,17]

Recent investigations revealed potential physiological and pathological roles of ELOVL6. Matsuzaka and co-workers recently reported that ELOVL6 deficient mice display improved insulin sensitivity and glucose homeostasis compared with wild-type mice when fed on a high-fat diet.[4] Moreover, mRNA levels of ELOVL6 are up-regulated in obese rodents by refeeding after fasting and by exposure to a high carbohydrate diet.[6,7,18] These observations suggest that ELOVL6 might be a new therapeutic target for diabetes. Despite the increasing information on the physiological and pathological roles of ELOVL6, a lack of useful chemical tools has made it difficult to address pharmacological roles of ELOVL6 and its therapeutic potential.

We previously reported the establishment of a homogeneous enzyme assay for ELOVL6, which is applicable to the ultrahigh throughput screening (UHTS) using a recombinant histidine-tagged acyl-CoA binding protein as a molecular trap for the detection of radioactive products of ELOVL6.[19] The UHTS of our company chemical library resulted in the identification of a novel 3-sulfonyl-8-azabicyclo[3.2.1]octane lead **1a**, which has an IC_{50} of 1750 nM. In this report, we describe the synthesis and preliminary SAR of the 3-sulfonyl-8-azabicyclo[3.2.1]octane class of ELOVL6 inhibitors and the discovery of the potent, selective, and orally available ELOVL6 inhibitor **1w**. Biological profiles of **1w** including cellular activity, off-target profiles, pharmacokinetic parameters, and in vivo efficacy in mice are also reported.

The synthesis of **1** and **2** described herein is outlined in Scheme 1. Commercially available Boc-tropinone **4** was reduced with sodium borohydride in the presence of cerium chloride followed by mesylation of the resultant alcohols **5** and **6** to give *exo*-mesylate **7** and *endo*-mesylate **8**, respectively. The *exo*-mesylate **7** was coupled with various arylthiols to give *endo*-sulfides **9**, which were subsequently oxidized with *m*-CPBA or $KMnO_4$ to afford sulfoxide **10** or sulfones **11**, respectively. Deprotection of the Boc group of **10** and **11** under acidic conditions gave amines **12**. Coupling of **12** with various phenyl arylcarbamates or acids furnished target **1**. The *exo*-isomer **2** was synthesized from the *endo*-mesylate **8** using the same procedures for the *endo*-derivatives **1**.

The newly synthesized compounds were first subjected to the human ELOVL6 enzyme assay. Selected potent compounds were further evaluated for inhibitory activities for mouse ELOVL6, human and mouse ELOVL3 enzymes, and their metabolic stability in mouse liver microsomes. ELOVL3 inhibitory activity was assessed because ELOVL6 and ELOVL3 show the highest homology to each other (44%) in the amino acid sequence.[6]

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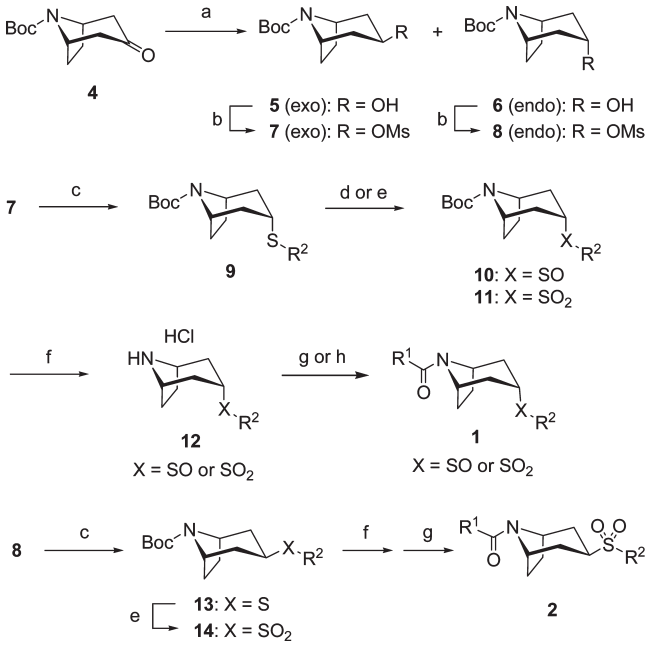
Scheme 1[a]

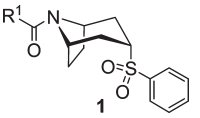


[a] Reagents and conditions: (a) $NaBH_4$, $CeCl_3 \cdot 6H_2O$, H_2O, EtOH; (b) MsCl, TEA, THF; (c) R^2SH, K_2CO_3, DMF; (d) *m*-CPBA, CH_2Cl_2; (e) $KMnO_4$, acetic acid, acetone; (f) HCl, MeOH; (g) phenyl arylcarbamate, TEA, $CHCl_3$; (h) R^1CO_2H, WSC·HCl, pyridine.

As lead **1a** exhibits only modest intrinsic ELOVL6 activity, we initiated SAR exploration of **1a** to obtain potent ELOVL6 inhibitors. First, the SAR around the left-hand phenyl moiety of **1a** was investigated (Table 1). Removal of the para-methyl group on the phenyl portion resulted in analogues devoid of potency as in **1b**, while the pyridine derivatives **1c**−**e** showed no improvement in potency. Given the important role of the substituent, the various para-substituted derivatives (**1f**−**j**) were synthesized and evaluated. While the para-fluoro and methoxy derivatives **1f** and **1g** were less active than **1a**, the para-chloro derivative **1h** was twice as potent as **1a**. Intriguingly, the para-trifluoromethyl derivative **1k** displayed a 22-fold increase in inhibitory activity (IC_{50} = 78 nM) relative to **1a**. With regard to larger substituents, the isopropyl derivative **1i** was the most potent (IC_{50} = 32 nM), whereas the phenyl derivative **1j** resulted in a marked loss of potency. The ortho- and meta-trifluoromethyl derivatives **1m** and **1l** were much less potent than the para-derivative **1k**. The potent derivatives **1i** and **1k** were evaluated for metabolic stability in mouse liver microsomes. The percent remaining of **1i** and **1k** after 30 min of incubation with mouse liver microsomes at 37 °C was 0% and 38%, respectively;[20] therefore, the trifluoromethyl derivative **1k** was selected for further optimization.

Next, the central 3-sulfonyl-8-azabicyclo[3.2.1]octane core was investigated (Table 2). With regard to the urea linkage moiety, replacement of the urea linkage with the amide (**1n**) or methyleneamide (**1o**) resulted in a marked loss of potency, indicating that the urea linkage is essential for ELOVL6 inhibitory activity. Regarding the 8-azabicyclo[3.2.1]-octane core, replacement of 8-azabicyclo[3.2.1]octane with piperidine resulted in a complete loss of potency, as in **3**. As for the sulfone linkage portion, replacement of the sulfone with a sulfoxide is detrimental to potency as in **1p**. The *endo* configuration of **1k** is essential for potency; the *exo*-isomer **2** is devoid of potency. These results indicate that the 3-*endo*-sulfonyl-8-azabicyclo[3.2.1]octane core is an essential structure for ELOVL6 inhibitory activity.

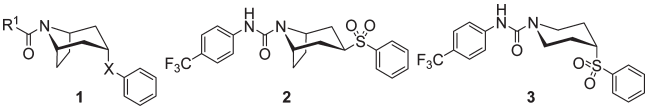
Table 1. SAR around Left-Hand Phenyl Moiety[a]



compd	R^1	human ELOVL6 enzyme IC_{50} (nM)
1a	4-Me-PhNH-	1750 ± 370
1b	PhNH-	> 10000
1c	2-pyridinyl-NH-	> 10000
1d	3-pyridinyl-NH-	> 10000
1e	4-pyridinyl-NH-	> 10000
1f	4-F-PhNH-	> 10000
1g	4-MeO-PhNH-	5070 ± 1090
1h	4-Cl-PhNH-	970 ± 360
1i	4-(*i*-Pr)-PhNH-	32 ± 3
1j	4-biphenyl-NH-	> 10000
1k	4-CF_3-PhNH-	78 ± 28
1l	3-CF_3-PhNH-	1400 ± 240
1m	2-CF_3-PhNH-	> 10000

[a] The values represent the mean ± SE for $n \geq 3$.

Table 2. SAR around Central 3-Sulfonyl-8-azabicyclo[3.2.1]octane Core[a]



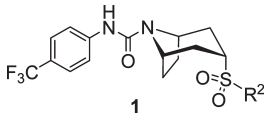
compd	R^1	X	human ELOVL6 enzyme IC_{50} (nM)
1k	4-CF_3-PhNH-	-SO_2-	78 ± 28
1n	4-CF_3-Ph-	-SO_2-	1750 ± 370
1o	4-CF_3-Ph-CH_2-	-SO_2-	> 10000
1p	4-CF_3-PhNH-	-SO-	> 10000
2			> 10000
3			> 10000

[a] The values represent the mean ± SE for $n \geq 3$.

Finally, the right-hand phenyl portion was optimized (Table 3). Meta-substitution with chloro (**1u**) and methoxy (**1r**) groups resulted in 8- to 11-fold decreased potency. These deleterious effects were more noticeable in the ortho- and para-substituted derivatives **1q**, **1s**, **1t**, and **1v**. These results suggest that introduction of substituents on the right-hand phenyl group is unfavorable. Subsequently, the phenyl group was replaced by a pyridine ring. As a result, the 2-pyridinyl derivative **1w** was tolerated, whereas the 3- and 4-pyridinyl derivatives **1x** and **1y** showed a 7-fold decrease in potency. Intriguingly, the 2-pyridinyl derivative **1w** exhibited improved mouse microsomal stability (% remaining = 67%) compared to **1k** (% remaining = 38%).[20] Given the potent ELOVL6 inhibitory activity and good microsomal stability, **1w** was selected as a candidate for further in vitro characterization.

Mouse ELOVL6 inhibitory activity, selectivity over the other ELOVL subtypes, and cellular activity of **1w** are summarized in Table 4. Compound **1w** showed comparable inhibitory activities for human and mouse ELOVL6s (IC_{50} of 79 and 94 nM, respectively). Regarding ELOVL subtype

Table 3. SAR around Right-Hand Phenyl Moiety[a]



1

compd	R^2	human ELOVL6 enzyme IC_{50} (nM)
1k	Ph-	78 ± 28
1q	2-MeO-Ph-	7970 ± 1560
1r	3-MeO-Ph-	840 ± 90
1s	4-MeO-Ph-	5630 ± 1500
1t	2-Cl-Ph-	3390 ± 150
1u	3-Cl-Ph-	600 ± 70
1v	4-Cl-Ph-	2850 ± 490
1w	2-pyridinyl	79 ± 10
1x	3-pyridinyl	560 ± 190
1y	4-pyridinyl	510 ± 120

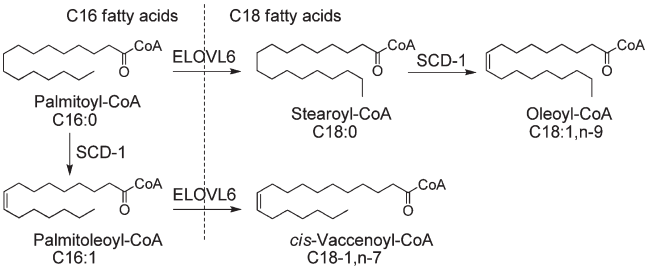
[a] The values represent the mean ± SE for $n \geq 3$.

Table 4. In Vitro Profiles of **1w**[a]

assay	IC_{50} (nM)
human ELOVL6 enzyme	79 ± 10
human ELOVL3 enzyme	6940 ± 680
human ELOVL1,2,5 enzyme	> 10000
mouse ELOVL6 enzyme	94 ± 19
mouse ELOVL3 enzyme	> 10000
cellular assay in mouse hepatocyte H2.35 cell	30 ± 9

[a] The values represent the mean ± SE for $n \geq 3$.

Scheme 2. Enzymatic Conversion of Palmitoyl-CoA with ELOVL6 and SCD-1



selectivity, **1w** showed excellent selectivity over the other human ELOVL subtypes (ELOVL1, -2, -3, and -5; $IC_{50} > 5\ \mu M$) and mouse ELOVL3 ($IC_{50} > 10\ \mu M$). In mouse, ELOVL6 selectivity over ELOVL3 of **1w** was more than 100-fold. Compound **1w** was also selective against the hERG K^+ channel ($IC_{50} > 10\ \mu M$). Furthermore, screening of **1w** by MDS Pharma Services (Panlabs) in a panel of 168 other receptor, enzyme, ion channel, and transporter assays revealed that **1w** has no significant activity at 10 μM. Encouraged by these results, we examined the cellular activity of **1w** in a mouse hepatocyte cell H2.35 assay. The ELOVL6 inhibitory activity in the H2.35 cell assay was assessed using [^{14}C]palmitic acid as a radiotracer. The enzymatic conversion of palmitic acid by ELOVL6 and SCD-1[21] is illustrated in Scheme 2. When ELOVL6 elongation takes place first, palmitoyl-CoA is elongated to give strearoyl-CoA followed by desaturation by SCD-1 to give oleoyl-CoA. However, when the desaturation process by SCD-1 takes place first, palmitoyl-CoA is converted to palmitoleoyl-CoA, which is elongated by ELOVL6 to yield *cis*-vaccenoyl-CoA. The elongation index is defined as follows and used as a surrogate readout for ELOVL6 inhibitory activity: elongation index = (C18 fatty acids)/(C16 fatty acids) = [stearoyl acid + oleic acid + *cis*-vaccenic acid]/[palmitic acid + palmitoleic acid]. As shown in Figure 1, **1w** effectively reduced the elongation index with an IC_{50} of 30 nM, indicating that **1w** is a potent inhibitor even at cellular levels. The suitable lipophilicity of **1w** ($\log D_{7.4} = 3.2$)[22] for good membrane penetration partly explains the potent cellular activity.

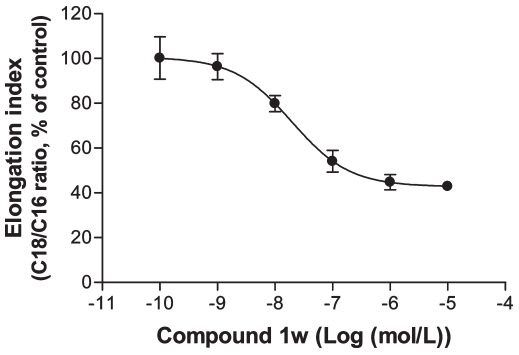


Figure 1. Inhibition of ELOVL6 activity by **1w** in the mouse hepatocyte cell line H2.35. The elongation index was determined by the radio-HPLC as described in Supporting Information and defined as % of control of the ratio of peak area [C18:0 + C18:1, $n-7$ + C18:1,$n-9$]/peak area [C16:0 + C16:1]. The results are taken from at least three independent tests and express the mean ± SEM.

Table 5. Plasma, Liver, and WAT Exposure of **1w** in C57BL/6J Mice[a]

dose (mg/kg)	time after dosing (h)	plasma (μM)	liver (nmol/g)	WAT (nmol/g)
10	2	0.46 ± 0.22	4.2 ± 1.4	2.7 ± 1.4
30	2	1.41 ± 0.46	10.0 ± 2.5	7.3 ± 3.7
	4	0.59 ± 0.10	6.0 ± 1.5	2.5 ± 0.2
	8	0.52 ± 0.23	5.9 ± 1.8	2.5 ± 1.1

[a] The reported data are an average generated after 10 or 30 mg/kg po doses in $n = 3$ animals.

Table 6. Pharmacokinetic Parameters of **1w** in SD Rats[a]

	iv			po			
compd	CL_p ((mL/min)/kg)	V_d (L/kg)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (μM·h)	C_{max} (μM)	T_{max} (h)	F (%)
1w	19	15.0	10.7	3.7	0.4	2.0	54

[a] The reported data are an average generated after 1 mg/kg iv and 3 mg/kg po doses in $n = 3$ animals/dose.

Next, the pharmacokinetic profile of **1w** was investigated. Compound **1w** exhibited sustained exposure in plasma, liver, and WAT in mice after oral dosing at 30 mg/kg, as shown in Table 5. The liver-to-plasma and WAT-to-plasma exposure ratios were 7−12 and 4−6, respectively, demonstrating that **1w** is penetrable to the liver and WAT. Compound **1w** also displayed good pharmacokinetic profiles in SD rats (Table 6).

Given the potent ELOVL6 inhibitory activity, selectivity, and appreciable pharmacokinetic profiles, **1w** was evaluated for its effects on the fatty acid profile in the liver in mice. The elongation index was used as a surrogate readout for ELOVL6 inhibitory activity in the liver using [^{14}C]palmitic acid as a radiotracer. After oral administration, **1w** potently and dose-dependently suppressed the elongation index in the liver in mice (Figure 2). On the basis of the potent in vivo activity, excellent selectivity, and good pharmacokinetic profiles in rodents, **1w** should be an attractive in vivo tool

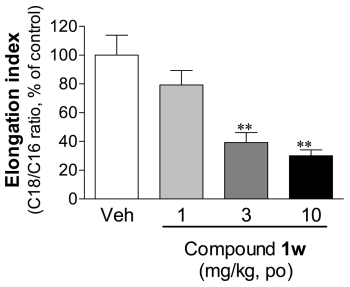


Figure 2. Effects of **1w** on ELOVL6 activity in the liver of C57BL/6J mice. Male C57BL/6J mice were orally administered vehicle (0.5% methylcellulose) and 1, 3, and 10 mg/kg **1w** (suspended in 0.5% methylcellulose), and 1 h later [1-^{14}C]palmitic acid was interperitoneally administered at 10 μCi/body. At 2 h postdosing of **1w**, fatty acids were extracted and measured by radio-HPLC to calculate the elongation index. The basal C18/16 ratio 2 h after oral administration of vehicle (0.5% methylcellulose) was ~0.15, which was set as a baseline (100%).

to probe the pharmacological effects of ELOVL6 inhibitor in rodents.

In conclusion, optimization of the lead **1a** led to the identification of the potent and selective ELOVL6 inhibitor **1w**. Compound **1w** displayed excellent selectivity against other ELOVL subtypes, hERG K^+ channel, and a panel of 168 unrelated biological targets. Compound **1w** potently reduced the elongation index in mouse hepatocyte cells H2.35. Furthermore, **1w** displayed sustained exposure in plasma, liver, and white adipose tissues in mice after oral dosing. Oral administration of **1w** potently and dose-proportionally suppressed the elongation index of fatty acids in the liver in mice. Taken together, these findings suggest that **1w** is a promising in vivo tool for the evaluation of pharmacological effects of ELOVL6 inhibition in rodents. Updated results of pharmacological studies using **1w** in rodents will be reported in due course.

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Supporting Information Available: Synthetic procedures and characterization data for all compounds; biological protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Fatty acyl-CoA elongation in microsomal fractions requires four sequential steps: (1) condensation between fatty acyl-CoA and malonyl-CoA to generate β-ketoacyl-CoA by ELOVLs, (2) reduction by β-ketoacyl-CoA reductase, (3) dehydrogenase by β-hydroxyacyl-CoA dehydrogenase, and (4) reduction by *trans*-2,3-enoyl-CoA reductase (see refs 9 and 10). ELOVL enzymes are responsible for the initial condensation reaction, which is rate-limiting in the four sequential steps (see refs 6 and 7).
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