Letters

Synthesis and Evaluation of a Novel Indoledione Class of Long Chain Fatty Acid Elongase 6 (ELOVL6) Inhibitors

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Abstract: Novel indoledione derivatives were synthesized and evaluated as long chain fatty acid elongase 6 (ELOVL6) inhibitors. Systematic optimization of an indole class of lead **1** led to the identification of potent ELOVL6 selective inhibitors. Representative inhibitor **37** showed sustained plasma exposure and good liver penetrability in mice. After oral administration, **37** potently inhibited ELOVL6 activity in the liver in mice.

The incidence of type 2 diabetes has dramatically increased over the past decade. Accumulated evidence suggests a strong correlation between insulin resistance and the development of type 2 diabetes mellitus. An increase in de novo lipid synthesis and fat storage in tissues such as liver leads to the dysfunction in those tissues, i.e., insulin resistance.[1] Although it is unclear how increased intracellular lipid content exacerbates tissue and whole body insulin sensitivity, it has been suggested that increased levels of long chain fatty acyl-CoA antagonize the metabolic actions of insulin. Interestingly, recent reports suggested that alternation of the specific fatty acid component (i.e., palmitoleate) has significant impact on the insulin sensitivity of liver and whole body.[2,3]

With regard to de novo lipid synthesis, the microsomal enzymes are responsible for the elongation of long chain fatty acids with chain length >C16 while FAS,[a] a cytosolic enzyme, is responsible for the de novo synthesis of the fatty acid with chain lengths up to C16.[4] Fatty acid elongation at microsomal fractions requires four sequential steps: (1) condensation between fatty acyl-CoA and malonyl-CoA to generate β-ketoacyl-CoA, (2) reduction by β-ketoacyl-CoA reductase, (3) dehydrogenation by β-hydroxyacyl-CoA dehydrogenase, and (4) reduction by *trans*-2,3-enoyl-CoA reductase.[4,5] ELOVL enzymes are responsible for the rate-limiting initial condensation reaction.[6,7]

So far, seven ELOVL enzymes have been identified in mammals and are designated ELOVL1−7.[6−11] Each ELOVL enzyme exhibits different fatty acid substrate preferences and tissue distributions, suggesting that they play different physiological roles in vivo.[12] Among ELOVL enzymes, ELOVL3 and ELOVL6 show the highest homology to each other (43.7%) and are expressed in the highly lipogenic tissues such as liver and adipose that play important roles in the regulation of lipid metabolism.[6,9] ELOVL6 regulates the synthesis of stearoyl-CoA (C18:0) and *cis*-vaccenoyl-CoA (C18:1).[6,7] Regarding the regulatory mechanism for expression, liver ELOVL6 expression is up-regulated by refeeding after fasting, by a high carbohydrate diet, and in obese rodents.[6,7,13] At the molecular level, ELOVL6 expression is directly and primarily regulated by sterol regulatory element-binding protein (SREBP)-1c.[14]

Recent investigations using gene-deleted mice for ELOVL6 suggest that ELOVL6 regulates the hepatic insulin sensitivity by modulating the fatty acid components.[3] Although increasing information on the physiological and pathological roles of ELOVL6 has drawn much attention, a lack of useful chemical tools makes it difficult to address the pharmacological roles of ELOVLs and their therapeutic potentials.

We recently reported the establishment of a homogeneous enzyme assay for ELOVL6, which is applicable to UHTS using

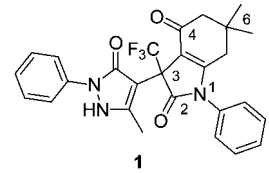
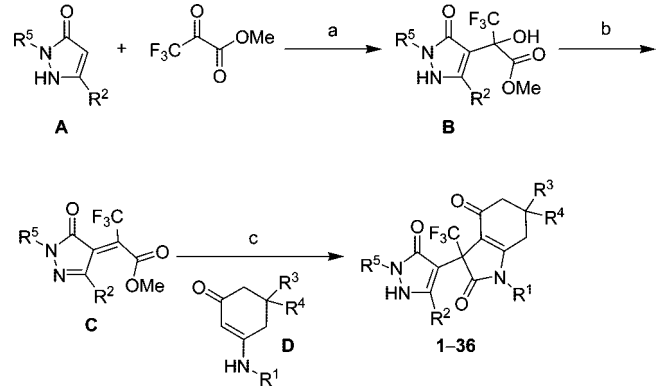


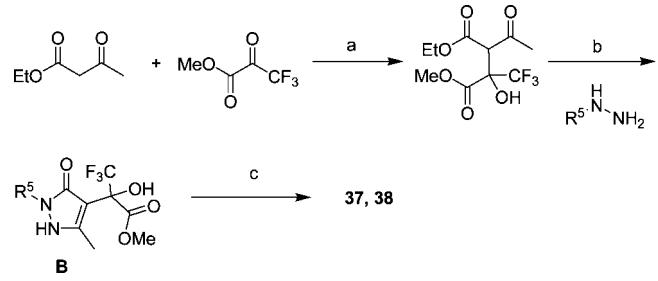
Figure 1. Potency and structure of lead **1**.

Scheme 1[a]



[a] Reagents and conditions: (a) $CHCl_3$, reflux, 2 h; (b) $SOCl_2$, toluene, reflux, 3 h; (c) $CHCl_3$, room temp, 14 h.

Scheme 2[a]



[a] Reagents and conditions: (a) neat, room temp, 14 h; (b) toluene, reflux, 2 h; (c) (i) $SOCl_2$, toluene, reflux, 3 h, (ii) 3-anilino-5,5-dimethylcyclohex-2-en-1-one, $CHCl_3$, room temp, 14 h.

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[a] Abbreviations: ELOVL, long chain fatty acid elongase; FAS, fatty acid synthase; UHTS, ultrahigh-throughput screen; SAR, structure−activity relationship; SCD-1, stearoyl-CoA desaturase 1.

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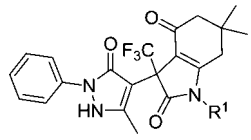
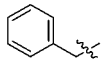
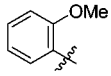
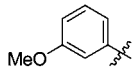
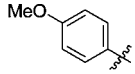
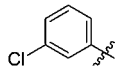
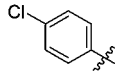
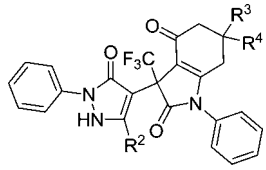
Table 1. Human ELOVL6 Inhibitory Activity of **1**−**17**[a]



compd	R^1	human ELOVL6[b] (IC_{50}, nM)
1	Ph	290 ± 61
2	H	1710 ± 308
3	Me	520 ± 148
4	Et	550 ± 185
5	*n*-Pr	185 ± 38
6	*i*-Pr	600 ± 155
7	cyclopropyl	144 ± 48
8	cyclobutyl	271 ± 67
9	cyclopentyl	411 ± 24
10	cyclohexyl	210 ± 60
11		879 ± 74
12		6340 ± 1360
13		226 ± 19
14		566 ± 130
15		6520 ± 2420
16		245 ± 53
17		603 ± 132

[a] The values represent the mean ± SE for $n \geq 3$. [b] Inhibitory activity of compounds on human ELOVL6 for palmitoyl-CoA elongation.

Table 2. Human ELOVL6 Inhibitory Activity of **18**−**23**[a]



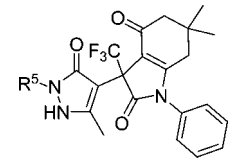
compd	R^2	R^3, R^4	human ELOVL6[b] (IC_{50}, nM)
18	Et	$R^3 = R^4 =$ Me	99 ± 28
19	*i*-Pr	$R^3 = R^4 =$ Me	>10000
20	Me	$R^3 = R^4 =$ H	4500 ± 190
21	Me	$R^3 =$ H, $R^4 =$ Me	704 ± 97
22	Me	$R^3 =$ H, $R^4 =$ Ph	6260 ± 428
23	Me	$-(CH_2)_3-$	123 ± 18

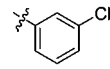
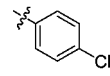
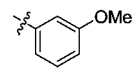
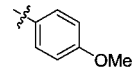
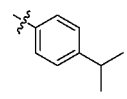
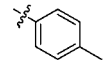
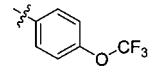
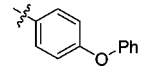
[a] The values represent the mean ± SE for $n \geq 3$. [b] Inhibitory activity of compounds on human ELOVL6 for palmitoyl-CoA elongation.

the recombinant histidine-tagged ACBP as a molecular probe for the detection of radioactive products of ELOVL6.[15] Our corporate chemical collection was screened against human ELOVL6, resulting in the identification of a novel indoledione class of lead **1**, which has an IC_{50} of 290 nM for human ELOVL6 (Figure 1). Systematic SAR studies of the indoledione lead **1**, aiming at optimization of ELOVL6 activity, resulted in the discovery of potent ELOVL6 inhibitors. In this report, preliminary results of the SAR studies of the indoledione class of ELOVL6 inhibitors and the discovery of the potent ELVOL6 selective inhibitor **37** are reported.

The synthesis of the indoledione ELOVL6 inhibitors **1**−**38** reported herein is outlined in Schemes 1 and 2. The substituted

Table 3. Human ELOVL3 and 6 Inhibitory Activity of **24**−**38**[a]



compd	R^5	human ELVOL6[b] (IC_{50}, nM)	human ELOVL3[c] (IC_{50}, nM)
24	Me	2020 ± 389	
25	*i*-Pr	>10000	
26		>10000	
27		4330 ± 2560	
28		44 ± 4	
29		12 ± 3	221 ± 15
30		58 ± 17	
31		43 ± 12	
32		17 ± 0.4	503 ± 69
33		44 ± 9	
34		14 ± 4	1100 ± 142
35		10 ± 2	59 ± 14
36		8.7 ± 3.0	375 ± 50
37		8.9 ± 2.7	337 ± 74
38		196 ± 88	

[a] The values represent the mean ± SE for $n \geq 3$. [b] Inhibitory activity of compounds on human ELOVL6 for palmitoyl-CoA elongation. [c] Inhibitory activity of compounds on human ELOVL3 for stearoyl-CoA elongation.

Table 4. Human ELOVL Subtype Selectivity and Mouse ELOVL3 and 6 Activity of **37**[a]

	enzyme						
	hELOVL1	hELOVL2	hELOVL3	hELOVL5	hELOVL6	mELOVL6	mELOVL3
activity (IC_{50})	>10 μM	>10 μM	337 ± 74 nM	>10 μM	8.9 ± 2.7 nM	31 ± 4 nM	194 ± 26 nM

[a] Inhibitory activity of **37** on ELOVLs for their respective substrates. The values are the mean ± SE for $n \geq 3$.

Table 5. Plasma Exposure of **37** in Mice

	time			
	2 h	4 h	8 h	24 h
plasma level (μM)[a]	3.4	2.8	1.4	0.4

[a] The plasma levels were determined after 1 mg/kg oral administration. The values represent the mean for $n = 3$ animals.

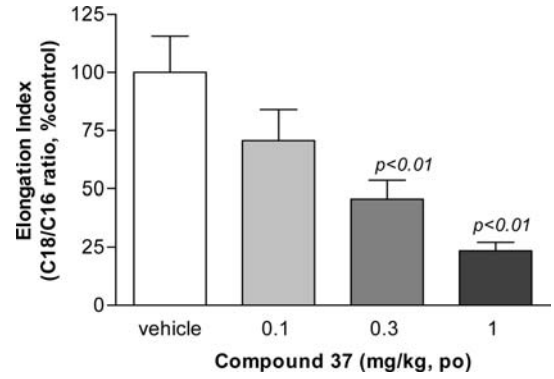


Figure 2. Effects of **37** on elongase activity in the liver of C57BL/6J mice. Male C57BL/6J mice were orally administered 0.1, 0.3, and 1 mg/kg **37** (dissolved in 0.5% methylcellulose), and 1 h later [1-^{14}C]palmitic acid was interperitoneally administered at 10 μCi/body. At 2 h postdosing of **37**, fatty acids were extracted and measured by radio-HPLC to calculate the elongation index (C16/C18 ratio).

pyrazol-3-one **A** and methyl 3,3,3-trifluoro-2-oxopropanoate were coupled to afford **B**, which was dehydrated using thionyl chloride to give enone **C**. Cyclocondensation of the enone **C** with the substituted 3-aminocyclohex-2-en-1-one **D** provided the desired indoledione derivatives **1**−**36**. The derivatives **37** and **38** were prepared by a slightly modified method (Scheme 2). Ethyl 3-oxobutanoate and methyl 3,3,3-trifluoro-2-oxopropanoate were coupled to give methyl 3,5-dideoxy-3-(ethoxycarbonyl)-2-*C*-(trifluoromethyl)pent-4-ulosonate, which was condensed with the appropriate substituted hydrazine to give **B**. Compound **B** was dehydrated with thionyl chloride followed by cyclocondensation with 3-anilino-5,5-dimethylcyclohex-2-en-1-one to afford **37** or **38**.

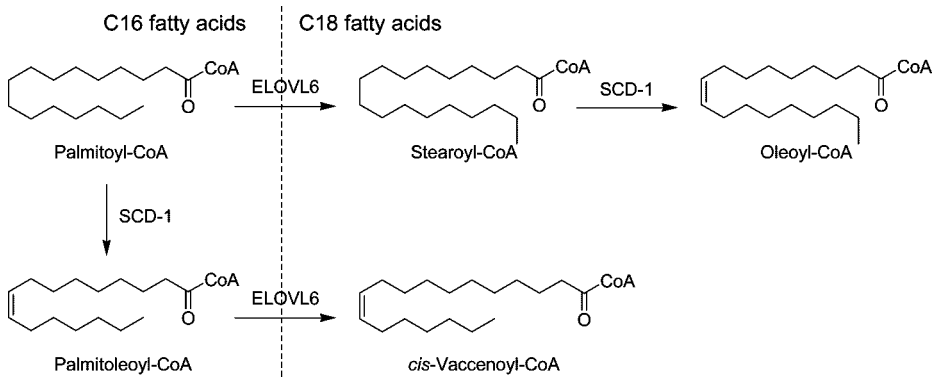
The compounds were optimized based on human ELOVL6 inhibitory activity, and the potent human ELOVL6 inhibitors were tested for human ELOVL3 inhibitory activity due to the high sequence homology among the ELOVL family members.

Substituent effects of R^1 of the lead compound **1** were investigated initially (Table 1). The R^1 substituent was essential for potency, as shown by a 6-fold potency decrease of **2**. The methyl and ethyl derivatives (**3** and **4**) also showed decreased activities, while the *n*-propyl derivative **5** was equipotent to lead **1**. The branched isopropyl derivative **6** displayed a decrease in activity. A series of cycloalkyl derivatives (**7**−**10**) were prepared and evaluated. Of them, the cyclopropyl derivative **7** was slightly more potent than the parent **1**. Benzyl substitution as in **11** was not tolerated. In light of the substituted phenyl derivatives (**12**−**17**), positional scanning was carried out with electron withdrawing chloro and electron donating methoxy groups. However, no noticeable substituent effect was identified, and the substitution at the meta-position was found to retain potency.

Next, R^2−R^4substituent effects were studied briefly (Table 2). The ethyl derivative **18** was slightly more potent than the parent **1**; however, the isopropyl derivative **19** showed a complete loss of potency. Because this portion of the molecule seems to be sensitive to steric effects, further investigation for the R^2 substituent was not attempted. Regarding the R^3 and R^4 substituents, desmethylation as in **20** was found to be detrimental to potency. The monomethyl derivative **21** was less potent than **1**, and the phenyl derivative **22** displayed a further decrease in potency. It was delightful to find that the cyclobutyl derivative **23** displayed an IC_{50} of 123 nM, which is twice as potent as lead **1**.

Finally, the substituent effect of the R^5 group was studied (Table 3). Methyl substitution as in **24** showed decreased activity, and the isopropyl and cyclohexyl derivatives (**25** and **26**) showed complete loss of potency. Hence, we decided to focus on substituted phenyl derivatives. Positional scanning with chloro and methoxy groups was carried out. As a result, substitution of the para-position appeared to be the most promising. A series of the para-substituted derivatives **33**−**38** were prepared and evaluated. With the exception of the phenoxy derivative **38**, all the para-substituted phenyl derivatives displayed potent ELOVL6 activities. The potent ELVOL6 inhibitors were tested for ELVOL3 inhibitory activity. The test compounds **29**, **32**, **34**−**37** showed moderate to good ELOVL6 selectivity over ELOVL3 as shown in Table 3. The isopropyl derivative **35** is the least selective where the selectivity (a ratio

Scheme 3. Enzymatic Reactions of Palmitoyl-CoA with ELOVL6 and SCD-1



of ELVOL3 to ELOVL6) is ~6-fold. Of them, compound **37** is a significantly more potent and selective inhibitor (Table 3). Compound **37** was shown to be selective over human ELOVL1, -2, and -5 enzymes and has a potent inhibitory activity for the mouse ELOVL6 enzyme as well (Table 4). The selectivity against mouse ELOVL3 is moderate where the ratio of mouse ELOVL3 to ELOVL6 is ~6-fold.

The plasma and liver levels 2 h following 10 mg/kg oral administration of **37** in mice were determined to be 30 and 50 μM, respectively; therefore, **37** is demonstrated to be highly liver penetrable (a liver-to-plasma ratio of 1.7). After oral dosing at 1 mg/kg in mice, **37** exhibited sustained plasma exposure as shown in Table 5.

Having demonstrated potent ELOVL6 activity and good exposure, **37** was evaluated for its effects on the fatty acid profile in the liver in mice. ELOVL6 is mainly responsible for the elongation of palmitoyl-CoA. Enzymatic reactions of palmitoyl-CoA involving ELVOL6 and SCD-1 are depicted in Scheme 3. When ELOVL6 elongation takes place first, palmitoyl-CoA is elongated to give strearoyl-CoA followed by desaturation by SCD-1 to give oleoyl-CoA. When the desaturation process by SCD-1 takes place first, palmitoyl-CoA is converted to palmitoleoyl-CoA, which is elongated by ELVOL6 to yield *cis*-vaccenoyl-CoA. We defined the elongation index as follows: elongation index = C18 fatty acids/C16 fatty acids = [stearoyl acid + oleic acid + *cis*-vaccenic acid]/[palmitic acid + palmitoleic acid]. 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, **37** potently and dose-proportionally suppressed the elongation index in the liver in mice (Figure 2).

In conclusion, new indoledione derivatives were synthesized and evaluated as ELOVL6 inhibitors. After systematic SAR studies of the R^1-R^5 substituents of the screen hit **1**, several para-substituted phenyl derivatives were identified as potent ELOVL6 inhibitors. Representative potent inhibitor **37** was selective for human ELOVL6 over human ELOVL1, -2, -3, and -5 enzymes. Compound **37** displayed potent inhibitory activity for mouse ELOVL6 as well; however, selectivity against mouse ELOVL3 was moderate. Sustained plasma exposure and appreciable liver penetration were observed after oral administration of **37** in mice. After oral dosing, **37** potently and dose-proportionally suppressed the elongation index of fatty acids in the liver in mice. The potent and selective inhibitor **37** would be a powerful tool to probe the pharmacology of the ELOVL6 enzyme inhibition in vivo. The updated results will be reported in due course.

Acknowledgment. We thank Hirokazu Ohsawa for collecting the high-resolution mass spectral data. We also thank Dr. Peter T. Meinke (Merck Research Laboratories, Rahway, NJ) for the editing of this manuscript.

Supporting Information Available: Synthetic procedures for the preparation of **1**−**38**, biological methods, HPLC retention times, and purity for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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