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Synthesis and evaluation of a novel 2-azabicyclo[2.2.2]octane class of long chain fatty acid elongase 6 (ELOVL6) inhibitors

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ABSTRACT

A series of novel 2-azabicyclo[2.2.2]octane derivatives was synthesized and evaluated as long chain fatty acid elongase 6 (ELOVL6) inhibitors. Screening of our corporate chemical collections against ELOVL6 resulted in the identification of lead **1**. Exploratory chemistry efforts were applied to lead **1** to identify the orally available, potent, and selective ELOVL6 inhibitor **28a**.

1. Introduction

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, that is, 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 alteration 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, microsomal enzymes are responsible for the elongation of long chain fatty acids with chain length $\geq$C16 while fatty acid synthase (FAS), 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] Long chain fatty acid elongase (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 feeding after fasting, 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 the 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.

Our laboratory recently reported the establishment of a homogenous enzyme assay for ELOVL6[15] and the discovery of an indoledione class of ELOVL6 inhibitors.[16] A novel 2-azabicyclo[2.2.2]octane class of lead **1** was also identified by screening of our corporate

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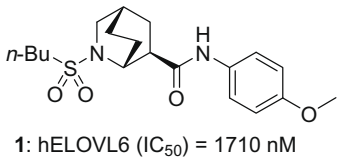


Figure 1. Potency and structure of lead **1**.

chemical collection against human ELOVL6 (Fig. 1). Exploratory chemistry was applied to this lead to improve intrinsic potency and metabolic stability with the goal of discovering orally active tool compounds. In this report, preliminary structure–activity relationships (SARs) of the novel 2-azabicyclo[2.2.2]octane derivatives and the discovery of the orally available, potent, selective ELOVL6 inhibitor **28a** are reported.

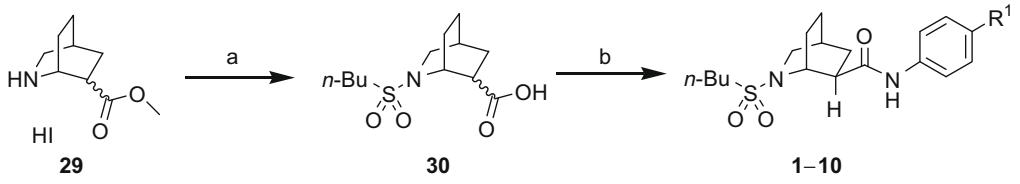
2. Chemistry

Synthesis of compounds **1**–**10** is illustrated in Scheme 1. Methyl 2-azabicyclo[2.2.2]octane-6-carboxylate hydroiodate (**29**)[17] was coupled with *n*-butylsulfonyl chloride followed by hydrolysis of the ester group under basic conditions to give **30**. The carboxylic acid group of **30** was coupled with the desired aniline to furnish target compounds **1**–**9** as a mixture of *endo*- and *exo*-isomers. The active *endo*-isomers were isolated by silica gel chromatography without difficulty. Substituting methyl piperidine-3-carboxylate for **29**, target compound **10** was prepared following Scheme 1. Scheme 2 outlines the synthesis of target compounds **11**–**28**. The amino group of **29** was protected as its *tert*-butylcarbamate. The ester group of **31** was saponified, followed by coupling with 4-isopropoxylaniline to give **32** as a mixture of *endo*- and *exo*-isomers, which were separated by silica gel chromatography. The *endo*-isomer of **32** was exposed to acidic conditions to remove the *tert*-butylcarbonyl group followed by coupling with the desired sulfonyl chloride to furnish target compounds **12**–**28**. Substituting methyl 8-azabicyclo[3.2.1]octane-2-carboxylate[18] for **31**, target compound **11** was prepared following Scheme 2. Compound **28** was resolved by CHIRALPAK AD, eluting with 40% *i*-PrOH in hexanes. The active isomer **28a** was obtained as the first-eluted enantiomer.

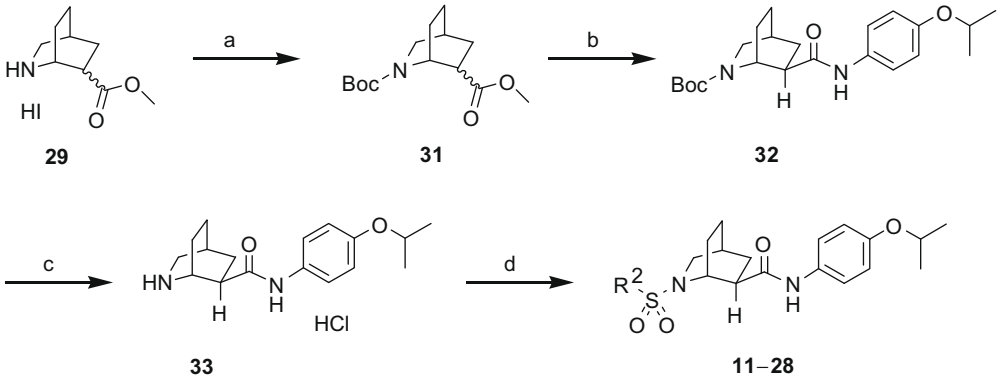
3. Results and discussion

All the derivatives tested in the present study are racemic mixtures of *endo*-isomers with exception of **28a** and **28b**. The corresponding *exo*-isomers were completely inactive for inhibition of the ELOVL6 enzyme. Substituent effects on the right-hand phenyl group of the lead compound **1** were investigated initially (Table 1). The isopropoxy derivative **2** was 10-fold more potent than lead **1**. The trifluoromethoxy and phenoxy derivatives (**3** and **4**) were slightly more potent, and the benzyloxy derivative **5** displayed a decreased in activity. The fluoro derivative **6** was devoid of potency. No noticeable improvement was observed with methyl, trifluoromethyl, and benzyl substitution as observed with **7**, **8**, and **9**. The 2-azabicyclo[2.2.2]octane ring was modified as shown in Figure 2. As a result, the corresponding piperidine derivative **10** (removal of the bridge head) and 8-azabicyclo[1.2.3]octane derivative **11** were much less potent than the 2-azabicyclo[2.2.2]octane derivative **2**.

Next, the substituent on the left-hand sulfonyl group was modified to improve potency using compound **2** as a template (Table 2). Additionally, metabolic stability of active derivatives was assessed by percent remaining of the parent after incubation of test compounds with human and mouse microsomes.[19] Smaller alkyl substituents, such as the methyl, ethyl, and propyl groups in compounds **12**–**15**, resulted only in decreases in potency. Larger alkyl groups were not tested since introduction of additional lipophilic groups is not preferred from a metabolic stability perspective. The phenyl derivative **16** was found to have increased activity; however, the microsomal stability of **16** was extremely

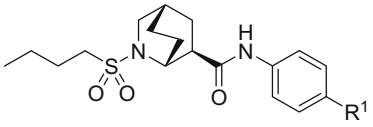


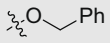
Scheme 1. Reagents and conditions: (a) (i) *n*-$BuSO_2Cl$, Et_3N, $CHCl_3$, rt, 13 h, 55%; (ii) 5 N aq NaOH, MeOH, rt, 17 h, 97%; (b) (i) 4-R^1PhCOOH, EDCI·HCl, $CHCl_3$, 14–24 h; (ii) separation of *endo*- and *exo*-isomers by silica gel chromatography, 30–55%.



Scheme 2. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, $CHCl_3$, rt, 24 h, 96%; (b) (i) 5 N aqueous NaOH, MeOH, rt, 3 h, 87%, (ii) 4-*i*-PrPhCOOH, EDCI·HCl, pyridine, rt, 14 h, (iii) separation of the *endo*- and *exo*-isomers by silica gel chromatography, 49%; (c) 4 N HCl–EtOAc, rt, 13 h, 100%; (d) R^2SO_2Cl, Et_3N, $CHCl_3$, rt, 12–24 h, 30–70%.

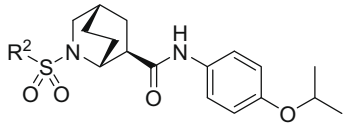
Table 1
Human ELOVL6 inhibitory activity of compounds **1**–**9**[a]



Compd	R^1	Human ELOVL6[b] (IC_{50}, nM)
1		1710 ± 640
2		120 ± 11
3		510 ± 240
4		710 ± 240
5		2900 ± 1100
6		>10,000
7		2300 ± 610
8		1300 ± 280
9		830 ± 280

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

Table 2
Human ELOVL6 inhibitory activity of compounds **18**–**23**[a]



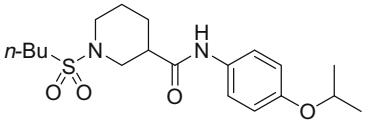
Compd	R^2	Human ELOVL6[b] (IC_{50}, nM)	Microsomal stability[d]	
			Human	Mouse
2	*n*-Bu	120 ± 11	35	10
12	Me	>10,000	[c]	[c]
13	120 ± 11	2900 ± 890	[c]	[c]
14	*n*-Pr	210 ± 38	61	14
15	*i*-Pr	620 ± 190	34	11
16	Ph	70 ± 17	13	0
17	4-MeOPh	610 ± 210	[c]	[c]
18	3-MeOPh	600 ± 220	[c]	[c]
19	4-CF_3Ph	3500 ± 790	[c]	[c]
20	4-*t*-BuPh	>10,000	[c]	[c]
21	4-FPh	190 ± 42	9	3
22	3-FPh	110 ± 13	8	1
23	2-FPh	130 ± 21	1	0
24	2-Thiophenyl	320 ± 62	6	1
25	3-Thiophenyl	67 ± 17	6	1
26	2-Pyridyl	120 ± 43	53	9
27	3-Pyridyl	80 ± 5	1	0
28		230 ± 65	88	86
28a		220 ± 38	88	75
28b		800 ± 95	91	71

[a] The values represent the mean ± SE for $n \geqslant 3$.
[b] Inhibitory activity of compounds on human ELOVL6 for palmitoyl-CoA elongation.
[c] Not determined.
[d] See Ref. 18 for the experimental conditions for the microsomal stability assay.

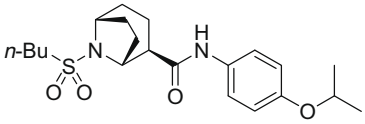
poor. Among the substituted phenyl derivatives prepared (**17**–**23**), only fluoro substitution was tolerated. Unfortunately, metabolic stability of the fluoro derivatives **21**–**23** also was very poor. Regarding heteroaromatic derivatives, common bioisosters, pyridine and thiophene rings, were tolerated as in the derivatives **24**–**27**, which again exhibited very poor metabolic stability. A hydrophilic imidazole ring was introduced to identify moderately potent but metabolically significantly stable derivative **28**. Compound **28** was resolved to give the enantiomers **28a** and **28b**. The active isomer **28a** displayed an IC_{50} value of 221 nM at ELOVL6 and retained excellent metabolic stability. Compound **28a** was selective over other human ELOVL sub-types (ELOVL1, 2, 3, and 5) and has potent inhibitory activity for mouse ELOVL6 as well (Table 3). Selectivity over human and mouse ELOVL3 is moderate probably due to the highest sequence homology of ELOVL6 and ELOVL3 enzymes among the ELOVL sub-types.

The plasma and liver levels 8 h following 30 mg/kg oral administration of **28a** in mice were determined to be 0.66 μM and 3.95 nmol/g, respectively, demonstrating compound **28a** to be highly liver penetrable (liver-to-plasma ratio: 6.2).

Given the potent ELOVL6 activity and appreciable plasma and liver exposure, compound **28a** 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 stearoyl-CoA desaturase 1 (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



10: hELOVL6 (IC_{50}) : 930 nM



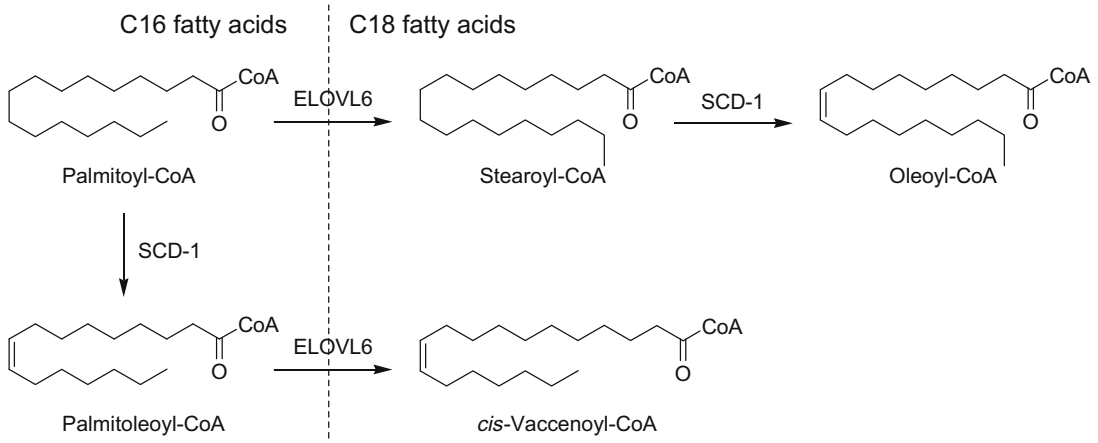
11: hELOVL6 (IC_{50}) : 5950 nM

Figure 2. Potency and structures of compounds **10** and **11**.

Table 3
Human ELOVL sub-type selectivity and mouse ELOVL3 and 6 activity of compound **28a**[a]

Enzyme	hELOVL1	hELOVL2	hELOVL3	hELOVL5	hELOVL6	mELOVL6	mELOVL3
Activity (IC_{50})	>10 μM	>10 μM	1510 nM	>10 μM	221 nM	73 nM	717 nM

[a] Inhibitory activity of compound **28a** on ELOVLs for their respective substrates. The values are the mean for $n \geqslant 3$.



Scheme 3. Enzymatic reactions of palmitoyl-CoA with ELOVL6 and SCD-1.

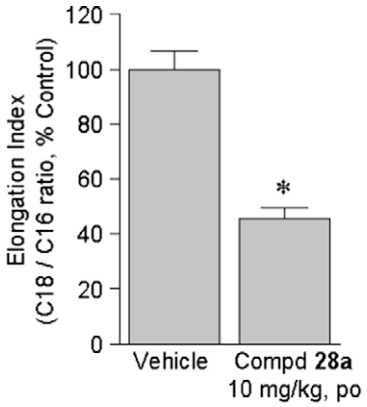


Figure 3. Effects of compound **28a** on ELOVL6 activity in the liver of C57BL/6J mice. Male C57BL/6J mice ($n = 5$) were orally administered 10 mg/kg of compound **28a** (dissolved in 0.5% methylcellulose) and 1 h later [1-^{14}C]-palmitic acid was interperitoneally administered at 10 μCi/body. At 2 h post-dosing of compound **28a**, fatty acids were extracted and measured by radio-HPLC to calculate the elongation index. $^{*}P < 0.05$ (ANOVA Dunnett) compared with the vehicle control.

desaturation process mediated 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, compound **28a** potently suppressed the elongation index in the liver in mice (Fig. 3).

4. Conclusion

A new series of 2-azabicyclo[2.2.2]octane derivatives was synthesized and evaluated as ELOVL6 inhibitors. After exploratory modification of the substituents on the right-hand phenyl group and the sulfonyl group of the screen hit **1**, orally available and selective inhibitor **28a** was identified. Appreciable liver penetration was observed after oral administration of **28a** in mice. After oral dosing, **28a** potently suppressed the elongation index of fatty acids in the liver in mice, suggesting that **28a** has considerable utility to probe the biology of the ELOVL6 enzyme inhibition in vivo. Further characterization of the biology of **28a** will be reported in due course.

5. Experimental

5.1. Chemistry

5.1.1. General procedures

Unless otherwise noted, all solvents, chemicals, and reagents were obtained commercially and used without purification. The ^{1}H NMR spectra were obtained at 400 MHz on a MERCURY-400 (Varian) or a JMN-AL400 (JEOL) spectrometer, with chemical shift (δ, ppm) reported relative to TMS as an internal standard. Mass spectra were recorded with electron-spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromass ZQ, micromass Quattro II or micromass Q-Tof-2 instrument. Flash chromatography was carried out with prepacked silica gel columns (KP-Sil™ silica) from Biotage. Preparative thin-layer chromatography (TLC) was performed on a TLC Silica Gel 60 F (Merck KGaA). Preparative HPLC purification was carried out on a YMC-Pack *Pro* C18 (YMC, 50 × 30 mm id), eluting with a gradient of CH_3CN/0.1% aqueous CF_3CO_2H = 10/90 to 50/50 over 8 min at a flow rate of 40 mL/min. Purity of the target compounds was determined by HPLC with the two different eluting methods as follows. Analytical HPLC was performed on a SPELCO Ascentis Express (150 × 4.6 mm id), eluting with a gradient of (A) 0.1% H_3PO_4/CH_3CN = 95/5 to 10/90 over 7 min followed by 10/90 isocratic over 1 min and (B) 10 mM potassium phosphate buffer (pH 6.6)/CH_3CN = 95/5 to 20/80 over 7 min followed by 20/80 isocratic over 1 min (detection at 210 nm). HPLC retention times and purity for the target compounds are available as Supplementary data. High resolution

mass spectra were recorded with electron-spray ionization on a micromass Q-Tof-2 instrument.

5.1.2. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-(4-methoxyphenyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (1)

To a solution of **30** (28 mg, 0.1 mmol) and 4-methoxyaniline (18.5 mg, 0.15 mmol) in pyridine (1 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (29.3 mg, 0.15 mmol), and the mixture was stirred at room temperature for 14 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and 1 N aqueous NaOH. The layers were separated, and the organic layer was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by preparative TLC (hexanes/ethyl acetate = 55/45) to give **1** (19.7 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.38–1.52 (2H, m), 1.56–1.67 (1H, m), 1.72–2.03 (6H, m), 2.07–2.16 (1H, m), 2.27–2.36 (1H, m), 2.92–3.13 (3H, m), 3.23–3.31 (1H, m), 3.51–3.59 (1H, m), 3.79 (3H, s), 3.93–3.98 (1H, m), 6.81–6.89 (2H, m), 7.39–7.47 (2H, m), 7.66 (1H, br s). ESI–MS m/z 381.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{29}N_2O_4S$, 381.1848; found, 381.1851.

5.1.3. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-[4-(propan-2-yloxy) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (2)

Compound **2** was prepared from **30** and 4-isopropoxyaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.31 (6H, d, J = 6.3 Hz), 1.40–1.53 (2H, m), 1.58–1.88 (6H, m), 1.93–2.04 (1H, m), 2.07–2.16 (1H, m), 2.27–2.39 (1H, m), 2.89–3.13 (3H, m), 3.22–3.31 (1H, m), 3.51–3.62 (1H, m), 3.92–3.99 (1H, m), 4.44–4.56 (1H, m), 6.81–6.87 (2H, m), 7.36–7.43 (2H, m), 7.48 (1H, br s). ESI-MS m/z 409.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{21}H_{33}N_2O_4S$, 409.2161; found, 409.2166.

5.1.4. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-[4-(trifluoromethoxy) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (3)

Compound **3** was prepared from **30** and 4-trifluoromethoxyaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.38–1.52 (2H, m), 1.57–1.69 (1H, m), 1.73–1.88 (5H, m), 1.91–1.99 (1H, m), 2.09–2.17 (1H, m), 2.28–2.37 (1H, m), 2.90–3.15 (3H, m), 3.23–3.31 (1H, m), 3.56–3.61 (1H, m), 3.93–3.97 (1H, m), 7.14–7.20 (2H, m), 7.55–7.61 (2H, m), 7.95 (1H, br s). ESI-MS m/z 435.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{26}N_2O_4SF_3$, 435.1565; found, 435.1562.

5.1.5. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-(4-phenoxyphenyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (4)

Compound **4** was prepared from **30** and 4-phenoxyaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (3H, t, J = 7.4 Hz), 1.21–1.29 (1H, m), 1.40–1.51 (2H, m), 1.59–1.89 (5H, m), 1.92–2.04 (1H, m), 2.09–2.16 (1H, m), 2.25–2.38 (1H, m), 2.90–3.15 (3H, m), 3.22–3.32 (1H, m), 3.55–3.61 (1H, m), 3.94–3.99 (1H, m), 6.94–7.02 (4H, m), 7.05–7.11 (1H, m), 7.30–7.35 (2H, m), 7.47–7.54 (2H, m), 7.72 (1H, br s). ESI-MS m/z 443.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{24}H_{31}N_2O_4S$, 443.2005; found, 443.2012.

5.1.6. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Benzyloxy)phenyl]-2-(butylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (5)

Compound **5** was prepared from **30** and 4-benzyloxyaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.41–1.50 (2H, m), 1.74–1.88 (6H, m), 1.93–2.01 (1H, m), 2.08–2.15 (1H, m), 2.26–2.37 (1H, m), 2.91–3.04 (2H, m), 3.05–3.11 (1H, m), 3.24–3.30 (1H, m), 3.54–3.60 (1H, m), 3.93–3.97 (1H, m), 5.05 (2H, s), 6.90–6.95 (2H, m), 7.30–7.46 (7H, m), 7.54 (1H, br s). ESI-MS m/z 457.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{25}H_{33}N_2O_4S$, 457.2161; found, 457.2169.

5.1.7. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-(4-fluorophenyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (6)

Compound **6** was prepared from **30** and 4-fluoroaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.38–1.52 (2H, m), 1.55–1.68 (1H, m), 1.73–1.90 (4H, m), 1.92–2.15 (3H, m), 2.27–2.37 (1H, m), 2.87–3.16 (3H, m), 3.21–3.30 (1H, m), 3.50–3.59 (1H, m), 3.89–4.00 (1H, m), 6.95–7.06 (2H, m), 7.45–7.54 (2H, m), 7.89 (1H, br s). ESI-MS m/z 369.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{18}H_{26}N_2O_3SF$, 369.1648; found, 369.1657.

5.1.8. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-(4-methylphenyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (7)

Compound **7** was prepared from **30** and 4-methylaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.41–1.53 (2H, m), 1.75–1.88 (6H, m), 1.92–2.02 (1H, m), 2.09–2.15 (1H, m), 2.28–2.36 (4H, m), 2.91–3.12 (3H, m), 3.24–3.29 (1H, m), 3.54–3.60 (1H, m), 3.93–3.97 (1H, m), 7.09–7.15 (2H, m), 7.38–7.43 (2H, m), 7.54 (1H, br s). ESI-MS m/z 365.3 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{29}N_2O_3S$, 365.1899; found, 365.1909.

5.1.9. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-*N*-[4-(trifluoromethyl) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (8)

Compound **8** was prepared from **30** and 4-trifluoromethylaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.3 Hz), 1.40–1.52 (2H, m), 1.58–2.00 (7H, m), 2.11–2.17 (1H, m), 2.27–2.37 (1H, m), 2.92–3.08 (2H, m), 3.11–3.18 (1H, m), 3.22–3.30 (1H, m), 3.55–3.64 (1H, m), 3.94–4.00 (1H, m), 7.53–7.61 (2H, m), 7.65–7.73 (2H, m), 8.09 (1H, br s) ESI-MS m/z 419.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{26}N_2O_3SF_3$, 419.1616; found, 419.1625.

5.1.10. (1*R**,4*S**,6*R**)-(±)-*N*-(4-Benzylphenyl)-2-(butylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (9)

Compound **9** was prepared from **30** and 4-benzylaniline using the procedure described for **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.2 Hz), 1.40–1.50 (2H, m), 1.55–2.02 (8H, m), 2.09–2.14 (1H, m), 2.26–2.35 (1H, m), 2.90–3.13 (3H, m), 3.23–3.30 (1H, m), 3.54–3.61 (1H, m), 3.94 (2H, s), 7.10–7.22 (6H, m), 7.41–7.48 (2H, m), 7.63 (1H, br s). ESI-MS m/z 441.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{25}H_{33}N_2O_3S$, 441.2212; found, 441.2212.

5.1.11. (±)-1-(Butylsulfonyl)-*N*-[4-(propan-2-yloxy)phenyl] piperidine-3-carboxamide (10)

Compound **10** was prepared from methyl piperidine-3-carboxylate and 4-isopropoxyaniline using the procedure described for **30** and **1**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (3H, t, J = 7.3 Hz), 1.31 (6H, d, J = 5.9 Hz), 1.40–1.51 (2H, m), 1.65–2.02 (6H, m), 2.54–2.63 (1H, m), 2.90–2.98 (2H, m), 2.99–3.08 (1H, m), 3.21–3.33 (1H, m), 3.53–3.64 (1H, m), 3.72–3.79 (1H, m), 4.44–4.54 (1H, m), 6.81–6.87 (2H, m), 7.39–7.45 (2H, m), 7.63 (1H, br s). ESI-MS m/z 383.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{31}N_2O_4S$, 383.2005; found, 383.2013.

5.1.12. (1*R**,2*R**,5*R**)-(±)-8-(Butylsulfonyl)-*N*-[4-(propan-2-yloxy) phenyl]-8-azabicyclo[3.2.1]octane-2-carboxamide (11)

Compound **11** was prepared from 8-*tert*-Butyl 2-methyl (1*R**,2*R**,5*R**)-(±)-8-azabicyclo[3.2.1]octane-2,8-dicarboxylate[19] and 1-butanesulfonyl chloride using the procedure described for **31**, **32**, **33**, and **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t,

J = 7.3 Hz), 1.31 (6H, d, *J* = 6.1 Hz), 1.40–1.52 (2H, m), 1.60–1.70 (1H, m), 1.72–1.85 (5H, m), 1.87–2.14 (4H, m), 2.20–2.31 (1H, m), 2.77–2.87 (1H, m), 2.93–3.01 (2H, m), 4.13–4.20 (1H, m), 4.31–4.39 (1H, m), 4.45–4.55 (1H, m), 6.80–6.88 (2H, m), 7.35–7.43 (3H, m). ESI-MS *m/z* 409.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{21}H_{33}N_2O_4S$, 409.2161; found, 409.2169.

5.1.13. (1*R**,4*S**,6*R**)-(±)-2-(Methylsulfonyl)-*N*-[4-(propan-2-yloxy) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (12)

To a solution of **33** (25 mg, 0.077 mmol) and triethylamine (14.4 μL, 0.31 mmol) in $CHCl_3$ (2 mL) was added methanesulfonyl chloride (10 μL, 0.13 mmol) at room temperature, and the mixture was stirred at room temperature for 15 h. The resulting mixture was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated. The residue was purified by preparative TLC (hexanes/ethyl acetate = 40/60) to give **12** (11.2 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (6H, d, *J* = 5.9 Hz), 1.55–1.66 (1H, m), 1.77–1.90 (3H, m), 1.93–2.03 (1H, m), 2.10–2.16 (1H, m), 2.29–2.38 (1H, m), 2.91 (3H, s), 3.02–3.10 (1H, m), 3.27–3.32 (1H, m), 3.48–3.54 (1H, m), 3.99–4.03 (1H, m), 4.44–4.54 (1H, m), 6.81–6.87 (2H, m), 7.37–7.43 (2H, m), 7.55 (1H, br s). ESI-MS *m/z* 367.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{18}H_{27}N_2O_4S$, 367.1692; found, 367.1698.

5.1.14. (1*R**,4*S**,6*R**)-(±)-2-(Ethylsulfonyl)-*N*-[4-(propan-2-yloxy) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (13)

Compound **13** was prepared from **33** and ethanesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (6H, d, *J* = 5.9 Hz), 1.37 (3H, t, *J* = 7.3 Hz), 1.55–1.66 (1H, m), 1.75–1.89 (3H, m), 1.94–2.05 (1H, m), 2.08–2.14 (1H, m), 2.27–2.36 (1H, m), 2.94–3.11 (3H, m), 3.24–3.30 (1H, m), 3.54–3.60 (1H, m), 3.92–3.97 (1H, m), 4.44–4.55 (1H, m), 6.80–6.88 (2H, m), 7.38–7.45 (2H, m), 7.56 (1H, br s). ESI-MS *m/z* 381.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{19}H_{29}N_2O_4S$, 381.1848; found, 381.1852.

5.1.15. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(propyl sulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (14)

Compound **14** was prepared from **33** and 1-propanesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07 (3H, t, *J* = 7.4 Hz), 1.31 (6H, d, *J* = 6.1 Hz), 1.52–1.66 (1H, m), 1.75–1.91 (5H, m), 1.94–2.04 (1H, m), 2.08–2.14 (1H, m), 2.27–2.36 (1H, m), 2.88–3.02 (2H, m), 3.04–3.11 (1H, m), 3.24–3.30 (1H, m), 3.53–3.60 (1H, m), 3.92–3.98 (1H, m), 4.45–4.55 (1H, m), 6.80–6.87 (2H, m), 7.37–7.43 (2H, m), 7.53 (1H, br s). ESI-MS *m/z* 395.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{20}H_{31}N_2O_4S$, 395.2005; found, 395.2011.

5.1.16. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(propan-2-ylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (15)

Compound **15** was prepared from **33** and isopropylsulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (6H, d, *J* = 6.1 Hz), 1.35–1.41 (6H, m), 1.57–1.66 (1H, m), 1.77–1.91 (3H, m), 1.94–2.04 (1H, m), 2.07–2.13 (1H, m), 2.25–2.35 (1H, m), 3.05–3.12 (1H, m), 3.14–3.22 (1H, m), 3.23–3.29 (1H, m), 3.64–3.71 (1H, m), 3.86–3.92 (1H, m), 4.45–4.53 (1H, m), 6.80–6.87 (2H, m), 7.37–7.46 (3H, m) ESI-MS *m/z* 395.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{20}H_{31}N_2O_4S$, 395.2005; found, 395.2009.

5.1.17. (1*R**,4*S**,6*R**)-(±)-2-(Phenylsulfonyl)-*N*-[4-(propan-2-yloxy) phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (16)

Compound **16** was prepared from **33** and benzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28–1.38 (7H, m), 1.42–1.52 (1H, m), 1.62–1.82 (3H, m), 2.02–2.08 (1H, m), 2.17–2.27 (1H, m), 2.82–2.91 (1H, m), 3.23–3.28 (1H, m), 3.44–3.50 (1H, m), 4.03–4.08 (1H, m), 4.46–4.53 (1H, m), 6.82–6.88 (2H, m), 7.34–7.42 (3H, m), 7.52–7.57 (2H, m), 7.59–7.64 (1H, m), 7.86–7.92 (2H, m). ESI-MS *m/z* 429.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{23}H_{29}N_2O_4S$, 429.1848; found, 429.1855.

5.1.18. (1*R**,4*S**,6*R**)-(±)-2-[(4-Methoxyphenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (17)

Compound **17** was prepared from **33** and 4-methoxybenzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.40 (7H, m), 1.43–1.53 (1H, m), 1.63–1.82 (3H, m), 1.99–2.06 (1H, m), 2.17–2.27 (1H, m), 2.84–2.93 (1H, m), 3.17–3.27 (1H, m), 3.39–3.47 (1H, m), 3.88 (3H, s), 3.99–4.05 (1H, m), 4.44–4.55 (0H, m), 6.81–6.88 (2H, m), 6.97–7.03 (2H, m), 7.36–7.42 (3H, m), 7.76–7.85 (2H, m). ESI-MS *m/z* 459.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{24}H_{31}N_2O_5S$, 459.1954; found, 459.1947.

5.1.19. (1*R**,4*S**,6*R**)-(±)-2-[(3-Methoxyphenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (18)

Compound **18** was prepared from **33** and 3-methoxybenzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26–1.43 (7H, m), 1.46–1.60 (1H, m), 1.65–1.84 (3H, m), 2.01–2.08 (1H, m), 2.17–2.26 (1H, m), 2.75–2.83 (1H, m), 3.26–3.33 (1H, m), 3.41–3.48 (1H, m), 3.87 (3H, s), 4.02–4.07 (1H, m), 4.44–4.55 (1H, m), 6.82–6.88 (2H, m), 7.11–7.17 (1H, m), 7.30–7.34 (1H, m), 7.37–7.42 (3H, m), 7.44–7.49 (2H, m). ESI-MS *m/z* 459.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{24}H_{31}N_2O_5S$, 459.1954; found, 459.1947.

5.1.20. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-{[4-(trifluoromethyl)phenyl]sulfonyl}-2-azabicyclo[2.2.2]octane-6-carboxamide (19)

Compound **19** was prepared from **33** and 4-(trifluoromethyl) benzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24–1.37 (7H, m), 1.38–1.49 (1H, m), 1.63–1.88 (3H, m), 2.04–2.11 (1H, m), 2.20–2.29 (1H, m), 2.93–3.01 (1H, m), 3.19–3.24 (1H, m), 3.49–3.55 (1H, m), 4.08–4.16 (1H, m), 4.45–4.56 (1H, m), 6.82–6.90 (2H, m), 7.35–7.44 (3H, m), 7.76–7.84 (2H, m), 7.96–8.03 (2H, m). ESI-MS *m/z* 497.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{24}H_{28}N_2O_4SF_3$, 497.1722; found, 497.1722.

5.1.21. (1*R**,4*S**,6*R**)-(±)-2-[(4-*tert*-Butylphenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (20)

Compound **20** was prepared from **33** and 4-*tert*-butylbenzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (6H, d, *J* = 5.9 Hz), 1.34 (9H, s), 1.44–1.55 (1H, m), 1.62–1.83 (3H, m), 1.94–2.05 (2H, m), 2.17–2.27 (1H, m), 2.86–2.94 (1H, m), 3.22–3.28 (1H, m), 3.42–3.48 (1H, m), 4.05–4.09 (1H, m), 4.44–4.54 (1H, m), 6.78–6.86 (2H, m), 7.36–7.42 (2H, m), 7.51–7.56 (3H, m), 7.76–7.83 (2H, m). ESI-MS *m/z* 485.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{27}H_{37}N_2O_4S$, 485.2474; found, 485.2480.

5.1.22. (1*R**,4*S**,6*R**)-(±)-2-[(4-Fluorophenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (21)

Compound **21** was prepared from **33** and 4-fluorobenzenesulfonyl chloride using the procedure described for **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.38 (7H, m), 1.39–1.49 (1H, m), 1.63–1.85 (3H, m), 2.02–2.09 (1H, m), 2.19–2.28 (1H, m), 2.90–2.99

(1H, m), 3.17–3.24 (1H, m), 3.44–3.51 (1H, m), 4.03–4.10 (1H, m), 4.44–4.57 (1H, m), 6.82–6.88 (2H, m), 7.18–7.24 (2H, m), 7.37–7.45 (3H, m), 7.85–7.92 (2H, m). ESI-MS *m/z* 447.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{23}H_{28}N_2O_4SF$, 447.1754; found, 447.1759.

5.1.23. (1*R**,4*S**,6*R**)-(±)-2-[(3-Fluorophenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (22)

Compound **22** was prepared from **33** and 3-fluorobenzenesulfonyl chloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.26–1.39 (7H, m), 1.41–1.52 (1H, m), 1.63–1.85 (3H, m), 2.03–2.10 (1H, m), 2.20–2.29 (1H, m), 2.87–2.97 (1H, m), 3.21–3.28 (1H, m), 3.45–3.53 (1H, m), 4.04–4.10 (1H, m), 4.45–4.55 (1H, m), 6.82–6.89 (2H, m), 7.28–7.34 (1H, m), 7.36–7.43 (3H, m), 7.50–7.60 (2H, m), 7.64–7.70 (1H, m). ESI-MS *m/z* 447.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{23}H_{28}N_2O_4SF$, 447.1754; found, 447.1759.

5.1.24. (1*R**,4*S**,6*R**)-(±)-2-[(2-Fluorophenyl)sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2]octane-6-carboxamide (23)

Compound **23** was prepared from **33** and 2-fluorobenzenesulfonyl chloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 6.3 Hz), 1.46–1.56 (1H, m), 1.62–1.88 (4H, m), 2.05–2.12 (1H, m), 2.24–2.34 (1H, m), 2.92–3.01 (1H, m), 3.36–3.47 (2H, m), 4.12–4.16 (1H, m), 4.44–4.55 (1H, m), 6.80–6.90 (2H, m), 7.21–7.32 (2H, m), 7.36–7.44 (3H, m), 7.53–7.62 (1H, m), 7.89–7.97 (1H, m). ESI-MS *m/z* 447.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{23}H_{28}N_2O_4SF$, 447.1754; found, 447.1755.

5.1.25. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(thiophen-2-ylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (24)

Compound **24** was prepared from **33** and 2-thiophenesulfonyl chloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 6.3 Hz), 1.39–1.47 (1H, m), 1.51–1.84 (4H, m), 2.05–2.11 (1H, m), 2.19–2.27 (1H, m), 2.75–2.83 (1H, m), 3.34–3.48 (2H, m), 4.04–4.09 (1H, m), 4.45–4.56 (1H, m), 6.82–6.88 (2H, m), 7.13–7.16 (1H, m), 7.20 (1H, br s), 7.35–7.42 (2H, m), 7.59–7.65 (2H, m). ESI-MS *m/z* 435.3 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{21}H_{27}N_2O_4S_2$, 435.1412; found, 435.1411.

5.1.26. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(thiophen-3-ylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (25)

Compound **25** was prepared from **33** and 3-thiophenesulfonyl chloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.25–1.42 (7H, m), 1.46–1.58 (1H, m), 1.66–1.85 (3H, m), 2.04–2.10 (1H, m), 2.19–2.27 (1H, m), 2.83–2.91 (1H, m), 3.25–3.32 (1H, m), 3.42–3.49 (1H, m), 4.04–4.09 (1H, m), 4.44–4.55 (1H, m), 6.82–6.88 (2H, m), 7.29 (1H, br s), 7.35–7.43 (3H, m), 7.44–7.48 (1H, m), 7.96–7.99 (1H, m) ESI-MS *m/z* 435.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{21}H_{27}N_2O_4S_2$, 435.1412; found, 435.1413.

5.1.27. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(pyridin-2-ylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (26)

Compound **26** was prepared from **33** and 2-pyridinesulfonyl chloride hydrochloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 5.9 Hz), 1.47–1.64 (2H, m), 1.69–1.85 (3H, m), 2.01–2.08 (1H, m), 2.24–2.33 (1H, m), 3.03–3.12 (1H, m), 3.25–3.32 (1H, m), 3.46–3.53 (1H, m), 4.28–4.32 (1H, m), 4.46–4.54 (1H, m), 6.82–6.89 (2H, m), 7.38–7.47 (2H, m), 7.52–7.57 (1H, m), 7.73 (1H, br s), 7.91–7.99 (1H, m), 8.02–8.07 (1H, m), 8.70–8.78 (1H, m). ESI-MS *m/z* 430.2 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{22}H_{28}N_3O_4S$, 430.1801; found, 430.1804.

5.1.28. (1*R**,4*S**,6*R**)-(±)-*N*-[4-(Propan-2-yloxy)phenyl]-2-(pyridin-3-ylsulfonyl)-2-azabicyclo[2.2.2]octane-6-carboxamide (27)

Compound **27** was prepared from **33** and 3-pyridinesulfonyl chloride hydrochloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.26–1.39 (7H, m), 1.41–1.51 (1H, m), 1.66–1.90 (3H, m), 2.04–2.11 (1H, m), 2.21–2.28 (1H, m), 2.89–2.97 (1H, m), 3.20–3.27 (1H, m), 3.47–3.52 (1H, m), 4.09–4.16 (1H, m), 4.44–4.55 (1H, m), 6.80–6.88 (2H, m), 7.37–7.43 (2H, m), 7.45–7.53 (2H, m), 8.12–8.18 (1H, m), 8.80–8.87 (1H, m), 9.07–9.12 (1H, m). ESI-MS *m/z* 430.1 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{22}H_{28}N_3O_4S$, 430.1801; found, 430.1808.

5.1.29. (1*R**,4*S**,6*R**)-(±)-2-[(1-Methyl-1*H*-imidazol-4-yl) sulfonyl]-*N*-[4-(propan-2-yloxy)phenyl]-2-azabicyclo[2.2.2] octane-6-carboxamide (28)

Compound **28** was prepared from **33** and 1-methylimidazole-4-sulfonyl chloride hydrochloride using the procedure described for **12**. ^1H NMR (400 MHz, $CDCl_3$): δ 1.32 (6H, d, J = 6.3 Hz), 1.41–1.52 (1H, m), 1.56–1.66 (1H, m), 1.70–1.98 (3H, m), 2.00–2.05 (1H, m), 2.15–2.24 (1H, m), 2.94–3.02 (2H, m), 3.43–3.48 (1H, m), 3.80 (3H, s), 4.28–4.31 (1H, m), 4.46–4.54 (1H, m), 6.82–6.88 (2H, m), 7.46–7.52 (3H, m), 7.59–7.63 (1H, m), 8.62 (1H, br s). ESI-MS *m/z* 433.3 $(M+H)^+$. HRMS $(M+H)^+$ calcd for $C_{21}H_{29}N_4O_4S$, 433.1910; found, 433.1909.

5.1.30. Optical resolution of (±)-28

Optical resolution of (±)-**28** (60 mg) was performed by chiral HPLC using Daicel CHIRALPAK AD (250 × 20 mm id), eluting with 40% 2-propanol in hexanes at flow rate of 1.0 mL/min. 26.6 mg of **28a** was obtained from the first eluate (t_R: 14.2 min) and 29.4 mg of **28b** was obtained from the second eluate (t_R: 30.7 min). $[\alpha]_D^{25}$ for **28a** = +282 (*c* 1.0, $CHCl_3$).

5.1.31. (1*R**,4*S**,6*R**)-(±)-2-(Butylsulfonyl)-2-azabicyclo[2.2.2] octane-6-carboxylic acid (30)

To a solution of **29** (298 mg, 0.989 mmol) and triethylamine (0.51 mL, 3.7 mmol) in $CHCl_3$ (5 mL) was added 1-butanesulfonyl chloride (0.23 mL, 1.84 mmol), and the mixture was stirred at room temperature for 13 h. The mixture was washed with water and saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 0→40% ethyl acetate in hexanes to give the corresponding sulfonamide (157 mg, 55%) as a 3:7 mixture of diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J = 7.6 Hz), 1.38–1.52 (2H, m), 1.58–1.93 (5H, m), 2.03–2.29 (2H, m), 2.69–2.75 (1H × 3/10, m), 2.85–3.00 (2H, m), 3.04–3.12 (1H × 7/10, m), 3.28–3.51 (2H, m), 3.71 (3H × 7/10, s), 3.73 (3H × 3/10, s), 4.05–4.10 (1H × 7/10, m), 4.14–4.18 (1H × 3/10, m). ESI-MS *m/z* 290.2 $(M+H)^+$. The sulfonamide obtained above (145 mg, 0.5 mmol) was dissolved in a mixture of 1.5 mL of 5 N aqueous NaOH and 5 mL of methanol, and the resultant mixture was stirred at room temperature for 17 h. The mixture was neutralized with 5 N aqueous HCl, and extracted with $CHCl_3$ three times. The combined organic extracts were dried over magnesium sulfate, and concentrated to give **30** (134 mg, 97%) as a 3:7 mixture of diastereomers. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88–0.99 (3H, m), 1.36–1.50 (2H, m), 1.60–1.86 (4H, m), 1.87–1.97 (1H, m), 2.06–2.24 (2H, m), 2.74–2.81 (1H × 3/10, m), 2.87–3.01 (2H, m), 3.10–3.18 (1H × 7/10, m), 3.28–3.53 (2H, m), 4.10–4.13 (1H × 7/10,H, m), 4.18–4.21 (1H × 3/10, m). ESI-MS *m/z* 276.1 $(M+H)^+$.

5.1.32. 2-*tert*-Butyl 6-methyl (1*R**,4*S**)-2-azabicyclo[2.2.2] octane-2,6-dicarboxylate (31)

To a solution of **29** (2.36 g, 7.94 mmol), *N*,*N*-dimethyl-4-aminopyridine (97 mg, 0.79 mmol), and triethylamine (4.4 mL, 31.8 mmol) in $CHCl_3$ (30 mL) was added di-*tert*-butyl carbonate

(3.47 g, 15.9 mmol), and the mixture was stirred at room temperature for 24 h. The mixture was washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 0→40% ethyl acetate in hexanes to give to give **31** (2.05 g, 96%) as a 3:7 mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (9H × 3/10, s), 1.48 (9H × 7/10, s), 1.61 (3H, s), 1.90–2.20 (2H, m), 2.61–2.97 (1H, m), 3.20–3.42 (2H, m), 3.65–3.74 (2H, m), 4.06–4.42 (2H, m). ESI-MS *m/z* 270.3 $(M+H)^+$.

5.1.33. *tert*-Butyl (1*R**,4*S**,6*R**)-(±)-6-{[4-(propan-2-yloxy)phenyl]carbamoyl}-2-azabicyclo[2.2.2]octane-2-carboxylate (32)

To a solution of **31** (2.05 g, 7.53 mmol) in 27 mL of methanol was added 5 N aqueous NaOH (15 mL), and the mixture was stirred at room temperature for 3 h. The mixture was neutralized with 5 N aqueous HCl, and extracted with $CHCl_3$ three times. The combined organic extracts were dried over magnesium sulfate, and concentrated to give the corresponding carboxylic acid (1.68 g, 87%) as a 3:7 mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38–1.49 (9H, m), 1.52–1.87 (3H, m), 1.91–2.17 (2H, m), 2.67–2.79 (1H × 3/10, m), 2.91–3.03 (1H × 7/10, m), 3.21–3.45 (2H, m), 4.23–4.46 (1H, m). ESI-MS *m/z* 256.3 $(M+H)^+$. To a solution of the carboxylic acid obtained above (1.68 g, 6.58 mmol) and 4-isopropoxyaniline (1.49 g, 9.87 mmol) in pyridine (20 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.89 g, 9.87 mmol), and the mixture was stirred at room temperature for 14 h. The resulting mixture was concentrated, and the residue was partitioned between ethyl acetate and water. The layers were separated, and the organic layer was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography with 0→25% ethyl acetate in hexanes to give **32** (1.26 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (6H, d, J = 5.9 Hz), 1.49 (9H, s), 1.54–2.05 (3H, m), 2.33–2.43 (1H, m), 2.86–2.96 (1H, m), 3.29–3.39 (2H, m), 4.21–4.28 (1H, m), 4.43–4.55 (1H, m), 6.78–6.90 (2H, m), 7.36–7.46 (2H, m), 7.94 (1H, br s).

5.1.34. *tert*-Butyl (1*S**,4*S**,6*R**)-(±)-*N*-(4-isopropoxyphenyl)-2-azabicyclo[2.2.2]octane-6-carboxamide hydrochloride (33)

To a solution of compound **32** (1.26 g, 3.24 mmol) in $CHCl_3$ (10 mL) was added 4 N aqueous HCl in ethyl acetate (8.1 mL), and the mixture was stirred at room temperature for 13 h and concentrated to give **33** (1.15 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (6H, d, J = 5.9 Hz), 1.61–1.72 (1H, m), 1.81–1.96 (2H, m), 2.00–2.10 (1H, m), 2.40–2.50 (1H, m), 3.17–3.26 (1H, m), 3.29–3.48 (2H, m), 4.08–4.14 (1H, m), 4.39–4.50 (1H, m), 6.74–6.80 (2H, m), 7.49–7.54 (2H, m), 8.92 (1H, br s), 9.23 (1H, br s), 9.47 (1H, br s). ESI-MS *m/z* 289.3 $(M+H)^+$.

5.2. Biological protocols

5.2.1. Enzyme assay

The full-length ELOVLs cDNAs were amplified from human or mouse liver cDNA library by polymerase chain reaction using the gene specific primers. Then COS-7 cells were transfected with the expression vector encoding the C-terminally V5-epitope tagged ELOVLs cDNA. After 48 h transfection, the cells were harvested and the microsome fractions were prepared as enzyme resources. The expression of each ELOVL was confirmed by immunoblot analysis using anti-V5-epitope antibody. For elongation reactions, 50 μL of the reaction mixture (1 μL of a diluted test compound in DMSO, 100 mM potassium phosphate buffer (pH 6.5), 200 μM BSA (fatty acid free), 500 μM NADPH, 1 μM rotenone, 20 μM malonyl-CoA, 833 kBq/mL [14C] malonyl-CoA and acyl-CoA), was used as the substrate mixture. The following long-chain acyl-CoAs were used as a preferential substrate for each ELOVL; ELOVL1, 10 μM stearoyl-CoA; ELOVL2, 10 μM arachidonoyl-CoA; ELOVL3, 10 μM stearoyl-CoA; ELOVL5, 40 μM arachidonoyl-CoA; ELOVL6, 40 μM palmitoyl-CoA . To start reaction, 50 μL of the ELOVL microsomal fraction was added to the substrate mixture, and then incubated for 1 h at 37 °C with gentle shaking. This reaction step was performed in a 96-well plate. After 1 h incubation, 100 μL of 5 N HCl was added for the hydrolysis of acyl-CoAs, and then the reaction mixture was filtered through a Unifilter-96, GF/C plate (PerkinElmer, Waltham, MA) using a FilterMate cell harvester (PerkinElmer, Waltham, MA). The 96-well GF/C filter plate was subsequently washed with distilled water to remove excess [^{14}C] malonyl-CoA and dried, after which 25 μL of MICROSCINT 0 was added to each well and radioactivity.

5.2.2. Plasma and liver exposure in mice

Plasma and liver concentrations: Pharmacokinetic characterizations of compound **28a** were conducted in male C57BL/6J mice following single oral administration. Single doses of compound **28a** at 30 mg/kg body weight were administered orally by gavage in a vehicle of 0.5% methylcellulose aqueous suspension. Blood samples from the abdominal vein and liver samples were obtained 8 h after the oral administration. Blood samples were centrifuged to separate the plasma. Liver samples were homogenized with phosphate-buffered saline (pH 7.4). Each sample was deproteinized with ethanol containing an internal standard. The respective compound and the internal standard were detected by liquid chromatography mass spectrometry/mass spectrometry (LC–MS/MS) in positive ionization mode using an electrospray ionization probe, and their precursor to production combinations were monitored using the Multiple Reaction Monitoring mode.

5.2.3. Effect of compound 28a on liver fatty acid elongation index

Male C57BL/6J mice (CLEA Japan) and SD rats (Charles River Japan) were individually housed in plastic cages with ad libitum access to normal rodent chow (CE2, CLEA Japan) and water. Mice were orally administered compound **28a** (dissolved in 0.5% methylcellulose) and 1 h later [1-^{14}C]-palmitic acid was interperitoneally administered at 10 μCi/body. At 2 h post-dosing of compound **28a**, animals were anesthetized with isoflurane (4%) and killed by blood collection from the vena cava. The liver was harvested, incubated in potassium hydroxide/ethanol at 70 °C for 1 h and the lipid fraction then obtained by collecting the ether phase after the addition of petroleum ether. After acidification with hydrochloric acid, the mixture was extracted with ether. Radio labeled palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), and vaccenic and oleic acids (18:1) were quantified by reversed-phase HPLC with a Packard Flow Scintillation Analyzer 500TR (radio-HPLC) (Amersham Biosciences, Piscataway, NJ). The identity of the labeled fatty acids was determined by comparing the retention times with known fatty acid standards. Elongation activity was monitored as the elongation index (EI), which was the ratio of radio labeled C18 (C18:0 + C18:1) to C16 (C16:0 + C16:1).All animal procedures were conducted according to protocols and guidelines approved by the Banyu Institutional Animal Care and Use Committee.

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Supplementary data

Supplementary data (HPLC retention times and purity for the target compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.042.

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19. Metabolic stability in liver microsomes: 1 μM compound was incubated at 37 °C in 0.25 mg/mL of human and mouse liver microsomes supplemented with 10 mM glucose-6-phospate, 1 mM β-nicotinamide-adenine dinucleotide phosphate, 1 U/mL glucose-6-phosphate dehydrogenase, 100 mM phosphate buffer and 3 mM magnesium chloride. Concentrations of respective compound were determined by LC–MS/MS. Metabolic stability was calculated from the ratio of respective compound concentration at 0 min to that at 30 min after the initiation of incubation.