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Short communication

Discovery of novel SCD1 inhibitors: 5-Alkyl-4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] analogs

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ABSTRACT

Expansion of the 6-membered ring and subsequent fine-tuning of the newly obtained 7-membered spiropiperidine structure resulted in the discovery of a series of novel and potent SCD1 inhibitors. Preliminary SAR was explored by modifying an alkyl chain on the azepine nitrogen and resulted in the identification of a highly potent SCD1 inhibitor: 6-[5-(cyclopropylmethyl)-4,5-dihydro-1′*H*,3*H*-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl]-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**9**). Compound **9** exhibited an IC_{50} value of 0.01 μM against human SCD1.

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1. Introduction

In many species, stearoyl-CoA desaturase 1 (SCD1) is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids from their saturated fatty acid precursors [1,2]. SCD1 introduces a double bond at the Δ9 position (between carbons 9 and 10) of stearoyl (C18:0) and palmitoyl-CoA (C16:0) [1,2] in conjunction with NAD(P)H, cytochrome b5 reductase, and cytochrome b5.

SCD1 has recently been shown to be a crucial factor in lipid metabolism and body weight control [3–6] because the products of SCD1, oleic (C18:1 n-9) and palmitoleic acids (C16:1 n-7) are the most abundant fatty acids found in phospholipids, cholesterol esters, and triglycerides. In humans, a higher desaturation index (the ratio of oleate to stearate or 18:1/18:0) strongly correlates with higher plasma triglyceride levels and (to a lesser extent) with plasma HDL levels [7]. The 18:1/18:0 ratio in plasma VLDL has recently been shown to be closely associated with the hepatic SCD1 expression in humans [8]. Even though the detailed mechanism by which SCD1 deficiency affects body weight and adiposity is not completely understood, inhibition of SCD1 may represent a novel approach for the treatment of metabolic syndrome.

Since Xenon Pharmaceuticals published the first example of small molecule SCD1 inhibitors (**1**, Fig. 1) in 2005 [9], numerous structures of SCD1 inhibitors have been reported in patents and scientific literature [10–15]. We have previously reported on the optimization of piperidine-based SCD1 inhibitors, which resulted in the discovery of highly potent spiropiperidines [16–18]. In continuing our SCD1 inhibitor project, spiropiperidine (**1**, Fig. 1) was chosen as a lead compound for further optimization. Since the X-ray structure of SCD1 has not been resolved and the structural information of this enzyme is very limited, our design of the novel SCD1 inhibitors mainly relied on the results obtained from our own SAR studies. Here in this article, we would like to disclose our recent efforts on the optimization of the spiropiperidine structures and the discovery of novel and highly potent SCD1 inhibitors.

2. Results and discussion

In the preliminary investigation (Fig. 1), expansion of the 6-membered ring via Beckmann rearrangement gave 7-membered spiropiperidine, 3*H*-spiro[1,5-benzoxazepine-2,4′-piperidin]-4(5*H*)-one (**2**). Subsequent reduction of the amide group in the newly

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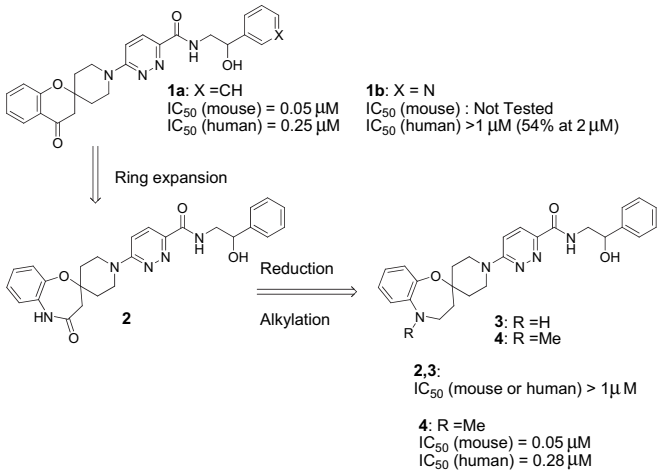


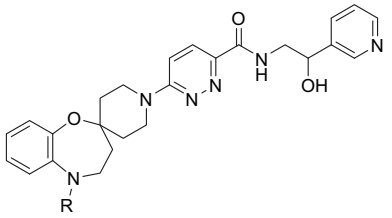
Fig. 1. Ring expansion of 6-membered spiropiperidine to 7-membered spiropiperidine.

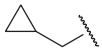
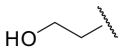
prepared spiropiperidine generated a secondary amine (**3**) with the 4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] structure. Although the inhibitory activity against SCD1 decreased during the course of these transformations, methylation of the secondary amine in **3** successfully led to **4**, which demonstrated potency comparable to **1a**. These results prompted us to further investigate optimal structures of the novel 7-membered spiropiperidines for better inhibitory activity against SCD1.

We prepared a series of spiropiperidine-based SCD1 inhibitors with 4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] as the key structural motif. Synthesis of the novel spiropiperidine-based SCD1 inhibitors shown in Table 1 is outlined in Schemes 1 and 2. Condensation of the *o*-hydroxyacetophenone (**11**) with 1-benzylpiperidin-4-one in the presence of pyrrolidine gave 1′-benzylspiro[chromene-2,4′-piperidin]-4(3*H*)-one (**12**) [19–21]. Treatment of **12** with hydroxylamine gave oxime that was subjected to reductive rearrangement (Beckmann rearrangement) to give 1′-benzyl-4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] (**13**) [19–21] with an overall yield of 64% from **11**. Introduction of the various alkyl group on the azepine nitrogen was carried out by reductive amination (Me (**14**) and cyclopropyl (**19**)), alkylation (Et, *i*Pr and cyclopropylmethyl), or epoxide opening (hydroxyethoxy). Deprotection of the benzyl group by hydrogenolysis proceeded smoothly for most of the tertiary amines (**15**–**18**, **20**–**21**) except for the cyclopropyl analog (**19**), which gave decomposed mixtures under the reaction conditions. Condensation between the secondary amines (**15**–**18**, **20**–**21**) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide [16] in BuOH provided the desired SCD1 inhibitors without complication (Scheme 2). For the preparation of cyclopropyl analog, **22** was subjected to reductive amination with [(1-ethoxycyclopropyl)oxy]trimethylsilane [22] to give **8** in 63% yield.

The results of the biological evaluation of 1′-alkyl-4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] analogs (**5**–**10**) are shown in Table 1. The inhibitory activity against enzymatic SCD1 was measured for both mice and humans. The sources of the SCD1 enzyme are the liver microsomes (for murine SCD1) and the microsome fractions prepared from the 293A cells transiently transfected with human SCD1 (for human SCD1). While other isoforms of SCD are known to exist in mouse liver, their expression levels are low. The right-hand phenyl was replaced with 3-pyridyl in this series of compounds in Table 1 to reduce lipophilicity even though a decrease in the inhibitory activity was observed by this structural modification. The phenyl analog (**4**)

Table 1
Evaluation of the 7-membered spiropiperidines for enzymatic SCD1 inhibitory activity.[a]

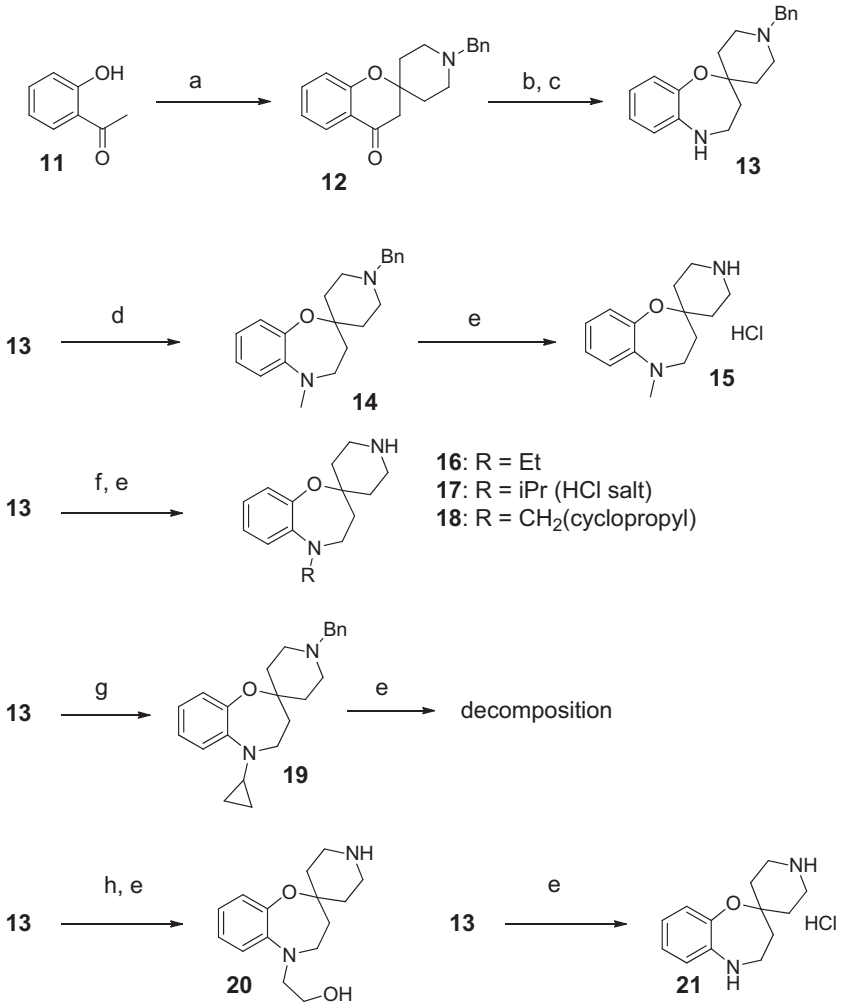


#	R	IC_{50} (μM) Mouse microsomal Δ9	IC_{50} (μM) Human cell (293A) microsomal Δ9	CLogP[b]
5	Me	0.3	0.7	1.5
6	Et	0.04	0.1	2.0
7		0.04	0.04	2.4
8		>2	Not tested	2.0
9		0.01	0.01	2.5
10		1.8	0.6	0.7

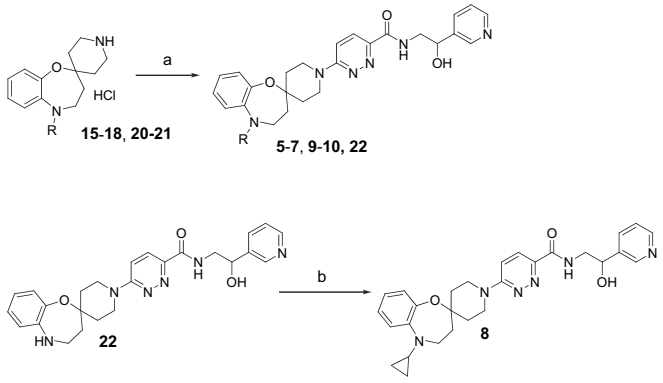
[a] Values are the arithmetic means of at least two experiments.
[b] The cLogP values were calculated by CLOGP software (Version 4.8.3, Daylight Chemical Information Systems, Inc.).

exhibited an IC_{50} value of 0.28 μM against human SCD1 while the 3-pyridyl analog (**5**) showed 0.7 μM in the same assay as shown in Fig. 1 and Table 1. As for lipophilicity, cLogP value for **4** is 3.0 while that for **5** is 1.5. We thought the less-lipophilic compound (**4**) was a good starting point in the structural optimization of spiropiperidine, in which addition of lipophilic groups was implemented. Elongation of the alkyl group on the azepine nitrogen significantly increased inhibitory activity against SCD1 with increase in lipophilicity (CLogP). One carbon elongation from methyl (**5**) to ethyl (**6**) resulted in a 7-fold increase in the inhibitory activity against human SCD1 with increase of lipophilicity (from 1.5 to 2.0). Secondary alkyl group, such as propyl (**7**), did not improve potency in a meaningful scale in spite of the increase in lipophilicity (from 2.0 to 2.4), indicating that there is a steric hindrance around the position. In addition, cyclopropyl (**8**) exhibited a sharp loss of activity with less than 50% inhibition even at 2 μM. From these data, it is assumed that the alkyl chain needs to rotate freely so that it can adapt an optimal interaction with the enzyme in the binding pocket. To examine this assumption, a methylene unit was inserted between cyclopropyl and the azepine nitrogen. This small modification remarkably increased inhibitory activity: cyclopropylmethyl analog (**9**) exhibited an IC_{50} value of 0.01 μM against human SCD1. This rise of inhibitory activity was accompanied with increase of lipophilicity as well (CLogP 2.5). Introduction of the hydrophilic functional group reduced lipophilicity in a considerable scale (CLogP 0.7) and was rather detrimental in terms of potency. Hydroxylethyl analog (**10**) showed 45 times weaker inhibitory activity against murine SCD1 than the ethyl analog (**6**).

Some of the potent compounds (**6**, **7** and **9**) were tested in the cellular assay. In cellular assay, whole transfected 293A cells were used for the evaluation of our SCD1 inhibitors. All of the compounds exhibited excellent potency presumably due to their good cell-membrane permeability. Among the tested compounds, **9** demonstrated strongest potency with an IC_{50} value of 0.01 μM Table 2.



Scheme 1. Reagents and conditions: (a) 1-benzylpiperidin-4-one, pyrrolidine, EtOH, reflux, 90%; (b) $NH_2OH{\cdot}HCl$, pyridine, EtOH, 88%; (c) DIBAL, CH_2Cl_2, 81%; (d) HCHO (aq), $NaBH_3CN$, CH_3CO_2H/THF, rt, 93%. (e) Pd/C, H_2, 1 N HCl, MeOH; (f) R-X, K_2CO_3, DMF or DMA, 50–70 °C; (g) (1-ethoxycyclopropoxy)trimethylsilane, $NaBH_3CN$, CH_3CO_2H/MeOH, reflux, quantitative yield; (h) oxirane, CH_3CO_2H/THF, 78%.



Scheme 2. Reagents and conditions: (a) 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl) pyridazine-3-carboxamide, Et_3N, n-BuOH, 100 °C; (b) (1-ethoxycyclopropoxy)trimethylsilane, $NaBH_3CN$, CH_3CO_2H, MeOH.

3. Conclusion

In summary, expansion of the 6-membered ring and subsequent fine-tuning of the newly obtained 7-membered spiropiperidine structure (benzoxazepine) resulted in the discovery of novel and potent SCD1 inhibitors. Preliminary SAR was explored by modifying alkyl chain on the azepine nitrogen, which resulted in the identification of a highly potent SCD1 inhibitor: 5-(cyclopropylmethyl)-1′-{6-[5-(pyridin-3-ylmethyl)-1,3,4-oxadiazol-2-yl]pyridazin-3-yl}-4,5-dihydro-3*H*-spiro[1,5-benzoxazepine-2,4′-piperidine] (**9**). Further structural optimization and biological evaluation of the novel spiropiperidine-based SCD1 inhibitors will be our future work.

4. Experimental

4.1. Chemistry

4.1.1. General procedures

^1H NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. The mass spectra (Low- or High-resolution mass) spectroscopy was

Table 2
Evaluation of the 7-membered spiropiperidines for cellular SCD1 inhibitory activity.[a]

Compound	IC_{50} (μM) Human cell (293A) Δ9
6	0.10
7	0.14
9	0.01

[a] Values are the arithmetic means of at least two experiments.

carried out with a JEOL GCmate, JEOL JMS-700 mass spectrometer or JEOL T100LC (AccuTOF). Thin-layer chromatography (TLC) was used routinely to monitor the progress and the purity of the compounds and was performed on Merck Kieselgel 60 F_{254} plates (0.25 mm thickness). For flash column chromatography, silica gel (Kieselgel 60, 230–400 mesh or Chromatorex NH Fuji Silysia Chemical Ltd. 200–300 mesh) or pre-packed silica gel column (KP-Sil™ silica) from Biotage was employed. The experimental procedures for the preparation of the intermediates in Schemes 1 and 2 are described in the supplementary information.

4.1.2. General synthetic procedures to obtain spiropiperidine-based SCD1 inhibitor (**5**–**10**) from benzoxazepine (**15**–**18**, **20**–**21**) and 6-chloro-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide [16]

A mixture of benzoxazepine (**15**–**18**, **20**–**21**, 1 equivalent), 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (1 equivalent), and triethylamine (2 equivalents) in n-BuOH was heated at 100 °C for 2 days, cooled to room temperature, and concentrated. Chromatography of the residue on SiO_2 (Chromatorex NH Fuji Silysia Chemical Ltd. 200–300 mesh, CH_2Cl_2/EtOAc) gave the desired product (**5**–**10**). The product was further purified by recrystallization when necessary.

4.1.3. N-(2-Hydroxy-2-pyridin-3-ylethyl)-6-(5-methyl-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl) pyridazine-3-carboxamide (**5**)

In accordance with the general procedures (Section 4.1.2), **15** (34.9 mg, 0.130 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (33.4 mg, 0.120 mmol) provided 32.2 mg of **5** (57%) as a brown amorphous. ^{1}H NMR (400M Hz, DMSO-d_6): δ 8.68 (1H, dd, J = 5.9 and 5.8 Hz), 8.53 (1H, s), 8.43 (1H, d, J = 4.7 Hz), 7.77 (1H, d, J = 9.8 Hz), 7.75 (1H, d, J = 6.2 Hz), 7.39–7.31 (2H, m), 6.97 (1H, dd, J = 7.7 and 7.7 Hz), 6.90 (1H, d, J = 9.0 Hz), 6.76 (1H, d, J = 9.4 Hz), 6.71 (1H, dd, J = 8.2 and 8.2 Hz), 5.75 (1H, d, J = 4.7 Hz), 4.89–4.83 (1H, m), 4.22 (1H, d, J = 13.3 Hz), 3.61–3.47 (4H, m), 3.17 (1H, t, J = 5.5 Hz), 2.77 (3H, s), 2.48 (2H, t, J = 1.9 Hz), 1.89 (2H, d, J = 13.3 Hz), 1.78 (2H, t, J = 5.5 Hz), 1.57 (2H, dt, J = 4.3 and 13.2 Hz). MS (ESI) m/z: 475 $(M + H)^+$; HRMS (ESI) m/z: 475.2450 (calcd for $C_{26}H_{31}N_6O_3$ 475.2458).

4.1.4. 6-(5-Ethyl-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl)-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**6**)

In accordance with the general procedures (Section 4.1.2), **16** (81.3 mg, 0.330 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (69.6 mg, 0.250 mmol) provided 88.4 mg of **6** (73%) as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (1H, dd, J = 5.9 and 5.9 Hz), 8.55 (1H, d, J = 2.0 Hz), 8.46 (1H, dd, J = 4.9 and 1.8 Hz), 7.79 (1H, d, J = 9.3 Hz), 7.78–7.77 (1H, m), 7.39 (1H, d, J = 9.8 Hz), 7.36 (1H, dd, J = 7.8 and 5.1 Hz), 6.96 (1H, dt, J = 10.4 and 3.8 Hz), 6.90 (1H, dd, J = 7.8 and 1.5 Hz), 6.80 (1H, dd, J = 8.0 and 1.4 Hz), 6.68 (1H, dt, J = 10.6 and 3.9 Hz), 5.78 (1H, s), 4.87 (1H, t, J = 5.3 Hz), 4.24 (2H, d, J = 12.2 Hz), 3.61–3.50 (3H, m), 3.33–3.27 (3H, m), 3.19 (2H, q, J = 7.0 Hz), 1.92 (2H, d, J = 14.1 Hz), 1.81–1.79 (2H, m), 1.63–1.55 (2H, m), 1.12 (3H, t, J = 6.8 Hz). MS (ESI) m/z: 489 $(M + H)^+$; HRMS (ESI) m/z: 489.2608 (calcd for $C_{27}H_{33}N_6O_3$ 489.2614).

4.1.5. N-(2-Hydroxy-2-pyridin-3-ylethyl)-6-(5-isopropyl-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl) pyridazine-3-carboxamide (**7**)

In accordance with the general procedures (Section 4.1.2), **17** (65.3 mg, 0.220 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (55.7 mg, 0.200 mmol) provided 81.8 mg of **7** (81%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.71 (1H, dd, J = 5.7 and 5.7 Hz), 8.55 (1H, d, J = 1.6 Hz), 8.46 (1H, dd, J = 4.5 and 1.8 Hz), 7.79 (1H, d, J = 9.8 Hz), 7.79–7.76 (1H, m), 7.38 (1H, d, J = 9.8 Hz), 7.37–7.34 (1H, m), 6.97–6.90 (2H, m), 6.82–6.80 (1H, m), 6.65 (1H, dd, J = 7.6 and 7.6 Hz), 5.77 (1H, d, J = 5.1 Hz), 4.87 (1H, q, J = 5.2 Hz), 4.23 (2H, d, J = 12.9 Hz), 3.80–3.68 (1H, m), 3.61–3.49 (4H, m), 3.33–3.21 (2H, m), 1.92 (2H, d, J = 12.9 Hz), 1.79–1.72 (2H, m), 1.62–1.55 (2H, m), 1.16 (6H, d, J = 6.6 Hz). MS (ESI) m/z: 503 $(M + H)^+$; HRMS (ESI) m/z: 503.2773 (calcd for $C_{28}H_{35}N_6O_3$ 503.2771).

4.1.6. 6-(4,5-Dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl)-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**22**)

In accordance with the general procedures (Section 4.1.2), **21** (62 mg, 0.24 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (55.9 mg, 0.200 mmol) provided 88 mg of **22** (96%) as a pale beige solid. ^{1}H NMR(400M Hz, DMSO-d_6): δ 8.71 (1H, dd, J = 6.0 and 6.0 Hz), 8.55 (1H, d, J = 2.4 Hz), 8.45 (1H, d, J = 4.7 Hz), 7.81–7.76 (2H, m), 7.38–7.34 (2H, m), 6.92 (1H, d, J = 9.0 Hz), 6.81 (1H, dd, J = 7.5 and 7.5 Hz), 6.71 (1H, d, J = 9.8 Hz), 6.58 (1H, dd, J = 4.4 and 4.5 Hz), 5.77 (1H, s), 5.44 (1H, t, J = 3.9 Hz), 4.87 (1H, dd, J = 11.8, 5.9 Hz), 4.24 (2H, d, J = 14.4 Hz), 3.62–3.45 (4H, m), 3.22–3.16 (2H, m), 1.95 (2H, d, J = 13.7 Hz), 1.85 (2H, t, J = 5.1 Hz), 1.64–1.53 (2H, m).

4.1.7. 6-(5-Cyclopropyl-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl)-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**8**)

To a solution of **22** (88.3 mg, 0.192 mmol) in MeOH (2 mL) were added acetic acid (0.11 mL, 1.9 mmol), powered molecular sieves (3A, 0.1 g), [(1-ethoxycyclopropyl)oxy]trimethylsilane (0.15 mL, 0.77 mmol), and sodium cyanoborohydride (36 mg, 0.57 mmol) at room temperature. The reaction mixture was heated to reflux for 2 h, filtered, and concentrated. The residue was diluted with 1 N HCl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was successively purified by chromatography on SiO_2 (CH_2Cl_2/MeOH) and preparative TLC (CH_2Cl_2/MeOH 10:1). The resulting material was triturated in hexane, collected by filtration and dried in vacuo to give 61 mg (63%) of **8** as a white amorphous. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (1H, dd, J = 5.6 and 5.7 Hz), 8.55 (1H, s), 8.45 (1H, d, J = 4.7 Hz), 7.82–7.74 (2H, m), 7.40–7.32 (2H, m), 7.13 (1H, d, J = 8.2 Hz), 7.00 (1H, dd, J = 7.9 and 7.9 Hz), 6.94 (1H, d, J = 7.5 Hz), 6.77 (1H, d, J = 6.6 Hz), 5.77 (1H, d, J = 4.7 Hz), 4.86 (1H, dd, J = 11.9 and 5.7 Hz), 4.23 (2H, d, J = 12.9 Hz), 3.62–3.48 (5H, m), 3.39–3.23 (2H, m), 1.88 (2H, d, J = 13.7 Hz), 1.83–1.77 (2H, m), 1.61–1.52 (2H, m), 0.74 (2H, d, J = 5.1 Hz), 0.39 (2H, s). MS (ESI) m/z: 501 $(M + H)^+$; HRMS (ESI) m/z: 501.2615 (calcd for $C_{28}H_{33}N_6O_3$ 501.2614).

4.1.8. 6-[5-(Cyclopropylmethyl)-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl]-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**9**)

In accordance with the general procedures (Section 4.1.2), **18** (48.0 mg, 0.176 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (41.8 mg, 0.150 mmol) provided 66.7 mg (87%) of **9** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (1H, dd, J = 6.0 and 6.0 Hz), 8.55 (1H, d, J = 1.9 Hz), 8.45 (1H, dd, J = 4.7 and 1.5 Hz), 7.79 (1H, d, J = 9.4 Hz), 7.77 (1H, d, J = 6.7 Hz), 7.38 (1H, d, J = 9.7 Hz), 7.35 (1H, dd, J = 7.7 and 4.9 Hz), 6.95 (1H, dd, J = 7.7 and 7.7 Hz), 6.90 (1H, dd, J = 7.8 and 1.6 Hz), 6.83 (1H, dd, J = 8.0 and 1.4 Hz), 6.68 (1H, dd, J = 11.9 and 4.5 Hz), 5.77 (1H, d, J = 4.7 Hz), 4.89–4.85 (1H, m), 4.24 (2H, d, J = 13.3 Hz), 3.59–3.52 (4H, m), 3.39 (2H, dd, J = 5.3 and 5.3 Hz), 3.02 (2H, d,

$J = 6.2$ Hz), 1.93 (2H, d, $J = 13.7$ Hz), 1.82 (2H, t, $J = 5.1$ Hz), 1.64–1.57 (2H, m), 1.04–0.98 (1H, m), 0.53–0.48 (2H, m), 0.22–0.19 (2H, m). MS (ESI) m/z: 515 $(M + H)^+$; HRMS (ESI) m/z: 515.2778 (calcd for $C_{29}H_{35}N_6O_3$ 515.2771).

4.1.9. 6-[5-(2-Hydroxyethyl)-4,5-dihydro-1′H,3H-spiro[1,5-benzoxazepine-2,4′-piperidin]-1′-yl]-N-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (**10**)

In accordance with the general procedures (Section 4.1.2), **20** (102 mg, 0.389 mmol) and 6-chloro-*N*-(2-hydroxy-2-pyridin-3-ylethyl)pyridazine-3-carboxamide (89.0 mg, 0.319 mmol) provided 86.4 mg (54%) of **10** as a pale brown solid. ^{1}H NMR (400M Hz, DMSO-d_6): δ 8.68 (1H, dd, $J = 5.7$ and 5.7 Hz), 8.53 (1H, s), 8.43 (1H, d, $J = 3.9$ Hz), 7.77 (1H, d, $J = 9.4$ Hz), 7.75 (1H, d, $J = 5.8$ Hz), 7.35 (1H, d, $J = 9.0$ Hz), 7.33 (1H, d, $J = 11.3$ Hz), 6.92 (1H, dd, $J = 7.7$ and 7.7 Hz), 6.86 (1H, d, $J = 7.4$ Hz), 6.81 (1H, d, $J = 7.4$ Hz), 6.62 (1H, dd, $J = 7.5$ and 7.5 Hz), 5.74 (1H, s), 4.85 (1H, dd, $J = 9.8$ and 4.7 Hz), 4.63 (1H, t, $J = 4.5$ Hz), 4.20 (2H, d, $J = 12.9$ Hz), 3.60–3.46 (5H, m), 3.38 (2H, t, $J = 4.5$ Hz), 3.30 (1H, s), 3.22 (2H, t, $J = 5.9$ Hz), 1.90 (2H, d, $J = 14.0$ Hz), 1.83–1.77 (2H, m), 1.56 (2H, t, $J = 10.6$ Hz). MS (ESI) m/z: 505 $(M + H)^+$; HRMS (ESI) m/z: 505.2569 (calcd for $C_{27}H_{33}N_6O_4$ 505.2563).

4.2. Biology

4.2.1. Desaturase enzymatic assay

The SCD1 activity was determined by measuring the conversion of stearate to oleate. In each reaction tube, test compounds were preincubated with 10 μL microsomes for 10 min at room temperature. The SCD1 reaction was started by the addition of 40 μL of a mixture containing 250 mM sucrose, 150 mM KCl, 40 mM NaF, 5 mM $MgCl_2$, 100 mM sodium phosphate, pH7.4, 1 mM ATP, 1.5 mM reduced glutathione, 0.06 mM reduced coenzyme A, 0.33 mM nicotinamide, 1.25 mM NADH and 0.01 μCi [^{14}C] stearate. After 60 min incubation at 37 °C, the reaction was stopped by adding 50 μL methanol containing 10% KOH and then the mixture was saponified at 80 °C for 30 min. The free fatty acids in the reaction were protonated by the addition of 5N HCl (15 μL) and extracted with 100 μL ethyl acetate. 30 μL of the ethyl acetate extracts of each reaction was charged to an $AgNO_3$–TLC plate (20 × 20 cm LK5D plates, 150 Å pore diameter, 250 μm thick) and differentiated in a solvent consisting of chloroform:methanol:acetate:water (90:8:1:0.8). [^{14}C] stearate and [^{14}C] oleate were quantified with BAS2500 (Fujifilm) and SCD1 activity was determined as the ratio of [^{14}C] oleate to [^{14}C] stearate. The IC_{50} values were calculated by linear regression using the straight line portions of the concentration–response curve. To measure the delta-6 desaturase activity, [^{14}C] linolenic acid was used as the substrate and the delta-6 desaturase activity was determined as the ratio of [^{14}C] C18:3 n-3 to [^{14}C] C18:4 n-3.

4.2.2. Cellular assay

A 293A cell-based desaturase assay was performed in a 96-well plate. Human SCD1 gene was cloned into the expression vector pCMV-script (Stratagene). 293A cells were transfected with the expression vector. The cells stably expressing human SCD1 were selected with G418. The 293A cells in 100 μL media (DMEM + 10% FBS) were seeded to each well of a 96-well plate and grown overnight to be confluent. The cells were preincubated with the test compound in fresh media for 30 min, after which 10 μL media containing 0.1 μCi [^{14}C] stearate was added to each well and incubated for another 4 h. Then the cells in each well were washed with cold PBS and the cellular lipids were saponified directly by adding 100 μL of 5% KOH in methanol:H_2O (1:1). The samples were processed as described for the SCD1 enzymatic assay to determine the SCD1 activity by quantifying the ratio of [^{14}C] oleate to [^{14}C] stearate.

Appendix. Supplementary material

Supplementary data associated with this article can be found in online version at doi:10.1016/j.ejmech.2011.02.002.

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