Bioorganic & Medicinal Chemistry Letters 23 (2013) 6773–6776

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

4-Bicyclic heteroaryl-piperidine derivatives as potent, orally bioavailable Stearoyl-CoA desaturase-1 (SCD1) inhibitors. Part 1: Urea-based analogs

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ARTICLE INFO

Article history:
Received 9 August 2013
Revised 25 September 2013
Accepted 30 September 2013
Available online 9 October 2013

Keywords:
Stearoyl-CoA desaturase
SCD1 inhibitors
Desaturation index
Obesity

ABSTRACT

A new series of urea-based, 4-bicyclic heteroaryl-piperidine derivatives as potent SCD1 inhibitors is described. The structure–activity relationships focused on bicyclic heteroarenes and aminothiazole–urea portions are discussed. A trend of dose-dependent decrease in body weight gain in diet-induced obese (DIO) mice is also demonstrated.

Stearoyl-CoA desaturase-1 (SCD1) is a critical microsomal enzyme that catalyzes the conversion of saturated fatty acid-CoAs to mono-unsaturated fatty acid-CoAs at C-9 position.[1] These mono-unsaturated fatty acid-CoAs, such as palmitoleic (C16)

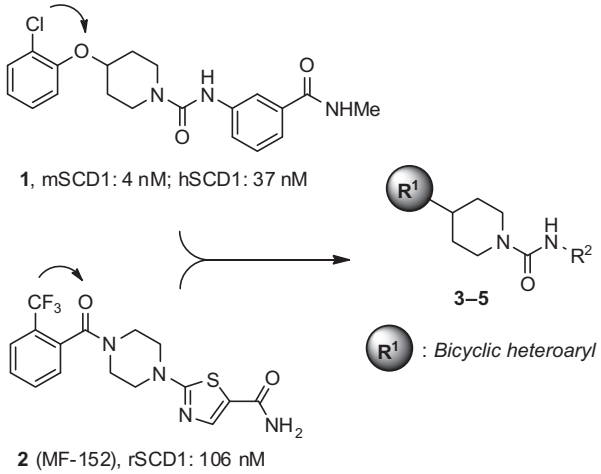
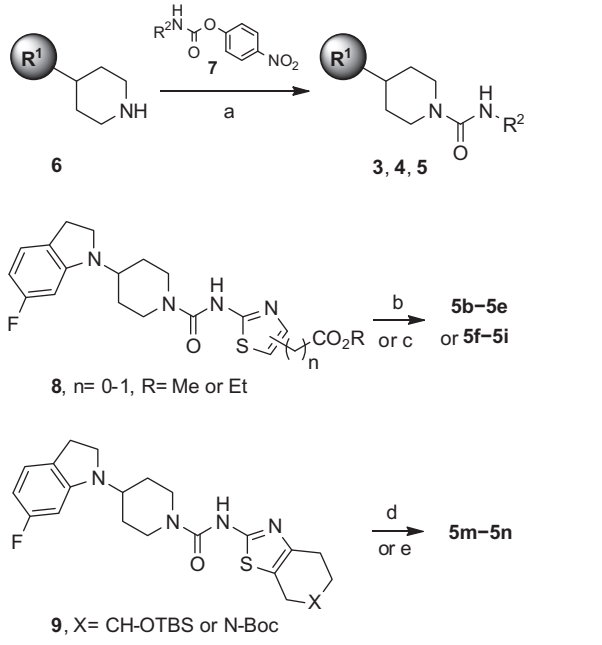


Figure 1. Representative piperidine- and piperazine-derived SCD1 inhibitors.



Scheme 1. R^1 represents bicyclic heteroaryl, such as indole, indoline, benzoisoxazole, etc. Reagents and conditions: (a) compound **7**, Et_3N, CH_2Cl_2, rt, 55–90%, or R^2NH_2, triphosgene, Et_3N, CH_2Cl_2, rt, 40–92%; (b) DIBAL-H, CH_2Cl_2, rt, **5b**–**5e** (45–78%); (c) $MeNH_{2(aq)}$ or $NH_{3(aq)}$, EtOH, 40–50 °C, **5f**–**5i** (59–97%); (d) for X = N-Boc, TFA, CH_2Cl_2, rt, **5m** (90%); (e) for X = CH-OTBS, TBAF, THF, rt, **5n** (92%).

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http://dx.doi.org/10.1016/j.bmcl.2013.09.096

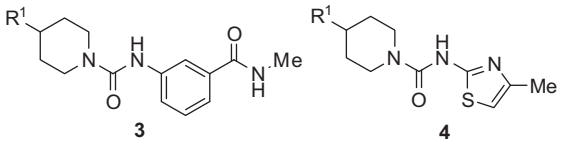
acid-CoA and oleic (C18) acid-CoA, are major building blocks in biosynthesis of lipids including phospholipids, triglycerides, cholesterol esters and wax esters.[2] Four SCD isoforms (SCD1–4) in rodents and two human genes (SCD1 and SCD5, also known as SCD2) have been identified and characterized. SCD1,[3] with ca. 85% identity across species,[4] is abundantly expressed in liver and adipose tissue and is regulated by several nutritional and hormonal factors, such as insulin, cholesterol, and poly-unsaturated fatty acids.[5] Moreover, SCD1 deficiency in mice, either naturally deficient Asebia mice[6] or laboratory-created SCD1 knockout ($SCD1^{-/-}$) mice,[7] has been shown to reduce body adiposity, increase insulin sensitivity, and impart resistance to diet-induced obesity.[8] The $SCD1^{-/-}$ mice also have lower levels of hepatic cholesterol esters and triglycerides.[9] These observations suggest inhibition of SCD1 activity may serve as a potential treatment for obesity, type-II diabetes, and other related metabolic disorders.

In recent years, piperidine and piperazine derived analogs, exemplified by **1**[10c] and **2**[10d] (MF-152), have been the major focus in the design of SCD1 inhibitors[10,11] (Fig. 1). These novel skeletons consist of a 4-phenoxy or 4-phenylcarbonyl motif have been proven to be potent inhibitors in vitro and efficacious in chronic in vivo studies. Furthermore, the *ortho*-substitution of phenyl ring, such as Cl or CF_3, is important to improving potency to low nanomolar range. In our medicinal chemistry program toward novel SCD1 inhibitors, we envisioned a ring constrained strategy by tethering the key *ortho*-substituted carbon to either the ether oxygen position or carbonyl group position (Fig. 1). The newly designed 4-bicyclic heteroaryl-piperidine templates open avenues to identify potential inhibitors by exploration of the right-hand portion of these molecules. Herein we report the initial discovery of urea-based 4-bicyclic heteroaryl-piperidine analogs (**3**–**5**) as potent and orally bioavailable SCD1 inhibitors.

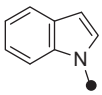
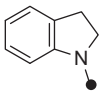
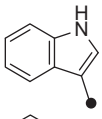
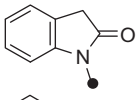
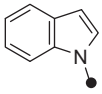
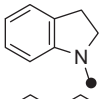
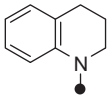
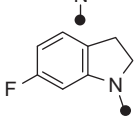
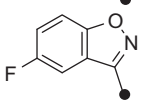
The synthesis of these urea-based analogs **3**–**5** is straightforward and shown in Scheme 1. Activated-nitrophenyl carbamate intermediate **7** was rapidly available by treating the corresponding R^2NH_2 with 4-nitrophenyl chloroformate. Treatment of **7** with 4-bicyclic heteroaryl-piperidine (**6**)[12] afforded target compounds **3**–**5** in good yield. Alternatively, compounds **3**–**5** could be obtained in a one-pot manner by activation of R^2NH_2 with triphosgene followed by addition of piperidine **6**. Elaboration of the ester group of urea intermediate **8** provided analogs **5b**–**5e** and **5f**–**5i** with an amide or hydroxyalkyl functionality. Further deprotection of **9** using TFA (X = N-Boc) or TBAF (X = CH-OTBS) gave **5m** or **5n**, respectively.

Compound activity was initially measured against rat SCD1 enzyme (rSCD1).[13] The results are compiled in Table 1 focusing on structure–activity relationships (SAR) at the bicyclic heteroaryl portion (R^1). With *N*-methyl benzamide substitution at urea portion, the 6,5-bicyclic rings, such as indole (**3a**) or indoline (**3b**), showed good potency below 200 nM. However, the reversed 3-substituted indole (**3c**) or 2-indolone (**3d**) resulted in a significant decrease in potency. Substituting the right hand portion of the urea with a 4-methylthiazole group led to a similar SAR that favored 6,5-ring system (**4b** vs **4c**). A dramatic improvement in potency to low nanomolar range was achieved by introducing a fluoro-substitution at the 6-position of the indoline (**4d**, 26 nM). Additionally, a 5-fluorobenzoisoxazole substituent (**4e**) provided good inhibitory activity at 140 nM. The cellular activity against human epithelial carcinoma A431 cells (using ^{13}C-palmitic acid and LC–MS method)[14] was added for further evaluation. In A431 assay, compounds **4d** and **4e** also exhibited good inhibitory activity at 21 and 54 nM, respectively. These results clearly illustrate the bicyclic

Table 1
SAR of bicyclic heteroaryl substituent R^1

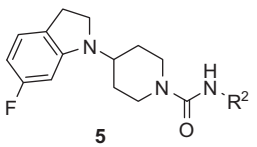


3 **4**

Compds	R^1	rSCD1 IC_{50}[a] (nM)
3a		170
3b		151
3c		15%[b]
3d		7%[b]
4a		538
4b		153
4c		491
4d		26
4e		140

[a] Single experiment or means of at least two runs.
[b] Inhibition percentage at 1 μM.

Table 2
SAR of urea portion R^2



5

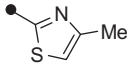
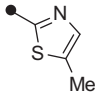
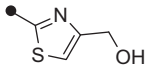
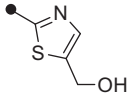
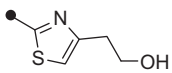
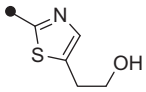
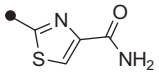
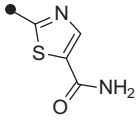
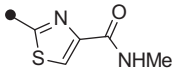
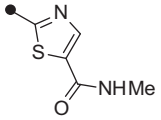
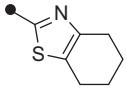
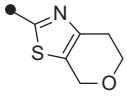
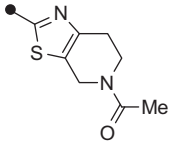
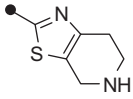
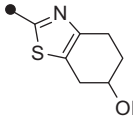
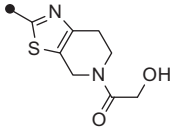
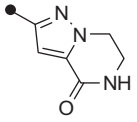
Compds	R^2	rSCD1 IC_{50}[a] (nM)	A431[b] (nM)
4d		26	21
5a		82	108
5b		52	42
5c		88	154
5d		20	18

Table 2 (*continued*)

Compds	R^2	rSCD1 IC_{50}[a] (nM)	A431[b] (nM)
5e		8	6
5f		682	—
5g		22	49
5h		20%[c]	—
5i		93	206
5j		347	76
5k		204	17
5l		46%[c]	—
5m		29	16
5n		14	1
5o		96	113
5p		18	30

[a] Single experiment or means of at least two runs.
[b] Using ^{13}C-palmitic acid and LC–MS method, see Ref. 14.
[c] Inhibition percentage at 1 μM.

heteroaryl-substituted piperidine is a suitable alternative to the original phenoxy-piperidine design.

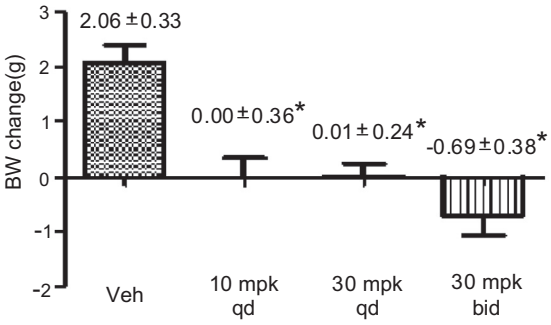


Figure 2. Dose-dependent effect of **4e** on BW change in DIO C57BL/6 mice (mean ± se, $^*P$ <0.05 vs vehicle-treated group, results were summarized from three experiments).

With suitable bicyclic heteroaryl moieties identified, we turned our attention to explore the SAR around the urea portion (R^2) by focusing the substituent effects on the thiazole ring. In general, a variety of substitutions such as methyl (**4d** and **5a**) or hydroxyalkyl (**5b**–**5e**) at the 4- or 5-position of the thiazole were well tolerated without significant loss of potency in both rSCD1 and A431 assay (Table 2). Specifically, the 4-methyl- and 4-hydroxymethyl-substituted thiazoles were about two to three fold more potent than the 5-substituted thiazole analogs (**4d** and **5b** vs **5a** and **5c**). The elongated hydroxyalkyl analogs (**5d**–**5e**) appeared to have slightly better inhibitory activity. However, attaching a primary amide at the 4-position (**5f**, 682 nM) was particularly disfavored that caused a 31-fold loss of potency as compared to the 5-substituted primary amide analog **5g** (22 nM). A similar trend that favored 5-substitution on the thiazole ring was observed in the methyl amide-substituted analogs **5h**–**5i**, though they were less potent than the corresponding primary amides **5f**–**5g**.

Based on the encouraging results observed with compounds **4d** and **5a**–**5e** that tolerated well for substitution at both 4- and 5-position, a series of di-substituted bicyclic thiazoles were next synthesized and evaluated (Table 2). Compounds **5j**–**5l** achieved modest potency (204 nM to ca. 1 μM) presumably due to the loss of hydrogen bonding donor capability. In fact, adding NH (**5m**) and OH (**5n**) groups gained the potency back to 14–29 nM with improved inhibitory activity against A431 cell (1–16 nM). A comparison between analogs **5l** (ca. 1 μM) and **5o** (96 nM) clearly demonstrated again the critical H-bonding interaction for SCD1 enzyme potency. Finally, a bicyclic pyrazole amide (**5p**) was found to be very potent against both rSCD1 enzyme and A431 cell (18 nM and 30 nM, respectively).

Among the compounds tested in vitro, analog **4e** was first selected for further evaluation. The pharmacokinetic (PK) profiles in mouse and rat are summarized in Table 3. The results reveal that **4e** has a low clearance level (CL: 0.5 mL/min/kg), high plasma concentration, and good bioavailability in rat (*F*%: 36.9%). An excellent exposure of **4e** in diet-induced obese (DIO) C57BL/6 mice (C_{max}: 14.9 μg/mL; $AUC_{24\ h}$: 169.3 h • μg/mL) was also obtained. With the favorable PK profile, **4e** was further evaluated for in vivo efficacy in DIO C57BL/6 mice at 10 mg/kg q.d. (once-daily), 30 mg/kg

Table 3
The PK profiles of **4e** in DIO mice and rats

Species	Administration[a]	$t_{1/2}$ (h)	C_{max}[b] (μg/mL)	$AUC_{24\ h}$ (h •μg/mL)	V_{ss} (L/kg)	CL (mL/min/kg)	*F*%
Rat	iv (2 mg/kg)	3.8	9.2	49.8	0.19	0.5	
Rat	po (10 mg/kg)	6.3	8.8	118.5			36.9
DIO mice	po (30 mg/kg)	3.1	14.9	169.3			—

[a] $n = 4$ for rat; $n = 3$ for DIO mice.
[b] $C_{max} = C_o$ ($t = 0$) for iv administration.

Table 4
Plasma liver enzyme levels after 10 days treatment in DIO mice

Treatment	ALT (U/L)	AST (U/L)
Vehicle	58 ± 5	99 ± 9
4e, 10 mg/kg q.d.	57 ± 7	89 ± 7
4e, 30 mg/kg q.d.	68 ± 12	93 ± 7
4e, 30 mg/kg b.i.d.	41 ± 3	102 ± 17

q.d., and 30 mg/kg b.i.d. (twice-daily) for 10 days (vehicle, 0.5% hypromellose).[15] As shown in Figure 2, **4e** displayed a trend of dose-dependent decrease in body weight (BW) gain in DIO C57BL/6 mice. In addition, plasma liver enzyme levels (ALT and AST) measured at the end of the study were not elevated in drug treated groups, indicating no potential overt liver toxicity (Table 4).

In summary, a new series of piperidine-urea analogs as potent SCD1 inhibitors have been discovered. Structurally, the bicyclic heteroaryl, such as 6-fluoroindoline, substituted piperidine demonstrated a viable alternative to the original phenoxy-piperidine motif. The selected analog **4e** with a favorable PK profile demonstrated a trend of dose-dependent in vivo efficacy in DIO C57BL/6 mice. Further exploration using the bicyclic heteroaryl scaffold to identify analogs with improved potency and efficacy will be reported in due course.

Acknowledgment

The authors thank the ADME team for bioanalysis support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.096.

References and notes

1. (a) Dobrzyn, A.; Ntambi, J. M. *Obes. Rev.* **2005**, *6*, 169; (b) Ntambi, J. M.; Miyazaki, M. *Curr. Opin. Lipidol.* **2003**, *14*, 255.
2. Miyazaki, M.; Gomez, F. E.; Ntambi, J. M. *J. Lipid Res.* **2002**, *43*, 2146.
3. (a) Miyazaki, M.; Jacobsen, M. J.; Man, W. C.; Cohen, P.; Asilmaz, E.; Friedman, J. M.; Ntambi, J. M. *J. Biol. Chem.* **2003**, *278*, 33904; (b) Wang, J.; Yu, L.; Schmidt, R. E.; Su, C.; Huang, X.; Gould, K.; Cao, G. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 735.
4. Zhang, L.; Ge, L.; Parimoo, S.; Stenn, K.; Prouty, S. M. *Biochem. J.* **1999**, *340*, 255.
5. (a) Waters, K. M.; Ntambi, J. M. *J. Biol. Chem.* **1994**, *269*, 27773; (b) Ntambi, J. M. *J. Lipid Res.* **1999**, *40*, 1549.
6. Zheng, Y.; Eilersten, K. J.; Ge, L.; Zhang, L.; Sundberg, J. P.; Prouty, S. M.; Stenn, K. S.; Parimoo, S. *Nat. Genet.* **1999**, *23*, 268.
7. Ntambi, J. M.; Miyazaki, M.; Stoehr, J. P.; Lan, H.; Kendziorski, C. M.; Yandell, B. S.; Song, Y.; Cohen, P.; Friedman, J. M.; Attie, A. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11482.
8. Ntambi, J. M.; Miyazaki, M. *Prog. Lipid Res.* **2004**, *43*, 91.
9. Miyazaki, M.; Kim, Y. C.; Gray-Keller, M. P.; Attie, A. D.; Ntambi, J. M. *J. Biol. Chem.* **2000**, *275*, 30132.
10. Representative SCD1 inhibitors, see: (a) Liu, G.; Lynch, J. K.; Freeman, J.; Liu, B.; Xin, Z.; Zhao, H.; Serby, M. D.; Kym, P. R.; Suhar, T. S.; Smith, H. T.; Cao, N.; Yang, R.; Janis, R. S.; Krauser, J. A.; Cepa, S. P.; Beno, D. W. A.; Sham, H. L.; Collins, C. A.; Surowy, T. K.; Camp, H. S. *J. Med. Chem.* **2007**, *50*, 3086; (b) Zhao, H.; Serby, M. D.; Smith, H. T.; Cao, N.; Suhar, T. S.; Surowy, T. K.; Camp, H. S.; Collins, C. A.; Sham, H. L.; Liu, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3388; (c) Xin, Z.; Zhao, H.; Serby, M. D.; Liu, B.; Liu, M.; Szczepankiewicz, B. G.; Nelson, L. T.; Smith, H. T.; Suhar, T. S.; Janis, R. S.; Cao, N.; Camp, H. S.; Collins, C. A.; Sham, H. L.; Surowy, T. K.; Liu, G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4298; (d) Li, C. S.; Belair, L.; Guay, J.; Murgasva, R.; Sturkenboom, W.; Ramtohul, Y. K.; Zhang, L.; Huang, Z. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5214; (e) Léger, S.; Black, W. C.; Deschenes, D.; Falgueyret, J.-P.; Gagnon, M.; Guiral, S.; Huang, Z.; Guay, J.; Leblanc, Y.; Li, C.-S.; Masse, F.; Oballa, R.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 499; (f) Ramtohul, Y. K.; Black, C.; Chan, C.-C.; Crane, S.; Guay, J.; Gurial, S.; Huang, Z.; Oballa, R.; Xu, L.-J.; Zhang, L.; Li, C.-S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1593; (g) Isabel, E.; Powell, D. A.; Black, W. C.; Chan, C.-C.; Crane, S.; Gordon, R.; Guay, J.; Guiral, S.; Huang, Z.; Robichaud, J.; Skorey, K.; Tawa, P.; Xu, L.; Zhang, L.; Oballa, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 479; For human SCD1-specific SCD inhibitors, see: (h) Powell, D. A.; Ramtohul, Y. Lebrun, M.-E.; Oballa, R.; Bhat, S.; Falgueyret, J.-P.; Guiral, S.; Huang, Z.; Skorey, K.; Tawa, P.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6366.
11. For liver-targeted SCD1 inhibitors, see: (a) Oballa, R. M.; Belair, L.; Black, W. C.; Bleasby, K.; Chan, C. C.; Desroches, C.; Du, X.; Gordon, R.; Guay, J.; Guiral, S.; Hafey, M. J.; Hamelin, E.; Huang, Z.; Kennedy, B.; Lachance, N.; Landry, F.; Li, C. S.; Mancini, J.; Normandin, D.; Pocai, A.; Powell, D. A.; Ramtohul, Y. K.; Skorey, K.; Sorensen, D.; Sturkenboom, W.; Styhler, A.; Waddleton, D. M.; Wang, H.; Wong, S.; Xu, L.; Zhang, L. *J. Med. Chem.* **2011**, *54*, 5082; (b) Koltun, D. O.; Zilbershtein, T. M.; Migulin, V. A.; Vasilevich, N. I.; Parkhill, E. Q.; Glushkov, A. I.; McGregor, M. J.; Brunn, S. A.; Chu, N.; Hao, J.; Mollova, N.; Leung, K.; Chisholm, J. W.; Zablocki, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4070; (c) Koltun, D. O.; Vasilevich, N. I.; Parkhill, E. Q.; Glushkov, A. I.; Zilbershtein, T. M.; Mayboroda, E. I.; Boze, M. A.; Cole, A. G.; Henderson, I.; Zautke, N. A.; Brunn, S. A.; Chu, N.; Hao, J.; Mollova, N.; Leung, K.; Chisholm, J. W.; Zablocki, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3050; (d) Leclerc, J. P.; Falgueyret, J.-P.; Girardin, M.; Guay, J.; Guiral, S.; Huang, Z.; Li, C. S.; Oballa, R.; Ramtohul, Y. K.; Skorey, K.; Tawa, P.; Wang, H.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6505; (e) Powell, D. A.; Black, W. C.; Bleasby, K.; Chan, C.-C.; Deschenes, D.; Gagnon, M.; Gordon, R.; Guay, J.; Guiral, S.; Hafey, M. J.; Huang, Z.; Isabel, E.; Leblanc, Y.; Styhler, A.; Xu, L.-J.; Zhang, L.; Oballa, R. M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7281; (f) Ramtohul, Y. K.; Powell, D.; Leclerc, J.-P.; Leger, S.; Oballa, R.; Black, C.; Isabel, E.; Li, C. S.; Crane, S.; Robichaud, J.; Guay, J.; Guiral, S.; Zhang, L.; Huang, Z. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5692; (g) Lachance, N.; Guiral, S.; Huang, Z.; Leclerc, J.-P.; Li, C. S.; Oballa, R. M.; Ramtohul, Y. K.; Wang, H.; Wu, J.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 623; (h) Lachance, N.; Gareau, Y.; Guiral, S.; Huang, Z.; Isabel, E.; Leclerc, J.-P.; Léger, S.; Martins, E.; Nadeau, C.; Oballa, R. M.; Ouellet, S. G.; Powell, D. A.; Ramtohul, Y. K.; Tranmer, G. K.; Trinh, T.; Wang, H.; Zhang, L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 980; (i) Deng, Y.; Yang, Z.; Shipps, G. W., Jr.; Lo, S.-M.; West, R.; Hwa, J.; Zheng, S.; Farley, C.; Lachowicz, J.; Heek, M. V.; Bass, A. S.; Sinha, D. P.; Mahon, C. R.; Cartwright, M. E. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 791.
12. Compound **6** are either commercially available or can be prepared according to literature procedures. Representative synthetic procedures for **3**–**5** are available in Supplementary data.
13. *Rat liver microsome preparation and SCD1 enzymatic assay:* Sprague Dawley rats from Charles River (200–225 g) underwent 2-cycle of fast-re-feeding (24-h fast followed by 24-h feeding with fat-free high carbohydrate diet, Research Diet, D00042802). Liver were removed and homogenized 1:4 (w/v) with pre-chilled homogenizing buffer (10 mM Tris–HCl, 0.25 M Sucrose, 1 mM EDTA, pH 7.4, supplemented with protease inhibitor cocktail). The homogenate was first spun at 12,000*g* for 15 min at 4 °C, followed by re-centrifugation of the supernatant at 100,000*g* for 60 min at 4 °C. The pellet was re-suspended in 2 mL pre-chilled 0.2 mM potassium phosphate buffer, pH 7.2, 10 mM EDTA at concentration of 20 mg/mL. The RLM preparation was stored at −80 °C. SCD1 enzymatic assay was done in a volume of 50 μL with 10 μg RLM in 96-well polypropylene plate (enzyme reaction buffer contains 0.1 M K-Phosphate Buffer, 10 mM ATP, 6 mM $MgCl_2$,1 mM CoA, 1 mM β-NADH, 1.6 mM L-glutathione, 20 μM Stearoyl-CoA). Stearoyl-[9,10-^{3}H]-CoA (ARC-0390, 1 mCi/mL, 60 Ci/mmol) was added at final concentration of 2 μCi/mL. [^3H_2O] generated was used as indicator of SCD1 activity.
14. *SCD1 assay in A431 cells:* Human epithelial carcinoma A431 cells were seeded at 100,000 cells/well in complete growth medium (DMEM, 4.5 g/L glucose, 10% FBS) in the presence of human insulin (Hannas Pharmaceutical Supply Co. Inc., Humanlin 100 U/mL, 4 mg/mL, final 0.05 mg/mL) in 96-well tissue culture plate. 24 h later, cells were washed extensively with Assay Buffer (DMEM, 4.5 g/L glucose, supplemented with 0.05 mg/mL Human Insulin and 0.1% Fatty Acid Free BSA). 100 μL of Assay Buffer was then added to each well including 20 μM U-^{13}C16 palmitic acid followed by addition of 5 μL of vehicle or Scd1 inhibitors. Cells were then returned to tissue culture incubator for 4 h. At the end of incubation, cells were washed three times with 200 μL/well Hanks buffer (HBSS) and then stored at −20 °C until extraction. For fatty acid extraction, after thawing out the plates, 40 μL of 1 N KOH (containing 5 μM of internal standard 7,7,8,8-D_4 palmitic acid) was added to each well and cell plates were then incubated at 37 °C for 30 min. Subsequently, 30 μL of cell lysate was transferred to 96-well plate and incubate at 85 °C for 30 min. Heated cell lysate was quickly spun at room temperature and 5 μL formic acid was added to neutralize the solution. 100 μL of Acetonitrile was then added to extract fatty acids followed by centrifugation at 3000 rpm for 10 min. Transfer 75 μL to new glass vial in 96-well cluster plate and inject 5 μL for LC/MS analysis for detection of ^{13}C-palmitic acid and ^{13}C-palmitoleic acid. The ratio of ^{13}C-palmitoleic acid to ^{13}C-palmitic acid was determined as index of SCD1 activity.
15. DIO C57BL/6 mice were orally dosed with either vehicle (0.5% hypromellose) or compound **4e** at 8 AM and 6 PM daily at 10 mL/kg. Dosing was performed as following: vehicle-treated group (8 AM and 6 PM with vehicle (0.5% hypromellose)); 10 mg/kg-treated q.d. group (8 AM with vehicle (0.5% hypromellose) and 6 PM with compound **4e** (dosing solution: 1 mg/mL)); 30 mg/kg-treated q.d. group (8 AM with vehicle (0.5% hypromellose) and 6 PM with compound **4e** (dosing solution: 3 mg/mL)); 30 mg/kg-treated b.i.d. group (8 AM and 6 PM with compound **4e** (dosing solution: 3 mg/mL)).