ARTICLE TYPE

Small-molecule Inhibitors of SREBP Activation – Potential for New Treatment of Metabolic Disorders

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Sterol regulatory element-binding proteins (SREBPs) are transcriptional factors that control lipid and cholesterol metabolism. Activation of SREBPs in response to a decrease in the cellular sterols results in acceleration of the synthesis of fatty acids, triglycerides, and cholesterol. Aberrant SREBP activity has been linked to metabolic disease states, such as obesity, fatty liver, insulin resistance, hyperlipidemia, and
10 atherosclerosis. Thus, inhibition of SREBP activation is a potential therapeutic approach to treating metabolic disorders. This review focuses on direct or indirect small-molecule inhibitors of SREBP activation.

Introduction

Sterol regulatory element-binding proteins (SREBPs) are one of
15 the most important families of transcription factors involved in lipid homeostasis. SREBPs control cellular metabolism in all tissues by regulating the expression of the genes related to biosynthesis and uptake of fatty acids, triglycerides, cholesterol, and phospholipids.[1,2] The central roles of SREBPs in lipid
20 metabolism link them tightly to metabolic syndromes. For example, high insulin levels, induced by high calorie diets or obesity, hyper-activate SREBPs, causing triglyceride accumulation and inducing fatty liver diseases.[3,4] Hyper-activation of SREBPs also increases cholesterol levels and
25 suppresses insulin receptor substrate (IRS)-2, leading to hyperlipidemia, arteriosclerosis, and insulin resistance.[5,6] Furthermore, activation of SREBPs is often correlated with the growth of cancers and the ability of hepatitis virus to cause fatty liver diseases.[7,8] The involvement of SREBP activation in
30 multiple diseases has made these transcription factors attractive pharmaceutical targets.[9] This review summarizes recent studies of small molecules that directly or indirectly suppress the cellular functions of SREBPs.

Regulation of SREBP pathway

35 ### The SREBP family

In mammals, the SREBP family consists of three isoforms, SREBP-1a, -1c, and -2, which play overlapping, but distinct, roles.[1] SREBP-1a, which usually exists in proliferating cells, promotes the synthesis of fatty acids and cholesterol. In contrast,
40 SREBP-1c is expressed mainly in the liver, and primarily stimulates fatty acid and triglyceride synthesis by inducing transcription of the genes necessary to convert acetyl CoA into fatty acids and triglycerides, e.g., fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD-1), and acetyl-CoA carboxylase

45 (ACC).[10] SREBP-2 is also expressed in the liver, and primarily activates genes involved in cholesterol synthesis and uptake, including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and low-density lipoprotein receptor (LDLR).[2]

The synthesis of SREBP

50 SREBPs are basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors. SREBP-1a and -1c are encoded by a single gene and generated by an alternative splicing of exon 1.[11] The expression of SREBP-1c gene is self-controlled, and is stimulated by both insulin and oxysterols through activation of the nuclear
55 hormone receptor, liver X receptor (LXR).[12-14] When stimulated by insulin, LXR heterodimerizes with retinoid X receptor (RXR). The heterodimer interacts with LXR-responsive elements located in the SREBP-1c promoter, stimulating transcription of the SREBP-1c gene.[13] SREBP-2 is encoded by another gene, whose
60 splicing variants have not been discovered.[11]

The proteolytic activation of SREBP

Unlike other transcription factors in the bHLH-LZ family, SREBPs are newly synthesized on the endoplasmic reticulum (ER) membrane as inactive precursors (Fig. 1).[14] On the ER, The
65 C-terminal domain of SREBPs binds to SREBP cleavage-activating protein (SCAP). The SREBP-SCAP complex is transported from the ER to the Golgi apparatus via COPII vesicles (Fig. 1A). In the Golgi apparatus, SREBPs are sequentially cleaved by two proteases: site-1 protease (S1P), a
70 membrane-bound serine protease also known as SKI-1; and site-2 protease (S2P), a Zn^{2+} metalloprotease, and the N-terminal domains of SREBPs, which are transcriptionally active fragments of SREBPs, are generated.

SCAP is an indispensable protein for SREBP activation, both
75 *in vitro* and *in vivo*. In addition to acting as an escort protein for SREBPs, physical association with SCAP seems to stabilize SREBPs. In SCAP-deficient CHO cells, both the precursor and the nuclear form of SREBPs disappear.[15] Without SCAP,
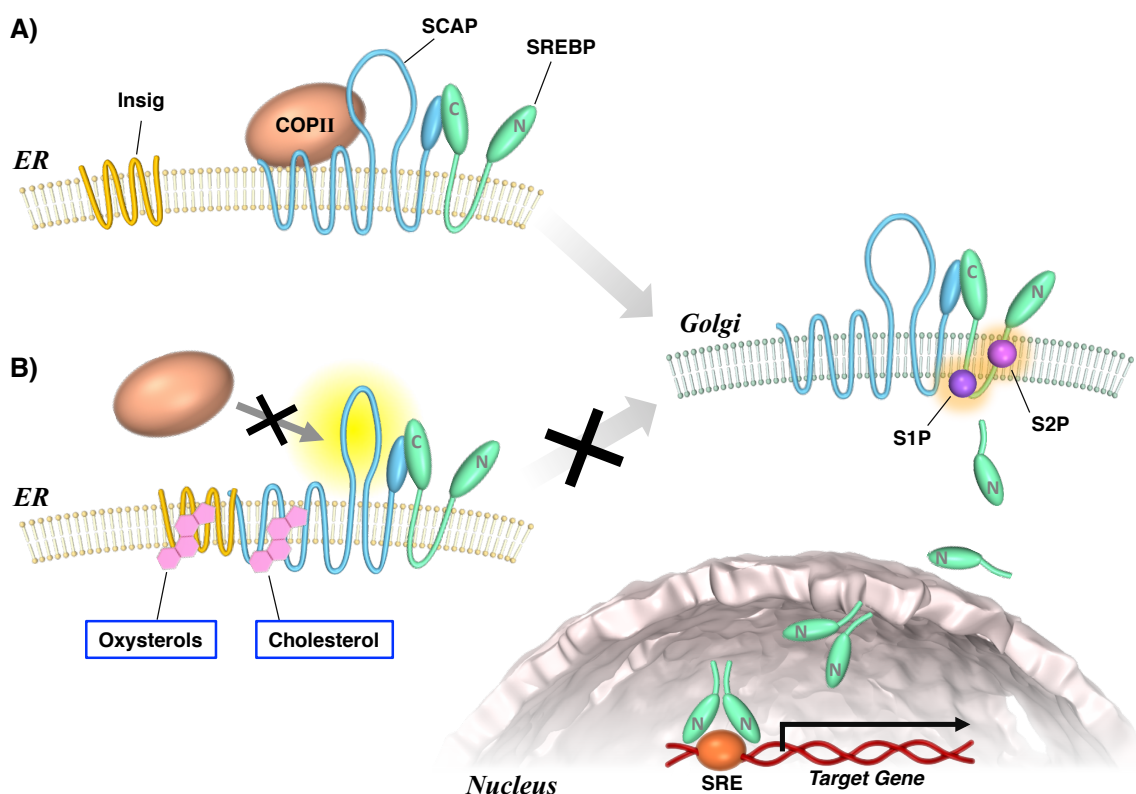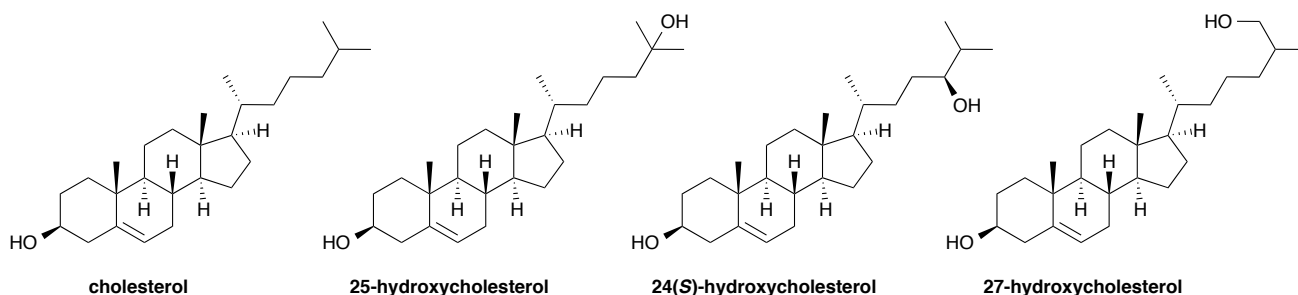
Fig. 1 The pathway of SREBP activation.

Fig. 2 Chemical structures of cholesterol and oxysterols.

SREBPs in the ER are unstable and cannot translocate to the Golgi apparatus. In hepatic SCAP-deficient mice, basal rates of cholesterol and fatty acid synthesis in the liver were reduced by 80%, due to decreases in mRNAs that encode multiple biosynthetic enzymes.[16]

The proteolytic activation of SREBP is tightly regulated by a negative feedback loop with cholesterol, in which the ER-resident proteins, Insig-1 and Insig-2, play an important role.[2,17] When cellular sterol levels are high, cholesterol (Fig. 2) binds to SCAP, and 25-hydroxycholesterol (Fig. 2), a metabolite of cholesterol, binds to the Insig proteins.[18,19] The binding of cholesterol to SCAP, or of 25-hydroxycholesterol to Insig proteins, stimulates formation of an SREBP-SCAP-Insig complex (Fig. 1B). The binding of Insig protein to SCAP blocks the interaction of SCAP with COPII, so that the SREBP-SCAP complex is retained in the ER.[20] Thus, excess sterols inhibit translocation of the SREBP-SCAP complex to the Golgi apparatus, and activation of SREBPs is blocked.

In addition to 25-hydroxycholesterol, other oxysterols that are generated under excess cholesterol conditions, such as 24(S)-hydroxycholesterol and 27-hydroxycholesterol (Fig. 2), block the processing of SREBPs, especially SREBP-2, by the same mechanism as that of 25-hydroxycholesterol.[19]

The regulation of the nuclear form of SREBP

The cleaved SREBP N-terminal domains (the nuclear form of SREBP) are released from the membrane of the Golgi apparatus and transferred to the nucleus, where they bind to the promoter
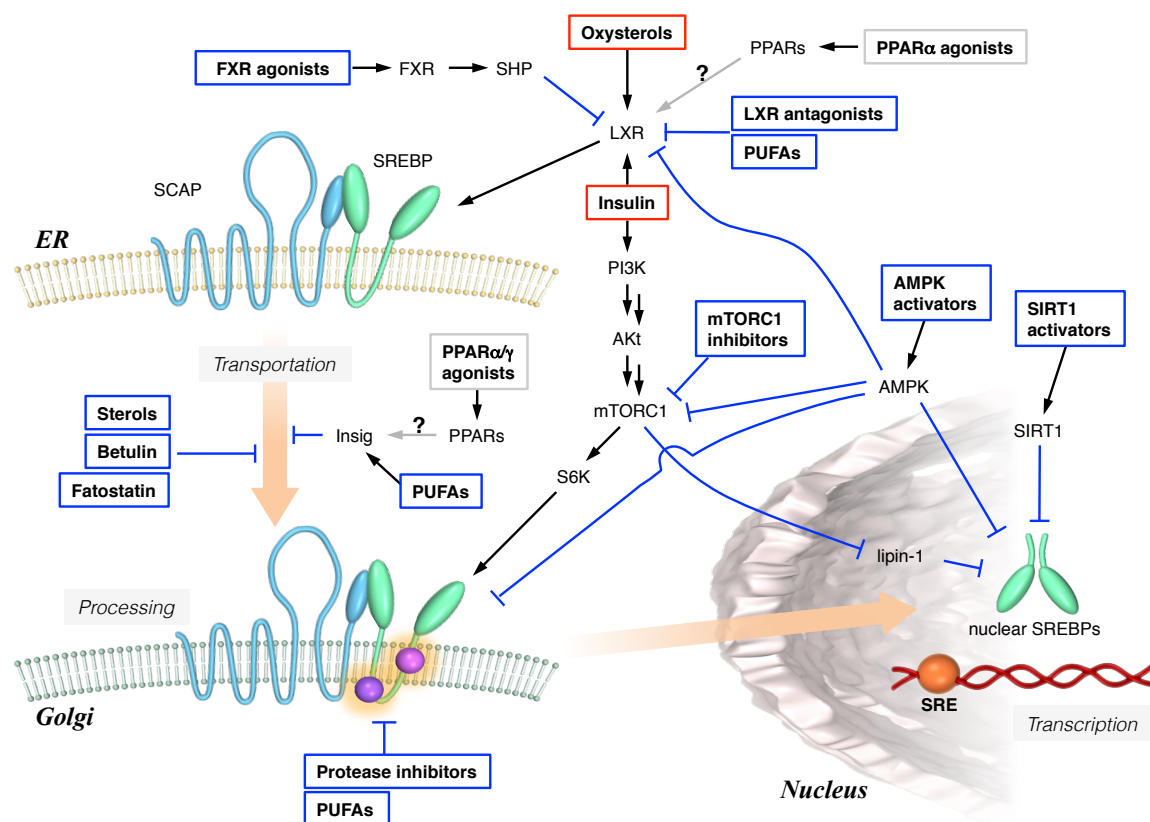
Fig. 3 Multiple regulation of SREBP activation by small molecules.

motifs known as sterol regulatory elements (SREs) as dimers.[11] They promote the expression of all genes encoding the enzymes required to convert acetyl CoA into fatty acids and cholesterol (Fig. 1).[2]

The translocation and stability of the nuclear form of SREBP are controlled by a number of enzymes: a phosphatase, a kinase, and a deacetylase. Insulin, the major activator of SREBP-1c in the liver, promotes the activation of SREBP-1c via multiple pathways.[21,22] One pathway is via the activation of Akt (also known as protein kinase B) and mammalian target of rapamycin (mTOR) kinase. Insulin activates phosphatidylinositol 3-kinase (PI3K) by inducing phosphorylation of IRS-1/2, promoting the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 binds to and activates Akt, which directly phosphorylates tuberous sclerosis complex 1/2 (TSC1/2) and PRAS40, leading to the activation of mTOR complex 1 (mTORC1). Activated mTORC1 phosphorylates lipin-1, a phosphatidic acid phosphatase, and inhibits its translocation to the nucleus. Dephosphorylated, nuclear, catalytically active lipin-1 promotes nuclear remodeling and decreases the nuclear abundance of SREBP-1c.[23] Therefore, mTORC1 activation would result in increased nuclear SREBP-1c (Fig. 3). In addition to the lipin-1-mediated regulation of SREBPs, mTORC1 activation has been reported to stimulate proteolytic SREBP-1c processing via activation of p70 S6-kinase (S6K), although the detailed mechanism remains unknown (Fig. 3).[24]

Another enzyme regulating the nuclear SREBP is adenosine 5'-monophosphate-activated protein kinase (AMPK), an important enzyme that monitors cellular energy status.[25] AMPK, activated in response to cellular metabolic stresses, phosphorylates and inactivates ACC and HMGCR, key enzymes involved in regulating *de novo* biosynthesis of fatty acids and cholesterol.[26] Interestingly, the latter two enzymes are encoded by the target genes of SREBPs, and the activation of AMPK and SREBP-1c in liver is inversely correlated.[27] Recent studies showed that AMPK directly controls SREBPs: AMPK binds directly to and phosphorylates SREBP-1c or -2, blocking both the cleavage processing and the nuclear translocation of SREBPs (Fig. 3).[28] AMPK also inhibits PI3K/Akt/mTOR signaling, which as mentioned earlier, increases the amounts of nuclear SREBP-1c (Fig. 3).[29] In addition, AMPK directly inhibits ligand-induced LXR activity on the SREBP-1c gene promoter (Fig. 3), although the detailed mechanism remains unknown.[30]

SIRT1 is an NAD^{+}-dependent deacetylase involved in both glucose and lipid metabolism.[31] Recent studies have shown that SREBPs are direct targets of SIRT1 (Fig. 3).[32,33] SIRT1 increases the amounts of deacetylated SREBPs, which are susceptible to ubiquitin-mediated degradation, decreasing the nuclear SREBPs levels, and, consequently, the expression levels of SREBP-responsive lipogenic genes.

Inhibitors of the synthesis of SREBP
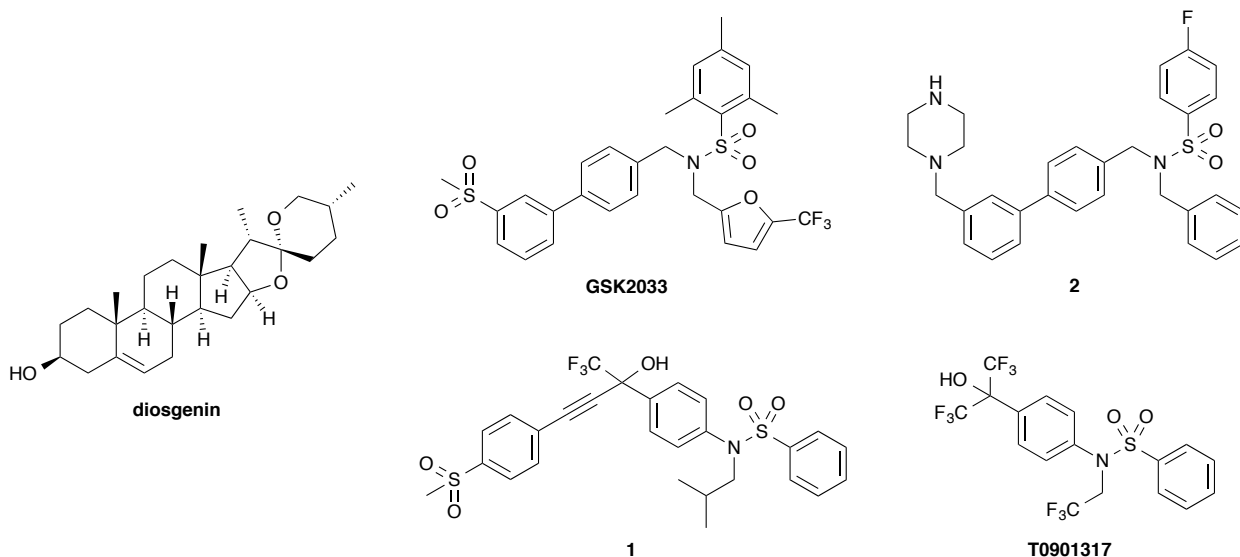
LXR antagonists

Fig. 4 Chemical structures of LXR ligands.

Diosgenin (Fig. 4), an aglycone of fenugreek that functions as an LXRα antagonist, down-regulates expression of SREBP-1c and
⁵ its downstream genes, including FAS, ACC, and SCD-1, and suppresses triglyceride accumulation in HepG2 cells.[34] Administration of fenugreek to KK-Ay mice reduced the expression of hepatic SREBP-1c and decreased triglyceride levels in the blood and liver. Similarly, the synthetic LXR antagonists,
¹⁰ GSK2033 and molecule **1** (Fig. 4), reduced the expression of SREBP-1c and triglyceride levels in cultured cells.[35,36] GSK2033 is a synthetic derivative of a hit molecule (**2**, Fig. 4) of a high-throughput screening (HTS),[35] and molecule **1** is an antagonist designed from the structure of the known LXR agonist,
¹⁵ T0901317 (Fig. 4).[36]

PUFAs

Polyunsaturated fatty acids (PUFAs) in fish oil have long been known to inhibit synthesis of fatty acids and triglycerides, and to lower plasma triglyceride levels.[37] In fact, eicosapentaenoate has
²⁰ been used clinically as a drug for hyperlipidemia. The lipid-lowering effects of PUFAs are mediated, in part, by down-regulation of the lipogenic function of SREBP-1c in the liver.

PUFAs suppress hepatic SREBP-1c transcriptionally (Fig. 3). Arachidonic acid, eicosapentaenoic acid, and docosahexaenoic
²⁵ acid (Fig. 5) down-regulate the expression of hepatic SREBP-1c by acting as antagonists of LXRα, thereby decreasing the expression levels of lipogenic enzymes.[38-40] Oral administration of fish oil into animal models reduced the mRNA expression of SREBP-1c, as well as triglyceride levels in the liver.[41]
³⁰ In addition to their effects on SREBP-1c, PUFAs are known to be ligands for peroxysome proliferator-activated receptor (PPAR) alpha,[42,43] a nuclear receptor that controls lipid and glucose metabolism. PUFAs also inhibit the hepatic maturation of carbohydrate-responsive element-binding protein (ChREBP),[44] a
³⁵ glucose-activated transcription factor involved in lipogenesis in the liver. Thus, inhibition of SREBP activation is probably only one of multiple mechanisms and targets by which PUFAs exert beneficial effects on metabolic diseases.
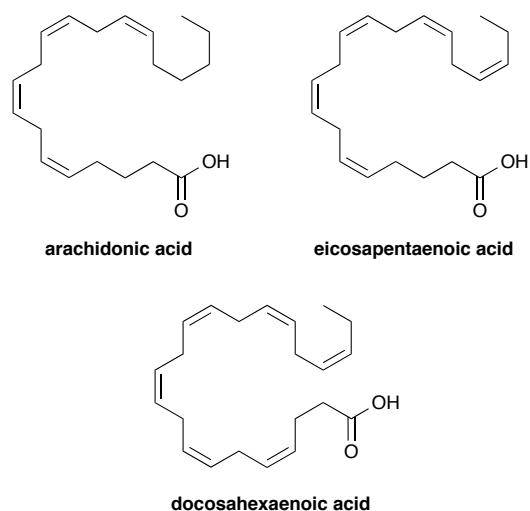

arachidonic acid   **eicosapentaenoic acid**

docosahexaenoic acid

⁴⁰ **Fig. 5** Chemical structures of PUFAs.

PPARα agonists

Recent investigation revealed that ligands of the PPAR family of nuclear receptors modulate SREBP activity (Fig. 3). Fibrates, anti-hyperlipidemia PPARα agonists, down-regulate triglyceride
⁴⁵ levels by promoting β-oxidation of fatty acids, and reduce very low-density lipoprotein (VLDL) levels by increasing adipose differentiation-related proteins.[45]

PPARα agonists appear to modulate the activity of SREBPs through LXR. Wy14,643 (Fig. 6) is a PPARα agonist that
⁵⁰ prevents LXR ligands from inducing SREBP-1c gene expression in primary rat hepatocytes and in mouse liver.[46] The activation of PPARα somehow blocks the ability of the LXR/RXR heterodimer to stimulate promoter activity of SREBP-1c.

In contrast, results of another research on PPARα agonists
⁵⁵ reveal a discrepancy. A report claimed that Wy14,643 and GW7647 (another PPARα agonist, Fig. 6) enhanced activity of the SREBP-1c gene promoter through LXR elements, thereby increasing endogenous SREBP-1c expression in cultured human
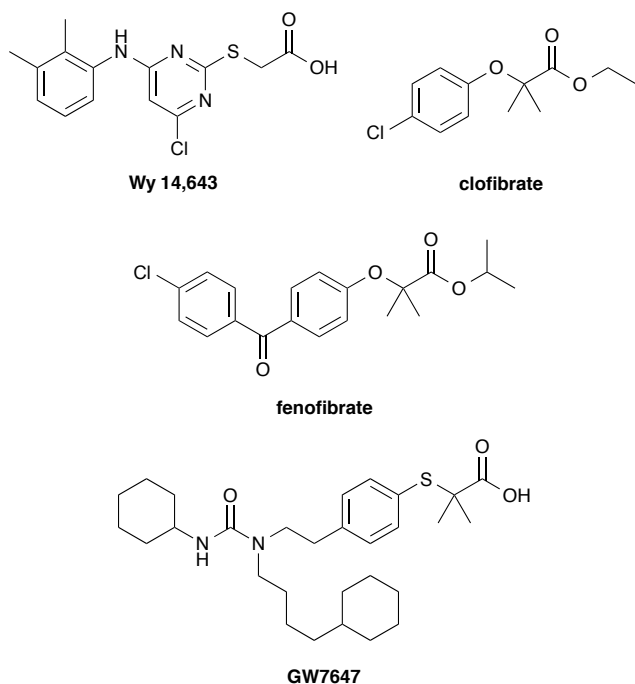
Fig. 6 Chemical structures of PPARα agonists.

hepatocytes.[47] Inconsistent results might be due to differences in the species and nutritional status of cells used in the experiments.[47]

FXR agonists

Farnesoid X receptor (FXR) is a nuclear receptor whose endogenous agonist is bile acid, a steroid acid composed primarily of cholic acid derivatives that are biosynthesized via oxidation of cholesterol.[48] The role of FXR in the metabolism of cholesterol, triglyceride, and glucose links it to SREBPs. Activation of FXR by FXR agonists (Fig. 7), such as cholic acid, chenodeoxycholic acid, or the synthetic agonist, GW4064, impairs the expression of SREBP-1c that is induced by high-fat diets or LXR ligands, ultimately decreasing the hepatic and renal levels of triglyceride.[49,50] The decrease in SERBP-1c expression seems to be mediated by the binding of small heterodimer partner (SHP) to LXRα or other transcription factors that stimulate SREBP-1c expression (Fig. 3). This mechanism could account for the observation that administration of bile acid decreases hepatic secretion of VLDL.[50]

Inhibitors of the proteolytic activation of SREBP

Sterols inhibit SREBP activation. However, sterols themselves might not be suitable as selective pharmaceuticals for treating metabolic disorders, considering their effects on SREBP-1c along with their other physiological functions.[51] Especially, oxysterols are endogenous ligands of LXRs,[52-54] and stimulate the transcription of SREBP-1c via activation of LXRα in the liver (Fig. 3).[55] By increasing SREBP-1c levels under sterol overload, the LXRs might ensure a sufficient supply of fatty acids to allow storage of excess cholesterol as cholesteryl ester.[55] Thus, administration of oxysterols could decrease blood cholesterol level by suppressing SREBP-2 activation, and also induce hypertriglyceridemia by accelerating SREBP-1c transcription.[56]
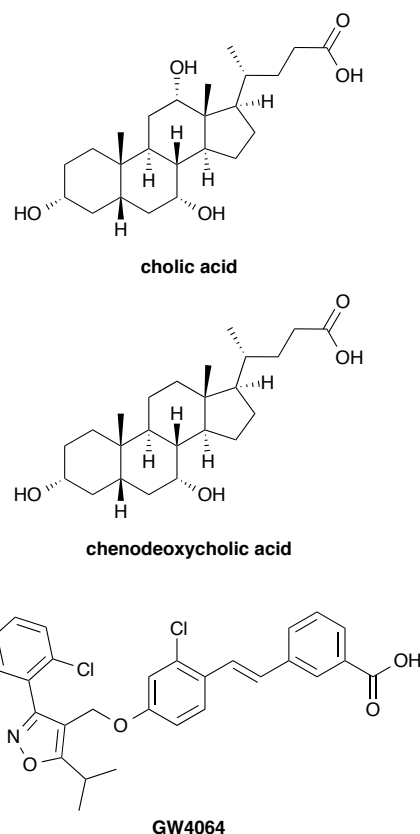

Fig. 7 Chemical structures of FXR agonists.

There have been a number of investigations for discovering non-sterol molecules that block the proteolytic activation of SREBP.

Protease inhibitors

S1P and S2P are essential for the proteolytic activation of SREBPs. Rates of fatty acid and cholesterol synthesis were reduced by 75% in the livers of mice with disrupted S1P gene.[57] The amounts of the nuclear form of SREBP and the expression levels of its target genes were also dramatically decreased. These observations suggested that S1P inhibitors could be effective as lipid-lowering drugs (Fig. 3).

S1P is inhibited by the general serine protease inhibitors, 3,4-dichloroisocoumarin and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) (Fig. 8).[58,59] AEBSF also suppresses generation of the nuclear form of SREBP-2.[58] However, these inhibitors are not selective for S1P. The peptide, decanoyl-Arg-Arg-Leu-Leu-chloromethylketone (Dec-RRLL-CMK, Fig. 8), is an irreversible S1P inhibitor that is more selective than AEBSF for S1P,[60] and impairs the maturation of SREBP-1/-2 in cultured cells.[61]

PF-429242 (Fig. 8), a non-peptidic, reversible, and highly selective inhibitor of S1P (IC_{50} = 0.17 μM) was discovered by a research group at Pfizer, Inc.[62] This aminopyrrolidineamide derivative was derived from a hit molecule (**3**) of a HTS using purified human S1P. PF-429242 inhibits the processing of SREBP-2 and suppresses the expression of SREBP target genes in cultured cells.[63] Hepatic rates of cholesterol and fatty acid synthesis were reduced in mice treated with PF-429242.

Inhibitors of S2P have also been reported. The metalloprotease inhibitor, 1,10-phenanthroline (Fig. 8), shows dose-dependent inhibitory effects on the protease activity of S2P.[64] Nelfinavir
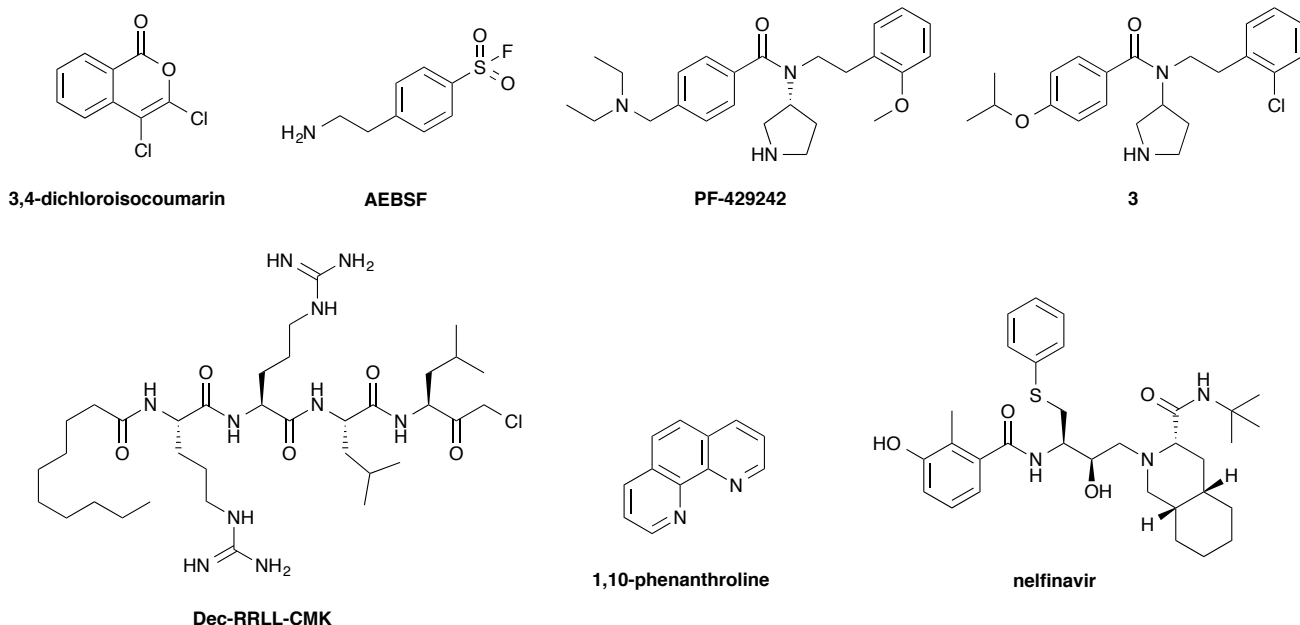
Fig. 8 Chemical structures of S1P and S2P inhibitors.

(Fig. 8), a human immunodeficiency virus (HIV) protease inhibitor, inhibits S2P activity, and also down-regulates the processing of SREBP-1 and the expression of FAS in cultured cells.[65,66]

S1P and S2P are major enzymes controlling cholesterol and fatty acid synthesis, but are also involved in several homeostatic functions, such as the ER stress response, bone mineralization, and targeting of proteins to lysosomes.[67] Dec-RRLL-CMK and nelfinavir exhibit cytotoxicity,[65,68] possibly due to impaired processing of activating transcription factor 6 (ATF6), another substrate of S1P and S2P that is involved in the stress response.[60, 65] Thus, chronic use of S1P and S2P inhibitors might cause a range of side effects.[67]

Inhibitors of SREBP-SCAP migration

Two non-endogenous molecules that selectively inhibit SREBP activation have been discovered through chemical library screening.[69,70] Both fatostatin and betulin (Fig. 9) inhibit migration of the SREBP-SCAP complex to the Golgi apparatus by directly binding to SCAP, and thereby down-regulate expression of the genes involved in cholesterol and fatty acid synthesis (Fig. 3).

Fatostatin was the first discovered non-endogenous molecule that inhibits SREBP-SCAP migration. Fatostatin, a synthetic small molecule with a non-steroidal structure, was originally described as an inhibitor of the insulin-stimulating differentiation of 3T3-L1 cells to adipocyte.[71] Administration of fatostatin by intraperitoneal injection blocked increases in body weight, blood glucose, and hepatic fat accumulation in *ob/ob* mice, even under uncontrolled food intake.[69] A structure-activity relationship study of fatostatin derivatives led to the discovery of FGH10019 (Fig. 9), a potent inhibitor of SREBP activation with drug-like physical properties.[72] *Ob/ob* mice fed normal chow containing FGH10019 exhibited reduced body weight, serum cholesterol levels, and triglyceride levels in the liver, compared to controls.
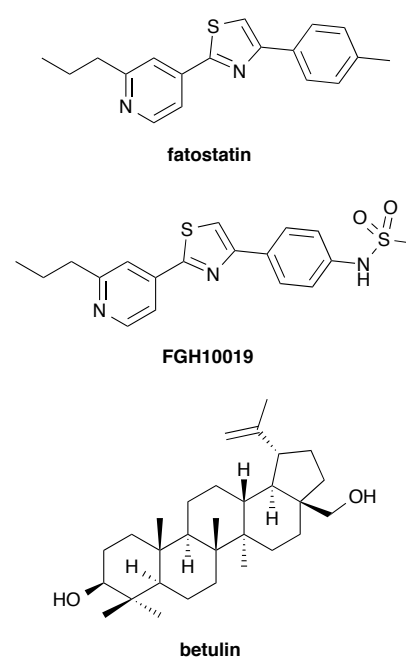

Fig. 9 Chemical structures of inhibitors of SREBP-SCAP migration.

More recently, betulin, a pentacyclic triterpene which is a natural product abundant in birch bark, was identified to inhibit SREBP processing in a human hepatoma cell line.[70] Mice fed a western-type diet, then treated with betulin through gastric irrigation, exhibited decreased lipid levels in serum and tissues, and increased insulin sensitivity. Administration of betulin to LDLR-knockout mice, an atherosclerosis disease model, reduced the size and improved the stability of atherosclerotic plaques.

The small molecules, including FGH10019 and betulin, that directly interact with the SREBP-SCAP complex and inhibit SREBP activation, improve lipid metabolism *in vivo*. Pharmacological inhibition of SREBP migration might prove to

be an effective approach for treating hyperlipidemia and associated metabolic diseases.

PUFAs

PUFAs have been reported to suppress hepatic SREBP-1c post-transcriptionally in addition to their effects on the SREBP synthesis. One possible mechanism by which PUFAs reduce lipid levels is inhibition of SREBP-1c maturation (Fig. 3). PUFAs, including eicosapentaenoic acid (Fig. 5), block the proteolytic processing of SREBP-1c and reduce amounts of the nuclear form of SREBP-1c, both in cells and *in vivo*.[73-75] Inhibition of SREBP-1c processing might be due to the loss of SCAP and/or S1P, which are required for proteolytic activation of SREBP-1c.[76] However, the mechanism by which eicosapentaenoic acid might induce the loss of SCAP and S1P remains unknown. On the other hand, arachidonic acid has been reported to inhibit the proteasomal degradation of Insig-1, potentiating the ability of sterols to block the proteolytic activation of SREBP-1c.[77]

PPARα/γ agonists

As mentioned earlier, PPARα agonists modulate the synthesis of SREBP through LXR activity. Interestingly, activation of PPARα by Wy14,643 or clofibrate (another PPARα agonist, Fig. 6) also promotes the expression of Insig-1/2, thereby decreasing nuclear levels of SREBP-1 and -2, and impairing lipid synthesis in Fao cells and rat liver.[78,79]

Glitazones, PPARγ agonists used in the treatment of type 2 diabetes mellitus, sensitize insulin response by promoting adipogenesis and glucose uptake.[80] PPARγ agonists have also been reported to modulate the amount of the nuclear SREBPs (Fig. 3). Troglitazone (Fig. 10), a PPARγ agonist, reduces nuclear levels of SREBP-2 in HepG2 and Caco-2 cells, thus, decreasing the expression of SREBP-2-responsive genes, including HMGCR and LDLR, without increasing the expression levels of Insig-2.[81] On the other hand, troglitazone promotes the expression of Insig-1/-2a in Fao cells, reducing the nuclear abundance of SREBP-1c and leading to a reduction in triglyceride synthesis.[79] Pioglitazone (Fig. 10), another PPARγ agonist, completely repressed nuclear translocation of SREBP-1c in the liver of wild-type mice fed the methionine and choline-deficient (MCD) diet, preventing MCD-induced steatohepatitis by an unknown mechanism.[82]

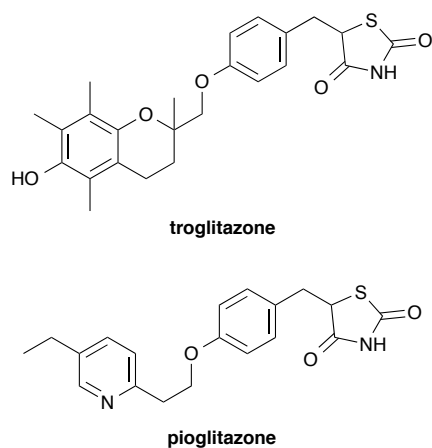Results of other research on PPAR agonists reveal some discrepancies as with the effects on the synthesis of SREBP. Two studies reported that administration of clofibrate, fenofibrate (another PPARα agonist, Fig. 6), or troglitazone did not affect either the amount of nuclear SREBPs in mouse liver or the expression of SREBPs, Insigs, and SREBP target genes in the liver and adipose tissue of pigs.[73,83] Another study reported that wild-type mice treated with Wy14,643 actually exhibited increased amounts of nuclear SREBP-1c and fatty acid synthesis in the liver.[84] Inconsistent results of the effects on the amount of the nuclear SREBP would also be due to differences in the species and nutritional status of cells and animals used in the experiments.[47,79] Thus, experimental effects of PPAR agonists on SREBPs should be interpreted with particular care.

Inhibitors of the nuclear form of SREBP

mTORC1 inhibitors

The nuclear translocation of SREBP is controlled by mTORC1 signaling pathway. Pharmacological inhibition of mTORC1 might impair the nuclear translocation of SREBP (Fig. 3). The best known inhibitor of mTORC1 is rapamycin (Fig. 11). Rapamycin binds to FKBP12, and the resulting complex binds to and inactivates mTORC1.[85] Treatment of hepatocytes and several other cell systems with this natural product impairs the insulin-induced accumulation of nuclear SREBP-1, decreasing the expression levels of SREBP-responsive genes, including FAS and SCD-1.[86,87] Similarly, *in vivo* administration of rapamycin blocks the diet-induced increase of hepatic SREBP-1c.[87] Another known mTOR inhibitor is Torin1 (Fig. 11), a synthetic kinase inhibitor that binds to the ATP-binding pocket of mTOR (IC$_{50}$ = 2 nM for mTORC1 in cells).[88,89] This selective mTOR inhibitor also decreases the expression of SREBP-responsive genes in NIH 3T3
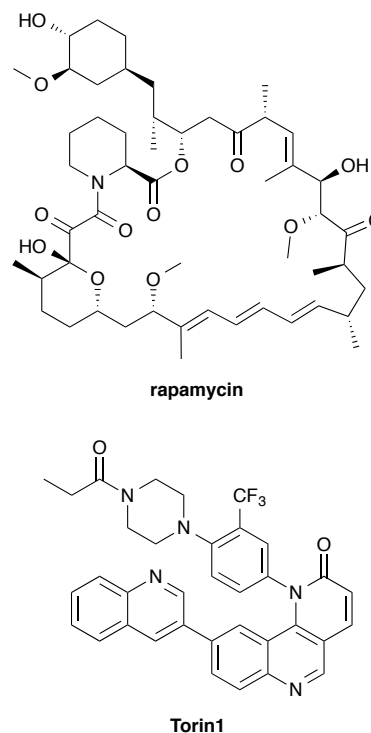
rapamycin

Torin1

Fig. 11 Chemical structures of mTORC1 inhibitors.

troglitazone

pioglitazone

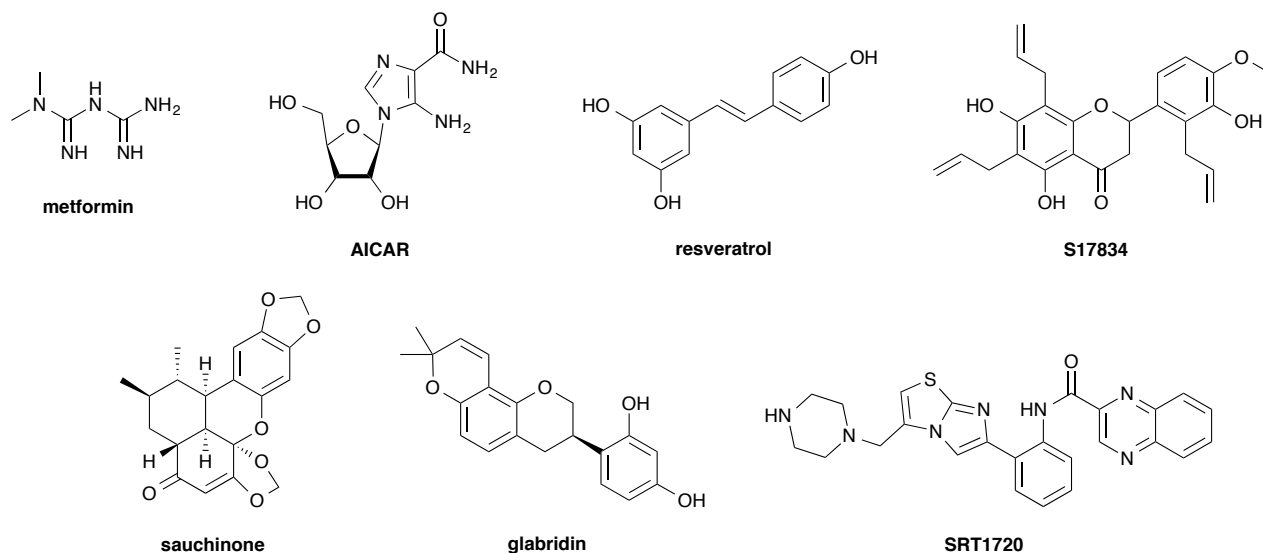**Fig. 10** Chemical structures of PPAR γ agonists.

Fig. 12 Chemical structures of AMPK and SIRT1 activators.

cells, which rapamycin fails to do.[23]

AMPK activators

Metformin (Fig. 12), an oral anti-diabetic drug in the biguanide class, activates AMPK.[90] Metformin requires the kinase, LKB1, which phosphorylates and activates AMPK in the liver, and lowers blood glucose levels.[91] Activation of AMPK mediates a reduction in expression of the hepatic gluconeogenic genes, PEPCK and Glc-6-Pase.[92] Metformin and other well known AMPK activators (Fig. 12), including 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), natural polyphenol resveratrol, and the synthetic polyphenol, S17834, inhibit the activation of SREBP-1c and -2 in cells and *in vivo*, leading to the improvement of alcohol-induced fatty liver disease in rats or diet-induced fatty liver diseases in diabetic mouse models.[28,90,93,94] The recently described AMPK activators, sauchinone and glabridin (Fig. 12), which are bioactive constituents of natural medicines, also suppress the expression levels of SREBP-1c and decrease triglyceride levels in liver and white adipose tissue of high fat diet-induced obese mice.[95,96]

These observations collectively suggest the exciting possibility that small-molecule AMPK activators might provide a pharmacological means of suppressing SREBPs, thereby impairing biosynthesis of fatty acids and cholesterols. A number of other AMPK activators have been discovered;[97] however, their effects on SREBPs have not yet been investigated. Further research might lead to the discovery of clinically useful AMPK activators.

SIRT1 activators

Small molecules that activate SIRT1, resveratrol and SRT1720 (Fig. 12), inhibit SREBPs in cells and *in vivo*, thereby decreasing levels of hepatic lipids and cholesterol, and improving alcoholic or non-alcoholic liver steatosis in mice.[32,33,98] As discussed above, resveratrol also activates AMPK, so that administration of resveratrol activates both SIRT1 and AMPK in mouse liver.[98] Due to the crosstalk of SIRT1 and AMPK, it is unclear which factor is located upstream.[99] Furthermore, although SRT1720 was

originally discovered as an activator of SIRT1,[100] its exact mechanism of action remains under debate.[101] Better understanding of the mechanisms by which resveratrol and SRT1720 activate SIRT1 and AMPK is required for the development of more effective drug-like activators. Nevertheless, it seems clear that small-molecule activators of AMPK and SIRT1 might serve as SREBP modulators.

Drugs with unknown mechanisms

A number of molecules have apparent indirect effects on SREBP expression through unknown mechanisms. For example, bortezomib (Fig. 13), a proteasome inhibitor used to treat multiple myeloma, suppresses the mRNA expression of SREBP-1c, thereby suppressing expression of downstream lipogenic genes and ultimately inhibiting alcohol-induced fatty liver.[102] The detailed molecular mechanism of this surprising effect remains unclear; however, decreased expression of C/EBPα, a transcription factor upstream of SREBP, might partly be responsible for decreasing SREBP expression levels. On the other hand, pharmacological inhibition of proteasome has been reported to stabilize SREBPs, which are susceptible to ubiquitin-proteasome degradation,[103] and to increase expression levels of SREBP-responsive genes.[103,104] The practical use of proteasome inhibitors for selective inhibition of SREBPs remains unclear and might not be appropriate for clinical applications.

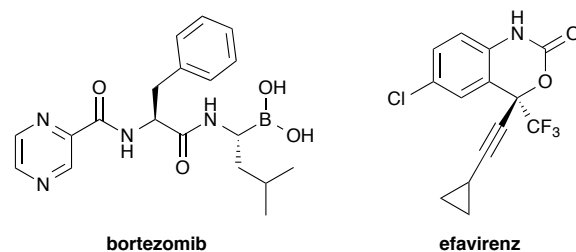Another example of a molecule that indirectly affects SREBP

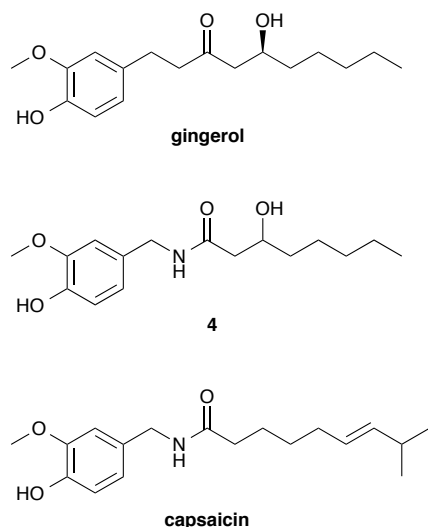**Fig. 13** Chemical structures of bortezomib and efavirenz.

Fig. 14 Chemical structures of gingerol, **4**, and capsaicin.

expression is efavirenz (Fig. 13), a non-nucleoside reverse transcriptase inhibitor used clinically to treat HIV. Efavirenz decreases levels of both SREBP-1c mRNA and its nuclear form in 3T3-L1 cells, reducing triglyceride accumulation during adipogenesis.[105] Such SREBP inhibition is not observed with all non-nucleoside reverse transcriptase inhibitors,[106] and the mechanism of efavirenz remains unknown.

A synthetic derivative of gingerol (Fig. 14), a bioactive natural product in ginger, provides another intriguing example. Ginger is used in natural medicine to treat a number of diseases, including diabetes, and an aqueous extract of ginger exhibited hypolipidaemic activity in rats.[107] An amide derivative of gingerol (**4**, Fig. 14) suppressed the expression of SREBP-1c in mouse liver, reducing the weight gain induced by high-fat diets.[108] Similarly to capsaicin (Fig. 14), which has anti-obesity activity, gingerol is known to activate transient receptor potential vanilloid 1 (TRPV1).[109,110] The effect of molecule **4** on TRPV1 is unknown, and the relationship between TRPV1 and SREBP-1c remains unclear. However, further investigation of one or more of these molecules that indirectly affect SREBP expression could lead to new approaches for treating metabolic diseases.

Conclusions

SREBP transcription factors have been widely investigated since their discovery twenty years ago. As master regulators of lipogenesis, SREBPs are likely to be key players in a broad range of signaling events. There is recent evidence that SREBPs play regulatory roles in a number of physiological processes, including cancer growth, ER stress, immune response, and autophagy.[14,21,22] However, despite increasing knowledge of their physiological roles in lipogenesis and their molecular interactions, the exact regulatory mechanisms of SREBPs *in vivo* remain unclear and merit further investigation. Small molecules that control SREBPs would serve as useful tools to better understand the various roles of SREBPs, and to develop new pharmacological interventions for metabolic disorders and cancers.

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