Drug Development in the Field of Sphingolipid Metabolism

12

Zhibei Qu and Lu Zhou

Abstract

Sphingolipids are the major lipid components on cellular membranes especially on lipid raft regions, intermediating various important biological functions for eukaryotic cells. Sphingolipid metabolism pathways can utilize sugar, protein, nucleic acid, and other metabolites participating lipid transport in the circulation, play an essential role in maintaining cell homeostasis and are related to a variety of different diseases including lysosomal storage disorders (LSDs), Gaucher disease, etc. The dynamic balance of sphingolipid levels in organisms is regulated by a series of sphingolipid synthases, hydrolases, and metabolic enzymes, such as sphingomyelinase (SMase), sphingomyelin synthase (SMS), serine palmitoyltransferase (SPT), ceramide synthase (CerS), glucosylceramide synthase (GCS), etc. Thus, sphingolipids and its related enzymes are potential targets for drug discoveries and receive great research interests by medicinal chemist. In this chapter, we will discuss the relationship between sphingolipids and the regulating enzymes involved in sphingolipid metabolisms, and systematically summarize the advances in the development of new drugs in the field.

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Keywords

Sphingolipid metabolism · Ceramide synthase · Sphingomyelin synthase · Serine palmitoyl transferase · Sphingomyelinase · Drug development

Abbreviations

AAL	Alternaria alternate lycopersici
CerS	Ceramide synthase
FB1	Fumonisin B1
GCS	Glucosylceramide synthase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LSD	Lysosomal storage disorders
SM	Sphingomyelin
SMase	Sphingomyelinase
SMS	Sphingomyelin synthase
SPT	Serine palmitoyltransferase

12.1 Background

Sphingolipids are essential lipids involved in regulating cell functions and maintaining metabolic homeostasis in organisms [1]. These lipids share a sphingoid base backbone which is *N*-acylated with various fatty acid chains. Sphingolipids can be divided into three structural classes [2], including sphingoid bases and derivates (i.e., sphingosine, sphingosine-1-

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phosphate), ceramides, and complex sphingolipids. Complex sphingolipids can be further divided into phosphosphingolipids (i.e., sphingomyelin, C1P), neural glycosphingolipids (Glc-Cer, Gal-Cer, Lac-Cer, etc.), and acidic glucosphingolipids (i.e., gangliosides).

From the perspective of macro-molecular metabolic pathways, the sphingolipid metabolism pathways can utilize sugar, protein, nucleic acid, and some metabolites in other lipid metabolism pathways (L-serine, Acyl-CoA, fatty acid, etc.) as raw materials for de novo synthesis of sphingolipids. In addition, some key active molecules (such as phosphoethanolamide, phosphor-choline, phosphor-inositol, DAG, etc.) generated by sphingolipid catabolism can participate in the anabolism of other substances, maintaining metabolic homeostasis of organisms [3].

The dynamic balance of sphingolipid levels in organisms is regulated by a variety of sphingolipid synthases, hydrolases, and metabolic enzymes. Functional deficiency or loss of some essential enzymes would directly break the balance, further leading to the occurrence of various diseases including lysosomal storage disorders (LSDs). LSDs are a class of inherited metabolic diseases. Typical LSDs are caused by mutations in genes that encode certain hydrolases and/or activators, preventing cells from producing these functional proteins abnormally, and then resulting in a large accumulation of related substrates in the cells. For example, Gaucher disease is a functional deficiency of glucocerebrosidase caused by mutation of GBA1 gene, resulting in abnormal accumulation of glucosylceramide. And glucosylceramide synthase inhibitors (e.g., Genz-112,638) were proved to alleviate the Gaucher disease.

Furthermore, abnormal sphingolipid metabolism is prevalent in many common diseases. Ceramide is a well-recognized signaling molecule mediating cell death. Although recent studies have shown that different types of ceramides could regulate cell growth and death differently [4], the lipotoxicity caused by ceramide in obesity and inflammation disease could not be ignored [5]. Therefore, studying the in vivo synthesis pathway of ceramide could lead new strategies for the treatment of related diseases. Ceramide can block Akt signaling pathway by activating PP2A and PKCζ, further regulating cell growth and other signals [6]. By inhibiting insulin stimulation of Akt [7] and activating the expression of some inflammatory factors (STAT3, etc.) [8], ceramide contributes to a variety of metabolic diseases.

In a high-fat diet, excessive fatty acid intake activates the intracellular palmitoylation metabolic pathway, in turn regulates the transcriptional activation of the de novo synthases of ceramide, Sptlc2 and CerS, further promotes the synthesis of ceramide and leads to obesity and its syndromes [9]. Interestingly, the sphingolipid metabolism of certain intestinal flora can also stimulate the synthesis of ceramide in the host, thereby promoting the development of inflammation and metabolic diseases [10]. Therefore, inhibition of ceramide synthase (such as CerS) is a potential therapeutical strategy.

Serine palmitoyl transferase (SPT) complex is the first enzyme in de novo biosynthesis of ceramide, which locates at the upstream of the entire sphingolipid synthesis pathways. In mammals, the SPT complex is composed of two large subunits, SPTLC1 and SPTLC2/3, and two small subunits, SSSPTA and SSSPTB [11]. SPT is very important in maintaining the balance of sphingolipid metabolism in eukaryotes. Homozygous SPTLC1- or SPTLC2-deficient mice are embryonic lethal, while heterozygous SPTLC1/2-deficient mice remain healthy [12, 13]. Compared with normal mice, the levels of sphingolipids (such as sphingosine, ceramide, and S1P) in tissues and plasma of SPTLC1/2-deficient mice are significantly reduced. Inhibiting SPT in cells using SPT inhibitors enables growth inhibition of some fungi and tumor cells [14–16], indicating that SPT activity is indispensable for the eukaryotic cell. The missense mutation of SPTLC1 gene is the main cause of the congenital disease hereditary sensory neuropathy type I (HSN1). High expression of SPTLC2 can promote the synthesis of ceramide in liver, activate the JNK signaling pathway, and then lead to insulin resistance [17, 18]. Inhibition

of SPT also has a certain effect on alleviating atherosclerosis and obesity metabolic syndrome [19, 20]. In addition, SPT can also affect the assembly of lipid rafts on biological membranes by regulating the synthesis of sphingolipids, thereby promoting the localization of the NS protein of HBV and the replication of viral nucleic acids [21]. Certain inflammatory factors (neutrophil elastase, etc.) can upregulate SPT activity to promote the synthesis of ceramide, which in turn lead to inflammation [22]. In recent years, the crystal structure of human SPT complex has been reported, laying an important structural foundation for the study of the mechanism of SPT and the development of inhibitors targeting SPT [23].

Sphingomyelin (SM) is an important direct metabolite of ceramide, which participates in the formation of cell (organelle) membranes and the conduction of various signals. The level of sphingomyelin in organisms is mainly regulated by sphingomyelin synthase (SMS) and sphingomyelinase (SMase). SMase catalyzes the decomposition of SM into ceramide, which plays a key role in maintaining the balance of ceramide. Based on the optimal working pH of enzyme, SMase can be divided into three types, including aSMase, nSMase, and alk-SMase. aSMase is expressed in almost all types of cells and has intracellular lysosomal form and extra-cellular secreted form depending on localization. The aSMase is normally located in the endosome/lysosome compartments. During cell stress and disease, aSMase can be preferentially transported to the outer lobes of the cell membrane and secreted into the extra-cellular space [24]. The activation of aSMase promotes the accumulation of a large amount of ceramide in the cell membrane, causing metabolic disorders, inflammatory reactions, or cell apoptosis, and ultimately leading to disease [25]. For example, in the pathological model of cystic fibrosis (CF) with Cftr gene defect, the defect or inhibition of aSMase activity can alleviate the CF caused by excessive accumulation of ceramide in respiratory cell [26]. The aSMase activation induces the accumulation of ceramide, which will promote the rapid death of tumor cells. Thus aMase could be a potential therapeutic target for tumors [27]. The nSMase located in the cell membrane has also been shown to regulate the release of inflammatory factors and the accumulation of Aβ by regulating the levels of SM and ceramide, thereby participating in the regulation of inflammation and neurodegenerative diseases [28]. Alk-SMase is expressed only in mammalian intestinal mucosa (also in human liver), and functions through the secretion of intestinal mucosal epithelial cells into the intestinal lumen, involved in the regulation of the metabolism and absorption of intestinal sphingolipids and then affecting the progression of diseases such as inflammation [29].

Interestingly, while SMase can improve the level of ceramide and perform certain physiological functions like ceramide, a SM synthase with the opposite function to SMase, SMS, also has physiological functions such as promoting the development of inflammation, cardiovascular disease, and metabolic syndrome. This may be related to the diversity of the SMS family's catalytic function and the difference in subcellular distribution. The SM generated by SMS1 and SMS2 can be transported to cell membrane to participate in the assembly of lipid rafts on cell membrane, promoting the function of lipid raft-related proteins. For example, SMS2 can stabilize the localization of CD36 to lipid rafts on cell membrane, thus promoting the cellular absorption of fatty acids [30]. SMS-produced DAG is considered to be a second messenger that activates the PKC-JNK axis, impairing insulin action and inducing insulin resistance [31]. SMS can maintain the CD14-TLR4 complex localization in cell membrane and promotes its function, thereby activating LPS-induced inflammatory signaling pathway downstream of TLR4, promoting the release of the inflammatory factor TNF-α, and aggravating the progression of associated inflammation [32]. In addition, SMS employing ceramide as a substrate, to somewhat alleviates the effect of ceramide overaccumulation in promoting apoptosis, and it can promote tumor growth and drug resistance by activation of certain signaling pathways (e.g., TGF-β/Smad) and promoting cytokines (e.g., BCL-2) expression [33].

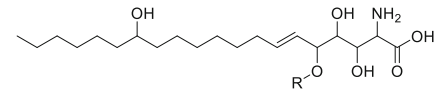
In summary, sphingolipid metabolism plays a vital role in maintaining homeostasis. Sphingolipid metabolism has its unique and complicated operating mechanism, involved in not only the formation of cells, but also the transformation or transmission of intra- and extra-cellular substances and signal transduction. The balance of sphingolipid metabolism plays a key role in modulating the normal development and growth of the body. The imbalance of sphingolipid metabolism caused by a variety of factors can directly or indirectly lead to the occurrence of diseases, suggesting that pharmaceutical intervention of sphingolipid metabolism may be a new way to treat certain diseases. However, in the complicated sphingolipid metabolism pathway, the effects of certain metabolites on diseases are subtle and sometimes even two-sided, suggesting the need for more comprehensive consideration and scrutiny when intervening in metabolic regulation.

12.1.1 Serine Palmitoyl Transferase Inhibitors

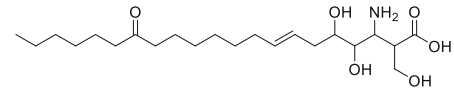
Serine palmitoyl transferase (SPT) complex is the first enzyme in de novo biosynthesis of ceramide, which locates at the upstream of the entire sphingolipid synthesis pathways [34]. SPT is very important in maintaining the balance of sphingolipid metabolism and inhibiting SPT in cells shows significant growth inhibition for eukaryotic cells. Inhibition of SPT also has a certain effect on alleviating atherosclerosis and obesity metabolic syndrome. Thus, SPT inhibitors are potential drugs for sphingolipid-related metabolic disorders.

SPT inhibitors can be divided into two subclasses. The first type was the substrate-mimics of SPT complexes. SPT has two natural substrates, palmitate and serine. Both mimics can be developed as potential SPT inhibitors.

In 1992, Marcia M. Zweerink and coworkers proved two known compounds, sphingofungin B and C, separated from *Aspergillus fumigatus*, showed the anti-fungi activity by inhibition of the sphingolipid synthetic pathways via SPT complexes [14]. In 1995, Yurika Miyake et al. proved myriocin as a highly efficient and selective SPT inhibitor using CTLL-2 cells as enzyme source, with a remarkably low IC50 of 0.3 nM [35].

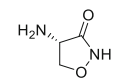


R = H sphingofungin B
R = Ac sphingofungin C

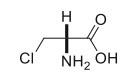


ISP-1/myriocin

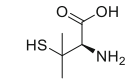
Moreover, a series of serine analogs such as L-Cycloserine [36], β-chloro-L-alanine [37], and L-penicillamine [38] were reported to show SPT inhibition activities but none was as good as myriocin and its derivates.



L-Cycloserine

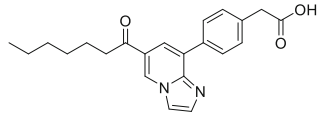


β-Chloro-L-alanine

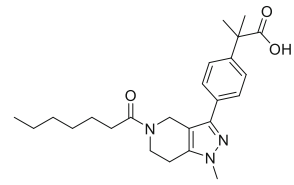


L-penicillamine

Medicinal chemists discovered a series of SPT inhibiting compounds which are non-analogs of SPT substrates. For example, Michael J. Genin screened two new imidazopyridine and pyrazolo-piperidine compounds (compound 1 and 2) [19], and found them showed good SPT inhibition properties in vitro (1: IC50 ~ 5 nM; 2: IC50 ~ 64 nM). In vivo tests showed that both compounds significantly reduced ceramide levels in DIO mice and promoted HDL levels.

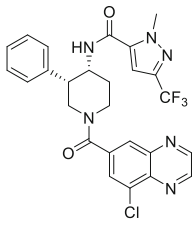


compound 1

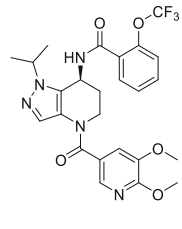


compound 2

Masahiro Yaguchi et al. discovered a new type of SPT inhibitor—compound 3, which has a significant effect on the disease of pl-21 acute myeloid leukemia PDX mice at an oral dose of 3 mg/kg [15]. And Ryutaro Adachi et al. obtained a new class of compounds with inhibitory SPT activity through enzyme-level activity screening, such as compound A (IC50 ~ 0.76 nM), which is effective for the growth of non-small cell lung cancer HCC4006 cells, with good in vitro activity (EC50 ~ 3.9 nM) [16].

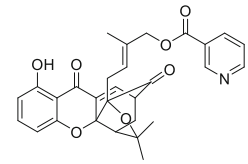


compound 3



compound A

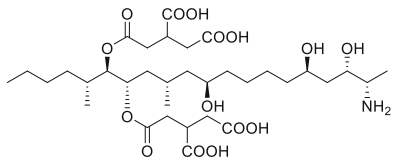
Dominic G. Hoch et al. obtained a class of gambogic acid and its structurally related xanthone derivatives (compound 18) by combining proteomics and metabolomics as a class of first-in-class mammalian SPT covalent inhibitor, and proved that its main mechanism is covalently bound to SPT small subunit B (SPTSSB) to destroy the formation of SPT complex [39].



compound 18

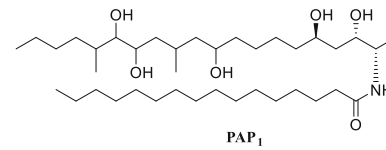
12.1.2 Ceramide Synthase Inhibitors

Ceramide is an intermediate in the biosynthetic pathway of lipids and a cell signaling molecule, catalytically produced by Ceramide synthase (CerS) [40]. CerS consists of six subtypes (CerS1–6) that distribute in different tissues and catalyze the synthesis of different ceramides. Elevated ceramide levels cause many disorders such as inflammation and obesity-related syndromes, and the development of CerS inhibitors is essential for potential therapeutical methods to metabolic diseases.



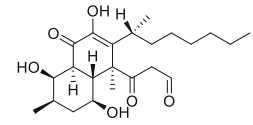
Fumonisin B_1

IC_{50} (CerS) = 0.1 μM



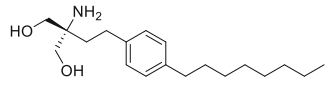
PAP_1

Fumonisin B_1 (FB_1), a mycotoxin isolated from *Fusarium moniliforme* by Gelderblom and coworkers, can inhibit CerS through substrate structural similarity (IC_{50}: 0.1 μM) [41], but is toxic that caused esophageal cancer, birth disability, and growth disorders [42]. Its derivatives, such as hydrolyzed esterification product PAP_1, had a better inhibitory effect but showed higher cytotoxicity [43].

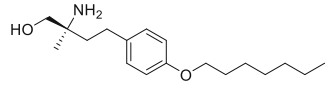


Australifungin
IC_{50} (CerS) = 0.093 μM

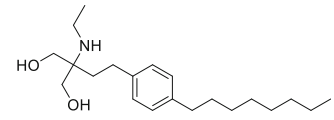
Australifungin, a toxin isolated from *Sporormiella australis* by Mandala and coworkers, had broad-spectrum antifungal activity against human pathogenic fungi [45], but the high chemical reactivity of the α-diketone and β-ketoaldehyde functional groups limited its use [46].



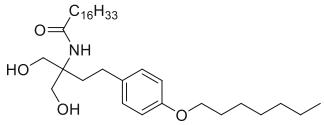
FTY720
IC_{50} (CerS) = 6.4 μM



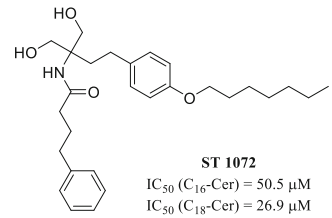
AAL(S)



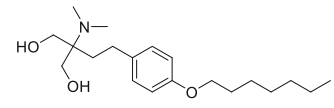
ST 1058
IC_{50} (C_{16}-Cer) = 25.7 μM
IC_{50} (C_{18}-Cer) = 50.7 μM
IC_{50} (C_{24}-Cer) = 60.6 μM



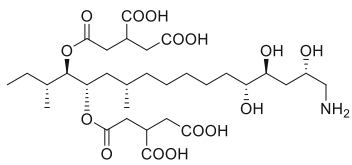
ST 1060
IC_{50} ($C_{24:1}$-Cer) = 61.3 μM
IC_{50} (C_{24}-Cer) = 63.6 μM



ST 1072
IC_{50} (C_{16}-Cer) = 50.5 μM
IC_{50} (C_{18}-Cer) = 26.9 μM
IC_{50} (C_{24}-Cer) = 81.3 μM



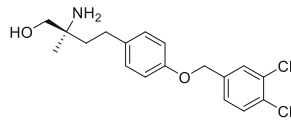
ST 1074
IC_{50} (C_{18}-Cer) = 21.4 μM
IC_{50} ($C_{24:1}$-Cer) = 28.7 μM



AAL-Toxin
IC_{50} (CerS) = 0.035-0.04 μM

Alternaria alternate lycopersici toxin (AAL-toxin), a fungal toxin isolated from *Alternaria alternata f.* sp. *lycopersici* by Bottini and coworkers, competitively inhibited ceramide synthase (IC_{50}: 0.04 μM) [44].

FTY720 was a synthetic analog of sphingosine and inhibited in a similar manner to FB_1 (IC_{50}: 6.4 μM), but the two had different inhibition efficiencies for long and short chain ceramide synthesis [47]. AAL(S) was an unphosphorylated FTY720 analog that could be used to study diseases associated with CerS1 [48]. The ST series of compounds were derivatives of FTY720 main chain modification with selective inhibitory effects [49]. P053, a small-molecule compound synthesized by Nigel Turner and coworkers, selectively inhibited CerS1 (IC_{50}: 0.5 μM), might be used to treat obesity [50].

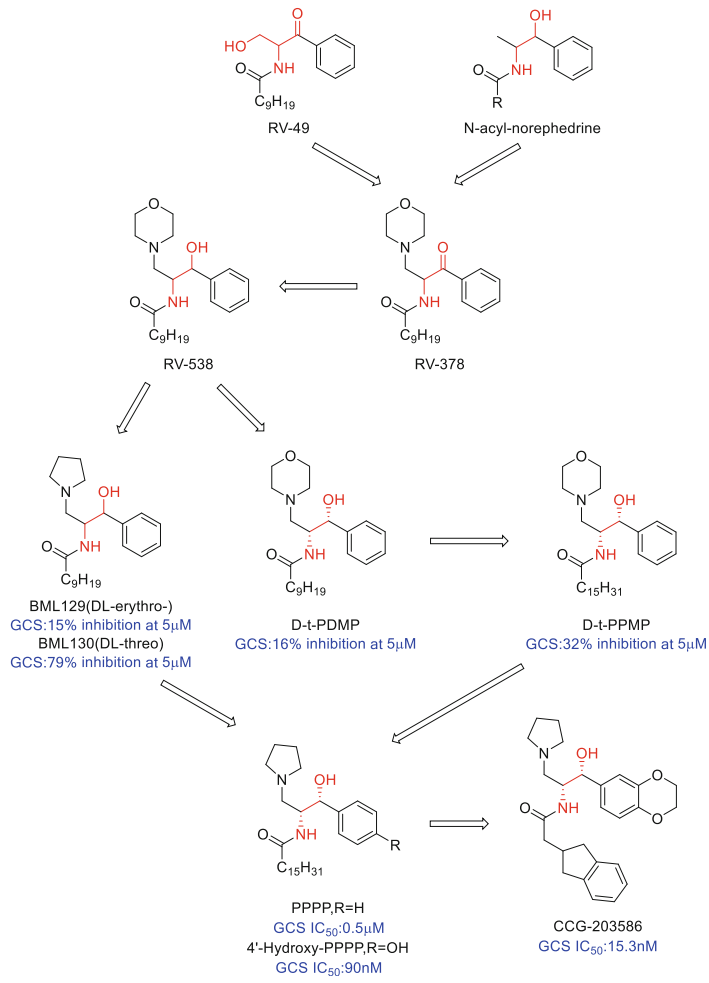


P053
IC_{50} (CerS1) = 0.5 μM

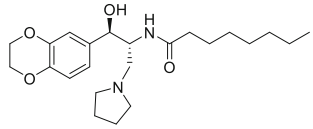
12.1.3 Glucosylceramide Synthase Inhibitors

Glucosylceramide synthase (GCS, also known as UCGC), catalyzes the conversion of ceramide to glucosyl ceramide [51]. GCS small-molecule inhibitors have been reported primarily for the treatment of lysosomal storage disorders—Gaucher's Disease and Fabry disease and emerged as promising studies related to type II diabetes and tumor resistance in recent years. They are generally divided into two categories by chemical structure.

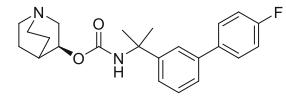
One class is ceramide analogs. The precursors of the PDMP family are RV-49 (70% inhibition at 0.3 mM) and *N*-acyl-norephedrine (82% inhibition of the most active compound at 0.3 mM). A non-competitive GCS inhibitor, RV-378 (72% inhibition at 150 mM), was synthesized by introducing a morpholine group at 1-position. The 3-position ketocarbonyl group of RV-378 is reduced to form a more potent analog RV-583 (84% inhibition at 37.5 mM) that is a competitive GCS inhibitor [52, 53]. RV-583 was originally a mixture of four stereoisomers. And only the D-threo-PDMP (1S,2R) is active against GCS [54]. By replacing the morpholinyl group of RV-538 with a pyrrolidinyl group, a pair of enantiomers BML-129 and BML-130 that showed growth inhibition of several kinds of cancer cells were produced [55]. PPMP, P4, and 4′-Hydroxy-P4 were prepared on the basis of structural modifications of D-threo-PDMP and BML-129/130 [56–58]. The most recent compound of the PDMP family is CCG-20358, which turns the alkyl chain linked to the *N*-acyl group into a benzocyclopentane to increase the rigidity of the entire molecule. It can cross blood–brain barrier [59].



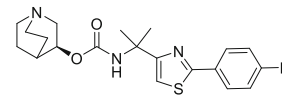
An effective GCS inhibitor eliglustat (Genz-112,638) is also a ceramide analog [60]. Further development on it revealed two brain-penetrant heterocyclic compounds Genz-667,161 and Genz-682,452 (venglustat) with reduced chiral centers [61–63]. Exelixis identified a more active GCS inhibitor (EXEL-0346) through high-throughput screening and hit optimization [64]. Recently, a novel CNS-permeable GCS inhibitor, T-036, was discovered [65].



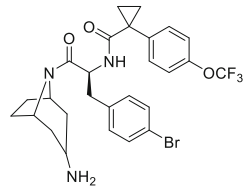
eliglustat(Genz-112638)
GCS IC_{50}=16nM
Cell IC_{50}=2nM



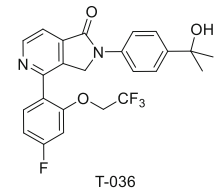
Genz-667161



venglustat(Genz-682452)

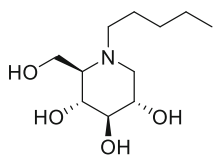


EXEL-0346
GCS IC_{50}=2nM

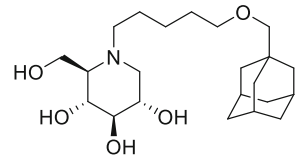


T-036
GCS IC_{50}=31nM

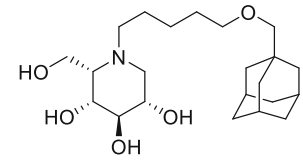
The other class is *N*-alkyl-deoxynojirimycins (DNMs). Miglustat (Zavesca), an alkyl iminosugar that mimics the transition state of the cationic intermediate in glycosylation reactions, is a competitive inhibitor of GCS. It is approved for the treatment of type I Gaucher's disease and Niemann–Pick disease. An optimized iminosugar (AMP-DNM) is a more effective GCS inhibitor [66]. Subsequently, it was found that ido-AMP-DNM, the C5-epimer of AMP-DNM, had a slightly stronger inhibitory effect on GCS [67]. Various iminosugar-based GCS inhibitors have been identified, which differ not only in the nature of the *N* substituent but also in the configuration of the piperidinyl iminosugar [68, 69]. However, DNMs are less selective for GCS and generally active against GBA1 and GBA2 as well. Some investigators designed a hybrid structure of two classes of GCS inhibitors, but with greatly reduced activity against GCS and active against GBA1 and GBA2 [70].



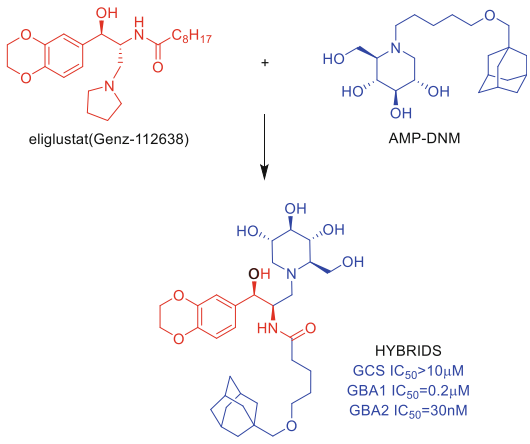
miglustat
GCS IC_{50}=50μM
GBA1 IC_{50}=400μM
GBA2 IC_{50}=230nM



AMP-DNM
GCS IC_{50}=150-220nM
GBA1 IC_{50}=0.2μM
GBA2 IC_{50}=1nM



L-ido-AMP-DNM
GCS IC_{50}=150nM
GBA1 IC_{50}=2μM
GBA2 IC_{50}<1nM

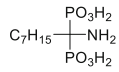


12.1.4 Sphingomyelinase Inhibitors

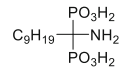
Sphingomyelinase (SMase) is an enzyme that hydrolyzes sphingomyelin to produce phosphocholine and ceramide. So far, at least 6 subtypes of SMase have been identified, mainly based on their optimal pH value and cofactors [71]. Among them, acid sphingomyelinase (A-SMase) is the most important subtype, and its biological activity accounts for 90% of the total SMase activity [72].

There are now growing evidence that the activation of SMase and the accumulation of ceramide play an essential role in the development of various human diseases. For example, the inhibitions of SMase activity prevent ischemic-stress-induced neuronal death [73], improve acute lung injury caused by repeated airway lavage [74], and reduce apoptosis in hepatic ischemia-reperfusion injury [75]. The A-SMase activity is also associated with major depression [76]. Therefore, the discovery of potent SMase inhibitors is of great significance for the development of drugs for the prevention and treatment of related diseases.

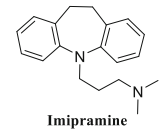
To date, a variety of inhibitors against A-SMase and N-SMase have been reported. The inhibitors of A-SMase are divided into two types, the direct inhibitors and the functional inhibitors. Direct inhibitors are characterized by not requiring high lysosomal drug concentrations as a prerequisite for inhibiting A-SMase, and there are few known examples of direct inhibitors [77]. For instance, several bisphosphonates have strong selective inhibition on A-SMase, among which the compound 7b and 7C have better inhibitory activity [78], imipramine can inhibit A-SMase4 [79]. Functional inhibitors are characterized as cationic amphiphilic substances, inducing the dissociation of A-SMase proteins from the endolysosomal membrane to inactivate A-SMase, including Astemizole and Amlodipine, etc. [80].



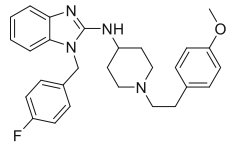
7b
IC_{50}(A-SMase)=0.04±0.01μM
(2009)



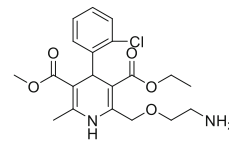
7c
IC_{50}(A-SMase)=0.02±0.00μM
(2009)



Imipramine
80-90% inhibition at 50μM
(2009)

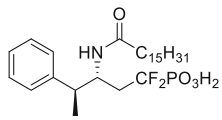


Astemizole

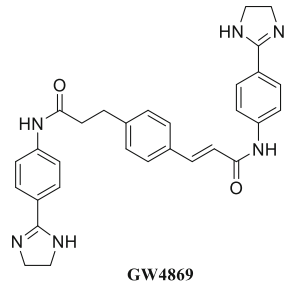


Amlodipine

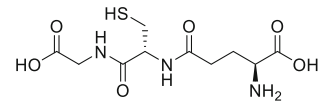
There are synthetic molecules and natural products as N-SMase inhibitors. Luberto and coworkers synthesized a series of compounds, among which GW4869 exhibited significant inhibitory activity against N-SMase both in vitro and cellular models. This compound is a non-competitive and selective inhibitor, which means GW6948 did not inhibit A-SMase [81]. Soeda synthesized a series of difluoromethyl analogs (SMAs) of sphingolipids, among which SMA-7 has better inhibitory activity. Liu found that N-SMase was inhibited in vitro by physiologically relevant concentrations of glutathione (GSH) and was activated in GSH-depleted cells [82], whereas glutathione acts as a natural inhibitor of N-SMase in the reduced (GSH) and oxidized (GSSG) forms [83].



SMA-7
IC_{50}(N-SMase):3.3 μM

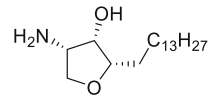


GW4869
IC_{50}(N-SMase):1 μM

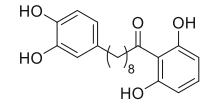


Glutathione
95% inhibition at 5mM

Microbial-derived natural products were screened as SMase inhibitors. Ryuji isolated a known compound, Alutenusin, from cultures of *Penicillium* sp. as a selective N-SMase inhibitor [84]. Tanaka isolated Schyphostatin from the mycelial extract of *Dasyscyphus mollissimus*, which was found to be a competitive inhibitor of N-SMase [85]. Schyphostatin was the most potent natural product against N-SMase [86], but unable to inhibit A-SMase activity [87]. Arenz discovered some analogs of Manumycin A are also the irreversible inhibitors of N-SMase, whose inhibitory ability is strongly influenced by their hydrophobic side chains [88].

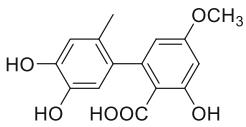


Jaspine B
EC_{50} (SMS) = 5 μM
(2009)

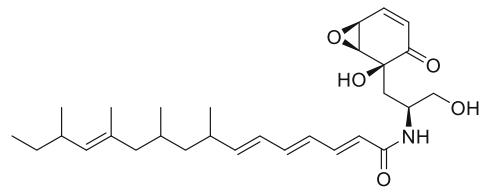


Malabaricone C
IC_{50} (SMS2) = 1.5 μM
selectivity (SMS2 vs SMS1) = 2
(2019)

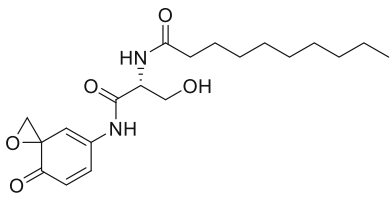
The first reported SMS inhibitor was D609, which had been known as a selective PC-PLC inhibitor before. Meng and coworkers found that



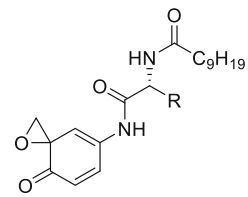
Alutenusin
IC_{50}(N-SMase)=28μM
(1999)



Schyphostatin
IC_{50}(N-SMase)=49.3μM
(1997)



2
88% inhibition at 100μM
(2001)



a:R=CH_3
b:R=$CH_2C_6H_5$

3a,b
3a:33% inhibition at 100μM
3b:23% inhibition at 100μM
(2001)

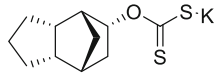
12.1.5 Sphingomyelin Synthase Inhibitors

With the increasing understanding of sphingomyelin synthase (SMS) family proteins, SMSs were found associated with the occurrence and development of various diseases [89]. A variety of SMSs inhibitors have been developed, and some of them have been evaluated in disease models.

D609 was capable of inducing U937 cell death by apoptosis, which was associated with the inhibition of SMS activity [90]. A significant increase in the intracellular level of ceramide and a decrease in that of sphingomyelin (SM) and diacylglycerol were observed, suggesting that SMS is a potential target of D609 and inhibition

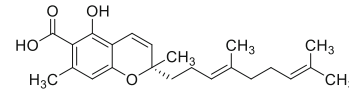
of SMS may contribute to D609-induced tumor cell death. However, D609 is very unstable under aqueous conditions due to the carbonodithioate structure, which restricted its further application in in vivo study.

New types of SMS inhibitors including natural products and synthetic molecules have been developed in the past 10 years. Jaspine B, an anhydrophytosphingosine derivative isolated from the marine sponge *Jaspis* sp. by Salma and coworkers, inhibited the activity of sphingomyelin synthase (IC_{50}: 5 μM) and induced cell death in SMS1-depleted cells but not SMS1-overexpressed cells [91].



D609
IC_{50} (SMS) = 177 ~ 600 μM
(2004)

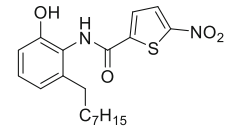
Malabaricone C, isolated from the fruits of *Myristica cinnamomea* King by Othman and coworkers, was reported as an SMS inhibitor [92]. It exhibited multiple efficacies, including reduction of weight gain, glucose tolerance improvement, and reduction of hepatic steatosis in high-fat diet-induced obesity mice models. However, Malabaricone C was also reported to inhibit α-Glucosidase [93] and cholinesterase [94] as well, suggesting that it was a multi-target natural product.



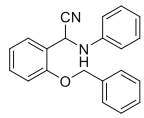
Daurichromenic acid
IC_{50} (SMS2) = 4 μM
selectivity (SMS2 vs SMS1) = 1.8
(2020)

Another natural product Daurichromenic acid (DCA), isolated from *Rhododendron dauricum* by Deepak and coworkers, was found as a sphingomyelin synthase inhibitor [95]. In addition, DCA was proved to inhibit amyloid β aggregation. Although these natural products showed moderate inhibition against SMS, they all acted on two or more targets. Thus, these compounds were not ideal chemical tools to study the potential roles of SMS in disease models.

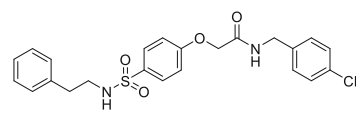
Medicinal chemists have put efforts into synthetic SMS inhibitors and made great progress in recent years. Swamy and coworkers designed a series of ceramide mimics based on ginkgolic acid which is a natural product and an SMS inhibitor. Among them, compound **5** showed moderate activity [96].



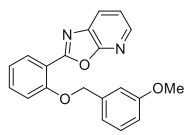
Compound 5
IC_{50} (SMS2) = 3 μM
selectivity (SMS2 vs SMS1) = 1.7
(2018)



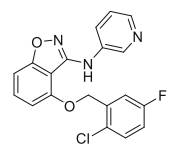
D2
IC_{50} (SMS2) = 14 μM
(2014)



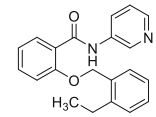
SAPA 1a
IC_{50} (SMS1) = 5.2 μM
(2015)



QY16
IC_{50} (SMS2) = 3 μM
selectivity (SMS2 vs SMS1) > 30
(2017)

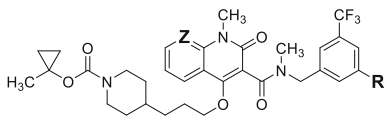


Compound 15w
IC_{50} (SMS2) = 100 nM
selectivity (SMS2 vs SMS1) = 560
(2018)



Ly93
IC_{50} (SMS2) = 91 nM
selectivity (SMS2 vs SMS1) = 1400
(2019)

A research group from Fudan University, China contributed to the development of SMS inhibitors. Deng and coworkers performed structure-based virtual screening in combination with chemical synthesis and bioassay [97]. They found a hit compound D2, which was the first small-molecule SMS inhibitor with potency close to the micromolar range. Based on the structure of lead compound D2, Qi and coworkers designed a series of oxazolopyridine derivatives with good selectivity [98]. Through a similar approach, Li from the same research group developed a new series of SAPA compounds, among which SAPA1a showed the best in vitro activity [99]. Progress of SMS inhibitors was made by Mo and coworkers in 2018 [100]. They developed 4-benzyloxybenzo[d]isoxazole-3-amine derivatives as potent and highly selective SMS2 inhibitors. Among them, compound 15w demonstrated good pharmacokinetics and attenuated chronic inflammation significantly in db/db mice. This was the first reported oral available selective SMS2 inhibitor. In the coming year, Li developed another oral available SMS2 inhibitor Ly93, with reported highest selectivity till then (1400-fold over SMS1) [101]. The 2-benzyloxybenzamide derivative Ly93 significantly decreased the plasma SM levels of C57BL/6 J mice and was capable of dose-dependently attenuating the atherosclerotic lesions in the root and the entire aorta in apolipoprotein E gene knockout mice. These preliminary molecular mechanism-of-action studies revealed SMS2 function in lipid homeostasis and inflammation process, which indicated that the selective inhibition of SMS2 would be a promising treatment for inflammation.



	Cmpd.	Z=	R=	IC_{50} (SMS2, nM)	selectivity (SMS2 vs SMS1)
(2017)	**3**	CH	CF3	6.5	150
(2020)	**37**	N	OMe	45	53

Another research group from Japan also made progress in this field. Adachi and coworkers developed a human SMS2 enzyme assay with a high-throughput mass spectrometry-based screening system and found a hit compound with the 2-quinolone scaffold. Further modification of the hit compound led to a potent and selective SMS2 inhibitor (compound 3, IC_{50}: 6.5 nM) [102]. Recently, the research group developed a new compound **37** as an effective in vivo tool for the study of the SMS2 enzyme. Compound **37** showed promising efficacy in reducing hepatic sphingomyelin levels in a mouse model [103].

12.2 Conclusion

Sphingolipid metabolism pathways play an essential role in maintaining cell homeostasis and are related to a variety of metabolic diseases, such as insulin resistance, metabolic syndrome, etc. The dynamic balance of sphingolipid levels in organisms is regulated by a variety of sphingolipid synthases, hydrolases and metabolic enzymes, such as sphingomyelinase (SMase), sphingomyelin synthase (SMS), serine palmitoyltransferase (SPT), ceramide synthase (CerS), glucosylceramide synthase (GCS), etc. The above-mentioned enzymes are potential targets for drug discoveries in metabolic disorders and receive a lot of research interests.

Medicinal chemists have discovered various natural products and synthetic small molecules targeting sphinogolipid metabolism-related enzymes. From the primary discovery of myriocin as an SPT inhibitor in 1990s, Fumonisin B1 as CerS inhibitor in early twenty-first century, to the very recent advances in SMS inhibitor recognition of Ly93 by Fudan University, hundreds of compounds were reported to be potential drugs to the perturbation of sphinogolipid metabolism and several of them come to preclinical stages. However, there are remaining challenges in drug development towards sphinogolipid metabolism. (1) The affinity of small-molecule inhibitors to target protein need great improvement. Except very few compounds mentioned in the chapter reaches a low IC50 to sub-100 nM level, the main inhibitors

to SMS, SMase and other related enzymes showed a weak affinity hindering their movements to clinical drugs. (2) Inhibitors with better selectivity are needed. Sphinogolipid metabolism is fundamental to the survival of cells and mammals, the lack of selectivity of sphinogolipid metabolism-related inhibitors will cause severe side effects or even death. (3) Distinguishing of clear indications of sphinogolipid metabolism-related drugs are favored. Due to the lack of understanding of sphinogolipid metabolism in the development of diseases, we do not have a lot of knowledge of the indications of sphinogolipid metabolism-related inhibitors. More clinical trials need to be done to clarify their therapeutic effects for human.

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