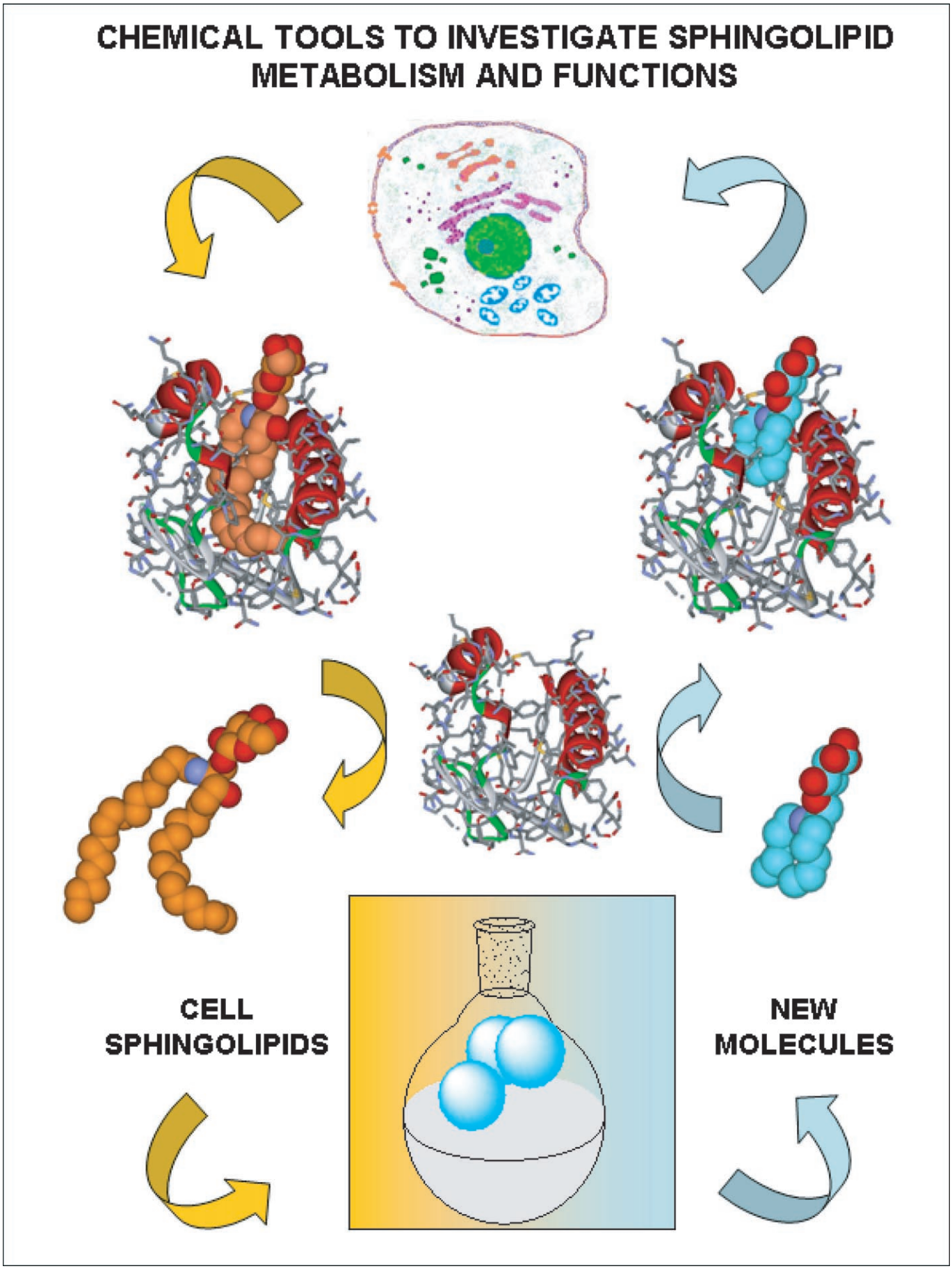

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Chemical Tools to Investigate Sphingolipid Metabolism and Functions

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Sphingolipids comprise an important group of biomolecules, some of which have been shown to play important roles in the regulation of many cell functions. From a structural standpoint, they all share a long 2-amino-1,3-diol chain, which can be either saturated (sphinganine), hydroxylated at C4 (phytosphingosine), or unsaturated at C4 (sphingosine) as in most mammalian cells. N*-acylation of sphingosine leads to ceramide, a key intermediate in sphingolipd metabolism that can be enzymatically modified at the C1-OH position to other biologically important sphingolipids, such as sphingomyelin or glycosphingolipids. In addition, both ceramide and sphingosine can be phosphorylated at C1-OH to give ceramide-1-phosphate and sphingosine-1-phosphate, respectively. To better understand the biological and biophysical roles of sphingolipids, many efforts have been made to design synthetic analogues as chemical tools able to unravel their structure–activity relationships, and to alter their cellular levels. This last approach has been thoroughly studied by the development of specific inhibitors of some key enzymes that play an important role in biosynthesis or metabolism of these intriguing lipids. With the above premises in mind, the aim of this review is to collect, in a systematic way, the recent efforts described in the literature leading to the development of new chemical entities specifically designed to achieve the above goals.*

1. Introduction

Sphingolipids (SLs) comprise ubiquitous natural products with essential roles in cell biology.[1] From a chemical perspective, mammalian SLs derive mainly from (*E*)-2-amino-4-octadecen-1,3-diol or sphingosine (Sph), although a minor portion arise from the saturated analogue sphinganine or its 4-hydroxy derivative phytosphingosine (PhytoSph). Ceramides (Cer) are afforded by *N*-acylation of these sphingoid bases with a variety of fatty acids, with those containing C18 and C20 carbon atoms being the most common in mammals. Complex SLs arise from functionalization at the C1-OH of Cer. Thus, glucosylation leads to glucosyl ceramide (GlcCer), which is further glycosylated to higher glycosphingolipids (GSLs), common components of cell membranes.[2] Esterification with phosphorylcholine leads to sphingomyelin (SM), whereas both Cer and Sph can also be phosphorylated in cells to the corresponding phosphate esters, ceramide-1-phosphate (CerP),[3] and sphingosine-1-phosphate (S1P),[4,5] respectively, which play important roles as signaling molecules (Figure 1). Fungal SLs exhibit some structural differences with respect to mammalian SLs, such as the presence of additional unsaturations and/or alkyl substituents or an extra hydroxyl group at the C4 position of the aliphatic chain. This is the case of phytosphingosine (PhytoSph, Figure 1), which, despite being an important component of epidermal ceramide in humans,[6] serves as the central scaffold for the synthesis of most fungal and plant SLs.[7] Additionally, many complex fungal SLs are derived from inositol phosphoceramide (IPC, Figure 1), which can be further glycosylated to the glycosylinositolphosphorylceramides (GIPCs), mannosyl inositolphosphoryl ceramide (MIPC), and mannosyl bis(inositolphosphoryl) ceramide ($M(IP)_2C$) (see section 2.5). These are also found in plants and protozoa but not in mammals or other higher animals, thus offering excellent opportunities for the development of new antifungal drugs.[8,9]

Cer plays a central role in the SL biosynthesis in mammals. Thus, it can be produced by the anabolic or de novo route,[10] and by degradation of SM or complex GSLs (Figure 2). The biological roles of SLs are complex and, in many instances, they are closely interrelated. In all cases, metabolic transformations are catalyzed by specific enzymes, whose modulation by means of specific agonists or antagonists represent an invaluable tool for a better understanding of SL-mediated cell regulation pathways. In addition, recent advances in the field have shed light on the role of specific extracellular receptors for some SLs, and on their implication in some metabolic disorders and pathologies.[11–14] In addition, SLs have also been recognized to play a structural role as cell membrane components. In this context, Cer, in association with other lipids and cholesterol, is involved in the formation of specific membrane do-

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mains, called rafts, which are important in determining the biophysical properties of the membranes and also to take part in processes such as protein concentration and oligomerization, cell adhesion, or cell resistance towards infection from particular viruses.[15,16]

Chemical approaches to gain insight into regulatory mechanisms and functions of SLs have been carried out based on two general approaches: 1) structural modifications of natural products acting as enzyme inhibitors of a particular biosynthetic step, and 2) chemical modifications of natural SLs to find structural analogues with better pharmacological properties (increased potency, selectivity, metabolic stability, cell membrane permeability, etc.).

2. Natural products and analogues

Natural products comprise a rich source of biologically active compounds, and their chemical modification represents a classical approach in the search of new hits for drug discovery. Thus, it is widely accepted that natural products are viable, biologically-validated starting points for library design.[17] Furthermore, with the advent of new and efficient techniques of high-throughput screening (HTS), alternative biochemical pro-

Antonio Delgado obtained the PhD in Pharmacy in 1986 from the University of Barcelona, where he is Associate Professor of Organic Chemistry since 1988. After two years as postdoctoral fellow of the Spanish Research Council (CSIC) at Cornell University, he became a staff member of RUBAM (Research Unit on BioActive Molecules, CSIC). His research interests are the design, synthesis, and biological evaluation of new pharmacological tools as modulators of sphingolipid metabolism with potential therapeutic value. He is also interested in synthetic methodology, as well as in the use of combinatorial chemistry for the generation of focused libraries. His academic activities have been mainly focused on Medicinal Chemistry, Organic Chemistry, and Synthetic Organic Chemistry, being the coauthor of three textbooks on these topics.

Josefina Casas obtained her PhD in Biochemistry from the Autonomous University of Barcelona (Spain) in 1987. She spent two years as a postdoctoral fellow at the University of California, in Davis with a Fulbright fellowship, working on insect biochemistry and biochemical toxicology. Then, she joined to the Spanish Research Council (CSIC) were currently works as senior research scientist. She has been collaborating, from the biochemical point of view, in different projects related to oxidizing enzymes from mammalian and plant sources. Nowadays her main research interests are in the areas of chemistry and biochemistry of sphingolipids

Amadeu Llebaria graduated at "Institut Químic de Sarrià" and obtained his PhD in Organic Chemistry at the "Universitat Autònoma de Barcelona" doing research at the CSIC (Spanish Research Council) on transition-metal mediated organic synthesis in Prof. J. M. Moretó's group. After a postdoctoral period in Göttingen with Prof. Armin de Meijere, he obtained a permanent position at CSIC where he is now staff research scientist and member of RUBAM (Research Unit on BioActive Molecules). His activities include the design and synthesis of biologically active molecules with an emphasis on lipid biological chemistry.

Jose Luis Abad obtained a masters degree in organic chemistry in 1991 and a PhD from the University of Barcelona in 1995 in the field of the bioorganic chemistry of steroidogenesis. Then he joined the group of Prof. Roger A. Jones (Rutgers University, NJ, USA) where he worked on the synthesis and applications of mass-labeled nucleotides. After 3 years at Rutgers, he returned to the CSIC and worked on the synthesis and use of stereospecifically deuterated fatty acids to decipher mechanistic aspects of acyl-CoA desaturase reactions. He is now a CSIC Ramon y Cajal Scientist and his research is currently focused on the synthesis of sphingolipid analogues. His research interests include the organic synthesis by both chemical and enzymatic methods of mass-labeled lipids and lipid analogues for both structural and biological studies.

Gemma Fabriàs obtained her PhD from the University of Barcelona in 1985 and became a staff scientist of the CSIC in 1987. After a two year Fulbright postdoctoral position at the NYSAES, Cornell University (USA), she joined the Institute of Bioorganic Chemistry (CSIC). Her research was focused on the biosynthesis of fatty acids and fatty acid derivatives, such as insect sex pheromones, with special interest in the mechanistics of fatty acyl-desaturases. Her current research is directed towards the study of metabolic pathways and functions of sphingolipids, with a particular awareness for dihydroceramide desaturase, from basic studies to the discovery of drugable inhibitors. She has published over 70 journal publications and is co-inventor of 6 patents.

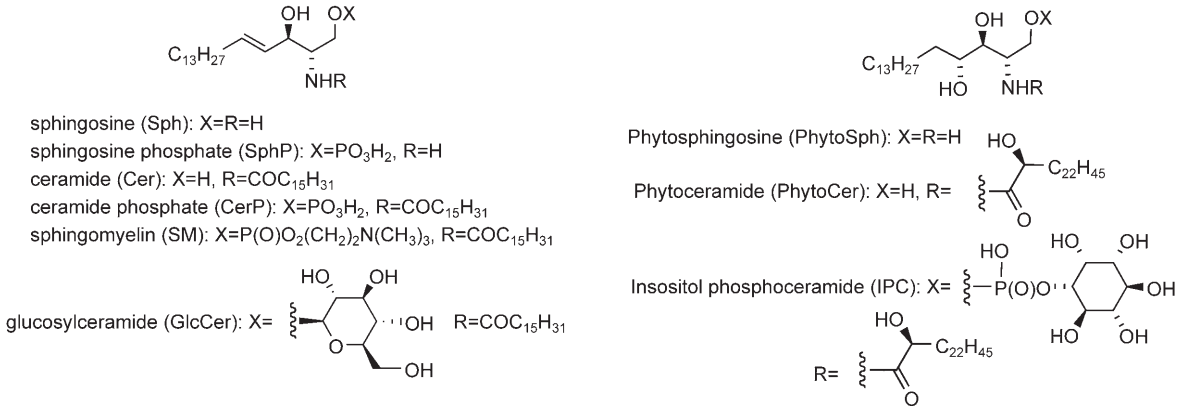


Figure 1. Some mammalian and fungal SL structures.

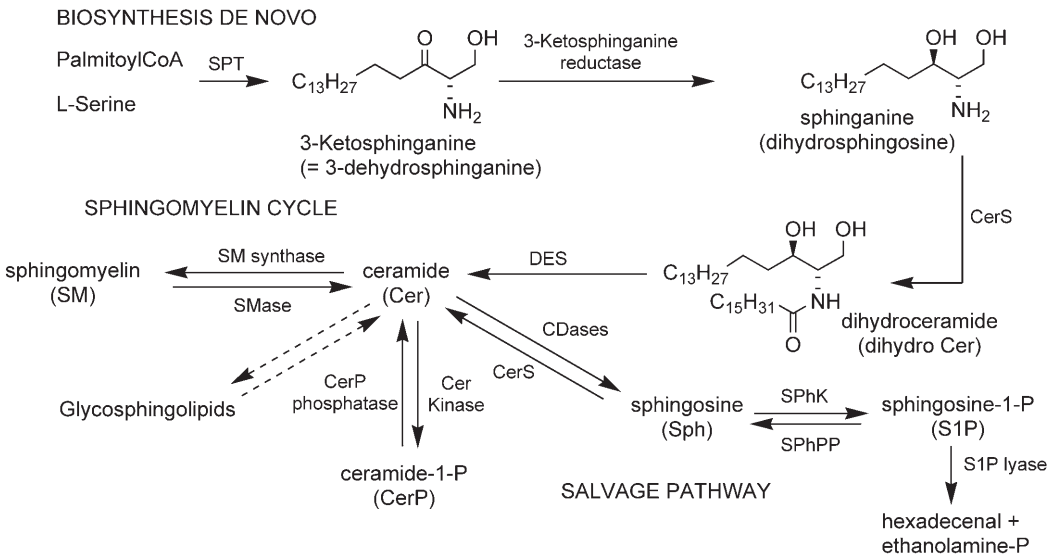


Figure 2. Biosynthesis of SLs in mammalian cells.

files for known natural products have been unraveled. This is the case of docetaxel and camptothecin, two antineoplasic agents that have been recently discovered as strong sphingosine kinase 1 inhibitors.[18]

In the following sections, different natural products with reported activities as inhibitors of enzymes involved in the biosynthesis and metabolism of SLs will be presented.

2.1. Inhibitors of serine palmitoyl transferase

In the first step of the de novo biosynthesis of ceramide, serine palmitoyltransferase (SPT) catalyzes formation of 3-ketodihydrosphingosine from serine and palmitoyl CoA (Figure 2). Several potent and selective SPT inhibitors have been identified from natural sources (Figure 3). Some are claimed to inhibit SPT by mimicking the natural intermediates of the enzymatic reaction,[19] such as the antifungal molecule sphingofungin B.[20] Structure–activity relationship studies conducted with this compound have led to the conclusion

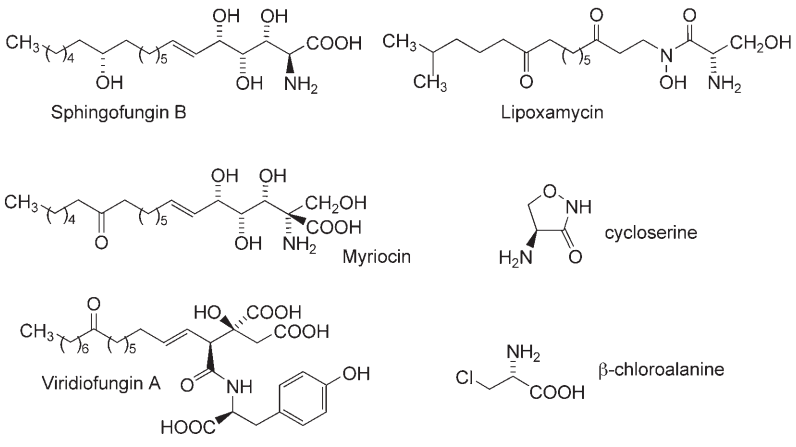


Figure 3. Inhibitors of SPT of natural origin.

that, although the C14 hydroxyl group confers potent SPT inhibition, its configuration is not essential for activity. Conversely, activity is dependent on the configurations of the stereogenic centers at positions α, β, γ, and ∂ with respect to the carboxyl group.[21]

The antifungal antibiotic myriocin (Figure 3) was initially described as a SPT inhibitor with some interesting immunosuppressant properties.[22] A preliminary structure–activity relationship study showed that the C4-OH, the C6 double bond, the C14-keto groups, and the C3-OH configuration, were not essential for biological activity.[23–25] Simplified myriocin analogues, such as **1** (Figure 4),[26] with enhanced water solubility

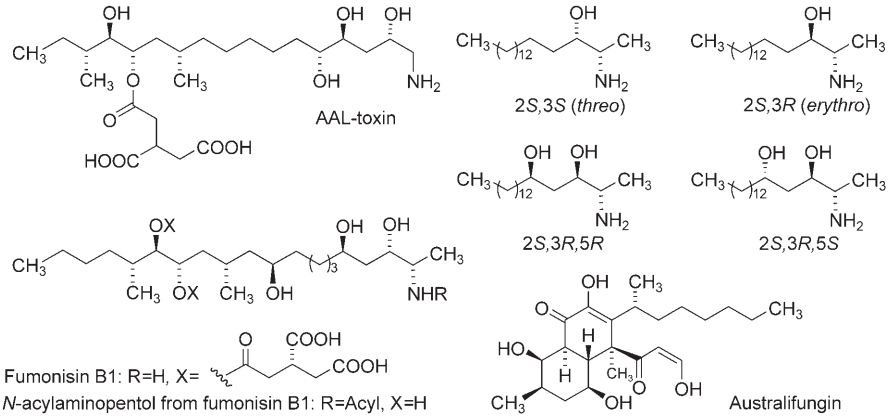


Figure 5. Inhibitors of CerS.

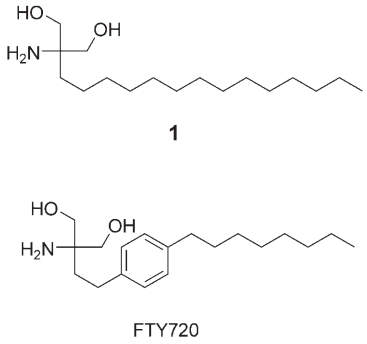


Figure 4. Synthetic analogues derived from simplification of myriocin.

and reduced toxicity were then designed to improve the immunosuppressant properties of the natural product. Structural modifications led to a series of conformationally restricted hydrocarbon chain analogues by incorporation of an aromatic ring, from which FTY720 (Figure 4) emerged as a new lead compound with immunosuppressant properties.[27] Recent studies have focused on the design and immunosuppressant properties of FTY720 analogues, which can also be considered as S1P analogues and will be described in detail in section 3.3.1.

2.2. Inhibitors of ceramide synthase

Several fungal metabolites have been isolated and identified as CerS inhibitors. Among them, fumonisins,[28] the fumonisin-related AAL-toxin,[29] and australifungins[30] have been more thoroughly studied (Figure 5).

The fumonisins family are produced by *Fusarium verticillioides* and *moniliforme*, Fumonisin B1 (FB1) being the most representative member of this class of compounds. FB1 contains an aminoicosapentol backbone with two hydroxyl groups esterified with 3-carboxy-1,5-pentanedioic acid. A similar structure is present in AAL-toxin, produced by the fungus *Alternaria alternata* var. *lycopersici*. Several studies[31] suggest that inhibition occurs because CerS recognizes the aminopentol moiety (Figure 5), which competes for binding to the sphingoid base substrate, and the dicarboxylic acid side chains, which act as analogues of fatty acyl-CoA phosphate groups and interfere with the binding of the acyl donor to the catalyst. The *O*-deacylated form of FB1 is a weak CerS inhibitor. However, it is also a substrate of CerS and its *N*-acylated forms are potent CerS inhibitors.[31] This sequence of metabolic transformations may occur in vivo and play a role in the diseases caused by fumonisins. In this regard, these *N*-acylated forms represent a new category of CerS inhibitors. Structure–function relationship studies showed that both *erythro*- and *threo*-2-amino-3-hydroxy-, and all (2*S*) stereoisomers of 2-amino-3,5-dihydroxy-octadecanes are CerS inhibitors and are acylated by CerS with the highest apparent V_{max}/K_M for the 2*S*,3*R* analogues (Figure 5).

FB1 inhibits fungal CerS in vitro, but its activity in whole cells is very weak, probably because of deficient internalization. In contrast, australifungin (Figure 5), a micotoxin isolated from *Sporormiella australis*, is a very potent inhibitor of fungal CerS from several species. However, both the alpha-diketone and beta-ketoaldehyde functional groups present in this compound confer australifungin with a low chemical stability, which limits its use.[30]

2.3. Inhibitors of sphingomyelinases.

Sphingomyelin (SM) hydrolysis can be carried out by different sphingomyelinases (SMase). Most mammalian cells are capable of signaling through the SM pathway, which can be activated both by specific receptors and by stress (UV, oxidants, radiation).[11] SM hydrolysis by SMases produces phosphorylcholine and the intracellular effector Cer. Generation of Cer in various cellular systems is currently recognized as critical to the initiation of vital cellular processes such as differentiation, cell proliferation, and apoptosis. SMases differ in their catalytic properties, subcellular location, and probably in their mode of regula-

tion. Thus, lysosomal degradation of SM takes place by the action of acid SMase, although a Zn-dependent secretary form has also been identified.[32] This second form is differentially glycosylated and processed, and is believed to function in inflammatory processes, including atherogenesis.[33] It is secreted by macrophages, human skin fibroblasts, and human vascular endothelial cells, and is the only SMase responsible for an extracellular hydrolysis of SM.[34,35] It has been reported that secreted SMase is required for infection by Neisseria gonorrhoea[36,37] and rhinoviruses,[38] and it has been suggested that this enzyme may play a critical role in the development of apoptosis and organ failure in sepsis.[39] On the other hand, neutral SMase is a Mg-dependent enzyme located in plasma membranes,[40] which was purified and characterized by Bernardo et al.[41] In addition, an Mg-independent neutral SMase is also found in cytosol[42] and an alkaline SMase has been identified in the gastrointestinal tract.[43]

Several natural products exhibit interesting inhibitory activities against SMases. This is the case of Manumycin A (Figure 6),

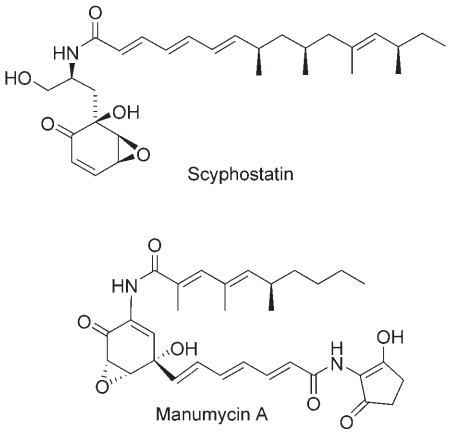


Figure 6. Natural products as SMase inhibitors.

an irreversible inhibitor of the neutral, but not the acidic, SMase,[44] and the structurally related scyphostatin (Figure 6). Primary kinetic data have shown that scyphostatin is a reversible inhibitor of both neutral SMase (IC_{50} 1 μM) and acidic SMase (IC_{50} 49 μM), but the precise type of inhibition has not been deciphered.[45]

Different scyphostatin analogues have been prepared and their activity has been investigated. Most of them present modifications both on the epoxycyclohexenone and acyl chain moieties. This is the case of spiroepoxide **2** (Figure 7), an irreversible and specific neutral SMase inhibitor that seems to bind to the enzyme active site.[46] Preliminary SAR studies showed the importance of the primary hydroxyl group for enzyme inhibition, as evidenced by the drastic decrease in inhibitory activity (2.5-fold and 3.8-fold reduction, respectively, after a 90 min preincubation with the analogue [100 μM]) upon its replacement by H or phenyl groups, respectively.[47]

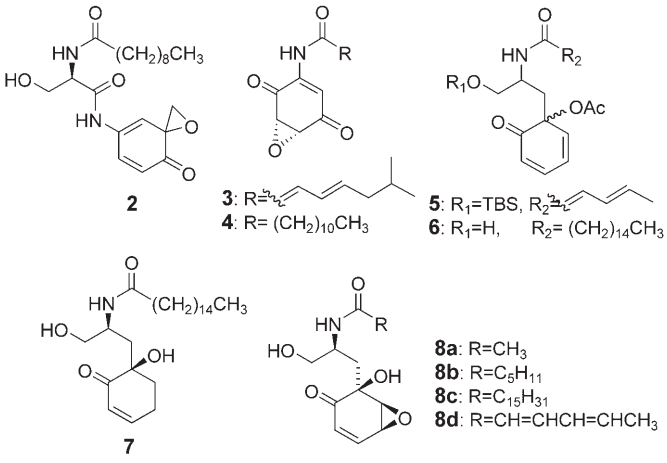


Figure 7. Synthetic analogues of scyphostatin.

In a related work,[48] analogues **3** and **4** (Figure 7) sharing the epoxy group and characteristic cyclohexenone structural features of Scyphostatin and Manumycin A were synthesized[48] and turned out to be selective irreversible inhibitors of neutral SMase. Additional SAR studies on related analogues confirmed that the presence of the cyclohexenone moiety was crucial for activity in these systems.[44] Analogues **5** and **6** (Figure 7), lacking the epoxide moiety, behaved as irreversible SMase inhibitors with activity comparable with spiroepoxide **2**. Interestingly, the epoxy moiety seems not to be a strict requirement for enzyme inhibition.[49] In this context, analogue **7** (Figure 7) has been reported as the first scyphostatin analogue with a reversible neutral SMase inhibitory activity.[50] Very recently, a series of *N*-acyl scyphostatin analogues have shown the dramatic influence of the fatty side chain on the mode of inhibition. Thus, unlike the natural product scyphostatin, compounds **8a**–**d** behaved as irreversible inhibitors of neutral Smase,[51] the palmitoyl analogue being the most potent of the series.

Recently, a new family of compounds, named sphingolactones, have been reported as irreversible inhibitors of neutral SMase (Figure 8) with negligible activity on acidic SMase. Com-

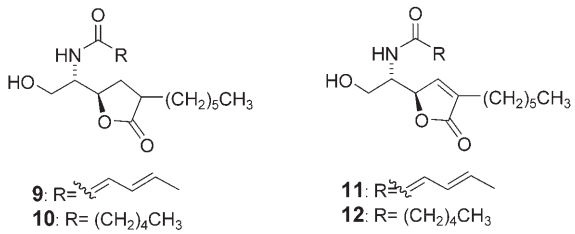


Figure 8. Sphingolactones, a new family of SMase inhibitors.

pounds with a saturated butyrolactone moiety (**9**–**10**) were better inhibitors than the corresponding unsaturated analogues (**11**–**12**), the (2*E*,4*E*)-hexadienoic acid derivative **9** being the most active one (data not indicated).[52]

2.4. Inhibitors of glucosylation and deglucosylation of ceramide

Glycosylation of Cer at the C1-OH position provides glycosphingolipids (GSLs), a wide structural group of cellular components that play important roles in biological systems as essential constituents of cell membranes. They differ in the nature and the number of sugar units linked to the Cer scaffold. As depicted in Figure 9, the biosynthesis of most GSL in mammals starts at the Golgi level with glucosylation of Cer, a process catalyzed by glucosyl ceramide synthase (GlcCerS, UDP-glucose:*N*-acylsphingosine glucosyltransferase), in which the α-glucosyl residue is transferred from UDP-glucose to Cer with inversion of configuration at the anomeric center of the sugar. The initially formed glucosyl ceramide (GlcCer) serves as a starting building block for the synthesis of several complex GSL families.[53] On the other hand, condensation of Cer with galactose leads to galactosylceramide, the starting point for the biosynthesis of other GSL series.[54]

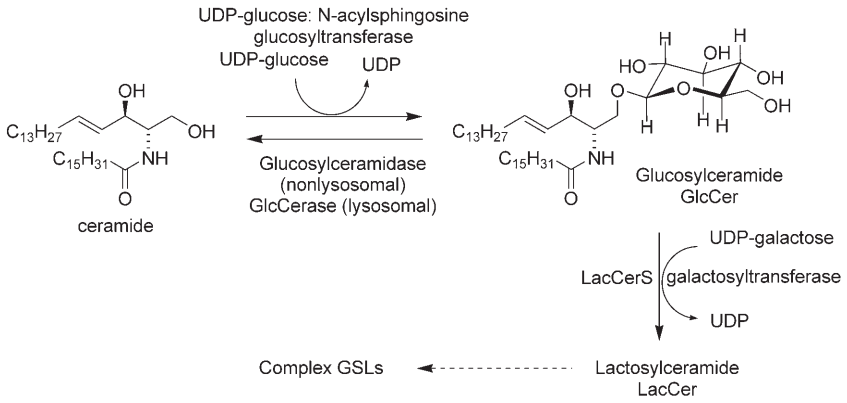


Figure 9. Biosynthesis of GlcCer and complex GSLs.

GSLs play a protective role in the cell against chemical and mechanical damage. In addition to these mechanical properties, GSLs are expressed as complex patterns, which have been recognized to take an active part in processes such as cell adhesion, cell growth regulation, and differentiation.[55] GSLs also play important roles within cellular membranes, where they form microdomains (rafts), which can regulate the activity of some receptors in the plasma membrane, and hence signal transduction.[16] Catabolic processes from GSLs take place in the lysosomes. These are specialized cell compartments where glycon-specific glycosidases with acid pH optima hydrolyze GSLs, arising from endocytosis from cell membranes.

Catabolism of GlcCer (Figure 9) in lysosomes is carried out by GlcCer β-glucosidase, a process that requires the participation of saposin C (Sap-C).[56] However, the existence of a nonlysosomal GlcCer β-glucosidase near the cell surface has also been documented.[57] This enzyme is an integral membrane protein that shows different specificity towards substrates and inhibitors, compared with the lysosomal enzyme.[58]

Metabolic disorders of GSLs metabolism constitute a rare group of inherited diseases, called sphingolipidoses, which have diverse, often neurodegenerative and severe phenotypes.[59] Many of these disorders are caused by a mutation in catabolic enzymes that reduce their efficiency, giving rise to increased lysosomal storage products. Recent advances in the treatment of some of these pathologies have been achieved through direct intervention on particular key enzymes involved in GSLs biosynthesis and/or degradation, as will be discussed below.

Iminosugars (polyhydroxypiperidines) comprise an important group of glycosylation enzymes inhibitors.[60,61] In particular, *N*-butyldeoxynojirimycin (NBDNJ) and *N*-butyldeoxygalactonojirimycin (NBDGJ, Figure 10), designed as synthetic analogues of the polyhydroxypiperidine alkaloid deoxynojirimycin (DNJ), have shown inhibitory activity against GlcCerS with IC_{50} values around 20 and 40 μM, respectively.[62,63] On the other hand, 1-deoxygalactonojirimycin (DGJ) is a potent competitive inhibitor of

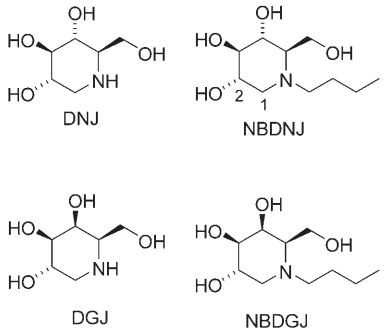


Figure 10. Iminosugars as glycosidase inhibitors.

α-galactosidase A (α-Gal A, IC_{50} 0.2 μM with 4-methylumbelliferyl-α-galactoside as substrate).[64]

Despite having been described as sugar analogues acting as transition state mimics of the glycosylation step, Butters et al have shown that inhibition of GlcCerS by NBDNJ is competitive for Cer and noncompetitive for UDP-glucose, indicating their putative mimicry with Cer.[65] Structure–activity studies carried out in iminosugars showed the importance of an *N*-alkyl chain for GlcCerS inhibition. Thus, a chain length of between three and six carbon atoms is required, whereas longer chains increase inhibition and toxicity. Modeling studies led to the assumption that NBDNJ analogues substituted at the C2 oxygen atom with a long alkyl chain should lead to potent inhibitors because of their closer similarity with Cer. However, biological evaluation of analogues designed according to the above hy-

pothesis failed to yield the expected results.[66] Some iminosugars have been approved for the treatment of certain sphingolipidoses. In particular, NBDNJ (miglustat, Zavesca)[67,68] is currently used for substrate reduction therapy[69] of both type I Gaucher disease and Fabry disease.

Catabolism of GlcCer can be modulated by the action of specific inhibitors. Thus, D-gluconolactone is a competitive and selective inhibitor of the nonlysosomal GlcCer β-glucosidase,[57] whereas conduritol B epoxide (CBE),[70,71] cyclophellitol,[72] and conduritol B aziridine (CBA)[73] are well-established irreversible inhibitors of lysosomal GlcCer β-glucosidase (Figure 11).

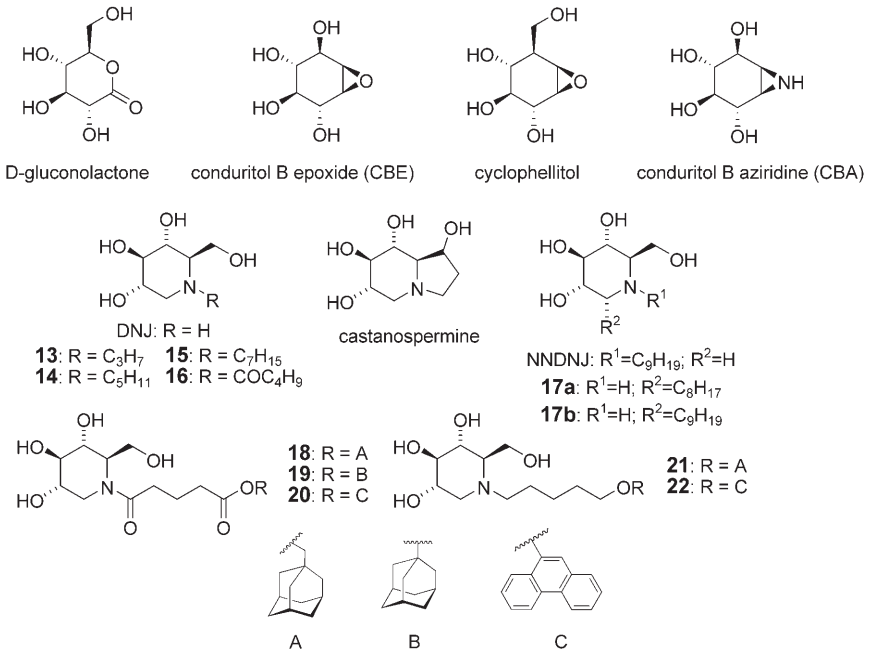


Figure 11. Natural and synthetic GlcCer β-glucosidase inhibitors.

The polyhydroxypiperidine alkaloids castanospermine[74,75] and DNJ[76–79] (Figures 10 and 11) have been described as GlcCer β-glucosidase inhibitors because of their structural similarity with the putative oxocarbenium ion that develops in the course of the hydrolysis step. A series of *N*-alkyl substituted DNJ analogues (NBDNJ, Figure 10, and compounds **13**–**16**, Figure 11) were described as GlcCer β-glucosidase inhibitors of lysosomal GlcCer β-glucosidase isolated from spleenic membrane suspensions, with IC_{50} values of 424, 332, 8.5, 13.5, and 83 μM, respectively.[58] These values are above and below those of DNJ in the same assay (141 μM). Interestingly, some analogues bearing a *N*-alkyl spacer and a large hydrophobic group (compounds **21** and **22**, Figure 11), showed activities at nanomolar concentrations (1.7 and 97 nM, respectively) against nonlysosomal GlcCer β-glucosidase. In this case, the hydrophobic groups were designed to favor the interaction with the cell membrane, taking into account the enzyme location. The nature of the spacer group seems important for inhibition, as evidenced by amido ester analogues **18**–**20**, with IC_{50} values of 461, 306, and 39 μM, respectively, in the same assay.[58]

More recently, the active-site specific chaperone activity,[80] initially described for the ability of DGJ (Figure 10) to enhance α-Gal A activity in Fabry lymphoblasts at subinhibitory concentrations,[64] has been noted in some polyhydroxypiperidine derivatives of natural and synthetic origin.[81] Among them, *N*-alkylated DNJ analogues have been recognized as GlcCer β-glucosidase inhibitors able to work as chemical chaperones of GlcCer β-glucosidase, and hence, to increase its cellular activity by assisting in the proper folding of the enzyme during the course of its maturation process in the endoplasmic reticulum. In this context, *N*-nonyldeoxynojirimycin (NNDNJ)[82,83] (Figure 11) and NBDNJ[84] (Figure 10) have shown promising chaperone effects on GlcCer β-glucosidase in different cell studies. In particular, 10 μM NNDNJ leads to a two-fold increase of GlcCer β-glucosidase activity,[82] whereas 10 μM NBDNJ leads to an increase of 1.3–9.9 times GlcCer β-glucosidase activity depending on the mutation under study.[84]

In recent years, a series of new α- and β-1-C-alkyl-1-DNJ analogues has emerged as selective inhibitors of some glycosidases.[85] Among them, some α-1-C-alkyl-DNJ derivatives (**17**, Figure 11) have been described as GlcCer β-glucosidase inhibitors with significant chaperone activity.[86,87] In particular, the C_α-octyl and C_α-nonyl DNJ analogues (**17a** and **17b**, Figure 11) have been described to be potent GlcCer β-glucosidase inhibitors (IC_{50} 0.50 and 0.27 μM, respectively). In addition, compound **17a** has been reported to increase GlcCer β-glucosidase activity by 1.7–2.0-fold in different Gaucher N370S cell lines.[87]

Isofagomine, a synthetic analogue of the alkaloid fagomine (Figure 12), has been described as a general inhibitor of β-glycosidases.[88–90] It was designed taking into account its close geometric and electronic similarities with the carbocationic intermediate that develops in the first step of the hydrolysis of a β-glycosidic bond. Isofagomine is also a potent GlcCer β-glucosidase inhibitor with IC_{50} and K_i values of 56 and 25 nM, respectively,[91] and shows a chaperone effect by increasing the activity of N370S mutant GlcCer β-glucosidase in Gaucher fibroblasts.[81,92]

Some isofagomine analogues bearing an alkyl chain at the C6 position (compounds **23**–**28**, Figure 12) have also been described as selective GlcCer β-glucosidase inhibitors, with IC_{50} values ranging between 160 nM (compound **24**) and 0.6 nM (compound **28**, the most potent GlcCer β-glucosidase known to date).[93,91] This finding led the authors to suggest the presence of a hydrophobic domain close to the GlcCer β-glucosi-

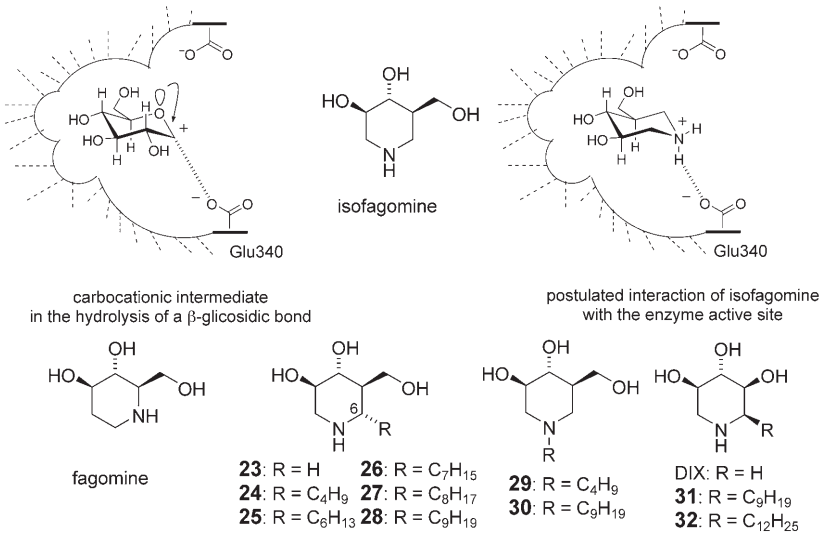


Figure 12. Isofagomine analogues and postulated model for the interaction of isofagomine with β-glucosidases.

dase active site. According to this hypothesis, binding interactions would be reinforced by an ionic bond between the protonated heterocyclic amine with one of the carboxylate residues (Glu 340) at the enzyme active site. This is consistent with the observation that the corresponding isomeric *N*-alkyl derivatives **29** and **30** (Figure 12) were markedly less potent inhibitors (IC_{50} greater than 40 μM). Some of the above analogues have been evaluated for their chaperone activity on human GlcCer β-glucosidase but no results have been published yet.[91]

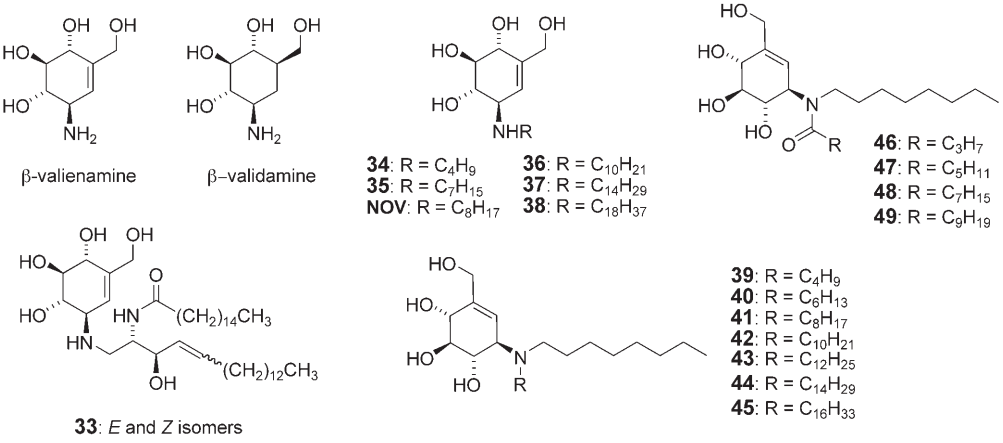


Figure 13. Aminocyclitols as GlcCer β-glucosidase inhibitors.

In a recent work,[86] a series of 1,5-dideoxy-1,5-imino-D-xylitol (DIX, Figure 12) and 1-alkyl substituted derivatives thereof (**31** and **32**, Figure 12) were synthesized and evaluated as GlcCer β-glucosidase inhibitors. Iminosugar **31** (K_i 2.2 nM) is a strong GlcCer β-glucosidase inhibitor able to increase GlcCer β-glucosidase activity 1.8-fold at extremely low concentrations (1 to 50 nM) when tested in N370S fibroblast cultures for four days, thus proving its chaperone effect.[86] These findings offer a new potential therapeutic alternative for the treatment of Gaucher disease, in addition to the current substrate reduction or enzyme replacement therapies.[94,95]

Aminocyclitols constitute another important group of natural products with GlcCer β-glucosidase activity. The polyhydroxylated alkaloids β-valienamine and its saturated analogue β-validamine are representative examples (Figure 13). Incorporation of these alkaloids as glucose surrogates in GlcCer afforded the first reported GlcCerase inhibitors (compounds **33**).[96,97] Interestingly, both *E* and *Z* isomers behaved as potent and selective GlcCer β-glucosidase inhibitors (IC_{50}, 0.3 and 0.1 μM for *E* and *Z* isomers, respectively), whereas β-validamine analogues were less potent.[98]

Some *N*-alkyl derivatives of β-valienamine[97] showed GlcCer β-glucosidase inhibitory activity (compounds **34**–**38**, Figure 13), the corresponding *N*-octyl derivative (*N*-octyl-β-valienamine, NOV) being the more potent of the series (IC_{50} = 0.03 μM). In a recent work,[99] NOV has been shown to increase the activity of a defective isoform of GlcCer β-glucosidase found in Gaucher disease. In particular, the maximum effect of NOV was observed in F213I homozygous cells in which NOV treatment at 30 μM for 4 days caused an approximately six-fold increase in the enzyme activity, up to approximately 80% of the activity in control cells. However, NOV was not effective in Gaucher cells with other mutations, such as N370S, L444P, 84CG, and RecNcil.

Tertiary *N*-alkyl (compounds **39**–**45**, Figure 13) and *N*-acyl NOV derivatives (compounds **46**–**49**, Figure 13) were also synthesized and tested as GlcCer β-glucosidase inhibitors to elucidate the role of the hydrophobic portion around the nitrogen atom (Figure 13).[100] It is worth noting that *N*-acyl derivatives lacked inhibitory activity, whereas the corresponding *N*-alkyl analogues were strong GlcCer β-glucosidase inhibitors, with IC_{50} values ranging between 1.5 and 4.2 μM. In a related work, a series of PDMP analogues (see also section 3.1.2) containing the β-valienamine and β-validamine moieties and all the possible configurations around the C2–C3 moiety of the PDMP framework were synthesized (Figure 14).[101] Valienamine derivatives **50a** were stronger GlcCer β-glucosidase inhibitors (IC_{50}

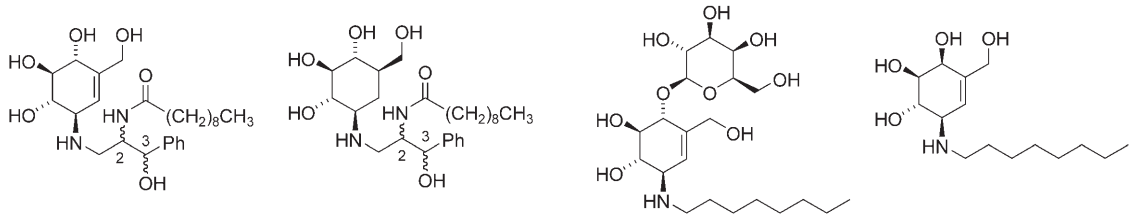


Figure 14. *N*-substituted β-valienamine (**50a** and **50c**), 4-epi-β-valienamine (NOEV), and β-validamine (**50b**) derivatives.

0.3–0.7 μM) than validamine counterparts **50b** (IC_{50} 5–20 μM). In a later work, a moderate GlcCer β-glucosidase activity was described for the 4-*O*-(β-D-galactopyranosyl) β-valienamine derivative **50c** (IC_{50} 20 μM, Figure 14). Its activity was rationalized by partial enzymatic hydrolysis to the potent inhibitor NOV (Figure 13).[102]

The galactose derivative *N*-octyl-4-epi-β-valienamine (NOEV, Figure 14) is a potent inhibitor of lysosomal β-galactosidase in vitro with potential application as a chemical chaperone in Morquio B disease, a human neurogenetic β-galactosidosis disease. Addition of NOEV to the culture medium increased mutant enzyme activity, around 20-fold, in cultured human or murine fibroblasts at low intracellular concentrations (0.2 μM), resulting in a marked decrease of intracellular substrate storage.[103]

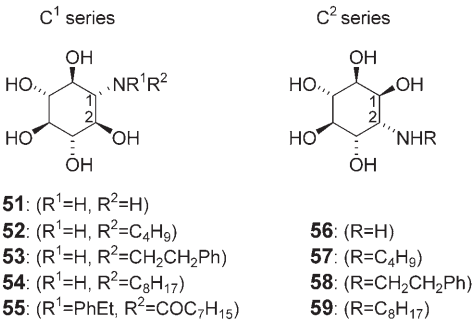


Figure 15. Inosamine derivatives as GlcCer β-glucosidase inhibitors.

Finally, a series of inosamine derivatives **51**–**59**, structurally related to aminocyclitols, have recently been described as selective GlcCer β-glucosidase inhibitors. (Figure 15) Interestingly, among the two diastereomeric series described in this work, only some analogues of the C1 series (compounds **51**–**55**) showed a significant inhibitory activity against recombinant GlcCer β-glucosidase, with IC_{50} values of approximately 10–15 μM.[104]

2.5. Natural inhibitors of fungal sphingolipid biosynthesis

SL are ubiquitous components of cellular membranes located in the external leaflet of the fungal cytoplasmic membrane. In contrast to mammalian cells, fungi have specific inositol phosphorylceramides composed of the long chain base phytoceramide (PhytoCer), characterized by the presence of an *N*-acyl group derived from a 2-hydroxy C24 fatty acid (Figure 1). Despite the similarities between SL biosynthesis in mammalian and fungal cells, several enzymes are found only in fungi. One of these enzymes is inositol phosphoryl ceramide synthase (IPC synthase). This essential enzyme in fungal metabolism catalyzes the transfer of phosphatidyl inositol to phytoceramide to afford IPC. This is further modified by reaction with GDP-mannose to yield mannosylinositolphosphorylceramide (MIPC) and mannosyldiinositoldiphosphorylceramide ($M(IP)_2C$) by the subsequent addition of one or two inositol phosphate residues, respectively (Figure 16).

The uniqueness of IPC synthase in fungal biosynthesis makes it an excellent target for the rational design and development of new, selective, and nontoxic antifungal

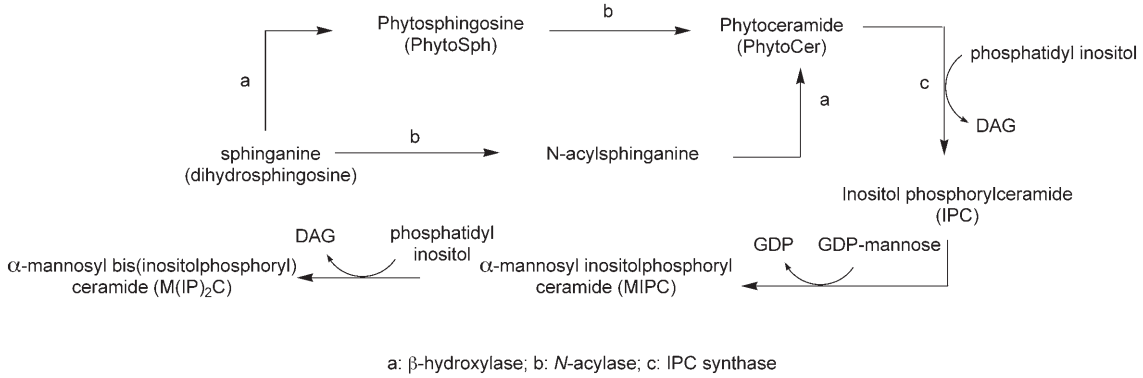


Figure 16. SL biosynthesis in fungi.

agents.[105–107] Three natural products have been described so far as efficient IPC synthase inhibitors: Aureobasidin A (AbA), Khafrefungin (Khf), and Galbonolide A (GbA, Rustmicin) (Figure 17)

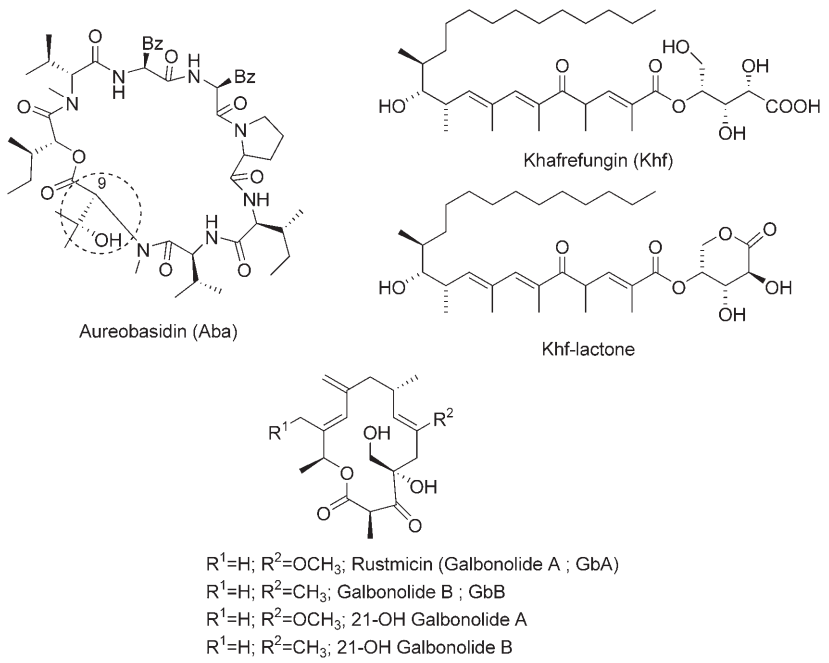


Figure 17. IPC synthase inhibitors of natural origin.

AbA is a cyclic depsipeptide isolated from the fermentation broth of *Aureobasidium pullulans*.[108] Its structure was initially determined by a combination of analytical techniques and the study of degradation products[109] and further confirmed by total synthesis[110,111] and X-ray analysis.[112] AbA presents a singular L-β-hydroxy-*N*-methylvaline fragment at position 9 (Figure 17) which is crucial for activity.[113] Modified analogues at different positions led to compounds with reduced antifungal activity against different species.[114]

Khf was isolated from the fermentation broth of a sterile fungal mycelium.[115] It consists of an aldonic acid unit esterified at the C4 position with a C22 linear polyketide acid (Figure 17). The absolute stereochemistry has been determined by total synthesis[116–118] and a series of modified analogues were synthesized and evaluated.[119] Thus, whereas most of the Khf derivatives lost antifungal activity, the lactone derivative (Khf-lactone, Figure 17) had almost the same activity as Khf, suggesting an equilibrium between natural Khf and its lactone form. In general, structure–activity relationships (SAR) revealed that the stereochemistry and the functional groups along the polyketide chain are crucial for the antifungal activity as well as the ester group linking the above chain with the aldonic acid unit. The requirement of an ester group for IPC synthase inhibition has been interpreted as indirect evidence of a nucleophilic attack upon the ester unit for enzyme inhibition. In addition, a macrocyclic Khf derivative structurally related to GbA was also synthesized, but the antifungal activity was lost. These results suggest that the structure of Khf might be strictly recognized by IPC synthase for an efficient inhibition.[119]

GbA (Rustmicin, Figure 17) was isolated as a fungal metabolite from *Micromospora chalcea* and from *Streptomyces galbus*, independently,[120–123] and later identified, together with related macrolides, such as galbonolide B (GbB) and their 21-hydroxy analogues (Figure 17) as the third class of IPC synthase inhibitors.[124] In a previous work,[125] the absolute stereochemistries of Gbs were determined by a combination of chemical modifications and total synthesis. GbA, GbB, and their 21-hydroxy analogues[126,127] were effective antifungal agents against several *Candida* species and also in an in vivo mouse model of cryptococciosis. From a chemical standpoint, Gbs are unstable both under basic and acidic conditions. This prompted the synthesis of synthetic analogues modified at different positions of the macrocycle.[128] However, all the analogues were devoid of antifungal activity except for the methylthio analogue at C6 ($R^1 =$ H and $R^2 = SCH_3$, see Figure 17), which showed moderate activity against *Cryptococcus neoformans* (MIC 0.5 mg mL^{-1}), and weak activity against *Candida albicans* and *Saccharomyces cerevisiae* (MICs 64 mg mL^{-1}).

3. Structural modifications of sphingolipids

SLs can be used as targets for the design of new compounds for modulation of their biosynthesis and metabolism. As, in general, the physiological roles of SLs are complex, the synthesis of analogues is generally driven by the need of more specific compounds that can be used as biochemical tools or as new hits for future drug development. In many instances, however, the design of SL analogues is oriented towards the search for selective enzyme inhibitors based on the similarities with the substrate or the product of a particular process.

3.1. Ceramide analogues

Cer is a cellular mediator that takes part in a variety of regulatory processes. Among the different physiological roles attributed to Cer, much attention has been focused on Cer metabolism and function because of its implication in regulation pathways involved in key biologic responses, such as cell stress, cell senescence, and apoptosis.[129] As a result, much effort has been put in the design of Cer analogues with improved antiproliferative properties and higher metabolic stability in the

search of new antitumor agents.[130,131] In this way, an efficient analogue would be able to mimic the apoptotic effects of Cer without taking part in the metabolic pathways leading to other well-recognized mitogenic SLs, such as CerP or S1P (Figure 1).[132] On the other hand, Cer analogues can also be designed as potential inhibitors of any of the enzymes involved in the biosynthesis and catabolism of Cer, thus altering the cellular levels of this lipid to allow a thorough study of its participation in cell regulatory processes. Finally, as Cer is an essential membrane component, incorporation of Cer analogues into artificial membranes and the study of their physical properties can offer new opportunities for disclosing the mechanism of several biological events. This is the case, inter alia, of several viral infections that require an initial binding of the infective cell to specific receptors on the host plasma membrane, a complex process where the participation of membrane microdomains or rafts have been shown.[131] In the use of analogues, however, one should bear in mind that the chemical analogues may have partly or entirely different transport and biochemical properties than the native compounds.

Some of the most relevant structural modifications carried out on the Cer skeleton are summarized in Figure 18.

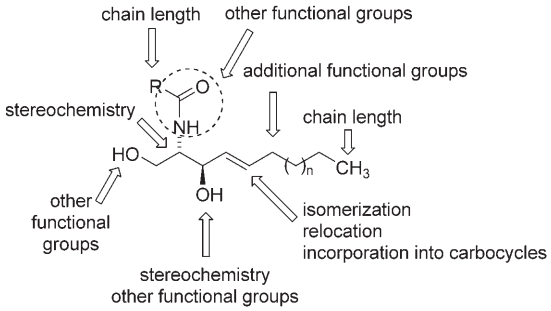


Figure 18. Some of the most significant structural modifications on Cer.

3.1.1. Modifications of the N-*acyl group*

Natural Cer consists of a long chain 2-amino-1,3-diol sphingoid base acylated at the nitrogen atom with a fatty acid acyl chain. As this highly lipophilic chain is usually responsible for the low cell permeability of Cer, analogues with shorter fatty acid acyl chains (typically two to eight carbon atoms),[133] a shorter sphingoid base,[134] or a cationic acyl chain[135–138] have been prepared to circumvent this problem and make them more suitable for biological purposes. In the latter case, several Cer analogues having a pyridium salt placed either internally or at the ω position of the *N*-acyl chain were prepared and their cytotoxicity against various human carcinomas was investigated. In general, these cationic analogues showed a faster cellular uptake and higher anticancer activities in cells than their corresponding uncharged counterparts. They inhibited the growth of cancer cells at low IC_{50} concentrations, whereas higher doses were needed to kill noncancerous cell lines. Structure–activity relationship studies in MCF-7 breast carcinoma cells revealed that the location of the pyridinium salt unit and its overall chain length affected markedly the cytotoxic effects of these compounds. All the terminal-pyridinium ceramides were more potent (IC_{50}/48 h: 0.6–8.0 μM) than their internal pyridinium analogues (IC_{50}/48 h: 8–20 μM). It appears that these analogues accumulate in mitochondria, resulting in a decrease of the mitochondrial membrane potential, release of mitochondrial cytochrome c, and activation of caspases. Inhibition of telomerase activity has also been shown to occur with these compounds.[135]

The *N*-adamantoyl analogue of Cer has also been prepared and it exhibited increased cytotoxicity in two drug-resistant breast tumor cell lines, SKBr3 and MCF-7/Adr (EC_{50}/24 h: 10.9 μM and 24.9 μM, respectively), compared to normal breast epithelial cells (EC_{50}/24 h: $>$ 100 μM).[139]

As Cer can be degraded to Sph by CDases with different subcellular localization and the replacement of an amide moiety by a sulfonamide group is an approach to enhance metabolic stability,[140] *N*-butyl and *N*-octylsulfamoyl Sph derivatives (Figure 19) have also been reported.[141] Both analogues inhibited glucosylceramide and sphingomyelin formation in cultured murine cerebellar neurons in a concentration-dependent manner. Concentration in the culture medium required for efficient inhibition was 10-fold lower for the *N*-octylsulfonamide analogue. A slight inhibition of recombinant human acid CDase was also produced by both compounds.

Replacement of the *N*-acyl moiety in Cer by *N*-alkyl groups has been also explored. This is the case of *N*,*N*-dimethylsphingosine (DMSph),[142] *N*,*N*,*N*-trimethylsphingosine (TMSph), and *N*-hexylsphingosine (HexSph, Figure 19). These analogues have shown proapoptotic,[143] anti-inflammatory,[144,145] and antileukemic properties,[130] respectively, in several experimental models.

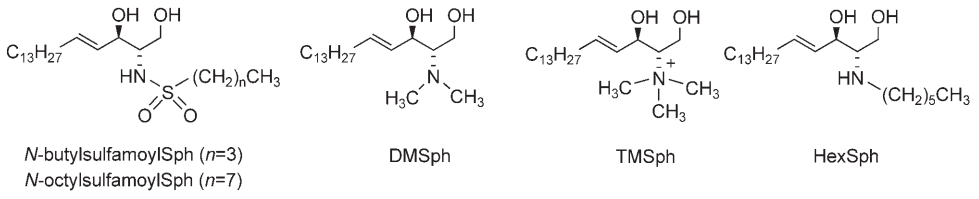


Figure 19. *N*-substituted Sph derivatives.

The Cer urea analogue **60** and the tertiary amide *N*-methyl-Cer (**62**) (Figure 20) have been prepared and evaluated as inhibitors of mitochondrial CDase.[146] The enzyme was inhibited by the urea analogues, but not by *N*-methylCer. Kinetic analysis indicated that the urea analogue was a competitive inhibitor of CDase with respect to the Cer substrate. On the other hand, specific short-chain *N*-acyl derivatives incorporating a fluorescent label into the fatty acid residue for intracellular membrane cellular trafficking studies[147] have been described (Figure 20). These include compound **61**, C6CerNBD, its C1 and C3-*O*-

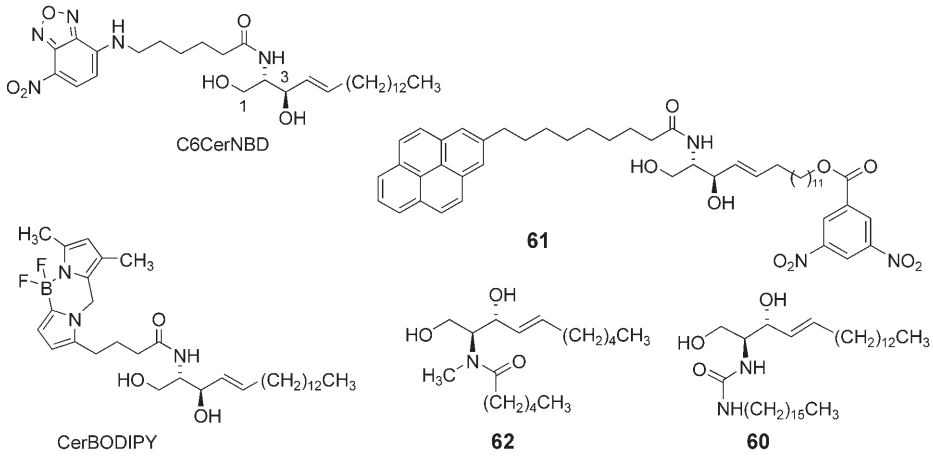


Figure 20. Cer analogues modified at the acyl chain.

methyl derivatives,[148] and the less polar CerBODIPY.[149] Interestingly, the nonfluorescent analogue **61** affords a fluorescent acid after hydrolysis of the amide bond, which represents an excellent pharmacological tool for the study of ceramidase (CDase) inhibitors.[150] Likewise, replacement of the amide moiety by a sulfonamido group has led to Cer derivatives with enhanced metabolic stability.[140] These analogues were evaluated as potential inhibitors of acid CDase.[141]

Biological studies on Cer binding proteins have also benefited from the rational design of specific Cer analogues modified at the *N*-acyl chain. Thus, photoaffinity and radiolabeled markers have been incorporated into the acyl chain to give reactive species upon irradiation, which allows the selective crosslinking of the label to the target protein with formation of a covalent bond with the adjacent receptor residues. Some Cer analogues, designed as carbene (**63** and **64**) or nitrene (**65**) precursors according to the above criteria are shown in Figure 21.[151]

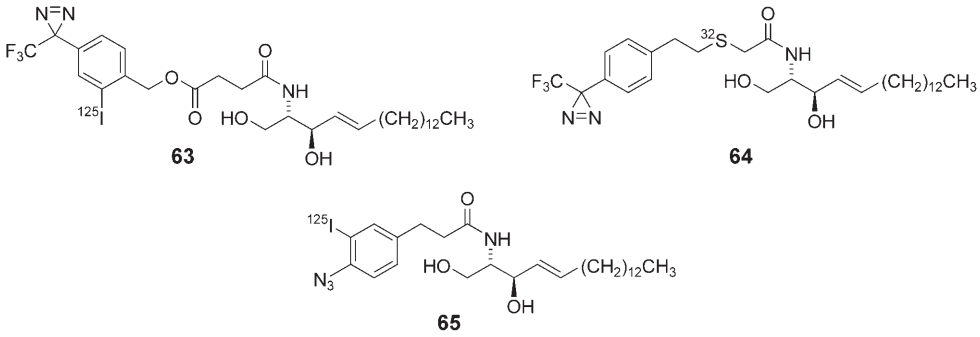


Figure 21. Cer analogues modified at the acyl chain with photoaffinity labels.

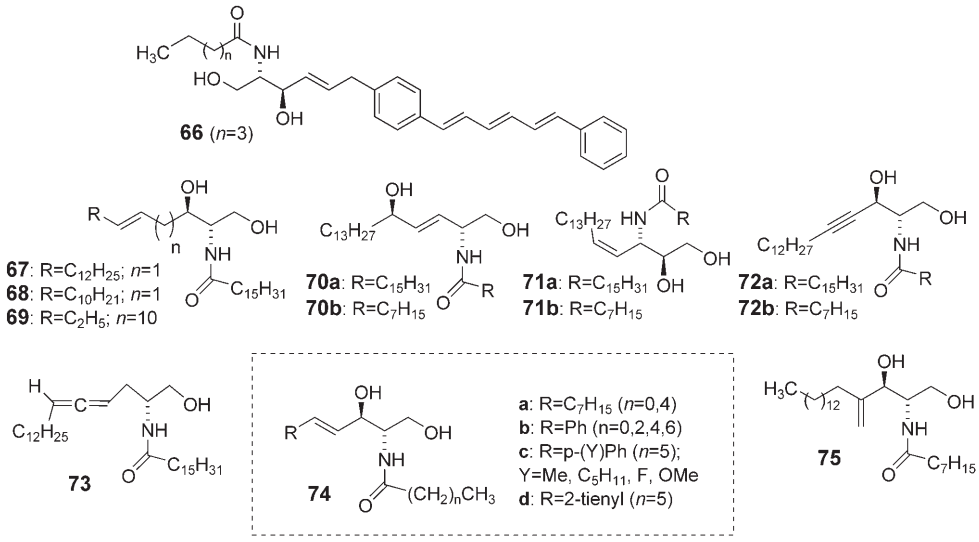


Figure 22. Cer analogues modified at the sphingoid chain.

Ceramide analogues with shorter sphingoid bases have also been reported.[134] A series of D-*erythro*- and L-*threo*-ceramide analogues has been synthesized and investigated for their ability to reverse the inhibitory effects of fumonisin B1 (FB1) on axonal growth in hippocampal neurons. The analogues (Figure 22) contained either a C7 chain, as in the DPH derivative **66**[152] (Figure 22) have also been synthesized and used to study their distribution and metabolism in cultured cells.

3.1.2. Modifications of the sphingoid base

In connection with the above modifications, Cer analogues incorporating a photoaffinity label as part of the sphingoid side chain (**74a**) or a phenyl group (**74b**) instead of the C13 residue present in naturally occurring ceramides, whereas the *N*-acyl chain length was reduced to two to eight carbon atoms. Only the D-*erythro*-analogues reversed the inhibitory effect of fumonisin B1 on the axonal growth of hippocampal

neurons. D-*erythro*-Cer analogues **74a** ($n=0$) and **74b** ($n=4$) (Figure 22) were most active in reversing the inhibitory effects of FB1 on axonal growth, although the mechanism remains unclear. Interestingly, substitution of the alkenyl chain of Cer by a styryl group did not result in a decrease of activity. The finding of a new active Cer analogue with an aromatic chain allows the study of further structure–activity relationships.

As indicated above, these shorter analogues are better candidates for biological studies because of their improved cell permeability. In another context, more profound variations in the nature of the sphingoid base, especially those concerning the double bond stereochemistry (*Z* or *E*), its position along the alkyl chain, and its replacement by other unsaturated or aromatic systems (Figure 22), have also been carried out. Most of the analogues **67**–**73** have been used for the preparation of artificial membranes and the study of their interfacial and other biophysical properties,[153] whereas aromatic derivatives **74c** and **74d** were examined for their influence on axonal growth in hippocampal neurons.[154] These analogues reversed the inhibitory effect of FB_1, but not of PDMP, on accelerated axonal growth in hippocampal neurons. Introduction of a methyl, pentyl, fluoro, or methoxy substituent in the *para* position of the phenyl ring in the sphingoid moiety yielded partly active compounds. Likewise, replacement of the benzene ring with a thienyl group did not abolish the ability to reverse the inhibition of accelerated axonal growth by FB_1. Compound **70b** was evaluated in several tumor cell lines, where it showed increased cytotoxicity (IC_{50} of approximately 20 μM) compared to that for the normal cells (IC_{50} 58.7 μM) after incubation for 24 h.[139] On the other hand, analogues **71b** and **72b** have been used as reference compounds to challenge a new family of dihydroceramide desaturase (DES) inhibitors.[155] More recently, a new Cer analogue **75** with an *exo*-methylene group at C4 has shown higher antiproliferative activity (IC_{50} of 5.9 μM) on mouse embryonic fibroblast cells than natural Cer (IC_{50} of 20 μM).[156] This result indicates that variations in the structural features of Cer can lead to significantly enhanced apoptogenic activity.

Replacement of the Cer 3-OH group by a fluorine atom has been carried out. Thus, both D-*erythro* and L-*threo* Cer short-chain analogues, having an allylic fluorine group instead of the natural allylic alcohol (compounds **76**–**78**, Figure 23), partly reversed FB1 inhibition.[154] In addition to the shortening of the sphingoid chain, double bond reduction to afford dihydroCer analogues, and incorporation of a phenyl group as part of the sphingoid base are some of the modifications carried out in these fluorinated systems.[157,158]

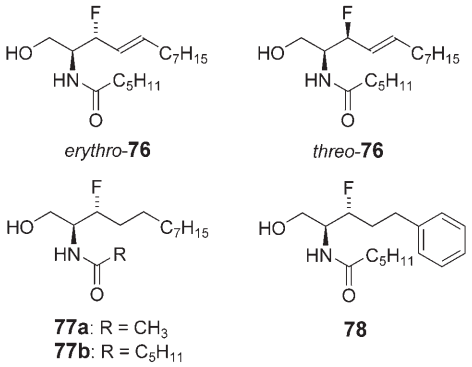


Figure 23. Fluorinated Cer analogues.

Amongst these analogues, compounds **77a** and **77b** showed a very slight activity as DES inhibitors (91% and 73% decrease of enzymatic activity, respectively, at equimolar concentrations of substrate and inhibitor).[159,158] The apoptogenic activity of compounds **76**–**78** (Figure 23) was examined in K-422 cancer cells and some structure–activity correlations were drawn.[157] Thus, shortening of the sphingoid base backbone led to a drastic decrease in apoptogenic activity. Replacement of the secondary hydroxyl group of the short-chain Cer analogues with a fluorine atom resulted in increased apoptogenic activity. Interestingly, in contrast to dihydroCer, fluorinated analogues exhibited apoptogenic activity.

The sphingoid base double bond has also been incorporated into a cyclopropene moiety in the search of DES inhibitors. In this context, the cyclopropene Cer analogue GT11 (Figure 24) has been described as the first inhibitor of this

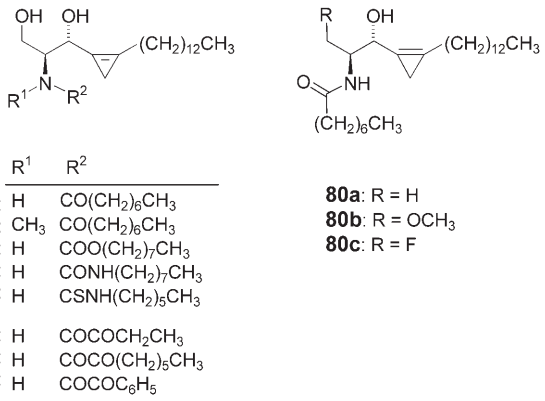


Figure 24. Cyclopropene derivatives with different *N*-acyl functionalities.

enzyme[160] with interesting activities in primary cultured neurons.[161] Kinetic analysis showed that GT11 was a competitive inhibitor (K_i of 6 μM). Modified GT11 analogues with different *N*-acyl chain lengths and functionalities (compounds **79a**–**d**) and metabolically stable substituents at C1 (compounds **80a**–**c**) have been also prepared and evaluated as DES inhibitors (Figure 24).[155,162] Structure–activity relationships could thus be established showing that the presence of a cyclopropene ring in place of the ceramide double bond and the natural 2*S*,3*R* stereochemistry are required for DES inhibition. The presence of a free hydroxyl group at C1 and a similar *N*-acyl chain length in both substrate and cyclopropene inhibitor seem to be essential for in vitro inhibitory activity. In addition, either *N*-methyl substitution (**79a**) or replacement of the amide α-carbonyl methylene by an oxygen atom (**79b**) resulted in inactive compounds. However, both urea (**79c**) and thiourea (**79d**) analogues of GT11, and α-ketoamides **79e**–**g**, did inhibit the desaturation of *N*-octanoylsphinganine to *N*-octanoylsphingosine, although with significantly lower potency than GT11. Further-

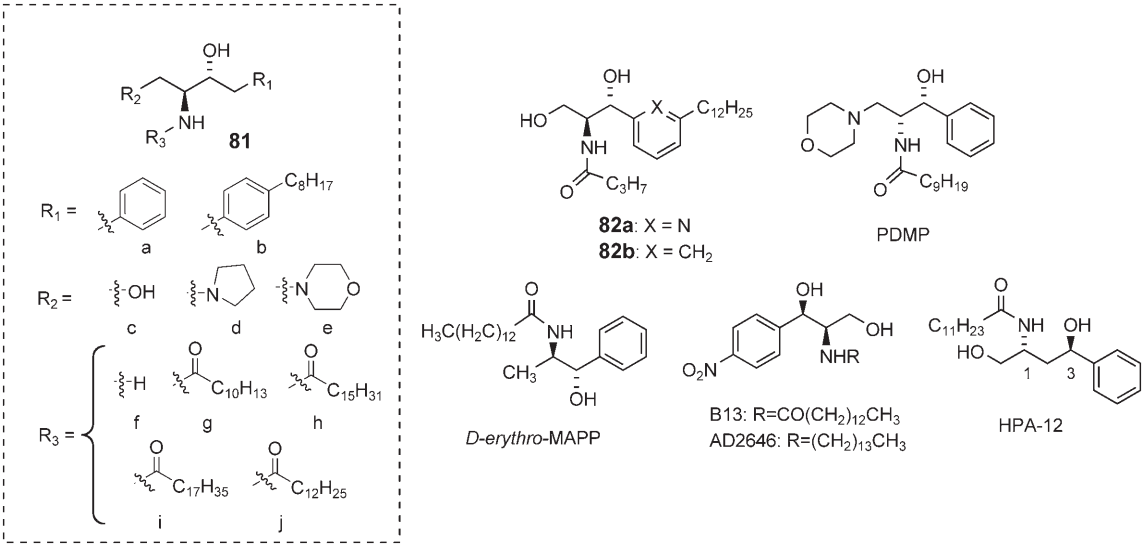


Figure 25. Cer analogues by incorporation of an aromatic ring into the sphingoid chain.

more, the α-ketoamides **79e–g** inhibit the acidic CDase with similar potencies (IC_{50} 52–83 μM). Inhibition of the neutral/alkaline CDase by these compounds requires around 20-fold higher concentrations.

Attempts to modify the biological profile of Cer, led to the incorporation of heteroatoms at particular positions of the sphingoid base, as in 7-oxasphingosine, 7-oxaceramide, its tioamide analogue, and *N*-methyloxaceramide.[163] The apoptosis-inducing properties of these analogues were compared to those of Sph and Cer using a human neuroblastoma (SK-*N*-BE) and a murine promyelocyte-derived (32d) cell line. There were no differences between the effects on cell viability of 7-oxoceramide, its tioamide analogue, and Cer. However, in the presence of the SphK inhibitor DMSph (Figure 19), Cer was more potent than the 7-oxatioamide analogue in 32d cells, whereas opposite results were found in SK-*N*-BE cells. In both types of cells, 7-oxasphingosine and Sph were equally toxic, even in the presence of DMSph.

In a different approach, the sphingoid alkenyl chain present in Cer has been replaced by an aromatic system (Figure 25). This is the case of compounds of general structure **81**, evaluated as selective inhibitors of sphingosine kinase (SphK).[164] Among the analogues tested, **81bcf** and **81adi** were selective inhibitors of human SphK2 over human SphK1. Furthermore, **81adi** did not affect PKC, thus being the first human SphK2-specific inhibitor. The cytotoxicity of Cer analogues **82** in the drug-resistant breast tumor cell lines SKBr3 and MCF-7/Adr, and normal breast epithelial cells has been reported (Figure 25).[139] Compound **82a** produced nonselective cytotoxicity across the three cell types (EC_{50}/24 h = 12.8–16.7 μM). However, **82b** exhibited increased cytotoxicity in the tumor cell lines compared to the normal breast epithelial cells, with EC_{50}/24 h for SKBr3, MCF-7/Adr, and normal breast epithelial cells, respectively, of 18.9, 45.5, and >100 μM.

Another example of replacement of the alkenyl side chain by an aromatic systems is found in HPA-12 and analogues, described as inhibitors of Cer trafficking from the ER to the site of SM synthesis. Among the four HPA-12 stereoisomers, 1*R*,3*R*, which resembles natural ceramide stereochemically, was found to be the most active.[165,166] In these studies, a series of HPA-12 analogues were synthesized in a diastereoselective and enantioselective manner by catalytic asymmetric Mannich-type reactions using a Cu^{II}-chiral diamine complex. Determination of the activity of these analogues on the de novo synthesis of SM in CHO cells showed that the optimal length of the amide side chain is 12 atoms (IC_{50} 50 nM; 70 nM for HPA-12). Likewise, both hydroxy groups are essential for inhibition of SM biosynthesis, as a 20–30-fold reduction of activity occurs by methylation of any of them. Finally, the presence of an additional hydroxy group at C2 position did not enhance the activity.

An aromatic group as sphingoid chain surrogate is also found in other kind of analogues, such as the CDase inhibitors D-*erythro*-MAPP,[167] active on the neutral ceramidase, B13,[168–170] AD2646,[171,172] and its analogues,[173] which inhibit the acidic CDase and have exhibited interesting activities in cancer chemotherapy, the GlcCerS inhibitor PDMP,[101,174] and some recently described analogues[175] (Figure 25). Interestingly, PDMP was the seed for several therapeutic concepts, such as the substrate reduction therapy for sphingolipidosis[176] and induction of apoptosis by ceramide build-up for cancer treatment.[174]

Finally, it is worth mentioning that B13, AD2646, and PDMP present a non natural *threo* configuration, whereas HPA-12 presents the 2-amino-1,4-butanediol core. These observations question the presumed structural equivalence of these analogues with Cer.

Compound HPA-12 is not the only reported example of Cer analogue containing the 2-amino-1,4-butanodiol core. Other 2-amino-1,4-butanediol and also 2-amino-1,5-pentanediol derivatives have been synthesized and evaluated as Cer analogues. Thus, compounds **83** and **84** (Figure 26) were prepared and their apoptotic activities were examined in HL-60 human leu-

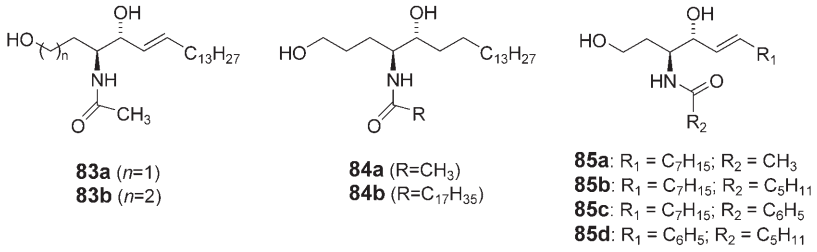


Figure 26. Homosphingolipids.

kemia cells. Interestingly, **83a** and **83b** had apoptotic activities comparable to *N*-acetylsphingosine.[177,178] Likewise, preliminary experiments showed that **83b** was more cytotoxic than *N*-acetylsphingosine in the above cells.[177,178] Homoceramides **85a–d** (Figure 26) have also been prepared and investigated for their potential to reverse the inhibitory effect of fumonisin B on axonal growth. None of the compounds showed any biological activity, probably because of the lack of metabolism to the corresponding glucosylhomoceramides.[179]

The C1-OH group of the sphingoid base has been replaced by other functional groups. Thus, in the context of the structural requirements of mitochondrial CDase inhibitors, the sphingoid base modified Cer analogues 1-*O*-methyl Cer, 3-*O*-methyl Cer and *cis*-D-*erythro* Cer were synthesized, but none of them inhibited the mitochondrial CDase isolated from rat brain.[146] Moreover, both the C1-OCH_3 and C3-OCH_3 analogues of C6-NBD-Cer were prepared and used to prove that Golgi labeling with C6-NBD-Cer is due to the formation of the fluorescent glucosylceramide and sphingomyelin metabolites rather than to the fluorescent ceramide analogue itself.[180]

Replacement of the C1-OH group by a methylthio or a thionium salt (compounds **86a** and **86b**, Figure 27) has been reported.[181,182] Compound **86a** decreased de novo Cer biosynthesis, and de novo formation of SM and of GSLs in a time- and concentration-dependent manner (90% reduction at 10 μM) This compound produced a 2.5-fold increase in the activity of sphinganine kinase, with the consequent depletion of newly formed free sphinganine in cells and reduced de novo SL biosynthesis. As a consequence of depletion of sphinganine levels, 1-methylthiodihydroceramide disrupted axonal growth in cultured hippocampal neurons. Interestingly, the D-*erythro* and the L-*threo* isomers were equally effective, but the corresponding free base and other structurally related compounds did not affect either sphingolipid biosynthesis or neuronal growth.[181,182] No activity data for **86b** has been reported, although the possibility that it acts as a suicide inhibitor because of the alkylating properties of the sulfonium group has been proposed.[181,182]

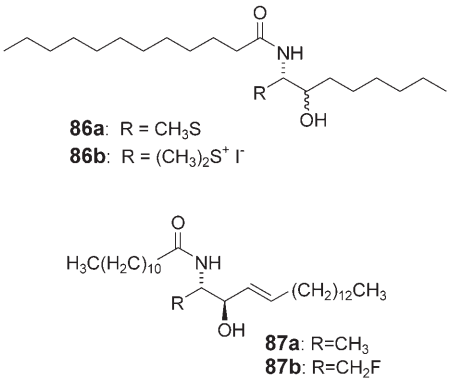
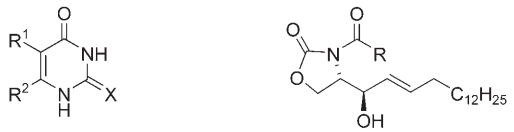


Figure 27. Cer analogues modified at C1 position.

With the aim of achieving metabolically stable derivatives, replacement of the CH_2OH group by methyl or fluorine has also been reported. Thus, compounds **87a** and **87b** (Figure 27) have been prepared from L-serine and D-galactose, respectively. Compound **87b** inhibited SM and GlcCer formation in cultured murine neurons, but only at high concentrations (100 μM) in the culture medium, and showed no proapoptotic properties in HaCaT cells.[141]

Following a combinatorial approach, a Cer library has been constructed and the library components have been screened for their effects on apoptosis in U937 leukemia cells.[183] In general, the most active members of the library had IC_{50} values in the low micromolar range The data revealed several important structural elements of ceramides that are important for apoptogenic acitivity The same Cer library was later reported and screened for their activity on IL-4 production by activated T cells.[184] Some ceramide derivatives with a lauroyl group showed strong inhibitory activities on IL-4 production in both phorbol 12-myristate 13-acetate-activated T cells and antigen-primed cells, suggesting that they can be used for the development of antiallergic agents.

Synthesis of conformationally constrained Cer analogues represents a relatively unexplored approach. Thus, incorporation of the terminal amino diol framework into a heterocyclic thiouracil or uracil system, such as in compounds **88a** and **88b**,[185] has been described (Figure 28). Compound **88a** was the most active at inhibiting proliferation of CCRF-CEM human leukemia cells, followed by compound **88b** (IC_{50} of 1.7 and 7.9 μM, respectively), whereas compounds **88c** and **88d** were inactive. Compounds **88a** and **88b** were able to trigger apoptosis, and the former exhibited antitumor activity in female



88a: $R^1=C_{14}H_{29}$; $R^2=C_2H_5$; X=S
88b: $R^1=C_{14}H_{29}$; $R^2=C_2H_5$; X=O
88d: $R^1=C_2H_5$; $R^2=C_{14}H_{29}$; X=S
88d: $R^1=C_2H_5$; $R^2=C_{14}H_{29}$; X=O

89a: R=CH_3
89b: R=C_2H_5
89c: R=C_6H_5
89d: R=$C_{15}H_{31}$

Figure 28. Conformationally constrained Cer analogues.

CD-1 nu/nu athymic mice bearing a WiDr human colon xenograft. Likewise, in a recent work,[186] cyclic carbamates **89a–d** (Figure 28) were synthesized and the 3-propionyl and 3-benzoyl derivatives (**89b** and **89c**, respectively) showed potent antileukemic activities against human leukemia HL-60 cells with good correlation between cell death and DNA fragmentation

Finally, a group of *N*-acylamino polyols have shown interesting biological profiles as Cer mimetic agents despite their structural dissimilarity with Cer. Most of them are derivatives of serinol, diethanolamine, or propanolamine acylated *N*-acylated with fatty acid residues (Figure 29).[187]

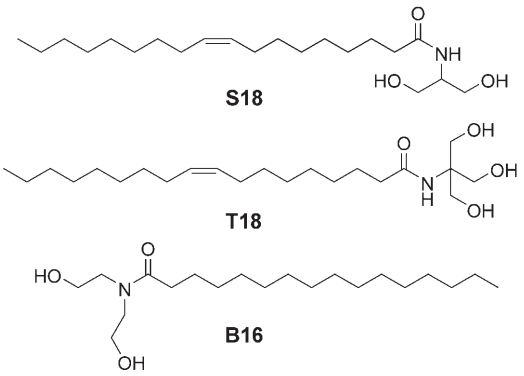


Figure 29. Cer analogues.

3.2. Sphingosine analogues

A structurally close Sph structural analogue is safingol (Figure 30) a modified dihydro Sph with the unnatural *threo* stereochemistry, which has been described as an inhibitor of protein kinase C (PKC)[188] and SphK (K_i 0.10 μM in human platelets for DL-*threo* isomer).[189] However, most of the hitherto described Sph analogues arise from variations on the carbon skeleton and/or functional groups. This is the case of both *Z* and *E* isomers of fluorescent D-*erythro* Sph, bearing the diphenyl-1,3,5-hexatrienyl (DPH) group as fluorophore (the *Z* and *E* deacylated analogues of compound **66** shown in Figure 22), which were synthesized and studied for their distribution and metabolism in cultured human skin fibroblasts. Both isomers were converted to Cer in vitro by microsomal protein from mouse brain although they were poorer substrates than natural D-*erythro* Sph. None of them was acylated to Cer in living cells.[152] Along this line, the *Z* and *E* isomers of both 4- and 5-methylsphingosine have been described. Cellular studies revealed that only (*Z*)-methylsphingosine (Z4MeSph) (Figure 30) was able to interfere with SPT activity in neuronal cultures.[190] The inhibitory effect of the Sph analogues on SPT is mediated by their respective 1-phosphate derivatives and the pronounced effect of Z4MeSph is caused by a high intracellular concentration of Z4MeSph 1-phosphate. In addition, Z4MeSph caused drastic changes in cell morphology of primary cerebellar neurons, and also is able to mimic the mitogenic effects of S1P.[191]

Another series of analogues are characterized by the incorporation of non natural functional groups on the sphingoid base skeleton. This is the case of the SPT inhibitor 2-azidosphingosine,[192] 2-carboxysphinganine,[193] the nitro Sph analogue **90a** (mixture of isomers), and the methyl substituted Sph derivative **91a**, amongst others[194] (Figure 30). Addition of 2-azidosphingosine of either the *E* or *Z*-configuration to primary cultured neurons inhibits de novo Sph and GSL biosynthesis in a concentration-dependent manner (5–50 μM range).[192] Two closely related structural Sph analogues, (*E*)-2-amino-4-octadecen-1-ol (50 μM) and methyl (2*R*,4*Z*)-2-amino-2-hydroxymethyl-3-keto-octadecenoate hydrochloride,[193] had no effect on serine incorporation into Sph and GSLs. Compounds **90a** and one of the diastereomers of **91a**, which was not characterized, inhibited ^{14}C-serine incorporation into SLs (**90a**, 30% reduction at 10 uM; **91a**, 70% reduction at 25 μM). In contrast, the other diastereomer of **91a** and compounds **90b** and **91b** were inactive.

The cytotoxic cyclic analogues **92**,[195] (Figure 30) have also been synthesized and their cytotoxicity in DU-145 (human prostate carcinoma) and HT-29 (human colon carcinoma) cell lines was examined. Some stereoisomers exhibited cytotoxicity against DU-145 cells and were uniformly more cytotoxic against HT-29 cells in comparison with D-*erythro* Sph. However, the range of biological activities among the various stereoisomers of cyclic analogues **92** was relatively small.

Some of the structural variations carried out for the design of Cer analogues have also been applied to Sph and/or dihydro Sph. These analogues arise from replacement of the C3-OH group by a fluorine atom, substitution of the sphingoid chain with an aromatic group, and modification of the natural stereochemistry of the amino alcohol moiety (compounds **93**–**96**,

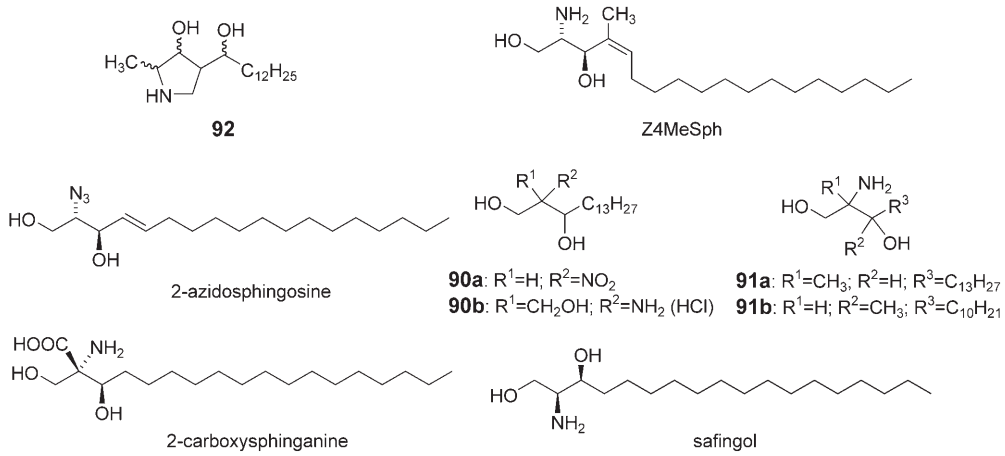


Figure 30. Sph analogues modified at the sphingoid backbone.

Figure 31).[159,158] These analogues were investigated as SphK inhibitors, although the precise enzyme, SphK1 or SphK2, is not specified. All the new compounds are stronger SphK inhibitors than DMS. Replacement of the alkyl chain by an aromatic residue or introduction of a fluorine atom for the 3-hydroxyl group leads, in general, to strong SphK inhibitors. The difference in activity between the *erythro*- and the *threo*-epimers is generally negligible, although the *threo* epimer is a better inhibitor in some cases. The presence of the *4,5*-trans double bond enhanced the capacity to inhibit SphK activity.[159,158]

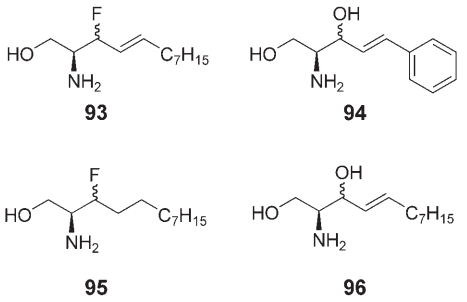


Figure 31. Sph analogues modified at C3 position and/or in the sphingoid chain.

S1P has been shown to bind a family of G-protein-coupled receptors called S1P receptors. Signaling by S1P is a complex process, as cells usually express a number of these receptors on their surfaces.[203] One approach to search for specific S1P receptor agonists or antagonists is the design of S1P analogues resulting from careful structure modification and thorough SAR studies. In a preliminary approach, the four possible S1P stereoisomers, and analogues **97**–**99** were synthesized and evaluated as S1P receptor ligands in binding experiments (Figure 33).[204] Interestingly, some *N*-aryl amide derivatives structurally related to **98**, albeit of opposite configuration at the α-carbon atom, have been recently described as selective S1P1 and S1P3 receptor antagonists. The lead compound in the series, VPC23019 (Figure 33), was found in broken cell and whole cell assays to behave as a competitive antagonist at the S1P1 and S1P3 receptors. A slight modification of the lead compound resulted in VPC25239, which was ten times more potent at the S1P3 receptor.[205]

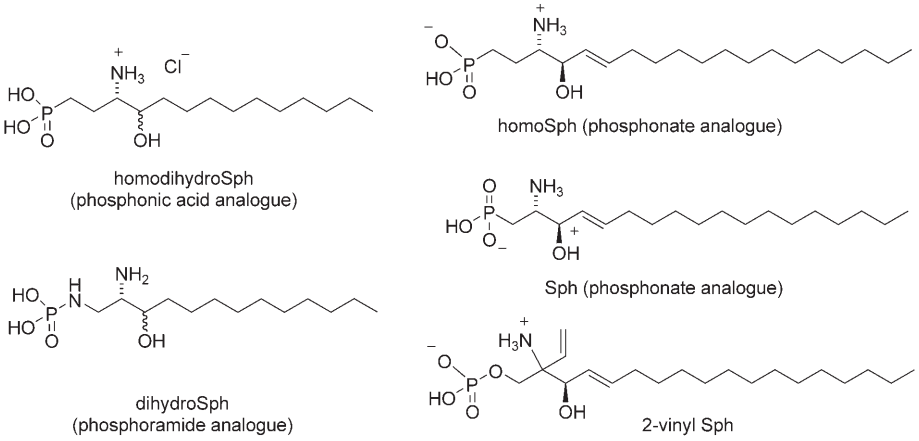


Figure 32. S1P analogues derived from modification of the phosphate group.

3.3. Sphingosine-1-phosphate analogues

As mentioned above, S1P is a SL metabolite formed by the action of SphK that has emerged as a potent bioactive agent for its ability to control numerous aspects of cell physiology both at intra- and extracellular levels.[196] Metabolically stable surrogates of Sph and dihydro Sph of different chain lengths, designed by replacement of the phosphate group by a phosphonate,[197–199] or a phosphoramide moiety[200] have been described in the literature (Figure 32). No biological activity has been reported for these compounds, except for the Sph phosphonate analogue (Figure 32), which is a highly toxic competitive inhibitor of S1P lyase (K_i 5 μM, determined from a tritium-labeled compound),[201] and the 2-vinyl Sph analogue (Figure 32, no stereochemistry is defined), which is also a potent inhibitor of S1P lyase (IC_{50} 2.4 μM).[202]

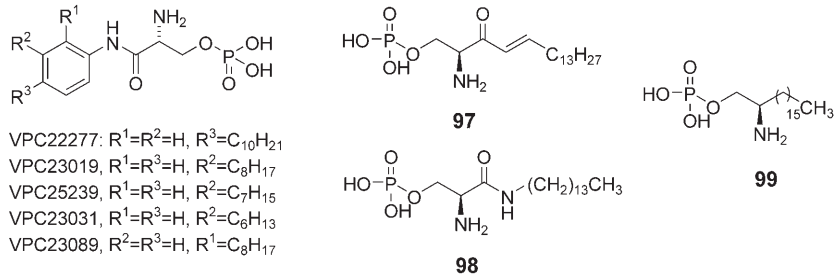


Figure 33. First generation of S1P analogues as selective S1P receptor ligands.

Docking experiments with S1P and a hypothetical S1P receptor model led to the design of four different families of S1P analogues[206] (**100**–**103**, Figure 34) whose binding to $S1P_1$, $S1P_2$, and $S1P_3$ receptors was investigated to conclude that: 1) the C3-hydroxyl group plays an important role in binding to S1Ps, specifically to $S1P_1$; 2) the tail portion (olefin and $nC_{13}H_{27}$)

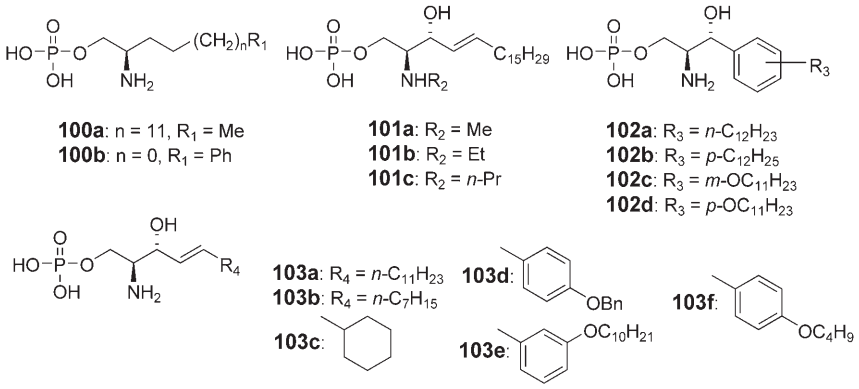


Figure 34. Second generation of S1P analogues as selective S1P receptor ligands.

of S1P can be replaced by *m*-alkylphenyl group without much impact on the binding affinity; 3) *N*-Me-S1P and *N*-Et-S1P show selective binding affinity for S1P1, thus providing a possibility to find selective agonists or antagonists to $S1P_1$ for the first time, and 4) a fine-tuning of the long alkyl chain may be possible in terms of the selectivity and the absolute affinity.

S1P analogues of unnatural *threo* stereochemistry (compounds **104**–**105**, Figure 35) have shown inhibition of S1P-induced Ca^{2+} ion increase in HL60,[207] suggesting that these compounds would compete with cell surface S1P receptors. It is worth mentioning the S1P receptor antagonist activity shown by some analogues arising from replacement of the phosphate group by a bromine atom (compounds **106**, Figure 35). These results show an apparent and hitherto unprecedented bioisosteric equivalence between the above moieties. However, the presence of a phosphate group seems not to be a strict requirement for S1P receptor inhibition. Thus, the natural product spisulosine (Figure 35) is an antiproliferative (antitumoral) compound of marine origin whose mechanism of action has been postulated to be related to antagonism at the S1P receptor.[208] On the other hand, some synthetic compounds, structurally unrelated to S1P, have been described as potent and selective S1P receptor antagonists. This is the case of JTE013 (Figure 35), a selective $S1P_5$ antagonist.[209,210]

Finally, S1P photoaffinity probes have been synthesized by incorporation of specific labels into the sphingoid chain for use in cell studies. Representative examples of ^{32}P-labeled photoreactive S1P analogues are shown in Figure 36.[211]

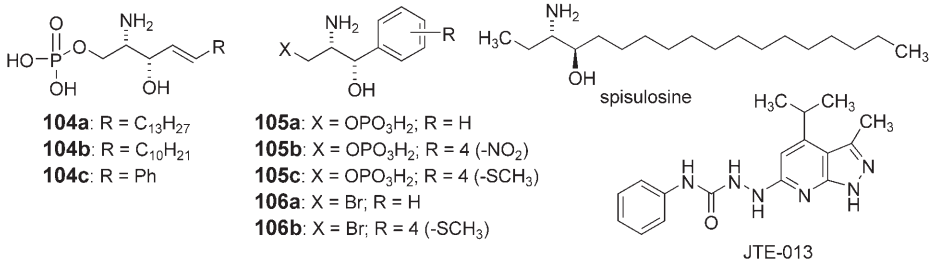


Figure 35. Some S1P receptor antagonists.

3.3.1. S1P receptor agonists based on FTY720 and analogues

As mentioned in section 2.1, structural modifications of the antifungal antibiotic myriocin, a SPT inhibitor, led to the new 2-amino-1,3-propanodiol derivative FTY720 (Figure 4) with interesting immunosuppressive properties.[27] Despite the fact that FTY720 does not inhibit SPT, it is metabolized in vivo to give an *O*-phosphorylated derivative.

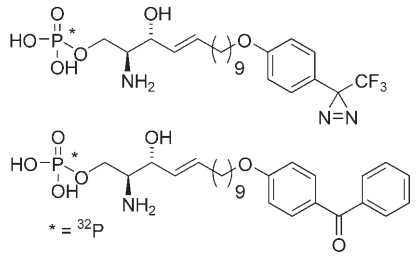


Figure 36. Photoaffinity probes derived from S1P.

This metabolite is an agonist of four of the five known S1P receptors acting through S1P signaling pathways to modulate chemotactic responses and lymphocyte trafficking. Agonism of S1P receptors by the phosphate ester of FTY720 appears to inhibit lymphocyte migration into lymphatic sinuses; this alteration in lymphocyte trafficking has been postulated to lead to the observed peripheral lymphopenia and immunosuppressive efficacy.[212,213] Recent studies[214] have shown that the (*S*)-enantiomer of FTY720-*O*-phosphate shows higher affinity than the (*R*)-enantiomer towards the S1P receptors, in contraposition to previous results.[212,215] Very recently, an azide derivative of FTY720 has been described as the first photoactivatable analogue of this ligand[216] and monoclonal antibodies against FTY720, together with a competitive enzyme immunoassay, have been developed.[217]

Some of the biological actions elicited by FTY720-*O*-phosphate parallel those of S1P. This finding has been rationalized at structural level, as shown in Figure 37. Thus, the three key structural motifs present in S1P (lipophilic tail, amino alcohol linker, and head group), can be also identified in FTY720-*O*-phosphate.

FTY720 is currently under phase II development as an immunomodulator. However, chemical modifications of FTY720 have been undertaken to gain insight into the therapeutic relevance of S1P receptors and provide chemical approaches to defining

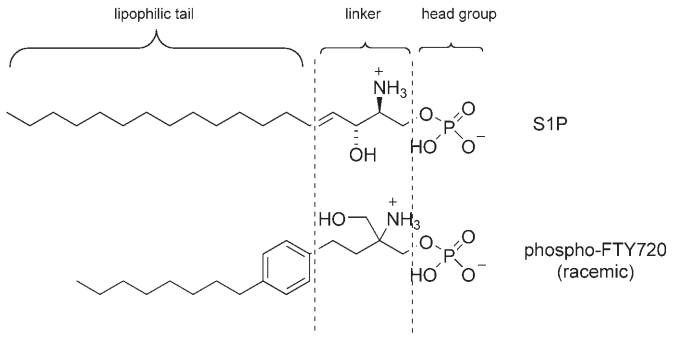


Figure 37. Structural similarities between S1P and FTY720-*O*-phosphate.

the contribution of distinct receptor subtypes to pathology, physiology, and treatment.[218] In this context, KRP-203 (Figure 38) was recently described as a FTY720 analogue with improved biological properties on allograft survival against both acute and chronic rejection in rat skin and heart transplantation.[219] In a recent study, the long-term effectiveness of KRP-203 treatment in combination with a subtherapeutic dose of cyclosporine A on rat renal allografts has been demonstrated.[220]

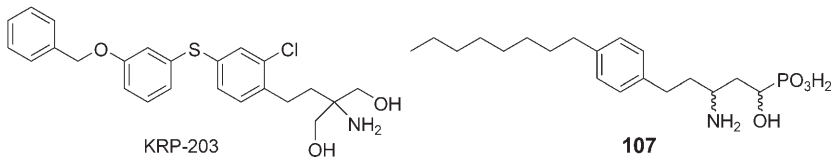


Figure 38. FTY720 analogues.

A series of S1P analogues incorporating an aromatic moiety, reminiscent of that present in FTY720, have been synthesized and tested to establish a SAR for selectivity towards S1P receptors (compounds **108**–**112**, Figure 39).[221,222]

A very important family of FTY720 analogues is that represented by nonhydrolyzable phosphonic acids or γ-amino-α-hydroxyphosphonic acids **107**, bearing a lipophilic chain with the characteristic 1,4-diphenylene unit (Figure 38).[223] This family includes potent agonists of the $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ receptor subtypes.

Further modifications led to simplified analogues derived from 3-(*N*-benzylaminopropyl)phosphonic acid.[224] Some of them showed high selectivity for $S1P_1$ receptors against $S1P_3$ receptors, with reduced cardiovascular toxicity in rodents. From a structural standpoint, it is worth mentioning the presence of additional substituents on the aromatic 1,4-diphenylene unit and that of heteroatoms into the lipophilic tail. From a series of analogues, a rationale for SAR was developed (Figure 40).

Combinatorial approaches to the synthesis of selective $S1P_1$ receptor agonists have also been undertaken.[225] Using high-throughput screening, Jo et al.[226] identified SEW2871 (Figure 41) as a structurally unique, nanomolar selective $S1P_1$ receptor agonist. SEW2871 binds to and activates the $S1P_1$ receptor and initiates a survival signaling pathway similar to that of S1P. In a combinatorial approach to the synthesis of selective $S1P_1$ agonists,[225] the common structural motif present in SEW analogues[226] was incorporated as a lipophilic tail surrogate in a new family of orally bioavailable amino acid FTY720 analogues (compounds **113**, Figure 41). From a structural standpoint, replacement of the phosphonic acid moiety by a carboxylic group and incorporation of the amino alkyl chain into a heterocyclic system are remarkable. Many of the new compounds showed potent selectivity for $S1P_1$ receptor subtypes, high oral bioavailability, and excellent pharmacokinetic profiles in rat, dog, and monkey.[227]

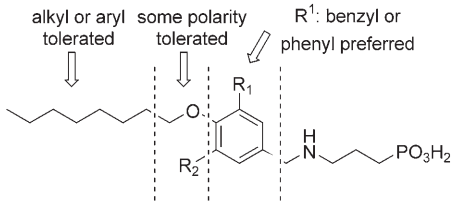


Figure 40. SAR for $S1P_1$ receptor selectivity.

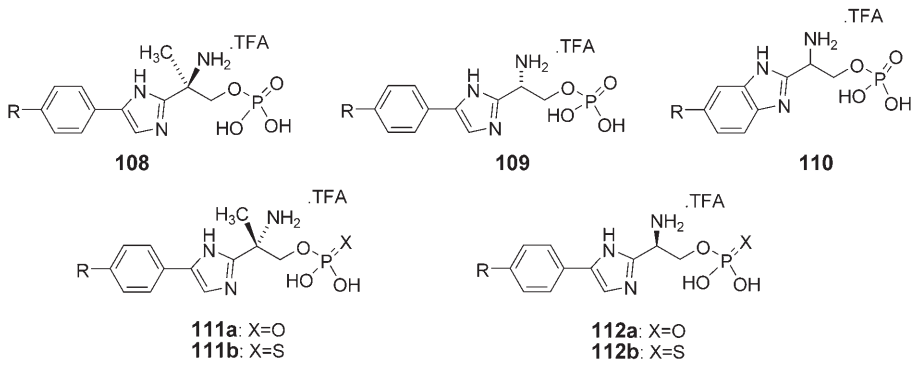


Figure 39. S1P analogues with an aromatic motif at the sphingoid chain.

3.4. Sphingomyelin analogues

Structural variations of SM have been undertaken in the search for modulators of the biological profile of this important sphingolipid. One of the first approaches described in the literature deals with SM analogues modified at the phosphocholine

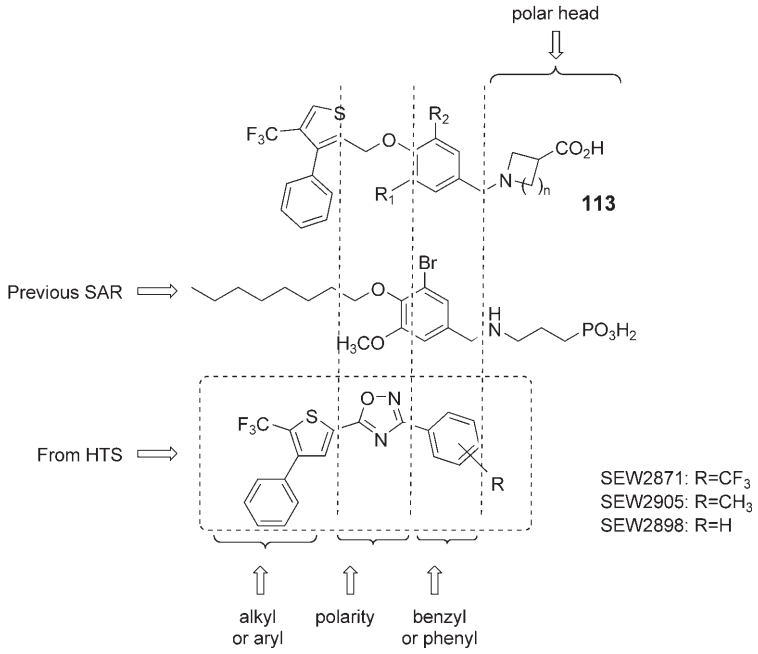


Figure 41. Development of selective $S1P_1$ ligands.

moiety or at the C3 position of the Sph backbone, which were tested as inhibitors of neutral and acid SMase.[228] No inhibitory activity was elicited by either CerP or Cer-1-phosphoethanol-*N*,*N*-dimethylamine, whereas the 3-*O*-methyl and the 3-*O*-ethyl analogues (compounds **114a** and **114b**) were selective inhibitors of neutral SMase (Figure 42) with IC_{50} values of 50 μM and 140 μM, respectively. The size of the alkyl group on the ether moiety was important, as shown by the inability of 3-*O*-tetrahydropyranyl-sphingomyelin to compete with the substrate of neutral SMase. However, when the rat brain acidic SMase was used, no inhibition by the 3-*O*-methyl analogue could be detected. Interestingly, the 3-deoxy analogue showed no ability neither to compete with SM nor to act as substrate of SMase, suggesting that the hydroxyl group is required for substrates.

Along the same line, approaches to SMase inhibitors based on hydrolytically stable SM analogues have been developed. Thus, the phosphodiester group has been replaced by other groups, such as phosphonic (compounds **114c** and **114e**),[229] and difluorophosphonic (compound **114d**)[230] acid monoesters, carboxylic ester (compound **115**),[231] phosphoramide (compound **116**),[232] and thiophosphate (compound **117**),[233] (Figure 42). Phosphonates **114c** and **114e**[229] exhibited moderate inhibitory activity toward SMase from *B. cereus* (IC_{50} 120 and 78 μM, respectively). On the other hand, difluorophosphonate **114d**[230] showed better inhibitory activity than the methylene **114c** and ethylene **114e** analogues and similar activity to phosphoramide **116**,[232] (**114d** and **116**: IC_{50} 57 and 53 μM, respectively). Although the carboxylic ester **115**[231] inhibits neutral SMase from rat brain microsomes (IC_{50} 31 μM), the inhibitory activity increased in the related carbamates, with compounds having an IC_{50} of 2–5 μM. Pyridylmethyl derivatives **118**–**121**,[234,231] with a pivaloylamide group were the most active within this series. Compound **118** prevented Cer generation and apoptotic neuronal cell death in a model of ischemia based on organotypic hippocampal slice cultures.[234] Finally, thiophosphate **117** (Figure 42) has been synthesized and proved to be a useful substrate for monitoring the enzyme activity of SMase by detecting the liberated thiol group with a thiol-sensitive reagent.[233]

Modifications at the 2-amido moiety have also been described, as in the thiourea analogue AD2765[235] (Figure 42). Cell cultures and in vitro studies also showed that this compound inhibited both the hydrolysis of BODIPY-conjugated SM and the synthesis of SM from BODIPY-conjugated Cer. This compound had no effect on other enzymes involved in Cer metabolism, such as acid CDase or GlcCerS. The overall effect of AD2765 on SM metabolism was concentration dependent, and treatment of normal human skin fibroblasts or cancer cells

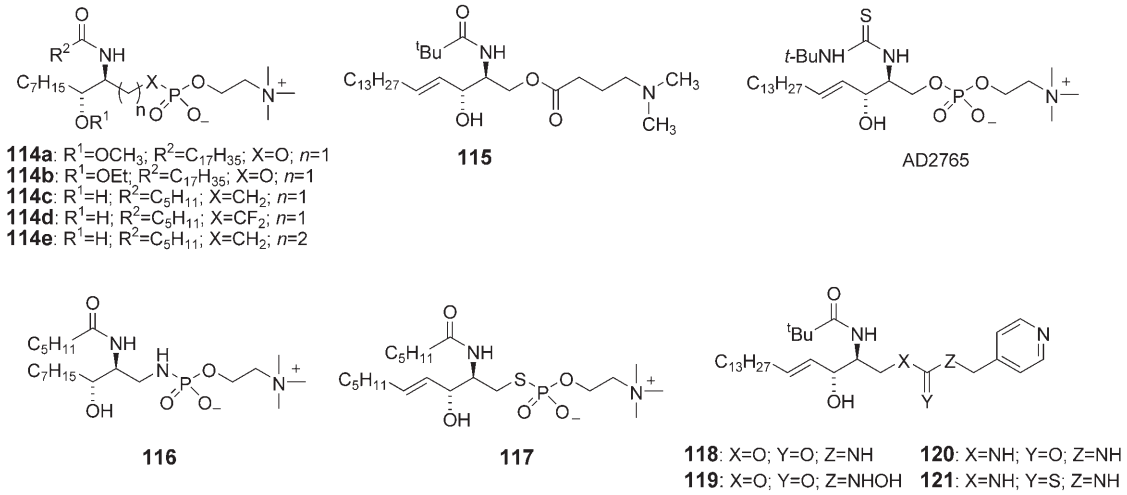


Figure 42. SM analogues.

with this compound (10 μM) led to an increase in cellular Cer and cell death.

Replacement of the long alkenyl chain by a phenyl group and an isosteric difluoromethylene phosphonic acid instead of the phosphodiester moiety are the key structural features of the des-choline derivatives **122**[236] and **123**,[237] which were synthesized as potential SMase inhibitors (Figure 43). Analogue

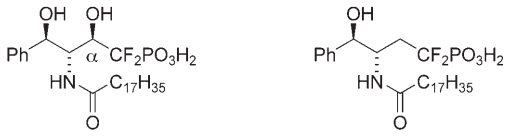


Figure 43. SM analogues by incorporation of an aromatic system into the sphingoid chain.

122 (initially tested as a diastereomeric mixture at the α carbon) produced a noncompetitive inhibition of the bovine brain neutral SMase (IC50 of 400 μM) and suppressed TNF-α-induced apoptosis of PC-12 neurons at a low concentration of 0.1 μM.

An interesting feature of **122** is the installation of additional hydroxyl functionality at the α position of the CF_2 unit. This framework was designed to improve the chelating properties of the analogues, which might favorably interact with Mg^{2+}-dependent neutral SMase. Further studies[237] showed that neutral SMase inhibition significantly increases in analogues with unnatural configurations on the sphingoid base. Thus, *ent*-**122** was approximately four-fold more potent than **122**. This trend was even more striking with deoxy derivatives (**123** versus *ent*-**123**), as *ent*-**123** was found to be approximately 60-fold more potent than **123**. These results reveal that the stereochemistry of the sphingoid chain backbone is critical for interaction with the enzyme.

The mode of inhibition for *ent*-**123** was determined to be noncompetitive (K_i 1.6 μM). This analogue is also a good inhibitor of acid SMase from bovine brain lysosomes, showing a 48% inhibition at a concentration of 3.3 μM.[237] These results are in line with those found for scyphostatin (see section 2.3 and Figure 6), one of the most potent small-molecule inhibitors of SMase.

Finally, as already mentioned for other SL analogues, a SM analogue incorporating a photoaffinity benzophenone-type probe in the long-chain base and a ^{14}C isotopic label in the choline methyl groups has been synthesized for the study of ligand-binding sites.[238]

4. Other active compounds

Some structurally unrelated compounds have shown interesting properties as modulators of SL metabolism and biosynthesis. This is the case of the S1P lyase inhibitor 4-desoxypyridoxine,[239] MS209 (Figure 44), a quinolone-derivative that has been reported as a SM synthase inhibitor,[240] and D609 (Figure 44),

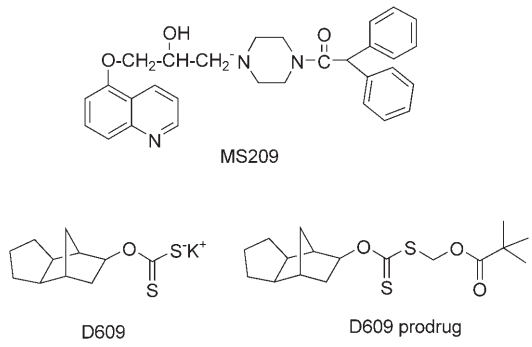


Figure 44. Miscellaneous SM synthase inhibitors.

another SM synthase inhibitor,[241–243] which was first reported as inhibitor of phosphatidylcholine-specific phospholipase C from *Bacillus cereus*.[244] As the xanthate group is chemically unstable in solution and is readily oxidized, chemically stable *S*-(acyloxymethyl) analogues of D609 have been reported. Among them, the pivaloyloxymethyl analogue (D609prodrug in Figure 44) inhibited SM synthase more efficiently than D609 in cells.[245]

There are a number of compounds, structurally unrelated to SM, that have found application as SMase inhibitors. This is the case of desipramine, SR33557, NB6, C11AG, and GW4869 (Figure 45).

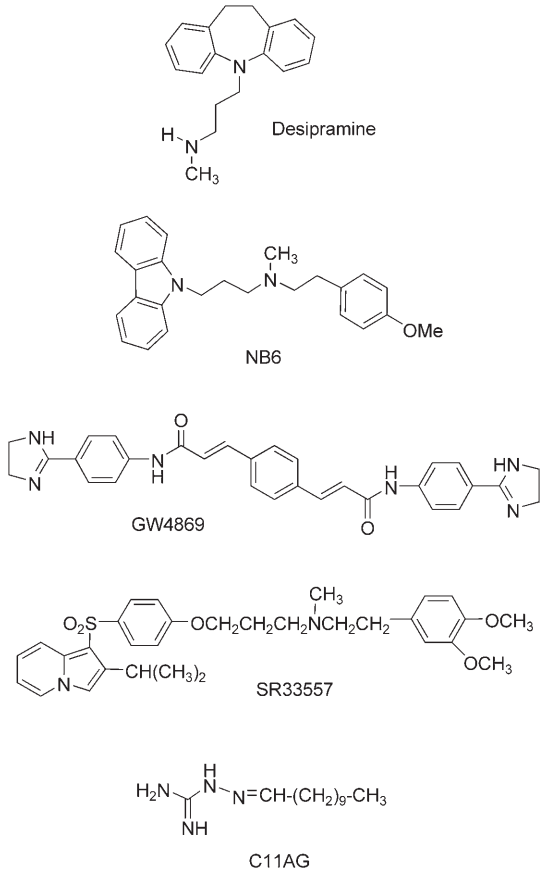


Figure 45. Miscellaneous compounds used as SMase inhibitors.

Although desipramine has been frequently used as an acid SMase inhibitor, it is not a real inhibitor and is rather unselective, as other hydrolases are also affected. This compound, and possibly also other similarly acting tricyclic antidepressants, induces proteolytic degradation of acid SMase[246] by interfering with the binding of the enzyme to the lipid bilayers and thereby rendering it susceptible to proteolytic cleavage by lysosomal proteases.[247] Compound SR33557 is also a specific acid SMase inhibitor[248] that avoids apoptosis induced by TNF in ML-1a cells. Recently, NB6 has been reported as an inhibitor of the SMase gene transcription[249] and it has been used in in vivo experiments to propose that activation of the plasmatic isoform of acid SMase may play a critical role in the development of apoptosis and organ failure in sepsis.[39] Amtmann et al. described a series of new guanidinium derivatives as inhibitors of neutral SMase. Lipophilicity was found to be correlated with the inhibitory potential of the compounds and the undecylidene aminoguanidine C11AG (Figure 45) was the most active.[250] Compound GW4869 (Figure 45) was discovered during high throughput screening on Mg^{2+} dependent neutral SMase. This compound acted as a noncompetitive inhibitor with the substrate SM and exhibited significant and specific inhibitory activity with no or minor inhibitory activity against other hydrolytic enzymes, such as bacterial phosphatidylcholine-PLC and bovine protein phosphatase 2A. Interestigly, GW4869 showed no inhibition of the human acid SMase.[251]

Finally, a screening of chemical libraries has led to the identification of some potent human SphK inhibitors, in particular the isoform 1, structurally unrelated to the SL structure. Some of these compounds exhibit SphK inhibitory activities in the nanomolar range and high selectivity towards SphK in front of other human lipid and protein kinases (compounds SKi and **124**–**126**, Figure 46).[252] Likewise, Gamble et al.[253] have reported that phenoxodiol (PXD), a synthetic analogue of genistein, inhibits SphK, and suggest that this activity may account in part for the proapoptotic and antiangiogenic properties exhibited by this compound in most cancer cells.

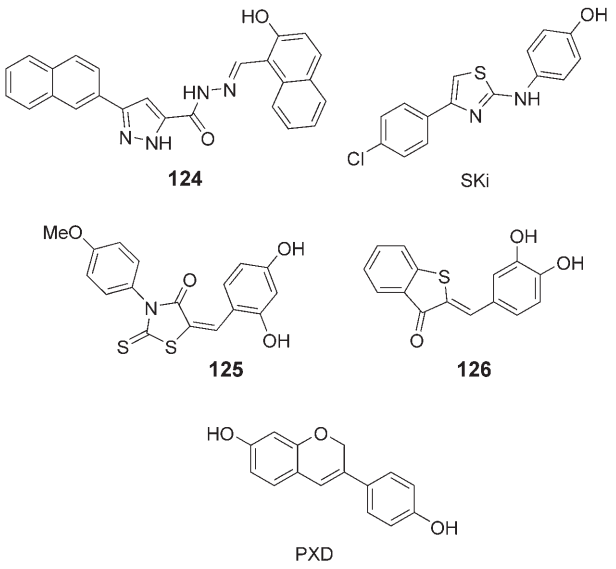


Figure 46. SphK inhibitors.

5. Perspectives and outlook

Over the last years, synthesis of SL analogues has consolidated as a useful complementary tool to unravel the biochemical functions of bioactive lipids. This endeavor requires a delicately orchestrated interplay between organic synthesis, molecular biology, physical biochemistry and, to some extent, the participation of genomics, proteomics, and the emerging field of lipidomics.[254] As in some other areas of medicinal chemistry, lipids can now be considered targets for therapeutic intervention in some diseases, such as sphingolipidoses, cancer, diabetes, atherosclerosis, and viral infections. Classical approaches rely on chemical manipulations of selected natural products known to interfere with SL biosynthesis and/or metabolism. In addition, modification of the SL structure can also provide interesting analogues with enhanced metabolic stability or selectivity towards specific receptors. However, one of the most promising approaches for the development of new chemical entities for the modulation of SL functions relies on the design of analogues able to interfere with their role as "dynamic" membrane components. As such, SLs take part in the formation of specific domains or rafts together with glycerolipids and cholesterol. The importance of rafts for protein recruiting and clustering in signaling processes, and also in infective events, has opened new avenues in this exciting field where chemistry and biophysics are still able to provide their utmost in the years to come.

Abbreviations

AbA	Aureobasidin A
CDase	ceramidase
Cer	ceramides
C6-NBD-Cer	*N*-[6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]hexanoyl]-D-*erythro*-sphingosine
CerP	ceramide-1-phosphate
CerS	sphinganine *N*-acyltransferases
DAG	1,2-diacylglycerol
DES	dihydroceramide desaturase
DMSph	*N*,*N*-dimethylsphingosine
FB1	Fumonisin B1
α-Gal A	α-galactosidase A
GbA	Galobonolide A
GIPC	glycosylinositolphosphorylceramide
GlcCer	glucosyl ceramide
GlcCer β-glucosidase	glucosylceramide β-glucosidase
GlcCerS	glucosyl ceramide synthase
GSLs	glycosphingolipids
HexSph	*N*-hexylsphingosine
HTS	high-throughput screening
IPC	inositol phosphorylceramide
Khf	Khafrefungin
LacCer	lactosylceramide

MIPC	mannosyl inositolphosphoryl ceramide
$M(IP)_2C$	mannosyl bis(inositolphosphoryl) ceramide
NBDGJ	*N*-butyldeoxygalactonojirimycin
NBDNJ	*N*-butyldeoxynojirimycin
PDMP	1-phenyl-2-decanoylamino-3-morpholino-1-propanol
PhytoCer	Phytoceramide
PhytoSph	Phytosphingosine
PKC	protein-kinase C
S1P	sphingosine-1-phosphate
SAP	saposine
SAR	structure-activity relationships
SL	Sphingolipid
SM	Sphingomyelin
SMases	sphingomyelinases
Sph	sphingosine
SphK	sphingosine kinase
SPT	serine palmitoyl transferase
SRT	substrate reduction therapy
TMSph	*N*,*N*-trimethylsphingosine
Z4 MeSph	(*Z*)-4-methylsphingosine

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